ABSTRACT

CAMPBELL L. TUSKEY. Unfolding the Pharmacokinetic and Exposure Variability From Overall Interindividual Variability for 1,2,3,4,6,7,8,9-Octachlorodibenzo-*p*-dioxin (OCDD) and Mercury. (Under the direction of Dr. Douglas J. Crawford-Brown)

There is variability in the level of environmental chemicals in individual's bodies. This variability is composed of exposure variability and pharmacokinetic parameter variability. In the present study, two compounds, 1,2,3,4,6,7,8,9-octachlorodibenzo-*p*-dioxin (OCDD) and mercury, were examined. Data from the National Health and Nutrition Examination Survey (NHANES) were used to represent overall variability. Models were used to predict pharmacokinetic variability. The variability in exposure was unfolded from the pharmacokinetic variability and the contribution of each type of variability to overall variability was determined. The results showed that most of the overall variability observed was due to exposure variability.

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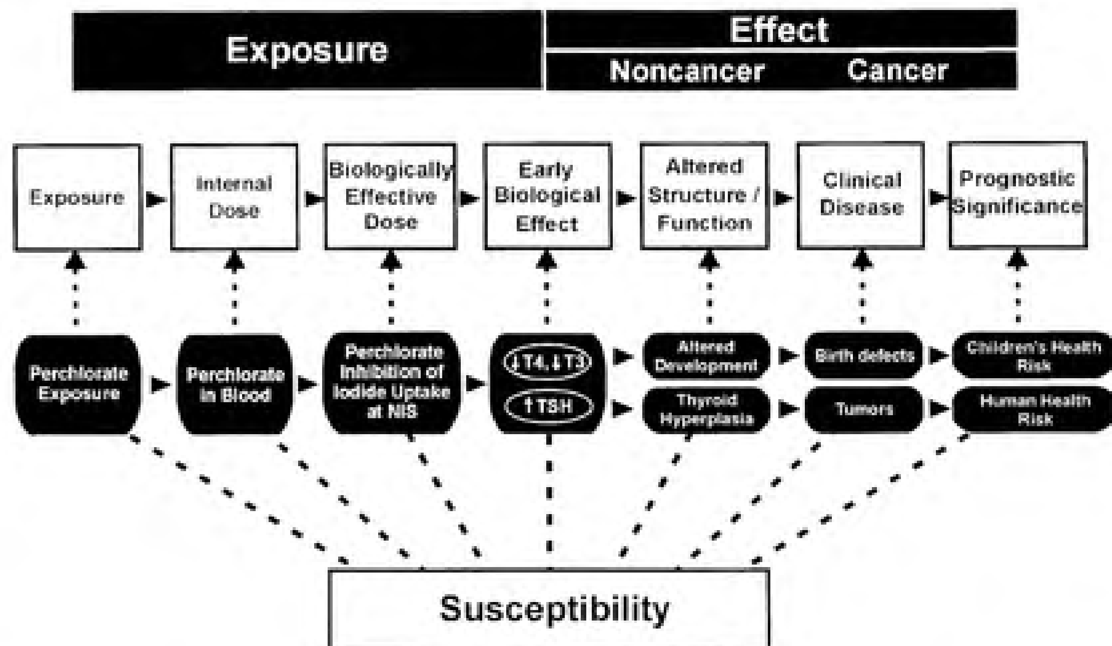
INTRODUCTION

In modern society, exposure to chemicals in the environment is inevitable. Factories emit a host of chemicals into the air in plumes. Industries dispense of their waste in streams, rivers, and lakes. And, on a smaller scale, individuals throw their household toxics into landfills and down the drain into water bodies. Once the chemicals are in the environment, they are taken up by people into their bodies.

While environmental chemicals are found in all of our bodies, there is variability in the concentration found in individuals in a population. Accounting for this variability can help to target the populations at greatest risk. As recognized by several researchers, this variability can be due to variability in exposure and variability in the relationship between exposure and dose (also described as variability in pharmacokinetics).

Figure 1 illustrates the mode of action of an environmental chemical developed by the US Environmental Protection Agency (EPA) (Farland and Jarabek 2003). This diagram is specific to perchlorate, but can be applied to all environmental chemicals. It includes most of the components that influence an individual's susceptibility to a chemical. At any stage as a chemical travels through the body, variability between individuals may be observed. The present study focuses on the first two components in this model, exposure and internal dose.

Figure 1
EPA Mode of Action Model



Clewell and Andersen (1996) clarify the first two types of variability shown in Figure 1: exposure and response. Exposure variability, as the name implies, refers to differences in the exposure of individuals to a substance. As discussed above, exposure to environmental chemicals is practically unavoidable, however, the amount and extent of that exposure is variable between people. A person may live one mile from a source and another person may live five miles from a source. Or, two people may both live one mile from a source, but person A was at home when an accidental spill occurred and person B was at work. Alternatively, response variability recognizes that under identical exposure conditions, individuals will show responses at different times and levels of severity, depending on pharmacokinetic factors (the relationship between exposure and the concentration in target organs), pharmacodynamic factors (the relationship between concentration and primary damage), or sensitivity to primary damage.

Hattis and Silver (1994) further subdivide what they call the susceptibility component (Clewell and Andersen's response component) into three types: uptake, pharmacokinetic, and response. Uptake variability refers to differences in the concentration needed in the environment to produce a given intake, usually due to differences in breathing rate, or eating habits. Pharmacokinetic variability refers to differences in the amount of uptake needed to produce a concentration-time product of toxicant in the blood or other organ, usually due to metabolic differences (shown as internal dose in Figure 1). These differences can include metabolism rate constants, partitioning coefficients, and half-lives. And finally, response variability refers to differences in the dose at the active site that produces a given level of response (shown as biologically effective dose in Figure 1).

Recognizing the different types of variability is not enough. It would be useful to understand the relative contributions of different sources of variability to overall variability in the relationship between exposure and response. A recent survey performed by the Centers for Disease Control (CDC), the National Health and Nutrition Examination Survey (NHANES), provided data on the combined effects of exposure and pharmacokinetic variability. This survey measured, among other things, levels of environmental chemicals in the blood, urine, and hair of a representative sample of the U.S. population.

Based on the toxicology of compounds and selected studies, there is information on the variability due to pharmacokinetics and pharmacodynamics of some of the compounds

measured. There has been a considerable amount of work done in the area of pharmacokinetic variability. Many of these studies have been performed with the goal of determining the effectiveness of a therapeutic drug for the majority of the population. These studies, along with the toxicological characteristics of a compound, can give us insights into the contribution of variability in the pharmacokinetics to the overall variability in individual concentrations of environmental chemicals in the blood and tissues. For example, there are data that quantify the variability of such parameters as the elimination half-life, area under the curve, and the peak concentrations (Jochemsen *et al.* 1982).

There has also been a considerable amount of work done in the area of exposure variability. For many different types of chemicals, researchers have used several techniques to quantify differences in exposure to environmental chemicals. These techniques include measuring the diversity of people's activities (Wallace 1989) including the National Human Activity Pattern Survey (NHAPS) (Klepeis *et al.* 2001), the compilation of this data into databases such as the Consolidated Human Activity Pattern Database (CHAD) (Klepeis *et al.* 2001), and field studies measuring the variability in concentrations of chemicals in various media (Garcia *et al.* 2001).

The data from the NHANES survey represents overall variability which includes contributions from both variability in pharmacokinetics and variability in exposure. By incorporating data measuring the variability in pharmacokinetic parameters into models of compounds measured in the NHANES survey, we can predict the expected variability

due to pharmacokinetic parameters alone. Then, by unfolding the variability due to pharmacokinetics from the overall variability measured by the NHANES survey, we will better be able to understand the variability in exposure alone. In this way, it will be possible to determine the relative contribution of pharmacokinetic and exposure variability to overall variability.

Specifically, this project focuses on two compounds, 1,2,3,4,6,7,8,9-octachlorodibenzo-*p*-dioxin (OCDD) and mercury. The NHANES data and the variability in pharmacokinetic parameters are available for both of these compounds. Using these data, the pharmacokinetic variability is unfolded from the overall variability to determine the exposure variability. Once the pharmacokinetic and exposure distributions have been established, the relative contribution of the two types of variability to overall variability can be concluded.

LITERATURE REVIEW

Variability

As discussed in the introduction, several types of variability are recognized. In general, there can be variability in the exposure to environmental chemicals and variability in an individual's susceptibility to environmental chemicals. The susceptibility variability can be further subdivided into uptake, pharmacokinetic, and response variability.

Just as there are several types of variability, there are also several causes of variability. Exposure variability can be caused by differences in activity patterns between people or variation in the time and concentration of an environmental release. As Pierce *et al.* (1996) explained, factors such as weight, body composition, alcohol consumption, nutrition, and interactions among toxicants have been shown to contribute to variability in the susceptibility of individuals to chemical exposures. A study by Sato (1991) discussed several factors that may influence the pharmacokinetics of organic solvents including the external environment, medications, cigarette smoking, eating and drinking habits, and simultaneous exposure to multiple chemicals. The study specifically looked at these effects by using a PBPK model. The effects of alcohol consumption on the pharmacokinetics of trichloroethylene (TCE) and the effects of metabolic interaction between benzene and toluene on the pharmacokinetics of each substance were assessed.

For the alcohol consumption scenario, alcohol was modeled to both inhibit and induce certain enzymes in the rate-limiting step of TCE metabolism (which is the catalyzation of TRI to chloral hydrate by cytochrome P-450). It was found that the lower the exposure to alcohol, the higher the inhibitory effects and conversely, enzyme induction is only seen at higher exposures to alcohol. For the benzene and toluene scenario, Sato assumed that the two compounds competitively inhibit the metabolism of the other. It was found that the metabolism of each compound was inhibited slightly at low concentrations and increasingly with growing exposure concentrations.

