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XIAOBEI ZHU

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A LONGITUDINAL STUDY OF THE VARIABILITY AND PREDICTORS OF URINARY MERCURY LEVELS IN 6 - 11 YEARS OLD CHILDREN

By

Xiaobei Zhu

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ABSTRACT

A LONGITUDINAL STUDY OF THE VARIABILITY AND PREDICTORS OF URINARY MERCURY LEVELS IN 6 - 11 YEARS OLD CHILDREN

By

Xiaobei Zhu

To exam the variability of urine mercury levels among children in three repeated measurements collected from three different areas in Germany during 1994-1997. Factors that affect urinary mercury levels among those children were also identified. Linear mixed model and Structure equation models were used to analyze the data. The Spearman correlation coefficients of the urinary mercury levels for the three mercury measurements were low, ranging from 0.212 to 0.307. In the present study, the intra-individual variation attributed more (approximately 72% - 74%) to the total variation compared with the inter-individual variation (approximately 26% - 28%) (ICC ranged from 0.26 to 0.28). The number of amalgam fillings had a significant effect on urinary mercury levels ($p<0.001$) controlling for other covariates. Residing in the toxic waste incinerator area posed a risk for increasing the urinary mercury level only in 1996, but seems to have a diminishing effect in 1997 from the results of both linear mixed model and SEMs. Age, the 24-hour total amount of urine, and urinary creatinine levels also have significant effect on urinary mercury levels.

DEDICATION

This work is dedicated to my beloved parents Shuyu Shi and Baofeng Zhu, my husband Yang Liang and my brother Yingnan Zhu.

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LIST OF ABBREVIATIONS

TWI Toxic Waste Incinerator group

RVC Rhine Valley Control group

OWC Odenwald Control group

ICC Intra-class correlation coefficient

CHAPTER 1

Introduction

Mercury has been widely used in dental amalgam, pesticides, batteries, paint, and thermometers and barometers by humans for a long time. The ubiquitous and persistent nature of mercury has made it an environmental and human health concern. Over the past decades there has been an increasing awareness throughout the world regarding the health and developmental risks associated with environmental exposure to mercury (WHO, 2003). Exposure to mercury could be occupational or residential, acute or chronic. Kidney and brain are the main targets of mercury exposure (Clarkson, 2002; Counter and Buchanan, 2004).

Mercury is part of the composition of the earth's crust and may be found in air, water, soil, aquatic sediments, living plants and animals. The general population is exposed to mercury in everyday life. According to Dr. Clarkson (1983), three forms of mercury are found in the environment, including elemental mercury (quicksilver, Hg0), organic mercury compounds (e.g., methyl mercury, phenyl mercury), and inorganic mercurial compounds (e.g., Hg_2Cl_2, $HgCl_2$) (Clarkson, 1983). Mercury combined with carbon is called organic mercury; methyl mercury is a common example of organic mercury. Mercury compounds that contain non-carbon substances such as chlorine, oxygen, or sulfur are called inorganic mercurials. Elemental mercury is usually referred to as metallic mercury or mercury vapor, which is nonflammable and has low solubility in both water and organic solvents. Elemental mercury vaporizes at room temperature and forms a heavy, shiny, silver-white, odorless liquid. The toxicities of mercury forms are

different depending on their specific property, such as their solubility, and chemical reactivity (Clarkson, 2002).

Mercury occurs naturally in the environment, and the levels are increased by certain human activities such as mining, the burning of fossil fuel by power plants, waste incineration, and other industrial activities (Trepka, Heinrich et al., 1997). These activities increase the amount of airborne mercury, which is eventually deposited in fresh and ocean waters (WHO, 1991). Inorganic mercury, present in water sediments, is subject to bacterial and microorganism conversion to methyl mercury compounds that bio-accumulate in the aquatic food chain, reaching the highest concentration in predatory fish (Clarkson, 1997). Methyl mercury compounds are found in seafood and freshwater fish.

Mercury intoxication can cause mental retardation, cerebral palsy, seizures, nephrotoxicity and death (WHO, 1990; Tominack, Weber et al., 2002). Studies on the background level of mercury exposure, resulting mainly from amalgam fillings or contaminated fish consumption, indicated an elevated mercury level among exposed people, but no significant association could be found between exposure and human health effects (Tulinius, 1995; Langworth, Bjorkman et al., 2002; Becker, Schulz et al., 2003; Schober, Sinks et al., 2003; Kingman, Albers et al., 2005). Animal studies indicate that long-term oral exposure to mercury may have adverse effects on the kidney, stomach, blood pressure, and heart rate (WHO, 1990; WHO, 2003). Hultman et al. (1994) did a study on mice and they reported that "accumulation of heavy metals, from dental amalgam and other sources may lower the threshold of an individual metal to elicit immunological aberrations" (Hultman, Johansson et al., 1994). In a review article, Tchounwou et al. (2003) indicated that a lower level of mercury exposure might be

unsafe among genetically susceptible populations and a noncytotoxic level of Hg^{2+} may pose a risk on a subset of populations with disease susceptibility (Tchounwou, Ayensu et al., 2003).

Amalgam fillings are one source of elementary mercury exposure in the general population (WHO, 1991; ATSDR, 1999; ATSDR, 2002; Schober, Sinks et al., 2003). Mercury is the principal metal in most dental fillings (approximately 50% by weight) (WHO, 1991; Nadarajah, Neiders et al., 1996). Mercury release increases with chewing, followed by absorption and uptake by body tissues, in particular, the brain and kidneys (WHO, 1991). Humans with amalgam fillings have significantly elevated mercury levels, with 3-5 times more mercury in their urine and 2-12 times more mercury in their tissues than those without amalgam fillings (Drasch, Schupp et al., 1994; Becker, Kaus et al., 2002; Zimmer, Ludwig et al., 2002; Becker, Schulz et al., 2003). Consumption of methyl mercury-contaminated food, especially fish, is a common route of acquiring methyl mercury.

In addition, living in an industrial area with a hazardous waste site or an incinerator may also add to mercury exposures (Kurttio, Pekkanen et al., 1998; ATSDR, 1999). Industry waste and the combustion of fossil fuels released tons of mercury into the environment from 1970 to 1990, although Mercury losses from incineration processes decreased by 47 percent between 1990 and 1996 in the United States (Sznopek and Goonan, 2000). The resulting mercury vapor exerts a significant vapor pressure. When inhaled, elemental mercury vapor is nearly completely absorbed across the alveolar membrane, with a resulting retention of 75–80%. Children also have higher minute ventilation, which increases most inhalation exposures (Forman, Moline et al., 2000). School-aged children

(6-12 years of age) may be exposed to more mercury vapor since they spend greater amounts of time outdoors.

Exposure assessment is an important part in epidemiological studies (Symanski, Sallsten et al., 2000). Usually it is not possible to measure the exposure directly, so biomarkers (such as mercury in urine) are used as surrogates and are measured during a limited time period (Brunekreef, Noy et al., 1987). Such measurements can only approximate the individual exposure level. In environmental epidemiological studies, the variability of exposure measurements is of critical importance and needs to be considered and addressed during study design and data analysis, since the variation of exposure could introduce biases in estimating the effect (Brunekreef, Noy et al., 1987; Symanski, Sallsten et al., 2000). Studies based on the occupational exposure to mercury suggest that the intra-individual variation of the exposure measurement seems to be an important issue and may induce errors if not appropriately corrected when assessing the exposure effect (Barregard, 1993; Symanski, Sallsten et al., 2000). Similar findings have been reported by Borel and his co-workers based on an iron-status assessment and the authors indicated that "day-to-day biological variation is a major component of the variability in the iron-status indicators and must be considered when assessing iron status" (Borel, Smith et al., 1991).

Unlike other environmental pollutants, such as polychlorinated biphenyls (PCB) and dichlorodiphenyl dichlorethylene (DDE), which have long half-lives, up to several years (Longnecker, Rogan et al., 1997), mercury has a very short half-life, up to approximately 2 months (Counter and Buchanan, 2004). The shorter half-life of mercury in the human body combined with other factors (such as diet and number of amalgam

fillings) may produce large intra-individual variations of mercury level over time. It is important to acquire knowledge about the magnitude of the variation in order to more accurately estimate the true exposure level. To date, most of the mercury exposure assessment studies or studies on the health effects due to mercury exposure were based on only one biological monitoring sample during the study period (Olstad, Holland et al., 1987; Schulte, Stoll et al., 1994; Tulinius, 1995; Batista, Schuhmacher et al., 1996; Khordi-Mood, Sarraf-Shirazi et al., 2001; Pesch, Wilhelm et al., 2002; Levy, Schwartz et al., 2004), which can not account for the temporal variation (usually called the 'time' effect). In addition, measurement error in cross sectional study can cause attenuation bias. Hence, a single measurement of the biomarkers is very likely to be a poor approximation of exposure.

The primary objective of the present study is to exam the variability of urine mercury levels in three consecutive measurements among children collected from three different areas in Germany during 1994-1997. The second objective is to identify factors that affect urinary mercury levels among those children. Our first hypothesis is that the intra-individual variation of mercury level among the children is higher than the inter-individual variation after controlling for confounders. Analysis of variance for repeated measurements will be used to investigate the intra-individual or "error" variance and the inter-individual or true variance (Brunekreef, Noy et al., 1987; Symanski, Sallsten et al., 2000). Our second hypothesis is that living in the region with a toxic waste incinerator will result in increased mercury levels among the study children.

In the south of the federal state of Hessen, Germany, an industrial toxic waste incinerator (TWI) was situated in the Rhine Valley with low mountains on each side.

Other industries such as a chemical plant were nearby. Besides the incinerator, the region was also used for agriculture, including the production of vegetables. Children from the TWI region comprise the exposure group. The first comparison group was 20 km north of the incinerator, in the Rhine Valley, and was also industrial and agricultural in nature (Rhine Valley control, RVC). Southeast of the incinerator region was the second comparison group, with low mountains separating it from the industrial areas (Odenwald control, OWC).

For the study population, we assume that mercury exposure via the breathing of the contaminated air from the TWI, the release of mercury vapor from dental amalgam, and fish consumption were the sources of mercury exposure. Hair, blood, and urine can be used to monitor exposure to mercury. Urinary mercury is considered to reflect the chronic exposure to inorganic mercury, while mercury in blood primarily reflects recent exposure and mercury in hair is used in monitoring long term methyl mercury exposure (Barregard, 1993; Pesch, Wilhelm et al., 2002; Gabrio, Benedikt et al., 2003). We used urine samples to monitor the mercury level in the study population. Mercury concentration in urine samples shows a circadian rhythm with highest concentrations in morning samples (Araki, Murata et al., 1983). For this reason, 24-hour urine samples were collected. In our analysis, three measures of urine mercury were used to test our hypotheses: 24-hour urinary mercury level, urinary mercury concentration, and the creatinine corrected urinary mercury.

CHAPTER 2

Population and methods

2.1 Study population

After approval by the data-protection agency in Hamburg, the Ministry for Cultural Affairs, and the local school committees, 1,091 second-grade school children in 18 townships were approached. The townships/primary schools were selected with respect to the willingness of the local school committees to cooperate. Informed consent according to requirements of the Ethical Committee of the Board of Physicians of the State of Hamburg and the data-protection agency in Hamburg was obtained from all participating parents. Children and their parents from nine townships/schools in the TWI, five in the RVC, and four in the OWC region were recruited for our study.

2.2 Questionnaires

Three questionnaires were used in 1994-5, and were repeated in 1996 and 1997. One questionnaire asked for the family's living conditions, including cigarette consumption in the home during the preceding year, place of residence, and fish consumption in the last year. From the questionnaire that was attached to the container for the 24-hour urine samples, the child's fish consumption in the two days preceding urine collection were obtained. Therefore, fish consumption was assessed first by questions of in general how often the child ate different type of fish, and second, by fish consumption in two days before the collection of the urine sample. In the third questionnaire the parents were asked for the child's gender, age, how many amalgam fillings the child had and when the most recent one was put in.

