Assessment of Chronic Mercury Exposure and Neurodegenerative Disease

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1. Executive Summary

Purpose

The purpose of this study was to determine suitable methods for assessment of chronic mercury exposure and associated neurodegenerative diseases.

Methods

This study combines an analytical review of the scientific literature with statistical analysis of cross sectional survey data (1999-2000 and 2001-2002 NHANES combined population, Women ages 16-49 years). From a review of toxicological evidence and chronic mercury exposure trials, blood I-Hg detection and blood CH_3Hg concentration are determined as the appropriate bioindicators for chronic mercury exposure. In the NHANES survey population, statistical analysis was employed to evaluate associations between both bioindicators for chronic mercury exposure (blood I-Hg and CH_3Hg) and the biochemical profile markers associated with the three main targets of mercury effect and deposition in the human body: the pituitary, immune system, and liver.

Background

Since the industrial revolution, global atmospheric levels of the neurotoxin mercury have risen beyond 300% of natural levels due primarily to emissions from the burning of coal. Mercury is released into the atmosphere from natural sources, degassing from the earth's crust, and from industrial sources, primarily from the burning of coal. Due to atmospheric transport, mercury is deposited locally and globally, in soil and water. There, microbial agents biotransform atmospheric mercury into more toxic, organic forms. Mercury returns from the environment bound for human absorption in our food chain, primarily in the consumption of fish. Other major sources of human exposure include dental amalgams and vaccines containing the preservative, Thimerosal (Thiomersal).

At this time, scientific consensus agrees that the global rate of mercury deposition may be increasing over time[1]. In addition, the latest studies suggest that as global mercury deposition increases, the incidence of the most closely associated neurodegenerative diseases such as Autism and Alzheimer's Disease are rising as well [2, 3]. Recent studies suggest that the incidence of Autism and Alzheimer's Disease may be rising in heavily industrialized countries around the world, in regions where unprecedented mercury levels have recently been found in women and children, fish and animals, rice and soil [4]. These trends support the theory that both chronic mercury exposure and associated risks of neurodegenerative disease may rise within the general U.S. population.

Toxicology

Acute exposure to mercury triggers a toxic response upon reaching a critical concentration, or threshold. This toxic threshold varies within populations as baseline mercury concentrations and rates of excretion vary within populations. Liver, microflora, immune system, and diet may become less effective means of mercury elimination as a result of previous exposure and rising mercury deposition themselves. If the rate of mercury deposition increases, risks of associated neurodegenerative disease, such as Alzheimer's Disease and Autism may increase rapidly, especially in the most susceptible populations.

There are three forms of mercury: elemental mercury (Hg), inorganic mercuric ions (I-Hg), and the organic compounds, methyl mercury (CH_3Hg) and dimethyl mercury. Elemental mercury vapor and the organic mercury compounds are lipophilic, readily absorbed by the human body, and cross the blood brain barrier and placental barriers. Elimination of organic mercury from the human body is dependent upon gastrointestinal microflora.

In tissue of the human body, organic mercury (CH_3Hg) is demethylated into inorganic mercury (I-Hg) and forms lasting deposits. Measurement of inorganic mercury (I-Hg) is subject to large variation due to loss of sample during storage and vaporization. Improvements in the measurement of inorganic mercury may enable accurate measurement of mercury deposition within the human body. Organic mercury levels reflect recent chronic mercury exposure. Taken together, the speciation of two blood mercury forms, Inorganic (I-Hg) and Organic (CH_3Hg), may best estimate chronic exposure and deposition. Assessment of chronic mercury exposure is necessary to ascertain individual baseline levels of mercury and susceptibility to exposure within populations.

Health Risks

Toxicological studies present strong evidence that chronic organic mercury exposure results in persistent, inorganic mercury deposits in target systems of the human body. The pituitary is a primary target for mercury deposition in the brain. The liver and immune system are main targets for mercury deposition in the body. A review of the scientific literature presents compelling evidence that mercury exposure and deposition are linked to neurodegenerative disease, particularly Alzheimer's Disease and Autism. The latest studies suggest the incidence of these most closely associated neurodegenerative diseases are rising as well [2, 3]. This rising incidence of neurodegenerative diseases that are most closely associated with mercury exposure may be occurring in heavily industrialized countries around the world, in regions where unprecedented mercury levels have recently been found in women and children, fish and animals, rice and soil [4].

Recently, the first clinical trial on autistic children showed an improvement of symptoms after treatment with mercury chelation[5]. This treatment was linked to low blood androgen levels in autistic children. Luteinizing Hormone is an androgen-regulating hormone that is produced in the pituitary, a main target of mercury deposition. As global mercury deposition increases, it is logical to assume increased deposition in the human pituitary and immune system may elevate risks of associated neurodegenerative disease.

Method For Assessment of Chronic Mercury Exposure

Previously published data from a study of chronic methyl mercury exposure on adult monkeys were analyzed to determine bioindicators for assessment of chronic exposure. Evidence for the demethylation of methyl mercury into inorganic mercury deposits in the brain, suggests that both inorganic mercury and methyl mercury should be used for assessment of chronic mercury exposure. The regression of I-Hg to CH_3Hg was a successful method for distinguishing between exposure groups. I-Hg determined long-term exposure levels and CH_3Hg determined time since last exposure. This study concluded that links between chronic organic mercury exposure and associated diseases should be investigated using the method for assessment of chronic mercury exposure presented in this study; one that utilizes both blood I-Hg and CH_3Hg concentrations as bioindicators of chronic mercury exposure.

Mercury and the Biochemical Profile

The National Health and Nutrition Survey (NHANES, 1999-2002) was used to investigate associations between bioindicators of chronic, organic mercury exposure and selected biochemical markers that profile the main target sites for inorganic mercury deposition. The reported association between organic and inorganic mercury levels in the blood are consistent with previous evidence that demethylation of organic mercury is a contributing source of I-Hg deposition [6]. This association provides evidence that organic mercury demethylates into inorganic mercury deposits within the human body. Cross-sectional analysis revealed that in the older population, the NHANES population-averaged means for pituitary, liver, and immune system biomarkers were all associated with bioindicators for chronic mercury exposure. For the first time in the general U.S. population, these results provide significant proof of a direct interaction between chronic, organic mercury exposure, inorganic mercury deposition, and effect on target systems within the human brain and body. For the first time in the general human population, this study provides strong evidence of a biological mechanism to link between chronic mercury exposure and Alzheimer's Disease. The interaction between mercury deposition and LH should be further investigated as a causative mechanism in the development of Alzheimer's Disease.

Policy Review

Government policy regarding the risks posed by mercury exposure is influenced by the many industries that profit from its sale, application, and emissions. In Spring, 2004, attorney generals from ten states and 45 senators asked the Environmental Protection Agency (E.P.A.) to scrap the new "clear skies" proposal, saying it was not strict enough. But instead, the Bush administration went ahead and set forth a new proposal to delay all mercury emission restrictions until 2018.

In 2004, the IOM concluded there was no evidence to link vaccines and Autism. Policy regarding chronic mercury exposure suffers from political and scientific uncertainty. In order to create effective public health policy regarding chronic mercury exposure, and to assess links with associated disease, a suitable method for assessment of chronic mercury exposure is needed. Links with associated disease are impossible to assess without a method for assessment of chronic mercury exposure to determine baseline exposure and deposition levels.

Conclusion

Due to the bioaccumulation of organic mercury in food sources, atmospheric deposition of mercury vapor ultimately deposits mercury in the human pituitary, liver, immune system, adrenals, and kidney. The accumulation of targeted mercury deposition may disrupt the endocrine and immune systems, damage the delicate balance between inflammation and suppression, and elevate risks of neurodegenerative disease. As global deposition of atmospheric mercury increases, it is logical to assume that the rate of chronic mercury exposure and deposition in target areas of the human body will increase as well.

Our analysis of chronic mercury exposure trials tested a hypothesis that a suitable method for assessment of chronic mercury exposure would observe the change in I-Hg that results from CH_3Hg exposure, demethylation, and deposition. The regression of I-Hg to CH_3Hg was effective at distinguishing different exposure groups in a trial of chronic mercury exposure. This is significant as it provides a method for assessment of chronic organic mercury exposure and inorganic mercury deposition.

This study concludes that blood I-Hg and methyl mercury levels should both be accurately measured in order to serve as bioindicators for the assessment of chronic mercury exposure. Our analysis of the NHANES population reports an association between organic and inorganic mercury levels in the blood. This is consistent with other studies that demonstrate demethylation of organic mercury as a contributing source of I-Hg deposition within the body. Data are sufficient to conclude there is an association between chronic, organic mercury exposure and inorganic mercury deposition in target systems of the human body. Our study reports evidence that chronic mercury exposure and resultant deposition are associated with changes in biochemical markers for the liver, immune system, and pituitary. Luteinizing hormone, white blood cell count, and bilirubin levels are biomarkers associated with chronic, organic mercury exposure. This is significant as it demonstrates for the first time within the U.S. population, that chronic, organic mercury exposure is associated with targets of inorganic mercury deposition. The reported associations between chronic mercury exposure and luteinizing hormone suggest a biological mechanism to link chronic mercury exposure and the development of neurodegenerative disease.

Keywords and Abbreviations

Chronic mercury exposure, neurodegenerative disease, National Health and Nutrition Survey (NHANES), inorganic mercury (I-Hg), methyl mercury (CH_3Hg), total mercury (T-Hg), mercuric ions (Hg++), elemental mercury (Hg), Luteinizing hormone (LH), White Blood Cell Count (W.B.C.), Alzheimer's Disease (AD), Autism.

Tables and Figures

2. Background

Introduction

In 1968, both Sweden and Japan restricted mercury containing pesticides and Nature published this scientific opinion; "We feel that the example set by these two countries should be followed elsewhere before concentrations of mercury reach a point where methyl mercury is being titrated in humans as well as fish" [7]. Now, forty years after the warning in Nature, mercury in the blood of the U.S. population is being titrated. Elevated blood mercury levels, beyond what was considered "without increased risk of adverse neurodevelopmental effects associated with methyl mercury exposure", were found in eight percent of the NHANES American survey population (1999-2000)[8].This study estimated that due to mercury exposure, 300,000 to 600,000 American children would be born with elevated risks of neurodevelopmental disorders during those years.

At this time, scientific consensus agrees that the global rate of atmospheric mercury deposition may be increasing over time[1]. The latest studies suggest that as the rate of global mercury deposition increases, the incidence of the most closely associated neurodegenerative diseases such as Autism and Alzheimer's Disease are rising as well [2, 3]. Recent studies suggest that the incidence of Autism and Alzheimer's Disease may be rising in heavily industrialized countries around the world, in regions where unprecedented mercury levels have recently been found in women and children, fish and animals, rice and soil [4]. These global trends support the theory that both chronic mercury exposure and risks of associated, neurodegenerative disease may rise within the general U.S. population.

The purpose of this study was to determine suitable methods for assessment of chronic mercury exposure and risks of associated neurodegenerative diseases. Currently, there is no method to measure long term, chronic mercury exposure. This inability to quantify chronic exposure makes it difficult to assess links with disease, and to develop effective policy and regulation concerning mercury exposure.

Source and Exposure

Since the industrial revolution, global atmospheric levels of the neurotoxin mercury have risen beyond 300% natural levels due primarily to emissions from the burning of coal. Mercury is released into the atmosphere from natural sources, one third from degassing of the earth's crust, and from industrial sources, two thirds primarily from the burning of coal. Due to atmospheric transport, mercury is deposited locally and globally, in soil and water. Oxidized forms of mercury that are released from point source plumes may deposit locally [1]. Upon deposition, microbial agents biotransform elemental mercury into more toxic, organic forms. Mercury returns from the environment bound for human absorption in the food chain, primarily from the consumption of fish. In the U.S., geographic variability in the mercury concentrations of fish affects the variability of mercury exposure between populations [9]. In a recent study on mercury concentrations in the fish from lakes around Western America, mercury concentration in fish was directly associated with the atmospheric transport and deposition of mercury vapor[10]. Other major sources of human exposure include dental amalgams and vaccines containing the preservative, Thimerosal (Thiomersal).

Mercury, commonly known as quicksilver, is the only metal that is liquid at room temperature. The element is volatile and vaporizes into a monatomic, mercury gas. Mercury volatility results in a global dispersion of mercury vapor. The natural source of mercury originates in the degassing of the elemental form from the earth's crust [11]. The gas condenses in clouds that travel through atmospheric transport for up to a year before it settles, deposits and revolatizes. Upon deposition, terrestrial and

aquatic microbes biotransform elemental and inorganic mercury into a methylated, organic form that is highly absorbable through digestion. The organic form, methyl mercury (CH_3Hg), is 100 times more toxic than inorganic mercury [11]. Mercury emissions return from the environment in the food chain as a biotransformed organic compound bound for human consumption. Global anthropogenic emissions of mercury are estimated to range between 2000 and 6000 metric tons per year. China alone is believed to emit about 1000 tons of mercury annually. In comparison, U.S. anthropogenic mercury emissions are estimated to be about 158 tons per year. A major source of atmospheric mercury that deposits in California originates from Chinese coal burning plants. Other industrial sources include natural gas, crude oils, the refining of petroleum products, sewage treatment facilities, batteries, light bulbs, thermometers, pesticides and fungicides [11 12 13 14].

For the human population, chronic mercury exposure originates primarily from fish consumption, dental amalgams, and vaccinations [11 15 16]. It is estimated that the average American receives chronic mercury exposure from food (20-75 ug/day), water (2 ug/day), and air (1 ug/day) [15]. Elemental mercury exposure is primarily from dental amalgams containing elemental mercury. The mercury vapor released from dental amalgams is presumed to account for the majority of mercury detected in human urine [11]. Consumption of fish is considered to be the major source of human exposure to organic mercury in the U.S. population [17]. In a population dependent on fish intake, studies found that mercury levels rose three-fold [11]. The U.S. Environmental Protection Agency (EPA) has lowered their advisory on "acceptable" levels of mercury exposure and advises pregnant mothers against eating more than three fish a month for fear of neurological effects on the unborn child. The infant and developing fetus are particularly susceptible to the effects of mercury and face chronic mercury exposure from the mother, mother's diet, and mother's dental amalgams [16]. After birth, the infant is further stressed due to the entire program of vaccines. Thimerosal, a vaccine preservative containing organic mercury accounts for an estimated 100% increase in additional infant mercury exposure. Mercury from vaccine preservative is injected directly into the blood stream and not subject to excretion by the gut. Before removal of timerosal from vaccines, the usual program of vaccinations resulted in more infant mercury exposure than is recommended by the EPA [18].

A dose response relationship analysis of methyl mercury and cases of poisoning reveals a toxic threshold in blood mercury levels that triggers disease response in acute exposure [19]. With rising baseline levels of chronic mercury exposure, the toxic threshold for poisoning may decrease. The Swedish Expert Group (1971) calculated the average long-term daily intake of methyl mercury associated with adverse health affects at 4.3 ug/kg/day or a steady state blood level of 200ug/L. The EPA's RfD for ingested CH_3Hg is 0.1 ug/kg/day which was reduced from 0.5 ug.kg/day due to increased concerns over adverse health risks [20].

According to a panel of scientific experts, the rate of atmospheric mercury deposition may be increasing due to industrial emissions and climactic changes[1]. This likely rise in the rate of mercury deposition may signal a significant increase in the origins of global mercury exposure.

Toxicology

On the basis of toxicological characteristics, there are three forms of mercury, elemental (Hg, metal and vapor), inorganic mercury (I-Hg, mercuric ions) and organic compounds (CH_3Hg, dimethyl mercury, and ethylmercury found in thimerosal vaccine preservative) (see figure 1.0). In 1968, the renowned scientific journal, Nature, published results from the biochemistry division of the University of Illinois. In this study, dimethyl mercury was shown to be the final product in the bacterial methylation of mercury. Dimethyl mercury is the most toxic form of mercury, "dimethyl mercury is the ultimate

product of this methyl transfer reaction…It therefore seems that dimethyl mercury could be the product of biological significance in mercury poisoning"[7]. In one research institute, a small dose of dimethyl mercury was spilled, absorbed through latex gloves and resulted in the death of at least one researcher [11].

Figure 2.0: Mercury Forms (Speciation)

CH_3 CH_3Hg ↔	CH_3Hg ↔	Hg++ ↔	Hg
Dimethyl Mercury	Methyl Mercury	Mercuric Ions	Elemental (atomic) Mercury
Lipid soluble	Lipid Soluble	Water Soluble	Liquid Metal/ Lipid Soluble Gas

Absorption

Metallic mercury (elemental mercury) volatizes to mercury vapor at room temperature and most human exposure is through inhalation. Elemental mercury in vapor form is lipophilic and 80% of inhaled elemental mercury is readily absorbed in the respiratory tract. Inhaled mercury vapor readily diffuses across the alveolar membranes and has an affinity for red blood cells. The vapor has high mobility and diffuses rapidly throughout the body and brain as a monatomic gas. Mercuric ions are water soluble and only 7-15% of the ingested dose is absorbed from the human GI tract [20]. In contrast, 95% of ingested organic mercury is absorbed by the human gastrointestinal tract (GI) [20] [15].Organic mercury forms are lipophilic and readily absorbed by cells and tissues.

Distribution

Elemenental mercury vapor (Hg) crosses the blood brain barrier and placental barriers. The half-life of elemental mercury in the human body is 45 days. Deposition occurs primarily in the kidney following inhalation [15]. Inorganic mercuric ions (I-Hg) are water soluble (69 g/l) and accumulate in the kidney. The half-life of I-Hg is 15-30 days. Organic mercury (CH_3Hg) has a half-life of 50-90 days in the human body. Ninety percent is found in red blood cells where it binds to water soluble molecules such as cysteine and glutathione [11]. Methylmercury accumulates in red blood cells and is slowly distributed to the organs of the body. Fifty percent is distributed to the liver and 10% to the brain. Organic mercury readily crosses the blood brain barrier and placental barriers. The cysteine complex of methyl mercury enters the endothelial cells of the blood brain barrier on the large neutral amino acid transporter (21). Ethyl mercury readily passes through the blood brain barrier as well [21]. Additionally, CH_3Hg exposure may compromise the blood brain barrier and other membrane structures resulting in increased brain uptake of CH_3Hg [22]. The half-life of mercury is longer in the brain than in the blood. CH_3Hg is demethylated into inorganic mercury deposits which have a half life on the order of years [6].

Excretion

Elimination of organic mercury from the human body is dependent upon gastrointestinal microflora. Elimination rates of organic mercury are dependent upon species, dose, sex, and animal strain. The excretion of mercury is almost exclusively through the gastrointestinal (GI) tract and is dependent on GI microbial flora populations[6]. Blood mercury passes through the liver to the bile and returns into the GI tract. Under healthy conditions, flora (microbes) in the GI tract demethylate organic mercury to the poorly absorbed, water soluble, inorganic mercury, which is excreted in feces. Approximately 1% of the human body burden of CH_3Hg is excreted daily in feces [11]. Much of the

organic mercury in bile is bound to glutathione and cysteine and is reabsorbed by the gut in a process of enterohepatic circulation [23]. Organic mercury is also excreted through breast milk and passes from mothers to infants in that manner. Inorganic mercury is primarily excreted through urine and feces. It is also excreted in saliva, bile, sweat, exhaled air and breast milk [11, 20]. Elemental mercury is excreted in exhaled air, sweat, saliva and as mercuric ions in feces and urine [11, 20].

Biotransformation

In tissue of the human body, organic mercury (CH_3Hg) is demethylated into inorganic mercury (I-Hg), which forms lasting deposits. Scientific studies provide evidence that organic forms of mercury and even dimethylmercury may be produced in the human GI tract by sulfate reducing bacteria (SRB) and methanogenic bacteria. The sulfate reducing bacteria, desulfovibrio, which actively methylates mercury, has been found both in the human colon and in the periodontal pocket of the human mouth in 58% of study subjects [24 25]. Desulfovibrio are ecologically the most significant group of sulfate reducing bacteria (SRB) in the human colon [25]. These flora exist as 66% of SRB in the healthy gut and 92% in persons with ulcerative colitis. These SRB may methylate mercurous ions into the more readily absorbed organic form, CH_3Hg. Scientists estimate that 9 micrograms of organic mercury may be formed per day in the gut of humans [11].

GI flora populations are variable and change with diet and the environment. SRB are in a competitive relationship with methanogenic flora populations [26]. The availability of dietary sulfates selects for an increase in SRB flora populations[27]. Sulfates are generally released into the environment through industrial pollution or as chemical byproducts. Methyltransferases involved in methionine synthesis may be involved in Hg methylation by the sulfate reducing bacteria, desulfovibrio. The enzyme responsible for methylating Hg in microbes, was found to transfer methyl groups from methyltetrahydrofolate to thiols such as homocysteine. This finding supports the hypothesis that mercury methylation may be a mistaken methylation of homocysteine [28].

GI flora don't regularly develop in infants until the ages of 2-3 years. During this time, chronic exposure to mercury or sulfates may select certain types of flora populations to develop. Microbial selection may predispose an infant to increased mercury absorption, impaired excretion, or increased biotransformation of mercury. Increased susceptibility to mercury exposure may occur after the administration of antibiotics as subsequent exposure to mercury or sulfates may select for certain flora populations.

Enterohepatic Circulation

Much of the methylmercury excreted from the liver in bile is reabsorbed in the gut, producing entero-hepatic circulation of methyl mercury. Mercury exposure disturbs GI function [23]. If demethylation rates in the GI tract are reduced, excretion of organic mercury may be impaired and methylmercury may be effectively trapped in enterohepatic circulation and retained by the body in a GI absorption/retention loop. CH_3Hg circulating through the body may create an elevated risk of biotransformation to dimethyl mercury, absorption by the brain, and resultant neurodegeneration. In addition, any increase in the half-life of mercury would result in a greater probability of mercury deposition in the brain. Thus the toxic dose of mercury (LD50) may vary within the human population due to variable microflora involved in biotransformation, and excretion of organic mercury. Available levels of cysteine and the rate of glutathione conjugation of mercury in the bile, may directly affect the rates of enterohepatic circulation. Changes in infant cysteine levels may thereby influence the absorption

rates of mercury due to enterohepatic circulation. Treatment for overexposure to organic mercury involves interrupting enterohepatic circulation with surgical drainage of the gallbladder or oral administration of a non absorbable thiol resin which binds mercury and enhances intestinal excretion [23].

Cellular Metabolism

CH_3Hg is slowly demethylated to mecuric ions in the tissues of the human body. The cellular demethylation of organic mercury results in the deposition of inorganic mercury in target tissues of the body. Autopsy studies on humans and primates both indicate that inorganic mercury deposits persist in the brain for years after exposure while organic mercury has a half life on the order of months [29]. There is reason to believe that inorganic mercury deposits (I-Hg) formed in the brain play an important role in CH_3Hg neurotoxicity [6]. A free radical mechanism is known to demethylate CH_3Hg in vitro [11].

The process of methylation and demethylation is well understood in bacteria [30 31 32 28 33]. In contrast, enzymes responsible for mercury biotransformation in mammalian cells are unknown. Thus, cellular metabolism of mercury within human tissue warrants further investigation.

Figure 2.1: Demethylation in Bacteria

$[(CH_3)_2Hg$ <> CH_3Hg <> Hg ++ <> Hg (metal and vapor)]
MerB (Organomercurial Lyase) > >>> MerA (Mercuric Reductase)>>>>>>>

The microbial process of mercury demethylation is ancient, well preserved and governed by three genes. Genetically, one promoter gene MerR activates both the enzyme coding genes MerB and MerA. Mercuric ion concentrations accumulate from the free radical demethylation of ingested methylmercury. Once a chemotactic threshold is passed, the MerB coding region is activated and transcribes organomercurial lyase, enzymes that demethylate organic mercury into mercuric ions. This process is meant to reduce the amount of organic mercury by rendering it water soluble. If mercuric ions continue to increase, the MerA coding region is activated and transcribes mercuric reductase, an enzyme that reduces mercuric ions into mercury vapor. This process is meant to further eliminate mercury by rendering it into a lipid soluble gas. It is possible that a similar or even identical, well conserved genetic model for the demethylation of mercury is at work in the tissue of the human body. Microbial demethylation occurs in a biphasic degradation with two distinct slopes, each one representing the expression of two enzymes, one for demethylation and one for reduction. In human tissue, the activated methyl donor, S-adenosylmethionine (SAM) provides a possible mechanism for intracellular methylation of mercuric ions into methylmercury (CH_3Hg).

Figure 2.3: Methylation in Human Tissue

Methionine + ATP→ Pi +PPi + S-adenosylmethionine→S-Adenosylhomocysteine →Homocysteine
H_20 → Adenosine

The activated methyl donor, S adenosylmethionine (SAM) provides a possible mechanism for intracellular methylation of mercury into organic mercury forms. The overall rate of cellular methylation, estimated by the ratio of SAM/SAH(S-adenosylhomocysteine), may be associated with the rate of mercury biotransformation. Methionine Synthase may play a role in the methylation of mercury.

Cellular Toxicity

Acute exposure to mercury triggers a toxic response upon reaching a critical concentration, or threshold. This toxic threshold may vary within populations as baseline mercury concentrations and rates of excretion vary within populations. The toxicological effects of mercury on cellular function are widespread and numerous. Mercury has particular affinity to sulfhydryl groups and thiol bonding. As a result, soluble complexes with cysteine, glutathione, and a variety of enzyme systems produce nonspecific cell injury or death. Every protein and amino acid chain in the body is a potential target of mercury.

Figure 2.4: Mercury and Thiol Bonding

$Hg+ + 2(S_2O_3)^{(2-)} \rightarrow Hg(S_2O_3)_2^{(3-)}$ (aq) k=2.4 x 10^13
Hg-S 217.1 +/- 22.2 D298/kJ mol-1
Hg-Hg 8 +/-2 D298/kJ mol-1

Methyl mercury (CH_3Hg) affects many areas of the cell. On the cell surface, mercury binds irreversibly and inhibits voltage sensitive calcium channels [34, 35]. Brain cells exposed to organic mercury respond with neurotransmission disruption, disruption of high affinity dopamine uptake, and cell-surface recognition disruption [20 15 36]. Chronic methylmercury exposure results in decreased ion currents in membrane channels of cultured cells [20 37]. Glutamate and acetylcholine receptors are impaired by chronic mercury exposure [15, 20, 36]. Inside the cell, mercury disrupts nuclear DNA and RNA synthesis, producing chromosome aberrations, and mitotic arrest. Similarly, mercury affects mitochondrial DNA synthesis. Studies have found a 50% decrease in protein synthesis in methyl mercury exposed rat brain [23, 38]. Both inorganic and organic mercury "progressively depressed oxygen uptake and carbon dioxide production with increasing concentration" in a study on guinea-pig brain slices [39]. Decreased mitochondrial respiration was also demonstrated in methylmercury exposed rats with neurological symptoms [38]. In this study, decreased protein synthesis was shown to precede the onset of neurological symptoms and it was concluded that the "inhibition of protein synthesis may have a direct bearing on the poisoning" [38]. Another study on rat brain cells found that impaired oxygen metabolism may be due to inhibition of cytochrome c oxidase by methylmercury and disruption of the mitochondrial membrane [40]. Mercury produces changes in cell and lipid bilayer permeability by affecting protein induction of lipid peroxidation. As mercury binds to tubulin, it prompts disassembly of microtubules and results in the disruption of the neuronal cytoskeleton. On a study of rat brain cells, mercury was shown to inhibit neurite outgrowth in PC 12 cells by disrupting nerve growth factor stimulated tyrosine kinase receptor (TrkA) activity[41 42]. In addition, chronic mercury exposure has been shown to inhibit neurite extension by perturbation of calcium regulation and impairment of microtubule assembly [37]. In summary, there is ample and consistent toxicological evidence that chronic methylmercury exposure may produce neurodevelopmental impairment.

Measurement and Error

In studies of hair mercury concentrations, no significant relationship was found between hair, tissue, and organ levels [43]. Blood mercury concentration is widely considered the appropriate indicator of absorbed dose that corresponds to target deposition [43 44]. Yet, as methylmercury has a half life in the

blood of a few months, any measurement of methylmercury can only be considered a measure of recent exposure and not of previous or long term, chronic mercury exposure.

The NHANES employed flow-injection cold vapour atomic absorption spectrometry in order to detect I-Hg (inorganic mercury in blood) and T-Hg (total mercury in blood) [45]. Inorganic mercury concentration is determined in the untreated sample by the reduction of inorganic mercury into elemental Hg. Total mercury is detected after the digestion and reduction of sample carried as atomic Hg. By subtraction, methyl mercury is estimated (T-Hg – I-Hg = CH_3Hg) [44]. Other organomercurials "may or may not be measured", including cysteine bound forms [44 45 46]. In addition, by this method, any elemental mercury in the sample is either lost or included in the inorganic mercury estimation.

Many sources of variation exist in the measurement of inorganic mercury including temperature, storage time, and experimental error [23, 43, 47, 48]. As storage time increases before measurement, so does sample variation around the mean [47]. Previous studies clearly and consistently demonstrate that proper methods for mercury detection limit storage time to two or three days before sampling [46 23, 47, 49]. Long periods of storage time before sampling is a common routine that increases the variance between measurements and underestimates the original mercury sample concentrations [46-49]. Inorganic mercury detection is particularly susceptible to sample loss over time [23 46-49].

Water soluble, I-Hg selective, spectroscopic sensors are a recent advance in the development of a practical assay for inorganic mercury detection [50]. Sequestration of inorganic mercury by the metal binding domain (MBD) of bacteria is a recent and novel method for measurement, chelation, and elimination of mercury from samples [51]. With advances in Hg detection, the different speciations of mercury in blood may serve with increasing accuracy as bioindicators to assess chronic, organic mercury exposure and inorganic mercury deposition.

3. Health Risks

The health risks posed by chronic mercury exposure are of particular concern because there are subtle neurobehavioral changes associated with low dose exposures. A prospective epidemiological study on a cohort of fish dependent Faroe island children followed from birth to age fourteen, found that chronic prenatal mercury exposure, as measured in cord blood, was associated with permanent deficits in motor, attention, and verbal tests [52]. In addition, maternal hair mercury levels at the time of birth were inversely associated with the eventual IQ of offspring[53]. Health risks associated with the neurotoxic effects of methylmercury were studied in several large human populations subject to acute and severe, chronic exposure. In 1953, inorganic mercury in the effluent of a vinyl chloride factory was discharged into Minamata Bay, Japan. Contaminated fish and shellfish caused an epidemic of medical disorders, termed "Minamata Disease", caused by the ingestion of methylmercury [54]. The clinical features of chronic mercury exposure were numbness, speech impairment, deafness, impaired vision, tremor, mental confusion, involuntary movement disorders, rise in gamma globulin levels in cerebrospinal fluid, and incontinence of urine and feces [54]. Upon autopsy, the pathology of chronic mehylmercury exposure cases revealed conspicuous signs of neurodegeneration [54]. The main targets for mercury deposition in acute and subacute cases were the liver, kidney and brain [54]. The same neurological symptoms were observed in local bird and fish populations as well as in experimental studies on rats [54]. Another methylmercury poisoning outbreak occurred in rural Iraq in 1971-2 due to the consumption of bread made with seeds that had been treated with organic mercury fungicides [19]. Scientific studies of this disaster confirmed there was a latent period between exposure and disease symptoms, wide variation in disease response between individuals, and symptoms including paresthesia, dysarthria, ataxia, visual impairments, and fatalities resulting from central nervous system failure [19]. In addition, these outbreaks demonstrated that prenatal methylmercury exposure, transferred from the mother's body burden, produces subtle neurodevelopmental disability and effects later neurobehavioural performance [20 19 52].

Studies across many species provide conclusive evidence of chronic organic mercury exposure's neurotoxic effects on developing organisms; to disrupt proliferation, migration, and differentiation of brain cells [41, 55]. Chronic mercury exposure has been linked with several neurodegenerative diseases. A convincing hypothesis has been made that Autism is caused by mercury poisoning[56]. In addition a hypothesis has been made that Alzheimer's Disease, Parkinson's Disease, and motoneurone disease are caused by environmental damage to specific regions of the central nervous system[57]. The main pathologic features of acute, organic mercury exposure include degeneration and necrosis of neurons in focal areas of the cerebral cortex [15]. In severe mercury poisoning, as seen during the Minamata and Iraqi episodes, infants exposed in utero were born with sever mental retardation, seizure disorders, cerebral palsy, blindness, and deafness [11]. Mercury vapor inhalation results in tremors, spasm, erethrism (excitability), loss of memory, depression, delirium and hallucination [15]. Hair mercury levels are associated with detectable alterations in performance tests of fine motor speed and dexterity, verbal learning, and memory in a dose dependent manner [14]. Chronic exposure to mercury vapor can also produce fatigue, anorexia, GI disturbances, muscle tremors and shaking [23]. Chronic organic mercury exposure may produce glomerulonephritis in the anti-basement of the kidneys progressing to interstitial immune complex nephritis [58, 59].

Along with temporary symptoms that characterize "mad hatter's" disease, permanent effects on the brain from mercury exposure are well documented. Ye, the risks of developing neurodegenerative disease in response to chronic mercury exposure are unknown. Associations between chronic mercury exposure and risks of neurodegenerative diseases such as Alzheimer's, Parkinson's, Autism, and

Multiple Sclerosis are difficult to assess due to the fact that there is no standard method that quantifies chronic mercury exposure.

Alzheimer's Disease (AD)

"Disturbances at any level of the hypothalamic-pituitary-adrenal axis or glucocorticoid action lead to an imbalance of this system and enhanced susceptibility to infection and inflammatory or autoimmune disease."

-The National Institute of Mental Health (NIMH), review article (Webster JI et al., 2002) [60].

Etiology of Neurodegenerative Disease

Neurodegeneration is characterized by abnormal protein dynamics, oxidative stress, mitochondrial dysfunction, and a process of neuroinflammation [42]. As this review will illustrate, chronic mercury exposure results in the targeted deposition of inorganic mercury that produces focal impairments in the brain, liver, kidney, adrenal, endocrine and immune systems. Chronic mercury exposure is associated with both the neurotoxic and immune response that classify neurodegenerative disease, particularly Alzheimer's Disease [11, 60-68]. Additionally, brain mercury concentration has been associated with all of the defining pathological and many genetic features of Alzheimer's Disease [42, 69].

Epidemiology

Studies in multiple species, as required by rigorous toxicological standards, corroborate associations between mercury and neurodegeneration [20, 23, 70-73]. Repeated studies have ascertained that mercury is associated with physiological features of AD [49, 69, 73-76]. Studies have shown that metallothioneins specific for removing mercury from the brain are depleted in the AD brain [73, 75]. Mercury deposits are associated with increased amyloid aggregation [75]. In a clinical observational study, Alzheimer's Disease patients had twice the total blood mercury concentrations compared to controls, Hock C et al., 1998 [49]. This significant association increased to 3 times the total mercury in early onset AD patients versus controls. The mercury levels associated with disease were below levels known to cause acute neurotoxicity. These results suggest a role for chronic mercury exposure and long term, mercury body burden in disease occurrence.

Deposition

"The elevation of mercury in AD (Alzheimer's Disease patients') nbM (nucleus basalis of Meynert) as compared to age matched controls is the largest trace element imbalance observed to date in the Alzheimer's Disease brain."

- Thompson CM et al, 1988 [76].

There is strong evidence that focal inorganic mercury deposits play a crucial role in organic mercury neurotoxicity and AD pathology [6, 66, 76]. Certain target organs act as a sink for mercury deposition and associated neurodegeneration. The liver, adrenal, and pituitary, trap and accumulate inorganic mercury despite the body's reaction to expel the foreign agent. As a result of mercury deposition and accumulation in the adrenal gland and pituitary gland, chronic exposure to mercury may lead directly to endocrine and immune system impairment.

"Pathological changes in the perforant pathway, by precluding normal hippocampal operation, account for some aspects of the memory impairment in Alzheimer's disease," Hyman BT, 1986 [77].

The transport of mercury from blood into brain occurs along hormonal-immune complexes that involve the flow of cytokines, hormones, and neuropeptides. Mercury binds selectively to cell surface receptors of the endocrine-immune complex, the acetylcholine neurotransmission pathway that regulates the brain's immune response to general infection. Circulating cytokines enter the brain by carrier-mediated transport mechanisms, and through areas with poorly developed blood brain barrier [78]. The circumventricular organs (CVO) include the pineal gland, the subfornical organ, the median emminence, the neural lobe of the pituitary, the area postrema, the subcommisural organ, and the organum vasculosum of the lamina terminalis. In CVO, the blood brain barrier is scarce and a probable route for entry of both cytokines and toxins [78]. Cytokines help recruit lymphocytes into the brain. They induce changes in endothelial cells via adhesion-signaling molecules, modulated to reduce adhesion and induce microglia to release more IL-1 cytokines in a positive feedback loop required for recruitment of lymphocytes into the central nervous system [78]. Specific binding to acetylcholine receptors may enable inorganic mercury to travel into the brain along this neuro-immunological pathway and result in targeted, focal deposition of mercury into AD associated brain regions [68]. Chronic deposition may accumulate and trigger an inflammation response leading to brain cell death[11].

Synaptic Failure

Synaptic failure, associated neurofibrillary tangles (NT) and amyloid Beta deposition, in the cholinergic pathways of the diancephalon region of the brain are defining characteristics of Alzheimer's Disease (AD) [65, 79]. A strong association between AD and synaptic failure may precede amyloid beta deposition in AD brain [42, 65]. Mercury is associated with accelerating amyloid fibril assembly [71]. Amyloid beta increases the rate of synaptic failure [65]. Inorganic mercury hyperphosphorylation of tau protein and involvement in the production of beta amyloid has been substantiated [49].

The molecular mechanism in neurodegenerative diseases characterized by neurofilament dysfunction is consistent with associated mercury deposition [69, 71, 76]. Alzheimer's Disease specific neurodegeneration is characterized by neurofibrillary tangles [63]. Mercury has been shown to disrupt microtubule assembly [11, 17, 23]. As mercury binds to tubulin, it prompts disassembly of microtubules and results in the disruption of the neuronal cytoskeleton. Brain cells exposed to organic mercury respond with neurotransmission disruption, disruption of high affinity dopamine uptake, and cell-surface recognition disruption [15].

Mercury has been shown in mice to cause alterations in the structure and processing of the nucleolar protein fibrillarin giving rise to fibrillarin fragments. Fibrillarin acts as an autoantigen and modification by mercury precedes a T-cell dependent immune response driven by modified fibrillarin in mice. A mercury induced autoimmune response was induced both by an acute dose of mercury and by chronic exposure to a lower dose. Chronic administration of subtoxic doses of mercury induce systemic autoimmune disease in mice, rat and rabbits [80-82]. Metals are involved in the aggregation of alpha-synuclein plaques which are associated with the etiologies of both Alzheimer's and Parkinson's disease [71].

Acetylcholine

"The cholinergic anti-inflammatory pathway is a neural pathway that utilizes A7 receptors to control cytokine synthesis."

Gallowitsch-Puerta M et al., 2005 [68].

The "cholinergic anti inflammatory pathway" includes alpha 7 (Ach7) acetylcholine receptors. Activation of Ach7 receptors prevents cytokine release and inflammation [68]. Acetylcholine is the principal neurotransmitter of the vagus nerve (10th accessory nerve). Acetylcholine transmission is associated with a T-2, anti-inflammatory, immune response that inhibits cytokine release [83]. Acetylcholine receptors expressed on lymphocytes and Ach secreting neurons of the parasympathetic nervous system suppress the acute inflammatory response, a function of the Vagus Nerve (10th accessory nerve)[78]. Resuscitating the anti-inflammatory T-2 response and the acetylcholine pathway is currently a prescribed treatment for AD[84].

The structure of the A7 acetylcholine receptor is similar to the MerR (mercury chelating) receptors of certain bacteria. A highly conserved disulfide bridge in the N-terminal domain region consisting of 2 cysteine residues is available for mercury binding [68]. In the brain, inorganic mercury inhibits ligand binding to acetylcholine receptors that are associated with impaired memory [67]. Synaptic dysfunction of the cholinergic system is correlated with dementia [65]. The greatest heavy metal imbalance in Alzheimer's Disease patients is Mercury deposition located in the Nucleus Basalis of Meynert, a cholinergic pathway associated with memory and AD pathology [76].

Liver

The Vagus nerve connects the hypothalmus with the liver to suppresses cytokine release and inflammation response in the liver and kidney [68, 83]. Impaired cholinergic pathways affect bile secretion, portal blood flow and liver regeneration [83]. In addition, endocrine disruption may decrease bilary flow via the vagal nerve or hormonal interactions. Hormones released by the hypothalmus, pituitary, and adrenal glands modulate hepatic function [83]. Mercury deposits in the liver and kidney are associated with necrosis. Cumulative liver damage and a reduced rate of bile excretion may lead to an increased rate of mercury absorption through enterohepatic circulation [15]. Thus, in an autocatalytic process, mercury exposure may lead to a rising rate of absorption, organ deposition, and disease risk.

Endocrine

"Endocrine abnormalities of the hypothalmic-pituitary-adrenal (HPA) system in patients with Alzheimer's and Parkinson's Disease have been described repeatedly," Hartman, 1997 [85].

Adrenal

Glucocorticoids are associated with the neurodegernative disease multiple sclerosis, and are essential for normal immune function. Adrenal secretion produces a shift from a T-1 immune response(inflammatory) to a T-2 immune response (anti-inflammatory) [78]. A balance between the T-1 immune response (inflammation) and the T-2 immune response (anti-inflammatory) characterizes a functioning immune system. An imbalance in the immune response may lead to a cycle of exposure, inflammation and disease [59].

The pituitary produces ACTH (adrenocorticotropic hormone) that stimulates the adrenal gland to produce corticosteroid hormones, involved in inflammation and immune responses. Glucocorticoids released by the adrenal gland play a role in transcription activation of Ach7 receptors. Glucocoroticoids released from the adrenal cortex have multiple effects on metabolism and also anti-inflammatory and

immuosuppressive effects [78]. Lymphocytes and other cells of the immune system also express adrenoceptors.