Having established that variability does exist in the population, it is necessary to discuss methods for further studying it. Dale Hattis has done much work in this area (Hattis *et al.* 1987; Hattis and Burmaster 1994; Hattis and Silver 1994; and Hattis *et al.* 1999). In several publications he discussed types of data on variability that are available, mathematical methods for treating these data, and conclusions to be drawn. Hattis and his colleagues have assembled a database of studies that measure interindividual variability. For the most part, these studies are from the pharmacology field. The parameters they measure are half-life, the area under the curve (AUC), and the peak concentration in blood (Hattis *et al.* 1987). When treating measurements of these parameters, several considerations must be made. In many cases, summary statistics are used to evaluate the measured data and quantify variability (Hattis *et al.* 1987 and Hattis and Burmaster 1994). To use summary statistics, the distributional form of the data must first be determined. While there are several distributional forms, Hattis and Silver (1994) found that many data are described by a lognormal distribution. This type of distribution

is expected when many factors contribute to the variation, acting multiplicatively. Assuming this, statistics that define the distribution are the mean and variance or the geometric standard deviation and geometric mean. In this study, the geometric standard deviation and geometric mean were calculated. One possible representation of variability is the log(geometric standard deviation) (Hattis *et al.* 1987), while the geometric standard deviation can also be used.

Similar Studies

Several studies were found that have explored overall interindividual variability, or pharmacokinetic and exposure variability individually. These studies are summarized below.

A study conducted by Pierrehumbert and his colleagues in 2002 quantified the effect of human variability in physiological and pharmacokinetic parameters on a distribution of values of biological indicator measurements. Specifically, the study used a general toxicokinetic model and applied it to toluene, phenol, mercury, and lead. While the exposure was held constant, the parameters were varied either randomly or scaled on body weight and 500 sets of parameters were generated by Monte Carlo simulation and introduced into the model. Therefore, for each chemical, 500 toxicokinetic profiles were obtained and the following statistics were calculated: geometric mean, the 95% confidence interval, and the variability extent index (VEI) which is equal to the ratio of 95% confidence interval limits. The VEI was used as an indication of variability.

The results from this study established the presence of variability in biological monitoring results and indicated that the variability in these results can be high (VEI ranged from 2.7 to 9.5). These results were compared with VEIs from pharmacokinetic studies, which had a median of 2.7 and air exposure studies, which had a VEI of 16. The authors concluded that the large interindividual variability should be taken into consideration when interpreting biological monitoring results.

Clewell and Andersen (1996) discussed a method to perform a Monte Carlo analysis to assess how interindividual variability in pharmacokinetic and metabolic parameters contributes to the variability in susceptibility to a chemical. A PBPK model should be run with parameter values sampled from a distribution that reflects the variability in one or more parameters in the human population. Generally, this type of distribution can be found in the literature. The model should be run at least 200 times with a different set of sampled parameter values each time. Each model run results in the dose metric of interest, and possibly the resulting risk metric.

This process was followed to determine the cancer risk of three chemicals and the noncancer risk of two chemicals. The distributions of parameter values were determined according to observed variation in the population obtained from the literature and used to run a PBPK model specific to each chemical. The models were run assuming that the parameters varied independently, however it is also possible that parameters are correlated with one another. Each model run was defined by a different set of parameter

values and resulted in various dose and risk metrics which were of interest in each case. Once the model had been run a sufficient number of times, a distribution of risk was generated. The cancer risk estimates varied from 3-fold to 12-fold between the 5th and 95th percentiles. The noncancer risk estimates both differed by just under 3-fold. These results are consistent with other measures of variability found in pharmacokinetic parameters (Hattis *et al.* 1987 and Hattis and Silver 1994).

A study by Pierce *et al.* (1996) used parameter values from individuals in a PBPK model for toluene and analyzed the effect of using person-specific values. Twenty-six men were exposed to controlled levels of toluene in the air. Blood samples were taken before and after the exposure to measure blood levels of toluene. A PBPK model was constructed that could incorporate subject-specific values of body weight, adipose tissue fraction, partition coefficient, exposure concentration, and alveolar ventilation rate. Once this was developed, a sensitivity analysis was performed to determine which parameters most influenced the results. To test whether the model results were improved by using subject-specific parameter values, the model goodness-of-fit was determined using subject-specific values, average values from the subjects, and average values from the literature. Results showed that by using subject-specific values in the model 91% of the variability in the data was explained, by using average values from the subjects 81% of the data variability was accounted for, and by using average values from the literature 53% of the data variability was explained. While this study controlled the level of exposure, it nevertheless showed that interindividual variability in physiological and pharmacokinetic parameters contributed to the variability that was seen.

In a study by Sweeney *et al.* (2003) a PBPK model was developed for the disposition of acrylonitrile (ACN) and its metabolite, cyanoethylene oxide (CEO) in humans. Once developed, the authors performed sensitivity and variability analyses on the model parameters for several exposure scenarios. The sensitivity analysis indicated which parameter values were the most important in calculating the dose metrics of interest. The variability analysis was performed to identify the parameters of importance in population variance. The parameters that contributed the most to the variability in blood and brain concentrations of CEO were the reaction rate of CEO with glutathione, the hydrolysis rate for CEO, and the blood:brain partition coefficient of CEO. For the variability in blood concentrations of ACN, the greatest contributor was the V_{max} for the conversion of ACN to CEO. The authors conclude that the human variability in the blood concentration predictions is modest and falls well within the default pharmacokinetic uncertainty factors that are used in risk assessment.

Finally, a study by Thomas *et al.* (1996) used a PBPK model to test the level of safety that is provided to the population of occupationally exposed people by the threshold limit values (TLV) that have been established for compounds. These levels were originally established without regard for interindividual variability. Therefore, Thomas and his colleagues used a PBPK model and Monte Carlo simulation techniques to perform a pharmacokinetic evaluation on a large number of individuals with varying physiological and metabolic parameters. For each parameter in the model, a mean and a dispersion was obtained from the literature. These were mainly in the form of percentages or standard deviations. From these measures, a coefficient of variation (standard deviation/mean)

was calculated for each parameter. Incorporating these values into the model and using an exposure concentration equal to the TLV yielded data from which the authors could determine the percentage of the population that was protected at various concentration levels. Based on the results of this study, the authors concluded that, for the compounds measured, there is an inconsistency in the percentage of the population protected under the current TLV. For example, end-expired indices for benzene protect 95% of the population while end-expired indices for methyl chloroform only protect 10%. Furthermore, the recommendation for phenol metabolite concentration protects 68% of the population and the recommended trichloroethanol concentration for methyl chloroform protects 97% of the population.

There are several points to summarize from the above studies that are important for the present study. Overall variability can be represented in biological monitoring results, as shown in Pierrehumbert *et al.* (2002). However, the variability found in monitoring of blood concentrations has been shown to be modest (Sweeny *et al.* 2003). Additionally, models can be used to determine the variability due only to pharmacokinetic factors. As demonstrated in studies by Clewell and Anderson (1996) and Thomas *et al.* (1996), using a method involving a Monte Carlo analysis can facilitate this determination. When this has been done, several studies have shown that while pharmacokinetic variability does contribute to overall variability (Pierce *et al.* 1996), it alone does not explain all of the variability (Pierrehumbert *et al.* 2002). Exposure variability has also been shown to be important.

NHANES DATASET AND SELECTED COMPOUNDS

NHANES

The dataset used in the current study was taken from the National Health and Nutrition Examination Survey (NHANES). The NHANES survey selected a sample of the noninstitutionalized population by using a complex, stratified, multistage, probability-cluster design (NCEH 2003). The process of selection of the sample was as follows: (1) Selection of 15 primary sampling units (PSUs) which are counties or groups of contiguous counties; (2) Selection of segments within the PSUs (ie. neighborhood blocks); (3) Selection of households within PSU segments; and (4) Selection of one or more participants within each household (CDC 2003). In order to collect the needed data, the NHANES survey included a home interview and a physical examination in a mobile examination center. The physical examination included taking a blood sample for participants older than 1 year and a urine sample for participants older than 6 years (NCEH 2003). Within the larger context of the NHANES survey, measurements were also made of the levels of environmental chemicals. This was done in randomly selected subsamples of the full survey within specific age groups. This was necessary to obtain a large enough quantity of sample for analysis and to accommodate mass-spectrometry methods (NCEH 2003).

In January of 2003, the Centers for Disease Control (CDC) released the results from the *Second National Report on Human Exposure to Environmental Chemicals*. This report gives the levels of 116 environmental chemicals in a sample of peple representative of the U.S. population measured during 1999-2000, as measured by the NHANES survey. The levels of chemicals and, where appropriate, their metabolites were measured in the blood and urine, and in one case the hair. It is planned that future reports of this nature will be released every two years with data for additional environmental chemicals. In the current report, the chemicals were selected on the basis of six criteria:

(1) scientific data showing exposure to Americans;

(2) seriousness of health effects resulting from exposure;

(3) need to assess efforts to reduce the exposure;

(4) availability of a reasonable biomonitoring analytical method;

(5) availability of adequate blood and urine samples;

(6) cost to perform the analysis (NCEH 2003).

The present analysis will focus on two of the compounds measured in the NHANES survey, OCDD and mercury. These compounds were chosen based on the following criteria: (1) the number of detectable data points available; (2) the availability of models in the literature that explain the pharmacokinetics and/or toxicokinetics of the compound and include the dominant method of exposure and a compartment modeling the concentration that was measured (generally blood or urine); and (3) the availability of

data in the literature on the distribution of the pharmacokinetic parameter to vary. Mercury and OCDD were both measured in the blood. OCDD was measured in male and female participants older than 12 years. Total (organic + inorganic) mercury was measured in male and female participants ages 1-5 and female participants ages 16-49 (NCEH 2003).