In 1997, to test the reliability of the questionnaire, the questionnaires were administered a second time. Fifty-two families, including 53 children, returned the questionnaires. For the question "when did the child receive the new amalgam", the Kappa is 0.85, for the question "whether the child has amalgam", the Kappa is 0.89. For smoking information the weighted Kappa is 0.78. A complete agreement for Rhine River fish consumption were also found.

2.3 Collection of urine samples and the determination of urinary mercury and creatinine

At the time of the physical examination one accompanying parent was instructed on how to collect a 24-hour urine sample. Flyers, suggested collecting the urine on a weekend, were also handed to their parents. Parents brought the urine sample to the clinic, where it was weighed, aliquoted, and frozen at -30°C. The samples were sent to the Institute for Toxicology, University of Kiel, Germany. Aliquots of 5 ml of urine were processed with 0.5 ml of 32% HCL and 0.2 ml of potassium-bromide/Potassium bromate (2 g KBr and 0.56 g $KBrO_3$ in 25 ml H_2O), and analyzed by flow injection atomic absorption spectroscopy (Perkin Elmer). The detection limit of Hg was 0.15 μg Hg/L. Urine creatinine (mg/L) was determined using the Jaffe-Method. The 24-hour mercury level was calculated by multiplying the urinary mercury concentration (μg/L) times the amount of urine collected in the 24-hour period (L/24-hour). The creatinine corrected urinary mercury (μg /g) was calculated using urinary mercury level (μg/L) divided by the urinary creatinine level (g/L).

2.4 Statistical methods

2.4.1. Data analysis

To approximate normality, geometric means and corresponding 95% confidence intervals were calculated. The log-transformed urinary mercury levels were used as the dependent variables. Exposures of interest were the place of residence (Toxic Waste Incinerator region, Rhine Valley comparison group, Odenwald comparison group) and amalgam fillings. Potential confounders, such as gender, age, fish consumption, passive smoking status, urinary creatinine, and 24-hour amount of urine were included in the models. The interactions between the time when the sample was collected (1994-5, 1996, 1997) and number of amalgam fillings, and between the time of sample collecting time ion and place of residence were also assessed. Gender, place of residence, and the time of sample collection were categorical variables in the model. Age, number of amalgam fillings, fish consumption (meals/month), smoking (number of cigarettes per day), urinary creatinine (mg/L) and 24-hour amount of urine (mL) were used as continuous variables. To describe the data, fish consumption was grouped as ≤ 0.05 (meals/month), 0.05 to ≤ 0.1 (meals/month), 0.1 to ≤ 0.15 (meals/month), and > 0.15 (meals/month). The number of cigarettes smoked per day by the children' parents were divided in to 4 groups (0 cigarette /day, 5 cigarettes /day, 15 cigarettes /day and 25 cigarettes /day). Urinary mercury levels in the three consecutive years could not be considered to be independent for the same child, which violates the "independent" assumption in the general linear model. Therefore, linear mixed modeling was used and restricted maximum likelihood estimates of the variance components were obtained using PROC MIXED, SAS software.

2.4.2. Linear mixed models:

The linear mixed model used is:

$$ln\,(y_{ij}) = X_{ij}\beta + b_i + \varepsilon_{ij},\ bi \sim N\,(0,\ \sigma^2_b),\ \varepsilon ij \sim N\,(0,\ \sigma^2_\varepsilon)$$

in here:

i = 1, 2, 3,.....n children;

$j = 1^{st}, 2^{nd}, 3^{rd}$ measurement index;

$ln\ (y_{ij})$ = the natural logarithm of the urinary mercury level;

X_{ij} = the fixed design vector for the *ith* child at the *jth* time point, and β is the corresponding slope. (X_{ij}: gender, age, place of residence, number of amalgam fillings, fish consumption and passive smoking status);

b_i = the random effect, which measure subject heterogeneity;

ε_{ij} = error term.

It follows that Var $(Y_{ij}) = \sigma^2_b + \sigma^2_\varepsilon$ for all *i, j.*

It is assumed that b_i and ε_{ij} are mutually independent and normally distributed with zero means and variances σ^2_b, and σ^2_ε, respectively. The term σ^2_b represents the variance between individuals; σ^2_ε measures time point variability and represents the variance within individuals. Thus, σ^2_b and σ^2_ε represent the inter-individual and intra-individual variance components, respectively. We used the linear mixed modeling procedure (PROC MIXED) in SAS to estimate the variance (σ^2_b) for inter-individual variation and (σ^2_ε) for the intra-individual variation.

The *intra-class correlation (ICC),* which is the ratio of the inter-individual variance to the total variance [ICC= $\sigma^2_b / (\sigma^2_b + \sigma^2_\varepsilon)$], was used to quantify the

proportion of total outcome variation that was due to inter-individual variation. The ratio of the variances ($\sigma^2_\varepsilon / \sigma^2_b$) is called variance ratio or $\hat{\lambda}$. Computation of the variance ratio is a useful technique to evaluate the potential bias in regression coefficients obtained with measures of exposure (Brunekreef, Noy et al., 1987). Therefore, we reported the variance ratio ($\hat{\lambda}$) in our results.

2.3 Structural Equation Models (SEMs) to detect the stability of urinary mercury

SEM is a generalization of regression, path analysis, and factor analysis, and provides a way to formulate a model that integrates mediating variables. In SEM multiple regression equations can be written simultaneously, and dependent variables in one regression can be independent variables in others (Lehert and Dennerstein, 2002). For the analysis, we transformed urinary mercury values to normally distribute by computing the normal scores from the ranks (Blom, 1958). SEM was performed to test our theoretical model. All the causal factors were selected according to the literature and our data. The number of changes in amalgam fillings in one year compared to the previous year was represented by Δ1 and Δ2 respectively. For example, Δ1 was calculated using the number of amalgam fillings in 1996 minus the number of amalgam fillings in 1995; Δ2 was calculated using: the number of amalgam fillings in 1997 minus the number of amalgam fillings in 1996. Since both the number of amalgam fillings at baseline and the changes in number of fillings (Δ1 and Δ2) will affect the mercury levels, both were included in the SEM. All analyses were conducted using the SAS System's CALIS procedure. These analyses used the maximum likelihood method of parameter estimation.

Chapter 3

Results

3.1 Study population

Of the 1,091 children in the second grade, 671 (61.5%) participated in the study. The characteristics of the study population are displayed in Table 1. Among them, 663 children had at least one urine sample during the three years. In 1994-5, 623 children provided urine samples. Sixty-six out of 623 children who had provided urine samples in 1994-5 did not give a urine sample in 1996. However, 37 additional urine samples were collected among the rest of the children resulting in a total of 594 urine samples available in 1996. In 1997, 68 out of 594 children who had provided urine samples in 1996 did not provide urine samples. Twenty-four children who gave urine sample, in 1994-5 but not in 1996, provided urine samples in 1997. Additionally, 3 children without urine samples in both 1994-5 and 1996 had provided urine samples in 1997. A total of 553 urine samples were available in 1997. Out of 663 children, 496 (74.8%) participated in all urine collection during the 3-year study period. The majority were around 8 or 9 years of age in 1994-5, 9 or 10 years of age in 1996 and > 10 years of age in 1997. Of 663 children, 362 (54.5%) resided in the toxic waste incinerator (TWI) region and 360 of these children were there during the entire study period. In 1994-5, 173 (26.1%) children had amalgam fillings and most of them (n=143) had between one to four amalgam fillings. Eighty-one (12.4%) children in 1996 and 45 (6.7%) children in 1997 had at least one amalgam filling. The percentage of amalgam fillings went down during the 3-year period. Fish consumption is low, with approximately 50% of children having less than 0.1 meal per month. Approximately half of the children had not been exposed to smoke during the 12 months before the urine sample was collected (Table 1).

3.2 Unadjusted urinary mercury (Hg) levels

Table 2 displays the median and geometric means of the urinary Hg concentration (μg/L), the creatinine corrected urinary Hg (μg/g creatinine), and 24-hour total urinary Hg (μg/24-hour) for the three years in the three different regions. For all children, both urinary Hg concentration and 24-hour urinary Hg increased during the three years. The creatinine corrected urinary Hg (μg/g creatinine) did not show this increasing trend (Table 2).

There was no apparent difference in the three urine mercury levels in 1994-5 among the three research regions. In 1996, children in the TWI group showed higher mercury level in all three outcomes compared with the other two regions. However, in 1997 children in the OWC group had the highest mercury levels for all three outcomes (Table 2).

Data on urinary mercury levels in children with and without amalgam fillings are shown in Table 2 with higher mercury levels in individuals with amalgam fillings. The urinary Hg levels for all three outcomes increased with each calendar year for both children with and without amalgam fillings.

3.3 Urinary creatinine and 24 hour total urine in three different years

In 1996, boys had a higher urinary creatinine level than girls (p-value = 0.02) (Table 3). Children with amalgam fillings had a higher urinary creatinine level than children without amalgam fillings in 1994-5 (p-value = 0.05), but not in 1996 and 1997. In 1997 the mean urinary creatinine was higher in the RVC group (1.09 mg/L) compared with the OWC group (0.98 mg/L).

The mean24-hour amount of urine increased in the three years from 639 mL in 1994-5 to 704 mL in 1996, and 779 mL in 1997. The increases were expected since the amount of urine increases with age. Similar trends were found for each region and for children with and without amalgam groups, respectively. Boys had a higher urine amount than girls in 1994-5 (p-value = 0.04) (Table 4).

3.4 The stability of the urinary mercury levels

The Spearman correlation coefficients of the urinary mercury levels for the three mercury measurements were low, ranging from 0.212 to 0.307 (Table 5). The low correlation coefficients indicate that there was substantial variation in mercury levels for each child over the three years. Adjusted estimates of the variance components on the log-transformed urinary mercury are shown in Table 6. There seems to be as much or greater variation within individuals as there is between individuals. A large variance ratio ($\hat{\lambda}$) indicates a larger intra-individual variation comparing to inter-individual variation. The intra-class correlation coefficient (ICC) is the proportion of the total mercury variance due to the variance between children. Hence a higher ICC indicates a higher inter-individual variation and a lower ICC indicates a higher intra-individual variation. A smaller ICC results in a larger $\hat{\lambda}$. For example, in table 6, if one uses the log-transformed urine concentration as the outcome, based on all children, $\hat{\lambda}$ = 2.75 and ICC = 0.28. A variance ratio ($\hat{\lambda}$) of 2.75 means that the intra-individual variance is 2.75 times the inter-individual variance. The ICC of 0.28 indicates that only 28% of the total variability was due to inter-individual variation and 72% can be attributed to other variances, including intra-individual variance. Similar patterns were shown when stratifying by

region and amalgam filling status. Children without amalgam fillings during the study period showed greater intra-individual variation (Table 6).

3.5 Associations between adjusted urinary mercury levels and other factors

Adjusted for the other covariates listed in Table 7, the number of amalgam fillings had a significant effect on the three log-transformed urine Hg measurements. There were also significant interactions between time of measurement and number of amalgam fillings, and time of measurement and living region. The effect of amalgam fillings varied depending on the time of measurement. For instance, in 1994-5, with an increase of one amalgam filling, the log- transformed urine mercury concentration will increase 19%:

$$(0.36 - 0.17 * \text{time}) * 100\% = 19\%, \text{ where time}=1.$$

Since there are significant interactions between the time when the sample was collected and number of amalgam fillings, the time when the sample was collected and living region, the changes of urinary mercury level between two years depend on both the region and the number of amalgam fillings (Table 7). For example: in TWI region, the log-transformed urine Hg concentration in 1994-5 versus 1997 is equal to

$$(-0.09) - 0.17 * \text{number of amalgam fillings} + 0.33 * \text{TWI, where TWI}=1.$$

According to the formula above, if the number of amalgam fillings is 1, the log urine Hg concentration in 1994-5 minus the log urine Hg concentration in 1997 = 0.07, indicating the log urine mercury concentration in 1994-5 is 7% higher than it was in 1997. When the number of amalgam fillings is 2 or more, the log urine mercury concentration in 1994-5 minus the log urine Hg concentration in 1997 will be

$(-0.09) - 0.17*2$ (or larger number) $+ 0.33 *\text{TWI} = -0.1$ or lower, which shows that the log urine Hg concentration in 1994-5 is 10% lower than that in 1997.