In a study of the adrenal glands and chronic mercury exposure stress (100-200ug/7-180 days), Hg deposits were found primarily in the zona glomerulosa and elsewhere dependent on dose and method of injection or digestion [86]. Necrotic cells were localized in cortical areas in both epinephric and norepinephric cells, in cortical lysosomes and in both the lysosomes and secretory granules of chromaffin cells. Thus, chronic mercury exposure may impair the adrenal, T-2, anti-inflammatory response, and promote an immune response shifting balance towards inflammation.

Pituitary

Associations between neurodegenerative disease and pituitary impairment may be explained by the targeted deposition of inorganic mercury in the pituitary [6, 74]. The effects of mercury accumulation in the pituitary and resultant endocrine disruption also provide a mechanism to explain the progression from chronic mercury exposure to neurodegenerative disease.

A study of infant monkeys exposed to ethyl and methyl mercury, by Burbacher et al., found that ethyl mercury left a higher proportion of inorganic mercury in the brain than methyl mercury (34% vs. 7%) [87]. This is significant because inorganic mercury remained in the brain for longer than a year (540 days in this study) while the organic form had a half life of 34 days. An increase of microglia and decrease in astrocytes was associated with the persistent inorganic mercury in the brain, 6 months after exposure had ended.

In another clinical study on Macaca Fascicularis monkeys, mercury concentration in different brain sites was measured following subclinical, chronic organic mercury exposure [6]. The one test monkey that died from mercury exposure, presumably from liver disease (another target organ), recorded an unusually high percentage of inorganic mercury in its pituitary, 81% as compared to the mean of 17% for other brain areas. In fact, after clinical administration of chronic methyl mercury exposure, the brain site with the highest population mean concentration of inorganic mercury was the pituitary, 200 - 300% higher than other brain sites. The inorganic form of mercury was found to deposit in the brain for almost two years, whereas the organic form had a half life in the brain of only one month [6]. These results show that the pituitary acts as a sink for inorganic mercury deposits and accumulation. This is explained physiologically as the pituitary is the one area in the brain that has no blood brain barrier and is therefore prone to absorbing molecules from the adjacent, main arterial supply to the head. The tissue of the pituitary is heavily vascularized and contains a lot of fatty tissue. The other paraventricular organs around the 3rd ventricle are at similar risk of mercury deposition (amygdala, hippocampus, nucleus basalis of meynert).

The pituitary hormone prolactin induces the expression of IL-2 receptors (inflammatory cytokines) on the surface of lymphocytes and is associated with autoimmune disease [88]. The pituitary produces TSH (thyroid stimulating hormone) to stimulate the thyroid gland. In the parafollicular cells of the thyroid gland, this triggers a release of calcitonin which in turn regulates Calcium (Ca++) concentrations in the body. Impaired calcium homeostasis has been implicated in studies as a proximal pathological role in the neurodegeneration associated with Alzheimer's Disease [89]. From repeated studies, it seems likely that chronic mercury exposure may lead to neurodegenerative disease through the selective disruption of the endocrine system.

Luteinizing Hormone (LH) is a gonadotropin secreted by the anterior pituitary that is involved in gonadotroph stimulation, mitogenisis, and immune regulation (for a review see,[90]). Studies have demonstrated that Alzheimer's Disease patients have elevated serum and neuronal levels of LH as

compared to controls, and brain regions affected by AD show elevated expression of LH receptors (for a review see,[90]). In cell cultures, LH stimulates amyloid beta production, a key element in oxidative stress leading to AD pathology. LH receptors are also found on immune cells where they are associated with neuroprotection and a role in the pro-inflammatory signaling process in the brain (for a review see,[91]). A disruption of the pituitary, such as one incurred by focal I-Hg deposition, may result in a cascade of events leading from oxidative stress to impaired neuroprotection, unbalanced neuro-immune response, inflammation, and neurodegenerative disease.

Immune Response

The brain can have widespread effects on the immune system[60]. Interactions between endocrine outflow (CRH, ACTH) and the production of lymphocytes work in concert with direct hormonal binding to lymphocyte receptors. In addition, the CNS can affect the immune system through sympathetic innervation of lymphoid organs [78].

Inflammation, in the forms of reactive astrocytes and microglia, may play an important role in Alzheimer's Disease (AD) where it correlates with brain atrophy and severity[92]. In AD neurodegeneration, there is impaired calcium homeostasis and chronic inflammation [42]. The accumulation of Amyloid Beta is metal dependent and triggers microglia to produce activated cytokines and the inflammatory cascade [72, 75]. Mercury exposure may create an immune imbalance by a twofold effect; initiation of a T-1 immune response (inflammatory reaction via amyloid beta) and suppression of a T-1 immune response (anti inflammatory response, acetylcholinergic receptors, adrenal gland). Thus focal, neurotoxic effects may trigger an autocatalytic cycle of exposure and inflammation proceeding to disease. Anti-inflammatory and anti cholinesterase drugs have been used to varying degrees in the treatment of AD and confirm the association of immune response, inflammation and AD neurodegeneration [84, 93].

The immune system is a main target for mercury deposition and toxic effect. Inorganic mercury (I-Hg) deposits are associated with neurotoxic and immune pathways implicated in neurodegeneration[20]. There is ample evidence for immunogenetic susceptibility to mercury exposure [20, 23, 82, 87]. A direct interaction between the immune system and mercury exposure leads to the suppression of white blood cell activation [66]. Even at sub acute, chronic mercury exposure levels, in vitro experiments demonstrate the immunomodulatory effects of mercury exposure[94]. An autoimmune reaction to mercury exposure and targeted deposition may underlie the mechanism from mercury exposure to disease.

Autocatalytic Origin of Disease

Alzheimer's and other neurodegenerative disease may be caused by an autocatalytic cycle wherein each exposure to mercury increases the rate and effect of future exposure. A functional endocrine reaction should induce the excretion of mercury from the body but an impaired endocrine-immune response may be ineffective to expel the body's mercury load. Chronic mercury exposure may further impair future mercury excretion through necrosis of the liver, disrupted biliary secretion, GI and GI flora disturbances, and necrosis of the kidney. Thus, mercury exposure may be a process involving increasing rates of absorption.

With both mercury neurotoxicity and AD, early life events impact future disease response. Early mercury exposure may determine the future rate and risk of disease. Indeed, in human life, early mercury exposure increases the risks of cognitive dysfunction and neurodegeneration associated with chronic exposure [36]. Similarly, with AD, early life events impact the future risk of disease. Early life

verbal cognition is associated with the risk of later developing Alzheimer's disease [95]. In the NUNN study, low linguistic ability in early life was a strong predictor of poor cognitive function, the risk of Alzheimer's Disease in later life (75-87 yrs), and more pathological neurofibrillary tangles in the hippocampus and neocortex [95]. These results imply an early origin for AD disease risk. Similarly, early mercury exposure can affect neurodevelopment and impart permanent changes in brain function [20, 52, 55].Crucial early, developmental exposure may increase the risk of disease by increasing the rate of absorption and deposition. The risks of chronic mercury exposure may be determined by exposure history. Early exposure may determine an individual's toxic threshold and account for the wide population variance in mercury tolerance.

Conclusion

This literature review supports the hypothesis that chronic mercury exposure is closely associated with both the pathology, and clinical symptoms of AD. Evidence from this analysis confirms that mercury concentrations are associated with all of the defining characteristics of AD; Apo-E levels, the formation of neurofibrillary tangles, plaques in the brain, inflammation, and neurodegeneration. A causative mechanism for mercury exposure and deposition in the development of AD is proposed in this report and should be further investigated.

Mercury Exposure from Vaccines and the Development of Autism

Recently, the first clinical trial on autistic children showed an improvement of symptoms after treatment with mercury chelation[5]. This treatment was linked to low blood androgen levels in autistic children. Luteinizing Hormone is an androgen regulating hormone that is produced in the pituitary, a main target of mercury deposition.

Epidemiology

Although the global prevalence rate of autism appears to rise over time, the precise rate of change is difficult to characterize due to broadening diagnostic criteria [96, 97]. It is now generally agreed that despite changing diagnostic criteria, the prevalence rate of Autism has undergone approximately a ten fold increase over the last 30 years [98, 99]. A recent report by the CDC on the U.S. population estimates an annual rate of 3-6 Autism cases/ 1,000 children[100].

With a comprehensive review of symptoms, traits, biological signs, and population characteristics of Autism in comparison to those of mercury exposure, Bernard et al. propose a hypothesis that Autism is a "novel form of mercury poisoning"[56]. The similarities between the two syndromes are too striking to ignore and too numerous to list here for the purpose of this study; but this excellent paper deserves attention. Based on a thorough correspondence of defining characteristics, this paper hypothesizes that the regressive form of autism is caused by mercury exposure. Are rising rates of global atmospheric mercury deposition, and chronic, organic mercury exposure, responsible for the global, rising rates of Autism?

To date, epidemiological studies of autism and ethyl mercury containing vaccines are inconsistent [101]. This review of published, scientific evidence suggests a complicated relationship between mercury exposure and the development of disease. The relationship between mercury exposure and autism may involve a subpopulation that possesses immunogenetic, metabolic, and environmental (biotransformation) susceptibility. If this is the case, epidemiological studies that find no link between autism and mercury exposure, may be explained by the existence of undefined, susceptible subpopulations. In addition, epidemiological studies that find a relationship between disease and exposure in the general population are in fact underestimating the true relationship between exposure and disease within the susceptible subpopulation.

Certainly there is known to be wide population variability in disease response to long term, chronic, and low dose mercury exposure[11, 19]. Acrodynia, or Pink disease, was an idiosyncratic response from infant exposures to mercurous chloride in calomel, a teething powder. Indeed, a variable response to mercury exposure within populations is a defining characteristic of mercury exposure. If mercury exposure causes autism in seemingly random individuals with no clear dose exposure relationship, this idiosyncratic reaction may not be detected by epidemiological studies. If there is immunogenetic susceptibility for disease in subpopulations, then epidemiological studies would have to be designed with that in mind to investigate the specific cohort that is susceptible. There is evidence for immunogenetic susceptibility to mercury exposure [80-82, 102] and autism [103, 104] which will discussed later in this review. Other sources of variation in disease response to mercury exposure include variable baseline levels of body burden and variable rates of excretion.

There is a limited ability for epidemiological studies to detect relative risk associations below 1.2. Because of the cumulative effect of many sources of mercury, only the sum of the exposures will measure the true relationship between exposure and disease and may have a detectable relative risk association above 1.2. Each individual exposures (thimerosal, dental amalgams, nutrition, air emissions,

human GI biotransformation) may only have a 1.2 or lower relative risk in the general population and therefore remain undetected in most epidemiological studies that investigate single exposures.

Ecological studies are effective at demonstrating a link between exposure and disease because they are able to look at large populations, thereby increasing the power of the study to investigate patterns of susceptible subpopulations. In a population assessment, Geir and Geir compared the incidence of autism in different birth cohorts with increasing vaccination rates [105]. This ecological study found a statistically significant increase in the incidence of autism (odds ratio = 6.0) associated with thimerosal containing childhood vaccines versus thimerosal free vaccines. The linear relationship began at 1981, with 135 ug as the average dose of mercury per child and 38 cases/ 100,000 children with autism, and ended in 1996, with 246 ug as the average dose of mercury per child and 278 cases/100,000 children with autism. An ecological study of Texas found a similar, significant increase in autism rates associated with increases in environmentally released mercury (from emissions in to the air)[106]. This study estimated that for every 1000lb of local mercury emissions, there was a 61%increase in the rate of autism. Oxidized forms of mercury released from point source plumes may deposit locally by the source and thereby influence geographic variability in risks of associated disease [1].

Bias

There is a problematic bias for scientists in researching the health effects of mercury exposure. Indeed, mercury is accepted by the health establishment (ADA, AMA) and widely used in medical practices (vaccine and dental amalgams). In 1999, concern was expressed concerning the safety of thimerosal containing vaccines by the American Academy of Pediatrics and the U.S. Public Health Service [59]. Within 18 months, all the mercury preservative was removed from all vaccines destined for use in the U.S.. Yet, the World Health Organization (WHO) "continues to recommend the use of vaccines containing thiomersal for global immunization programs since the benefits of using such products far outweigh any theoretical risk of toxicity" [107].

Researchers studying mercury are frequently funded by pharmaceutical companies that profit from its sale and are liable if their product is linked to disease. The Lancet published a breach of their financial disclosure agreement by one scientist who failed to disclose a conflict of interest ("Mercury Vaccines and Potential Conflict of Interest" Lancet. Vol. 364. Oct2, 2004) with 12 unmentioned industry sponsorships and financial agreements. His study published in Lancet (2002) was the only published experimental design to measure mercury levels after vaccination in a clinical trial of infants [108]. Bias exists throughout the experimental design of this study. Cases and controls were from different states, ensuring different background rates of mercury. The blood samples were taken 3-28 days after vaccination (a wide range) and stored frozen "until assessment". Due to leeching of mercury into the plastic over time, final blood mercury levels would be lower than original values. This study was designed to find the lowest possible levels of blood mercury as previously published guidelines regarding the measurement of mercury insist on immediate sample measurement due to loss of mercury into the container (see "Measurement and Error", p. 15). Despite these design flaws, the change in mercury concentration in infants blood was still dramatic, with a wide range of variability. In this study, controls were not given thimerosal and had no detectable levels of blood mercury. In infants receiving thimerosal at age 2 months, the levels rose to 4.5-20.55 nmol/L. And in infants 6 months of age, the levels rose to 2.85-6.90 nmol/L.

Toxic Dose of Ethyl Mercury

Thimerosal contains 49.6% mercury by weight and is metabolized to ethyl mercury (EtHg) and thiosalicylate [20]. The vaccination dose of ethyl mercury (CH3CH2-HG-S-C6H4-COOH) is 12.5-25 ug of mercury, intravenous, per dose. There is an additive effect in mercury blood levels with previous vaccinations [108]. It was estimated that an infant undergoing the usual U.S. program of vaccines from birth to six months of age would receive more than the recommended dose of organic mercury (0.1 ug/kg/day)[18].

Most children are subjected to a variable, chronic mercury exposure from other, background sources. Mother's mercury from dental amalgams and diet all pass to the developing fetus. Throughout infancy, the mother's mercury body burden is passed to the infant through breast feeding. Due to the entire program of vaccination, thimerosal in vaccines accounts for an average of 50% of the mercury exposure during infancy. In one study of thimersoal and mercury levels, prevaccination blood levels were 0.04-.5 ug Hg/L. Preterm infant levels rose to an average value of 7.4 ug Hg/L whereas the levels in term infants rose to 2.2 ug Hg/L [18]. Thus, vaccinations contribute to an immense and sharp rise in mercury exposure.

Ethyl mercury (the organic form of mercury found in vaccines, also called Thimerosal) is considered to be as toxic as methyl mercury (the organic form of mercury found in fish) but the exact mechanisms of toxicity may differ. The estimated half life of ethyl mercury is 7 days whereas the half life for methyl mercury is on the order of one to several months. [11, 108]. Ethyl mercury demethylates to inorganic mercury more readily then methylmercury [21, 102, 108]. Ethylmercury causes more severe renal damage in rats than methylmercury and accumulate less in the brain [21]. The precise differences in human toxicology have not been properly studied and may differ from animal models considerably. Yet, the unproven assumption that Ethyl- and Methyl mercury are equivalent is the basis for Ethylmercury dosage, standards and regulation [21]. This assumption of equivalence between ethyl and methyl mercury has been challenged by a recent scientific study. In a study of infant monkeys exposed to ethyl and methyl mercury, Burbacher et al. found that ethyl mercury left a higher proportion of inorganic mercury in the brain than methyl mercury (34% vs. 7%) [87]. This is significant as inorganic mercury remained in the brain for longer than a year (540 days in this study) while the organic form had a half life of 34 days. An increase of microglia and decrease in astrocytes was associated with the persistent inorganic mercury in the brain, 6 months after exposure had ended. This study concludes that "CH_3Hg is not a suitable reference for risk assessment from exposure to thimerosal derived Hg."

Autoimmune Response

There are three properties that are believed to be involved in an autoimmune response. 1. The antigen is an immunogenic peptide of the AcH receptor. 2. An antigen specific T-cell receptor is involved. 3. Class 2 molecules of the major histocompatability complex (MHC 2) ([109], p.241) are involved. It is possible that mercury may act as the antigen that triggers an autoimmune response.

As discussed earlier, mercury has been shown in mice to cause alterations in the structure and processing of the nucleolar protein fibrillain giving rise to fibrillarin fragments [82]. Fibrillarin acts as an autoantigen and modification by mercury precedes a T-cell dependent immune response driven by modified fibrillarin in mice [81]. This autoimmune response is characterized by a T cell dependent polyclonal B cell acrivation with increased serum levels of IgG1 and IgE antibodies (serum immunoglobulins), production of autoantibodies and the formation of IgG deposits in the kidney [66]. The response is H-2 restricted, T cell dependent and differs for males and females. This mercury induced autoimmune response is induced both by an acute dose of mercury and by chronic exposure to a

lower dose. Chronic administration of subtoxic doses of mercury induce systemic autoimmune disease in mice, rat and rabbits [80]. In mice, chronic mercury exposure induced a delayed autoimmune response [81]. A susceptible haplotype (of H-2 domains and other genetic regions) was a prerequisite for autoimmune induction by mercury.

MHC 1,2 genes regulate risk of mercury induced autoimmunity in mice [82, 102]. Mercury may bind to sulfur residues on aminoterminal domains of the alpha chain of MHC complex glycoproteins. Certain genetic variations of MHC 2 molecules may contain crucial sulfide residues, which undergo conformational change when bound to mercury, or double bound to Dimethyl mercury. It is possible that dimethyl mercury creates disulfide bonds between T-cell receptors and MHC 2 molecules to trigger an autoimmune response. An autoimmune response may lead to inflammation, cell death, and a process of neurodegeneration that progresses to the development of Autism.

Antithesis

In 2004, the Institute of Medicine (IOM) reviewed recent epidemiological studies and concluded that "the body of epidemiological evidence favors rejection of a causal relationship between thimerosal containing vaccines and thimerosal"[110]. After the IOM's 1999 request for more evidence, several epidemiological studies were published with conclusions to suggest vaccines were not related to the childhood risk of autism.

Synthesis

In the same publication in which the FDA summarized the 2004 IOM report as finding "no link between vaccines and autism" , it also encouraged companies to comply with the systematic removal of thimerosal from U.S. vaccines[101]. In fact, as recently as 2007, the FDA announces that it "continues to work with…vaccine manufacturers to reduce or eliminate thimerosal from vaccines"[111]. If thimerosal is not a health risk, they why remove it from vaccines?

In reaching the conclusion that there was no causal association between thimerosal containing vaccines and autism, the committee did not review studies by Geier et al. [105] that did find associations between vaccines and disease, citing "serious methodological flaws" and "nontransparent" methods". Yet, studies that the IOM did cite, review, and support, presented results with the same deficiencies and methodological flaws. A retrospective study of Danish children published in The New England Journal of Medicine concluded that there was "strong evidence against the hypothesis that MMR vaccination causes autism"[112]. This study only analyzed associations of autism with one, thimerosal free vaccine (MMR) while neglecting to take into account or mention the possible effects of other thimerosal containing vaccinations and variability in background rates of mercury exposure. This study analyzed data from 1991-1998. In 1992, Denmark discontinued the use of thimerosal in its vaccines. The effect of this dramatic change in infant mercury exposure during the middle of the study period was not discussed or mentioned. This study design was unbalanced, with 5000 missing children due to death or emigration, and a population allegedly skewed to find fewer older, thimerosal exposed children than younger exposed children.

To prevent conflict of interest and bias, the 2004, IOM committee was made up of scientists with no financial ties to the vaccine manufacturers. Yet, the epidemiological studies they cite in reaching their conclusion were primarily funded by vaccine manufacturers and associated scientists. By their own logic, this implies that the studies may have suffered from bias and conflict of interest. The article in the New England Journal of Medicine was funded by the National Vaccine Program Office and National Immunization Program. The second author Dr. Hvid and colleagues had affiliations with the Statens

Serum Institute in Denmark, responsible for manufacturing vaccines for Denmark and countries around the globe, with annual revenues of $120 million. This epidemiological study was designed to find no association by studying only one, thimerosal free vaccine, with no regard to ethyl-mercury containing vaccines, and other sources of mercury exposure.

Another interesting epidemiological study on the Danish registry compared Autism rates before and after 1992, the year thimerosal was phased out of vaccinations[113]. This ecological study found that Autism rates continued to increase after thimerosal was removed and implied non-causation. This study had flawed methodolgy as the population changed in the middle of the study period. In 1995, outpatients were included whereas only inpatients were contained in the study before 1995. By their own admission, this mid-study population change would "exaggerate the incidence rates" of Autism and thereby explain the observed rising trend. Of course there is another simple explanation to the observed trend. As the rate of atmospheric deposition increases, the rate of chronic mercury exposure and the background rates of mercury deposition within the human body may increase as well. Therefore, even if one source of exposure is decreased (thimerosal), the other increasing sources of a child's exposure (diet, transference of mother's exposure, air, water, dental amalgams) may result in overall rising incidence rates of associated diseases. In fact, the incidence rates of Autism only continued to rise until 1999. In 2000, rates began to decrease again. This suggests that removal of thimerosal from vaccines may have had a delayed effect in lowering Autism rates, perhaps obfuscated by confounding factors such as study design, boradening diagnostic criteria and disease awareness, and changing rates in other sources of mercury exposure. This study was performed by scientists affiliated with the Statens Serum Institute. Bias and conflict of interest may have exerted a strong influences on their interpretation of results and study design.

None of the epidemiological studies cited by the IOM attempted full mercury exposure assessment or even acknowledged the possible confounding effects of multiple sources of mercury exposure. Indeed, while companies are purportedly taking thimerosal out of some vaccines, some of the new influenza vaccines have 25 ug ethylmerciry/dose. If each possible source of mercury exposure provides a partial risk, with an OR of 1.2 or less, then links with disease may not be detected when studied in isolation from other sources of exposure. In fact, epidemiological studies that did find associations between vaccination and Autism and were not included in the IOM review were the only studies to quantify multiple sources of exposure (vaccination regimen)[105]. Thus, it could be argued that studies that did find an association between exposure and disease were the only ones that did not suffer from methodological flaws; studies by Geier et al. quantified multiple sources of exposure.

In another epidemiological study authored by an expert witness for the vaccine manufacturers, similar and admitted methodological flaws are again employed. One study that did look at multiple sources of ethyl-mercury exposure from thimerosal concluded there was no assocation between vaccines and Autism rates [114]. Yet, in this study, Dr. Fombonne employed flawed methods, flawed study design, and then misinterpreted his results. In another paper that he authored, Dr. Fombonne describes the very flaws he later employs. In his review of epidemiological studies, Fombonne concludes that Autism prevalence rates are hard to compare over time due to variance and error in estimation of rates, and due to changes in the definition of disease, and diagnostic criteria [115]. Yet, in a subsequent study Dr. Fombonne then studies the time trends in Autism prevalence within a population whose diagnostic criteria and exposures drastically change during the course of the study [114]. Further, acknowledged methodological problems include errors due to ecological estimations of prevalence and lack of individual data. In addition, the exposures of thimerosal and MMR vaccines were analyzed separately and not as a cumulative risk. He asserts that risk of autism from thimerosal and MMR are not related but as this thesis will show, any mercury exposure increases the risk of immune dysregulation such as an

imbalanced immune response to MMR vaccine. Fombonne concluded that the broadening of diagnostic criteria were responsible for the increase in prevalence of disease during the period of his study and not due to vaccines. Upon careful analysis, the syllogism that supports his conclusion is due to a profound mininterpretation of results. In his discussion, he reports that the classification of disease faced two events of broadening diagnostic criteria, in 1992 and 1994. Dr. Fombonne found no association between thimerosal or MMR vaccine and Autism prevalence because he took endpoints across three different populations of broadening diagnostic criteria. By his own assessment, this was a methodology flaw which prevents accurate time trend analysis [115]. In his own words, "unless comparisons also control rigorously for changing case definitions, interpretation of differences in prevalence rates over time and across surveys is virtually impossible"[96]. Indeed, if you heed his advice, and analyze his study results separately, for each population of uniform diagnostic criteria, a strong, direct relationship between thimerosal exposure and Autism prevalence is evident. In years when diagnostic criteria did not change, a clear relationship between thimerosal burden and Autism prevalence is evident (Fig. 2). Thimerosal levels rose sharply between 1992 and 1994, diagnostic criteria did not change, and the rate of Autism prevalence rose as well. When thimerosal load rose in 1990, the prevalence of Autism rose as well. Thus, with the proper methodology, one that Dr. Fombonne recommends and yet he does not adopt, the opposite conclusions are drawn from his misinterpretation and an association between thimerosal load and Autsim prevalence is evident. By his own assessment, his study, and similar epidemiological studies reviewed by the IOM that found now association between vaccines and Autism were fundamentally flawed in design and methodolgy due to changing diagnostic criteria.

The IOM had mixed opinions about thimerosal. Although the IOM concluded that epidemiological evidence does not suggest causality, they admit that toxicological data may support a biological mechanism of causation, that there may be a genetically susceptible subpopulation to mercury exposure, and that there is evidence of immune dysregulation in the serum of autistic patients[110]. If there exists a genetically susceptible subpopulation to exposure, no epidemiological study would be expected to detect an association between exposure and disease unless the study was designed with that subpopulation in mind.

Risk and Susceptible Subpopulations

Autism is a pervasive neurodevelopmental disorder with symptoms apparent usually during the first three years of life [103]. Autism is a rare disease that affects roughly 0.04% of children (1/2,500). Bernard et al. claim that autism is a "novel form of mercury poisoning ", that may occur in a subpopulation of infants with genetic susceptibility [29, 56]. From a genetic screen in a human population, it is purported that susceptibility to Autism has a multi-locus etiology[104].

In the adult brain, CH_3Hg damage is focal, yet in the developing brain, the damage is more diffuse [20]. In utero mercury exposure may disrupt microtubule assembly and impair cellular migration during brain development. This is consistent with the findings that abnormal regulation of brain growth in autism results in unusual brain growth patterns [116].

There is evidence of immunogenetic susceptibililty to autoimmune disorders among patients with autism [104, 117]. A study from Johns Hopkins concluded that there is familial clustering of autoimmune disorders associated with autistic patients [118]. This study concludes that an "increased number of autoimmune disorders suggests that in some families with autism, immune dysfunction could interact with various environmental factors to play a role in autism pathogenesis". More evidence to suggest that autism is linked to genetic susceptibility is that boys are more susceptible to autism than

girls (4-5 times). This may be related to the fact that Y chromosomes are completely lacking in immune genes [119].

In studies on genetically susceptible strains of rodents, subtoxic organic mercury exposure results in systemic autoimmunity and enhanced allergic inflammation[120]. Thimerosal has been shown to affect the immune system by interfering with cytokine secretion by human dendritic cells [121]. The mechanism underlying this process is not well understood but it may be linked to glutathione depletion (GSH) [121]. The autoimmunogen effect of ethylmercury (Thimerosal) is thought to be caused by the inorganic mercury (I-Hg) formed in the body [120]. Because of an autoimmune response present in both mercury exposure and Autism, the possibility of a genetic subpopulation that is most susceptible to autoimmune disease in response to exposure is biologically plausible. The major histocompatability complex, on the short arm of chromosome 6, comprises a number of genes that control the function and regulation of the immune system. One of these genes, the CB4 gene, encodes a product that is involved in eliminating pathogens, viruses and bacteria, from the body. Two studies have confirmed the findings of an increased frequency of the CB4 null allele in autism [117, 122]. A specific link between autism and MHC has been made in other studies where "an association of autism with the major histocompatability complex has been reported with an increased frequency of the extended haplotype B44-SC30-DR4 in autistic subjects, their mothers, or both (40%) as compared to controls (2%) [104]. MHC genes exhibit "extremely high" levels of polymorphisms "relating to its role in presenting antigens" [119]. This high rate of polymorphism may be related to a variable disease response to mercury exposure within populations.

The defining characteristics of a high risk subpopulation may not rely entirely on genetic predisposition. A susceptible subpopulation may be defined by having an elevated baseline of chronic mercury exposure. Higher levels of baseline chronic mercury exposure may reduce the toxic dose to achieve threshold and neurotoxic effect. Another high risk subpopulation may de defined by liver function, GI motility, GI flora populations and the overall rate of methylmercury excretion. Subpopulations with impaired excretion of methylmercury will suffer from body burdens with longer half-lives, and a resultant increased proportion of demethylation and deposition. Variability among populations with regards to genetic response, baseline exposure levels, and rate of excretion may all contribute to define subpopulations that are high risk to exposure and disease response. Exposure and mercury deposition may be hereditary in that previous exposure is passed down from mother to child during gestation and milking. Geographic clustering and variability may also play a factor in exposure as atmospheric deposition is related to both local plume sources, and global wind patterns.

Conclusions

To date, due to poor methodology and flawed design, epidemiology studies of Autism and mercury exposure are inconclusive and err by misinterpretation. By the authors' own admissions, there are many factors that are unaccounted for: changing diagnostic criteria, variance in estimates of Autism prevalence rates, multiple sources of exposure, an undefined, susceptible subpopulation, and a rising time trend in baseline, chronic mercury exposure. Due to the significant limitations of such studies, the IOM has no evidence to refute the theory that ethyl-mercury (thimerosal) containing vaccines elevate the rate and risk of autism. In fact, in results from the Fombonne study (Fig. 2) during a time period with unchanging diagnostic criteria (1992-1994), there is evidence to prove a direct, causal relationship between cumulative thimerosal load and prevalence rate of Autism [114].

In the 2004 report, the IOM offers recommendations that included "increased efforts to quantify the level of prenatal and postnatal exposure to thimerosal and other forms of mercury in infants, children, and pregnant women"[110]. The proper assessment of mercury exposure and risk of disease requires quantifying background, chronic exposure, rate of deposition, and acute exposure from the

cumulative dosage of multiple vaccines. This thesis hopes to define susceptible subpopulations by presenting and testing a method for the assessment of chronic mercury exposure. A method for the assessment of chronic mercury exposure may help define background levels of exposure, rates of inorganic mercury deposition, and define subpopulations most susceptible to mercury exposure.

4. Method for Assessment of Chronic Mercury Exposure

For human populations, the vast majority of chronic mercury exposure is presumed to be organic mercury from ingested food [11]. Consumption of contaminated fish is considered the major source of human exposure to organic, methyl mercury (CH_3Hg) in the United States [8]. Demethylation of Methyl Mercury to water soluble, mercuric ions (Hg++) is a necessary step for excretion from the gastrointestinal tract (GI) and kidney [19]. Under normal conditions, the GI tract excretes the vast majority of ingested organic mercury (95%). In human tissue and organs, this demethylation process, crucial to excretion, also results in the accumulation of inorganic mercury. In the brain, inorganic mercury deposits play an important role in mercury neurotoxicity[6]. While organic mercury has a half life in the brain of approximately one month, inorganic mercury deposits have a half life on the order of years[6, 22]. Animal studies on monkeys reveal that methylmercury is demethylated into inorganic mercury in the brain and other tissues upon long term, chronic exposure [123]. As a result of inorganic mercury deposition, the percentage of inorganic mercury relative to total mercury concentration in the brain increases after long term exposure to organic mercury [6]. Because of tissue demethylation of CH_3Hg and resultant I-Hg deposition in the brain, Rice et al. recommend that "health effects of methylmercury should focus on long-term exposure" [123].

In a population of microbial samples, Schaefer et al. found a strong log linear relationship between the proportion of organic mercury and total mercury levels [124]. These results describe a controlled, metabolic response to mercury exposure that maintains specific proportions of inorganic mercury and organic mercury at different levels of total mercury. The Schaefer study contends that this data represents a highly conserved and tightly controlled enzymatic response to mercury exposure that biotransforms mercury into a readily eliminated form. Studies on animals and humans have demonstrated that in the brain and tissue, organic mercury is slowly demethylated into inorganic mercury (for a review see, [125]). As methylmercruy is eliminated more slowly from the brain than inorganic mercury, the result of chronic mercury exposure is a shift in the conserved relationship between inorganic mercury and methylmercury in the brain towards a higher percentage of inorganic mercury [6 125].

Hypothesis

1. Assessment of chronic mercury exposure is best characterized by both recent exposure and longer term exposure. Demethylation of methylmercury into I-Hg and resultant deposition of I-Hg in brain and tissue suggests that I-Hg may serve as a bioindicator for long term, chronic methyl mercury exposure. If this hypothesis is correct, longer exposure groups will have greater I-Hg deposition and display a greater percentage of I-Hg relative to T-Hg (total mercury = CH_3Hg + I-Hg). Therefore, different chronic mercury exposure groups may be distinguished and characterized by comparing I-Hg and CH_3Hg.

This study applied statistical analysis to a data set of brain mercury levels from a toxicological trial of organic mercury exposure in monkeys [6, 22]. In this study, groups of five M. fascicularis (7-14 years of age, 2.4-6 kg) were given daily doses of methyl mercury in apple juice for 6, 12, and 18 months. The daily dose of CH_3Hg was 50 ug/kg body weight. One group of monkeys was administered CH_3Hg for 12 months, after which the group was unexposed for 6 months (in our analysis this group is labeled 12.6). One group was not administered mercury and served only as controls (group labeled 0).

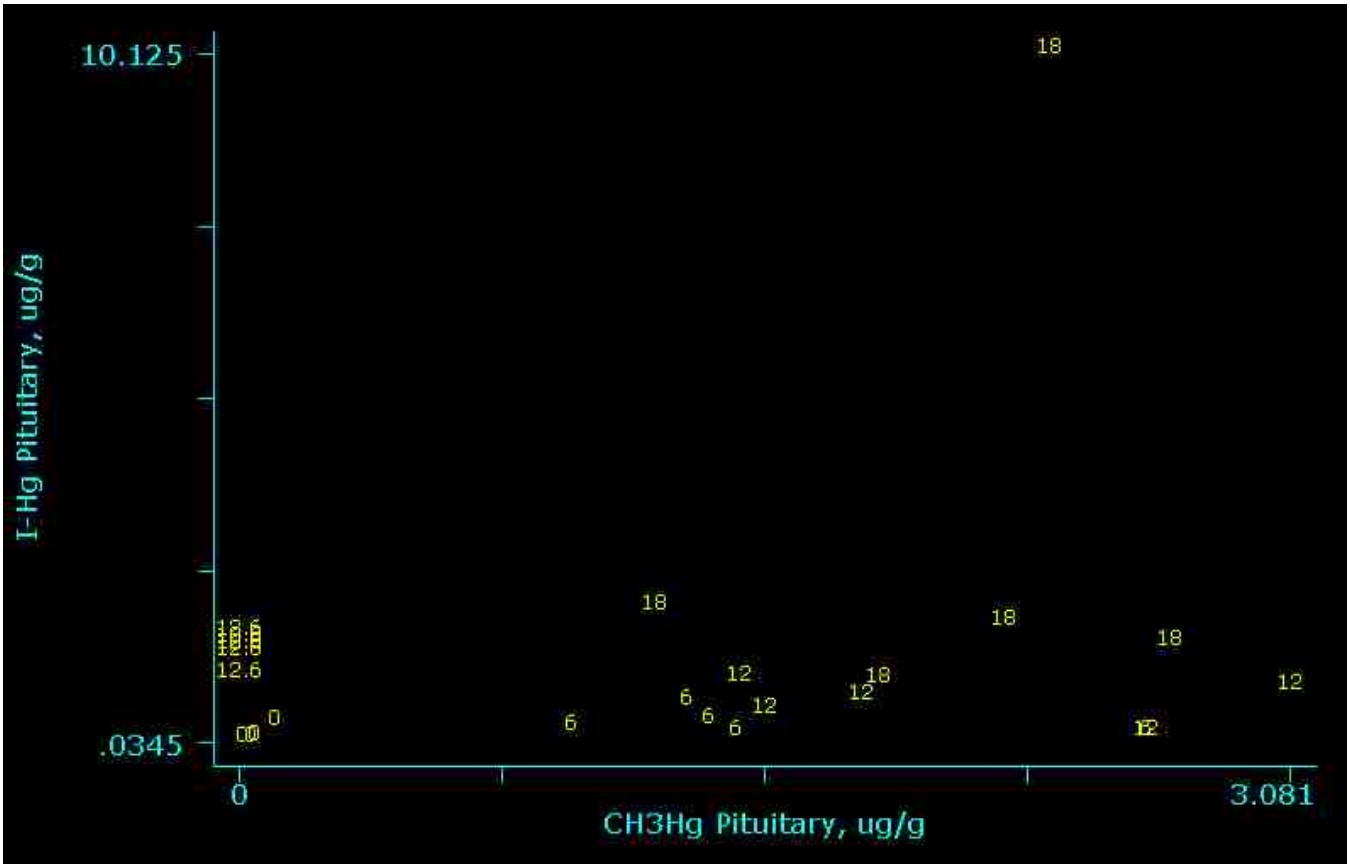
Brain samples from different brain regions were analyzed for total, inorganic, and organic mercury forms. After clinical administration of chronic methyl mercury exposure, the brain site with the highest percentage of mercury found in the inorganic form was the pituitary; 200 - 300% higher than other brain sites [22]. We estimated the mean value of whole brain concentrations. To look at the relationship between exposure, I-Hg, and methylmercury we illustrated the exposure groups with a linear regression comparing I-Hg to CH_3Hg with both the mean values of whole brain and for pituitary levels in particular. We also compared boxplots of mean values for I-Hg and CH_3Hg of the different exposure groups.

Results

From the graph of observed points (Figure 4.0 and 4.01), the process of deposition is illustrated. The graph can be divided into four quadrants. On the top, far right quadrant (high I-Hg and high CH_3Hg) are the observed points with the highest I-Hg deposition and methyl mercury exposure. On the bottom, far right (high CH_3Hg, low I-Hg) are the observed points with recent methylmercury exposure but low I-Hg deposition. On the left top (High I-Hg, low CH_3Hg) are the observed points with high deposition of I-Hg but little recent exposure. On the bottom left (low levels for both) are the observed points with little or no exposure or deposition. Thus, in the regression of I-Hg and CH_3Hg is a depiction of both recent exposure (CH_3Hg) and deposition (I-Hg), and a method to distinguish between different exposure groups. Rising inorganic mercury concentrations distinguish rising exposure groups and can differentiate between the low exposure group (6 months) and the high exposure group (18 months). Organic mercury levels differentiate time since exposure as illustrated by the differences in methylmercury concentrations between 12 month exposure and 12 months exposure followed by 6 months rest (group 12.6). After 6 months rest, organic mercury is eliminated and only inorganic mercury, from the demethylation process remains. From figure 4.01, we can see that methylmercury levels do not distinguish between exposure groups as 6 months exposure reaches a higher level of CH_3Hg than 18 months exposure. From figure 4 and 4.01, it is evident that CH_3Hg levels reach a peak value and then slowly demethylates into I-Hg over time. In fact, I-Hg levels are the best bioindicator of chronic mercury exposure. CH_3Hg serves as a bioindicator for time since exposure (compare groups 12 and 12.6) but can not distinguish between different chronic exposure groups with equivalent time since exposure (groups 6, 12, 18). This is due to the fact that blood methylmercury concentrations reach a steady state concentration with chronic mercury exposure. As the rate of elimination of methylmercury is more rapid than that of I-Hg deposits, I-Hg levels represent a more reliable assessment of chronic mercury exposure and effect.

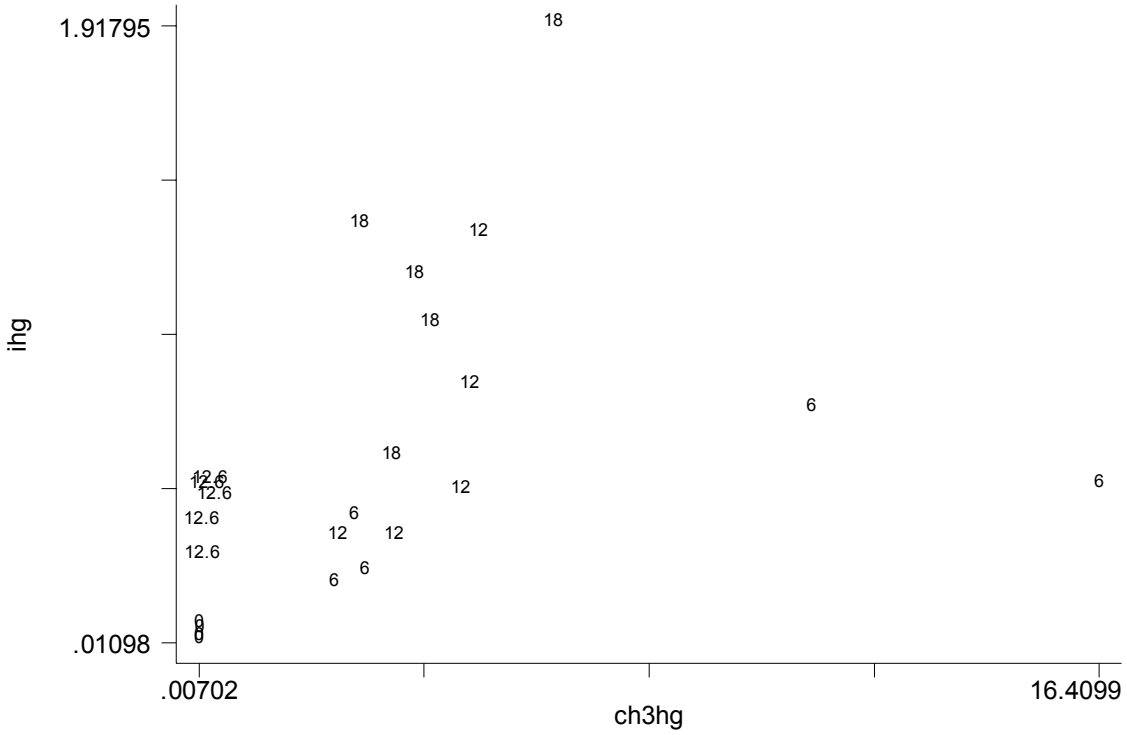
The outlier monkey in the highest exposure group (18 months) with the highest and most extreme levels of I-Hg deposition was diagnosed with liver disease and died. This unique fatality recorded an unusually high percentage of inorganic mercury in its pituitary, 81% as compared to the mean of 17% for other brain areas [6]. After 18 months exposure, the pituitary had an overall 100% higher mean inorganic percentage than the overall brain mean percentage, yet total mercury remained equivalent to the mean value for whole brain. The inorganic form of mercury was found to deposit in the brain for almost two years, whereas the organic form had a half life in the brain of only one month. Thus the amount and percentage of inorganic mercury is a measure of mercury deposition and neurotoxic effect. An explanation for the death of this monkey may be that the liver damage caused poor excretion of organic mercury. A longer organic mercury half life may have resulted in more demethylation and deposition of inorganic mercury. Thus, what seems to be an idiosyncratic response, the death of one monkey among five, may be the result of impaired excretion, increased deposition, and the neurotoxic effects of inorganic mercury deposits. From these results, it seems that inorganic mercury levels may be

Figure 4: Relationship between Inorganic Mercury (I-Hg) and Methylmercury (CH_3Hg) in Macaque Monkey Pituitary samples following Chronic Mercury Exposure. Chronic exposure groups are labeled, O months, 6 months, 12 months, 18 months and 12 months exposure followed by 6 months rest (labeled group 12.6).