In the present study, for OCDD, detectable data points for subjects ages 20-85 were used. One measurement was made in each subject. Table 1 presents several summary statistics for this data set.

Table 1
OCDD Measured Data Summary Statistics

Statistic	Value
Subjects	995
Minimum	603.4 fg/g
Maximum	49612.7 fg/g
Mean	3296.4 fg/g
Median	2361.7 fg/g
Standard Deviation	3503.0
Interquartile Range	2431.2

For mercury, detectable data points for female subjects ages 16-49 were used. One measurement was made in each subject. Table 2 presents several summary statistics for this data set.

Table 2
Mercury Measured Data Summary Statistics

Statistic	Value
Subjects	1611
Minimum	0.2 µg/L
Maximum	38.9 µg/L
Mean	1.8 µg/L
Median	1.0 µg/L
Standard Deviation	2.7
Interquartile Range	1.4

Biological Markers

The measurements made in the NHANES survey were of biological markers. Also referred to as biomarkers, these have been used to assess the levels of exposure to chemicals. They have traditionally been thought to represent molecular and/or cellular changes in the body that occur over time as a chemical is processed through the body. Specifically, they reflect the internal dose of a chemical or the amount that has crossed physiological barriers and entered the body. The magnitude of a biomarker depends on several factors including the route of exposure, physiological characteristics of the receptor, and chemical characteristics of the substance. Depending on the substance, measurement of the internal dose of a substance may include measurement of the substance itself or of a metabolite, if that metabolite is critical to the toxic effects. Criteria for the choice of a particular biomarker include biological relevance, sensitivity, specificity, accessibility, cost, and feasibility. Because a biomarker should be accessible, measurement usually occurs in the blood or urine as these procedures are less invasive than measurement in other tissues (Paustenbach 2001).

There are available biomarkers for dioxins. The predominant storage sites for dioxin in the body are adipose tissue and the liver. Several studies have shown the relationship between blood levels of dioxin and levels in adipose tissue or the liver. For OCDD, there is a constant 2:1 ratio between serum levels and adipose tissue fractions (Schecter *et al.* 1990). Therefore, blood levels of OCDD are a reasonable biomarker to use.

Blood and urine measurements are the most common biomarkers used for mercury. It has been shown that blood levels of mercury peak immediately following a short-term exposure (Cherian *et al.* 1978), however, for chronic exposure to mercury, mercury levels in the blood will remain elevated long after cessation of exposure (Lindstedt *et al.* 1979). Realizing this, blood levels of mercury are a reasonable biomarker for both evaluation of acute and chronic exposures.

Exposure to and Toxicology of Dioxins

Polychlorinated dibenzodioxins (dioxins) are a class of chlorinated hydrocarbons. In general, the structure is a dibenzo-*p*-dioxin molecule with two benzene rings joined by two oxygen bridges at adjacent carbons on each benzene ring. The most studied dioxin is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) which is also the most toxic dioxin. However, the present study explores variation in blood levels of 1,2,3,4,6,7,8,9-octachlorodibenzo-*p*-dioxin (OCDD), which, as the name implies has eight chlorine molecules attached at various positions. Dioxins are found in the environment as the result of man-made releases and contamination. Specifically, dioxins are formed when

chlorine compounds are burned along with hydrocarbons. This combination is found frequently in hazardous waste incinerators and paper mills. Exposure to humans is mainly through foods such as dairy products, eggs, and foods containing animal fats. The presence of dioxins in food is the result of dioxins in the environment that accumulate up the food chain.

Dioxins are highly persistent lipophilic compounds. They are resistant to biodegradation and have the potential to bioaccumulate. Therefore, a single chemical analysis of levels in the blood represents a measure of past cumulative exposure (ATSDR 1998). Dioxins are well absorbed through oral exposure. The liver and adipose tissue are the major storage sites for dioxins. The primary routes of excretion are the bile and feces and to a much smaller extent, the urine. Data on the metabolism of dioxins is available from animal studies only. Phase I processes that work in the metabolism of dioxin include oxidation and reductive chlorination and oxygen bridge cleavage. After this, conjugation catalyzed by phase II enzymes follows. This facilitates excretion by adding more polar groups to the molecule. Since dioxin metabolites are generally not detected in tissues, it seems that dioxin is excreted soon after metabolism (ATSDR 1998). The elimination half-life of OCDD, specifically, has been shown to be 6.7 years in one study and 10 years in another (Flesch-Janys *et al.* 1996 and Rohde *et al.* 1997). Effects of dioxin are thought to be controlled through an interaction with the aryl hydrocarbon receptor (AHR), particularly the induction of gene expression for cytochromes P450, CYP1A1, and CYP1A2 (NCEH 2003). TCDD is the most potent of the dioxins. Because of this, all dioxin congeners can be assigned a toxic equivalency factor (TEF) relative to TCDD.

Multiplying the TEF by the concentration of the specific congener yields a toxic equivalency (TEQ) value for that specific compound. The sum of the TEQ values is known as the total TEQ. In general, the congeners that have less potency are found in higher environmental concentrations. Therefore, relative contributions of individual congeners to the total TEQ are roughly equal (NCEH 2003).

Exposure to and Toxicology of Mercury

Mercury is a naturally occurring metal found in metallic, inorganic, and organic forms. For the purposes of this study, only inorganic and organic mercury are discussed. The main exposure route to mercury, in the form of methyl mercury, is through food. Mercury is released into the environment through the use of organomercury fungicides, and the use of mercury in plastics, paper, and battery manufacture. Once released, mercury is biotransformed into methyl mercury in water and soil by microorganisms. This form of mercury concentrates in the food chain. Fish consumption is the major source of methyl mercury exposure for humans. In blood measurements, current analytical techniques provide for measuring levels of total mercury and levels of inorganic mercury. Levels of organic mercury cannot be measured directly from the blood. Therefore, the difference between total mercury and inorganic mercury in the blood represents the organic mercury level in the blood (Miles 1977).

Organic mercury, usually in the form of methyl mercury, is readily absorbed in exposure through food. After absorption through the gastrointestinal tract, it distributes throughout

the body with the highest accumulation in the kidneys. The ability of organic mercury to cross the blood-brain barrier and the placental barrier also allows for accumulation in the brain and potentially the fetus. The distribution of organic mercury in the body is due to its ability to cross diffusion barriers and penetrate membranes easily (Aberg *et al.* 1969). Tissue concentrations tend to remain constant relative to blood levels. Therefore, blood levels are a good indicator of tissue concentrations independent of dose (ATSDR 1999). Metabolism of methyl mercury is first caused by the degradation to inorganic mercury. This occurs through a reaction with hydroxyl radicals produced by cytochrome P-450 reductase (ASTDR 1999). Once reduced, the mercury cation enters an oxidation-reduction cycle. The elimination of methyl mercury is primarily through the fecal pathway in the inorganic form. There is evidence of biliary reabsorption of methyl mercury as the result of a complex with nonprotein sulfhydryl compounds in the bile (Urano *et al.* 1990). This cycle may contribute to the long half-life of methyl mercury (ATSDR 1999).

Models

As will be discussed in more detail in the Methods section, models were utilized in this study to describe the activity of a chemical compound in the body. These models come in several types and can be as simple as a one-compartment model or as complex as a multi-compartment model which mimics the human body. For the most part, models are first developed in animal species that have a lot of available data. From these animal models, there are several techniques to extrapolate this information to humans. Specific

types of models are physiologically based pharmacokinetic and toxicokinetic models (PBP/TK). This type of modeling involves the computer simulation of the uptake and disposition of a chemical, taking into account its blood and tissue solubility, metabolism and protein binding in various tissues, and the physiology of the organism (Andersen and Krishnan 1994). They are used to simulate the kinetic behavior of the chemical in a species and to predict the tissue dosimetry of the toxic component (Andersen and Krishnan 1994). The steps of model development include model representation, obtaining the numerical values of the various parameters, model simulations, and model validation. For a model to be validated, it must correctly predict the behavior of a chemical over a variety of exposure situations using data other than those used to produce the parameter values for the model.

Pharmacokinetic and toxicokinetic models have traditionally been used for population-level applications and therefore have used average estimates of biological parameters (Pierce *et al.* 1996). However, it has been recognized that using average population estimates of parameters, instead of distributions that represent the range of interindividual variability may not develop the full range of risk estimates, suggesting that distributions should instead be used (Andersen and Krishnan 1994). On an individual-level, a few previous studies have developed and used PBPK models using subject-specific parameter values (Pierce *et al.* 1996 and Thomas *et al.* 1996). Using subject-specific values in models rather than average values generally yielded predicted concentrations closer to measured concentrations.

METHODS

Steps in Methodology

The following steps were used in the analysis for each compound. In the two sections that follow (OCDD and Mercury), the first four steps will be explained in detail specifically related to the two compounds. And in the Convolution section, steps five through twelve will be discussed in more detail.

1. Establish an appropriate model for each compound, building it and validating that it accurately predicts concentrations in various tissues.
2. Identify pharmacokinetic parameter(s) in the model appropriate to vary.
3. Develop a measure of variability for each parameter that will be varied from information found in the literature.
4. Run a Monte Carlo analysis varying the parameters chosen to simulate the pharmacokinetic distribution.
5. Verify that lognormal is a reasonable description for each of the pharmacokinetic and NHANES distributions.
6. Determine the measure of overall variability for the NHANES measured distribution and the measure of variability for the pharmacokinetic distribution for each compound.