Figure 1 shows the changes of the adjusted means of log 24-hour total urine mercury in different years with different number of amalgam fillings at the mean values of all the other covariates. When the number of amalgam fillings increases, the changes in the log 24-hour total urinary mercury became larger from year to year. In 1996, the adjusted mean was higher among children living in the TWI area compared to the OWC group (p-value = 0.0023). However the TWI adjusted mean decreased in 1997 becoming the lowest of the three regions (Figure 1).

Age had a significant effect on the three log urinary mercury outcomes (Table 7). Girls had higher urinary mercury levels when using log urine mercury/creatinine (μg/g) as the outcome (p = 0.0033). The 24-hour total amount of urine had a significant effect on the three outcomes. The results did not change when restricted to the children with all three measurements during the study period (data not shown). Urine creatinine also had a significant effect on log urine Hg concentration ($p < 0.0001$) and on log 24-hour urine Hg levels (p = 0.02).

Path coefficients (pcoe) are shown in Path Diagrams 1, 2, and 3. The number of amalgam fillings (pcoe = 0.48, p-value < 0.05) and gender (pcoe = 0.12, p-value < 0.05) had a significant effect on the urine mercury/creatinine (μg/g) during the study period. The change in number of amalgam fillings in 1996 had an effect on the urine mercury/creatinine (μg/g), with pcoe = 0.25 (p-value < 0.05). The number of fish meals had a significant effect in 1995 (pcoe = 0.81, p-value < 0.05), but not in 1996 or 1997. We did not find a fish meal effect in our mixed model. Urinary mercury (Hg) level divided by urine creatinine in 1995 had a significant effect on the level in 1996 (pcoe = 0.14, $p < 0.05$) and 1997 (pcoe = 0.13, $p < 0.05$), and the urine mercury/creatinine (μg/g)

in 1996 also affected the level in 1997 (pcoe = 0.22, $p < 0.05$). Living in the TWI area had no effect on the urine mercury/creatinine ($\mu g/g$) in 1995 (pcoe = 0.10, p-value > 0.05), but had a significant risk effect in 1996 (pcoe = 0.33, p-value < 0.05), and a significant but diminishing effect in 1997 (pcoe = - 0.21, $p < 0.05$) (Path Diagram 1), which confirms the findings from the linear mixed model. The amount of total urine also significantly affected the urinary mercury level divided by urine creatinine (pcoe = 0.22, $p < 0.05$).

When using normal-transformed 24-hour total urine mercury as the dependent variable, our findings were similar to those when we used normal-transformed urine mercury/creatinine ($\mu g/g$) as the outcome, with several differences: there was no gender effect, but age and urine creatinine showed significant effects on the outcome. The age effect was weak with pcoe=0.04, $p <0.05$, based on the one-sided t-test, which is consistent with our mixed model using the two-sided test. The path coefficients for urine creatinine were pcoe=0.09, ($p <0.05$) in 1994-5, pcoe=0.13, ($p <0.05$) in 1996 and pcoe=0.13, ($p <0.05$) in 1997 (Path Diagram 2). Path Diagram 3 displayed the factors affecting the normal-transformed urinary mercury concentration.

CHAPTER 4

Discussion

4.1 Study population

Of the 1,091 eligible second grade children, 61.5% participated in the study (n = 671), and, therefore, it is unlikely that a selection bias can explain our findings. The distributions of factors, which may affect the mercury level in children, were similar among the three regions during the study period (data not shown). The reproducibility of the information was high. Kappas on questions of amalgam fillings, fish consumption, and smoking status were higher than 75%. Hence, we do not suspect an information bias could explain our findings.

4.2 The variability of mercury levels in urine

The importance of assessing the intra- and inter-individual variation in biomarkers of exposure has been recognized in occupational and environmental epidemiology for a long time (Brunekreef, Noy et al., 1987; Rappaport, Symanski et al., 1995; Symanski, Sallsten et al., 2000; Symanski, Bergamaschi et al., 2001; Symanski and Greeson, 2002). We are not aware of any studies to date that have quantified the variability in urinary mercury levels in children. In the present study, we investigated the variability of the mercury levels among school-aged children residing in three different areas, who participated in a longitudinal study in Hesse, Germany. The low Spearman correlation coefficients of the urinary mercury levels (0.212 to 0.307) indicate that the mercury level in the human body was not stable. After adjusting for the other covariates in Table 7, the 'time' variable showed a significant effect on the three mercury measurements, which denotes that at least in two years the mercury levels were statistically different. The

adjusted point estimates of the variance components in the log-transformed urinary mercury showed a greater variation within children than between children. Children without amalgam fillings showed larger variance ratios than children with amalgam fillings. Since children with amalgam fillings had higher urinary Hg levels, the increase of number amalgam fillings posed a relatively smaller impact on urinary Hg levels comparing to children without amalgam fillings whose urinary Hg levels were 2 –3 times lower. In addition, in each year more boys (56.7%) gave urine samples among children without amalgam fillings, meanwhile approximately 50% of children who provided urine sample with amalgam fillings are boys. Since gender has significant effect on urine mercury levels, the greater within children variation may be caused by gender.

Due to the large intra-individual variation, a single measurement of urinary mercury may not be adequate for estimating the "true" individual exposure level. Symanski et al. quantified the intra- and inter-individual variation in urinary levels of mandelic acid and phenylglyoxylic acid among workers exposed to styrene (Symanski, Sallsten et al., 2001). They found that due to the intra-individual variation, estimates of workers' exposures that rely on single measurements would perform poorly in a regression analysis designed to examine effects resulting from chronic exposure. However, the bias in an observed slope coefficient would be diminished if a second or third urine sample were collected. Brunekreef et al. (Brunekreef, Noy et al., 1987) indicated that when the intra-individual variation is large, repeated measurements for each individual could reduce the bias in a regression coefficient. The authors also suggested that the average of a number of measurements for the same subject was more reliable than one measurement. However, we think the average of several measurements

may also produce bias in estimating the "true" level because one outlier could drag the average level away from the "true" level. Instead of taking the average of several repeated measurements of exposure, one can use statistical methods for repeated exposure measurements to analyze the data, such as the mixed effect model, which can check if there is outlier problem by exploring the residual distribution. In case there are several exposure measurements in a long period and only one outcome measured at the end of the study, one could use other methods to handle the repeated exposure measurements, such as using "area under the curve" to estimate the cumulative exposure status.

Given the advantages of repeated measurements, the following questions should be considered: 1) How large should the inter-measurement interval be? 2) How many measurements are needed?

Since the half-life of urinary mercury is approximately two months and some factors among children, such as the number of amalgam fillings and diet, may change within one half-life, we suggest that at least three 24-hour urine samples should be collected in the first two-month period in order to monitor the variation of urinary mercury within one half-life. Then, two or more urinary mercury samples should be collected in subsequent two-month periods. By using this strategy we expect to monitor the variation of urinary mercury within one half-life and between two or three additional half-lives. Our suggestion is consistent with the recommended biological mercury monitoring strategy in the medical guideline produced in 1996 by the Health & Safety Executive (HSE) in the UK, which suggests using a usual urinary sampling frequency of

between 1 to 3-month, and more frequent sampling for subject close to the health guideline value (Mason, Hindell et al., 2001).

A strength of our study is the large sample size (N=663), which increases the power to detect a large intra-individual variation.

A limitation in our study is the long interval (approximately 1 year apart) between measurements. Symanski et al., in one study investigating the variance of aggregated data from five different data sets based on 53 workers with 123 measurements, indicated an increasing variability with the increased interval between measurements after controlling for factors that were likely to contribute to variability (Symanski and Rappaport, 1994). In our study the observed large intra-individual variation could be caused by the long interval between measurements. We hope that future studies will incorporate a greater number of repeated measurements with shorter measurement intervals in order to provide detailed information on the choice of the optimal number of measurements and time intervals.

4.3 Factors that affect urinary mercury levels

Among the study population, the number of amalgam fillings was strongly associated with the urinary mercury levels. After adjusting for place of residence, gender, age, fish consumption, smoking status, urine creatinine and 24-hour urine amount, the number of amalgam fillings had the most significant effect on the urinary mercury levels in both the linear mixed model and the structural equation model. Our results were consistent with previous findings that people with amalgam fillings have higher urinary mercury level compared to people without amalgam fillings (Olstad, Holland et al., 1987; Olstad, Holland et al., 1990; Schulte, Stoll et al., 1994; Trepka, Heinrich et al., 1997;

Counter and Buchanan, 2004). Kingman et al estimated that 10 amalgam surfaces would raise urinary concentration by 1 μg of mercury per liter, which is twice the normal environmental background concentration (Kingman, Albertini et al., 1998). In our study, the increase in log mercury levels due to one amalgam filling is approximately 36%. Alternatively, an increase in 1 unit of the log urinary mercury concentration is associated with an increase of 2.7 amalgam fillings (without considering the interaction between the time of measurement and the number of amalgam fillings).

Given the operation of the incinerator and other industrial activities in the TWI region, it was expected that the mercury would be higher in the TWI area than in the OWC area. Compared to the mercury levels in 1994/5, residing in the toxic waste incinerator area posed a risk of increased urinary mercury levels in 1996. However in 1997, the mercury levels were lower than those in 1994/5. The reason for this could be due to the percentage of children having at least one amalgam fillings were different in three regions in different years. In 1994/5, the percentage of children having at least one amalgam fillings is 25.9% in TWI, 30.2% in RVC, and 30.1% in OWC. In 1997, the percentage decreased to 5% in TWI, 3.6% in RVC, and 16.8% in OWC. The higher percentage of children having amalgam fillings in OWC in 1997 may produce the higher urinary Hg levels compare to children in TWI region. Other possible explanation may be that parents were aware of the possible risk of increasing the internal body burden of mercury in the incinerator area. So they tried to protect their child from mercury exposure, for instance, they may have suggested that their child not play outdoors, etc. The finding of decreased mercury levels in 1997 may also have occurred by chance.

Consistent with this result, Trepka et al. also failed to find higher mercury level in the polluted compared to the control region (Trepka, Heinrich et al., 1997).

In the present study, gender showed a significant effect on mercury level when using the log creatinine corrected urine mercury as the outcomes. However, using the log creatinine corrected urine mercury as the dependent variable may produce bias and thus an artificial difference in urinary mercury levels between boys and girls can be generated because the urine creatinine level differed by gender (Mage, Allen et al., 2004; Barr, Wilder et al., 2005). We found that boys had a significantly higher creatinine concentration compared to girls in 1996. Recently, Schisterman et al evaluated four statistical methods for the analysis of PCB exposure, serum lipid and health risk (Schisterman, Whitcomb et al., 2005). They suggested that lipid standardization (the division of serum PCB concentration by serum lipids) is highly prone to bias. Hence, simple division by creatinine may also produce a bias. It is likely that the observed gender effect on urinary mercury levels results from a different creatinine concentration in boys and girls. A solution for this problem is not to divide urinary mercury level by urinary creatinine level, but to use urinary creatinine levels included in the model as a confounder. Hence the statistical significance of the independent variables would not be due to an association with the urinary creatinine level.

4.4 Structural Equation Models (SEMs)

Using SEMs, we found that residing in the toxic waste incinerator area posed a risk of increasing the urinary mercury level in 1996 compared to 1995; the risk seems to be diminished in 1997. These results are consistent with those from the linear mixed model. Path Diagrams 1-3 showed that the urinary mercury level in 1995 can predict 10 -

16% of the increase in mercury levels in 1996 and 13 -17% of the increase in 1997; and the mercury levels in 1996 predicted an 18 – 22% increase of the mercury levels in 1997. Those results indicated a low stability with a 10 – 22% fluctuation in the urinary mercury levels among the study population, which is in agreement with the results from mixed linear model.