(Data points taken from Vahter et al. Reference[6])

Figure 4.01: Relationship between Mean Inorganic Mercury (I-Hg) and Methylmercury (CH_3Hg) levels in macaque monkey whole brain samples (raw data taken from reference [6]). Chronic exposure groups are labeled, O months, 6 months, 12 months, 18 months and 12 months exposure followed by 6 months rest (labeled group 12.6).



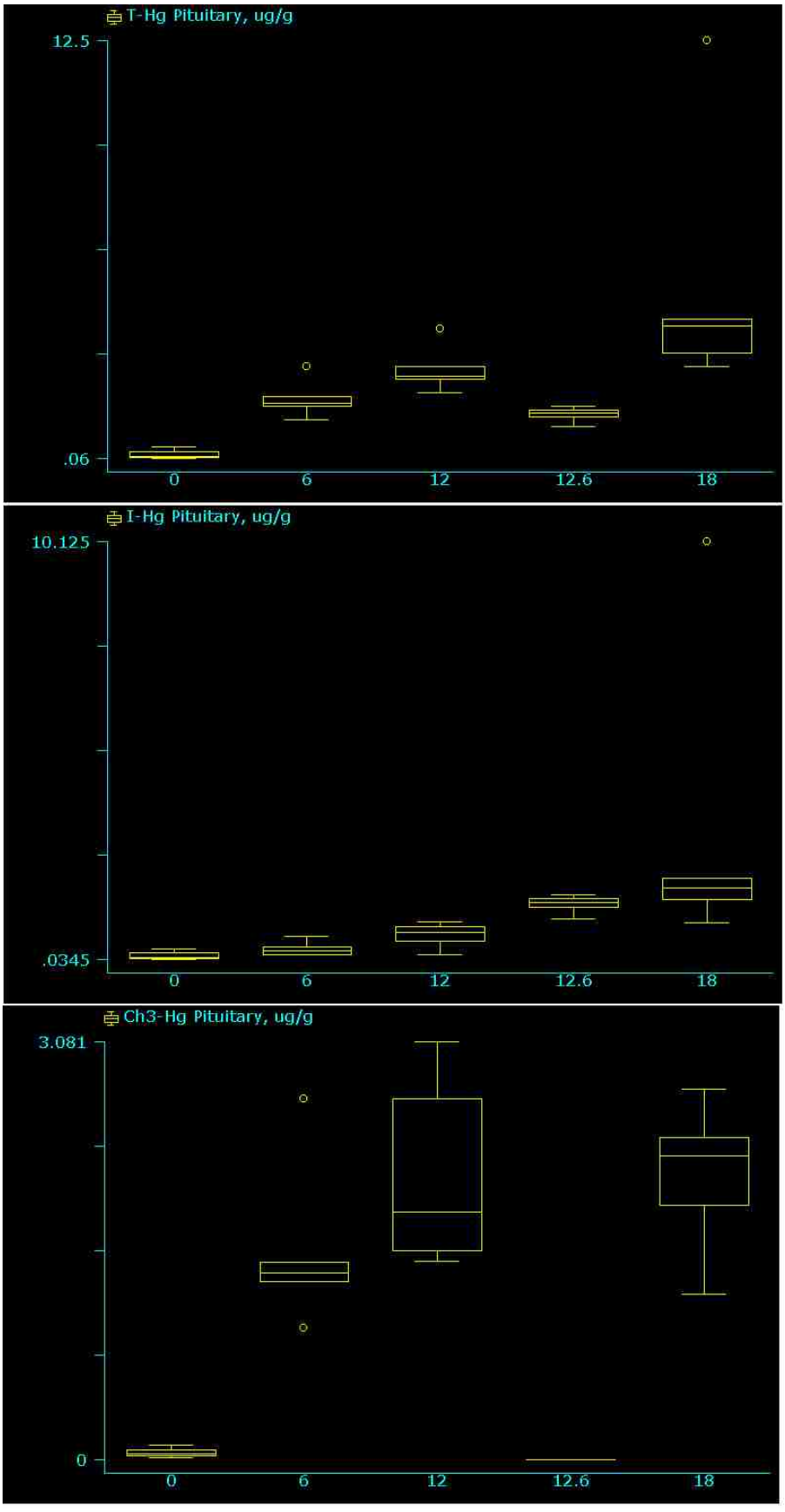


Figure 4.1: Boxplots of mercury forms by exposure (months) in Pituitary from brain data clinical trials on macaque monkeys. Group 12.6 indicates 12 months exposure followed by 6 months no exposure. (A) Total Mercury. T-Hg increases with exposure and decreases partially after 6 months rest. (B) Inorganic Mercury. I-Hg increases with exposure and accumulates in the pituitary even after 6 months rest. The increase in I-Hg after 6 months rest relative to 12 months exposure indicates impaired excretion of CH_3Hg and demethylation into I-Hg. (C) Methyl Mercury (CH_3Hg). CH_3Hg reaches a maximum at 12 months and disappears with 6 months rest. After 12 months exposure, demethylation of CH_3Hg to I-Hg must account for the rise in I-Hg during the 6 months rest.

a more significant indicator of chronic mercury exposure and risk of disease, than methylmercury levels. Inorganic mercury levels can differentiate levels of exposure and even detect chronic exposures after 6 months of no exposure (group 12.6). In contrast, methylmercury levels are less definitive in separating different exposure groups and are unable to detect past exposures (group 12.6 has no methyl mercury). Careful analysis of the different brain regions clearly demonstrates that the pituitary is the main target site for inorganic mercury deposition [6]. After 12 months exposure with 6 months rest (group 12.6) the mean inorganic mercury level in the pituitary is higher than immediately after 12 months exposure (Figure 4.1B). These results describe a demethylation reaction with the fact that organic mercury levels disappear after exposure has ended for 6 months (in the 12.6 group). Although some organic mercury is excreted, Figures 4.1B and 4.1C present evidence that CH_3Hg is also demethylated into I-Hg deposits in the pituitary. These inorganic mercury deposits accrue and persist for many months if not years after exposure has ended. This analysis provides evidence that inorganic mercury levels may best represent chronic, organic mercury exposure while methylmercury represents recent exposure; but only taken together, can one distinguish between all of the different exposure groups.

Discussion

To study the effects of chronic mercury exposure, scientists must be able to identify different exposure populations. It is evident that both I-Hg and Ch3-Hg in brain must be analyzed in order to distinguish different exposure groups with different times since exposure, as one would expect in a typical diagnostic test. The question arises, is inorganic mercury an important bioindicator for chronic exposure in blood? In blood tests on these animals, the concentration of I-Hg in blood "increased in a fashion comparable to that of methylmercury" [22]. Yet, unlike in the brain, inorganic mercury in blood does not deposit with a longer half life than the organic forms. With the one trial monkey that died, blood organic mercury concentrations reached a steady state at 12 months while blood inorganic mercury concentrations continued to rise during the entire trial period [22]. In contrast to all the other animals, where I-Hg in blood remained a fraction of the total mercury, in the one monkey that died, I-Hg levels rose to a level beyond organic mercury. Thus, inorganic mercury in blood was the best bioindicator of disease response and mercury poisoning.

These results show that the pituitary acts as a sink for inorganic mercury deposits and accumulation. This is explained physiologically as the pituitary is the one area in the brain that has no blood brain barrier and is therefore prone to absorbing molecules from the adjacent, main arterial supply to the head. The tissue of the pituitary is heavily vascularized and contains a lot of fatty tissue, making it difficult for water soluble mercuric ions (I-Hg) to be eliminated. Dose response relationship analysis of methyl mercury poisoning cases reveals a toxic threshold in blood mercury levels that triggers disease response in acute exposure [19]. As local concentrations of inorganic mercury rise in the pituitary from chronic exposure, the risks of reaching a toxic threshold rise as well. The effects of I-Hg accumulation in the pituitary and resultant endocrine disruption provide a mechanism to explain the progression from chronic mercury exposure to neurodegenerative disease.

Previous experiments have shown that the macaque neuropathology data in response to mercury exposure corresponds highly with that of human (for a review see, [55]). Therefore the applicability of these results from monkey to human seems to be a fair assumption. A shorter half life of mercury in blood of monkeys than in humans would indicate that for humans facing equivalent exposures, blood levels would be higher [126].

If we assume that blood I-Hg in humans comes primarily from the demethylation of organic mercury, then a blood test for I-Hg may be the best practical method for assessment of chronic methyl

mercury exposure. This assumption that CH_3Hg is the prime source of I-Hg is plausible as methylmercury from the consumption of fish is overwhelmingly the largest source of chronic mercury exposure.

Conclusion

Data are sufficient to conclude that both I-Hg and organic mercury concentrations are associated with chronic, organic mercury exposure. Results from this analysis illustrate that chronic exposure and mercury deposition are associated with changes in the proportion of inorganic mercury to methyl mercury. In the pituitary, levels of total, organic or inorganic mercury were insufficient by themselves to distinguish between all the exposure groups. Only by looking at both inorganic mercury and organic mercury levels in brain tissue, can one best characterize the exposure groups in terms of both the level and time course of chronic methyl mercury exposure and deposition. In blood, while inorganic mercury is a reliable sign of chronic exposure, it is unable to distinguish between different exposure groups. Yet, in the one test animal that died, blood inorganic mercury was the best bioindicator of deposition and the risk of disease response. Therefore it is recommended that in any method for assessment of chronic mercury exposure that uses blood levels, blood inorganic mercury (I-Hg) should be used as the primary bioindicator. Blood methylmercury levels should be used to confirm and in order to characterize the time since exposure. The regression of I-Hg to CH_3Hg may be the most effective method for assessment of chronic mercury exposure as it can distinguish between different chronic exposure group, determine time since exposure, and illustrate the rate of I-Hg deposition. I-Hg may best determine susceptibility to future exposures. By lowering the amounts of future exposures needed to reach the critical threshold concentration for toxic effect, I-Hg deposits may explain the focal neurotoxicity typical of mercury poisoning in adults. Each I-Hg deposit may act as a critical area where the reservoir of I-Hg deposited requires only subtoxic, or chronic future doses to reach the toxic threshold and lead towards a disease response.

5.Mercury and the Biochemical Profile

Abstract

The purpose of this study was to assess chronic, organic mercury exposure within the general U.S. population. To assess links with neurodegenerative disease, this study looked at associations between chronic, organic mercury exposure and main targets of inorganic mercury deposition and effect within the human body: the pituitary, immune system, and liver. From a review of toxicological evidence, blood I-Hg detection and blood CH_3Hg concentration were determined to be the appropriate bioindicators of chronic, organic mercury exposure. This epidemiological study analyzed data from the NHANES (1999-2000 and 2001-2002) surveys to evaluate relationships between chronic, organic mercury exposure and main target sites of inorganic mercury deposition and effect. In a cross sectional analysis, the biochemical profile markers luteinizing hormone (pituitary), white blood cell count (immune system), and bilirubin (liver), were all significantly associated with both blood I-Hg and CH_3Hg levels in the general U.S. population, specifically within the subpopulation of women ages 30-39 years. These results demonstrate a significant relationship between chronic, organic mercury exposure and biochemical changes related to the main targets of inorganic mercury deposition and effect. These associations provide links between exposure and the biological mechanism leading to neurodegenerative disease. Associations with the immune system (white blood cell) and pituitary (luteinizing hormone) establish links with Autism and Alzheimer's Disease. Associations with the liver (bilirubin) reiterate concern that mercury deposition may increase enterohepatic circulation, raise the absorption rate of mercury, and thereby elevate susceptibility to future exposure such as from vaccines containing ethyl mercury. The reported, direct association between I-Hg deposition and chronic organic mercury exposure suggests that the demethylation of organic mercury within the body is a contributing source of I-Hg deposition. This study reported a significant difference in the proportion of I-Hg detection between the two survey populations: 3% I-Hg detection in 1999-2000, and 6% I-Hg detection in 2001-2002. Whether this change in I-Hg detection was due to geographical differences in exposure, measurement error, or to a time dependent rise in the rate of chronic mercury exposure is unclear. While results from this study do not verify a time dependent increase in chronic mercury exposure and deposition, they do support this possibility. A rise in mercury deposition within the U.S. population may elevate susceptibility to further exposure and forecast a rise in the population risks of associated neurodegenerative diseases. Continued monitoring of these biomarkers in the NHANES population is necessary for full assessment of chronic mercury exposure and associated risks of neurodegenerative disease within the U.S. population.

Hypothesis

1. Previous studies suggest that chronic, organic mercury exposure is best characterized by elevated blood concentrations of both blood I-Hg and CH_3Hg. Therefore, both these bioindicators for chronic, organic mercury exposure and I-Hg deposition may be associated with the biochemical profile markers for main targets of mercury deposition and effect within the human population: the pituitary (luteinizing hormone), liver (bilirubin), and immune system (white blood cell count). The null hypothesis is that bioindicators for chronic, organic mercury exposure (blood I-Hg and CH_3Hg) are not associated with biochemical profile markers for main targets of inorganic mercury deposition and effect within the NHANES survey population. The alternative hypothesis is that chronic, organic mercury exposure is associated with main targets of deposition and effect.
2. As the rate of global mercury deposition is rising over time, the risks of chronic mercury exposure, deposition, and associated public health risks within the general U.S. population may be rising over time as well. The null hypothesis is that bioindicators for chronic mercury exposure and I-Hg deposition are not rising over time. The alternative hypothesis is that bioindicators for chronic mercury exposure and deposition are rising in a time dependent manner.

Purpose

The purpose of this study was to assess chronic mercury exposure within the general U.S. population using data from NHANES, 1999-2002. To analyze links with neurodegenerative disease this study quantified associations between chronic mercury exposure and the targets of mercury deposition and effect within the human body: the pituitary, immune system, and liver. In addition, this study investigated changes in the rate of I-Hg deposition to determine time trends of chronic mercury exposure and population averaged risks of associated neurodegenerative diseases such as Alzheimer's and Autism.

Model and Methods

This study analyzed the NHANES, national survey 1999-2000 and 2001-2002 data sets after converting SAS files to STATA format, and using STATA software to perform the analysis. The NHANES data set for blood mercury levels contained a sample of American Women, ages 16-49 years. This population was chosen due to the availability of data, as no men were tested for mercury levels and children were excluded to focus on an adult population. Three biochemical profile markers were chosen to represent main targets for mercury deposition and effect: bilirubin (liver) (for review see thesis sections "Distribution" (p.11), "Deposition" (p.17), "Liver" (p.19)), luteinizing hormone (LH) (pituitary) (see thesis section "Pituitary" (p.20)), and white blood cell count (immune system) (see thesis section "Immune Response" (p.21)). Blood inorganic and organic mercury as well as urine mercury were compared with these selected biochemical profile markers. As total mercury (T-Hg) and inorganic mercury (I-Hg) were measured, blood organic mercury was estimated by the simple formula T-Hg – I-Hg = CH_3Hg (see "Measurement and Error" section, p. 14). Data were analyzed as a raw population for internal validity and again as a survey weighted population for external validity.

The NHANES target population is the civilian, non-institutionalized U.S. population. NHANES 1999-2000 includes over-sampling of low-income persons, African Americans, and Mexican Americans. In addition to analyzing the NHANES population as a raw population of individuals, this study employed recommended survey analysis to reflect the complex survey design and sample

weighting methodology of the NHANES datasets (as described in the Analytic and Reporting Guidelines, Sept., 2006 version, and the NHANES Public Data Release File Documentation). In this study, only associations that were significant in the raw population were re-analyzed as a survey population. Survey weighted population analysis lends external validity to results in terms of reflecting Women, Ages 16-49 in the U.S. population.

The NHANES is a continuous survey beginning in 1999 and data are released in two year increments. This study looked at two of these increments, 1999-2000 and 2001-2002. There were no reported changes in the methodology for measuring mercury detection or biochemical profiles between these two survey groups. However, I-Hg (NHANES code: lbxihg) values below the limit of detection (0.4 ug/L) were assigned estimate values that dropped from 1999-2000 (0.3 ug/L = assigned estimate for values below LOD) and 2001-2002 (0.28 ug/L = assigned estimate for values below LOD). This unexplained change in estimates may have artificially stabilized the Mean I-Hg blood concentration between survey groups; pulling down the 2001-2, mean I-Hg concentration to the same value as 1999-2000 survey group.

Due to the complex survey design and sampling methodology, each measurement has its own weighting, primary sampling unit (PSU), and stratum. The sampling weights are used to produce unbiased national estimates and reflect the unequal probabilities of selection, non-response adjustments, and adjustments to independent population controls. The PSU's generally represent single counties in America although some small counties are combined.

The appropriate weight variable for our analysis in the combined years 1999-2002 is WTMEC4YR, in the NHANES dataset. The stratum variable is SDMVSTRA and the PSU variable is SDMVPSU. The PSU and stratum help estimate variances that would have been estimated using the true design structure. To estimate sampling error, a Taylor series linearization method was employed. In STATA the 1999-2002 NHANES data set is processed by:

svyset[pw=wtmec4yr], psu(sdmvpsu) strata(sdmvstra).

The stated purposes of the NHANES survey include estimating the percent of persons in the U.S. population that possess certain risk factors for disease. As the NHANES survey is a continuous survey, it is difficult to determine if differences between NHANES survey populations are due to geographical variance, variance in methodology, or actual time dependent changes over the survey years.

Associations reported in this study are rigorous and persistent in raw, adjusted, and survey populations and in both naïve and robust models. The data were adjusted for potential confounders such as age and race. Associations were considered significant if p values were less than or equal to 0.05.

Approximately 95% of this population had undetectable levels of inorganic mercury (below the limit of detection , 0.4ug/L (LOD)). Due to indeterminate storage times for up to a year, the measurement of inorganic mercury was subject to great measurement error and variance. 95% of the population below the LOD were all given a constant estimate value by NHANES. Because of these factors, I-Hg could not be treated as a continuous variable. For the purposes of this study, I-Hg detection was transformed into a binary variable: 0 for non detect (below the LOD), 1 for detect (above the LOD). To identify associations between I-Hg detect and the biochemical profile markers, t-tests, and logistic regression analysis was performed. The lincom STATA function was used to derive an odds ratio of I-Hg detection for a one standard deviation change in the biomarkers of interest (lincom (Std. Dev.)*(Biomarker)). To test for associations between CH_3Hg and the biochemical profile markers, linear regression analysis was performed.

Marginal Models:

Linear Regression*:

$$Y= B0 +B1(x) + B2(x) + Bi(x)$$

Y= Continuous outcome variable (CH_3Hg, Urinary Hg)
B1= Continuous explanatory variable for biochemical profile markers (LH, WBC, Bilirubin).
B2= Age, as a continuous variable by year.
Bi= Categorical variables for race.

Logistic Regression*:

$$Logit[P(Y=1| B0, Bi, X=x)] = B0 + B1(x) + B2(x) + Bi(x)$$

Exp(B1) = O.R.
Y= Binary outcome variable for detection of inorganic mercury in blood (1=detection, 0=non detection).
B1= Continuous explanatory variable for biochemical profile markers (LH, WBC, Bilirubin).
B2= Age, as a continuous variable by year.
Bi= Categorical variables for race.

Logistic Regression*:

$$Logit[P(Y=1| B0, Bi, X=x)] = B0 + B1(x) + B2(x) + Bi(x)$$

Exp(B1) = I.R.R.
Y= Binary outcome variable for survey group years (1999-2000 =0, 2000-2001=1).
B1= Binary explanatory variable for I-Hg detection.
B2= Age, as a continuous variable by year.
Bi= Categorical variables for race.

*None of the reported results contained an Odds Ratio or relative risk with a confidence interval that spanned 1 or a slope with a confidence interval that spanned 0.
Summary description and units for mercury forms and biochemical profile markers are provided in table 5.0 below.

Results

Table 5.0: Summary Description of Mercury Forms and Biochemical Profile Markers	
I-Hg Detection	Blood Inorganic Mercury (I-Hg) 0 = below the limit of detection (LOD= 0.4 ug/L) 1= above the limit of detection.
Total Hg	Blood total Hg: 1999-2000: LOD=0.05 ug/L 2001-2002: LOD=0.05 ug/L and 0.015 ug/L
CH_3Hg	Blood Methyl Mercury (ug/L) CH_3Hg = T-Hg - I-Hg
Urinary Mercury	(ng/ml) 1999-2000: LOD=0.05 ug/L 2001-2002: LOD=0.05 ug/L and 0.015 ug/L
Bilirubin	Total blood (umol/L) Liver Function Biomarker
White Blood Cell Count (WBC)	(SI) Immune System Biomarker
Luteinizing hormone (LH)	(mIU/mL) Pituitary/Endocrine System Biomarker

Table 5.3: **Mean Values for Mercury forms and Biochemical Profile Markers in the NHANES combined population, 1999-2002.**

Form	**Observations**	**Mean**	**Std. Dev.**	**Min**	**Max**
I-Hg Detection	3616	4.6% Detection	0.21	0	1
CH_3Hg	3613	1.15 (ug/L)	2.1	0	29.5
Urinary Mercury	3531	1.4 (ng/ml)	2.7	.1	68.51
Bilirubin	3569	8.45 (umol/L)	4.3	1.7	72
White Blood Cell Count	3614	7.8 (SI)	2.3	2.6	20.1
Luteinizing hormone (LH)	1133	13.4 (mIU/mL)	17.5	.1	129

The combined NHANES 1999-2002 population consisted of 3600 women, divided in two groups based on their years of survey. 1708 women in the survey group for the years 1999-2000 and 1908 in the survey group for the years 2001-2 group. The survey weighting design extends inferences based on this data with external validity to 66 million American women; 31 million for the years 1999-2000, and 35 million for the years 2001-2. The biochemical profile marker, Luteinizing hormone (LH), was measured only in a subsample of this population; 1133 Women, restricted to the ages 35-49 Years. In order to be concise, only certain, key table and figures are provided in the results section. Other figures and tables are provided after the conclusions as a supplemental section.

Scatterplots of age (years) versus the biochemical profile markers are illustrated in figure 5.0 (p.59). From these rough graphs, a possible association between LH and age is evident (figure 5.0 D). From the tables of descriptive statistics, we see the trend of a rise in I-Hg detection with age (table 5.02, p.99). There is also a slight rising trend of methylmercury with age (table 5.03, p.100). Blood LH follows this rising trend in mean values with age (table 5.05, p. 101) but the other biochemical markers do not seem to be affected by age (tables 5.04, p.100, table 5.06, p.101, table 5.07, p.102). A rising trend with age is illustrated in figures 5B, p.59 (methyl mercury) and 5E (LH). From logistic regression analysis, Age was found to be significantly associated in a direct relationship with I-Hg detection (table 5.93, p. 123). From linear regression analysis, Age was found to be significantly associated in a direct relationship with organic mercury concentration (table 5.94, p. 124).

The raw data and survey weighted data provide evidence of an increase in the proportion of I-Hg detection in the 2001-2002 survey populations as compared to the baseline, 1999-2000 survey population (Table 5.22, p.46, Table 5.21, p.103). From the results of the T-Test, there is evidence to reject the null hypothesis that the mean values of I-Hg detection are equivalent for the two survey groups (Table 5.21A, p.103). The two survey populations possess slightly different age and race distributions (Figure 5.01, p.64, Figure 5.02, p.66). To control for these differences, comparisons were adjusted for age and race. From both marginal models (logistic regression) and longitudinal regression analysis (Table 5.21B,C,D,E, p.103), there is evidence of a significant increase in the proportion of detectable blood inorganic mercury concentrations (I-Hg) over the years 1999-2002. Survey participants in the 2001-2 years were 320% more likely to have detectable levels of inorganic mercury in their blood than participants of the same race and age in the earlier survey group (1999-2000) (Table 5.22, p. 46, Tables 5.21F,G, p. 103).

To ensure the validity of these results in assessing the risks of I-Hg detection between survey populations, the analysis was repeated with categorical explanatory variables for age (Table 5.23, p.107). The transformed values were: age1= 16-19 years, age2= 20-29 years, age 3= 30-39 years, age4= 40-49 years. All logistic regressions were repeated with these dummy variables, and compared to the baseline population, age3= 30-39 years. In the raw population, both naive and robust estimates were performed. The analysis was repeated on the survey weighted population. The odds ratio for the estimated risk of I-Hg detection in Survey population 2001-2002 as compared to the baseline survey population 1999-2000, is comparable to the previous estimate using age as a continuous variable. In the raw population, the youngest age group (16-19 years) displayed a reduced risk of I-Hg detection as compared to the baseline (30-39 year old) population. In addition, race1 (Mexican American) and race 4 (African American) had an increased risk of I-Hg detection as compared to the baseline white population (table 5.23(A,B), p. 107). In the survey weighted population , the youngest age group (16-19) had a reduced chance of I-Hg detection as compared to the baseline (30-39 year old) population (table 5.23 (C), p.107). In addition, race 4 (African American) had an increased risk of I-Hg detection as compared to the baseline white population. In the survey weighted population, race1 (Mexican

American) did not have a significant difference in I-Hg detection as compared to the baseline white population.

From logistic regression analysis, there is evidence that I-Hg detection was significantly associated with Bilirubin (directly) in the full, survey weighted population (Table 5.5, p.47, Figure 5.03 (A), p. 100). An odds ratio of 1.28 indicates that there was a 28% increase in the population averaged risk of I-Hg detection associated with a one standard deviation rise in bilirubin, among people of the same race and age by year (Table 5.51, p. 47). CH_3Hg concentration was also directly associated with I-Hg detection (Table 5.5, p. 47) (Fig. 5.03(D), p. 48, Fig. 5.11, p. 73). An Odds Ratio of 1.48 indicates that there was a 48% increase in the population averaged risk of I-Hg detection associated with a one standard deviation rise in blood organic mercury, among people of the same race and age by year (Table 5.51, p. 47). White Blood Cell Count and Luteinizing Hormone were inversely associated with I-Hg Detection in the raw population but not in the survey weighted, full population. However, when a subpopulation is restricted by age (30-39 years for WBC, 35-39 years for LH), both LH and WBC were significantly associated in an inverse relationship with I-Hg Detection in both the raw and survey weighted populations (Table 5.5, p. 47, Fig. 5.03(B) and (C), p. 48). These associations were adjusted for age in years as a continuous variable (Fig. 5.2(D), p.75, Fig. 5.3(D), p. 79). An Odds Ratio of 0.654 indicates that there was a 35% decrease in the population averaged risk of I-Hg detection associated with a one standard deviation rise in white blood cell count, among people of the same race and age by year (Table 5.5, p. 47). An Odds Ratio of 0.469 indicates that there was a 53% decrease in the population averaged risk of I-Hg detection associated with a one standard deviation rise in luteinizing hormone, among people of the same race and age by year (Table 5.5, p. 47). These associations had low correlation coefficients so they are considered significant but weak. These results were confirmed by T-Tests (Fig. 5.1B, p. 70 and Fig. 5.3B, p. 79) that give evidence to reject the null hypothesis that the mean levels of these biochemical profile markers were the same for both groups of I-Hg detection. Therefore, the alternative hypothesis, that mean values of these biochemical profile markers were different for the two groups of I-Hg detection is accepted. I-Hg detection was also directly associated with urinary mercury. These results were also confirmed by T-Tests (Figure 5.11B, p. 73).

To test the results of the logistic regressions of I-Hg detection for rigor, explanatory variables were transformed into categorical variables. First, age in years (ridageyr) was transformed into categorical variables for age in decades (Fig. 5.31(A), p.82). The baseline population was age3= 30-39 years. Logistic regression was performed with biochemical profile markers as continuous variables and age and race as categorical variables. CH_3Hg concentrations were associated with I-Hg detection using categorical variables for age, and race in both raw and survey weighted populations with equivalent results to the analysis using age as a continuous variable. Bilirubin was associated with I-Hg detection in the logistic regression using race and age as categorical variables in both the raw and survey weighted populations with equivalent results to the analysis using age as a continuous variable. Luteinizing hormone (LH) was associated with I-Hg detection in the raw population when race and age are transformed as categorical variables. However this association does not persist in the survey weighted population. With age as a categorical variable and race as a categorical variable, I-Hg detection was associated with white blood cell (WBC) in the raw population but not in the survey weighted population.

Next, the biochemical profile markers were transformed into binary, categorical variables: 0= low levels, and 1=high levels (defined as 1 std. deviation above the mean). After transforming blood CH_3Hg concentrations into binary, categorical variables for low (orgmerc2=0, < 1 std. deviation above the mean, <3.3 ug/L) and high levels (orgmerc2=1, >= 3.3 ug/L), a significant, direct association with I-Hg detection is evident. An odds ratio of 3.7 indicates that there was a 270% increase in the population averaged risk of I-Hg detection among those with high levels of organic mercury as compared to those

with low organic mercury levels, among people of the same race and age by decade (Table 5.51, p. 47). After bilirubin was transformed into a binary, categorical variable (bili2=high), a significant, direct association with I-Hg detection was evident. An odds ratio of 1.95 indicates that there was a 95% increase in the population averaged risk of I-Hg detection among those with high levels of bilirubin as compared to those with low bilirubin levels, among women of the same race and age by decade (Table 5.51, p.47). With LH (lbxlh) transformed as a binary categorical variable, there were no significant associations with I-Hg detection, even in subpopulations restricted by age. With WBC as a binary, categorical variable, there was no significant relationship with I-Hg detection, even in subpopulations restricted by age.

When a subpopulation is restricted to the roughly 5% of the population with detectable levels of I-Hg, and I-Hg is then treated as a continuous variable, linear regressions indicate that there are no significant associations with the markers of interest. This may be due to the fact that this population is already in the top 5% of I-Hg levels, the population is small, and measurement error and variance are significant factors in I-Hg determination. Converting this population restricted to I-Hg detection into high and low values for blood I-Hg concentration, likewise produces no significant associations with biomarkers of interest (Fig. Fig. 5.33, p. 53).

After linear regression analysis, marginal models provide evidence that blood methyl mercury (CH_3Hg) was significantly associated, in a direct relationship with bilirubin (Table 5.6, p.51, Figure 5.4(A), p.51, table 5.72 A, p. 112) and in an inverse relationship with both white blood cell counts (Table 5.6, p.51, Fig 5.4C, p.51, table 5.72 C, p.112) and luteinizing hormone (LH) (Table 5.6, p.51, Table 5.72 B, p.112).

To test these weak associations for rigor, subpopulations were created by age (decade). The association between methyl mercury and bilirubin was associated with ages 40-49 years (Table 5.73A, p. 115) and ages 30-39 years (table 5.73 C, p.115) (Figure 5.5B, p. 89). The Association between methylmercury and white blood cell count was associated with ages 40-49 (Table 5.73 B, p. 115) and ages 30-39 Years (Table 5.73 D, p.115). The association between methylmercury and LH was associated with ages 35-39 Years (Table 5.73 E, p.115). To test the sensitivity of these associations, outliers were dropped from the population and we repeated the regressions. When the outliers were dropped, white blood cell count was still associated with methyl-mercury (Figure 5.5A, p.89).

Analysis of race and mercury concentration indicates that African American's have an elevated risk of I-Hg detection as compared to the baseline white population (table 5.92, p.120). In addition, African Americans and Mexican Americans had elevated population averaged mean urinary mercury concentration as compared to the baseline white population (table 5.92, p.120). In the subpopulation restricted to I-Hg detection (>0.4 ug/L, LOD, n=160), the races with the highest levels of blood inorganic mercury (>1 std. dev. above the mean I-Hg for those with detectable levels) were African American and Hispanic (Fig. 5.33, p.53). When the subpopulation was restricted to African Americans, the race with the greatest risk of I-Hg detection, I-Hg detection and CH_3Hg were both inversely associated with luteinizing hormone in the full population (LH) (Table 5.52, p.54, Table 5.53, p. 54, Fig. 5.8, p.92, Fig. 5.9, p.97). For African American women, an odds ratio of 0.509 indicates that there was a 49% reduction in the full population averaged risk of I-Hg detection associated with a one standard deviation rise in LH (Table 5.52, p.54, Fig 5.8(A), p.92). These results were adjusted for age, as a continuous explanatory variable by year, and confirmed by t-tests (Figure 5.8(A), p. 92). In contrast to LH, White Blood Cell Count and Bilirubin were not associated with either bioindicators of chronic mercury exposure within the African American subpopulation (Table 5.52, p54, 5.53, p.54, Fig. 5.8(B)(C), p.92, Figure 5.9(B)(C), p. 97). Within the African American subpopulation, I-Hg detection

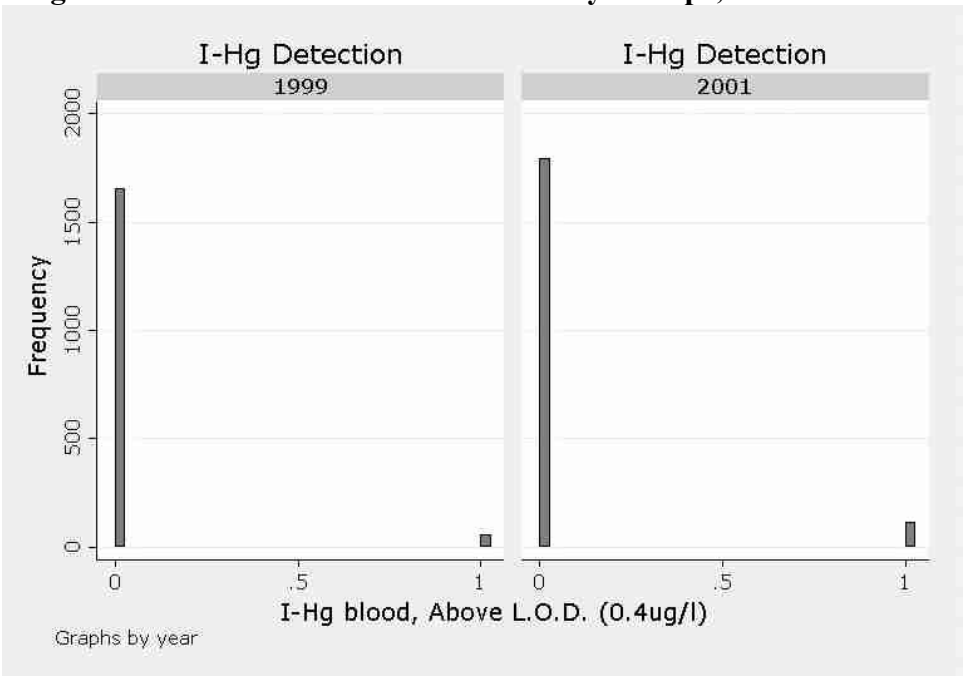
was strongly associated with both CH_3Hg, and Urinary Mercury (U-Hg) (Table 5.52, p. 54, Fig. 5.8(D), Fig. 5.8(E), p. 92).

In the full population, urinary mercury levels were significantly associated with white blood cell count in an inverse relationship but only in the raw population and not in the survey weighted population. Urinary mercury levels were associated with bilirubin levels in a direct relationship in both the raw population and the survey weighted population (table 5.91, p. 119). There was no significant relationship between urinary mercury levels and LH in the full population.

Table 5.22: Risk of I-Hg Detection for Survey Group 2001-2002 as compared to the baseline 1999-2000 survey group, in the NHANES Combined Survey Population (1999-2002). I-Hg detection is a binary variable: non-detect=0, I-Hg detection=1. Results of logistic regression presented are adjusted for age (continuous variable) and race (categorical variable). Only associations that were significant in the raw population are presented here with further adjustments for survey weights.

Survey Group	Population (Number of Observations)	Association With I-Hg Detection	Odds Ratio	Std. Error	P-Value	[95% Confidence Interval]
2001-2002 (year2)	Full (3616)	Direct	3.19	0.850	<0.001	1.85 5.50

I-Hg Detection Rates for each of two Survey Groups; NHANES 1999-2000 and 2001-2002. (A) Histogram




```
Summary for variables: ihgdetect
     by categories of: year
    year |      mean        sd         N
---------+------------------------------------
    1999 |  .0322014  .1765863        1708
    2001 |  .0592243  .2361059        1908
---------+------------------------------------
   Total |  .0464602  .2105086        3616
```

Table 5.5: Associations Between I-Hg Detection and Biochemical Profile Markers in the NHANES combined survey population (1999-2000 and 2001-2002). Odds ratios for I-Hg detection (binary variable: non-detect=0, I-Hg detection=1) are calculated for a one standard deviation change in the explanatory variables using STATA command: lincom (Std. Dev.) (X) after logistic regression. Results presented are adjusted for age (continuous variable) and race (categorical variable). Only associations that were significant in the raw population are presented here with further adjustments for survey weights.

Biochemical Profile Marker	Population (Number of Observations)	Association With I-Hg Detection	Odds Ratio of I-Hg Detection for a 1 Std. Dev. Change in Marker	Std. Error	P-Value	[95% Confidence Interval]
Bilirubin (lbdstbsi)	Full (3569)	Direct	1.28	0.139	0.029	1.03 1.60
White Blood Cell (lbxwbc)	Full Population (3614)					
	Raw	Inverse	0.813	0.077	0.028	0.676 0.978
	Survey Weighted	Not Significant	0.768	0.104	0.061	0.582 1.01
	Subpopulation Restricted by Age: 30-39 Years. (855)	Inverse	0.654	0.135	0.049	0.428 0.998
Luteinizing Hormone (lbxlh)	Full Population (1133)					
	Raw	Inverse	0.689	0.115	0.026	0.496 0.956
	Survey Weighted	Not Significant	0.744	0.158	0.175	0.481 1.14
	Subpopulation Restricted by Age: 35-39 Years. (391)	Inverse	0.469	0.164	0.039	0.229 0.961
CH_3Hg (organic mercury)	Full (3613)	Direct	1.48	0.097	<0.001	1.30 1.70

Table 5.51: Associations Between I-Hg Detection and Biochemical Profile Markers in the NHANES combined survey population (1999-2000 and 2001-2002) as Categorical Variables. Biochemical profile markers are transformed into binary variables for low (0) and hi levels (1) (hi = greater than one std. deviation above the mean). Survey weighted logistic regression are adjusted for age as categorical variables by decade, with the baseline population age3 = thirty years olds. The survey weighted logistic regression is also adjusted for race, as categorical variables, with the baseline population race3 = whites. Only associations that were significant in the raw population are presented here with further adjustments for survey weights.

Biochemical Profile Marker (BPM)	Population (Number of Observations)	Association With I-Hg Detection	Odds Ratio of I-Hg Detection for Hi vs. Low (BPM)	Std. Error	P-Value	[95% Confidence Interval]
Bilirubin (binary variable)	Full (3616)	Direct	1.95	0.575	0.031	1.07 3.56
CH_3Hg (binary variable)	Full (3616)	Direct	3.70	0.953	<0.001	2.18 6.26

<u>Figure 5.03</u>: Graphs of Probabilities for I-Hg Detection (Blood Inorganic Mercury) vs. Concentration of Biochemical Profile Markers in NHANES 1999-2002. (A) Bilirubin, full population, survey weighted. (B) White Blood Cell Count, survey weighted, subpopulation restricted by age: 30-39 Years. (C) Luteinizing Hormone, survey weighted, subpopulation restricted by age: 35-39 Years. (D) CH_3Hg, full population, survey weighted.
I-Hg Detect is a transformed binary value (0 = non-detect 1= detection). Race is adjusted against baseline white population (race=3); race1= Mexican American, race2=Hispanic, race3=White, race4= African American, race5=Other. Age is a continuous variable by decade.