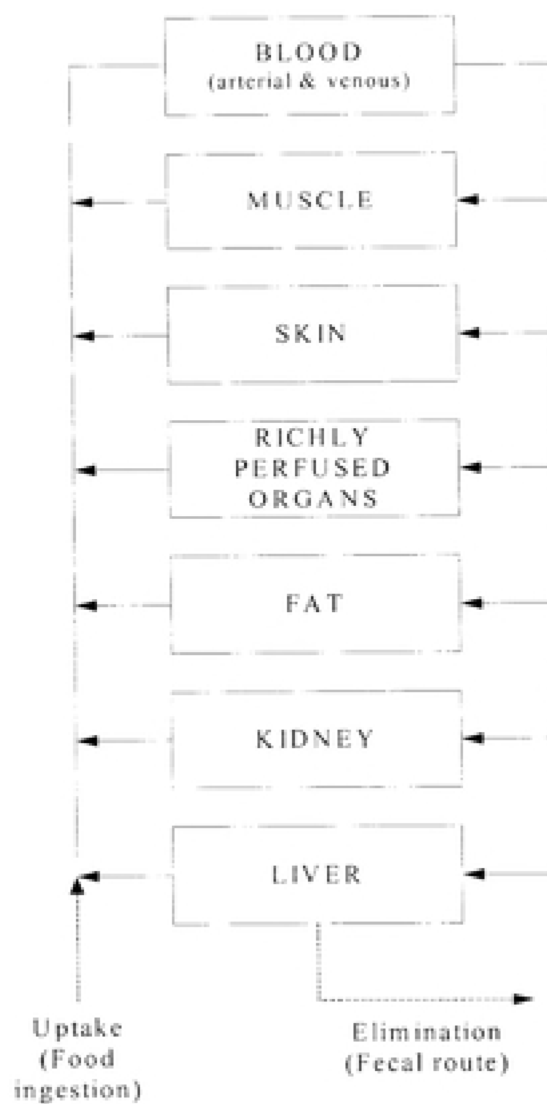
7. Simulate the variability in the exposure distribution by convolving with the pharmacokinetic distribution.
8. Compare the resulting distribution (convolved distribution) against the overall, measured distribution from NHANES.
9. If the convolved distribution is wider, reduce the variability in exposure and if narrower, increase the variability in exposure.
10. Repeat this procedure until the resulting, convolved distribution is the same as the measured NHANES distribution.
11. Determine the measure of exposure variability for the resulting exposure distribution.
12. Calculate the relative contribution of pharmacokinetic and exposure variability to overall variability.

<u>OCDD</u>

Exposure to dioxin is generally through food ingestion. Once it has entered the gastrointestinal tract, dioxin enters the liver, however, it only passes through the liver and into the blood. Once in the blood, it is distributed to other organs in the body and both phase I and phase II metabolism occurs. After metabolism, dioxin is eliminated mainly through the bile and feces. This process is described in more detail in the chapter entitled, "NHANES Dataset and Selected Compounds."

A model developed by Maruyama *et al.* (2003) to quantify the relationship between exposure and concentration in body tissues of dioxins was established for OCDD. This model was developed to be used for a full range of dioxins, although for the purposes of this study it was only applied to OCDD. A schematic diagram of the model is shown in Figure 2. The compartments contained within this model represent the following organs: the liver, kidney, fat, blood, muscle, richly perfused tissue, and skin. This model assumes that the rate of flow in a compartment is proportional to the perfusion of that compartment. The exposure route was assumed to be solely food ingestion and the elimination route the liver.

Figure 2
OCDD Model Schematic Diagram from Maruyama *et al.* (2003)



Flow between the compartments was described by mass balance equations which determine the concentration in a specific tissue at a given time. The equations are as follows:

The concentration of OCDD in the fat, muscle, richly perfused tissue, and skin compartments (represented by C_i) at a given time is represented by the following equation:

$dC_i/dt = Q_i * (C_{blood} - C_i/R_i)/V_i$

where:

Q_i the blood perfusion rate for each tissue. This was calculated by Maruyama *et al.* (2003) from Snyder *et al.* (1975) using tissue volumes and the blood content in each tissue.

C_{blood} the initial OCDD concentration in blood. This was calculated by Maruyama *et al.* (2003) from dioxin concentration data in Japanese breast milk reported in Ogaki *et al.* (1987). These calculations were transmitted to the author in a personal communication (Maruyama 2004a).

C_i the initial OCDD concentration in each tissue. This was calculated by Maruyama *et al.* (2003) from dioxin concentration data in Japanese breast milk reported in Ogaki *et al.* (1987). These calculations were transmitted to the author in a personal communication (Maruyama 2004a).

R_i the tissue-blood partition coefficient. These were determined through various means by Maruyama *et al.* (2003). For the fat, muscle, and richly perfused tissue they were calculated using either dioxin concentration data in human tissues, if available (Iida *et al.* 1999a) or based on structural information of the chemicals (Parham *et al.* 1997). For the skin, the partition coefficient was calculated using dioxin concentrations in serum and blood (Iida *et al.* 1999b).

V_i the tissue volume. These were calculated from the tissue weight (Snyder *et al.* 1975) and tissue density (Kissel and Robarge 1988) cited in Maruyama *et al.* (2003).

The concentration of OCDD in the blood compartment (represented by C_{blood}) at a given time is represented by the following equation:

$$dC_{blood}/dt = \{(Q_{liver} * C_{liver}/R_{liver}) + (Q_{fat} * C_{fat}/R_{fat}) + (Q_{kidney} * C_{kidney}/R_{kidney}) + (Q_{muscle} * C_{muscle}/R_{muscle}) + (Q_{rich} * C_{rich}/R_{rich}) + (Q_{skin} * C_{skin}/R_{skin}) - C_{blood} * (Q_{liver} + Q_{fat} + Q_{kidney} + Q_{muscle} + Q_{rich} + Q_{skin}) + D * Abs\} / V_{blood}$$

where:

Q_i the blood perfusion rate for each tissue ("*i*" representing the specific tissue). This was calculated by Maruyama *et al.* (2003) from Snyder *et al.* (1975) using tissue volumes and the blood content in each tissue.

C_i the initial OCDD concentration in each tissue ("*i*" representing the specific tissue). This was calculated by Maruyama *et al.* (2003) from dioxin concentration data in Japanese breast milk reported in Ogaki *et al.* (1987). These calculations were transmitted to the author in a personal communication (Maruyama 2004a).

R_i the tissue-blood partition coefficient. These were determined through various means by Maruyama *et al.* (2003). For the liver, fat, kidney, muscle, and richly perfused tissue they were calculated using either dioxin concentration data in human tissues, if available (Iida *et al.* 1999a) or based on structural information of the chemicals (Parham *et al.* 1997). For

the skin, the partition coefficient was calculated using dioxin concentrations in serum and blood (Iida *et al.* 1999b).

D the daily intake of OCDD. This was obtained from Liem *et al.* (2000).

Abs the intestinal absorption rate. This was set according to a previous report (McLachlan 1993).

V_{blood} the tissue volume of blood This was calculated from the tissue weight (Snyder *et al.* 1975) and tissue density (Kissel and Robarge 1988) cited in Maruyama *et al.* (2003).

The concentration of OCDD in the kidney compartment (represented by C_{kidney}) at a given time is represented by the following equation:

$dC_{kidney}/dt = Q_{kidney} * (C_{blood} - C_{kidney}/R_{kidney})/V_{kidney}$

where:

Q_{kidney} the blood perfusion rate for the kidney. This was calculated by Maruyama *et al.* (2003) from Snyder *et al.* (1975) using tissue volume of the kidney and the blood content in the kidney.

C_{blood} the initial OCDD concentration in blood. This was calculated by Maruyama *et al.* (2003) from dioxin concentration data in Japanese breast milk reported in Ogaki *et al.* (1987). These calculations were transmitted to the author in a personal communication (Maruyama 2004a).

C_{kidney} the initial OCDD concentration in the kidney. This was calculated by Maruyama *et al.* (2003) from dioxin concentration data in Japanese breast

milk reported in Ogaki *et al.* (1987). These calculations were transmitted to the author in a personal communication (Maruyama 2004a).

R_{kidney} the partition coefficient between the kidney and blood. This was calculated using either dioxin concentration data in human tissues, if available (Iida *et al.* 1999a) or based on structural information of the chemicals (Parham *et al.* 1997).

V_{kidney} the tissue volume of the kidney. This was calculated from the tissue weight (Snyder *et al.* 1975) and tissue density (Kissel and Robarge 1988) cited in Maruyama *et al.* (2003).

The concentration of OCDD in the liver compartment (represented by C_{liver}) at a given time is represented by the following equation:

$dC_{liver}/d_t = \{Q_{liver} * (C_{blood} - C_{liver}/R_{liver}) - C_{liver} * V_{liver} * K_1\}/V_{liver}$

where:

Q_{liver} the blood perfusion rate for the liver. This was calculated by Maruyama *et al.* (2003) from Snyder *et al.* (1975) using the tissue volume of the liver and the blood content in the liver.

C_{blood} the initial OCDD concentration in blood. This was calculated by Maruyama *et al.* (2003) from dioxin concentration data in Japanese breast milk reported in Ogaki *et al.* (1987). These calculations were transmitted to the author in a personal communication (Maruyama 2004a).

C_{liver} the initial OCDD concentration in the liver. This was calculated by Maruyama *et al.* (2003) from dioxin concentration data in Japanese breast

milk reported in Ogaki *et al.* (1987). These calculations were transmitted to the author in a personal communication (Maruyama 2004a).