4.5 Summary

Analyses using both linear mixed models and SEMs showed that the number of amalgam fillings had a significant effect on urinary mercury levels. Residing in the toxic waste incinerator area posed a risk for increasing the urinary mercury level only in 1996, but seems to have a diminishing effect in 1997 from the results of both linear mixed model and SEMs. Based on our data we found that the intra-individual variation attributed more (approximately 72% - 74%) to the total variation compared with the inter-individual variation (approximately 26% - 28%). Since the large intra-individual variance may induce a biased estimation of the effect, repeated determinations of the biomarkers of the exposure with a shorter interval between two measurements may be one method that could be used to reduce the likelihood of the attenuation biases. Because the half-life of urine mercury is approximately two months, we recommend taking at least three measurements within one half-life (approximately two months), and then taking two additional measurements in at least two-month periods. We suggest that a repeated measurement approach with shorter time intervals between two measurements should be used to increase the accuracy of exposure assessments. Considering the characteristics and living conditions of different populations, some factors might only be generalized to children aged 8-11 years in Germany. However, the information regarding the intra-

individual and inter-individual variation provided in our study and our suggestions for future study designs should be useful to other investigators planning prospective studies for assessing mercury exposure among school aged children.

Table1. Descriptive characteristics of the study population (n=663)

Variable	Categories	94/95 n (%)	96 n (%)	97 n (%)
Gender of the Child	Male	336 (50.7)	317 (47.8)	298 (45.0)
	Female	292 (44.0)	275 (41.5)	254 (38.3)
	Missing	35 (5.3)	71 (10.7)	111 (16.7)
Age	≤ 8	250 (37.7)	0	0
	> 8 - 9	349 (52.6)	225 (34)	0
	> 9 - 10	27 (4.1)	346 (52.2)	153 (23.1)
	> 10	3 (0.5)	24 (3.6)	399 (60.2)
	Missing	34 (5.1)	68 (10.2)	111 (16.7)
Place of resident at each year	TWI[a]	360 (54.3)	360 (54.3)	362 (54.6)
	RVC[b]	117 (17.7)	116 (17.5)	108 (16.3)
	OWC[c]	168 (25.3)	155 (23.4)	139 (21.0)
	Missing	18 (2.7)	32 (4.8)	54 (8.1)
Number of amalgam fillings at each year	0	448 (67.6)	510 (77.0)	497 (75.0)
	1	35 (5.3)	41 (6.2)	31 (4.7)
	2	53 (8.0)	25 (3.8)	9 (1.4)
	3	31 (4.7)	7 (1.1)	1 (0.2)
	4	24 (3.6)	5 (0.8)	1 (0.2)
	> 4	30 (4.5)	3 (0.5)	3 (0.5)
	Missing	42 (6.3)	72 (10.9)	121 (18.3)
Fish consumption (Meals per month)	≤ 0.05	141 (21.3)	156 (23.5)	174 (26.2)
	> 0.05 - 0.1	260 (39.2)	247 (37.3)	247 (37.3)
	> 0.1 - 0.15	97 (14.6)	102 (18.4)	50 (7.5)
	> 0.15	129 (19.5)	86 (13.3)	80 (12.1)
	Missing	36 (5.4)	72 (10.9)	112 (16.9)
Number of cigarettes per day (smoking by the parents during the last 12 months)	0	338 (51)	347 (52.3)	318 (48.0)
	5	142 (21.4)	130 (19.6)	136 (20.5)
	15	87 (13.1)	74 (11.2)	58 (8.8)
	25	62 (9.4)	45 (6.8)	41 (6.2)
	Missing	34 (5.1)	67 (10.1)	110 (16.6)

a: Toxic Waste Incinerator group (TWI)

b: Rhine Valley Control group (RVC)
c: Odenwald Control group (OWC)

Table 2. Crude urinary mercury level:

Time	Urine Hg concentration (μg/L)				The creatinine corrected urinary Hg[a] (μg/g creatinine)				Urine Hg over 24-hour (μg/24h)			
	n	Median (5^{th}-95^{th} pct)	GM[b]	CI-GM[c]	n	Median (5^{th}-95^{th} pct)	GM[b]	CI-GM[c]	n	Median (5^{th}-95^{th} pct)	GM[b]	CI-GM[c]
1994-5	625	0.075 (0.075-1.130)	0.193	0.179-0.209	624	0.157 (0.058-1.348)	0.149	0.138-0.160	623	0.069 (0.025-0.623)	0.086	0.079-0.093
1996	596	0.130 (0.050-0.990)	0.182	0.166-0.199	596	0.153 (0.042-1.267)	0.143	1.131-0.157	594	0.078 (0.021-0.635)	0.091	0.083-0.100
1997	553	0.160 (0.050-1.470)	0.186	0.170-0.204	552	0.179 (0.037-1.480)	0.163	0.150-0.178	553	0.109 (0.021-0.809)	0.110	0.104-0.124
						1994-5						
TWI	342	0.075 (0.075-1.340)	0.154	0.138-0.171	342	0.156 (0.060-1.508)	0.197	0.177-0.219	341	0.069 (0.027-0.623)	0.088	0.079-0.098
RVC	117	0.075 (0.075-0.620)	0.133	0.115-0.154	117	0.163 (0.055-0.939)	0.177	0.151-0.207	117	0.072 (0.021-0.412)	0.078	0.067-0.092
OWC	166	0.075 (0.075-1.160)	0.150	0.129-0.174	165	0.156 (0.056-1.230)	0.200	0.170-0.234	165	0.065 (0.025-0.699)	0.086	0.072-0.101
						1996						
TWI	325	0.130 (0.050-1.030)	0.166	0.146-0.189	325	0.199 (0.043-1.331)	0.210	0.185-0.239	325	0.095 (0.022-0.768)	0.103	0.091-0.118

Table 2 (cont'd)

RVC	116	0.110 (0.050-0.560)	0.108	0.092-0.127	116	0.116 (0.035-0.788)	0.133	0.112-0.159	114	0.064 (0.021-0.479)	0.068	0.058-0.081
OWC	155	0.110 (0.050-1.070)	0.130	0.109-0.154	155	0.148 (0.042-1.596)	0.169	0.142-0.200	155	0.076 (0.019-1.015)	0.086	0.072-0.102
						1997						
TWI	306	0.130 (0.050-1.040)	0.147	0.130-0.145	305	0.165 (0.038-1.380)	0.174	0.154-0.196	306	0.100 (0.020-0.696)	0.103	0.091-0.117
RVC	108	0.170 (0.050-0.660)	0.157	0.133-0.186	108	0.184 (0.028-0.961)	0.163	0.136-0.197	108	0.103 (0.020-0.561)	0.166	0.089-0.127
OWC	139	0.200 (0.050-2.300)	0.212	0.175-0.258	139	0.216 (0.041-2.353)	0.239	0.198-0.290	139	0.135 (0.022-1.513)	0.146	0.121-0.177
						1994/95						
With amalgam	171	0.250 (0.075-2.180)	0.276	0.230-0.330	171	0.293 (0.064-3.069)	0.341	0.285-0.407	171	0.132 (0.025-0.285)	0.154	0.128-0.185
Without amalgam	447	0.075 (0.075-0.480)	0.117	0.109-0.125	446	0.136 (0.056-0.625)	0.155	0.144-0.167	445	0.057 (0.025-0.285)	0.068	0.063-0.074
						1996						
With amalgam	81	0.260 (0.050-3.950)	0.331	0.226-0.429	81	0.406 (0.066-3.596)	0.409	0.303-0.551	80	0.143 (0.038-1.838)	0.199	0.147-0.270

Table 2 (cont'd)

Without amalgam	510	0.120 (0.050-0.750)	0.127	0.117-0.139	510	0.138 (0.040-1.000)	0.160	0.146-0.175	509	0.067 (0.021-0.500)	0.081	0.074-0.088
1997												
With amalgam	46	0.385 (0.050-3.960)	0.462	0.298-0.715	46	0.468 (0.051-3.663)	0.465	0.309-0.698	46	0.272 (0.040-2.398)	0.289	0.194-0.430
Without amalgam	497	0.174 (0.036-0.152	0.149	0.137-0.163	496	0.150 (0.050-0.850	0.173	0.159-0.190	497	0.108 (0.020-0.626	0.105	0.096-0.115

a: The creatinine corrected urinary Hg: calculated by urinary mercury concentration (μg/L) divided by the urinary creatinine level (g/L).

b: Geometric mean (GM).

c: 95% confidence interval for GM (CI-GM).

Table 3. Crude urinary creatinine levels in three years.

	Urine creatinine in 1994-5 (mg/L)				Urine creatinine in 1996 (mg/L)				Urine creatinine in 1997 (mg/L)			
	n	Mean (SD [a])	F- test	p-value	n	Mean (SD)	F- test	p-value	n	Mean (SD)	F- test	p-value
All children	626	0.84 (0.35)	-	-	596	0.89 (0.44)	-	-	552	0.97 (0.43)	-	-
Boys	335	0.83 (0.33)	0.39	0.54	317	0.93 (0.41)	5.86	0.02*	297	1.0 (0.43)	2.03	0.15
Girls	291	0.85 (0.37)			291	0.84 (0.39)			254	0.94 (0.44)		
TWI	343	0.83 (0.34)			325	0.90 (0.47)			305	0.93 (0.39)		
RVC	117	0.80 (0.36)	0.30	0.74	116	0.91 (0.43)	1.06	0.35	108	1.09 (0.50)	5.61	0.004*
OWC	166	0.84 (0.36)			155	0.85 (0.37)			139	0.98 (0.45)		
With amalgam	172	0.89 (0.37)	3.74	0.05*	81	0.85 (0.43)	0.76	0.38	45	1.08 (0.44)	3.29	0.07
Without amalgam	447	0.82 (0.34)			510	0.90 (0.44)			496	0.96 (0.43)		

a: Standard deviation.

Table 4. Crude 24 - hour total amount of urine samples in three consecutive years.

	Amount of urine in 1994-5 (L)				Amount of urine in 1996 (L)				Amount of urine in 1997 (L)			
	n	Mean (SD [a])	F- test	p-value	n	Mean (SD)	F- test	p-value	n	Mean (SD)	F- test	p-value
All children	62 6	639 (304)	-	-	594	704 (344)	-	-	553	779 (380)	-	-
Boys	33 5	661 (313)	4.23	0.04*	316	722 (369)	1.54	0.21	298	786 (401)	0.23	0.63
Girls	29 1	612 (291)			274	687 (313)			254	770 (355)		
TWI	34 2	638 (306)			325	695 (352)			306	789 (385)		
RVC	11 7	653 (297)	0.19	0.83	114	713 (362)	0.29	0.75	108	706 (370)	0.26	0.77
OWC	16 7	630 (305)			155	718 (311)			139	772 (378)		
With amalgam	17 3	634 (327)	0.15	0.70	80	716 (357)	0.09	0.76	45	731 (394)	0.93	0.33
Without amalgam	44 6	644 (296)			509	703 (342)			497	788 (380)		

a: Standard deviation.

Table 5. Spearman Correlation Coefficients of urinary mercury levels:

	HG_24U96 [a]	HG_24U97 [a]	HG_UCr96 [b]	HG_UCr97 [b]	HG_Con96 [c]	HG_Con97 [c]
HG_24U94/95[a]						
Corr.	0.212	0.280	–	–	–	–
p-value	<.0001*	<.0001*				
n	557	520				
HG_24U96						
Corr.	1.000	0.277	–	–	–	–
p-value		<.0001*				
n	594	526				
HG_UCr 94/95[b]						
Corr.	–	–	0.234	0.267	–	–
p-value			<.0001*	<.0001*		
n			560	520		
HG_UCr96						
Corr.	–	–	1.000	0.306	–	–
p-value				<.0001*		
n			596	527		
HG_Con94/95[c]						
Corr.	–	–	–	–	0.216	0.271
p-value					<.0001*	<.0001*
n					561	522
HG_Con96						
Corr.	–	–	–	–	1.000	0.241*
p-value						<.0001
n					596	528

a: 24-hour urinary Hg levels in 1994-5, 1996 and 1997 respectively;
b: The urinary Hg levels adjusted for creatinine in 1994-5, 1996 and 1997 respectively;
c: The urinary Hg concentration in 1994-5, 1996 and 1997 respectively.