<u>(A)</u>:

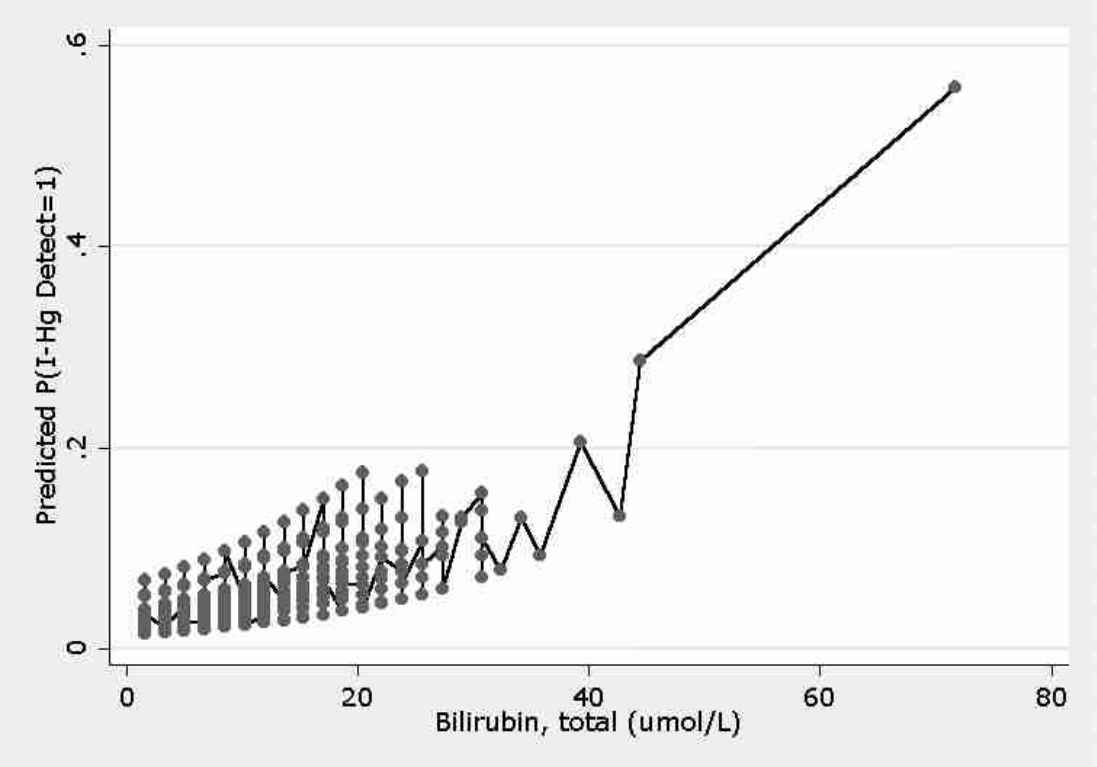
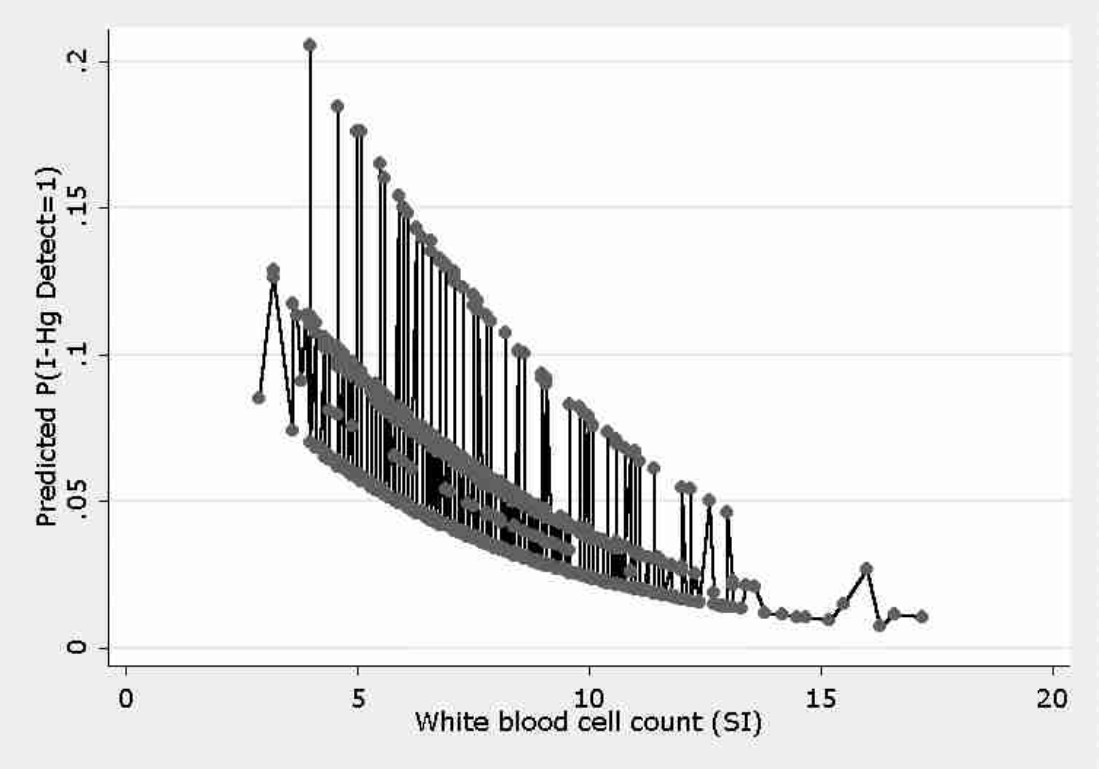
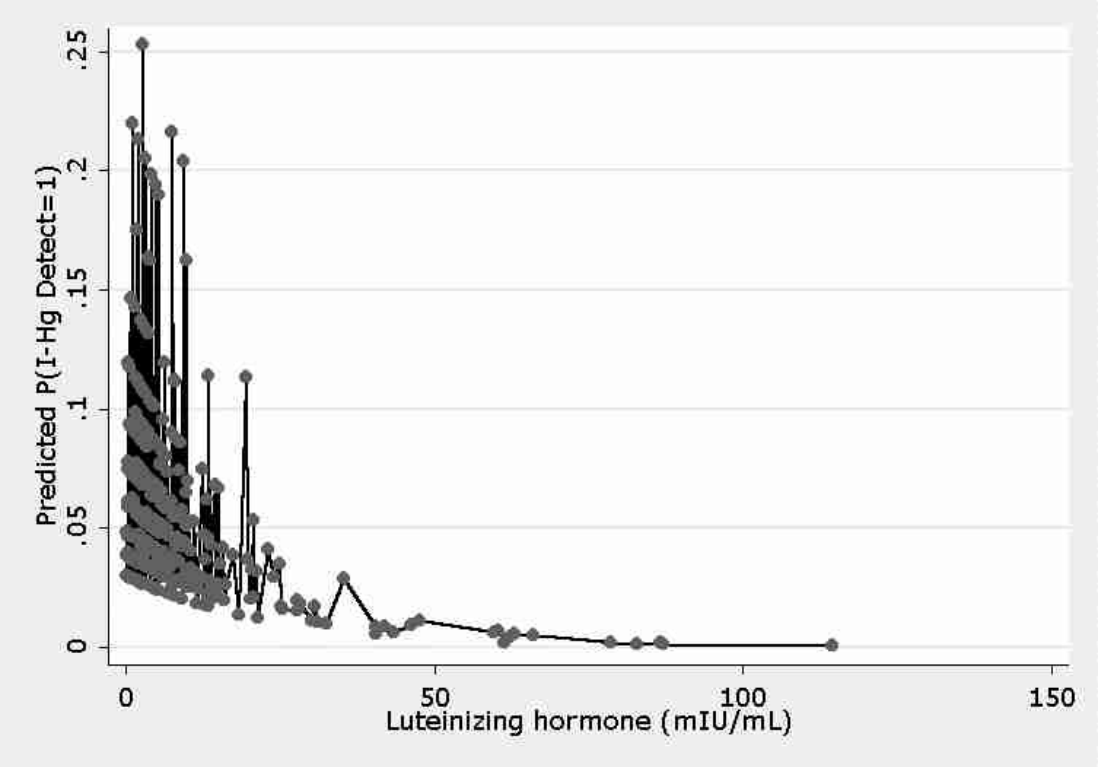


Figure 5.03 (B):



(C):



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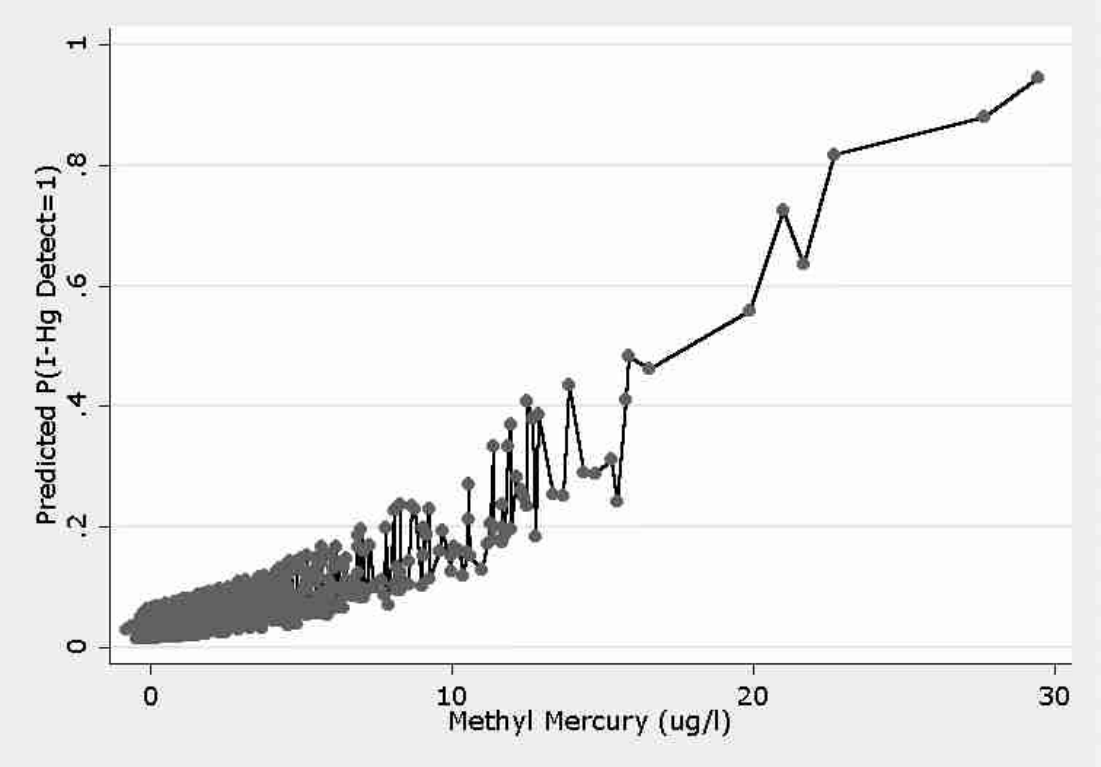


Table 5.6: Associations Between CH_3Hg and Biochemical Profile Markers in the NHANES combined survey population (1999-2000 and 2001-2002). Results presented are slopes for linear regression, adjusted for age (continuous variable) and race (categorical variable). Only associations that were significant in the raw population are presented here with further adjustments for survey weights.

Biochemical Profile Marker	Population (Number of Observations)	Association With CH_3Hg	Slope	Std. Error	P-Value	[95% Confidence Interval]
Bilirubin (lbdstbsi)	Full (3566)	Direct	0.044	0.018	0.018	0.008 0.80
White Blood Cell (lbxwbc)	Full (3611)	Inverse	-0.065	0.028	0.026	-0.121 -0.008
Luteinizing Hormone (lbxlh)	Full Population 35-49 Years. (1132)	Inverse	-0.011	0.004	0.006	-0.018 -0.003

Figure 5.4: Observed Points and Fitted lines for Linear Regressions of Blood Methyl Mercury (ug/L) versus biochemical profile markers in Women ages 16-49 years, combined 1999-2000 and 2001-2002 NHANES. **(A)** Blood Bilirubin **(B)** Blood Luteinizing Hormone * **(C)** White Blood Cell Count
*LH measured in subpopulation, Ages 35-49 years.

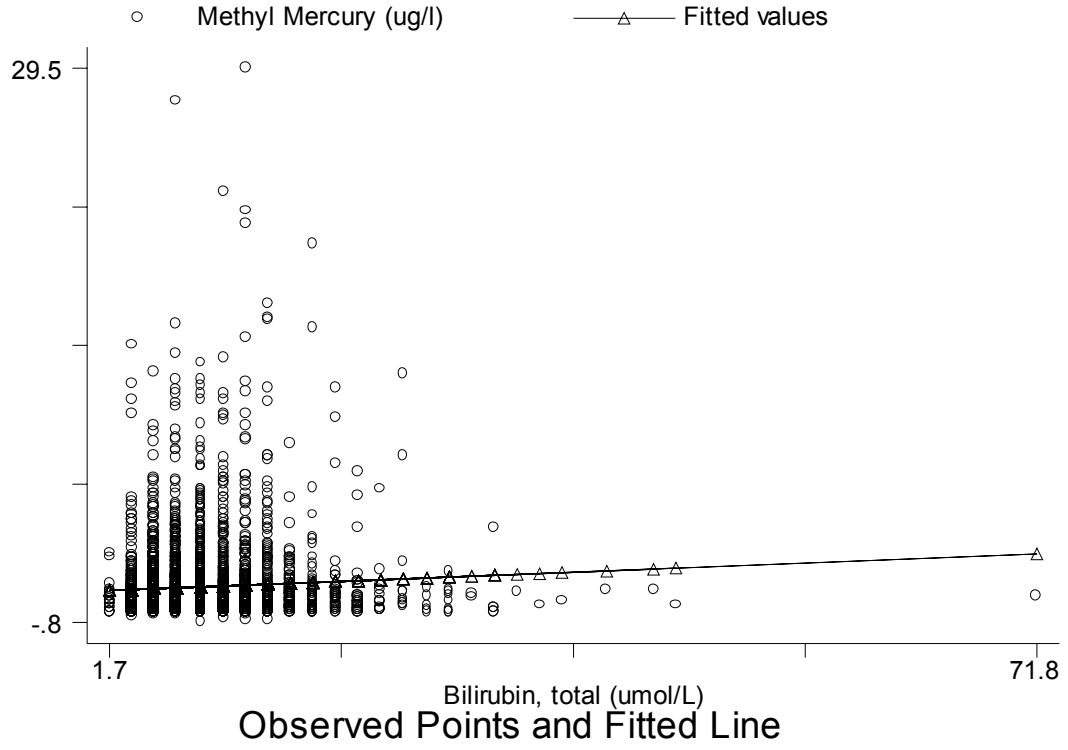


Figure 5.4 (B)

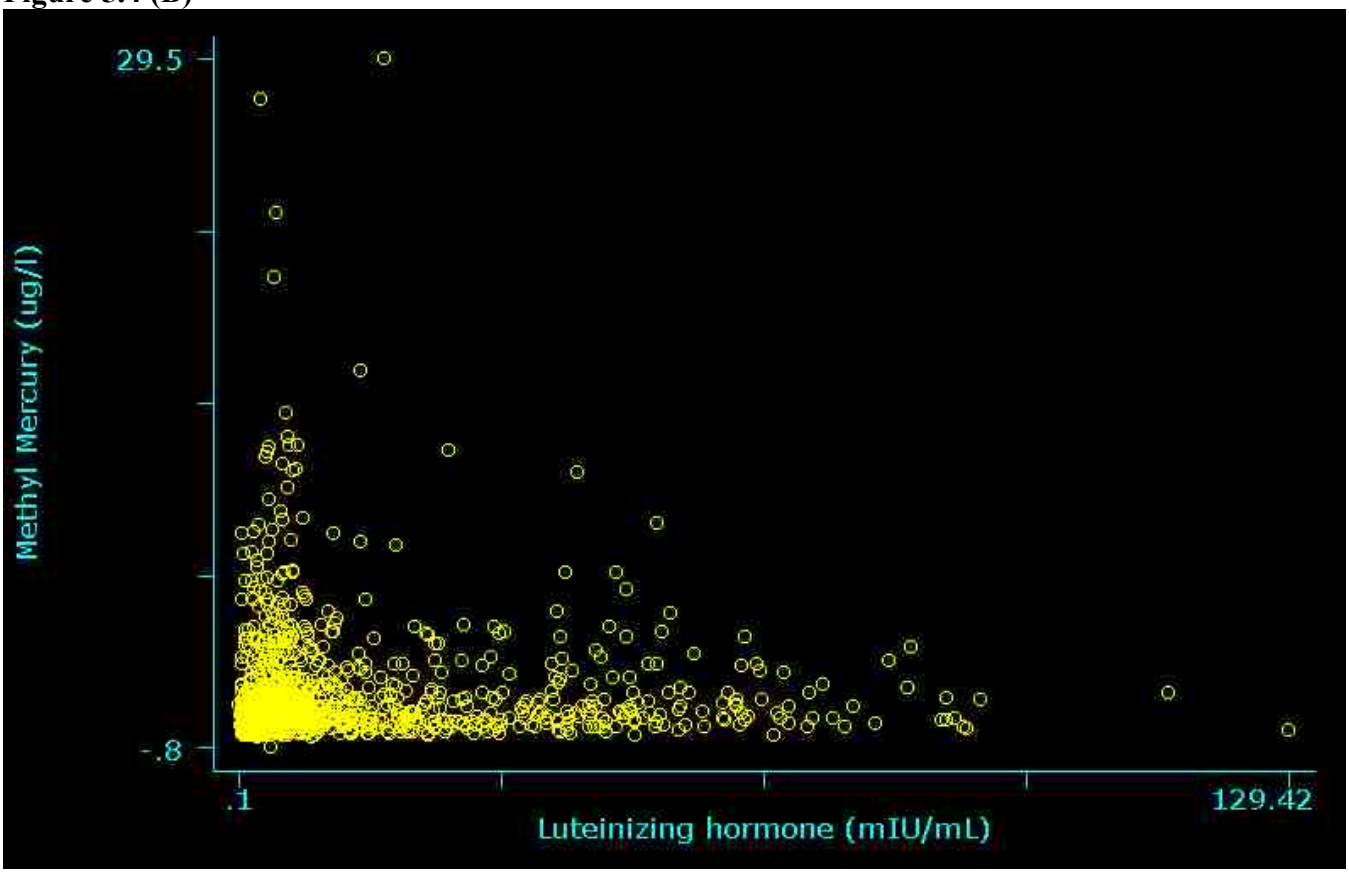


Figure 5.4 (C)

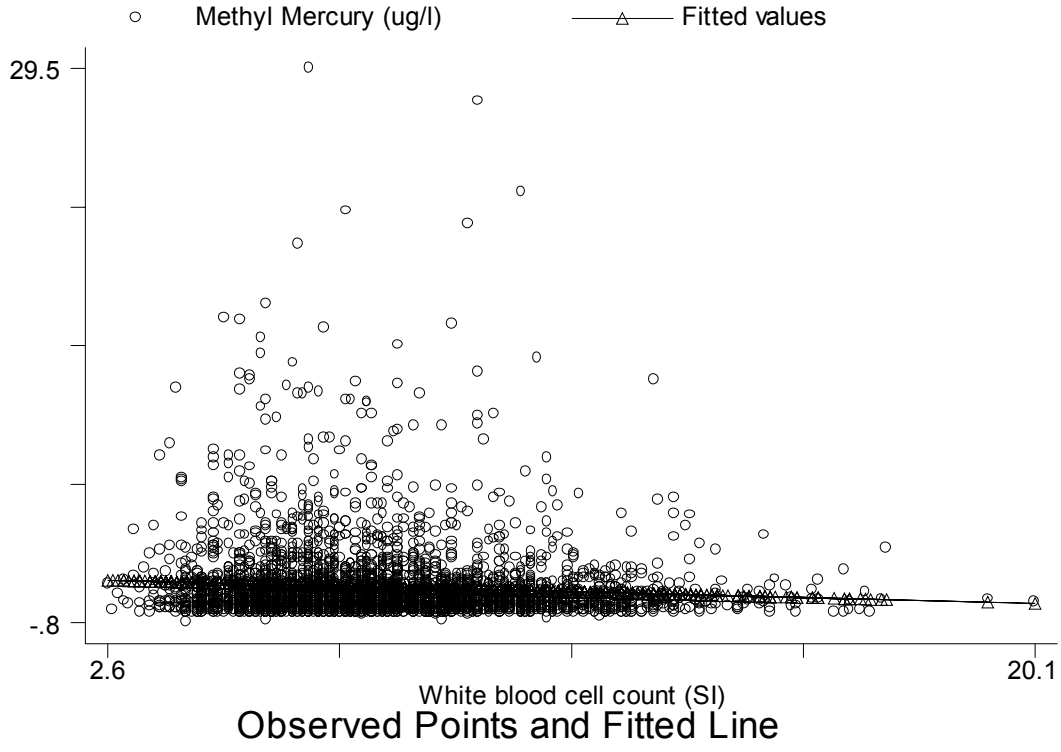
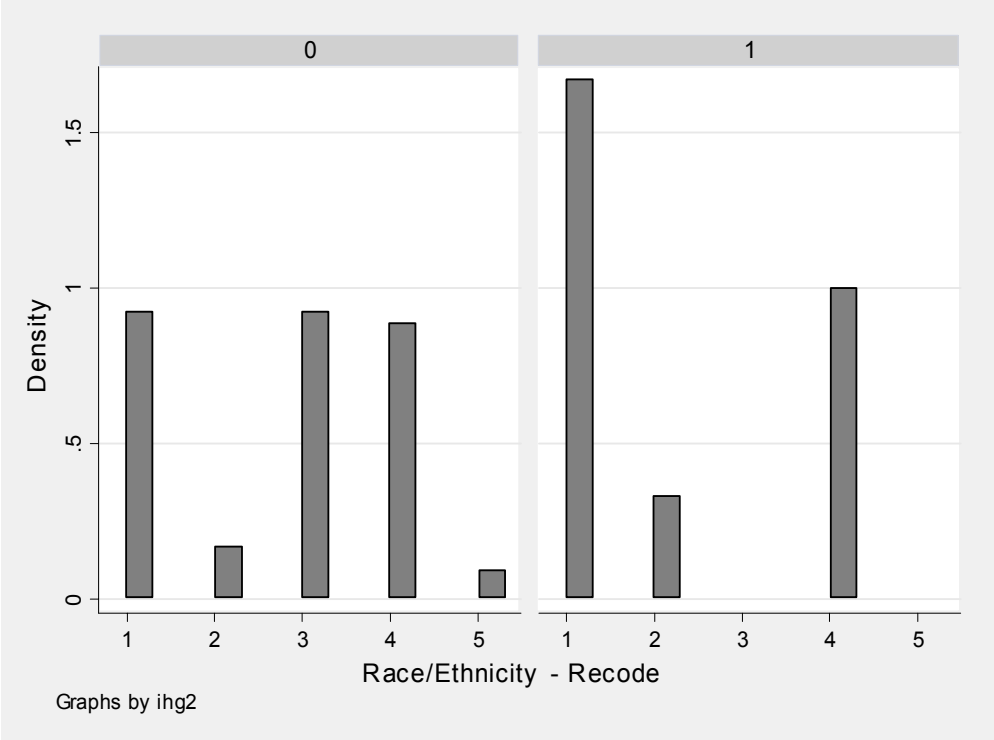


Figure 5.33: Subpopulation Restricted by I-Hg Detection (>0.4 ug/L, LOD), NHANES 1999-2003.

5.33(A): Binary variable for Low (<1 std. dev. above the mean, ihg2=0, n=159) and Hi (> 1 std. dev. above the mean, ihg2=1, n=9) in the NHANES subpopulation restricted to I-Hg detection (>0.4 ug/L, LOD, n=168).

```
. tabulate ihg2, gen(ihg2)

       ihg2 |      Freq.     Percent        Cum.
------------+-----------------------------------
          0 |        159       94.64       94.64
          1 |          9        5.36      100.00
------------+-----------------------------------
      Total |        168      100.00
```

5.33(B): Histogram of race in Low (<1 std. dev. above the mean, ihg2=0) and Hi (> 1 std. dev. above the mean, ihg2=1) in the NHANES subpopulation (n= 168) restricted to I-Hg detection (>0.4 ug/L, LOD).



Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other

Table 5.52: Subpopulation Restricted by race (African American). Associations Between I-Hg Detection and Biochemical Profile Markers in African American women in the NHANES combined survey population (1999-2000 and 2001-2002). Odds ratios for I-Hg detection (binary variable: non-detect=0, I-Hg detection=1) are calculated for a one standard deviation change in the explanatory variables using STATA command: lincom (Std. Dev.) (X) after logistic regression. Results presented are adjusted for age in years (continuous variable).

Biochemical Profile Marker	Population (Number of Observations)	Association With I-Hg Detection	Odds Ratio of I-Hg Detection for a 1 Std. Dev. Change in Marker	Std. Error	P-Value	[95% Confidence Interval]
Bilirubin (lbdstbsi)	Full (775) Raw	Not Significant (P>0.5)				
White Blood Cell (lbxwbc)	Full Population (789) Raw	Not significant (P>0.5)				
Luteinizing Hormone (lbxlh)	Full Population (253) Raw	Inverse	0.509	0.148	0.020	0.288 0.900
CH_3Hg (organic mercury)	Full Population (789) Raw	Direct	1.88	0.199	<0.001	1.53 2.31
Urinary Mercury (urxuhg)	Full Population (763) Raw	Direct	3.26	0.681	<0.001	2.17 4.91

Table 5.53: Subpopulation Restricted by race (African American). Associations Between CH_3Hg and Biochemical Profile Markers in African American women in the NHANES combined survey population (1999-2000 and 2001-2002). Results presented are slopes for linear regression, adjusted for age (continuous variable in Years).

Biochemical Profile Marker	Population (Number of Observations)	Association With CH_3Hg	Slope	Std. Error	P-Value	[95% Confidence Interval]
Bilirubin (lbdstbsi)	Full (774)	Not Significant (P>0.5)	0.0188	0.017	0.282	-0.015 0.053
White Blood Cell (lbxwbc)	Full (788)	Not Significant (P>0.5)	-0.007	0.040	0.862	-0.072 0.086
Luteinizing Hormone (lbxlh)	(252)	Inverse	-0.014	0.006	0.027	-0.026 -0.0015
Urinary Hg (urxuhg)	(762)	Direct	0.197	0.056	<0.001	0.087 0.307

Discussion

In the marginal models, statistical analysis provides significant evidence to reject the null hypothesis that bioindicators for chronic mercury exposure (blood I-Hg and CH_3Hg) and the selected biochemical profile markers are not associated (Table 5.5, p.47, Table 5.6, p.51, Table 5.22, p. 46). The alternative hypothesis is accepted, that bioindicators for chronic mercury exposure and selected biochemical profile markers for the targets of mercury deposition and effect are associated, specifically within women, ages 30-49 years, in the general U.S. population. Associations presented in this study were rigorous and conserved in both raw and survey weighted populations and in both naïve and robust statistical estimations. Results were adjusted for the potential confounders of race and age, and yet, residual confounding may remain an issue. Significant results from the logistic regressions were confirmed by non-parametric T-Tests.

In the NHANES survey, I-Hg concentrations were determined after an indeterminate storage time of up to one year. Previous research has shown that storage times greater than 3 days result in sample loss and increased variance between measurements. Due to these limiting factors, I-Hg concentrations in the NHANES study may underestimate true concentration levels. In this study, I-Hg was transformed into a binary variable, I-Hg detection. This transformation helps address the issues of measurement error, variance, and estimate values below the LOD by grouping the population into low I-Hg levels (below the lod) and high levels (above the lod).

Low correlation coefficients for the logistic regressions of I-Hg detection demonstrate that these associations were weak. Weak associations are often typical of biological systems however large sample comparisons may produce such results with no biological importance. The fact that these associations persisted in smaller subpopulations restricted by age and year of survey suggests that these results were not a statistical anomaly. Tests for rigor involved dropping outliers and investigating subpopulations grouped by age. Results from these tests yielded more evidence that these associations were significant. In addition, as these results were persistent with both bioindicators for chronic mercury exposure (Blood I-Hg and Blood CH_3Hg) the reported associations with biochemical profile markers are even more unlikely due to random chance. Confirmation of results in the survey weighted population and in subpopulations restricted by age, extends an external validity to these associations in the U.S. population, women ages 30-39 years. The biochemical profile markers had the most enduring associations with blood organic mercury, then blood I-Hg and least of all urinary mercury. These findings help confirm previous evidence that blood mercury levels are the most suitable bioindicators of body burden and chronic mercury exposure.

As this is a cross sectional study, it is impossible to determine cause and effect. In addition, these are population averaged results and therefore, conclusions can not be drawn on individuals. This makes it difficult to interpret these associations but previous research offers possible interpretations. Previous research indicates that I-Hg detection may be an important biomarker for organic mercury demethylation and resultant I-Hg deposition in tissues of the human body[22]. In contrast, CH_3Hg in blood is a biomarker that reflects time since recent organic mercury exposure[6]. Taken together, both mercury forms (I-Hg and CH_3Hg in blood) serve as biondicators for chronic, organic mercury exposure. As the biochemical profile markers (bilirubin, LH, WBC) were associated with both blood I-Hg and CH_3Hg, it may be reasonable to assume that they are also associated with chronic, organic mercury exposure.

From the results of logistic regressions using transformed categorical explanatory variables, it is evident that the most robust, direct association exists between I-Hg detection and CH_3Hg concentration in the blood. This supports previous research that maintains demethylation of CH_3Hg into I-Hg is a main source of I-Hg in the body [6]. The direct associations of both blood inorganic and organic mercury with

age suggest that chronic mercury exposure and I-Hg deposition may be a process of accumulation related to the aging process.

Robust associations between bioindicators for chronic mercury exposure (I-Hg and organic mercury) with bilirubin are persistent in the NHANES population. The role of bilirubin in the enterohepatic circulation of organic mercury may help explain this direct association between bilirubin and I-Hg. Associations between chronic mercury exposure and bilirubin reflect a physiological connection between exposure and the rate of excretion. The bile is an important step for excretion of mercury from the liver. Impaired excretion of methyl mercury and increased enterohepatic circulation may elevate the rate of I-Hg deposition and risks of associated neurodegenerative disease.

Associations between chronic mercury exposure and biochemical profile markers were associated with subpopulations in the older age groups. This may be explained as chronic mercury exposure is directly related to the duration of exposure, and thus, the accumulation of I-Hg deposition is expected to be associated with age. To prevent the possible confounding by age, results were adjusted for age in years as a continuous variable and age in decades as a categorical variable. Associations between I-Hg detection and bilirubin, while significant in the full population (16-49 years), were tied to subpopulations above the age of thirty. In like manner, both LH and WBC were related to I-Hg detection in subpopulations restricted to the third decade of life, even after adjusting for age in years as a continuous, explanatory variable (Fig. 5.2(C), p. 75, Fig 5.3(C), p. 79)..

The inverse association between mercury exposure and white blood cell count provides more evidence to link chronic mercury exposure, I-Hg deposition, and effects on the immune system within the general U.S. population. The immune system is a main target for mercury deposition and toxic effect. Inorganic mercury (I-Hg) deposits are associated with neurotoxic and immune pathways implicated in neurodegeneration[20]. There is ample evidence for immunogenetic susceptibility to mercury exposure [20, 23, 82, 87]. Even at sub acute, chronic mercury exposure levels, in vitro experiments have demonstrated the immunomodulatory effects of mercury exposure[94]. Previous studies have demonstrated that a direct interaction between the immune system and mercury exposure leads to the suppression of white blood cell activation [66]. Our results support this inverse association within the general U.S. population.

Results from this study suggest a link between chronic mercury exposure and pituitary function (LH) that is most evident in the third decade of life (35-39 years). This is in accordance with previous research that has demonstrated that the pituitary is a main target for I-Hg deposition [6]. This interaction may explain a pathway between chronic mercury exposure, target deposition, and a disease process leading to neurodegeneration. The disease process leading from chronic mercury exposure to neurodegenerative disease may include impaired liver function, increased I-Hg deposition, immune system damage, and focal deposition of I-Hg in the pituitary. Luteinizing Hormone (LH) is a gonadotropin secreted by the anterior pituitary that is involved in gonadotroph stimulation, mitogenisis, and immune regulation (for a review see,[90]). Studies have demonstrated that Alzheimer's Disease patients have elevated serum and neuronal levels of LH as compared to controls [90, 91]. Brain regions affected by AD show elevated expression of LH receptors (for a review see,[90]). In cell cultures, LH stimulates amyloid beta production, a key element in oxidative stress leading to AD pathology. LH receptors are also found on immune cells where they are associated with neuroprotection and a role in the pro-inflammatory signaling process in the brain (for a review see,[91]). A disruption of the pituitary, such as one incurred by focal I-Hg deposition, may result in a cascade of events leading from oxidative stress to impaired neuro-protection, unbalanced neuro-immune response, inflammation, and neurodegenerative disease.

These results demonstrate relationships between chronic organic mercury exposure and biochemical changes related to the main targets of inorganic mercury deposition and effect. These associations provide links between exposure and a biological mechanism leading to neurodegenerative disease. Associations with the immune system (white blood cell) and with the pituitary (luteinizing hormone) establish links between mercury exposure, deposition, and the risks of Autism and Alzheimer's Disease. Associations with the liver (bilirubin) reiterate concern that mercury deposition may increase enterohepatic circulation, raise the absorption rate of mercury, and thereby elevate susceptibility to future exposures such as from vaccines containing ethyl mercury.

In the full population, associations between I-Hg detection and LH, and between I-Hg detection and WBC, were significant in the raw population but not in the survey weighted population (Table 5.5, p. 47). This may be due to oversampling of African Americans who faced the highest risk of I-Hg detection. In fact, there were no whites in the 0.2% of the NHANES population with the highest blood I-Hg levels (Fig. 5.33, p. 53). This finding suggests that African Americans and Mexican Americans face the greatest risk of chronic mercury exposure, I-Hg deposition, and highest risks of associated disease. There was a significant, inverse association between chronic mercury exposure and luteinizing hormone (LH) in the subpopulation of African Women (Table 5.52, p.54, Table 5.53, p.54). Persistent results from the raw population, the survey weighted subpopulation (35-39), and the African American subpopulation, suggest a strong link between chronic organic mercury exposure and targeted inorganic mercury deposition in the pituitary.

A significant difference in the proportion of I-Hg detection between the two survey populations was reported in this study; 3% I-Hg detection in 1999-2000, to 6% I-Hg detection in 2001-2002. This difference in I-Hg detection may be interpreted in several ways. I-Hg detection variability may reflect error and variance due to the methods of I-Hg measurement. I-Hg detection may also reflect real differences in I-Hg deposition between geographical regions. A recent study of human mercury exposure from fish in the U.S. established that geographic variability in Hg concentrations, in different species of fish, affects per capita exposure [9]. Another source of geographic variability may be the amount of local coal burning power plants. Oxidized forms of mercury released from point source plumes may deposit locally by the source[1].

Alternatively, these differences in I-Hg detection may be explained by a longitudinal analysis of the American population, wherein the percentage of people with detectable levels of inorganic mercury in their blood is dramatically increasing over time. According to a panel of scientific experts, the rate of atmospheric mercury deposition may be increasing due to industrial emissions and climactic changes[1]. This likely rise in the rate of mercury deposition may signal a significant increase in the origins of global mercury exposure. A rising rate in detection of inorganic mercury within the U.S. population over time may indicate an increase in the rate of mercury deposition within the U.S. population. A rise in I-Hg detection may indicate increased susceptibility to further exposure. If this is a time dependent increase in I-Hg detection, the public health risks of associated neurodegenerative diseases may be rising over time as well. This possibility is of great concern and warrants continued monitoring of the NHANES population over time in order to determine the time trend of I-Hg detection rates within the U.S. population.

Conclusion

Data are sufficient to conclude there is an association between chronic, organic mercury exposure and inorganic mercury deposition in target systems of the human body. Results from this study present evidence to reject the null hypothesis that bioindicators for chronic, organic mercury exposure (blood I-Hg and CH_3Hg) are not associated with biochemical profile markers for the main targets of

inorganic mercury effect and deposition within the NHANES survey population. Therefore, the alternative hypothesis, that chronic, organic mercury exposure is associated with the main targets of deposition and effect, is accepted. The NHANES survey weighting design extends this inference with external validity to the general U.S. women's population, specifically ages 30-39 years.

In the 2004, IOM report on vaccines, their assessment of a biological mechanism concluded that there was no human evidence of an immune response that associates thimerosal exposure with autism [110]. This thesis presents human evidence within the U.S. population for a biological mechanism to a causative relationship between mercury exposure, immune dysregulation, and the risk of Autism. Associations of mercury exposure with the immune system (white blood cell) and with the pituitary (luteinizing hormone) establish links with Autism and Alzheimer's Disease. Mercury's neurotoxic effect on these targets would increase the risks of immune dysregulation, autimmune disorders, inflammation, impaired cell migration, and neurodegeneration. Associations with the liver (bilirubin) reiterate concern that mercury deposition may increase enterohepatic circulation, raise the absorption rate of mercury, and thereby elevate susceptibility to future exposure such as from vaccines containing ethyl mercury. The reported, direct association between I-Hg and organic mercury suggests that the demethylation of organic mercury within the body is a contributing source of I-Hg deposition.

In this study, African Americans were found to have the highest risk for I-Hg detection. In fact, there were no whites in the population with the highest blood I-Hg levels (Fig. 5.33). This finding suggests that African Americans and Mexican Americans face the greatest chronic mercury exposure, I-Hg deposition, and highest risks of associated disease. There was a significant, inverse association between chronic mercury exposure and luteinizing hormone (LH) in the subpopulation of African Women (Table 5.52, p. 54, Table 5.53, p. 54). Persistent results from the raw population, the survey weighted subpopulation (35-39), and the African American subpopulation, suggest a strong link between chronic organic mercury exposure, inorganic mercury deposition, and disruption of the pituitary.

This study presents associations between I-Hg deposition and biochemical profile markers (bilirubin, luteinizing hormone, and WBC) that are specific to the older population (above thirty). Links between mercury exposure, targets of deposition and effect, and the aging process are consistent with a process of exposure over decades. Cumulative I-Hg deposition in target sites within the body help explain a biological mechanism for disease. The link between chronic mercury exposure, deposition in the pituitary, and LH should be further investigated for a causal role in the development of AD.

Data are suggestive that there is a rising time trend in chronic mercury exposure and inorganic mercury deposition in target systems of the human body. Evidence presented in this study suggests that I-Hg detection rates were elevated in the later survey group (2001-2002) as compared to the baseline survey group (1999-2000). A rise in the rate of I-Hg detection may be associated with a rise in chronic mercury exposure, deposition, and associated risks for neurodegenerative diseases. Whether the rise in I-Hg detection was due to geographical differences in exposure, measurement error, or to a time dependent rise in the rate of chronic mercury exposure is unclear. However, this trend should be monitored in future NHANES surveys and further investigated in order to determine the overall trend of exposure, assess the related public health risks of mercury exposure, and form effective policy objectives. As emissions of mercury into the global environment and food chain continue to rise over time due to rising coal burning capacity worldwide, the public health threats of rising chronic mercury exposure, deposition, and associated diseases may be rising over time as well. While results from this study do not verify a time dependent increase in chronic mercury exposure and deposition, they do support this possibility. Rising rates of chronic mercury exposure and deposition may pose a devastating public health threat and warrants further study of chronic mercury exposure and effect within future NHANES populations.

Supplemental Figures for NHANES study

Figure 5.0: **Scatterplots of Age (Years) versus Biochemical Profile Markers**, in Women ages 16-49 years, combined 199-2000 and 2001-2002 NHANES. **(A)** Blood Methyl- Mercury **(B)** Urinary Mercury **(C)** Blood Bilirubin **(D)** Blood Leutenizing Hormone* **(E)** White Blood Cells
*LH measured in subpopulation, Women ages 35-49 years.