R_{liver} the partition coefficient between the liver and blood. This was calculated using either dioxin concentration data in human tissues, if available (Iida *et al.* 1999a) or based on structural information of the chemicals (Parham *et al.* 1997).

V_{liver} the tissue volume of the kidney. This was calculated from the tissue weight (Snyder *et al.* 1975) and tissue density (Kissel and Robarge 1988) cited in Maruyama *et al.* (2003).

K_1 the elimination constant. This was calculated according to the following equation: $K_1 = BILE * L_{bile}/L_{liver}/V_{liver}$, where L_{bile} is the lipid content of the bile and L_{liver} is the lipid content of the liver. The lipid content of the bile was obtained from Snyder *et al.* (1975) and the lipid content of the liver was obtained from Iida *et al.* (1999a).

Table 3 shows the population mean values of the model variables used. Although the original source is noted above, most parameter values were used as cited in Maruyama *et al.* (2003).

Table 3: OCDD Model Parameters

Daily intake (D)			**1170.00 pg/day**
Intestinal absorption (Abs)			**76.00%**
Biliary excretion rate (BILE)			**255.00 mL/day**
Lipid content of liver (L_{liver})			**6.50%**
Lipid content of bile (L_{bile})			**1.90%**
Tissue volume (V)			**mL**
Muscle	23076.92	Fat	11180.43
Skin	2026.36	Kidney	253.33
Richly perfused organs	1747.12	Liver	1483.65
Blood	4204.72		
Blood perfusion rate (Q)			**mL/day/g tissue**
Muscle	43.20	Fat	25.92
Skin	34.56	Kidney	316.80
Richly perfused organs	675.36	Liver	283.68
Tissue-blood partition coefficients (R)			
Muscle	26.0	Fat	55.0
Skin	14.0	Kidney	2.8
Richly perfused organs	16.0	Liver	56.0
Initial dioxin concentration (C)			**pg/g tissue**
Muscle	1.798	Fat	75.714
Skin	1.798	Kidney	2.177
Richly perfused organs	3.786	Liver	5.679
Blood	0.426		

The model was validated to ensure that results similar to those reported in the literature were obtained. This was done by running the model with all of the parameter values as reported in Maruyama *et al.* (2003), for the time period reported. As shown in Figures 3 and 4, our model (smooth line) showed a small overestimation of model predictions (squares) at younger ages and a small underestimation of model predictions at older ages from Maruyama (2004b) in the blood and kidneys. For the purposes of this study, it was

important to capture the relative variation between two individuals with different parameter values. Eventhough there is a systematic bias in either direction in our model, the relative values between individuals will be represented correctly and thus the model was determined to be validated.

Figure 3
Concentration-Time Profile for OCDD in Blood

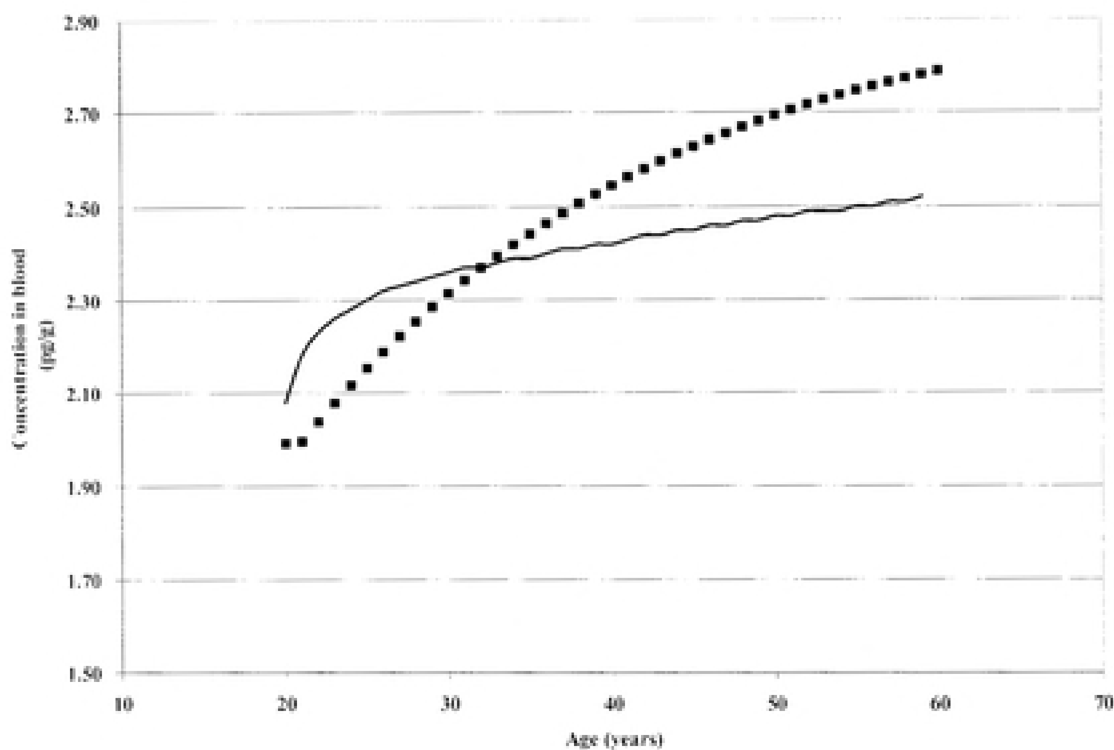
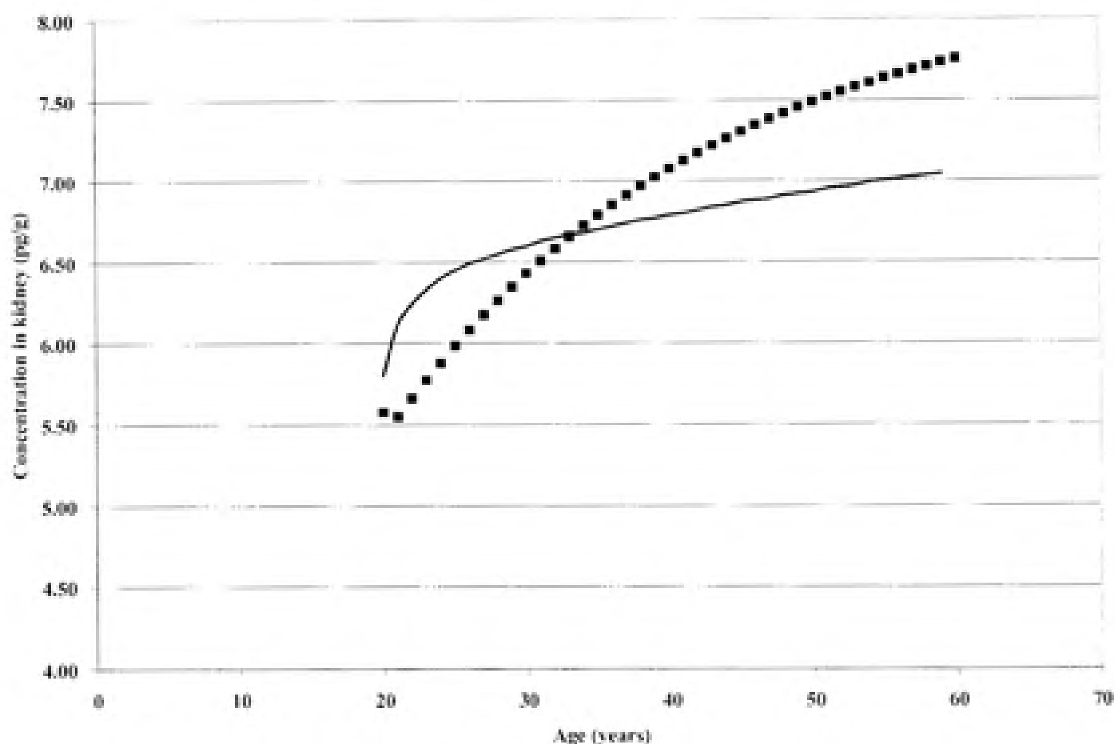


Figure 4
Concentration-Time Profile for OCDD in Kidneys



For OCDD, three parameters were identified to vary: the tissue-blood partition coefficients, the absorption rate, and the biliary excretion rate. Studies measuring interindividual variability in these parameters were identified in the literature. From these data, the coefficient of variation was calculated. This is the ratio of the standard deviation to the mean and is recognized as a measure of relative variability (Pagano and Gauvreau 2000). A study by Dills *et al.* (1994) measured the interindividual differences in partition coefficients between seventy-three people in four compounds: acetone, 1,1,1-trichloroethane, toluene, and styrene. The coefficient of variation was calculated for each compound. The average value of the coefficients of variation was 0.13. Therefore the partition coefficients were varied by thirteen percent on either side of the population

mean. This parameter is dependent in part on the lipid content of blood and the solubility of the chemical (Poulin and Krishnan 1995). Consequently, the six partition coefficients were assumed to be correlated. To account for this in the model, a parameter, called the multiplier, was added which facilitated the correlation. This variable was initially set equal to one and varied by thirteen percent. Each individual partition coefficient was set equal to the multiplier times the population mean value and thus varied with the multiplier. In this way all of the partition coefficients varied in the same direction and by the same amount. A study by Schlummer *et al.* (1998) measured the absorption rate for several dioxins and PCBs in seven individuals. The coefficient of variation was calculated for each compound that an absorption was found for. Because OCDD was not one of these compounds, the average was taken. An average coefficient of variation of 0.32 was found. Therefore, the absorption rate was varied by thirty-two percent on either side of the population mean. A study by Arvidsson *et al.* (1988) measured the interindividual variability in ceftriaxone in six individuals. A coefficient of variation of 0.42 was found. Therefore, the biliary excretion rate was varied by forty-two percent on either side of the population mean.