Table 6. Adjusted inter- and intra-individual variations ($\hat{\sigma}^2_b$ and $\hat{\sigma}^2_\varepsilon$) for log-transformed urinary mercury.

	n	$\hat{\sigma}^2_b$ (Adjusted inter-individual variance)	$\hat{\sigma}^2_\varepsilon$ (Adjusted intra-individual variance)	$\hat{\lambda} = \sigma^2_\varepsilon / \sigma^2_b$ (Variances ratio)	ICC = $\sigma^2_b / (\sigma^2_b + \sigma^2_\varepsilon)$
Log-transformed urine concentration (μg/L) [a]					
All children	663	0.26	0.71	2.75	0.28
Regions					
TWI	360	0.28	0.72	2.55	0.28
RVC	101	0.15	0.51	3.41	0.23
OWC	125	0.16	0.71	4.51	0.18
Amalgam fillings					
With	205	0.70	0.81	1.15	0.56
Without	456	0.05	0.56	12.31	0.08
Log-transformed urine Hg divided by urine creatinine (μg/g creatinine) [b]					
All children	663	0.24	0.67	2.81	0.26
Regions					
TWI	360	0.28	0.73	2.59	0.28
RVC	101	0.16	0.50	3.19	0.24
OWC	125	0.14	0.72	5.19	0.16
Amalgam fillings					
With	205	0.72	0.80	1.10	0.48
Without	456	0.04	0.58	14.50	0.06
Log-transformed 24-hour urinary mercury (μg/24h) [a]					
All children	663	0.24	0.69	2.9	0.26
Regions					
TWI	360	0.29	0.75	2.55	0.28
RVC	101	0.16	0.5	3.23	0.24

Table 6 (cont'd)

OWC	125	0.14	0.74	5.48	0.15
Amalgam fillings					
With	205	0.68	0.84	1.23	0.45
Without	456	0.05	0.58	10.79	0.08

a: Adjusted for number of amalgam fillings, residence, gender, age, fish consumption, smoking status at home, urine creatinine, and urine amount.

b: Adjusted for number of amalgam fillings, residence, gender, age, fish consumption, smoking status at home, and urine amount.

Table 7. Adjusted effects estimates and *P*-values for log urinary mercury (Hg) level:

	Log urine Hg concentration (μg/L)			Log urine Hg divided by urine creatinine (μg/g creatinine)			Log urine Hg over 24-hour (μg/24h)		
Covariates	Estimate	SE [a]	P-value (t-test)	Estimate	SE [a]	P-value (t-test)	Estimate	SE [a]	P-value (t-test)
Intercept	-3.5	0.61	< .0001*	-3.2	.063	< .0001*	-4.6	0.62	< .0001*
# of amalgam fillings	0.36	0.06	< .0001	0.35	0.07	< .0001*	0.37	0.07	< .0001*
TWI vs. OWC	-0.29	0.10	0.004*	-0.25	0.10	0.01*	-0.30	0.1	0.003*
RVC vs. OWC	-0.21	0.12	0.08	-0.24	0.13	0.06	-0.21	0.13	0.10
Girls vs. Boys	0.10	0.06	0.09	0.17	0.06	0.003*	0.08	0.06	0.15
Age	0.16	0.06	0.005*	0.12	0.06	0.05*	0.16	0.06	0.01*
Fish meals	0.29	0.19	0.13	0.30	0.20	0.13	0.25	0.19	0.2
Smoking	0.004	0.003	0.18	0.004	0.003	0.21	0.004	0.004	0.22
Time 94/95 vs. 97	-0.09	0.16	0.57	0.06	0.16	0.73	-0.13	0.16	0.41
Time 96 vs. 97	-0.35	0.12	0.004*	-0.28	0.12	0.03*	-0.36	0.12	0.004*
Urinary creatinine	0.30	0.07	< .0001*	_	_	_	0.18	0.07	0.02*
Urine amount	-0.0003	0.00009	0.003*	0.0004	0.00007	< .0001*	0.0009	0.00008	< .0001*
# of amalgam fillings *time 94/95 vs. time 97	-0.17	0.07	0.01*	-0.18	0.07	0.009*	-0.19	0.07	0.006*
# of amalgam fillings *time 96 vs. time 97	0.03	0.08	0.74	0.04	0.08	0.66	0.01	0.08	0.86
TWI*time 94/95 vs. time 97	0.33	0.12	0.005*	0.26	0.12	0.03*	0.34	0.12	0.005*

Table 7 (cont'd)

TWI*time 96 vs. time 97	0.60	0.12	< .0001	0.59	0.12	< .0001	0.59	0.12	< 0.001
RVC*time 94/95 vs. time 97	0.19	0.15	0.2004	0.19	0.15	0.2068	0.19	0.15	0.2164
RVC*time 96 vs. time 97	0.16	0.15	0.2860	0.18	0.15	0.2436	0.12	0.15	0.4161

a: standard errors.

Figure1. Estimated adjusted means of log 24-hour urine mercury versus time in three different regions:

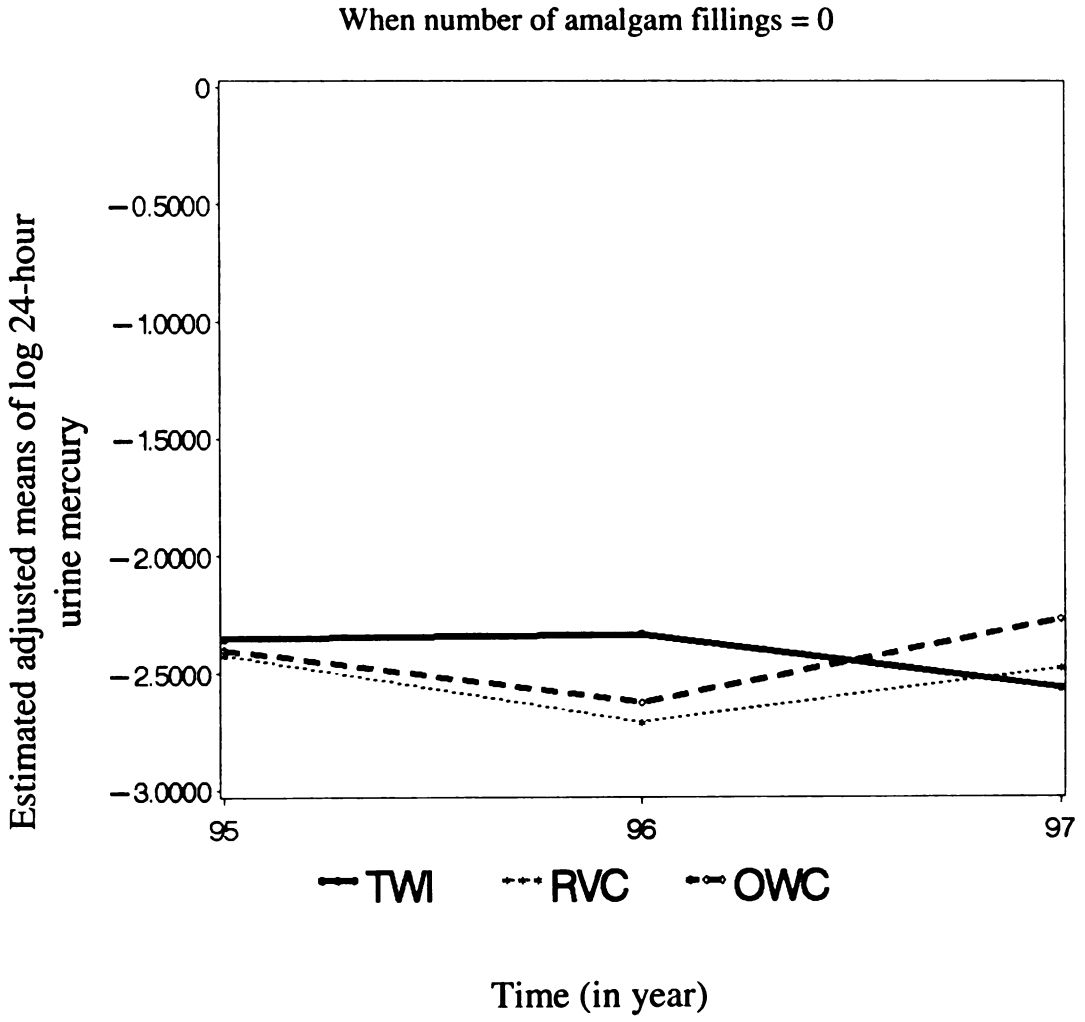


Figure1. Estimated adjusted means of log 24-hour urine mercury versus time in three different regions (cont'd):

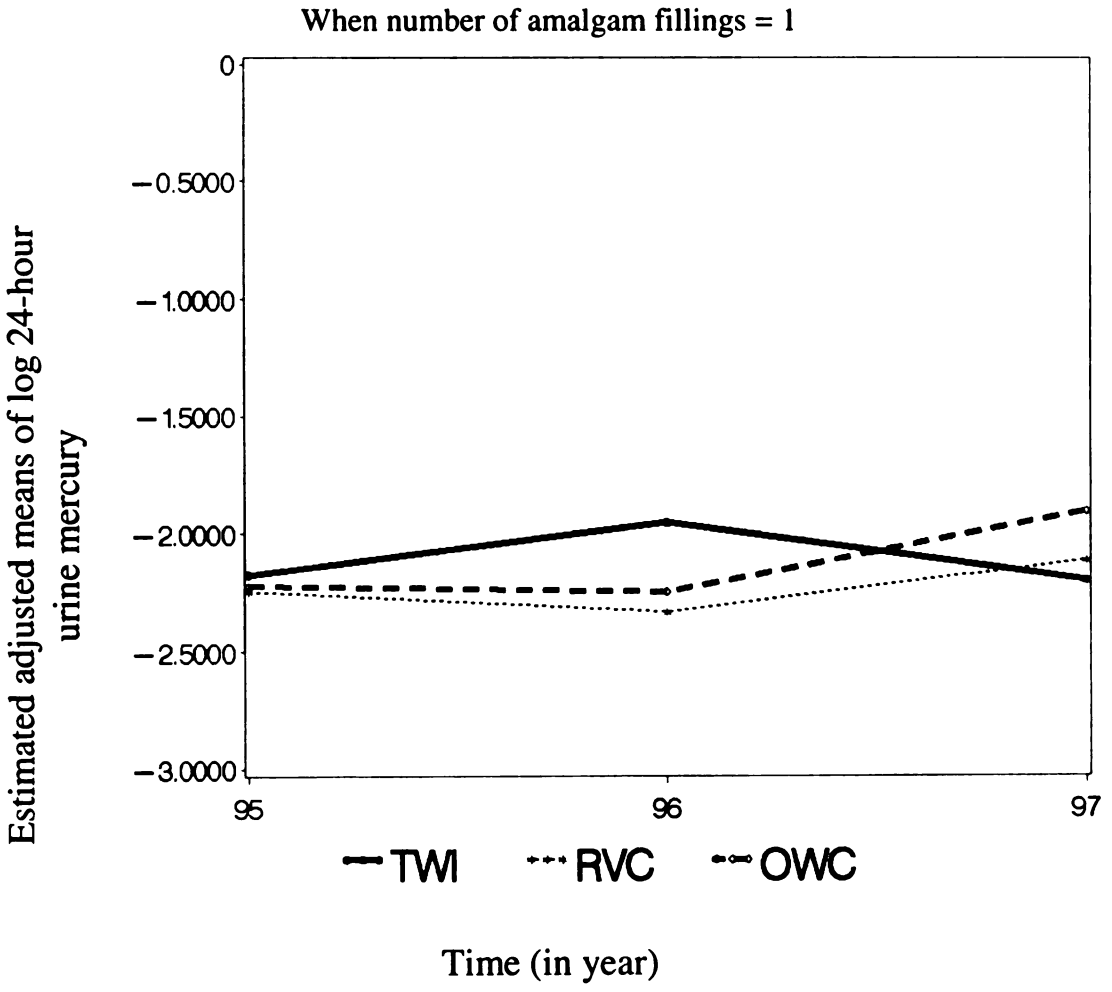


Figure1. Estimated adjusted means of log 24-hour urine mercury versus time in three different regions (cont'd):