(A)

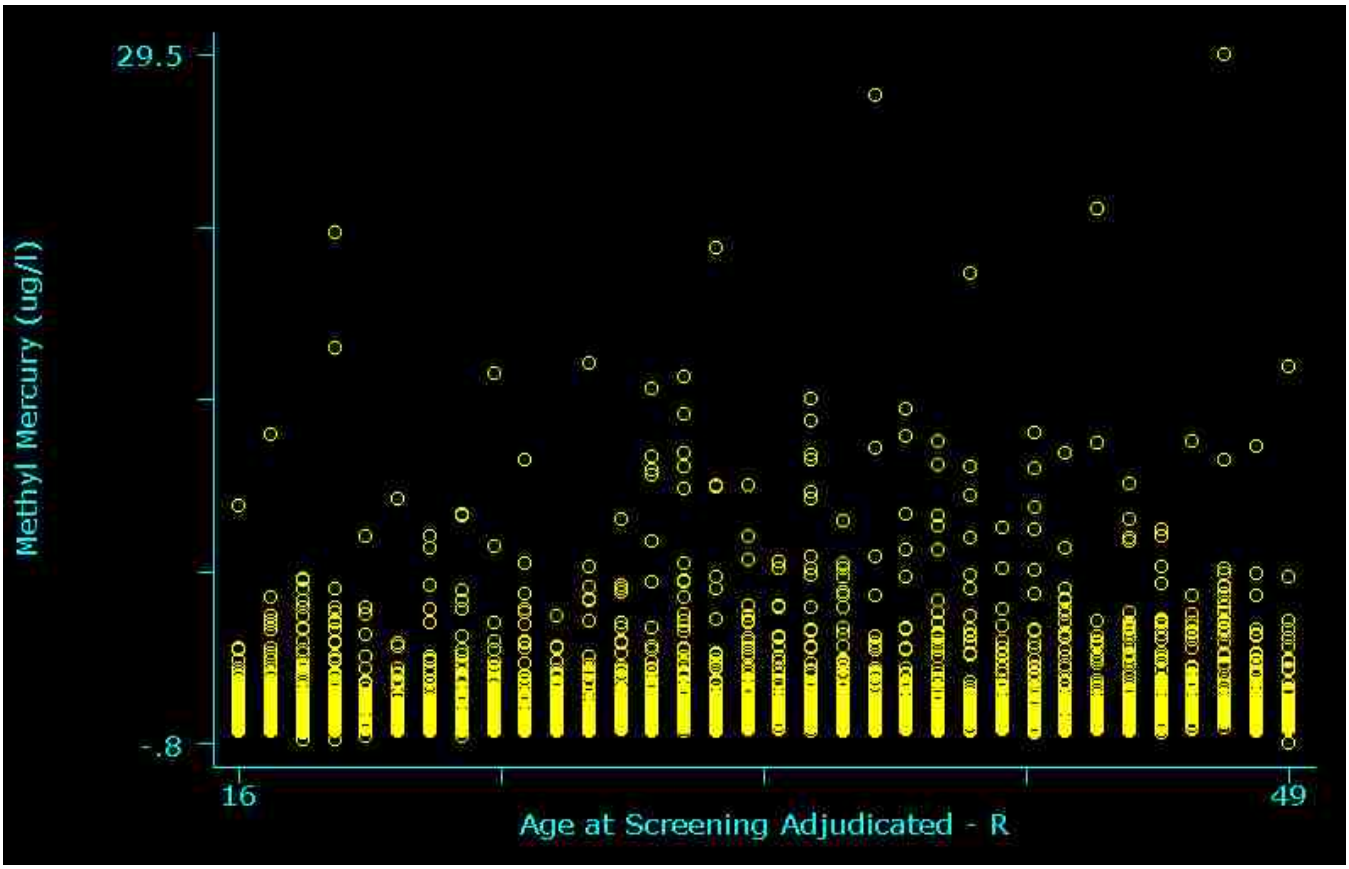


Figure 5.0: **(B)**

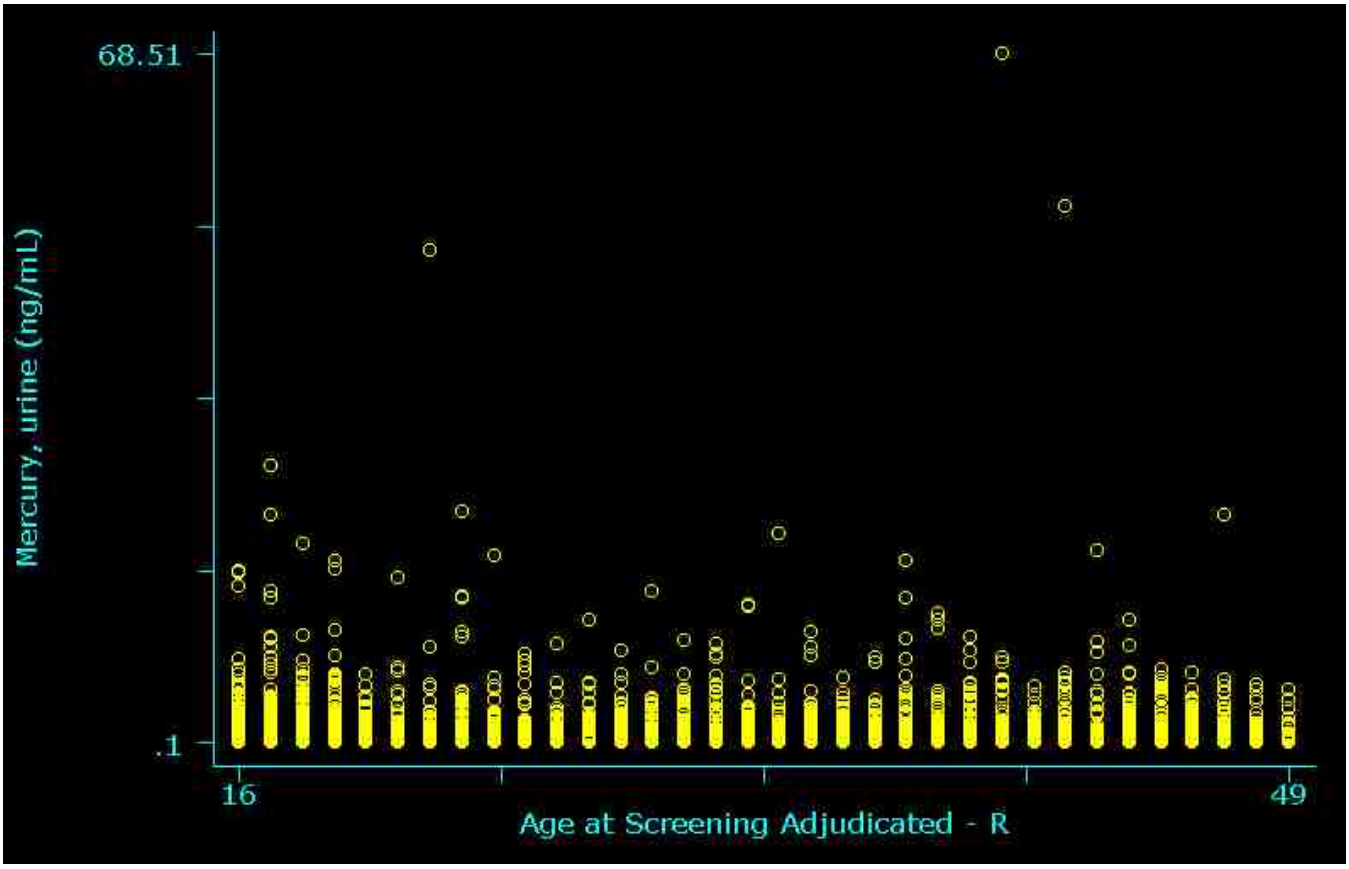

68.51
.1
Mercury, urine (ng/mL)
16
49
Age at Screening Adjudicated - R

Figure 5.0: **(C)**

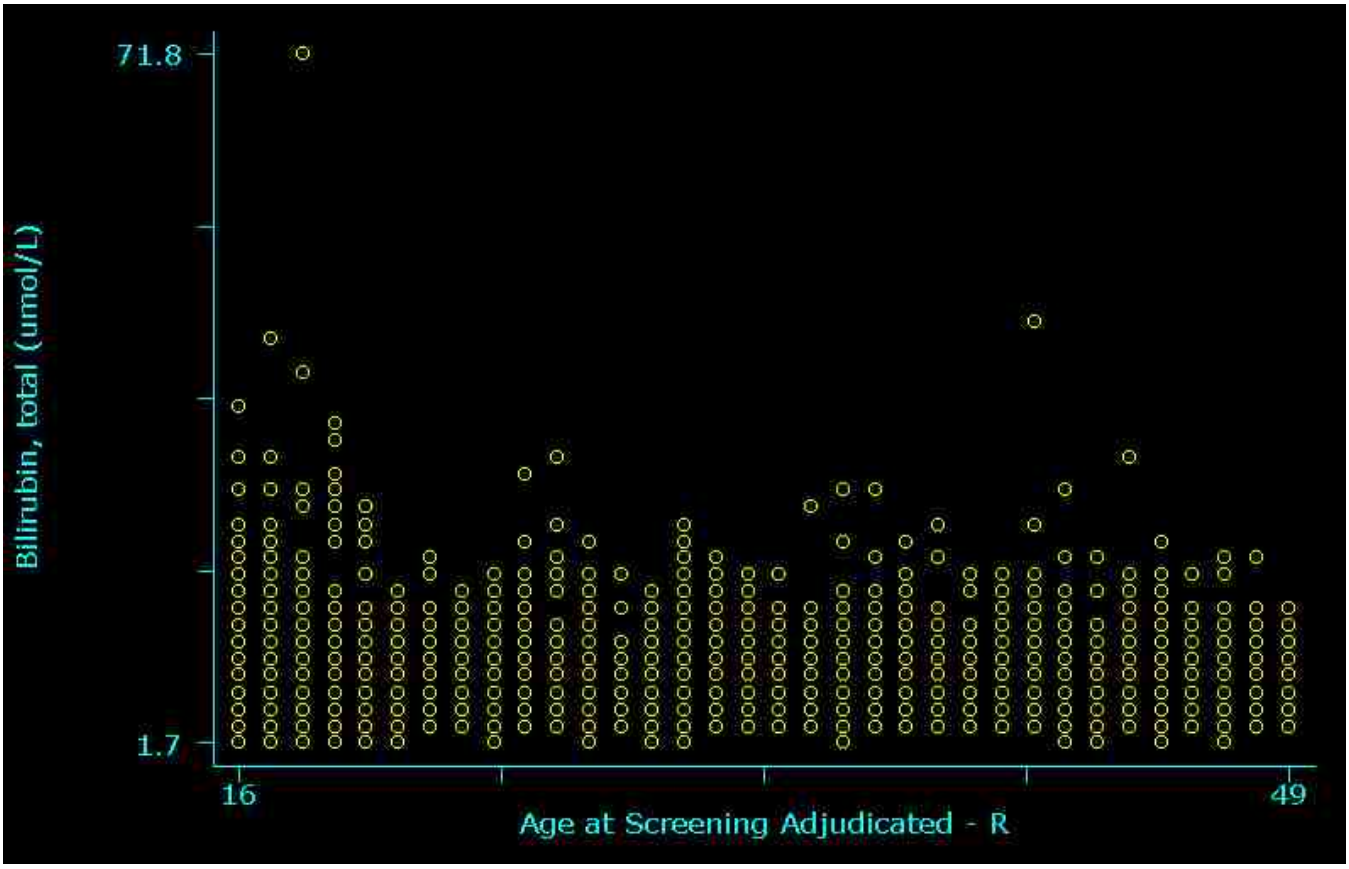

71.8
1.7
Bilirubin, total (umol/L)
16
49
Age at Screening Adjudicated - R

Figure 5.0: **(D)**

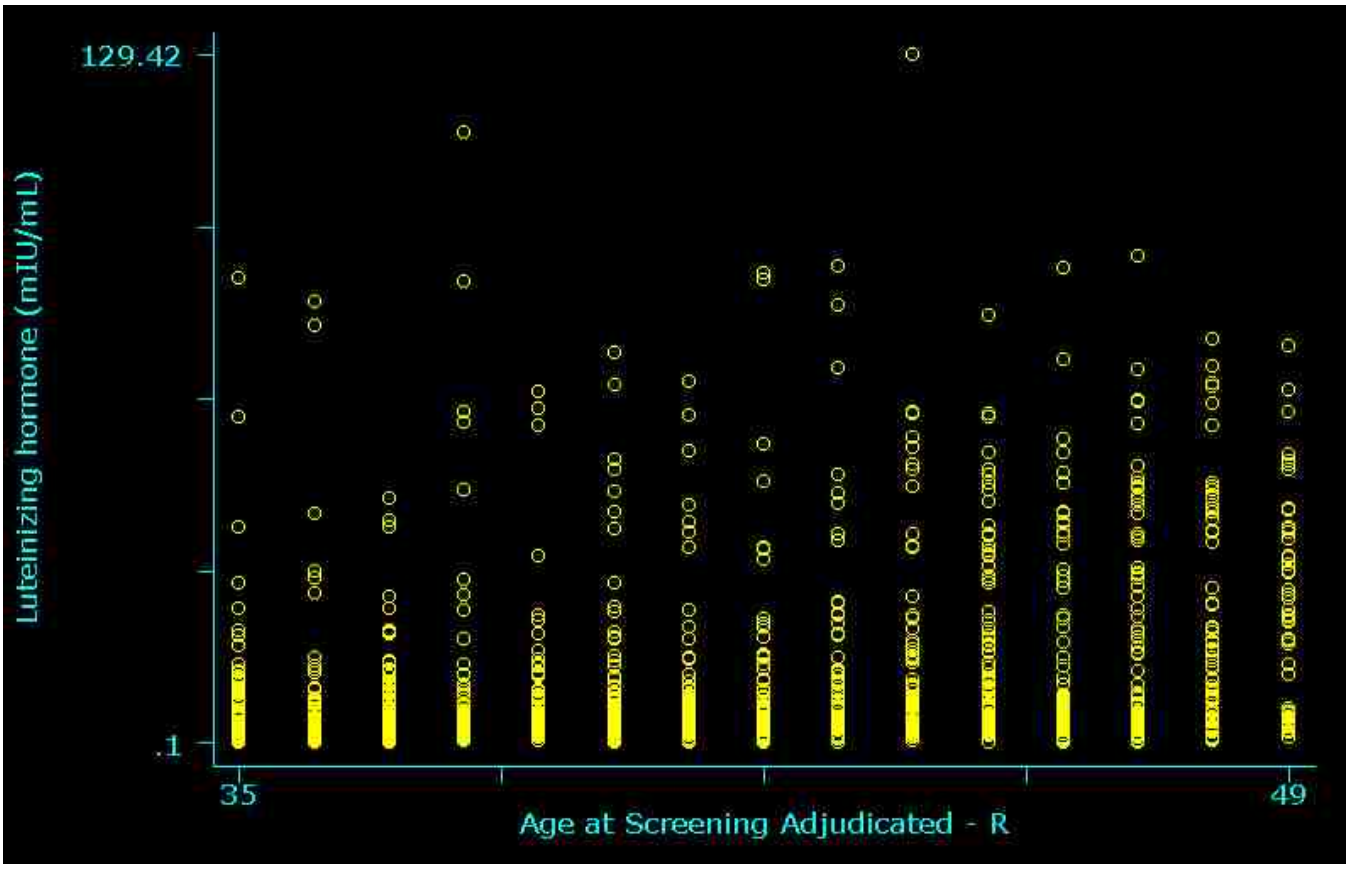

129.42
.1
Luteinizing hormone (mIU/mL)
35
49
Age at Screening Adjudicated - R

Figure 5.0: **(E)**

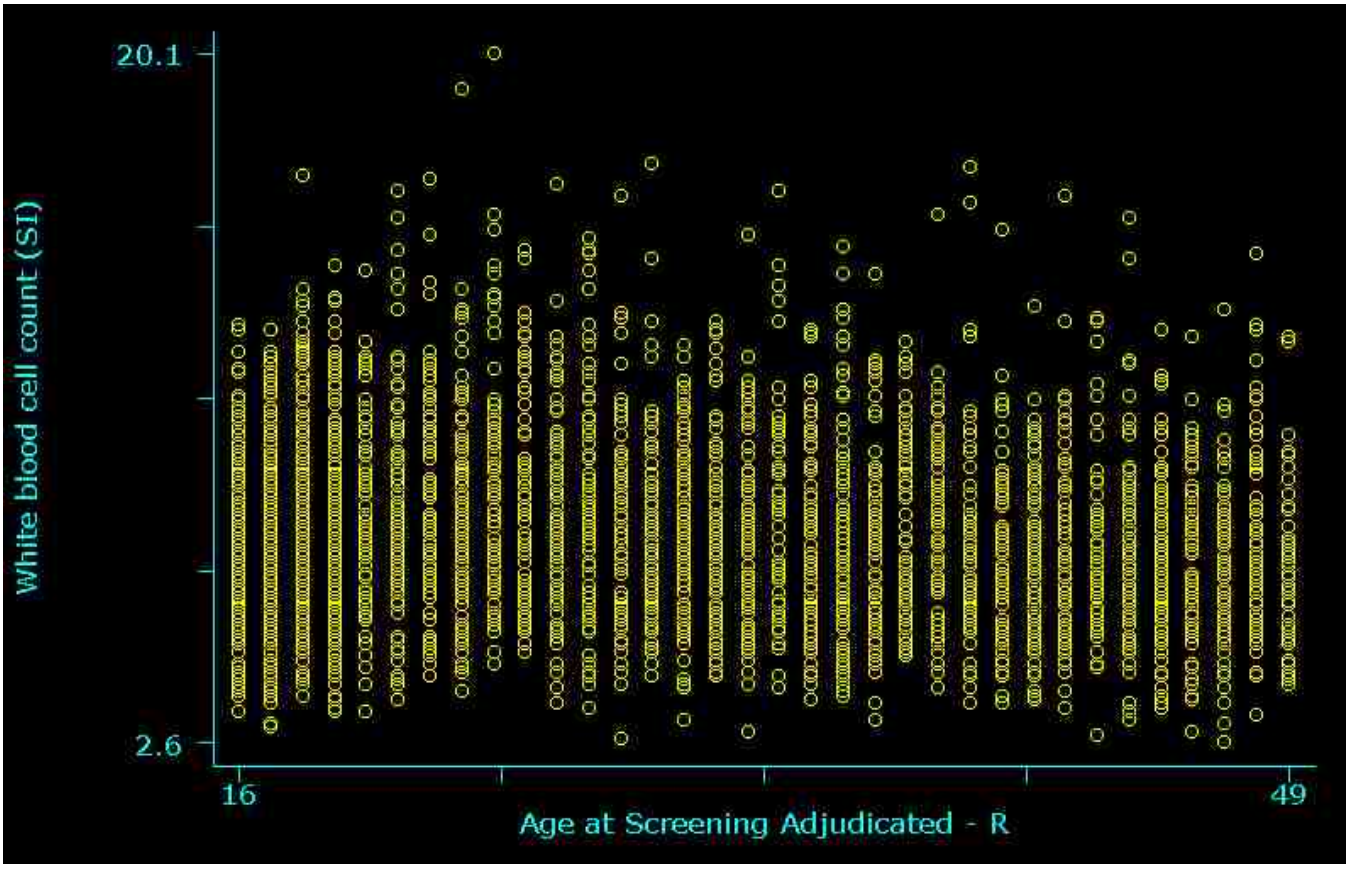

20.1
2.6
White blood cell count (SI)
16
49
Age at Screening Adjudicated - R

Figure 5.01: **Age distribution by decade for each of the two survey groups**; NHANES 1999-2000 and NHANES 2001-2002. (A) Histogram (B) Table (C) Mean Estimates (D) T-Test of Mean Values for age in years (ridageyr)

Figure 5.01 (A):

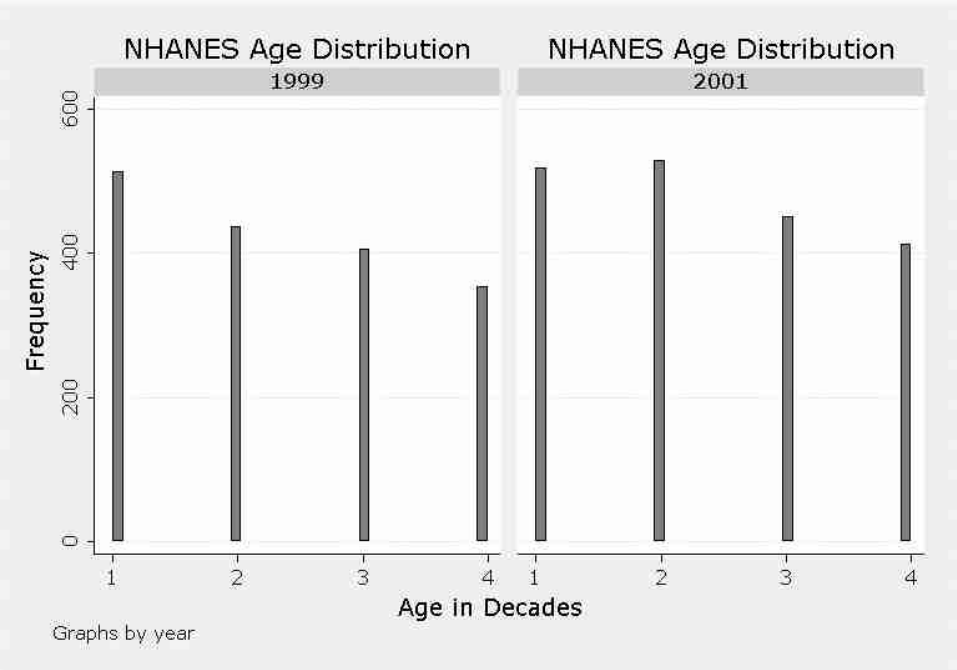


Figure 5.01 (B) : NHANES age distribution by survey group

- . tab age year
-

age	year 1999-2000	2001-2002	Total
16-19	513	518	1,031
20-29	436	528	964
30-39	405	450	855
40-49	354	412	766
Total	1,708	1,908	3,616

Figure 5.01 (C):

```
tabstat ridageyr, stats (mean sd iqr n) by (year)

Summary for variables: ridageyr
     by categories of: year

    year |      mean        sd       iqr         N
---------+--------------------------------------------
1999-2000|  28.77518  10.28321        19      1708
2001-2002|  29.13836  10.27533        19      1908
---------+--------------------------------------------
   Total |  28.96681  10.27923        19      3616
```

Figure 5.01 (D):

```
. ttest ridageyr,by(year) unequal

Two-sample t test with unequal variances

------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------1999-
1999-2000|    1708    28.77518    .2488197    10.28321    28.28715     29.2632
2001-2002|    1908    29.13836    .2352374    10.27533    28.67702    29.59971
---------+--------------------------------------------------------------------
combined |    3616    28.96681     .170941    10.27923    28.63166    29.30196
---------+--------------------------------------------------------------------
    diff |           -.3631891    .3424148               -1.034537    .3081591
------------------------------------------------------------------------------
Satterthwaite's degrees of freedom:  3569.55

                   Ho: mean(1999) - mean(2001) = diff = 0

     Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
       t =  -1.0607                 t =  -1.0607                 t =  -1.0607
   P < t =   0.1445           P > |t| =   0.2889           P > t =   0.8555
```

*No evidence to suggest that mean ages are different between survey groups.

Figure 5.02: **Race Distribution for each of the two survey groups**; NHANES 1999-2000 and NHANES 2001-2002. Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other. (A) Histogram (B) Table (C) T-Test of Mean Values for Mexican Americans (race1) in years (ridageyr) (D) T-Test of Mean Values for Hispanic (race2) in years (ridageyr) (E) T-Test of Mean Values for White (race3) in years (ridageyr) (F) T-Test of Mean Values for African American (race4) in years (ridageyr). (G) T-Test of Mean Values for Other (race5) in years (ridageyr).

Figure 5.02 (A):

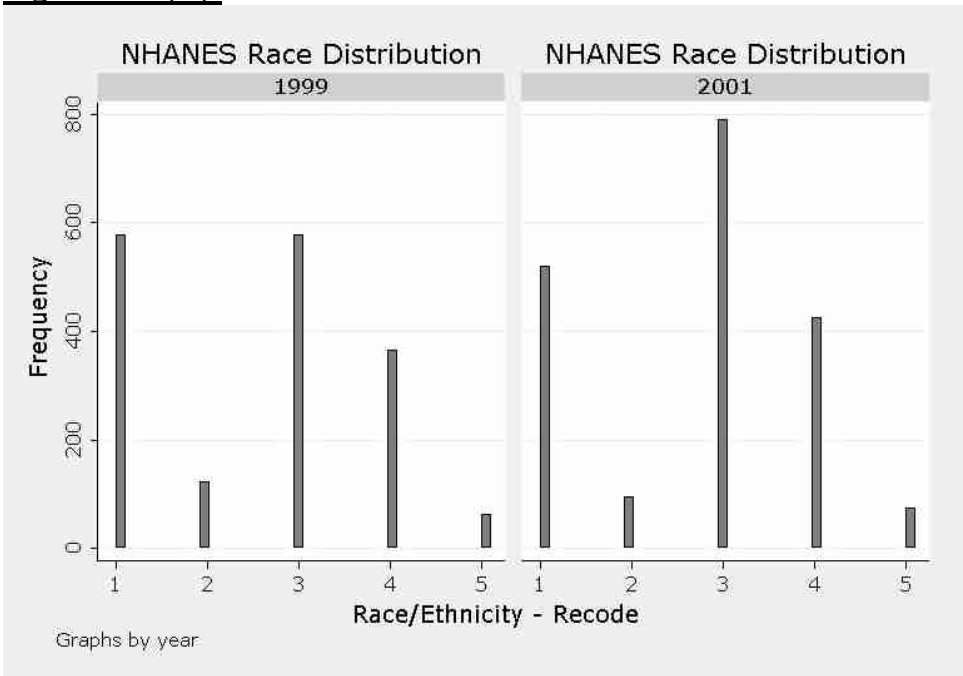


Figure 5.02(B): Distribution of race by survey group. Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.

```
. tab ridreth1 year

Race/Ethni |
    city - |         year
    Recode |   19990-2000  2001-2 |     Total
-----------+----------------------+----------
         1 |        578        521 |     1,099
         2 |        123         96 |       219
         3 |        578        791 |     1,369
         4 |        365        425 |       790
         5 |         64         75 |       139
-----------+----------------------+----------
     Total |      1,708      1,908 |     3,616
```

Figure 5.02(C):

```
. ttest race1,by(year) unequal

Two-sample t test with unequal variances

------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
1999-2000|    1708    .3384075    .0114525    .4733065    .3159452    .3608698
2001-2001|    1908    .2730608    .0102024    .4456486    .2530517    .2930699
---------+--------------------------------------------------------------------
combined |    3616     .303927    .0076499    .4600151    .2889284    .3189256
---------+--------------------------------------------------------------------
    diff |            .0653467    .0153378                .0352748    .0954186
------------------------------------------------------------------------------
Satterthwaite's degrees of freedom:  3511.71

                   Ho: mean(1999) - mean(2001) = diff = 0

     Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
       t =   4.2605                 t =   4.2605                 t =   4.2605
   P < t =   1.0000           P > |t| =   0.0000           P > t =   0.0000

* More Mexican Americans in NHANES 1999-2000 as compared to 2001-20002.
```

Figure 5.02(D):

```
. ttest race2,by(year) unequal

Two-sample t test with unequal variances

------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
1999-2000|    1708    .0720141    .0062569    .2585869     .059742    .0842861
2001-2002|    1908    .0503145    .0050057    .2186504    .0404973    .0601316
---------+--------------------------------------------------------------------
combined |    3616    .0605642    .0039672    .2385621    .0527859    .0683424
---------+--------------------------------------------------------------------
    diff |            .0216996    .0080129                .005989    .0374102
------------------------------------------------------------------------------
Satterthwaite's degrees of freedom:  3359.47

                   Ho: mean(1999) - mean(2001) = diff = 0

     Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
       t =   2.7081                 t =   2.7081                 t =   2.7081
   P < t =   0.9966           P > |t| =   0.0068           P > t =   0.0034

.
* More Hispanic in NHANES 1999-2000 as compared to 2001-20002.
```

Figure 5.02(E)

```
. ttest race3,by(year) unequal

Two-sample t test with unequal variances

------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
    1999 |    1708    .3384075    .0114525    .4733065    .3159452    .3608698
    2001 |    1908    .4145702    .0112814    .4927768    .3924451    .4366953
---------+--------------------------------------------------------------------
combined |    3616    .3785951    .0080672     .485104    .3627785    .3944118
---------+--------------------------------------------------------------------
    diff |           -.0761627    .0160757               -.1076811   -.0446444
------------------------------------------------------------------------------
Satterthwaite's degrees of freedom:  3596.13

                 Ho: mean(1999) - mean(2001) = diff = 0

     Ha: diff < 0               Ha: diff != 0                 Ha: diff > 0
       t =  -4.7378               t =  -4.7378                  t =  -4.7378
   P < t =   0.0000           P > |t| =   0.0000          P > t =   1.0000

Group 1999 = NHANES survey group 1999-2000
Group 2001 = NHANES survey group 2001-2002
* More white in NHANES 2001-2001 as compared to 1999-2000.
```

Figure 5.02(F):

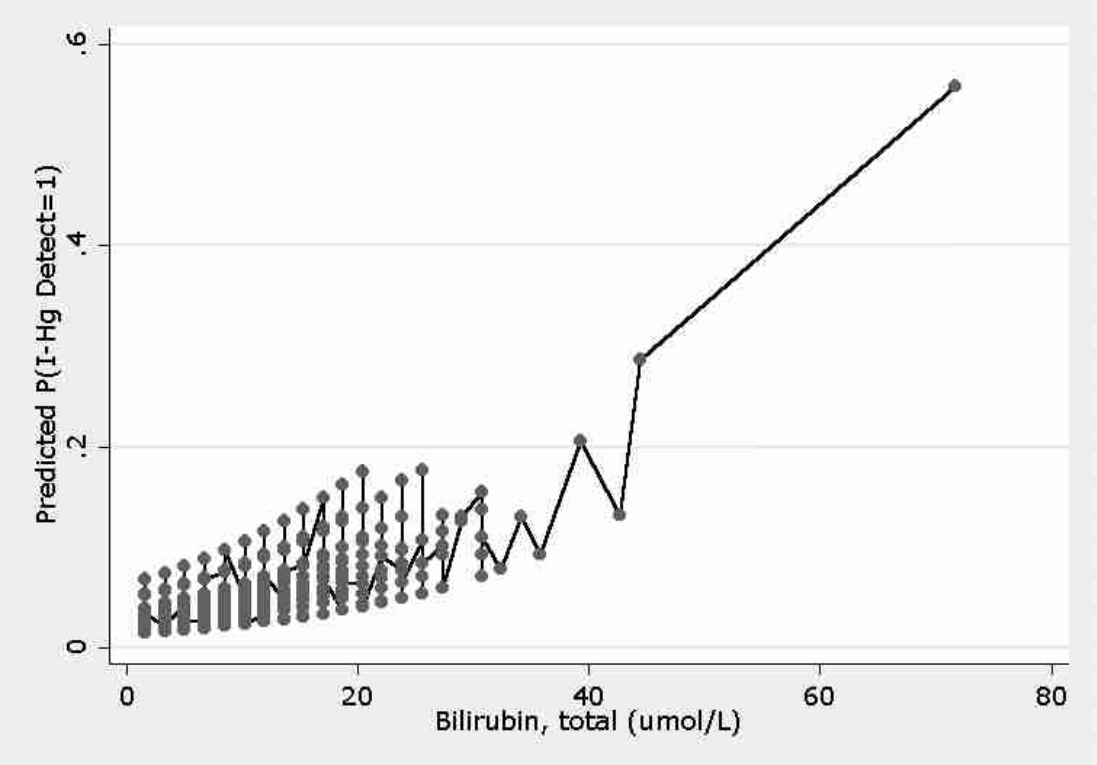
```
. ttest race4,by(year) unequal

Two-sample t test with unequal variances

------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
    1999 |    1708    .2137002    .0099216    .4100377    .1942405    .2331599
    2001 |    1908    .2227463    .0095282    .4161985    .2040595    .2414331
---------+--------------------------------------------------------------------
combined |    3616    .2184735    .0068725    .4132675     .204999    .2319479
---------+--------------------------------------------------------------------
    diff |           -.0090461    .0137559               -.0360162     .017924
------------------------------------------------------------------------------
Satterthwaite's degrees of freedom:  3581.05

                 Ho: mean(1999) - mean(2001) = diff = 0

     Ha: diff < 0               Ha: diff != 0                 Ha: diff > 0
       t =  -0.6576               t =  -0.6576                  t =  -0.6576
   P < t =   0.2554           P > |t| =   0.5108          P > t =   0.7446
Group 1999 = NHANES survey group 1999-2000
Group 2001 = NHANES survey group 2001-2002
* No Difference in African American.
```

Figure 5.02(G):

```
. ttest race5,by(year) unequal

Two-sample t test with unequal variances

------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
    1999 |    1708    .0374707    .0045966    .1899679    .0284552    .0464863
    2001 |    1908    .0393082      .00445    .1943781    .0305808    .0480355
---------+--------------------------------------------------------------------
combined |    3616    .0384403    .0031976    .1922832    .0321709    .0447096
---------+--------------------------------------------------------------------
    diff |           -.0018375    .0063977               -.014381    .0107061
------------------------------------------------------------------------------
Satterthwaite's degrees of freedom:   3586.3

                 Ho: mean(1999) - mean(2001) = diff = 0

     Ha: diff < 0               Ha: diff != 0                 Ha: diff > 0
       t =  -0.2872               t =  -0.2872                  t =  -0.2872
   P < t =   0.3870           P > |t| =   0.7740          P > t =   0.6130
Group 1999 = NHANES survey group 1999-2000
Group 2001 = NHANES survey group 2001-2002
* NO difference in Other.
```

Figure 5.1: Association Between Blood Inorganic Mercury (I-Hg Detection) and Blood Bilirubin (NHANES code= lbdstbsi) in Women Age 16-49, NHANES 1999-2002. (A) Graph of Probability of I-Hg Detection vs. Bilirubin concentration in survey weighted, full population. (B) T-Tests (C) Logistic Regression (D) Subpopulation Restricted by Age Group.
Blood Inorganic Mercury Detection (I-Hg Detect) is a transformed binary value (0 = non-detect 1= detection). Race is adjusted against baseline white population (race=3); race1= Mexican American, race2=Hispanic, race3=White, race4= African American, race5= Other. Age is a continuous variable by decade.

(A)



(B): T-Test of I-Hg Detection vs. Bilirubin (lbdstbsi): Group 0 = no I-Hg detection, Group 1=I-Hg detection. `ttest lbdstbsi, by(ihgdetect) unequal`

```
Two-sample t test with unequal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       0 |    3404    8.419448    .0743729    4.339198    8.273628    8.565268
       1 |     165    9.115152    .3208505    4.121399    8.481621    9.748682
---------+--------------------------------------------------------------------
combined |    3569    8.451611    .0725009    4.331283    8.309464    8.593758
---------+--------------------------------------------------------------------
    diff |           -.6957038    .3293575               -1.345552   -.0458556
------------------------------------------------------------------------------
Satterthwaite's degrees of freedom:  182.072
                    Ho: mean(0) - mean(1) = diff = 0
     Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
       t =  -2.1123                 t =  -2.1123                 t =  -2.1123
   P < t =   0.0180           P > |t| =   0.0360           P > t =   0.9820
```

Figure 5.1 (C): Logistic Regression of IHg Detection vs. Bilirubin (lbdstbsi)in raw population, not survey weighted, adjusted for age(as a continuous variable by decade) and race (as categorical variables): **logistic ihgdetect lbdstbsi age race1 race2 race4 race5, ro**

```
Logistic regression                                     Number of obs   =       3569
                                                        Wald chi2(6)    =      25.52
                                                        Prob > chi2     =     0.0003
Log pseudo-likelihood = -656.42986                      Pseudo R2       =     0.0178
```

ihgdetect	Odds Ratio	Robust Std. Err.	z	P>|z|	[95% Conf.	Interval]
lbdstbsi	1.037123	.0137183	2.76	0.006	1.010581	1.064362
age	1.279832	.0902787	3.50	0.000	1.114576	1.46959
race1	1.514024	.3061603	2.05	0.040	1.018605	2.250399
race2	1.332001	.4771241	0.80	0.424	.6600905	2.687855
race4	1.90719	.3989699	3.09	0.002	1.265695	2.873816
race5	1.099776	.5246159	0.20	0.842	.4317813	2.801206

Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.

Survey Weighted Logistic Regression, Full Population.

```
. svylogit ihgdetect lbdstbsi ridageyr  race1 race2 race4 race5, eform

Survey logistic regression

pweight:  wtmec4yr                                      Number of obs    =      3569
Strata:   sdmvstra                                      Number of strata =        28
PSU:      sdmvpsu                                       Number of PSUs   =        57
                                                        Population size  =  64701840
                                                        F(   6,     24)  =      3.39
                                                        Prob > F         =    0.0144
```

ihgdetect	exp(b)	Std. Err.	t	P>|t|	[95% Conf.	Interval]
lbdstbsi	1.059576	.0267187	2.29	0.029	1.006315	1.115655
ridageyr	1.026967	.011736	2.33	0.027	1.003243	1.051253
race1	1.602393	.3844635	1.97	0.059	.9809683	2.61748
race2	1.570496	.5416908	1.31	0.201	.7756581	3.179824
race4	2.088547	.5296835	2.90	0.007	1.243302	3.508421
race5	1.064267	.5493903	0.12	0.905	.3702819	3.058926

Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.

Odds Ratio of I-Hg Detection for a one Std. Dev. Change in bilirubin (4.3)

```
1 std. dev. (4.3) of lbdstbsi
. lincom 4.3*lbdstbsi, or
 ( 1)  4.3 lbdstbsi = 0
```

ihgdetect	Odds Ratio	Std. Err.	t	P>|t|	[95% Conf.	Interval]
(1)	1.28253	.1390654	2.29	0.029	1.027439	1.600954

Figure 5.1(D): Subpopulations Restricted by Age Groups (Decade): 40-49 Years
T-Test of I-Hg Detection v Bilirubin (lbdstbsi): Group 0 = no I-Hg detection, Group 1=I-Hg detection

```
ttest lbdstbsi, by(ihgdetect) unequal
Two-sample t test with unequal variances

------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       0 |     703    8.554908    .1514943    4.016742    8.257471    8.852344
       1 |      47    10.22766    .5821878    3.991279    9.055776    11.39954
---------+--------------------------------------------------------------------
combined |     750    8.659733    .1472627    4.032956    8.370637     8.94883
---------+--------------------------------------------------------------------
    diff |           -1.672752    .6015756               -2.879669    -.465835
------------------------------------------------------------------------------
Satterthwaite's degrees of freedom:  52.4247

                     Ho: mean(0) - mean(1) = diff = 0

     Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
       t =  -2.7806                 t =  -2.7806                 t =  -2.7806
   P < t =   0.0038           P > |t| =   0.0075           P > t =   0.9962
```

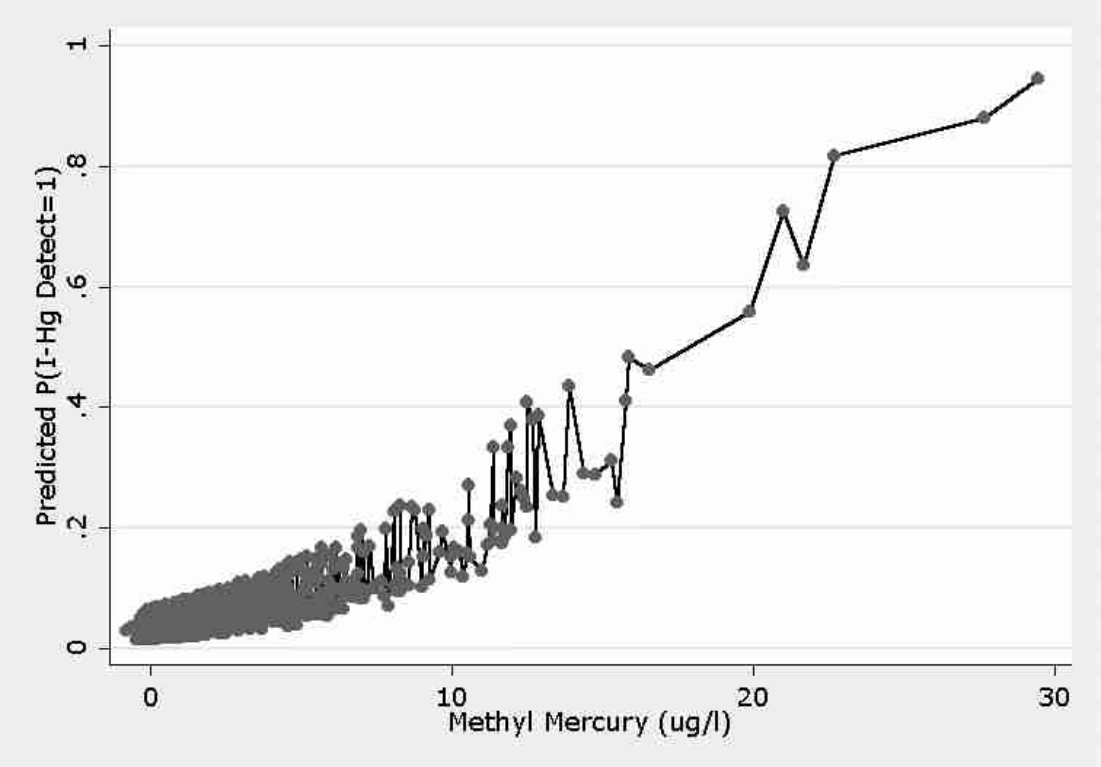
Figure 5.1(D): Subpopulations Restricted by Age Groups (Decade): 40-49 Years

```
Logistic Regression of IHg Detection vs. Bilirubin (lbdstbsi),

Adjusted for race (as categorical variables):

logistic ihgdetect lbdstbsi race1 race2 race4 race5, ro


Logistic regression                               Number of obs   =        750
                                                  Wald chi2(5)    =      13.97
                                                  Prob > chi2     =     0.0158
Log pseudo-likelihood = -169.95246                Pseudo R2       =     0.0326

------------------------------------------------------------------------------
             |               Robust
   ihgdetect | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
    lbdstbsi |   1.086716   .0304171     2.97   0.003     1.028706    1.147998
       race1 |   .9498615   .3738514    -0.13   0.896     .4391774    2.054379
       race2 |     .45774   .4780005    -0.75   0.454      .059121    3.544021
       race4 |   1.901341   .6930022     1.76   0.078     .9307081    3.884245
       race5 |   .7126669   .7252421    -0.33   0.739     .0969763    5.237304
```

Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.

Figure 5.11: Association Between Blood I-Hg and Blood Methyl Mercury in Women Ages 16-49, NHANES 1999-2002. (A) Graph of Probability of I-Hg Detect vs. CH_3Hg in survey weighted, full population. (B) T-Tests (C) Logistic Regression

(A)



(B):T-Test of I-Hg Detection vs. CH_3Hg: `ttest ch3hg, by(ihgdetect) unequal`

```
Two-sample t test with unequal variances

------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       0 |    3445    1.057074    .0298966    1.754756    .9984571    1.115691
       1 |     168    3.178036    .3927474    5.090589    2.402646    3.953426
---------+--------------------------------------------------------------------
combined |    3613    1.155696     .034633    2.081726    1.087794    1.223598
---------+--------------------------------------------------------------------
    diff |           -2.120962    .3938837               -2.89853   -1.343394
------------------------------------------------------------------------------
Satterthwaite's degrees of freedom:  168.941

                    Ho: mean(0) - mean(1) = diff = 0

     Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
       t =  -5.3847                 t =  -5.3847                 t =  -5.3847
   P < t =   0.0000           P > |t| =   0.0000           P > t =   1.0000
```

Group 0 = no I-Hg detection, Group 1=I-Hg detection

Figure 5.11(C): Logistic Regression of I-Hg Detection vs. CH3Hg, adjusted for age (continuous variable by decade) and race (as categorical variable), in the raw population (not adjusted for survey weights).

```
. logistic ihgdetect ch3hg age  race1 race2 race4 race4, ro
note: race4 dropped due to collinearity
Logistic regression                                     Number of obs   =       3613
                                                        Wald chi2(5)    =     109.05
                                                        Prob > chi2     =     0.0000
Log pseudo-likelihood = -628.97526                      Pseudo R2       =     0.0744

------------------------------------------------------------------------------
             |               Robust
   ihgdetect | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       ch3hg |   1.248848   .0287297     9.66   0.000     1.193789    1.306446
         age |   1.159068   .0861953     1.98   0.047     1.001863     1.34094
       race1 |   1.843868     .38285     2.95   0.003     1.227415    2.769928
       race2 |   1.218353   .4844549     0.50   0.619     .5588677    2.656056
       race4 |   1.893347   .3976641     3.04   0.002     1.254441    2.857657
```

Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.