Specifically, to vary these parameters and simulate the pharmacokinetic distribution, a Monte Carlo type analysis was used. The OCDD model was run through fifty iterations. For each iteration, a different value of the partition coefficient multiplier (between 0.87 and 1.13), the absorption rate (between 0.517 and 0.99), and the biliary excretion rate (between 148 and 362) were used. Each iteration can be thought of as a person with a unique set of pharmacokinetic parameters. Because the initial OCDD concentrations

used in the model corresponded to the initial concentrations of a twenty year-old, the model was run for 7300 to 31025 days (20-85 years). The concentration of OCDD for each modeled subject at the beginning of each year was used to determine the distribution in the population of the blood concentration of OCDD due to variability in pharmacokinetic parameters.

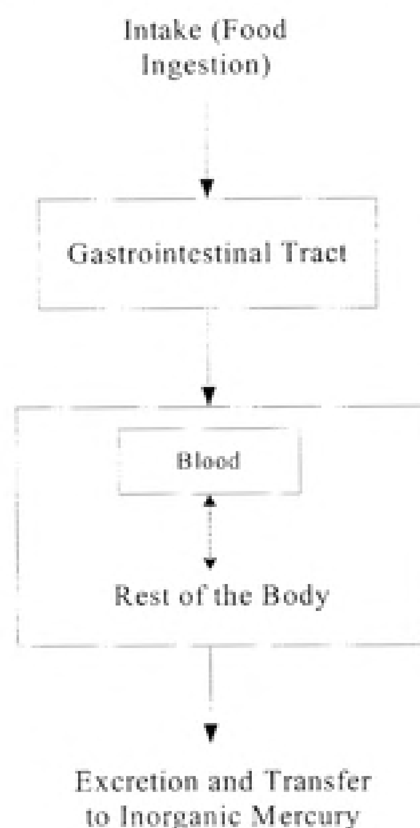
Mercury

Exposure to organic mercury is generally through food ingestion. It is readily absorbed into the gastrointestinal tract and from here distributes throughout the body. Distribution of organic mercury is made possible by its ability to cross diffusion barriers and penetrate membranes easily. Once distributed, tissue concentrations tend to remain constant relative to blood levels. Organic mercury is first metabolized by degradation into inorganic mercury. The small amount of organic mercury that is not metabolized is eliminated in the feces or hair. This process is described in more detail in the chapter entitled, "NHANES Dataset and Selected Compounds."

The model established for mercury was developed by Carrier *et al.* (2001a). It was first built to explain the tissue distribution and elimination of mercury in rats and in a later publication adapted to humans. The model as explained by Carrier *et al.* (2001a) is actually two separate models, one for organic mercury and one for inorganic mercury. For the purposes of this study, only the organic component was utilized. Mercury levels in the blood, the measurement used in this study, have been shown to be predominantly

composed of organic mercury (Kershaw *et al.* 1980; Sherlock *et al.* 1984 Birke *et al.* 1972; and Smith *et al.* 1994). Figure 5 shows a schematic diagram of the model as used in this study. The compartments contained within this model represent the following organs: gastrointestinal tract, the blood, and the rest of the body. This is a non-perfusion model that describes the rate of flow in a compartment. The exposure was assumed to occur only through oral intake of food.

Figure 5
Organic Mercury Model Schematic Diagram



Flow between the compartments was described by mass balance equations which determine the concentration in a specific tissue at a given time. The equations are as follows:

The concentration of mercury in the gastrointestinal tract (represented by GI) at a given time is represented by the following equation:

$dGI(t)/dt = (-k_{abs} * GI(t)) + g(t)$

where:

k_{abs}	the oral absorption rate constant. This was based on an absorption half-life of 3 hours, which is approximately the time it takes to digest a meal.
GI	the initial amount of mercury in the gastrointestinal tract. This was assumed to be zero since for this scenario, exposure began at birth.
g	the daily intake. This was obtained from MacIntosh *et al.* (1996).

The concentration of mercury in the rest of the body (represented by Q) at a given time is represented by the following equation:

$dQ(t)/dt = k_{abs} * GI(t) - k_{elim} * Q(t)$

where:

k_{abs}	the oral absorption rate constant. This was based on an absorption half-life of 3 hours, which is approximately the time it takes to digest a meal.
GI	the initial amount of mercury in the gastrointestinal tract. This was assumed to be zero since for this scenario, exposure began at birth.
K_{elim}	the elimination rate constant. This represents the sum of excretion rates of organic mercury and the metabolism rate of organic mercury into inorganic mercury. Therefore, this value was dependent on the determination of the excretion rates and metabolism rate.

Q the initial amount of mercury in the rest of the body. This was assumed to be zero since for this scenario, exposure began at birth.

As shown by Farris *et al.* (1993), the concentration-time profiles for organic mercury in blood, kidney, liver, and brain are parallel and the proportions between blood and the other organs remain constant. Hence, there is assumed to be a constant partition coefficient (K) between the blood and internal organs for organic mercury (Carrier *et al.* 2001b).

The concentration of mercury in the blood (represented by B) at a given time is represented by the following equation:

$dB(t)/dt = Q(t)/K$

where:

Q the initial amount of mercury in the rest of the body. This was assumed to be zero since for this scenario, exposure began at birth.

K the ratio of whole body to blood mercury concentrations. This was based on several pieces of information. The absorption fraction of mercury has been shown to be near 100% (Aberg *et al.* 1969 and Miettinen *et al.* 1971). Smith *et al.* (1994) has shown that 7.7% of an intravenous dose was in the blood after the rapid tissue distribution. Carrier *et al.* (2001a) thus estimated K to be 100/7.7.

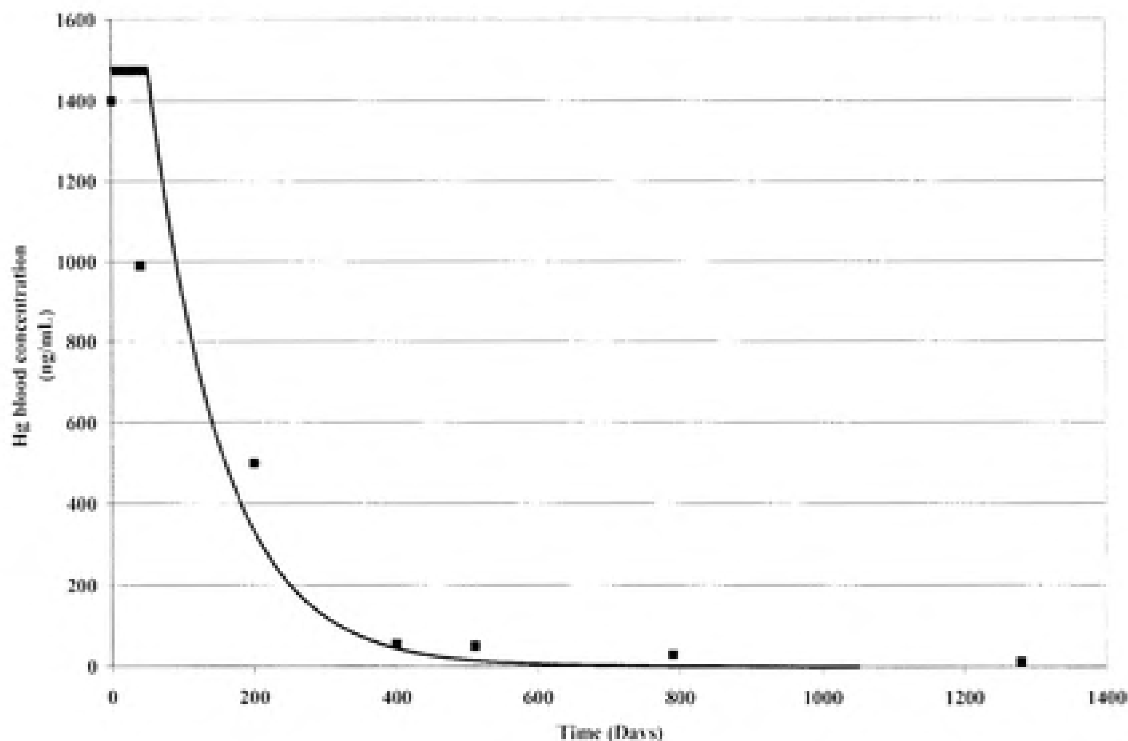
Table 4 shows the values of the model variables. Except for daily intake, the population mean values of model parameters were taken from Carrier *et al.* (2001a).