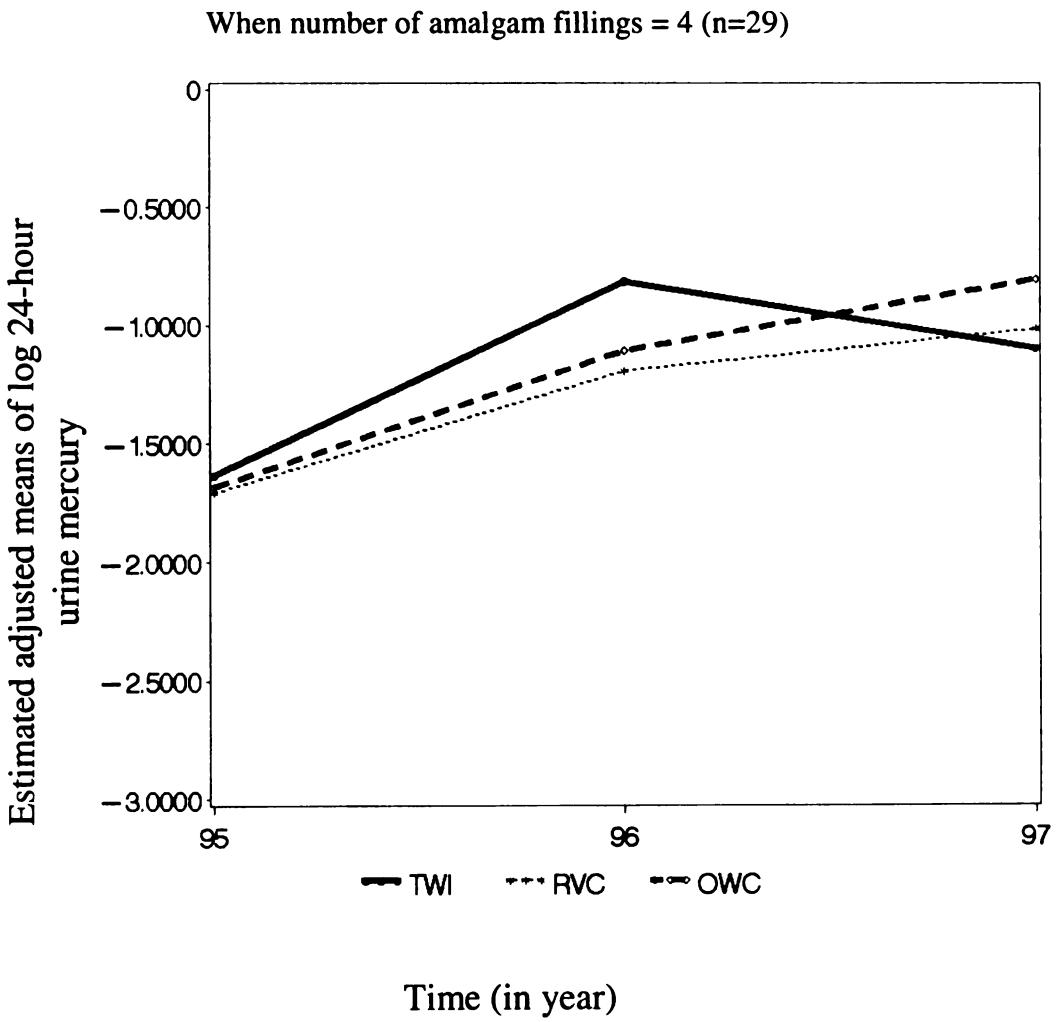


Figure1. Estimated adjusted means of log 24-hour urine mercury versus time in three different regions (cont'd):

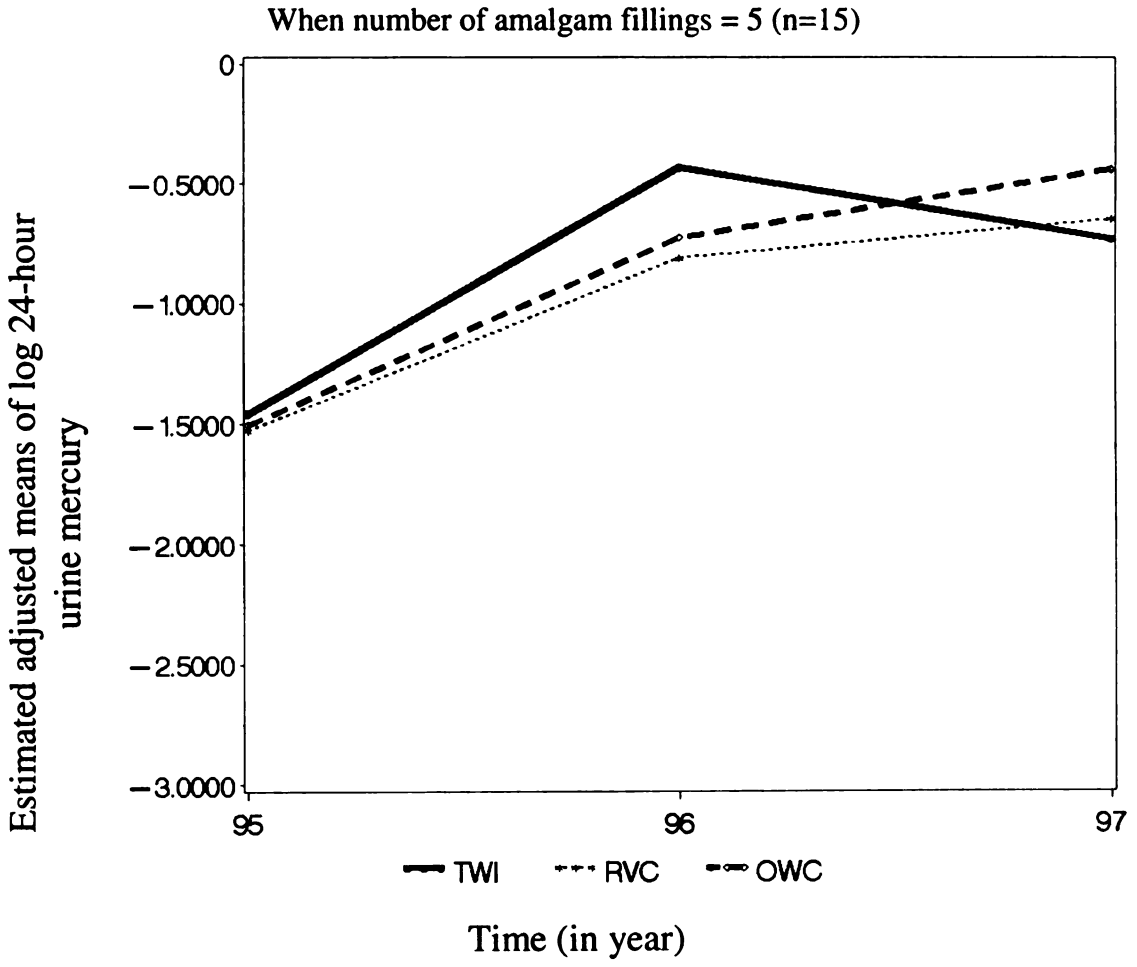


Figure2. Estimated adjusted means of log urine mercury divided by urine creatinine versus time in three different regions:

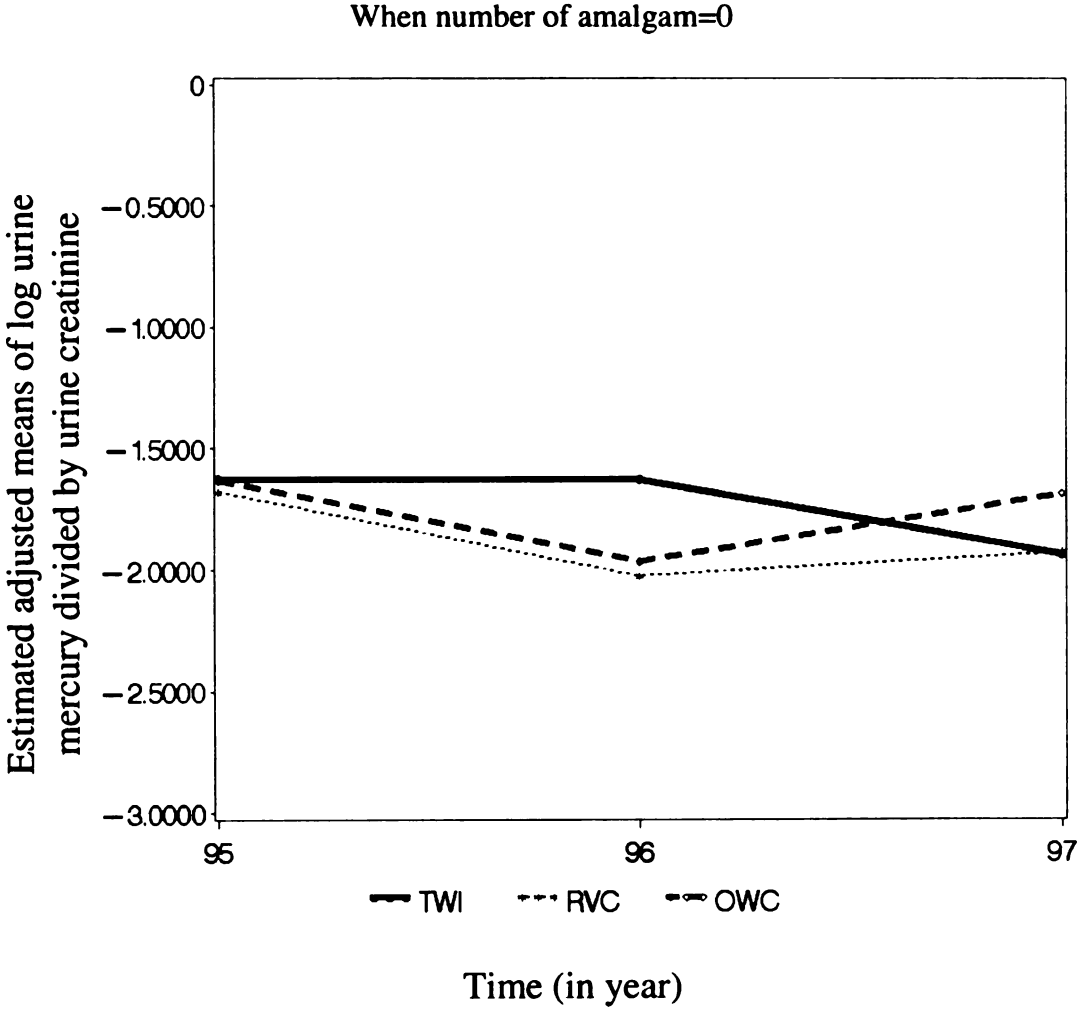


Figure2. Estimated adjusted means of log 24-hour urine mercury versus time in three different regions (cont'd):

When number of amalgam=4 (n=29)