Survey Weighted Logistic Regression, Full Population

```
svylogit ihgdetect ch3hg ridageyr  race1 race2 race4 race5, eform

Survey logistic regression

pweight:  wtmec4yr                                      Number of obs    =      3613
Strata:   sdmvstra                                      Number of strata =        28
PSU:      sdmvpsu                                       Number of PSUs   =        57
                                                        Population size  =  65606783
                                                        F(   6,     24)  =      6.71
                                                        Prob > F         =    0.0003

------------------------------------------------------------------------------
   ihgdetect |     exp(b)   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       ch3hg |   1.207132   .0375719     6.05   0.000     1.132684    1.286474
    ridageyr |    1.01788   .0118487     1.52   0.139     .9939333    1.042405
       race1 |   1.807365   .4494428     2.38   0.024     1.086843    3.005559
       race2 |   1.404565   .5511767     0.87   0.394     .6294826    3.134008
       race4 |    1.95556     .47571     2.76   0.010     1.189049    3.216197
       race5 |   .7499263   .4211956    -0.51   0.612     .2377627     2.36534
------------------------------------------------------------------------------
```

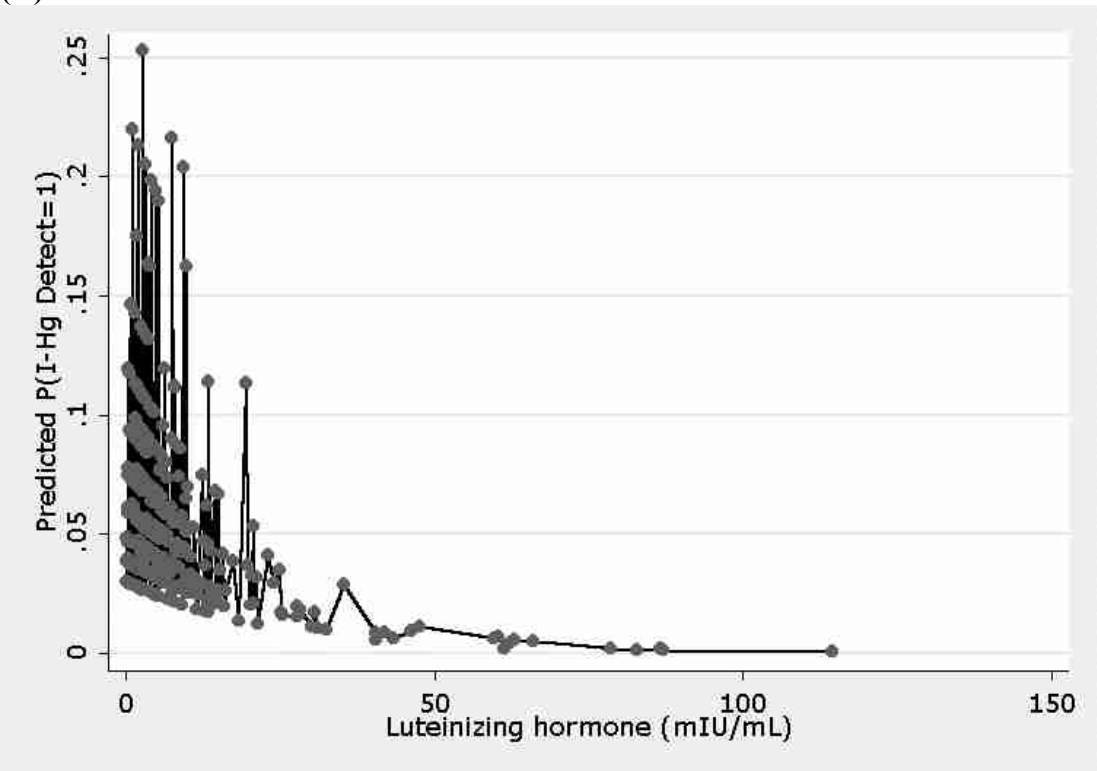
Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.

Odds Ratio of I-Hg Detection for a one Std. Dev. Change in CH_3Hg

```
. lincom 2.1*ch3hg, or
 ( 1)  2.1 ch3hg = 0

------------------------------------------------------------------------------
   ihgdetect | Odds Ratio   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
         (1) |   1.484859   .0970539     6.05   0.000     1.299057    1.697236
------------------------------------------------------------------------------
```

Figure 5.2: Association Between Blood Inorganic Mercury (I-Hg Detection) and Blood Leutenizing Hormone (LH) (NHANES* code= lbxlh) in Women Age 16-49, NHANES 1999-2002. (A) Graphs of probability I-Hg Detection vs. LH (B) T-Tests (C) Logistic Regression (D) Associations by Age Group. *LH was measured only in Women ages 35-49 years. Blood Inorganic Mercury Detection (I-Hg Detect) is a transformed binary value (0 = non-detect 1= detection). Race is adjusted against baseline white population (race=3); race1= Mexican American, race2=Hispanic, race3=White, race4= African American, race5=Other. Age is a categorical variable by decade.

(A)



(B) T-Test of I-Hg Detect vs. Luteinizing Hormone (LH, lbxlh):

ttest lbxlh, by(ihgdetect) unequal: Two-sample t test with unequal variances

Group	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
0	1065	13.69863	.5448433	17.7806	12.62954	14.76772
1	68	9.510882	1.375317	11.34115	6.765739	12.25603
combined	1133	13.44729	.519489	17.48603	12.42802	14.46656
diff		4.187747	1.479307		1.24864	7.126854

Satterthwaite's degrees of freedom: 89.5415

Ho: mean(0) - mean(1) = diff = 0

Ha: diff < 0	Ha: diff != 0	Ha: diff > 0
t = 2.8309	t = 2.8309	t = 2.8309
P < t = 0.9971	P > |t| = 0.0057	P > t = 0.0029

Group 0 = no I-Hg detection, Group 1=I-Hg detection

Figure 5.2 (C): Logistic Regression of IHg Detection vs. Leutenizing Hormone (lbxlh), adjusted for age, and race (both as categorical variables): logistic ihgdetect lbxlh age race1 race2 race4 race5, ro

```
Logistic regression                                     Number of obs   =       1133
                                                        Wald chi2(6)    =       9.35
                                                        Prob > chi2     =     0.1548
Log pseudo-likelihood = -251.89383                      Pseudo R2       =     0.0207
```

ihgdetect	Odds Ratio	Robust Std. Err.	z	P>|z|	[95% Conf.	Interval]
lbxlh	.9789097	.0093745	-2.23	0.026	.9607075	.9974569
age	1.233232	.3275048	0.79	0.430	.7328151	2.075368
race1	1.153093	.3827386	0.43	0.668	.6016318	2.21003
race2	1.937406	.9206802	1.39	0.164	.7633412	4.917253
race4	1.840847	.5810744	1.93	0.053	.9915867	3.417469
race5	.5507826	.5672278	-0.58	0.563	.0731752	4.14569

Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.

```
. lincom 17.5*lbxlh,or

 ( 1)  17.5 lbxlh = 0
```

ihgdetect	Odds Ratio	Std. Err.	z	P>|z|	[95% Conf.	Interval]
(1)	.6886464	.1154087	-2.23	0.026	.4958444	.9564168

Figure 5.2 (D): Survey Weighted Logistic Regression of IHg Detection vs. Leutenizing Hormone (lbxlh), adjusted for age, and race (both as categorical variables): `. svylogit ihgdetect lbxlh race1 race2 race4 race5 age,eform`

```
Survey logistic regression

pweight:  wtmec4yr                                      Number of obs    =      1133
Strata:   sdmvstra                                      Number of strata =        28
PSU:      sdmvpsu                                       Number of PSUs   =        57
                                                        Population size  =  29935892
                                                        F(   6,     24)  =      0.79
                                                        Prob > F         =    0.5848
```

ihgdetect	exp(b)	Std. Err.	t	P>|t|	[95% Conf.	Interval]
lbxlh	.9832166	.0119696	-1.39	0.175	.9590383	1.008004
race1	1.20121	.5533314	0.40	0.694	.4682302	3.081616
race2	2.171677	1.01612	1.66	0.108	.8340547	5.65452
race4	2.006589	.8146042	1.72	0.097	.8747172	4.603087
race5	.8077572	.7287655	-0.24	0.815	.1276175	5.112712
age	1.248843	.3151451	0.88	0.386	.7453542	2.092439

Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.

(D): Subpopulation Restricted by Age (Decade)

Age: 40 year olds

T-Test of I-Hg Detection vs. Leutenizing Hormone(LH, lbxlh): Group 0 = no I-Hg detection, Group 1=I-Hg detect

`ttest lbxlh, by(ihgdetect) unequal`

```
Two-sample t test with unequal variances

------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       0 |     686    16.30109    .7279622     19.0665    14.87179     17.7304
       1 |      45    11.45467     1.99966    13.41413    7.424617    15.48472
---------+--------------------------------------------------------------------
combined |     731    16.00275    .6952312    18.79697    14.63786    17.36764
---------+--------------------------------------------------------------------
    diff |            4.846427    2.128044                .584063     9.10879
------------------------------------------------------------------------------
Satterthwaite's degrees of freedom:  56.3716

                     Ho: mean(0) - mean(1) = diff = 0

     Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
       t =   2.2774                 t =   2.2774                 t =   2.2774
   P < t =   0.9867          P > |t| =   0.0266          P > t =   0.0133
```

Figure 5.2 (D): Subpopulation Restricted by Age (Decade)

Age: 40 year olds

Logistic Regression of I-Hg Detection vs. LH(lbxlh), Adjusted for Race

```
logistic ihgdetect lbxlh race1 race2 race4 race5, ro
Logistic regression                               Number of obs   =        731
                                                  Wald chi2(5)    =       7.89
                                                  Prob > chi2     =     0.1624
Log pseudo-likelihood = -164.72771                Pseudo R2       =     0.0255

------------------------------------------------------------------------------
             |               Robust
   ihgdetect | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       lbxlh |   .9810055   .0104006    -1.81   0.070      .960831    1.001604
       race1 |   .9456699   .3814593    -0.14   0.890       .42893    2.084936
       race2 |   .4776339   .4986906    -0.71   0.479     .0617118    3.696769
       race4 |   1.925861   .7184311     1.76   0.079     .9270204    4.000927
       race5 |   .7187846   .7531396    -0.32   0.753     .0921962    5.603821
------------------------------------------------------------------------------
```

Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.

Subpopulation Restricted by Age (Decade)
Age: 35-39 Years

T-Test of I-Hg Detection vs. Leutenizing Hormone(lbxlh): Group 0 = no I-Hg detection, Group 1=I-Hg detection

```
. ttest lbxlh, by(ihgdetect) unequal
```

Two-sample t test with unequal variances

Group	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
0	379	8.9881	.7202012	14.02082	7.571998	10.4042
1	23	5.707826	.6250205	2.997493	4.411613	7.004039
combined	402	8.800423	.6809104	13.65221	7.461823	10.13902
diff		3.280274	.9535934		1.390109	5.170439

```
Satterthwaite's degrees of freedom:  108.113

                     Ho: mean(0) - mean(1) = diff = 0

     Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
       t =   3.4399                 t =   3.4399                  t =   3.4399
   P < t =   0.9996           P > |t| =   0.0008           P > t =   0.0004
```

Figure 5.2 (D): Subpopulation Restricted by Age (Decade)
Age: 35-39 Years
Logistic Regression of I-Hg Detection vs. LH (lbxlh), Adjusted for Race

```
. logistic ihgdetect lbxlh ridageyr race1  race2 race4 race5, ro
note: race5 != 0 predicts failure perfectly
      race5 dropped and 11 obs not used
Logistic regression                               Number of obs   =        391
                                                  Wald chi2(5)    =      10.13
                                                  Prob > chi2     =     0.0716
Log pseudo-likelihood = -82.106098                Pseudo R2       =     0.0614
```

ihgdetect	Odds Ratio	Robust Std. Err.	z	P>|z|	[95% Conf.	Interval]
lbxlh	.9671564	.015385	-2.10	0.036	.9374677	.9977854
ridageyr	1.319748	.2117593	1.73	0.084	.9636335	1.807465
race1	1.656802	.9566202	0.87	0.382	.5343129	5.137425
race2	5.02426	3.131271	2.59	0.010	1.481077	17.04381
race4	1.522713	.9159575	0.70	0.485	.4683759	4.950412

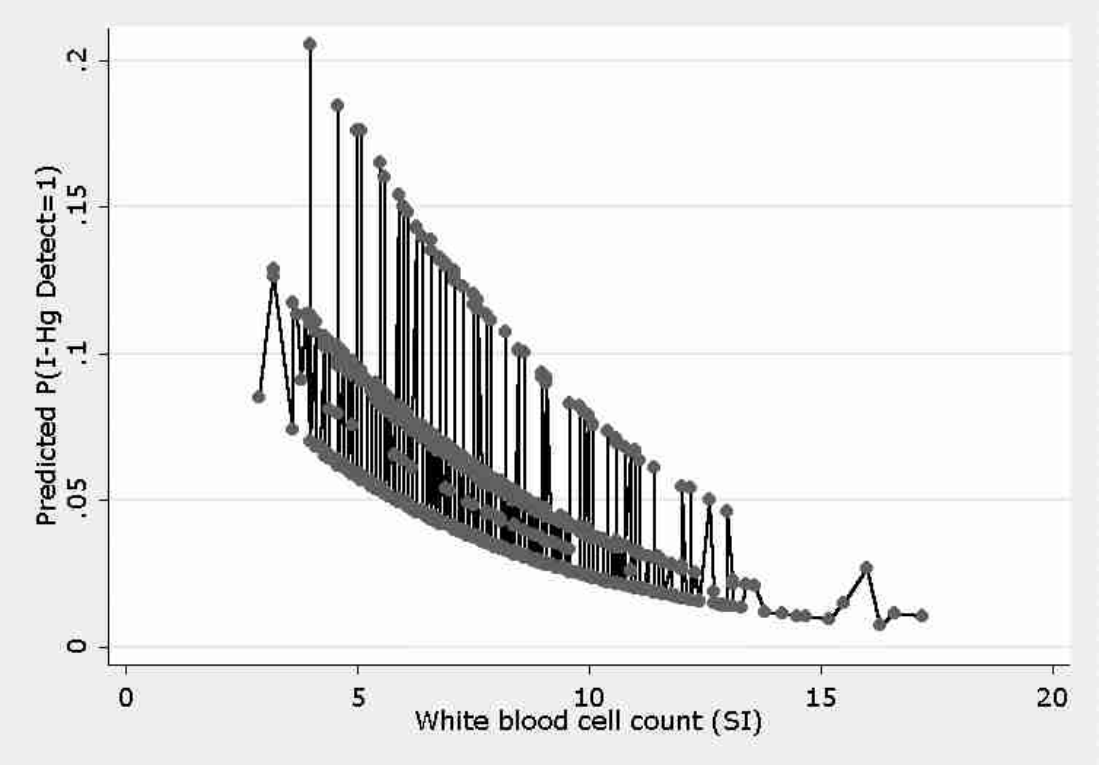
Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other

```
lincom 17.5*lbxlh, or
 ( 1)  17.5 lbxlh = 0
```

ihgdetect	Odds Ratio	Std. Err.	t	P>|t|	[95% Conf.	Interval]
(1)	.4692136	.1645339	-2.16	0.039	.2290362	.9612513

<u>Figure 5.3</u>: Association Between Blood Inorganic Mercury (I-Hg Detection) and Blood White Blood Cell Count (NHANES code= lbxwbc) in Women Age 16-49, NHANES 1999-2002. (A) Graph of Probability of I-Hg Detection vs. WBC concentration in survey weighted population restricted by age (30-39 Years). (B) T-Tests in full population. (C) Logistic Regression (D) Associations by Age Group. Blood Inorganic Mercury Detection (I-Hg Detect) is a transformed binary value (0 = non-detect 1= detection). Race is adjusted against baseline white population (race=3); race1= Mexican American, race2=Hispanic, race3=White, race4= African American, race5=Other. Age is a continuous variable by decade.

(A)



<u>(B)</u>: T-Test of I-Hg Detection vs. White Blood Cell Count (lbxwbc): Group 0 = no I-Hg detection, Group 1=I-Hg detection

```
 ttest lbxwbc, by(ihgdetect) unequal
Two-sample t test with unequal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       0 |    3446    7.827829    .0393217    2.308286    7.750733    7.904926
       1 |     168     7.27619    .1729646    2.241878    6.934711     7.61767
---------+--------------------------------------------------------------------
combined |    3614    7.802186    .0383898    2.307864    7.726918    7.877454
---------+--------------------------------------------------------------------
    diff |            .5516389     .177378                .2016912    .9015866
------------------------------------------------------------------------------
Satterthwaite's degrees of freedom:  184.684
                     Ho: mean(0) - mean(1) = diff = 0
     Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
       t =   3.1100                 t =   3.1100                 t =   3.1100
   P < t =   0.9989           P > |t| =   0.0022           P > t =   0.0011
```

<u>Figure 5.3 (C)</u>: Raw Population (not survey weighted), Logistic Regression of I-Hg Detection vs. White Blood Cell Count (lbxwbc), Adjusted for Age, and Race:

```
. logistic ihgdetect lbxwbc age  race1 race2 race4 race5, ro

Logistic regression                                     Number of obs   =       3614
                                                        Wald chi2(6)    =      26.53
                                                        Prob > chi2     =     0.0002
Log pseudo-likelihood = -666.48533                      Pseudo R2       =     0.0192

------------------------------------------------------------------------------
             |               Robust
   ihgdetect | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
    lbxwbcsi |   .9141298   .0374174    -2.19   0.028     .8436577    .9904885
         age |   1.252531   .0869212     3.24   0.001     1.093246    1.435023
       race1 |   1.507857   .3034488     2.04   0.041     1.016389     2.23697
       race2 |   1.390447   .4952777     0.93   0.355     .6917612    2.794813
       race4 |   1.743535   .3699397     2.62   0.009     1.150336    2.642633
       race5 |   1.062462    .509891     0.13   0.900     .4147721    2.721557
------------------------------------------------------------------------------
```

Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.

Odds Ratio of I-Hg Detection for a one Std. Deviation Change in WBC (2.3).

```
. lincom 2.3*lbxwbc,or
 ( 1)  2.3 lbxwbcsi = 0

------------------------------------------------------------------------------
   ihgdetect | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
         (1) |   .8134261   .0765793    -2.19   0.028     .6763671    .9782587
------------------------------------------------------------------------------
```

<u>Fig. 5.3(D)</u>: Subpopulation Restricted by Age (Decade): 30-39 Years.

Group 0 = no I-Hg detection, Group 1=I-Hg detection

T-Test of I-Hg Detection vs. White Blood Cell Count (lbxwbc):

```
ttest lbxwbc, by(ihgdetect) unequal
Two-sample t test with unequal variances

------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       0 |     808     7.85297    .0812448     2.30941    7.693494    8.012446
       1 |      47    7.178723    .3089168    2.117827    6.556906    7.800541
---------+--------------------------------------------------------------------
combined |     855    7.815906    .0787704    2.303277      7.6613    7.970513
---------+--------------------------------------------------------------------
    diff |            .6742469    .3194218                .0334454    1.315048
------------------------------------------------------------------------------
Satterthwaite's degrees of freedom:  52.5692

                    Ho: mean(0) - mean(1) = diff = 0
     Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
       t =   2.1108                 t =   2.1108                 t =   2.1108
   P < t =   0.9802           P > |t| =   0.0396           P > t =   0.0198
```

Fig. 5.3(D): Survey Weighted Logistic Regression in Subpopulation Restricted by Age: 30-39 Years.

```
 svylogit ihgdetect lbxwbc race1 race2 race4 race5 ridageyr, eform
Survey logistic regression
pweight:  wtmec4yr                                Number of obs    =       855
Strata:   sdmvstra                                Number of strata =        28
PSU:      sdmvpsu                                 Number of PSUs   =        57
                                                  Population size  =  20051629
                                                  F(   6,     24)  =      1.75
                                                  Prob > F         =    0.1515

------------------------------------------------------------------------------
   ihgdetect |     exp(b)   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
    lbxwbcsi |   .8312639   .0748512    -2.05   0.049     .6914455    .9993551
       race1 |   1.610803    .637804     1.20   0.238     .7167102    3.620274
       race2 |   3.429905    1.85777     2.28   0.030     1.132872    10.38444
       race4 |   1.676024   .8050182     1.08   0.291      .627552    4.476213
       race5 |   1.288658   1.256531     0.26   0.797     .1754082    9.467287
    ridageyr |    .996067   .0759816    -0.05   0.959     .8521829    1.164245
------------------------------------------------------------------------------
```

Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.

Odds Ratio of I-Hg Detection for a one Std. Deviation Change in WBC (2.3).

```
. lincom 2.3*lbxwbc, or

 ( 1)  2.3 lbxwbcsi = 0

------------------------------------------------------------------------------
   ihgdetect | Odds Ratio   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
         (1) |   .6537316   .1353903    -2.05   0.049     .4279996    .9985174
------------------------------------------------------------------------------
```

Figure 5.31: Logistic Regression of I-Hg using Categorical Explanatory Variables.

(A) Age in years (ridageyr) was transformed into dummy variables for age in decades:

Age as a categorical variable (by decade)

```
. tabulate age, generate(age)

        age |      Freq.     Percent        Cum.
------------+-----------------------------------
      16-19 |      1,031       28.51       28.51
      20-29 |        964       26.66       55.17
      30-39 |        855       23.64       78.82
      40-49 |        766       21.18      100.00
------------+-----------------------------------
      Total |      3,616      100.00
```

In regressions, dummy variables for age are compared to age 30-39 years as baseline.

(B)

```
. logistic ihgdetect lbxwbc race1 race2 race4 race5 age1 age2 age4, ro

Logistic regression                               Number of obs   =       3614
                                                  Wald chi2(8)    =      26.45
                                                  Prob > chi2     =     0.0009
Log pseudo-likelihood = -666.20121                Pseudo R2       =     0.0197

------------------------------------------------------------------------------
             |               Robust
   ihgdetect | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
    lbxwbcsi |   .9127369   .0373821    -2.23   0.026     .8423328    .9890255
       race1 |   1.529616   .3112128     2.09   0.037     1.026593    2.279115
       race2 |   1.389981   .4941141     0.93   0.354     .6925028    2.789949
       race4 |   1.750913   .3738802     2.62   0.009     1.152136    2.660881
       race5 |   1.064738   .5115529     0.13   0.896      .415225    2.730248
      16-19  |   .5612148   .1278824    -2.54   0.011      .359061    .8771825
      20-29  |     .71706    .161101    -1.48   0.139     .4616544    1.113766
      40-49  |   1.076266   .2301155     0.34   0.731     .7078235    1.636493
------------------------------------------------------------------------------
```

Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.

Figure 5.31 (B):

```
. svylogit ihgdetect lbxwbc race1 race2 race4 race5 age1 age2 age4, eform
Survey logistic regression

pweight:  wtmec4yr                                Number of obs    =      3614
Strata:   sdmvstra                                Number of strata =        28
PSU:      sdmvpsu                                 Number of PSUs   =        57
                                                  Population size  =  65607099
                                                  F(   8,     22)  =      3.73
                                                  Prob > F         =    0.0068

------------------------------------------------------------------------------
   ihgdetect |     exp(b)   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
    lbxwbcsi |   .8904419   .0524145    -1.97   0.058     .7894437    1.004361
       race1 |   1.600802   .4033067     1.87   0.072     .9562166    2.679904
       race2 |   1.666735   .5495869     1.55   0.132     .8491453     3.27153
       race4 |   1.862183   .5308828     2.18   0.037     1.039441    3.336147
       race5 |   1.031224   .5312303     0.06   0.953     .3595709    2.957478
       16-19 |   .4877849    .158218    -2.21   0.035     .2512588    .9469681
       20-29 |   .6417564   .2156002    -1.32   0.197      .322824    1.275777
       40-49 |   1.097802   .2880124     0.36   0.725     .6419382    1.877392
------------------------------------------------------------------------------
```

Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.

Figure 5.31 (C):

```
logistic ihgdetect ch3hg race1 race2 race4 race5 age1 age2 age4, ro

Logistic regression                               Number of obs   =       3613
                                                  Wald chi2(8)    =     109.17
                                                  Prob > chi2     =     0.0000
Log pseudo-likelihood = -628.89562                Pseudo R2       =     0.0745

------------------------------------------------------------------------------
             |               Robust
   ihgdetect | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       ch3hg |     1.2496   .0293225     9.50   0.000     1.193431    1.308414
       race1 |   1.809426   .3877436     2.77   0.006     1.188874    2.753884
       race2 |   1.196198   .4790682     0.45   0.655     .5456367    2.622424
       race4 |   1.855304   .4003774     2.86   0.004     1.215415    2.832081
       race5 |    .839737    .428099    -0.34   0.732     .3091717    2.280798
       16-19 |   .7563162   .1849112    -1.14   0.253     .4683751    1.221274
       20-29 |    .842621   .1976134    -0.73   0.465     .5321163    1.334314
       40-49 |    1.1629   .2571563     0.68   0.495     .7538988     1.79379
------------------------------------------------------------------------------
```

Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.

Figure 5.31 (C):

```
. svylogit ihgdetect ch3hg race1 race2 race4 race5 age1 age2 age4, eform

Survey logistic regression

pweight:  wtmec4yr                                Number of obs    =      3613
Strata:   sdmvstra                                Number of strata =        28
PSU:      sdmvpsu                                 Number of PSUs   =        57
                                                  Population size  =  65606783
                                                  F(   8,     22)  =      5.41
                                                  Prob > F         =    0.0008

------------------------------------------------------------------------------
   ihgdetect |     exp(b)   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       ch3hg |   1.206252   .0367387     6.16   0.000     1.133405     1.28378
       race1 |   1.845432   .4589492     2.46   0.020     1.109685    3.068998
       race2 |   1.427256   .5493598     0.92   0.363     .6495548    3.136085
       race4 |   1.965843     .47388     2.80   0.009     1.200699    3.218572
       race5 |   .7542242    .415037    -0.51   0.612     .2447492    2.324233
        age1 |   .6397023    .217165    -1.32   0.198     .3194791    1.280894
        age2 |   .7502604   .2618714    -0.82   0.417      .367437    1.531938
        age4 |   1.211545   .3804675     0.61   0.546     .6373877    2.302902
------------------------------------------------------------------------------
```

Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.
Age1=16-19 years, Age2=20-29 years, Age3=30-39 years, Age4=40-49 years

Figure 5.31 (D):

```
. sum ch3hg

    Variable |       Obs        Mean    Std. Dev.       Min        Max
-------------+--------------------------------------------------------
       ch3hg |      3613    1.155696    2.081726        -.8       29.5

. gen orgmerc=1

. replace orgmer=2 if ch3hg>4
(233 real changes made)

. tabulate orgmerc, gen(orgmerc)

    orgmerc |      Freq.     Percent        Cum.
------------+-----------------------------------
          1 |      3,383       93.56       93.56
          2 |        233        6.44      100.00
------------+-----------------------------------
      Total |      3,616      100.00
```

Orgmerc1= Low CH_3Hg < 3.0 ug/L, Orgmerc2= High CH_3Hg >= 3.0 ug/L

Figure 5.31 (D): Orgmerc1= Low CH_3Hg (< 3.0 ug/L), Orgmerc2= High CH_3Hg (>= 3.0 ug/L)

```
. logistic ihgdetect orgmerc2, ro
Logistic regression                                     Number of obs   =       3616
                                                        Wald chi2(1)    =      55.53
                                                        Prob > chi2     =     0.0000
Log pseudo-likelihood =  -657.8553                      Pseudo R2       =     0.0321
------------------------------------------------------------------------------
             |               Robust
   ihgdetect | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
    orgmerc2 |   4.500692   .9085179     7.45   0.000     3.030081    6.685046
------------------------------------------------------------------------------

. logistic ihgdetect orgmerc2  age1 age2 age4  race1 race2  race4 race5, ro
Logistic regression                                     Number of obs   =       3616
                                                        Wald chi2(8)    =      67.53
                                                        Prob > chi2     =     0.0000
Log pseudo-likelihood = -649.08423                      Pseudo R2       =     0.0450
------------------------------------------------------------------------------
             |               Robust
   ihgdetect | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
    orgmerc2 |   4.374734   .9096862     7.10   0.000     2.910395    6.575841
        age1 |   .6662871   .1541942    -1.75   0.079     .4233248    1.048695
        age2 |   .7622536   .1735688    -1.19   0.233     .4878393    1.191028
        age4 |   1.091314   .2342531     0.41   0.684     .7165347    1.662119
       race1 |   1.675724   .3502589     2.47   0.014     1.112461    2.524179
       race2 |   1.377718    .490328     0.90   0.368     .6858337     2.76759
       race4 |   1.947021   .4100741     3.16   0.002     1.288528    2.942032
       race5 |   .8537122   .4264922    -0.32   0.752     .3206812    2.272738
------------------------------------------------------------------------------
```

Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.
Age1=16-19 years, Age2=20-29 years, Age3=30-39 years, Age4=40-49

```
. svylogit ihgdetect orgmerc2  age1 age2 age4  race1 race2  race4 race5, eform
Survey logistic regression
pweight:  wtmec4yr                                      Number of obs    =      3616
Strata:   sdmvstra                                      Number of strata =        28
PSU:      sdmvpsu                                       Number of PSUs   =        57
                                                        Population size  =  65642103
                                                        F(   8,     22)  =      4.89
                                                        Prob > F         =    0.0014
------------------------------------------------------------------------------
   ihgdetect |     exp(b)   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
    orgmerc2 |   3.699288   .9526756     5.08   0.000     2.184602    6.264177
        age1 |   .5780129   .1951648    -1.62   0.115     .2897529    1.153047
        age2 |   .6984534   .2433829    -1.03   0.312     .3424715    1.424461
        age4 |   1.144424   .3251146     0.47   0.638     .6401073    2.046072
       race1 |   1.736706   .4478806     2.14   0.041     1.024848     2.94302
       race2 |   1.607539   .5296208     1.44   0.160     .8194534    3.153545
       race4 |   2.050218   .5220258     2.82   0.009     1.217976     3.45113
       race5 |    .773155   .4239733    -0.47   0.642     .2518772    2.373255
------------------------------------------------------------------------------
```

Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.
Age1=16-19 years, Age2=20-29 years, Age3=30-39 years, Age4=40-49

Figure 5.31 (E):

```
. logistic ihgdetect lbdstbsi race1 race2 race4 race5 age1 age2 age4, ro

Logistic regression                                     Number of obs   =       3569
                                                        Wald chi2(8)    =      25.56
                                                        Prob > chi2     =     0.0012
Log pseudo-likelihood = -656.21209                      Pseudo R2       =     0.0182

------------------------------------------------------------------------------
             |               Robust
   ihgdetect | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
    lbdstbsi |   1.037694   .0138795     2.77   0.006     1.010844    1.065257
       race1 |    1.53539   .3148467     2.09   0.037     1.027239    2.294912
       race2 |   1.330836   .4756784     0.80   0.424     .6605122     2.68144
       race4 |   1.922521   .4069107     3.09   0.002     1.269727    2.910931
       race5 |   1.100892   .5255784     0.20   0.840     .4318885    2.806196
        age1 |    .543783   .1274973    -2.60   0.009     .3434392    .8609966
        age2 |   .7151661   .1619664    -1.48   0.139     .4588091    1.114761
        age4 |   1.119878   .2395275     0.53   0.597     .7363926    1.703067
------------------------------------------------------------------------------
Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.
Age1=16-19 years, Age2=20-29 years, Age3=30-39 years, Age4=40-49

. svylogit ihgdetect lbdstbsi race1 race2 race4 race5 age1 age2 age4, eform

Survey logistic regression

pweight:  wtmec4yr                                      Number of obs    =      3569
Strata:   sdmvstra                                      Number of strata =        28
PSU:      sdmvpsu                                       Number of PSUs   =        57
                                                        Population size  =  64701840
                                                        F(   8,     22)  =      4.78
                                                        Prob > F         =    0.0017

------------------------------------------------------------------------------
   ihgdetect |     exp(b)   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
    lbdstbsi |   1.059481   .0256805     2.38   0.024     1.008239    1.113327
       race1 |   1.623043   .3904945     2.01   0.053     .9922627    2.654809
       race2 |   1.576212   .5402249     1.33   0.195     .7819614    3.177195
       race4 |   2.089179   .5250606     2.93   0.007     1.249515    3.493088
       race5 |   1.058846    .537079     0.11   0.911     .3752277    2.987935
        age1 |   .4659508   .1525191    -2.33   0.027     .2385592    .9100891
        age2 |   .6482956   .2225899    -1.26   0.217     .3212195    1.308411
        age4 |    1.15946   .3066337     0.56   0.580     .6750742    1.991406
------------------------------------------------------------------------------
Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.
Age1=16-19 years, Age2=20-29 years, Age3=30-39 years, Age4=40-49
```

Figure 5.31 (F):

```
logistic ihgdetect bili2 age1 age2 age4  race1 race2  race4 race5, ro

Logistic regression                                     Number of obs   =       3616
                                                        Wald chi2(8)    =      23.60
                                                        Prob > chi2     =     0.0027
Log pseudo-likelihood = -667.58879                      Pseudo R2       =     0.0178

------------------------------------------------------------------------------
             |               Robust
  ihgdetect | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       bili2 |   1.485919   .3131436     1.88   0.060      .983133    2.245835
        age1 |   .5576656   .1278516    -2.55   0.011     .3558161    .8740215
        age2 |   .6930073   .1558995    -1.63   0.103     .4459136    1.077023
        age4 |   1.110222   .2351186     0.49   0.621     .7330704    1.681412
       race1 |   1.555099   .3169455     2.17   0.030     1.042976    2.318686
       race2 |   1.344317   .4780955     0.83   0.405     .6695436    2.699135
       race4 |   1.945344   .4064932     3.18   0.001     1.291612    2.929955
       race5 |   1.051052   .5019454     0.10   0.917     .4122116    2.679958
------------------------------------------------------------------------------
```

Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.
Age1=16-19 years, Age2=20-29 years, Age3=30-39 years, Age4=40-49

```
Survey Weighted logistic regression:
. svylogit ihgdetect bili2  age1 age2 age4  race1 race2  race4 race5, eform

Survey logistic regression
pweight:  wtmec4yr                                      Number of obs    =      3616
Strata:   sdmvstra                                      Number of strata =        28
PSU:      sdmvpsu                                       Number of PSUs   =        57
                                                        Population size  =  65642103
                                                        F(   8,     22)  =      5.54
                                                        Prob > F         =    0.0007

------------------------------------------------------------------------------
  ihgdetect |     exp(b)   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       bili2 |   1.951656   .5745968     2.27   0.031     1.068795     3.56379
        age1 |   .4760667   .1523347    -2.32   0.028     .2474264    .9159876
        age2 |   .6318899   .2144321    -1.35   0.187       .31566    1.264921
        age4 |   1.134137   .3006678     0.47   0.638     .6594603    1.950483
       race1 |   1.622397   .4016845     1.95   0.060     .9777825     2.69198
       race2 |    1.53679   .5413768     1.22   0.232     .7476706    3.158776
       race4 |   2.092386   .5263652     2.93   0.006     1.250824    3.500156
       race5 |   .9854922   .4914865    -0.03   0.977     .3553632    2.732964
------------------------------------------------------------------------------
```

Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.
Age1=16-19 years, Age2=20-29 years, Age3=30-39 years, Age4=40-49

Figure 5.31 (G):

```
. logistic ihgdetect lbxlh  luteage2  luteage3 race1 race2 race4 race5, ro

Logistic regression                                     Number of obs   =       1133
                                                        Wald chi2(7)    =       9.68
                                                        Prob > chi2     =     0.2077
Log pseudo-likelihood = -251.94157                      Pseudo R2       =     0.0205

------------------------------------------------------------------------------
             |               Robust
   ihgdetect | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       lbxlh |    .980807   .0092647    -2.05   0.040     .9628155    .9991347
    luteage2 |   1.174513   .3223157     0.59   0.558     .6859095     2.01117
    luteage3 |   .9338205   .3336956    -0.19   0.848     .4635443    1.881203
       race1 |   1.153476   .3800244     0.43   0.665     .6047437    2.200116
       race2 |   1.937199   .9160431     1.40   0.162     .7667728    4.894201
       race4 |   1.842123   .5835344     1.93   0.054     .9901052    3.427332
       race5 |   .5719054   .5928205    -0.54   0.590     .0749883    4.361691
------------------------------------------------------------------------------
```

Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.
Age1=16-19 years, Age2=20-29 years, Age3=30-39 years, Age4=40-49

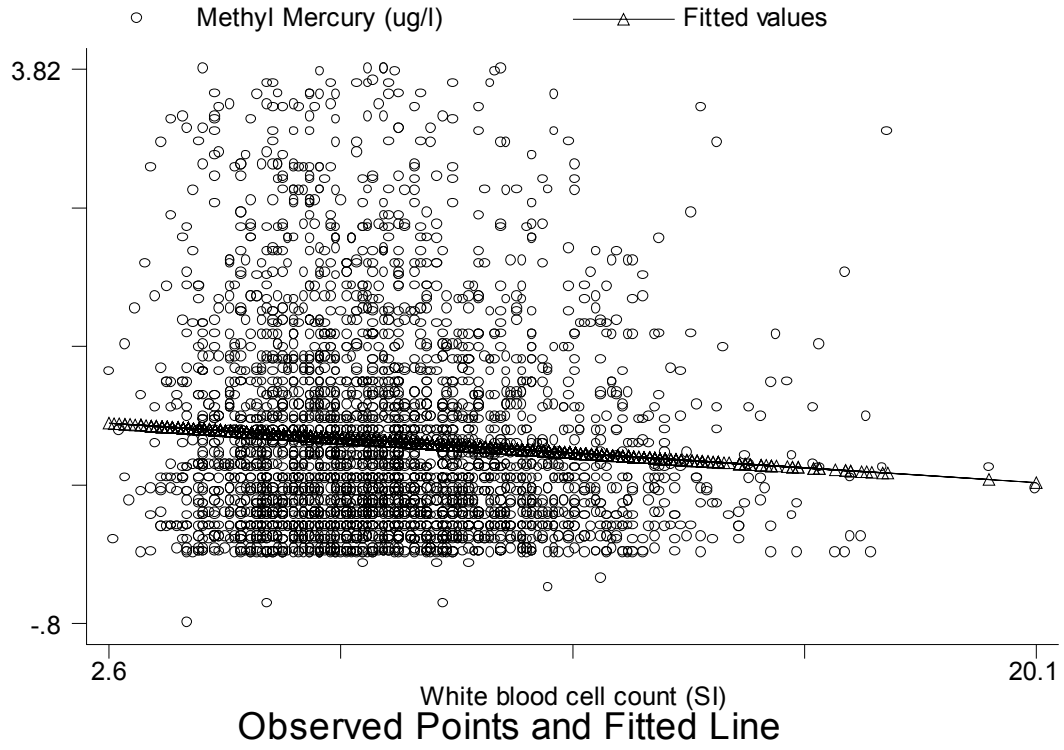
Survey Weighted Logistic Regression:

```
. svylogit ihgdetect lbxlh  luteage2  luteage3 race1 race2 race4 race5, eform

Survey logistic regression

pweight:  wtmec4yr                                      Number of obs    =      1133
Strata:   sdmvstra                                      Number of strata =        28
PSU:      sdmvpsu                                       Number of PSUs   =        57
                                                        Population size  =  29935892
                                                        F(   7,     23)  =      0.70
                                                        Prob > F         =    0.6718

------------------------------------------------------------------------------
   ihgdetect |     exp(b)   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       lbxlh |   .9846182   .0119633    -1.28   0.212     .9604519    1.009392
    luteage2 |   1.282988   .3215149     0.99   0.328     .7684808    2.141964
    luteage3 |   .9578207   .3252018    -0.13   0.900     .4783099    1.918046
       race1 |   1.182553   .5487151     0.36   0.720     .4577973    3.054697
       race2 |   2.165573   1.027591     1.63   0.114     .8205325    5.715442
       race4 |   2.004461   .8160743     1.71   0.098     .8717113    4.609169
       race5 |   .8596245   .8001178    -0.16   0.872     .1281043    5.768378
------------------------------------------------------------------------------
```

Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.
Age1=16-19 years, Age2=20-29 years, Age3=30-39 years, Age4=40-49

Figure 5.5: Test of Rigor: Outliers Removed for Linear Regressions of Blood Methyl Mercury (ug/L) Versus Biochemical Profile Markers, Combined 1999-2000 and 2001-2002 NHANES. (A) CH_3Hg vs. White Blood Cell (lbxwbc) (B) * Subpopulation Restricted by Age: 30-39 Years: CH_3Hg vs. Bilirubin (lbdstbsi).
*Adjusted for age (years) as a continuous variable (ridageyr) and race.

(A) CH_3Hg vs. White Blood Cell (lbxwbc)



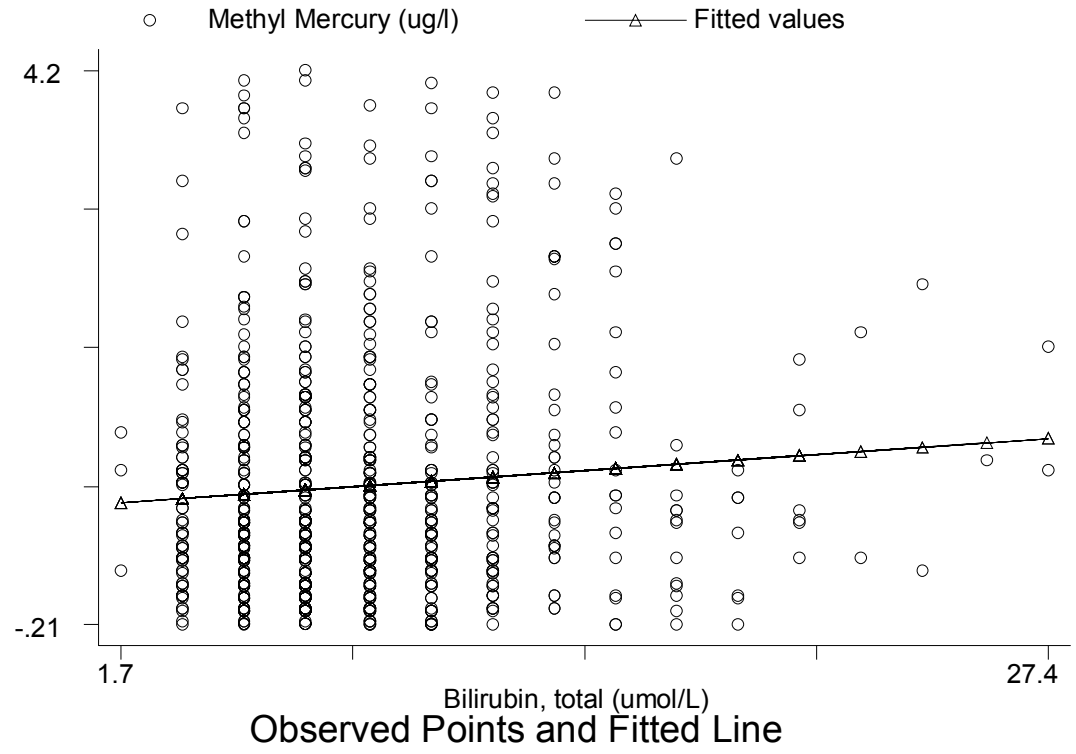

```
. regr ch3hg lbxwbc

      Source |       SS       df       MS              Number of obs =    3367
-------------+------------------------------           F(  1,  3365) =   20.27
       Model |   14.470942     1   14.470942           Prob > F      =  0.0000
    Residual |  2401.91396  3365  .713793154           R-squared     =  0.0060
-------------+------------------------------           Adj R-squared =  0.0057
       Total |  2416.38491  3366  .717880246           Root MSE      =  .84486

------------------------------------------------------------------------------
       ch3hg |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
    lbxwbcsi |  -.0283393    .006294    -4.50   0.000    -.0406798   -.0159989
       _cons |   .9439173   .0514406    18.35   0.000     .8430593    1.044775
------------------------------------------------------------------------------
```

Figure 5.5: Survey Weighted Regression of organic mercury to white blood cell (lbxwbc): Adjusted for age (ridageyr) as a continuous variable and race as a categorical variable.

```
. svyreg ch3hg lbxwbc race1 race2 race4 race5 ridageyr

Survey linear regression

pweight:  wtmec4yr                              Number of obs    =      3611
Strata:   sdmvstra                              Number of strata =        28
PSU:      sdmvpsu                               Number of PSUs   =        57
                                                Population size  =  65571779
                                                F(   6,     24)  =     10.65
                                                Prob > F         =    0.0000
                                                R-squared        =    0.0398

------------------------------------------------------------------------------
       ch3hg |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
    lbxwbcsi |  -.0648348   .0276852    -2.34   0.026    -.1214575   -.0082121
       race1 |  -.3514437   .1381687    -2.54   0.017    -.6340306   -.0688569
       race2 |   .2687287   .3777198     0.71   0.482     -.503795    1.041252
       race4 |   .2552534   .1904777     1.34   0.191    -.1343171     .644824
       race5 |   1.149271   .3585909     3.20   0.003     .4158703    1.882672
    ridageyr |   .0336289   .0057833     5.81   0.000     .0218007    .0454572
       _cons |   .6797867   .2997267     2.27   0.031     .0667767    1.292797
------------------------------------------------------------------------------
```

Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.
Ridageyr= age by year

Figure 5.5 (B) : Subpopulation Restricted by Age: 30-39 Years: CH_3Hg vs. Bilirubin (lbdstbsi).