Table 4
Mercury Model Parameters

Daily intake (g)	**8.2 μg/day**
Ratio of mercury concentration in body to blood (K)	**12.9870**
Rate Constants	**$days^{-1}$**
Oral absorption (K_{abs})	5.5440
Whole body elimination – organic Hg (K_{elim})	0.0138
Initial mercury amount	**μg**
GI tract	0
Rest of body	0

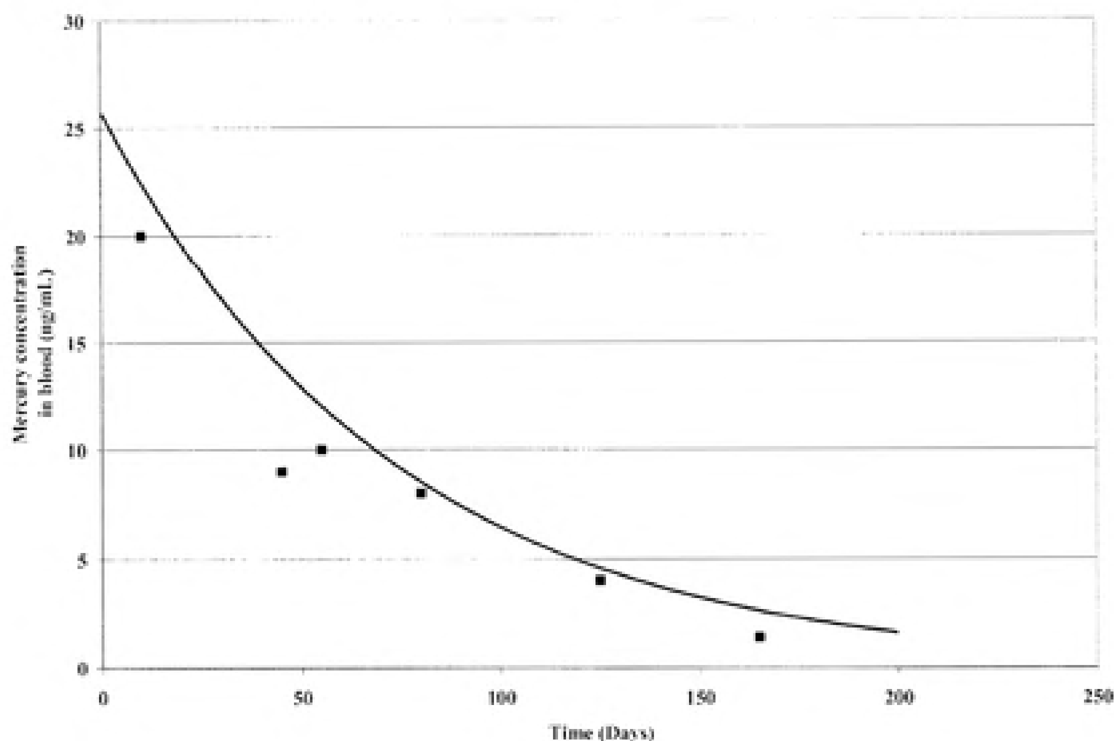
The model was validated to ensure that results similar to those reported in the literature were obtained. Data from two studies were used. The first, Birke *et al.* (1972), measured the time course of total mercury in the blood over approximately 1000 days after several years of exposure to 800 μg of methyl mercury per day through consumption of fish. Figure 6 shows the concentration-time profile of organic mercury obtained from our model (smooth line) with the data for total mercury from Birke *et al.* (1972) (squares). Since, as discussed above, organic mercury accounts for most of the total mercury in the blood, and as shown in Carrier *et al.* (2001a), the total and organic mercury curves practically overlap, our predicted results for organic mercury were assumed to be nearly the same as the measured results for total mercury.

Figure 6
Concentration-Time Profile for Mercury



The second study, Kershaw *et al.* (1980), experimentally determined the amount of mercury in the blood over approximately 165 days after volunteers were orally exposed to approximately 20 μg of methyl mercury per kg of body weight through fish consumption. Figure 7 shows the concentration-time profile for organic mercury obtained from our model (smooth line) with the data for total mercury from Kershaw *et al.* (1980) (squares). Since, as discussed above, organic mercury accounts for most of the total mercury in the blood, and as shown in Carrier *et al.* (2001a), the total and organic mercury curves practically overlap, our modeled results for organic mercury were assumed to be nearly the same as the measured results for total mercury.

Figure 7
Concentration-Time Profile for Mercury



For Mercury, the elimination rate constant was identified as an appropriate pharmacokinetic parameter to vary. A study by Al-Shahristani *et al.* (1976) measured the elimination half-life of mercury in forty-eight individuals. The half-life of mercury is assumed to be related to the elimination rate constant by the following equation, ½ life =0.693*elimination rate constant. From the data measured in Al-Shahristani *et al.* (1976) the individual values of the elimination rate constants were calculated. A coefficient of variation of 0.32 was calculated for these elimination rate constants. Therefore, in our model, the elimination rate constant was varied by thirty-two percent on either side of the population mean.

Specifically, to vary the elimination rate constant and simulate the pharmacokinetic distribution, a Monte Carlo type analysis was used. The mercury model was run through fifty iterations. For each iteration, a different value of the elimination rate constant was used between 0.0094 and 0.0182. Each iteration can be thought of as a person with a unique elimination rate constant. The model was run for 0-17885 days (0-49 years). Only the data from ages 16-49 were used in the analysis. The amount of mercury for each modeled subject at the beginning of each year was used to determine the distribution in the population of the concentration of organic mercury in the blood due to variability in the elimination rate constant.

Convolution

An important step in analyzing data for the purposes of interindividual variability is determining the distributional form of the data (Hattis *et al.* 1987; Hattis and Burmaster 1994; Hattis and Silver 1994; and Hattis *et al.* 1999). For the present study, it was expected that all of the distributions would be lognormal. It has been observed previously that distributions of interindividual variability are usually lognormal (Hattis and Silver 1994 and Hattis *et al.* 1999). Furthermore, a lognormal distribution is expected when many factors contribute to the overall variability, the factors work multiplicatively, and there are no large dependencies between factors (Hattis *et al.* 1999). To show that a lognormal distribution was reasonable for the simulated pharmacokinetic distributions and the measured, NHANES distributions, a goodness-of-fit procedure was done in Crystal Ball 2000 (Decisioneering, Inc., Denver, CO). For each of the measured

NHANES distributions and the simulated pharmacokinetic distributions, a mathematical fit was performed against each continuous distributional form. Three goodness-of-fit tests were used, the Chi-Square test, the Kolmogorov-Smirnov test, and the Anderson-Darling test. The Chi-Square test divides the distribution into areas of probability and compares data points in that area with expected data points. A *p*-value of > 0.5 indicates a close fit. The Kolmogorov-Smirnov test calculates the largest vertical distance between two cumulative distributions. A value of < 0.3 indicates a close fit. The Anderson-Darling test is similar to the Kolmogorov-Smirnov test, but gives more weight to the differences in two distributions at the tails rather than at the mid-point. A value of < 1.5 indicates a close fit (Decisioneering 1998).

Once the distributional forms were verified, several statistics were calculated to determine a measure of overall variability for the NHANES measured distribution and a measure of pharmacokinetic variability for the pharmacokinetic distribution for each compound. For lognormal distributions, the geometric mean and the geometric standard deviation were calculated. The geometric mean is equal to the median (50th percentile) of a distribution. The geometric standard deviation of the measured NHANES distribution was calculated by dividing the 84th percentile by the 50th and dividing the 50th percentile by the 16th. If the data are distributed lognormally, the results of these calculations will be the same. However, usually there is a slight difference in these calculations. In this case, the mean of the two values was calculated. For the pharmacokinetic distributions, the geometric standard deviation was calculated using the following equations: 84th percentile=Median*GSD and 16th percentile=Median/GSD. Again, where there was a

slight difference in these calculations, the average of the two values was calculated. The geometric standard deviation was used as an indication of the amount of variability in each of the distributions (Griffin *et al.* 1999).

In order to quantify the level of variation in blood levels found due only to exposure and those due only to pharmacokinetic factors, a method called convolution was used. The distribution of values simulated from the model (referred to as the pharmacokinetic distribution) was due to variability in pharmacokinetic factors and the distribution of values measured in the NHANES survey (referred to as the measured distribution) was due to overall variability. A third distribution was created to capture the variability due only to exposure factors (referred to as the exposure distribution). The pharmacokinetic and exposure distributions were then combined to yield a fourth distribution (referred to as the convolved distribution). The results of the convolved distribution were compared to the NHANES distribution. If the variability in the convolved distribution was wider, the variability in the exposure distribution was decreased, and if narrower, the variability was increased. This procedure was repeated until the convolved distribution was equal to the measured distribution of NHANES values. Once these two distributions were equal, the variability in the exposure distribution could be determined. Table 5 shows a description of all of the distributions used in this analysis.

Table 5
Distribution Descriptions

Distribution Name	Description
Measured, NHANES	The distribution of values of blood concentrations of OCDD or mercury measured in the NHANES survey. It represents overall interindividual variability.
Pharmacokinetic	The distribution of values simulated through the varying of one or more pharmacokinetic factors in the model. It represents the pharmacokinetic variability component of overall interindividual variability.
Exposure	A simulated distribution created through convolution with the pharmacokinetic distribution to represent the exposure variability component of overall interindividual variability.
Convolved	A distribution created by combining the pharmacokinetic and exposure distributions to yield a distribution equal to the measured distribution.

Specifically, the following equation was used: $GSD_3=e^{[[(\ln(GSD_1))^2]+[\ln(GSD_2))^2]]^{0.5}}$. The exposure distribution was created as GSD_1 with an initial geometric standard deviation smaller than the measured distribution. The geometric standard deviation of the pharmacokinetic distribution was defined as GSD_2. The equation was then solved for GSD_3 which is defined as the geometric standard deviation of the convolved distribution. If this found that the geometric standard deviation of the convolved distribution was larger than the measured distribution, the geometric standard deviation of the exposure distribution was lowered, and conversely, if the convolved distribution was smaller than the measured distribution, the geometric standard deviation of the exposure distribution was increased. The geometric standard deviation of the exposure distribution was adjusted until the geometric standard deviation of the convolved distribution was equal to that of the measured distribution.

Once the convolved distribution had been created to equal the measured distribution, the variability in exposure can be quantified by the final geometric standard deviation used in the convolution. To further quantify the level to which exposure and pharmacokinetic factors contribute to overall variability, the contribution to variance calculation was also performed in Crystal Ball. This calculation represents a measure of the relative importance of variability in exposure and pharmacokinetic factors within the overall variability, as represented by the measured, NHANES distribution.