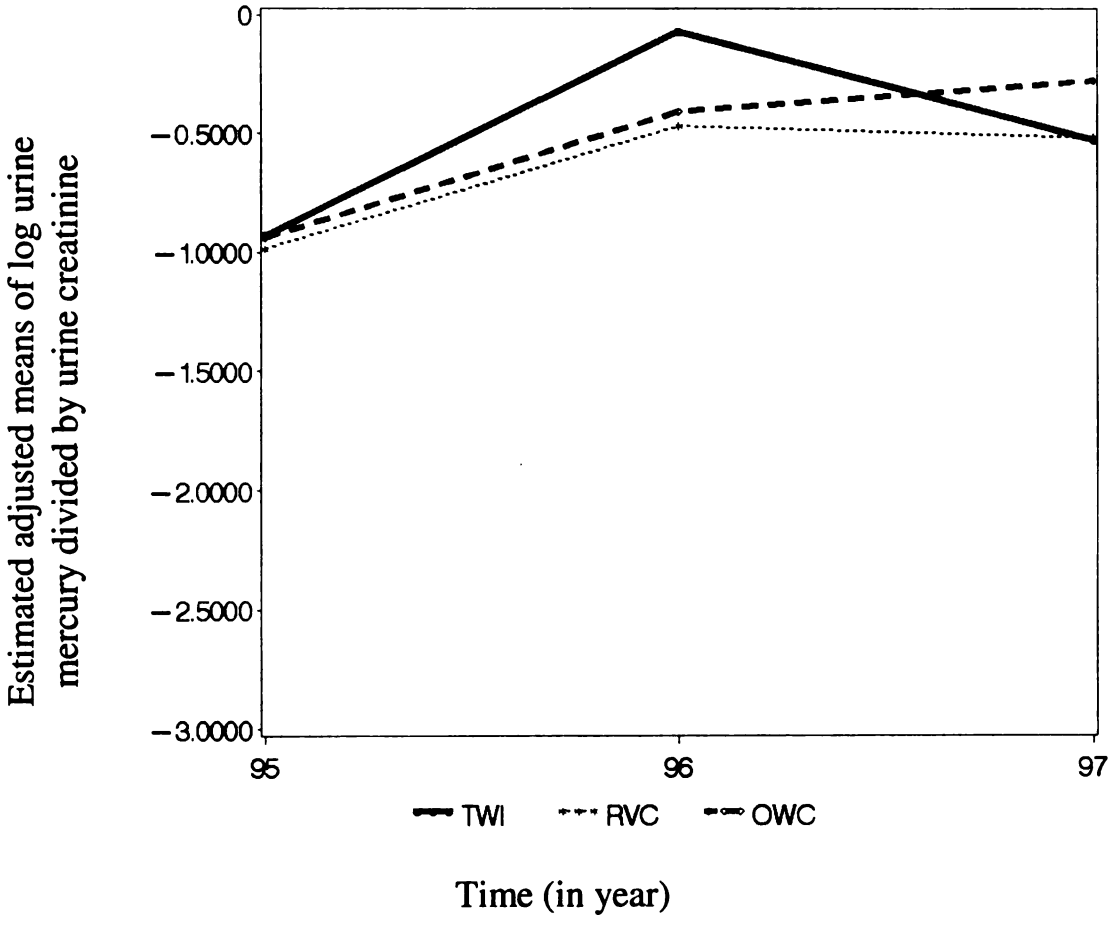


Figure3. Estimated adjusted means of log urine mercury concentration versus time in three different regions:

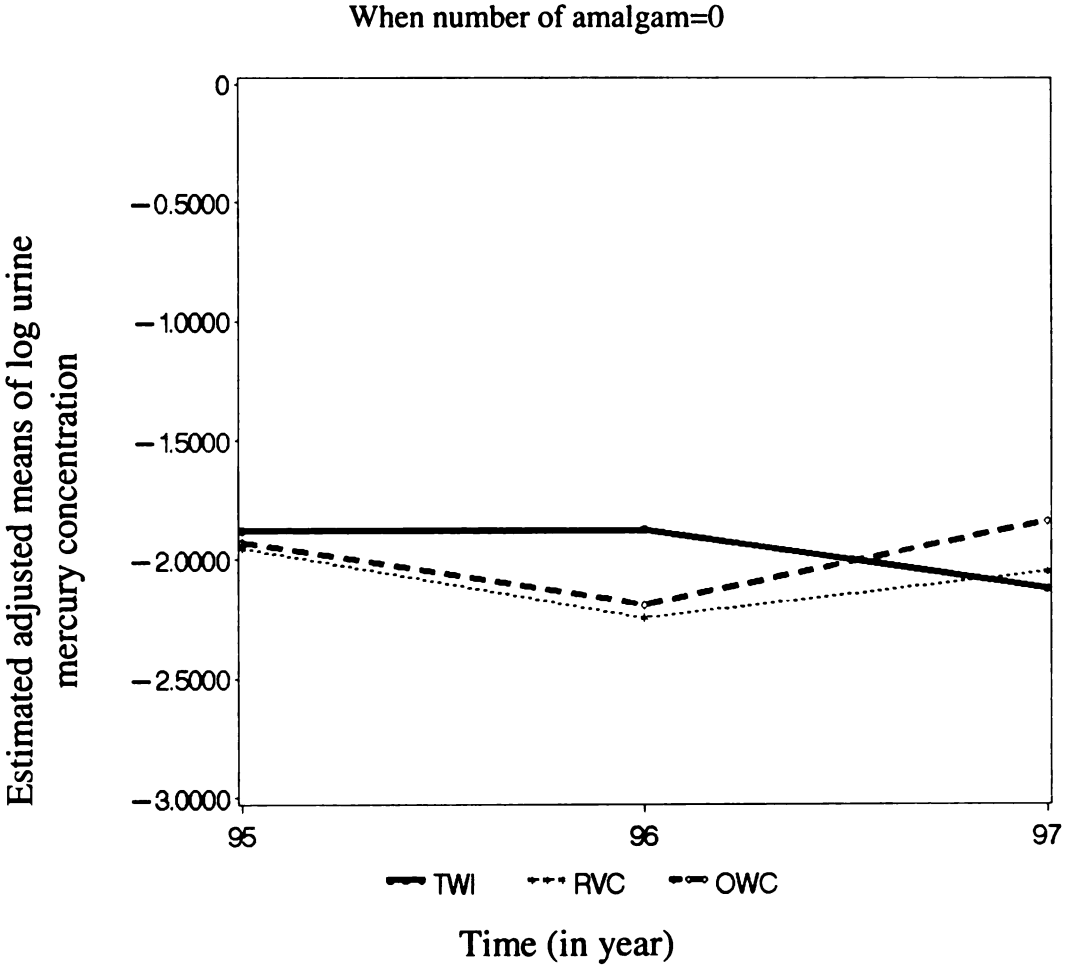
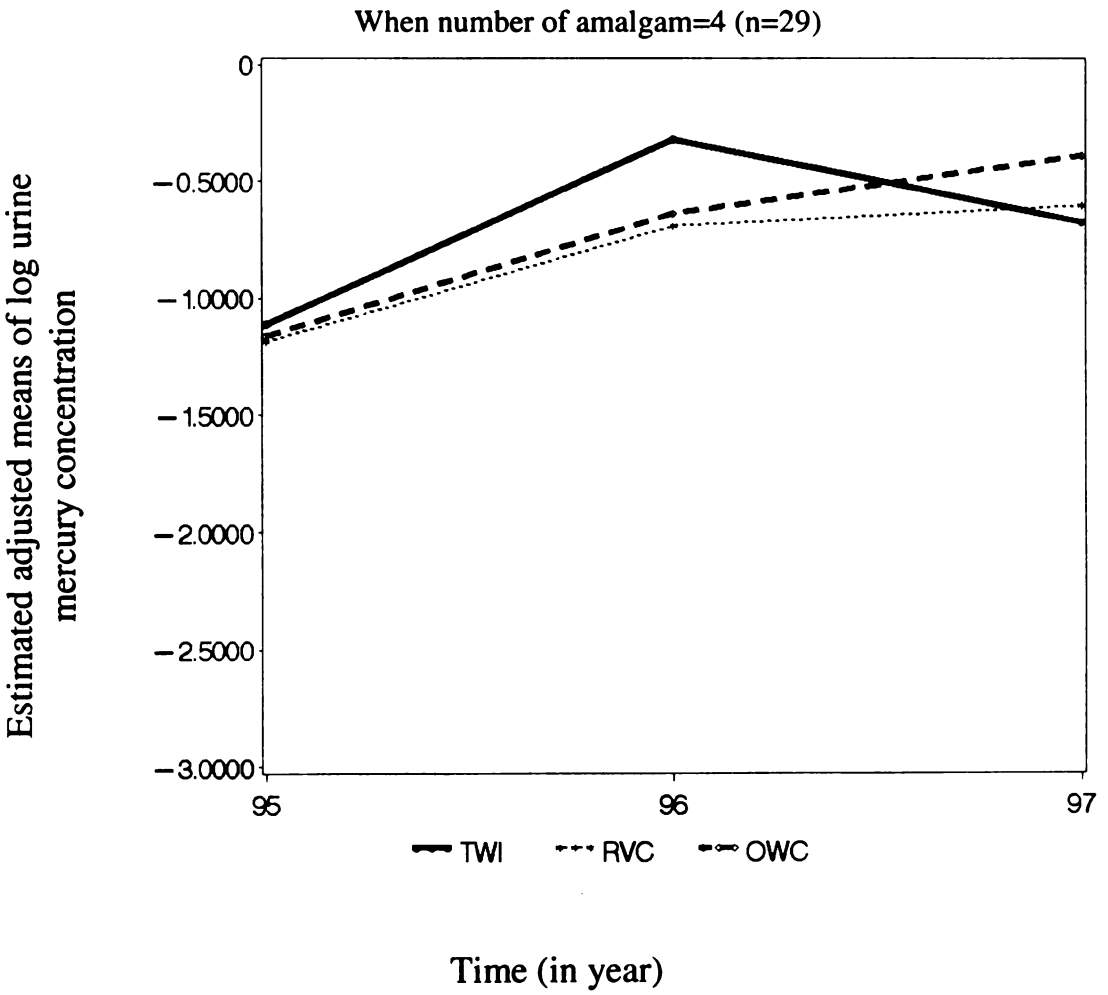
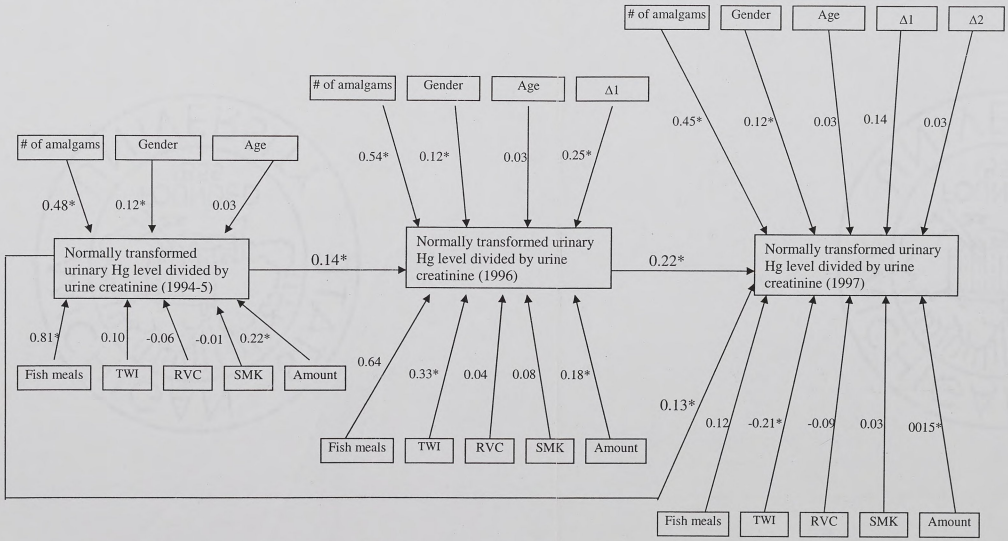


Figure3. Estimated adjusted means of log urine mercury concentration versus time in three different regions (cont'd):