Regression with robust standard errors

Number of obs	= 771
F(6, 764)	= 5.03
Prob > F	= 0.0000
R-squared	= 0.0389
Root MSE	= .93249

ch3hg	Coef.	Robust Std. Err.	t	P>|t|	[95% Conf.	Interval]
lbdstbsi	.0217671	.008588	2.53	0.011	.0049082	.038626
ridageyr	.0209049	.011866	1.76	0.079	-.0023889	.0441987
race1	-.0575541	.0793951	-0.72	0.469	-.2134125	.0983044
race2	.1327266	.1394807	0.95	0.342	-.1410844	.4065375
race4	.3646939	.0917469	3.97	0.000	.1845879	.5447998
race5	.2688041	.2050045	1.31	0.190	-.1336349	.6712431
_cons	-.0896855	.4110679	-0.22	0.827	-.8966421	.7172711

Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.
Ridageyr= age by year

Figure 5.8: Subpopulation restricted by Race: African American. Associations Between Blood Inorganic Mercury (I-Hg Detection) and biochemical profile markers in African American Women Age 16-49, NHANES 1999-2002.

(A) Luteinizing Hormone (lbxlh) (B) White Blood Cell Count (lbxwbc) (C) Bilirubin (lbdstbsi) (D) Blood Organic Mercury (CH_3Hg) (E) Urinary Mercury (Urxuhg)

Blood Inorganic Mercury Detection (I-Hg Detect) is a transformed binary value (0 = non-detect 1= detection). Age is a continuous variable by decade.

Fig 5.8 (A):

Logistic Regression of I-Hg Detection vs. Luteinizing Hormone (lbxlh) : Group 0 = no I-Hg detection, Group 1=I-Hg detection.

```
. logistic ihgdetect lbxlh ridageyr, ro

Logistic regression                                     Number of obs   =        253
                                                        Wald chi2(2)    =       6.02
                                                        Prob > chi2     =     0.0494
Log pseudo-likelihood = -70.217792                      Pseudo R2       =     0.0297
------------------------------------------------------------------------------
             |               Robust
  ihgdetect | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       lbxlh |   .9651255   .0147596    -2.32   0.020     .9366263    .9944918
    ridageyr |   1.069743   .0530011     1.36   0.174     .9707474    1.178834
------------------------------------------------------------------------------
```

O.R. of I-Hg Detection for a one std. dev. change in Luteinizing Hormone (lbxlh): Group 0 = no I-Hg detection, Group 1=I-Hg detection.

```
. lincom 19*lbxlh, or
 ( 1)  19 lbxlh = 0
------------------------------------------------------------------------------
   ihgdetect | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf.Interval]
-------------+----------------------------------------------------------------
         (1) |   .5094385   .1480259    -2.32   0.020     .2882444    .9003732
------------------------------------------------------------------------------
```

T-Test of I-Hg Detection vs. Luteinizing Hormone (lbxlh) : Group 0 = no I-Hg detection, Group 1=I-Hg detection. . ttest lbxlh, by(ihgdetect) unequal

```
Two-sample t test with unequal variances
------------------------------------------------------------------------------
 Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]-------
---+--------------------------------------------------------------------
     0 |     232    15.86849    1.292604    19.68836    13.32169    18.41529
     1 |      21    9.347143    1.781708    8.164812    5.630565    13.06372
---------+--------------------------------------------------------------------
combined|     253    15.32719    1.199258    19.07536    12.96535    17.68904
---------+--------------------------------------------------------------------
    diff|            6.521349    2.201206                2.089242   10.95346
------------------------------------------------------------------------------
Satterthwaite's degrees of freedom:  45.5023
                     Ho: mean(0) - mean(1) = diff = 0
     Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
       t =   2.9626                 t =   2.9626                  t =   2.9626
   P < t =   0.9976             P > |t| =   0.0048              P > t =   0.0024
```

Fig 5.8 (B):

Logistic Regression of I-Hg Detection vs. White Blood Cell Count (lbxwbc): Group 0 = no I-Hg detection, Group 1=I-Hg detection.

```
. logistic ihgdetect lbxwbc ridageyr, ro
Logistic regression                                     Number of obs   =        789
                                                        Wald chi2(2)    =       8.02
                                                        Prob > chi2     =     0.0181
Log pseudo-likelihood = -182.54683                      Pseudo R2       =     0.0202

------------------------------------------------------------------------------
             |               Robust
  ihgdetect | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
   lbxwbcsi |   .9155473   .0843254    -0.96   0.338     .7643317    1.096679
   ridageyr |   1.033485   .0133414     2.55   0.011     1.007665    1.059968
------------------------------------------------------------------------------
```

T-Test of I-Hg Detection vs. White Blood Cell Count (lbxwbc): Group 0 = no I-Hg detection, Group 1=I-Hg detection.

```
. ttest lbxwbc, by(ihgdetect) unequal

Two-sample t test with unequal variances

------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf.Interval]
---------+--------------------------------------------------------------------
       0 |     739    6.933694    .0769922    2.092997    6.782544   7.084844
       1 |      50       6.552    .3436804    2.430188    5.861348   7.242652
---------+--------------------------------------------------------------------combined
|     789    6.909506     .075341    2.116265    6.761613   7.057399
---------+--------------------------------------------------------------------
   diff |            .3816942    .3521989               -.3244123    1.087801
------------------------------------------------------------------------------
Satterthwaite's degrees of freedom:  54.0326

                    Ho: mean(0) - mean(1) = diff = 0

     Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
       t =   1.0837                 t =   1.0837                 t =   1.0837
   P < t =   0.8584             P > |t| =   0.2833             P > t =   0.1416
```

Fig 5.8 (C):

Logistic Regression of I-Hg Detection vs. Bilirubin (lbdstbsi): Group 0 = no I-Hg detection, Group 1=I-Hg detection.

```
logistic ihgdetect lbdstbsi ridageyr, ro

Logistic regression                                     Number of obs   =        775
                                                        Wald chi2(2)    =       6.01
                                                        Prob > chi2     =     0.0495
Log pseudo-likelihood = -176.83903                      Pseudo R2       =     0.0176

------------------------------------------------------------------------------
             |               Robust
  ihgdetect | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
   lbdstbsi |   1.036297   .0274212     1.35   0.178     .9839225     1.09146
   ridageyr |   1.033173   .0139897     2.41   0.016     1.006115     1.06096
------------------------------------------------------------------------------
```

T-Test of I-Hg Detection vs. Bilirubin (lbdstbsi): Group 0 = no I-Hg detection, Group 1=I-Hg detection.

```
. ttest lbdstbsi, by(ihgdetect) unequal

Two-sample t test with unequal variances

------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       0 |     727    8.034388    .1559011    4.20355    7.728317    8.340459
       1 |      48    8.591667     .489996    3.394792    7.605922    9.577411
---------+--------------------------------------------------------------------
combined |     775    8.068903    .1493725    4.158355     7.77568    8.362126
---------+--------------------------------------------------------------------
    diff |           -.5572788    .5141996               -1.586961    .4724039
------------------------------------------------------------------------------
Satterthwaite's degrees of freedom:  56.9596

                     Ho: mean(0) - mean(1) = diff = 0

     Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
       t =  -1.0838                 t =  -1.0838                 t =  -1.0838
   P < t =   0.1415         P > |t| =   0.2830         P > t =   0.8585
```

Fig 5.8 (D):

Logistic Regression of I-Hg Detection vs. Blood Organic Mercury (CH_3Hg): Group 0 = no I-Hg detection, Group 1=I-Hg detection.

```
logistic ihgdetect ch3hg ridageyr, ro
Logistic regression                               Number of obs   =        789
                                                  Wald chi2(2)    =      42.83
                                                  Prob > chi2     =     0.0000
Log pseudo-likelihood = -164.55709                Pseudo R2       =     0.1168

------------------------------------------------------------------------------
             |               Robust
  ihgdetect | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       ch3hg |   1.316371   .0603964     5.99   0.000     1.203163    1.440231
    ridageyr |   1.012119   .0150793     0.81   0.419     .9829913     1.04211
------------------------------------------------------------------------------
```

O.R. of I-Hg Detection for a one std. dev. change in Blood Organic Mercury (CH_3Hg): Group 0 = no I-Hg detection, Group 1=I-Hg detection.

```
lincom 2.3*ch3hg, or
 ( 1)  2.3 ch3hg = 0

------------------------------------------------------------------------------
  ihgdetect | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
         (1) |   1.881786   .1985778     5.99   0.000     1.530192    2.314165
------------------------------------------------------------------------------
```

T-Test of I-Hg Detection vs. Blood Organic Mercury (CH_3Hg): Group 0 = no I-Hg detection, Group 1=I-Hg detection.

```
ttest ch3hg, by(ihgdetect) unequal
Two-sample t test with unequal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       0 |     739    1.188769    .0589973    1.603816    1.072946    1.304591
       1 |      50       4.324    .8892811    6.288167    2.536923    6.111077
---------+--------------------------------------------------------------------
combined |     789    1.387452    .0831262    2.334945    1.224277    1.550628
---------+--------------------------------------------------------------------
    diff |           -3.135231     .891236               -4.925841   -1.344622
------------------------------------------------------------------------------
Satterthwaite's degrees of freedom:  49.4322

                    Ho: mean(0) - mean(1) = diff = 0

     Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
       t =  -3.5178                 t =  -3.5178                 t =  -3.5178
   P < t =   0.0005           P > |t| =   0.0009           P > t =   0.9995
```

Fig 5.8 (E):

Logistic Regression of I-Hg Detection vs. Urinary Mercury (Urxuhg): Group 0 = no I-Hg detection, Group 1=I-Hg detection.

```
logistic ihgdetect urxuhg ridageyr, ro

Logistic regression                                     Number of obs   =        763
                                                        Wald chi2(2)    =      36.98
                                                        Prob > chi2     =     0.0000
Log pseudo-likelihood = -139.59274                      Pseudo R2       =     0.2211

------------------------------------------------------------------------------
             |               Robust
  ihgdetect | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
      urxuhg |    1.41625   .0868427     5.68   0.000     1.255872    1.597109
    ridageyr |   1.031682    .016913     1.90   0.057     .9990597    1.065369
------------------------------------------------------------------------------
```

O.R. of I-Hg Detection for a one std. dev. change in Urinary Mercury (Urxuhg): Group 0 = no I-Hg detection, Group 1=I-Hg detection.

```
lincom 3.4*urxuhg, or

 ( 1)  3.4 urxuhg = 0

------------------------------------------------------------------------------
  ihgdetect | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
         (1) |   3.264946   .6806883     5.68   0.000     2.169773    4.912898
------------------------------------------------------------------------------
```

T-Test of I-Hg Detection vs. Urinary Mercury (Urxuhg): Group 0 = no I-Hg detection, Group 1=I-Hg detection.

```
ttest urxuhg, by(ihgdetect) unequal

Two-sample t test with unequal variances

------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       0 |     715    1.376196    .0703363    1.880755    1.238105    1.514286
       1 |      48    7.092917    1.523573    10.55563    4.027883    10.15795
---------+--------------------------------------------------------------------
combined |     763    1.735832    .1260135    3.480803    1.488457    1.983207
---------+--------------------------------------------------------------------
    diff |           -5.716721    1.525196               -8.784675   -2.648767
------------------------------------------------------------------------------
Satterthwaite's degrees of freedom:  47.2005

                    Ho: mean(0) - mean(1) = diff = 0

     Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
       t =  -3.7482                  t =  -3.7482                  t =  -3.7482
   P < t =   0.0002          P > |t| =   0.0005          P > t =   0.9998
```

Figure 5.9: Subpopulation restricted by Race: African American. Associations Between Blood Organic Mercury (CH_3Hg) and biochemical profile markers in African American Women Age 16-49, NHANES 1999-2002.
(B) Luteinizing Hormone (lbxlh) (B) White Blood Cell Count (lbxwbc) (C) Bilirubin (lbdstbsi)
(D) Urinary Mercury (Urxuhg)
Age is a continuous explanatory variable by decade.

5.9(A): Luteinizing Hormone (lbxlh)

```
regr ch3hg lbxlh ridageyr, ro

Regression with robust standard errors                Number of obs =     252
                                                      F(  2,   249) =    2.68
                                                      Prob > F      =  0.0708
                                                      R-squared     =  0.0125
                                                      Root MSE      =  3.0524

------------------------------------------------------------------------------
             |               Robust
       ch3hg |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       lbxlh |  -.0136017   .0061208    -2.22   0.027    -.0256568   -.0015466
    ridageyr |   .0738498   .0477687     1.55   0.123    -.0202325     .167932
       _cons |  -.7797043   1.868877    -0.42   0.677    -4.460527    2.901119
------------------------------------------------------------------------------
```

5.9(B): White Blood Cell Count (lbxwbc)

```
regr ch3hg lbxwbc ridageyr, ro

Regression with robust standard errors                Number of obs =     788
                                                      F(  2,   785) =   23.04
                                                      Prob > F      =  0.0000
                                                      R-squared     =  0.0723
                                                      Root MSE      =  2.2532

------------------------------------------------------------------------------
             |               Robust
       ch3hg |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
    lbxwbcsi |   .0070445   .0404868     0.17   0.862    -.0724307    .0865197
    ridageyr |   .0592879   .0087853     6.75   0.000     .0420424    .0765334
       _cons |  -.3602701   .3649487    -0.99   0.324    -1.076661    .3561208
------------------------------------------------------------------------------
```

5.9(C): Bilirubin (lbdstbsi)

```
regr ch3hg lbdstbsi ridageyr, ro

Regression with robust standard errors                Number of obs =     774
                                                      F(  2,   771) =   24.71
                                                      Prob > F      =  0.0000
                                                      R-squared     =  0.0746
                                                      Root MSE      =  2.2329

------------------------------------------------------------------------------
             |               Robust
       ch3hg |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
    lbdstbsi |   .0188002   .0174643     1.08   0.282    -.0154831    .0530834
    ridageyr |   .0596691   .0090406     6.60   0.000     .0419219    .0774163
       _cons |  -.4775358   .2991514    -1.60   0.111    -1.064784     .109712
------------------------------------------------------------------------------
```

5.9(D): Urinary Mercury (urxuhg)

```
regr ch3hg urxuhg ridageyr, ro

Regression with robust standard errors                Number of obs =     762
                                                      F(  2,   759) =   26.54
                                                      Prob > F      =  0.0000
                                                      R-squared     =  0.1556
                                                      Root MSE      =  2.1768

------------------------------------------------------------------------------
             |               Robust
       ch3hg |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
      urxuhg |   .1969935   .0560783     3.51   0.000     .0869065    .3070805
    ridageyr |    .054267   .0087737     6.19   0.000     .0370434    .0714905
       _cons |  -.4911032   .2136444    -2.30   0.022    -.9105073   -.0716991
------------------------------------------------------------------------------
```

Table 5.01: Descriptive statistics of Age (years) for women 16-49 years of age, by race/ethnic group, combined 1999-2000 and 2001-2002 NHANES.

	Count	Mean	Std. Error of Mean	Std. Deviation
Total	3616	29.0	.171	10. 3
Race Ethnicity				
Mexican American	1099	27.3	.311	10.3
Other Hispanic	219	29.0	.681	10.1
White	1369	30.6	.267	9.89
Black	790	28.7	.377	10.6
Other	139	28.4	.850	10.0

Table 5.02: Descriptive Statistics of I-Hg Detection (proportion of detection) for women 16-49 years of age, by age and race/ethnic group, combined 1999-2000 and 2001-2002 NHANES.

	Count	Mean *	Std. Error of Mean
Total	3616	.047	.004
Race Ethnicity			
Mexican American	1099	.049	. 007
Other Hispanic	219	.046	.014
White	1369	.036	.005
Black	790	.063	.009
Other	139	.036	.016
Age(years)			
16-19	1031	.035	.006
20-29	964	.038	.006
30-39	855	.055	.008
40-49	766	.063	.009

- I-Hg Detection is defined as I-Hg concentrations above 0.4 ug/L. Mean Detection is presented as a proportion of detection within the population (i.e. 0.046 = 4.6% of the population had I-Hg detection with levels above the LOD. of 0.4 ug/L).
- The vast majority of samples had I-Hg levels below the limit of detection. NHANES gave a standard estimation of 0.3 ug/L for 1999-2000 and 0.28 ug/L for 2001-2 for all non-detects. As a result, there is no distribution of mean I-Hg levels that assume a standard estimate value in approximately 95% of the population.

Table 5.03: Descriptive statistics of blood CH_3Hg (ug/L) for women 16-49 years of age, by age and race/ethnic group, combined 1999-2000 and 2001-2002 NHANES.

	Count	Mean	Std. Deviation
Total	3613	1.16	2.08
Race Ethnicity			
Mexican American	1099	.803	1.30
Other Hispanic	218	1.42	2.85
White	1368	1.20	2.18
Black	789	1.39	2.33
Other	139	1.80	2.67
Age(years)			
16-19	1030	.680	1.34
20-29	963	.964	1.74
30-39	855	1.56	2.64
40-49	765	1.59	2.41

Table 5.04: Descriptive statistics of Blood Bilirubin levels (umol/L) for women 16-49 years of age, by age and race/ethnic group, combined 1999-2000 and 2001-2002 NHANES.

	Count	Mean *	Std. Deviation
Total	3569	8.45	4.33
Race Ethnicity			
Mexican American	1088	8.30	3.94
Other Hispanic	216	8.91	5.87
White	1356	8.73	4.46
Black	775	8.07	4.16
Other	134	8.41	3.92
Age(years)			
16-19	1017	8.97	5.21
20-29	955	7.79	3.78
30-39	847	8.40	3.89
40-49	750	8.66	4.03

Table 5.05: Descriptive statistics of Blood Luteinizing Hormone (LH) levels (mIU/ml) for women 35-49 years of age, by age and race/ethnic group, combined 1999-2000 and 2001-2002 NHANES.

	Count	Mean *	Std. Deviation
Total	1133	13.4	17.5
Race Ethnicity			
Mexican American	300	12.2	15.2
Other Hispanic	67	12.0	15.9
White	478	13.5	18.0
Black	253	15.3	19.1
Other	35	11.7	18.6
Age(years)			
16-19	0		
20-29	0		
30-39	402	8.80	13.7
40-49	731	16.0	18.8

Table 5.06: Descriptive statistics of White Blood Cell Count (SI) for women 16-49 years of age, by age and race/ethnic group, combined 1999-2000 and 2001-2002 NHANES.

	Count	Mean *	Std. Deviation
Total	3614	7.80	2.16
Race Ethnicity			
Mexican American	1099	8.00	2.10
Other Hispanic	218	8.28	2.30
White	1369	8.04	2.43
Black	789	6.91	2.12
Other	139	8.17	2.43
Age(years)			
16-19	1031	7.64	2.16
20-29	962	8.39	2.47
30-39	855	7.82	2.30
40-49	766	7.27	2.12

Table 5.07: Descriptive statistics of Urinary Mercury (ng/ml) for women 16-49 years of age, by age and race/ethnic group, combined 1999-2000 and 2001-2002 NHANES.

	Count	Mean	Std. Deviation
Total	3531	1.41	2.68
Race Ethnicity			
Mexican American	1083	1.47	3.20
Other Hispanic	213	1.58	2.11
White	1336	1.16	1.57
Black	763	1.74	3.48
Other	136	1.27	1.93
Age(years)			
16-19	1003	1.32	2.36
20-29	943	1.28	2.51
30-39	843	1.53	2.13
40-49	742	1.56	3.65

Table 5.1: Rate of Detection for Inorganic Mercury (I-Hg) in NHANES population by year.

Forms	**Population**	**Years**	**Observations**	**Mean**	**Std. Dev.**	**Min.**	**Max**
I-Hg Detect	Raw	1999-2000	1708	3% Detection	0.18	0	1
I-Hg Detect	Raw	2001-2	1908	6% Detection	0.24	0	1
I-Hg Detect	Survey Weighted	1999-2000	Inference to 31 million	2.2% Detection		0	1
I-Hg Detect	Survey Weighted	2001-2	Inference to 35 million	6.7% Detection		0	1

Table 5.21: Comparison of I-Hg Detection between the two Survey Groups, NHANES 1999-2000 (year1) and NHANES 2001-2002 (year2). (A) T-Test (B) Logistic Regression, raw populations. (C) Logistic Regression adjusted for age (ridageyr, continuous variable) and race (categorical variable), naïve estimate.
(D) Logistic Regression adjusted for age and race, robust estimate (E) Random Effects model, longitudinal analysis (F) Survey Weighted MEANS (G) Survey weighted logistic regression.

Table 5.21: (A): I-Hg detection by survey group. Group 1999 = NHANES survey group 1999-2000, Group 2001 = NHANES survey group 2001-2002

```
. ttest ihgdetect,by(year) unequal

Two-sample t test with unequal variances

------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
    1999 |    1708    .0322014    .0042728    .1765863    .0238209    .0405819
    2001 |    1908    .0592243    .0054053    .2361059    .0486234    .0698252
---------+--------------------------------------------------------------------
combined |    3616    .0464602    .0035007    .2105086    .0395966    .0533237
---------+--------------------------------------------------------------------
    diff |           -.0270229    .0068901               -.040532   -.0135138
------------------------------------------------------------------------------
Satterthwaite's degrees of freedom:  3505.66

                 Ho: mean(1999) - mean(2001) = diff = 0

     Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
       t =  -3.9220                 t =  -3.9220                 t =  -3.9220
   P < t =   0.0000             P > |t| =   0.0001             P > t =   1.0000
```

Table 5.21: (B) Logistic Regression, I-Hg Detection for NHANES 2001-2002 (year2) as compared to baseline population, NHANES 1999-2000 (Year1):

```
. logistic  ihgdetect  year2

Logistic regression                               Number of obs   =       3616
                                                  LR chi2(1)      =      15.22
                                                  Prob > chi2     =     0.0001
Log likelihood = -672.04233                       Pseudo R2       =     0.0112

------------------------------------------------------------------------------
   ihgdetect | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       year2 |   1.892013    .317686     3.80   0.000     1.361444    2.629351
------------------------------------------------------------------------------
```

Table 5.21: (C) Logistic Regression of I-Hg Detection for NHANES 2001-2002 (year2) as compared to baseline population NHANES 1999-2000 (Year1); adjusted for race, as a categorical vaiable, and age as a continuous variable in years(ridageyr): naïve estimate:

```
. logistic  ihgdetect  year2  race1 race2 race4 race5 ridageyr

Logistic regression                                     Number of obs   =       3616
                                                        LR chi2(6)      =      36.09
                                                        Prob > chi2     =     0.0000
Log likelihood = -661.60694                             Pseudo R2       =     0.0266

------------------------------------------------------------------------------
   ihgdetect | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       year2 |   1.939744   .3277433     3.92   0.000     1.392911    2.701253
       race1 |   1.606922   .3280307     2.32   0.020     1.077045    2.397484
       race2 |   1.451428    .518192     1.04   0.297     .7209376    2.922088
       race4 |   1.960732   .4070237     3.24   0.001     1.305327    2.945216
       race5 |   1.083835   .5200382     0.17   0.867     .4231998    2.775753
    ridageyr |   1.025262   .0077308     3.31   0.001     1.010222    1.040527
------------------------------------------------------------------------------
```

year2= NHANES survey years 2001-2002
Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.
Ridageyr= age by year

Table 5.21: (D) Logistic Regression of I-Hg Detection for NHANES 2001-2002 (year2) as compared to baseline population NHANES 1999-2000 (Year1); adjusted for race, as a categorical variable, and age as a continuous variable in years(ridageyr): robust estimate:

```
. logistic ihgdetect year2  race1 race2 race4 race5 ridageyr, ro

Logistic regression                                     Number of obs   =       3616
                                                        Wald chi2(6)    =      41.79
                                                        Prob > chi2     =     0.0000
Log pseudo-likelihood = -661.60694                      Pseudo R2       =     0.0266

------------------------------------------------------------------------------
             |               Robust
   ihgdetect | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       year2 |   1.939744     .32328     3.98   0.000     1.399208    2.689098
       race1 |   1.606922   .3206966     2.38   0.017     1.086723    2.376133
       race2 |   1.451428   .5157998     1.05   0.294     .7232702    2.912664
       race4 |   1.960732   .4043851     3.26   0.001     1.308774    2.937457
       race5 |   1.083835   .5172405     0.17   0.866     .4253463    2.761745
    ridageyr |   1.025262   .0073359     3.49   0.000     1.010984    1.039742
```

year2= NHANES survey years 2001-2002
Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.
Ridageyr= age by year

Table 5.21: (E) Random Effects model, longitudinal analysis of I-Hg detection for year2 as compared to year1, adjusted for race (ridreth1) as a categorical variable.

```
. xtlogit  ihgdetect year2, or i(ridreth1) re

Fitting comparison model:

Iteration 0:   log likelihood =  -679.6543
Iteration 1:   log likelihood = -672.19317
Iteration 2:   log likelihood = -672.04241
Iteration 3:   log likelihood = -672.04233

Fitting full model:

tau =  0.0     log likelihood = -672.04233
tau =  0.1     log likelihood = -671.34223
tau =  0.2     log likelihood = -672.18373
Iteration 0:   log likelihood = -671.34223
Iteration 1:   log likelihood = -671.06527
Iteration 2:   log likelihood =  -670.8494
Iteration 3:   log likelihood = -670.72637
Iteration 4:   log likelihood = -670.72327
Iteration 5:   log likelihood = -670.72326

Random-effects logistic regression              Number of obs      =      3616
Group variable (i): ridreth1                    Number of groups   =         5

Random effects u_i ~ Gaussian                   Obs per group: min =       139
                                                               avg =     723.2
                                                               max =      1369

                                                Wald chi2(1)       =     15.09
Log likelihood  = -670.72326                    Prob > chi2        =    0.0001

------------------------------------------------------------------------------
   ihgdetect |         OR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       year2 |   1.924639   .3244336     3.88   0.000     1.383131    2.678151
-------------+----------------------------------------------------------------
    /lnsig2u |   -3.34755   1.142699                     -5.587199   -1.107902
-------------+----------------------------------------------------------------
     sigma_u |   .1875377   .1071496                      .0612005    .5746747
         rho |   .0105774    .011959                      .0011372    .0912266
------------------------------------------------------------------------------
Likelihood-ratio test of rho=0: chibar2(01) =     2.64 Prob >= chibar2 = 0.052
```

year2= NHANES survey years 2001-2002

Table 5.21: (F) Survey Weighted MEANS of I-Hg Detection, by Survey Group:

```
. svymean ihgdetect, by(year)

Survey mean estimation

pweight:  wtmec4yr                                Number of obs    =      3616
Strata:   sdmvstra                                Number of strata =        28
PSU:      sdmvpsu                                 Number of PSUs   =        57
                                                  Population size  =  65642103

--------------------------------------------------------------------------------
Mean   Subpop. |   Estimate    Std. Err.   [95% Conf. Interval]         Deff
---------------+----------------------------------------------------------------
ihgdetect      |
    year==1999 |   .0223738    .0044452    .0132823    .0314653    1.540761
    year==2001 |   .0667028    .0113226    .0435454    .0898602    3.932265
--------------------------------------------------------------------------------
```

Table 5.21: (G) Survey weighted logistic regression

```
. svylogit ihgdetect year2 race1 race2 race4 race5 ridageyr, eform

Survey logistic regression

pweight:  wtmec4yr                                Number of obs    =      3616
Strata:   sdmvstra                                Number of strata =        28
PSU:      sdmvpsu                                 Number of PSUs   =        57
                                                  Population size  =  65642103
                                                  F(   6,     24)  =      7.28
                                                  Prob > F         =    0.0002

------------------------------------------------------------------------------
   ihgdetect |     exp(b)   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       year2 |   3.192289   .8497688     4.36   0.000     1.852082    5.502302
       race1 |   1.613499   .4257961     1.81   0.080     .9405196    2.768023
       race2 |   1.651324   .6109662     1.36   0.186      .774817    3.519373
       race4 |   2.095875   .5439684     2.85   0.008     1.232626    3.563684
       race5 |   1.033402   .5465218     0.06   0.951     .3503658    3.048013
    ridageyr |   1.025802   .0110067     2.37   0.024     1.003536    1.048562
------------------------------------------------------------------------------
```

year2= NHANES survey years 2001-2002
Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.
Ridageyr= age by year

Table 5.22: Risk of I-Hg Detection for Survey Group 2001-2002 versus 1999-2000

Analysis	Model Type	Correlation	O.R.	Std. Error	P- Value
Logistic	Survey	Wtmec4yr	3.2	0.85	<0.001

Table 5.23: Risk of I-Hg Detection for Survey Group 2001-2002 (year2) versus 1999-2000 (year1) Using Categorical variables for Age.

(A): Naïve estimate:

```
. logistic ihgdetect year2  race1 race2 race4 race5 age1 age2 age4

Logistic regression                                     Number of obs   =       3616
                                                        LR chi2(8)      =      37.24
                                                        Prob > chi2     =     0.0000
Log likelihood =  -661.0346                             Pseudo R2       =     0.0274

------------------------------------------------------------------------------
   ihgdetect | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       year2 |   1.943959   .3286112     3.93   0.000      1.39572    2.707548
       race1 |   1.623991   .3331982     2.36   0.018     1.086277    2.427877
       race2 |   1.452066   .5185198     1.04   0.296     .7211571    2.923768
       race4 |   1.967366   .4103545     3.24   0.001     1.307197    2.960938
       race5 |   1.069029   .5130146     0.14   0.889     .4173569    2.738238
        age1 |   .5667871   .1303831    -2.47   0.014     .3610858    .8896709
        age2 |   .6684062   .1514851    -1.78   0.075     .4286741    1.042206
        age4 |   1.109508   .2362925     0.49   0.626     .7308862    1.684268
------------------------------------------------------------------------------
```

year2= NHANES survey years 2001-2002
Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.
Age1=16-19 years, Age2=20-29 years, Age3=30-39 years, Age4=40-49

Table 5.23 (B): Robust estimate:

```
. logistic ihgdetect year2  race1 race2 race4 race5 age1 age2 age4, ro

Logistic regression                                     Number of obs   =       3616
                                                        Wald chi2(8)    =      41.65
                                                        Prob > chi2     =     0.0000
Log pseudo-likelihood =  -661.0346                      Pseudo R2       =     0.0274

------------------------------------------------------------------------------
             |               Robust
   ihgdetect | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       year2 |   1.943959    .324671     3.98   0.000     1.401275    2.696813
       race1 |   1.623991   .3279605     2.40   0.016     1.093165    2.412578
       race2 |   1.452066   .5154446     1.05   0.293     .7241567    2.911657
       race4 |   1.967366   .4096737     3.25   0.001     1.308084    2.958931
       race5 |   1.069029   .5097315     0.14   0.889     .4198767    2.721805
        age1 |   .5667871   .1291647    -2.49   0.013     .3626104    .8859305
        age2 |   .6684062   .1510955    -1.78   0.075     .4291642    1.041016
        age4 |   1.109508   .2359994     0.49   0.625     .7312647    1.683397
------------------------------------------------------------------------------
```

year2= NHANES survey years 2001-2002
Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.
Age1=16-19 years, Age2=20-29 years, Age3=30-39 years, Age4=40-49

Table 5.23 (C): Survey Weighted Population:

```
. svylogit ihgdetect year2  race1 race2 race4 race5 age1 age2 age4, eform

Survey logistic regression

pweight:  wtmec4yr                                Number of obs    =      3616
Strata:   sdmvstra                                Number of strata =        28
PSU:      sdmvpsu                                 Number of PSUs   =        57
                                                  Population size  =  65642103
                                                  F(   8,     22)  =      5.84
                                                  Prob > F         =    0.0005

------------------------------------------------------------------------------
   ihgdetect |     exp(b)   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       year2 |   3.227128   .8597735     4.40   0.000     1.871427    5.564928
       race1 |   1.639983   .4345437     1.87   0.072     .9538624    2.819634
       race2 |   1.664271   .6081792     1.39   0.174     .7881971    3.514092
       race4 |   2.094948   .5402282     2.87   0.008     1.236298    3.549958
       race5 |   1.012761   .5312893     0.02   0.981     .3463735    2.961208
        age1 |   .4668655   .1488628    -2.39   0.024     .2432058    .8962098
        age2 |   .5854915   .2036276    -1.54   0.135     .2874771    1.192444
        age4 |   1.085844   .2896951     0.31   0.760     .6292077    1.873878
------------------------------------------------------------------------------
```

year2= NHANES survey years 2001-2002
Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.
Age1=16-19 years, Age2=20-29 years, Age3=30-39 years, Age4=40-49

Table 5.3: Mean Values for Mercury forms and Biochemical Profile Markers in the NHANES combined population, 1999-2002.

Form	**Observations**	**Mean**	**Std. Dev.**	**Min**	**Max**
I-Hg Detection	3616	4.6% Detection	0.21	0	1
CH_3Hg	3613	1.15 (ug/L)	2.1	0	29.5
Urinary Mercury	3531	1.4 (ng/ml)	2.7	.1	68.51
Bilirubin	3569	8.45 (umol/L)	4.3	1.7	72
White Blood Cell Count	3614	7.8 (SI)	2.3	2.6	20.1
Luteinizing hormone (LH)	1133	13.4 (mIU/mL)	17.5	.1	129

Table 5.4: Marginal Model Associations With Mercury by Form American Women, Ages 16-49 (NHANES, 1999-2002).

Biochemical Profile Variable	**Association with Mercury**	**I-Hg Detection**	**CH_3Hg**	**Urinary Mercury**
Bilirubin	Yes, Direct	Yes, Direct	Yes, Direct	Yes, Direct
White Blood Cell Count	Yes, Inverse	Yes, Inverse 30-39 Years	Yes, Inverse	Yes, Inverse Association in Raw population
Luteinizing hormone	Yes, Inverse	Yes, Inverse 35-39 Years	Yes, Inverse	No Association

Table 5.5: I-Hg detection and Bilirubin

Analysis	**Model Type**	**Correlation**	**O.R.**	**Std. Error**	**P Value**
Logistic	Adjusted, Raw Population	Robust	1.04	0.014	0.006
Logistic	Survey Weighted	Wtmec4yr	1.06	0.027	0.029

Table 5.6: I-Hg detection and White Blood Cell Count

Analysis	**Model Type**	**Correlation**	**O.R.**	**Std. Error**	**P Value**
Logistic	Adjusted, Raw Population	Robust	0.92	0.037	0.029
Logistic	Survey Weighted	Wtmec4yr	0.89	0.053	0.059

Table 5.61: I-Hg detection and Luteinizing hormone (LH)

Analysis	**Model Type**	**Correlation**	**O.R.**	**Std. Error**	**P Value**
Logistic	Adjusted, Raw Population	Robust	0.979	0.009	0.026

Table 5.62: Blood I-Hg Detection and Urinary Mercury

Analysis	Model Type	Correlation	O.R.	Std. Error	P Value
Logistic	Adjusted, Raw Population	Robust	1.39	0.039	<0.001
Logistic	Survey Weighted	Wtmec4yr	1.33	0.0631	<0.001

Table 5.63: Blood I-Hg Detection and CH_3Hg

Analysis	Model Type	Correlation	O.R.	Std. Error	P Value
Logistic	Adjusted, Raw Population	Robust	1.25	0.029	<0.001
Logistic	Survey Weighted	Wtmec4yr	1.21	0.0376	<0.001

Table 5.7: Organic Mercury (CH_3Hg) and Luteinizing hormone (LH)

Analysis	Model Type	Correlation	Slope	Std. Error	P- Value
Regression	Survey	Wtmec4yr	-0.011	0.004	0.006

Table 5.71: Blood Organic Mercury (CH_3Hg) and Bilirubin

Analysis	Model Type	Correlation	Slope	Std. Error	P- Value
Regression	Raw, adjusted	robust	0.029	0.008	<0.001
Regression	Survey Weighted	Wtmec4yr	0.044	0.018	0.018

Table 5.712: Blood Organic Mercury (CH_3Hg) and White Blood Cell Count

Analysis	Model Type	Correlation	Slope	Std. Error	P- Value
Regression	Raw, adjusted	Robust	-0.052	0.014	<0.001
Regression	Survey Weighted	Wtmec4yr	-0.065	0.028	0.026

Table 5.72: Linear Regressions of Blood Organic Mercury (CH_3Hg) to Biochemical Profile Markers, Women Age 16-49 Years, NHANES 1999-2000 & 2001-2002 Combined Population. (A) Bilirubin (lbstbsi) (B) Leutenizing Hormone (lbxlh) * (C) White Blood Cells (lbxwbc). Adjusted for Age in years (ridageyr) as a continuous variable. Dummy variables for race are compared to the baseline, white population; race1=Mexican American, race2=Hispanic, race3=White, race4=African American, race5= Other.
*LH measured in subpopulation, Ages 35-49 years.

(A)

```
 regr ch3hg lbdstbsi ridageyr  race1 race2 race4 race5, ro

Regression with robust standard errors                Number of obs =    3566
                                                      F(  6,  3559) =   26.42
                                                      Prob > F      =  0.0000
                                                      R-squared     =  0.0517
                                                      Root MSE      =  2.0253

------------------------------------------------------------------------------
             |               Robust
       ch3hg |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
    lbdstbsi |   .0294591   .0084044     3.51   0.000     .0129811     .045937
    ridageyr |   .0370246    .003375    10.97   0.000     .0304074    .0436417
       race1 |  -.2619715    .069337    -3.78   0.000    -.3979157   -.1260272
       race2 |   .2789491   .2030637     1.37   0.170    -.1191838     .677082
       race4 |   .2745151   .1011532     2.71   0.007     .0761911    .4728391
       race5 |   .6317864   .2282889     2.77   0.006     .1841962    1.079377
       _cons |  -.1901475   .1250077    -1.52   0.128    -.4352414    .0549463
------------------------------------------------------------------------------
```

Ridageyr= age by year(continuous variable)
Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.