Contribution to variance is calculated using a rank correlation method. While a simulation is running, a rank correlation coefficient is calculated between all parameters and the outcome. This coefficient provides a measure of the degree to which the parameter and outcome change together. A value greater than one indicates a positive relationship while a value less than one indicates a negative relationship. The larger the absolute value of this measure, the stronger the relationship. Once this measure has been calculated, the rank correlation coefficient is squared and normalized to 100% to yield the contribution to variance measure. The method outlined above will only yield an approximation of the variance and is not an absolute decomposition of the variance (Decisioneering 1998).

RESULTS

Simulated Pharmacokinetic Data

By varying the tissue-blood partition coefficients, the biliary excretion rate, and the absorption rate, a distribution of concentrations of OCDD in the blood for individuals at ages 20-85 was obtained. In all, 3300 data points were generated. The distribution represents the variability due to pharmacokinetic factors. Statistics of this distribution are given in Table 6.

Table 6
OCDD Pharmacokinetic Data

Statistic	Value
Maximum	3.98 pg/g
Minimum	0.43 pg/g
Geometric Mean	2.95 pg/g
Geometric Standard Deviation	1.21

The results of the goodness-of-fit tests performed for the lognormal distribution were as follows: Chi-Square p=0.00, Kolmogorov-Smirnov=0.1416, and Anderson-Darling=168.3659. Since the results of the Kolmogorov-Smirnov test were well below the cutoff value of 0.3, a lognormal description was determined to be reasonable.

By varying the elimination rate constant a distribution of mercury in the blood for individuals at ages 16-49 was obtained. In all, 1700 data points were generated.

The distribution represents the variability due to a pharmacokinetic factor. Statistics of this distribution are given in Table 7.

Table 7
Mercury Pharmacokinetic Data

Statistic	Value
Maximum	68.79 µg
Minimum	33.04 µg
Geometric Mean	45.66 µg
Geometric Standard Deviation	1.25

The results of the goodness-of-fit tests performed for the lognormal distribution were as follows: Chi-Square p=0.00, Kolmogorov-Smirnov=0.0691, and Anderson-Darling=15.8562. Based on the result of the Kolmogorov-Smirnov test (which was well below the cutoff value of 0.3), a lognormal description was determined to be reasonable.

Measured NHANES Data

For OCDD, the data obtained from the NHANES survey was measured in the blood of survey participants. There were 1203 detectable data points for people ages 12 and older (non-detectable values were not used). In the present study, 995 data points were used for participants ages 20-85. Table 8 shows the statistics for the measured data.

Table 8
OCDD Measured Data

Statistic	Value
Maximum	49.61 pg/g
Minimum	0.60 pg/g
Geometric Mean	2.47 pg/g
Geometric Standard Deviation	2.04

The distribution fitting function in Crystal Ball found that a lognormal distribution was the closest fit for the measured OCDD data. The results of the goodness-of-fit tests performed were as follows: Chi-Square p=0.00, Kolmogorov-Smirnov=0.0428, and Anderson-Darling=3.7088. Therefore, a lognormal description was determined to be reasonable.

For mercury, the data obtained from the NHANES survey was measured in the blood of survey participants. Measurements of mercury were also made in the urine and hair, however these data were not used. There were 2184 data points for male and female participants ages 1-5 and female participants ages 16-49. In the present study, 1611 detectable data points for females ages 16-49 were used for analysis (non-detectable values were not used). Table 9 shows the statistics for the measured data.

Table 9
Mercury Measured Data

Statistic	Value
Maximum	38.9 µg/L
Minimum	0.2 µg/L
Geometric Mean	1.00 µg/L
Geometric Standard Deviation	3.06

The results of the goodness-of-fit tests performed for the lognormal distribution were as follows: Chi-Square p=0.00, Kolmogorov-Smirnov=0.0510, and Anderson-Darling=6.3050. Based on the results of the Kolmogorov-Smirnov test (which was well below the cutoff value of 0.3), a lognormal description was determined to be reasonable.

Convolution

Using the results explained above for the measured and pharmacokinetic distributions for OCDD, the convolved distribution was generated to find the variability due to exposure alone. The geometric standard deviation of the exposure distribution for OCDD was found to be 1.99.

Similarly, using the results for mercury, the geometric standard deviation of the exposure distribution was found to be 2.99.

Contribution to Variance

For both OCDD and mercury, variability in exposure was found to be the major contributor to overall variability, as shown in the contribution to variance calculation in Table 10.

Table 10
Contribution to Variance

Compound	Pharmacokinetic Variance	Exposure Variance
OCDD	7.5%	92.5%
Mercury	4.2%	95.8%

DISCUSSION

The results of this study quantify the level of overall interindividual variability due to pharmacokinetic parameters and the level due to exposure parameters. These data show that interindividual variability is predominantly made up of variability in exposure for both OCDD and mercury. The overall variability was greater in the measured data for mercury than for OCDD. This carried through to the pharmacokinetic and exposure variability – both were greater for the mercury distributions than for the OCDD distributions. While pharmacokinetic variability should not be discounted, it accounts for a far lower proportion of overall variability in both of these compounds.

However, the amount of variability seen in pharmacokinetic parameters for both OCDD and mercury was only simulated for a few parameters (in the case of OCDD) or one parameter (in the case of mercury). There is variability in all other parameters represented in the models chosen and also in the parameters not represented in the models. It is likely that total pharmacokinetic interindividual variability is larger than the results of this study showed. Nevertheless, due to the major contribution exposure variability makes to overall variability, even if these other sources of pharmacokinetic variability had been accounted for, it is likely that exposure variability would still have been dominant.

Furthermore, there could be physiological reasons to explain the low variability found in pharmacokinetic parameters and the differences found in OCDD and mercury. OCDD showed lower variability in pharmacokinetic factors than did mercury. Dioxins are known to be heavily retained in the fat and liver. When the partition coefficients, biliary excretion rate, and absorption rate were varied individually, the absorption rate made a larger difference in the blood concentrations of OCDD than did the other two. This is likely due to the fact that there is not much dioxin moving between tissues in the body (accounting for the partition coefficients) and not much being excreted (accounting for the biliary excretion rate). On the other hand, varying the elimination rate constant of mercury, resulted in more variability. While mercury does accumulate in the kidneys and to some extent, the brain, it is not held in the body as strongly as dioxins are, and therefore interindividual differences in pharmacokinetic factors have more of an influence over the concentrations found in blood.

Factors other than pharmacokinetic and exposure differences could also contribute to the overall variability measured. Differences in lifestyle characteristics such as diet, smoking and drinking habits, and exposure to multiple chemicals were not taken into consideration in this study, but can also add to interindividual variability. Additionally, uncertainty was not accounted in this study. Uncertainty is an error in the measurement of a parameter due to the imperfection in the information about the parameter or in the measurement techniques, while variability is the interindividual differences within a population that remain after the application of perfect measurement techniques (Clewell and Andersen 1996 and Hattis *et al.* 1999). As explained in the *Exposures Factor Handbook*,

uncertainty can be characterized in three ways (EPA 1997). The first is scenario uncertainty which is caused by missing or incomplete information due to descriptive errors, aggregation errors, or errors in professional judgment. The second type, incomplete analysis is due to parameter measurement errors, sampling errors, variability, or surrogate data. And finally, model uncertainty is due to relationship errors or modeling errors. The measurements used in this analysis were taken from raw data. A consequence of not considering the three types of uncertainty discussed above is that the true variability may be overstated (Hattis *et al.* 1987). Some of the "variability" found could actually be due to uncertainty and not true interindividual variability.

Placing the results of this study in the context of exposure to environmental chemicals shows that current efforts to control the exposure of populations to environmental chemicals are well placed. Interindividual differences in exposure can be controlled while interindividual differences in pharmacokinetic and pharmacodynamic parameters cannot readily be controlled. If it is possible to further identify high risk behaviors leading to high exposures and restrict these, this study has shown that variability in biomarker measurements will be reduced which will, in turn, reduce overall levels of environmental chemicals in individuals.

The results of this study are consistent with findings from previous studies. Pierce *et al.* (1996) found that when exposure was controlled for, pharmacokinetic variability could not account for the total variability found. As shown in this study, the total variability is also due to exposure variability. Additionally, Pierrehumbert *et al.* (2002) found

variability extent indexes (VEIs) between 2.7 and 9.5 for pharmacokinetic parameters and reported that VEIs for exposure had been found to be 16. In the present study, the variability in the pharmacokinetic parameters was also found to be much lower than the exposure variability.

It is important to note that the interindividual differences found in pharmacokinetic factors are likely to be less than those found in an individuals response to environmental chemicals. As a chemical travels through the body, in addition to variability in pharmacokinetic factors, there will be variability in the other stages in the mode of action of a compound shown in Figure 1. Therefore, it is hard, if not impossible, to correlate the data used in this study (concentrations in blood) with responses or risks to environmental exposures. These differences may increase the amount of interindividual variability seen in response parameters (Hattis *et al.* 1999).

While this project did answer the original question that was asked, it also highlights several additional questions. Future studies should measure the amount of variability in pharmacokinetic and pharmacodynamic factors resulting from exposure to environmental chemicals. In this way, the modeling of this variability can be based on actual data for a wider range of compounds, in addition to biological principles. Studies should also be performed that are similar to the present study, but for additional compounds. Once a larger number of compounds have been investigated in this way, overall conclusions and patterns may be drawn. Finally, studies should be performed that unfold the exposure variability and pharmacokinetic variability and response variability in measurements of

response or risk to environmental chemicals. In this way, determinations can be made as to whether the current policies dictating how interindividual variability is accounted for in risk assessment are sufficient.

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