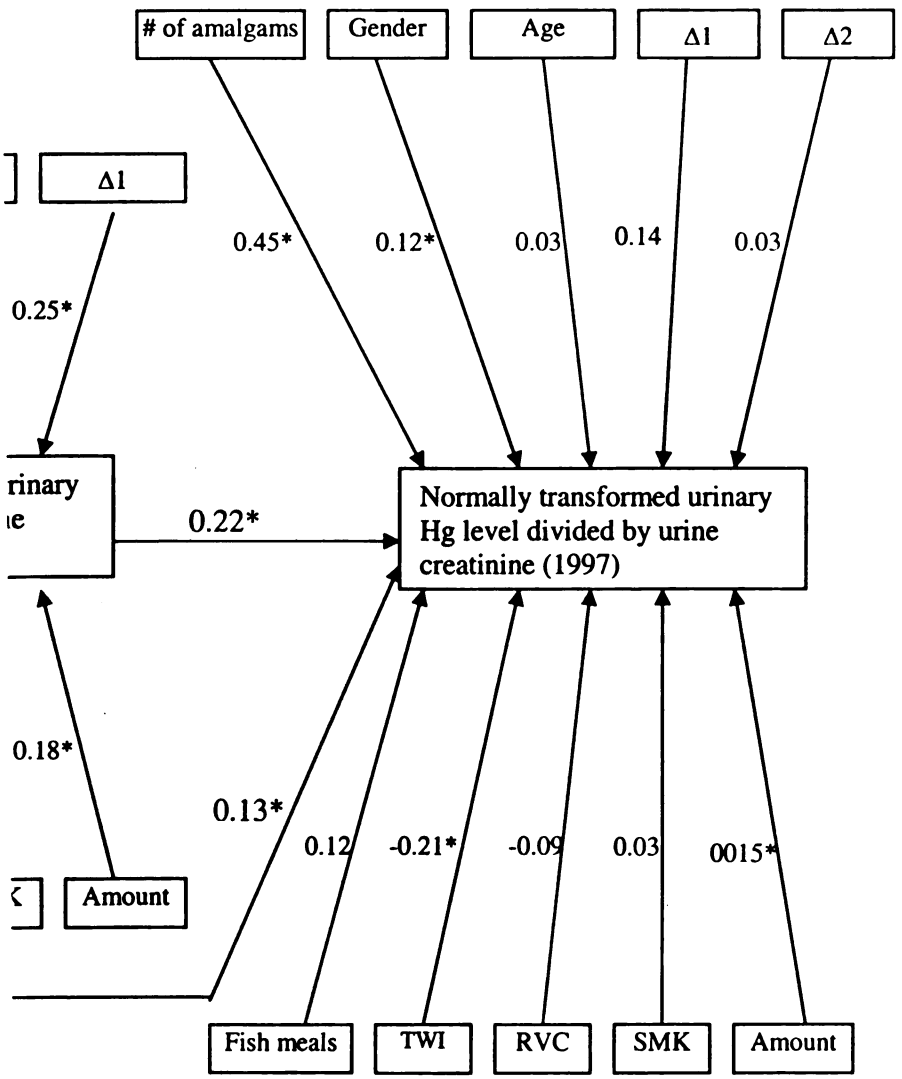


Path Diagram 1: The possible causal model for normally transformed creatinine corrected urinary mercury (Hg) levels:



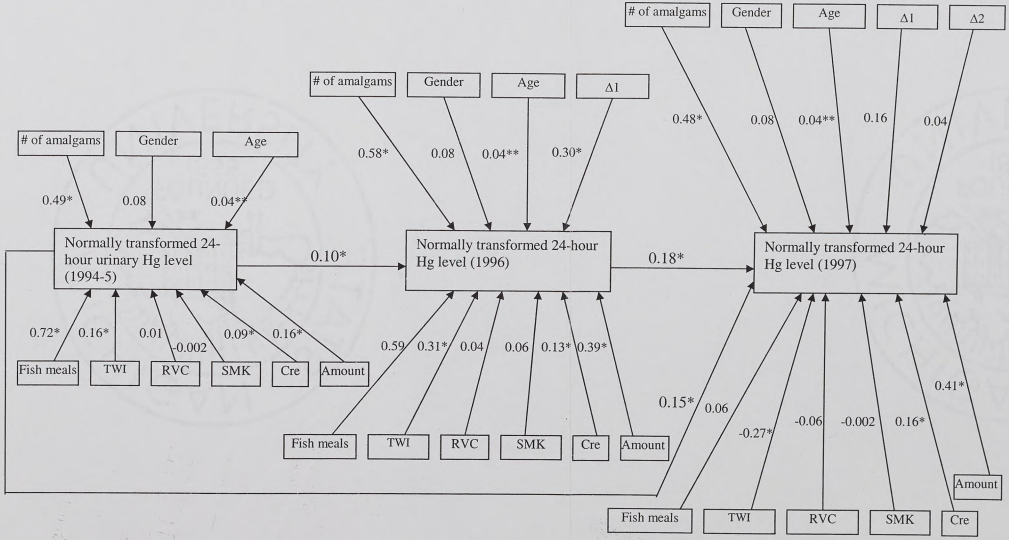
*: $p < 0.05$ two-sided test. Δ1 = the number of amalgam fillings in 1996 minus the number of amalgam fillings in 1995; Δ2 = the number of amalgam fillings in 1997 minus the number of amalgam fillings in 1996; TWI: Toxic Waste Incinerator Group; RVC: Rhine Valley Control Group; Cre: creatinine in urine; Amount: 24-hour total urine amount. SMK: Number of cigarette smoked by their parents.

tinine corrected urinary mercury (Hg) levels:



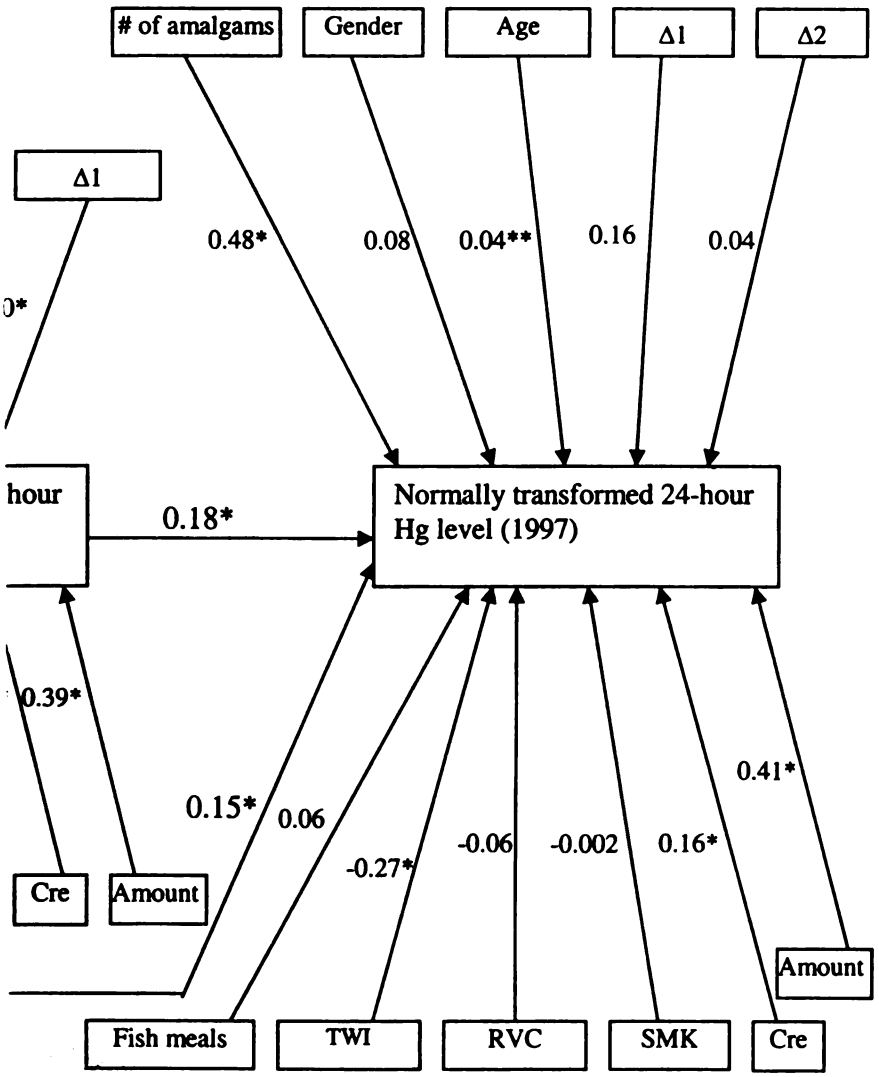
ıinus the number of amalgam fillings in 1995; Δ2 = the
. in 1996; TWI: Toxic Waste Incinerator Group; RVC:
:al urine amount. SMK: Number of cigarette smoked by

Path Diagram 2: The possible causal model for normally transformed 24-hour urinary mercury (Hg) levels:



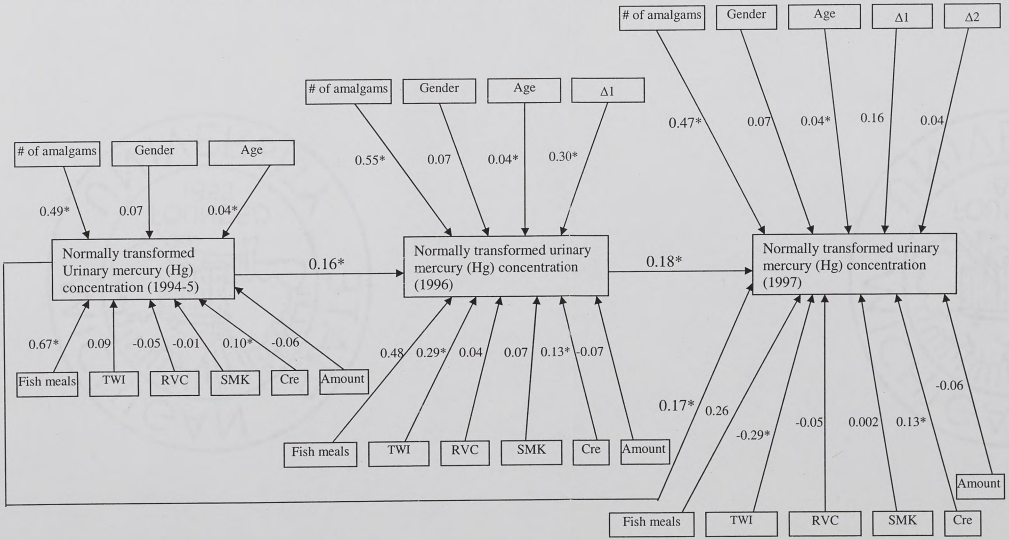
**: $p < 0.05$ one-sided test; *: $p < 0.05$ two-sided test. Δ1 = the number of amalgam fillings in 1996 - the number of amalgam fillings in 1995; Δ2 = the number of amalgam fillings in 1997 - the number of amalgam fillings in 1996; TWI: Toxic Waste Incinerator Group; RVC: Rhine Valley Control Group; Cre: creatinine in urine; Amount: 24-hour total urine amount; SMK: Number of cigarette smoked by their parents.

ur urinary mercury (Hg) levels:



nalgam fillings in 1996 - the number of amalgam fillings
;am fillings in 1996; TWI: Toxic Waste Incinerator
: 24-hour total urine amount; SMK: Number of cigarette

Path Diagram 3: The possible causal model for normally transformed urinary mercury (Hg) concentrations:



*: $p < 0.05$ two-sided test. Δ1 = the number of amalgam fillings in 1996 - the number of amalgam fillings in 1995; Δ2 = the number of amalgam fillings in 1997 - the number of amalgam fillings in 1996; TWI: Toxic Waste Incinerator Group; RVC: Rhine Valley Control Group; Cre: creatinine in urine; Amount: 24-hour total urine amount; SMK: Number of cigarette smoked by their parents.

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