Survey Weighted Regression:

```
svyreg ch3hg lbdstbsi race1 race2 race4 race5 ridageyr
Survey linear regression
pweight:  wtmec4yr                              Number of obs    =      3566
Strata:   sdmvstra                              Number of strata =        28
PSU:      sdmvpsu                               Number of PSUs   =        57
                                                Population size  =  64666519
                                                F(   6,     24)  =      8.32
                                                Prob > F         =    0.0001
                                                R-squared        =    0.0406
------------------------------------------------------------------------------
       ch3hg |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
    lbdstbsi |   .0441854   .0176249     2.51   0.018     .0081384    .0802324
       race1 |  -.3377264   .1376161    -2.45   0.020    -.6191828   -.0562699
       race2 |   .2415385   .4007183     0.60   0.551    -.5780224    1.061099
       race4 |   .3370606   .1775659     1.90   0.068    -.0261025    .7002238
       race5 |   1.042425   .3823843     2.73   0.011     .2603611    1.824488
    ridageyr |   .0352444   .0062074     5.68   0.000     .0225488      .04794
       _cons |  -.2629977   .2428089    -1.08   0.288    -.7595976    .2336021
------------------------------------------------------------------------------
```

Ridageyr= age by year(continuous variable)
Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other

Table 5.72 (B):

```
. regr ch3hg lbxlh ridageyr race1 race2 race4 race5, ro

Regression with robust standard errors                Number of obs =    1132
                                                      F(  6,  1125) =    7.22
                                                      Prob > F      =  0.0000
                                                      R-squared     =  0.0350
                                                      Root MSE      =  2.4835

------------------------------------------------------------------------------
             |               Robust
       ch3hg |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       lbxlh |  -.0085914   .0031118    -2.76   0.006    -.0146969   -.0024858
    ridageyr |   .0266999   .0181733     1.47   0.142    -.0089575    .0623572
       race1 |  -.5135549   .1404104    -3.66   0.000    -.7890507   -.2380592
       race2 |   .3219232   .4711148     0.68   0.495    -.6024392    1.246286
       race4 |   .5608793    .221245     2.54   0.011       .12678    .9949786
       race5 |    1.38178   .5115431     2.70   0.007     .3780946    2.385466
       _cons |   .5471325   .7468238     0.73   0.464    -.9181918    2.012457
------------------------------------------------------------------------------
Ridageyr= age by year(continuous variable)
Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.
```

Survey Weighed Regression:

```
. svyreg ch3hg lbxlh race1 race2 race4 race5 ridageyr

Survey linear regression

pweight:  wtmec4yr                            Number of obs    =      1132
Strata:   sdmvstra                            Number of strata =        28
PSU:      sdmvpsu                             Number of PSUs   =        57
                                              Population size  =  29923099
                                              F(   6,     24)  =      4.94
                                              Prob > F         =    0.0020
                                              R-squared        =    0.0247

------------------------------------------------------------------------------
       ch3hg |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       lbxlh |  -.0108912   .0036663    -2.97   0.006    -.0183897   -.0033927
       race1 |  -.6052247   .2029188    -2.98   0.006     -1.02024   -.1902092
       race2 |    .343313    .788399     0.44   0.666    -1.269144     1.95577
       race4 |   .5046719   .2783463     1.81   0.080    -.0646102    1.073954
       race5 |   1.330827   .6130145     2.17   0.038     .0770721    2.584583
    ridageyr |     .01893   .0321906     0.59   0.561    -.0469071    .0847672
       _cons |   .9352752   1.268819     0.74   0.467    -1.659752    3.530302
------------------------------------------------------------------------------
Ridageyr= age by year(continuous variable)
Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.
```

Table 5.72 (C):

```
. regr ch3hg lbxwbc ridageyr  race1 race2 race4 race5, ro

Regression with robust standard errors                Number of obs =    3611
                                                      F(  6,  3604) =   29.31
                                                      Prob > F      =  0.0000
                                                      R-squared     =  0.0519
                                                      Root MSE      =  2.0292

------------------------------------------------------------------------------
             |               Robust
       ch3hg |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
    lbxwbcsi |  -.0521023   .0141182    -3.69   0.000    -.0797828   -.0244217
    ridageyr |   .0357987   .0033716    10.62   0.000     .0291882    .0424092
       race1 |  -.2784288   .0697395    -3.99   0.000    -.4151616   -.1416961
       race2 |   .2924682   .2009245     1.46   0.146    -.1014688    .6864052
       race4 |   .1994858   .1059372     1.88   0.060    -.0082171    .4071886
       race5 |   .6920375   .2263871     3.06   0.002      .248178    1.135897
       _cons |   .5222849   .1760035     2.97   0.003     .1772084    .8673613
------------------------------------------------------------------------------
Ridageyr= age by year(continuous variable)
Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.
```

Survey Weighted Regression:

```
. svyreg ch3hg lbxwbc race1 race2 race4 race5 ridageyr

Survey linear regression

pweight:  wtmec4yr                              Number of obs    =      3611
Strata:   sdmvstra                              Number of strata =        28
PSU:      sdmvpsu                               Number of PSUs   =        57
                                                Population size  =  65571779
                                                F(   6,     24)  =     10.65
                                                Prob > F         =    0.0000
                                                R-squared        =    0.0398

------------------------------------------------------------------------------
       ch3hg |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
    lbxwbcsi |  -.0648348   .0276852    -2.34   0.026    -.1214575   -.0082121
       race1 |  -.3514437   .1381687    -2.54   0.017    -.6340306   -.0688569
       race2 |   .2687287   .3777198     0.71   0.482     -.503795    1.041252
       race4 |   .2552534   .1904777     1.34   0.191    -.1343171     .644824
       race5 |   1.149271   .3585909     3.20   0.003     .4158703    1.882672
    ridageyr |   .0336289   .0057833     5.81   0.000     .0218007    .0454572
       _cons |   .6797867   .2997267     2.27   0.031     .0667767    1.292797
------------------------------------------------------------------------------
Ridageyr= age by year(continuous variable)
Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.
```

Table 5.73: Linear Regressions of Blood Organic Mercury (CH_3Hg) to Biochemical Profile Markers, in subpopulations of Women grouped by Age in Years, NHANES 1999-2000 & 2001-2002 Combined Population. (A) Ages 40-49 Years; CH_3Hg vs Bilirubin (lbstbsi) (B) Ages 40-49 Years; CH_3Hg vs. White Blood Cell Count (lbxwbc) (C) Ages 35-39; CH_3Hg vs Bilirubin (lbdstbsi) (D) Ages 30-39; CH_3Hg vs. White Blood Cells (lbxwbc). (E) Ages 30-39; CH_3Hg vs. Luteinizing Hormone (LH) (lbxlh). Adjusted for Age in years (ridageyr) as a continuous variable. Dummy variables for race are compared to the baseline, white population; race1=Mexican American, race2=Hispanic, race3=White, race4=African American, race5= Other.

(A) Ages 40-49 Years; CH_3Hg vs Bilirubin (lbstbsi)

```
regr ch3hg lbdstbsi ridageyr race1 race2 race4 race5, ro

Regression with robust standard errors                Number of obs =     749
                                                      F(  6,   742) =    4.00
                                                      Prob > F      =  0.0006
                                                      R-squared     =  0.0378
                                                      Root MSE      =  2.3737

------------------------------------------------------------------------------
             |               Robust
       ch3hg |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
    lbdstbsi |   .0336363   .0217365     1.55   0.122     -.009036    .0763087
    ridageyr |   .0431208   .0308483     1.40   0.163    -.0174395    .1036811
       race1 |  -.4031824   .1665817    -2.42   0.016      -.73021   -.0761547
       race2 |  -.3321979   .2180767    -1.52   0.128    -.7603186    .0959229
       race4 |   .6507238   .2979537     2.18   0.029     .0657912    1.235657
       race5 |   1.168833   .6060024     1.93   0.054    -.0208509    2.358516
       _cons |  -.6696384   1.431362    -0.47   0.640     -3.47964    2.140364
------------------------------------------------------------------------------
```

Ridageyr= age by year(continuous variable)
Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.

(B) Ages 40-49 Years; CH_3Hg vs. White Blood Cell Count (lbxwbc)

```
. regr ch3hg lbxwbc race1 race2 race4 race5, ro
Regression with robust standard errors                Number of obs =     765
                                                      F(  5,   759) =    5.57
                                                      Prob > F      =  0.0000
                                                      R-squared     =  0.0358
                                                      Root MSE      =  2.3672

------------------------------------------------------------------------------
             |               Robust
       ch3hg |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
    lbxwbcsi |  -.0648807   .0431991    -1.50   0.134    -.1496846    .0199231
       race1 |  -.4284151   .1659874    -2.58   0.010    -.7542641   -.1025662
       race2 |  -.2735482   .2156087    -1.27   0.205    -.6968083     .149712
       race4 |    .559665   .2917654     1.92   0.055    -.0130981    1.132428
       race5 |   1.21452   .5871536     2.07   0.039     .0618823    2.367158
       _cons |   2.02657   .3513744     5.77   0.000     1.336789    2.716351
------------------------------------------------------------------------------
```

Ridageyr= age by year(continuous variable)
Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.

Table 5.73 (C): Ages 30-39; CH_3Hg vs Bilirubin (lbdstbsi)

```
. regr ch3hg  lbdstbsi race1  race2 race4 race5, ro

Regression with robust standard errors                Number of obs =     847
                                                      F(  5,   841) =    8.60
                                                      Prob > F      =  0.0000
                                                      R-squared     =  0.0381
                                                      Root MSE      =  2.5801

------------------------------------------------------------------------------
             |               Robust
       ch3hg |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
    lbdstbsi |   .0836107   .0264533     3.16   0.002     .0316884    .1355329
       race1 |  -.6452009   .1596354    -4.04   0.000    -.9585315   -.3318703
       race2 |   .5378101   .5665725     0.95   0.343     -.574252    1.649872
       race4 |    .405491   .2375085     1.71   0.088    -.0606879    .8716699
       race5 |   .5536321   .4558753     1.21   0.225    -.3411548    1.448419
       _cons |   .8431566   .2243168     3.76   0.000     .4028702    1.283443
------------------------------------------------------------------------------
```

Ridageyr= age by year(continuous variable)
Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.

Survey Weighted Regression:

```
. svyreg ch3hg lbdstbsi race1 race2 race4 race5 ridageyr

Survey linear regression

pweight:  wtmec4yr                            Number of obs    =       847
Strata:   sdmvstra                            Number of strata =        28
PSU:      sdmvpsu                             Number of PSUs   =        57
                                              Population size  =  19914496
                                              F(   6,     24)  =      5.87
                                              Prob > F         =    0.0007
                                              R-squared        =    0.0423

------------------------------------------------------------------------------
       ch3hg |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
    lbdstbsi |   .1143499   .0259214     4.41   0.000     .0613347    .1673651
       race1 |  -.6280074   .2281058    -2.75   0.010    -1.094536   -.1614786
       race2 |    .872882   .8786158     0.99   0.329    -.9240891    2.669853
       race4 |   .3136023   .3039881     1.03   0.311    -.3081232    .9353278
       race5 |   .6200551   .6932843     0.89   0.378    -.7978705    2.037981
    ridageyr |   .0235316   .0310718     0.76   0.455    -.0400173    .0870806
       _cons |  -.2746783   1.154181    -0.24   0.814    -2.635243    2.085886
------------------------------------------------------------------------------
```

Ridageyr= age by year(continuous variable)
Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.

Table 5.73 (D): Ages 30-39; CH_3Hg vs. White Blood Cells (lbxwbc).

```
. regr ch3hg  lbxwbc race1  race2 race4 race5, ro

Regression with robust standard errors                Number of obs =     855
                                                      F(  5,   849) =    8.62
                                                      Prob > F      =  0.0000
                                                      R-squared     =  0.0270
                                                      Root MSE      =  2.6123

------------------------------------------------------------------------------
             |               Robust
       ch3hg |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
    lbxwbcsi |  -.0723845    .034076    -2.12   0.034    -.1392676   -.0055014
       race1 |  -.6988591   .1641941    -4.26   0.000    -1.021133   -.3765851
       race2 |   .5749902   .5620753     1.02   0.307    -.5282298     1.67821
       race4 |   .2581545   .2548571     1.01   0.311    -.2420693    .7583782
       race5 |   .5429921   .4732027     1.15   0.252    -.3857922    1.471776
       _cons |   2.155493   .3399598     6.34   0.000     1.488233    2.822754
------------------------------------------------------------------------------
```

Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.

Table 5.73 (E): Ages 35-39; CH_3Hg vs. Luteinizing Hormone Hormone (LH) (lbxlh).

```
. regr ch3hg  lbxlh ridageyr race1  race2 race4 race5, ro

Regression with robust standard errors                Number of obs =     402
                                                      F(  6,   395) =    3.90
                                                      Prob > F      =  0.0009
                                                      R-squared     =  0.0517
                                                      Root MSE      =  2.6266

------------------------------------------------------------------------------
             |               Robust
       ch3hg |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       lbxlh |  -.0135734   .0054831    -2.48   0.014    -.0243531   -.0027937
    ridageyr |   .1148142    .090477     1.27   0.205    -.0630625    .2926908
       race1 |  -.5843171   .2416931    -2.42   0.016    -1.059483   -.1091514
       race2 |    1.18767   1.032845     1.15   0.251    -.8428913     3.21823
       race4 |    .484276   .3134363     1.55   0.123     -.131936    1.100488
       race5 |   1.983898   .9693392     2.05   0.041     .0781892    3.889607
       _cons |   -2.64772   3.252748    -0.81   0.416    -9.042583    3.747143
------------------------------------------------------------------------------
```

Ridageyr= age by year(continuous variable)
Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.

Table 5.73 (E): Ages 35-39

Survey Weighted Regression:

```
svyreg ch3hg lbxlh race1 race2 race4 race5 ridageyr

Survey linear regression

pweight:  wtmec4yr                                Number of obs    =       402
Strata:   sdmvstra                                Number of strata =        28
PSU:      sdmvpsu                                 Number of PSUs   =        57
                                                  Population size  =  10138006
                                                  F(   6,     24)  =      2.27
                                                  Prob > F         =    0.0705
                                                  R-squared        =    0.0590

------------------------------------------------------------------------------
       ch3hg |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       lbxlh |  -.0210409   .0075129    -2.80   0.009    -.0364064   -.0056753
       race1 |  -.6864714   .3147743    -2.18   0.037    -1.330257   -.0426857
       race2 |   1.608213    1.49481     1.08   0.291    -1.449016    4.665442
       race4 |   .4258422   .4332328     0.98   0.334    -.4602183    1.311903
       race5 |   1.972353   1.334098     1.48   0.150    -.7561838    4.700891
    ridageyr |   .1712217   .1470553     1.16   0.254    -.1295403    .4719836
       _cons |   -4.64519   5.214143    -0.89   0.380    -15.30931    6.018929
------------------------------------------------------------------------------
```

Ridageyr= age by year(continuous variable)
Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.

Table 5.91: Urinary Mercury and Bilirubin

Analysis	Model Type	Correlation	Slope	Std. Error	P- Value
Linear Regression	Survey	Wtmec4yr	0.26	0.010	0.017

```
regr urxuhg  lbdstbsi race1 race2 race4 race5 ridageyr, ro
Regression with robust standard errors                Number of obs =    3490
                                                      F(  6,  3483) =    6.66
                                                      Prob > F      =  0.0000
                                                      R-squared     =  0.0108
                                                      Root MSE      =  2.6346
------------------------------------------------------------------------------
             |               Robust
      urxuhg |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
    lbdstbsi |   .0251078   .0095258     2.64   0.008      .006431    .0437845
       race1 |   .3403985   .1078064     3.16   0.002     .1290285    .5517685
       race2 |    .427708   .1519776     2.81   0.005     .1297337    .7256822
       race4 |   .6191392   .1364855     4.54   0.000     .3515396    .8867389
       race5 |    .164533   .1753975     0.94   0.348    -.1793592    .5084252
    ridageyr |   .0120985   .0045938     2.63   0.008     .0030916    .0211053
       _cons |   .5700113   .1501655     3.80   0.000       .27559    .8644325
------------------------------------------------------------------------------
Ridageyr= age by year(continuous variable)
Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.

Survey Weighted Population: svyreg urxuhg  lbdstbsi race1 race2 race4 race5
ridageyr
Survey linear regression
pweight:  wtmec4yr                              Number of obs    =      3490
Strata:   sdmvstra                              Number of strata =        28
PSU:      sdmvpsu                               Number of PSUs   =        57
                                                Population size  =  63099012
                                                F(   6,     24)  =      4.22
                                                Prob > F         =    0.0049
                                                R-squared        =    0.0165
------------------------------------------------------------------------------
      urxuhg |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
    lbdstbsi |    .025844     .01025     2.52   0.017     .0048803    .0468076
       race1 |    .325975   .1020661     3.19   0.003     .1172263    .5347237
       race2 |   .3833382   .1894089     2.02   0.052    -.0040466    .7707229
       race4 |   .7141062   .1949674     3.66   0.001     .3153531    1.112859
       race5 |    .125043   .1474088     0.85   0.403    -.1764419    .4265279
    ridageyr |   .0078998   .0033859     2.33   0.027      .000975    .0148247
       _cons |   .6516055   .1483139     4.39   0.000     .3482695    .9549415
------------------------------------------------------------------------------
Ridageyr= age by year(continuous variable)
Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.
```

Table 5.92: Race Effects and Mercury Forms: The reported Odds Ratio (O.R.) or slope is relative to the baseline, white population.

Mercury Form	Race	Analysis	Model Type	Correlation	Odds Ratio (OR)	Std. Error	P- Value
I-Hg Detection	Black	Logistic	Adjusted, Raw	Robust	1.9	0.393	0.002
I-Hg Detection	Black	Logistic	Survey Weighted	wtmec4yr	2	0.534	0.011

Raw Population, Age as a continuous variable:

```
logistic ihgdetect  race1 race2 race4 race5 ridageyr, ro
Logistic regression                               Number of obs   =       3616
                                                  Wald chi2(5)    =      21.78
                                                  Prob > chi2     =     0.0006
Log pseudo-likelihood = -669.72823                Pseudo R2       =     0.0146
------------------------------------------------------------------------------
             |               Robust
   ihgdetect | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       race1 |    1.50747   .3026857     2.04   0.041     1.017034    2.234405
       race2 |   1.340228   .4760842     0.82   0.410     .6680507    2.688734
       race4 |   1.902568   .3932167     3.11   0.002     1.268868     2.85275
       race5 |    1.06084   .5084355     0.12   0.902     .4146572    2.714005
    ridageyr |   1.025318   .0073151     3.50   0.000      1.01108    1.039756
------------------------------------------------------------------------------
Ridageyr= age by year(continuous variable)
```

Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.

Raw Population, Age as a categorical variable:

```
. logistic ihgdetect  race1 race2 race4 race5  age1  age2 age4, ro

Logistic regression                               Number of obs   =       3616
                                                  Wald chi2(7)    =      21.56
                                                  Prob > chi2     =     0.0030
Log pseudo-likelihood = -669.20221                Pseudo R2       =     0.0154
------------------------------------------------------------------------------
             |               Robust
   ihgdetect | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       race1 |   1.525309   .3106727     2.07   0.038     1.023261     2.27368
       race2 |   1.344033   .4770771     0.83   0.405     .6702985    2.694957
       race4 |   1.911501   .3991809     3.10   0.002     1.269454    2.878272
       race5 |   1.054577   .5055032     0.11   0.912     .4121562     2.69833
        age1 |   .5663149   .1291213    -2.49   0.013     .3622277    .8853892
        age2 |   .6852332   .1542384    -1.68   0.093     .4408008    1.065208
        age4 |   1.121734   .2381172     0.54   0.588      .739946    1.700511
------------------------------------------------------------------------------
```

Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.
Age1=16-19 years, Age2=20-29 years, Age3=30-39 years, Age4=40-49

Table 5.92: (continued):

Survey Weighted Population, age as a continuous variable:

```
 svylogit ihgdetect  race1 race2 race4 race5 ridageyr, eform

Survey logistic regression

pweight:  wtmec4yr                                Number of obs    =      3616
Strata:   sdmvstra                                Number of strata =        28
PSU:      sdmvpsu                                 Number of PSUs   =        57
                                                  Population size  =  65642103
                                                  F(   5,     25)  =      2.84
                                                  Prob > F         =    0.0367

------------------------------------------------------------------------------
   ihgdetect |     exp(b)   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       race1 |   1.549632   .3936259     1.72   0.095     .9217359    2.605257
       race2 |   1.547931   .5331532     1.27   0.215     .7652765    3.131014
       race4 |   2.034373   .5361414     2.69   0.012      1.18671    3.487518
       race5 |    1.03489   .5475319     0.06   0.949     .3507158    3.053749
    ridageyr |   1.025682   .0109394     2.38   0.024      1.00355    1.048301
------------------------------------------------------------------------------
```

*Survey weighted population: Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.

ridageyr= age in years as a continuous variable.

Survey Weighted Population, age as a categorical variable:

```
. svylogit ihgdetect  race1 race2 race4 race5  age1 age2 age4, eform

Survey logistic regression

pweight:  wtmec4yr                                Number of obs    =      3616
Strata:   sdmvstra                                Number of strata =        28
PSU:      sdmvpsu                                 Number of PSUs   =        57
                                                  Population size  =  65642103
                                                  F(   7,     23)  =      3.71
                                                  Prob > F         =    0.0078

------------------------------------------------------------------------------
   ihgdetect |     exp(b)   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       race1 |   1.575972    .400624     1.79   0.084     .9370297    2.650598
       race2 |   1.560918   .5324564     1.31   0.202     .7769422    3.135967
       race4 |   2.033236   .5315736     2.71   0.011      1.19115    3.470636
       race5 |   1.033539   .5386299     0.06   0.950     .3559783     3.00075
        age1 |   .4887826   .1583454    -2.21   0.035     .2519795    .9481262
        age2 |   .6262808   .2146998    -1.37   0.183     .3106475    1.262613
        age4 |   1.145636   .2995942     0.52   0.607     .6710672    1.955813
------------------------------------------------------------------------------
```

Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.

Age1=16-19 years, Age2=20-29 years, Age3=30-39 years, Age4=40-49 years

Table 5.92 (Continued): Race Effects and Mercury Forms: The reported Odds Ratio (O.R.) or slope is relative to the baseline, white population.

Mercury Form	Race	Analysis	Model Type	Correlation	Slope	Std. Error	P- Value
Urinary Mercury	Black	Linear Regression	Adjusted, Raw	Robust	0.6	0.14	<0.001
Urinary Mercury	Black	Linear Regression	Survey Weighted	wtmec4yr	0.7	0.194	<0.001
Urinary Mercury	Mexican	Linear Regression	Adjusted, Raw	Robust	0.35	0.11	<0.001
Urinary Mercury	Mexican	Linear Regression	Survey Weighted	wtmec4yr	0.33	0.10	0.002

Survey Weighted Population:

```
svyreg urxuhg race1 race2 race4 race5 ridageyr

Survey linear regression

pweight:  wtmec4yr                              Number of obs    =      3531
Strata:   sdmvstra                              Number of strata =        28
PSU:      sdmvpsu                               Number of PSUs   =        57
                                                Population size  =  63947268
                                                F(   5,     25)  =      3.34
                                                Prob > F         =    0.0191
                                                R-squared        =    0.0143

------------------------------------------------------------------------------
      urxuhg |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       race1 |   .3245899   .1006417     3.23   0.003     .1187545    .5304253
       race2 |   .3872116    .181142     2.14   0.041     .0167347    .7576885
       race4 |   .6961877   .1932283     3.60   0.001     .3009914    1.091384
       race5 |   .1161961   .1491957     0.78   0.442    -.1889434    .4213356
    ridageyr |   .0078836   .0033209     2.37   0.024     .0010916    .0146756
       _cons |   .8795611    .125314     7.02   0.000     .6232653    1.135857
------------------------------------------------------------------------------
```

Ridageyr= age by year (continuous variable)

Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other (categorical variables)

Table 5.93: Age Effect and I-Hg Detection.
(No reported Slopes contain 0 and no reported O.R.'s contain 1 in their confidence intervals.)

Mercury Form	Variable	Analysis	Model Type	Correlation	Odds Ratio (OR)	Std. Error	P- Value
I-Hg Detection	Age (year)	Logistic	Adjusted, Raw	Robust	1.03	0.007	<0.001
I-Hg Detection	Age (year)	Logistic	Survey Weighted	wtmec4yr	1.03	0.01	0.024

Survey Weighted Population, Age as a continuous variable:

```
. svylogit ihgdetect  race1 race2 race4 race5 ridageyr, eform
Survey logistic regression
pweight:  wtmec4yr                                Number of obs    =      3616
Strata:   sdmvstra                                Number of strata =        28
PSU:      sdmvpsu                                 Number of PSUs   =        57
                                                  Population size  =  65642103
                                                  F(   5,     25)  =      2.84
                                                  Prob > F         =    0.0367
------------------------------------------------------------------------------
   ihgdetect |     exp(b)   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       race1 |   1.549632   .3936259     1.72   0.095     .9217359    2.605257
       race2 |   1.547931   .5331532     1.27   0.215     .7652765    3.131014
       race4 |   2.034373   .5361414     2.69   0.012      1.18671    3.487518
       race5 |    1.03489   .5475319     0.06   0.949     .3507158    3.053749
    ridageyr |   1.025682   .0109394     2.38   0.024      1.00355    1.048301
------------------------------------------------------------------------------
```

Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.
ridageyr= age in years as a continuous variable.

Survey Weighted Population, Age as a categorical variable:

```
. svylogit ihgdetect  race1 race2 race4 race5  age1 age2 age4, eform
Survey logistic regression
pweight:  wtmec4yr                                Number of obs    =      3616
Strata:   sdmvstra                                Number of strata =        28
PSU:      sdmvpsu                                 Number of PSUs   =        57
                                                  Population size  =  65642103
                                                  F(   7,     23)  =      3.71
                                                  Prob > F         =    0.0078
------------------------------------------------------------------------------
   ihgdetect |     exp(b)   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       race1 |   1.575972    .400624     1.79   0.084     .9370297    2.650598
       race2 |   1.560918   .5324564     1.31   0.202     .7769422    3.135967
       race4 |   2.033236   .5315736     2.71   0.011      1.19115    3.470636
       race5 |   1.033539   .5386299     0.06   0.950     .3559783     3.00075
        age1 |   .4887826   .1583454    -2.21   0.035     .2519795    .9481262
        age2 |   .6262808   .2146998    -1.37   0.183     .3106475    1.262613
        age4 |   1.145636   .2995942     0.52   0.607     .6710672    1.955813
------------------------------------------------------------------------------
```

Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.Age1=16-19 years, Age2=20-29 years, Age3=30-39 years, Age4=40-49 years

Table 5.94: Age Effect and CH_3Hg

Mercury Form	Variable	Analysis	Model Type	Correlation	Slope	Std. Error	P- Value
Organic Mercury	Age (year)	Linear Regression	Adjusted, Raw	Robust	0.37	0.003	<0.001
Organic Mercury	Age (year)	Linear Regression	Survey Weighted	wtmec4yr	0.35	0.006	<0.001

```
. regr ch3hg  race1 race2 race4 race5 ridageyr, ro

Regression with robust standard errors                Number of obs =    3613
                                                      F(  5,  3607) =   31.96
                                                      Prob > F      =  0.0000
                                                      R-squared     =  0.0487
                                                      Root MSE      =  2.0318

------------------------------------------------------------------------------
             |               Robust
       ch3hg |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       race1 |  -.2727954   .0696737    -3.92   0.000    -.4093993   -.1361916
       race2 |    .279263   .2004799     1.39   0.164    -.1138023    .6723282
       race4 |    .260343   .1009071     2.58   0.010     .0625023    .4581837
       race5 |   .6875855   .2276118     3.02   0.003     .2413248    1.133846
    ridageyr |   .0368248   .0033456    11.01   0.000     .0302655    .0433842
       _cons |   .0717908   .1063471     0.68   0.500    -.1367157    .2802973
------------------------------------------------------------------------------
```

*Raw population: Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other. ridageyr= age in years as a continuous variable.

Survey Weighted Population, Age as a continuous variable:

```
. svyreg ch3hg  race1 race2 race4 race5 ridageyr
Survey linear regression
pweight:  wtmec4yr                              Number of obs    =      3613
Strata:   sdmvstra                              Number of strata =        28
PSU:      sdmvpsu                               Number of PSUs   =        57
                                                Population size  =  65606783
                                                F(   5,     25)  =     10.56
                                                Prob > F         =    0.0000
                                                R-squared        =    0.0363
------------------------------------------------------------------------------
       ch3hg |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       race1 |  -.3575219   .1427765    -2.50   0.018    -.6495327   -.0655111
       race2 |   .2340007   .3941081     0.59   0.557    -.5720409    1.040042
       race4 |   .3088219   .1822552     1.69   0.101    -.0639318    .6815757
       race5 |   1.152467   .3608042     3.19   0.003     .4145401    1.890395
    ridageyr |   .0349455    .005872     5.95   0.000     .0229359    .0469551
       _cons |   .1385998   .1628108     0.85   0.402    -.1943857    .4715853
------------------------------------------------------------------------------
```

Race1=Mexican American, Race2=Hispanic, Race 3=White, Race 4= African American, Race5=Other.
ridageyr= age in years as a continuous variable.

6. Policy Review

U.S. and global policy regarding chronic mercury exposure suffers from political and scientific uncertainty. In order to create effective public health guidelines regarding chronic mercury exposure, a suitable method for assessment of chronic mercury exposure is needed to establish links with associated disease risks.

On March 15th, 2005, the Environmental Protection Agency (E.P.A.) approved the deceptively titled, "Clear Skies" initiative. The "Clear Skies" Act, regarding the regulation of mercury emissions from power plants, was first put to Congress in July 29, 2002 by Republican Senator Robert Smith, from New Hampshire. This bill was designed to revise the more stringent regulatory plans of the "Clean Air" Act put in place by the Clinton administration. Analysis of this policy to deregulate mercury emissions reveals a repeated trend: conflict of interest, corporate politics, and negligence in regards to safeguarding public health.

The government policy surrounding the health risks posed by mercury exposure is defined by the many industries that profit from its sale, application, and emissions. The NRDC, founded in 1970, claims to be the first public interest law firm to work on national environmental issues. To gain access to the "energy task force", the NRDC sued the Department of Energy(D.O.E.) and pursued litigation until some "energy task force" papers were released. Even then the D.O.E. violated the freedom of information act, illegally withheld documents with no legal justification and censored public documents in order to conceal details of the new energy plan. From those released documents, details revealed how energy companies had authored their own regulations and proposed revisions on previous EPA rulings.

Industries that profit from the manufacture and sale of the mercury commodity exerted immense power and influence on rewriting the policy towards deregulation. A thorough and documented conflict of interest between industry and government policy remains at the source of poor mercury regulations. The NRDC notes that the Bush administration deceptively claims that the pollution trading rule makes the United States "the first country in the world to regulate mercury emissions from utilities." As noted above, the rule does not directly reduce mercury pollution until 2018. This rhetorical argument diverts attention away from the fact that the administration threw out the Clean Air Act's requirement that power plants make deep cuts in their mercury emissions over the next three years, substituting it with a scheme that delays any mercury reductions for at least 13 years. Indeed, for the United States to be first in any meaningful sense, other countries must refrain from regulating the toxin for the next 13 years. For now, the NRDC is still considering what future actions to take. From the D.O.E. documents attained by the NRDC, there is ample evidence to support a case of a conflict of interest between industry, policy and the public health. According to NRDC communication director, Craig Noble, the new EPA rule was made behind closed doors with industry while the conservation community had no seat at the table.

EPA and Bush administration officials stressed that they could not require tighter controls on mercury pollution because the cost to industry was much higher than the benefits to public health. They did not disclose that an EPA-funded, peer-reviewed Harvard University study concluded just the opposite. The Harvard study estimated health benefits 100 times as great as the EPA did, and, according to the Washington Post, top EPA officials deleted any mention of the analysis from public documents. The Harvard analysis—and a recent study by the Mount Sinai School of Medicine—both show that

more stringent controls on power plant mercury pollution are necessary to protect public health. (See http://www.washingtonpost.com/wp-dyn/articles/A55268-2005Mar21.html.)

The threat of rising mercury levels was clearly reviewed and outlined by government scientists and made available to regulatory officials. In 2000, the National Research Council published "The Toxicological Effects of Methylmercury"[11]. This comprehensive report on mercury hazards clearly detailed the growing health threat from utility emissions of mercury. The Bush administration edited this scientific document to downplay the health risks of mercury exposure.

"This is a pattern of undermining and disregarding science on political considerations," said Senator Hillary Rodham Clinton, citing a letter by the Union of Concerned Scientists, signed by 60 scientists, including 20 nobel laureates, which criticized the Bush administration's handling of science issues (New York Times, April 7, 2004). In July 2001, one third of congress wrote a letter to the President, urging him not to revise the original EPA plans for immediate regulation of mercury by "maximum achievable control technology."

When EPA originally proposed these D.O.E., "energy task force" rules to deregulate industry in December 2003, the proposal contained whole paragraphs taken directly from memos provided to the agency by Latham & Watkins, a law and lobbying firm that represents large coal-fired utilities. An enormous public outcry followed release of the proposal. Forty-five U.S. senators sent a letter to then-EPA Administrator Mike Leavitt, urging him "to take prompt and effective action to clean up mercury pollution from power plants," and noted that EPA's "current proposals … fall far short of what the law requires, and … fail to protect the health of our children and our environment." One-hundred-eighty U.S. representatives also publicly opposed the proposal.The attorneys general of New Jersey, California, Connecticut, Maine, Massachusetts, New Hampshire, New York, Vermont and Wisconsin, the chief counsel of the Pennsylvania Department of Environmental Protection, and the New Mexico environment secretary condemned the rules. The association of state and local air protection officials and NESCAUM likewise denounced the proposal.

In Spring 2004, attorney generals from ten states and 45 senators asked the E.P.A. to scrap the new "Clear Skies" proposal, saying it was not strict enough. But instead, the Bush administration went ahead and set forth the new proposal to delay any mercury restrictions until 2018. The ruling on March 15, 2005 that ratified the Bush proposal effectively revised the scientific assessment of the serious health risks posed by mercury exposure. The new proposal that passed contained an act to revise previous EPA regulatory findings that it was "appropriate and necessary" to regulate mercury emissions. Now apparently, it is not. This revision was originally suggested to the energy task force by a Southern Company lobbyist (source: NRDC).

In 1999, concern was expressed over the safety of thimerosal containing vaccines by the American Academy of Pediatrics and the U.S. Public Health Service [29]. Within 18 months, mercury preservative was purportedly removed from vaccines destined for use in the U.S.. This policy restriction did not last and was never put into full effect. In fact, the World Health Organization (WHO) "continues to recommend the use of vaccines containing thiomersal for global immunization programs since the benefits of using such products far outweigh any theoretical risk of toxicity" [107]. The Institute of Medicine (I.O.M.) recommended that more studies were necessary to determine what role thimerosal may play in autism.

At a global level, the Bush administration recently blocked international efforts to limit mercury pollution and trade at a United Nations Environmental Program (UNEP) conference in Nairobi (www.nrdc.org/media/pressreleases/050225a.asp.). While world mercury production is rising and chronic mercury exposure may be affecting the health of everyone on the planet, government agencies regulate the many sources of mercury with ambivalence and contradictions. On one hand, the National Research Council published a report on the growing risks of mercury exposure. On the other hand, the Energy Task Force dismantles the regulatory actions scheduled by the Clean Air act. On one hand, the EPA got rid of mercury in thimerosal vaccines throughout America in a rapid response to health concerns. On the other hand vaccines still contain mercury and the World Health Organization (WHO) claims that the benefits outweigh the risks for thimerosal vaccines in developing countries. On one hand, the EPA has lowered the acceptable level of mercury exposure and advises pregnant mothers against eating more then three fish a month because of high mercury levels. On the other hand, background levels of mercury are rising and human exposure from the medical establishment is still largely unrestricted regarding vaccines and dental amalgams.

7. Conclusion

At this time, a scientific consensus suggests that the global rate of atmospheric mercury deposition may be increasing over time[1]. In addition, the latest studies suggest that as global mercury deposition increases, the incidence of the most closely associated neurodegenerative diseases such as Autism and Alzheimer's Disease are rising as well [2, 3]. Recent studies suggest that the incidence of Autism and Alzheimer's Disease may be rising in heavily industrialized countries around the world, in the same regions where unprecedented mercury levels have recently been found in women and children, fish and animals, rice and soil [4]. These trends support the theory that both chronic mercury exposure and associated risks of neurodegenerative disease may rise over time within the general U.S. population.

Due to the bioaccumulation of organic mercury in food sources, atmospheric deposition of mercury vapor ultimately deposits mercury in the human pituitary, liver, immune system, adrenals, and kidney. The accumulation of targeted mercury deposition may disrupt the endocrine and immune systems, damage the delicate balance between inflammation and suppression, and elevate risks of neurodegenerative disease. As emissions of mercury into the atmosphere increase on a global scale, it is logical to assume that the rate of chronic mercury exposure and deposition in target areas of the human body will increase as well.

Our analysis of chronic mercury exposure trials tested a hypothesis that a suitable method for assessment of chronic mercury exposure would observe the change of I-Hg that results from CH_3Hg exposure, demethylation, and deposition. The regression of I-Hg to CH_3Hg was effective at distinguishing different exposure groups in a trial of chronic mercury exposure. This is significant as it provides a method for assessment of chronic, organic mercury exposure by the rate of inorganic mercury deposition.

From the NHANES study, data are sufficient to conclude there is an association between chronic, organic mercury exposure and inorganic mercury deposition in target systems of the human body. This study concludes that blood I-Hg and methyl mercury levels should both be accurately measured in order to serve as bioindicators for the assessment of chronic mercury exposure. Our analysis of the NHANES population reports an association between organic and inorganic mercury levels in the blood. This is consistent with other studies that demonstrate demethylation of organic mercury as a contributing source of I-Hg deposition within the body. Our study reports evidence that chronic mercury exposure and resultant deposition are associated with changes in biochemical markers for the liver, immune system, and pituitary. Luteinizing hormone, white blood cell count, and bilirubin levels are all biomarkers associated with chronic, organic mercury exposure. This is significant as it demonstrates for the first time within the U.S. population, that chronic, organic mercury exposure is associated with targets of inorganic mercury deposition. Blood I-Hg was the most accurate bioindicator to characterize susceoptible subpopulations, women ages 35-39 years, and African American women ages 16-49 years. The method presented here for the assessment of chronic mercury exposure should be further tested as a method to define subpoplations most susceptible to further mercury exposure.

Biological Mechanism for Mercury Exposure and the Risks of Neurodegenerative Disease

The 2004, IOM report on vaccines gave an assessment of a biological mechanism regarding vaccines and Autism and concluded that there was no human evidence of an immune response that associates thimerosal exposure with autism [110]. Results from this thesis report provide human evidence, within the U.S. population, of a biological mechanism and a causative relationship between mercury exposure, immune dysregulation, and the risk of Autism.

Reported, direct associations between I-Hg and organic mercury suggest that demethylation of organic mercury within the body is a contributing source of I-Hg deposition. The process of I-Hg deposition occurs in targets of the endocrine and immune system and may lead to an elevated risk of neurodegeneration. As Webster et al. discuss in their review, "disturbances at any level of the hypothalamic-pituitary-adrenal axis or glucocorticoid action lead to an imbalance of this system and enhanced susceptibility to infection and inflammatory or autoimmune disease[60]". I-Hg deposition in white blood cells would produce a similar effect on the immune system, with resulting immune imbalance leading to increased risks of autoimmune disease.

Associations with the liver (bilirubin) reiterate concern that mercury deposition may increase enterohepatic circulation, raise the absorption rate of mercury, and thereby elevate susceptibility to future exposure such as from vaccines containing ethyl mercury. As the rate of mercury deposition accelerates with exposure, so do the risks of disease. I-Hg deposits accumulate over years in targets of the immune and endocrine system. Infants are particularly susceptible to exposure as they have no microflora in their GI tract to help eliminate mercury. During gestation, a hereditary burden of exposure would include inheritance of mercury exposure from the mother's mercury burden. After birth, exposure would include diet, mother's milk, and a regimen of vaccines. Geographic clustering of direct exposure from point source plumes is another possible influence on the rate of deposition.

According to the biological mechanism presented here, the subpopulation most susceptible to mercury exposure and the risks of disease would be characterized by quantifying the rate of I-Hg deposition. Proper assessment of chronic mercury exposure and neurodegenerative disease would consist of the most accurate measurement of blood I-Hg to serve as bioindicator for chronic mercury exposure and targeted deposition. The sum of all thimerosal-containing vaccines would then predict the relative risks of a disease response for each subpopulation characterized by I-Hg deposition.

In 2004, the IOM concluded that toxicological data may support a biological mechanism of causation, that there may be a genetically susceptible subpopulation to mercury exposure, and that there is evidence of immune dysregulation in the serum of autistic patients[110]. Results from our NHANES analysis present evidence that African Americans face the highest risk of I-Hg deposition and associated effect on the pituitary (LH). Due to increased risk of chornic mercury exposure and targeted I-Hg deposition in the endocrine system, the African American subpopulation may face elevated risks of associated neurodegenerative disease. Indeed, several epidemiological studies have found a higher prevalence of Autism in African American children than in white children [127, 128].

Associations of chronic mercury exposure with the immune system (white blood cell) and pituitary (luteinizing hormone) within the general U.S. population establish links with mercury deposition, Autism and Alzheimer's Disease. Deposition in target areas may decrease the amount of future exposure or acute dose (e.g. vaccines) needed to surpass a threshold concentration and trigger mercury's targeted, neurotoxic effect. Once the critical threshold concentration is reached, simultaneous neurotoxic events may cause focal damage in the pituitary, adrenals, immune system, and liver and induce a cascade of inflammation, autoimmune responses, neurodegeneration, and disease.

Policy recommendations

- Limit all sources of mercury exposure.
- Reduce dietary intake of contaminated fish.
- Monitor and measure mercury content in food and diet.
- Remove mercury from vaccines and dental amalgams.
- Regulate coal burning power plant emissions to limit mercury emissions into the atmosphere.
- Restrict mining of mercury.
- Chronic mercury exposure should be measured with the method for assessment of chronic mercury exposure presented in this paper.
- Chronic mercury exposure should be monitored in the general population and within susceptible populations such as the elderly, expectant mothers, and newborn infants.
- Continued research in the fields of mercury speciation, detection and elimination therapy should be developed.

Future Research

- Future research should adopt the method for assessment of mercury exposure presented in this study that relies on both blood I-Hg and methylmercury as bioindicators of chronic mercury exposure.
- Bioindicators for chronic mercury exposure within the U.S. population should be monitored to assess risks of disease.
- Accurate methods of inorganic and dimethyl mercury detection should be developed.
- A causative role for chronic mercury exposure and neurodegenerative disease may be linked through impairment of the pituitary, and secretion of Luteinizing Hormone. Investigate a causative role for mercury deposition and the process from LH disruption to Alzheimer's Disease and Autism.
- Investigate the cellular mechanism for the demethylation of organic mercury in mammalian tissue. This mechanism is unknown and may be an important area for future research in molecular biology.
- Study the effects of liver function, the role of bilirubin, gastric motility, and the role of variable flora populations on the rate of mercury elimination.
- Perform clinical research on mercury elimination and chelation therapy to apply towards susceptible populations such as pregnant mothers, children, and elderly populations.
- Present and Test the following hypothesis:

Hypothesis 1: In this biological mechanism, chronic, organic mercury exposure is linked to elevated risks of neurodegenerative disease, specifically types of Autism, and Alzheimer's Disease. Elevated risks of neurodegenerative disease may be due to immune and endocrine disruption caused by targeted I-Hg deposition in the liver, pituitary, and white blood cells. A process of focal I-Hg accumulation may lead to elevated risks for mercury's neurotoxic effect. Once this neurotoxic concentration is surpassed, resultant endocrine and immune system impairment may instigate a cascade of neuroinflammatory reactions, autoimmune disorders, impaired cell migration and neural development, neurodegeneration, and associated disease.

Hypothesis 2: Accurate assessment of blood I-Hg concentration will define the subpopulation most susceptible to cumulative mercury exposure (vaccines, diet, amalgams) and at highest risk of associated neurodegenerative disease (Autism and Alzheimer's Disease).

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