A MARGIN OF EXPOSURE APPROACH TO ASSESSMENT OF NON-CANCER RISKS OF DIOXINS BASED ON HUMAN EXPOSURE AND RESPONSE DATA

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Introduction

Existing non-cancer risk assessments for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds are generally based on animal data, with interspecies extrapolation based on estimated whole body concentration ("body burden") as the dose metric of interest. In the most recent WHO/FAO JECFA evaluation, reviewers noted that interspecies extrapolation presented several issues and uncertainties, but concluded that the current database of human data was insufficient to serve as the basis of a quantitative risk assessment (*1*). Since that time, the body of available data examining human exposure-response relationships has expanded substantially, with a particular focus on potential responses in infants and with exposure assessments based on measured serum lipid or maternal breast milk lipid-adjusted concentrations. At the same time, increasingly comprehensive datasets have been developed to characterize general population serum lipid concentrations in the US and other countries. These data can be examined in the context of earlier, more limited data to assess changes in human exposures to dioxin (as reflected in lipid-adjusted concentrations) over the past 30 years.

This paper explores the use of such human data sets on exposure and non-cancer endpoints in the context of a margin of exposure approach to risk assessment for PCDD/Fs and TEQ-contributing PCB compounds. Exposure and response data in this effort are based on the most commonly used metric in such studies: serum lipid concentration of TCDD toxic equivalents (TEQs) as estimated using the WHO toxic equivalency factors (TEFs) (*2*,*3*). Reliance on circulating serum lipid concentrations avoids issues associated with estimating "body burden" (which is highly influenced by assumptions regarding body fat content and degree of liver sequestration), provides an exposure metric that can be assessed directly, and is of high biological relevance to a variety of potential target tissue responses. Three example data sets from the literature are analyzed here using a benchmark dose approach for comparison to the current exposure data.

Methods

Exposure characterization

For this analysis, two data sets were evaluated to estimate serum lipid concentrations of PCDDs and PCDFs. US EPA conducted the National Human Adipose Tissue Survey (NHATS) from 1970 to 1987 to monitor chemicals in adipose tissue in a statistically representative sample of US residents. Seventeen PCDD/Fs were measured in a subset of NHATS tissue sampled between 1971 and 1982 from 36 Vietnam veterans, 79 non-Vietnam veterans, and 80 civilian men who were born between 1936 and 1954 and were between 20 and 45 years of age at the time of sampling (this subset was not necessarily statistically representative) (*4*). No differences in any congener concentrations were found among these groups, so the entire study group of 195 was used in this effort to characterize adipose tissue lipid-adjusted concentrations of PCDD and PCDF compounds during the 1970s. No PCB compounds were measured in this study. The lipid-adjusted adipose tissue concentrations measured in this study are assumed to reflect the lipid-adjusted concentrations in serum in these individuals (*5*).

To determine how congener levels have changed over time, the results from the 1971-1982 samples analyzed by Kang et al. (1990) were compared with recent data from serum collected in 2001-2002 and analyzed for 17 PCDD/Fs from a subsample of participants in the US National Health and Nutrition Examination Survey (NHANES). That study used a complex, multistage, probability sampling design to select participants representative of the civilian, non-institutionalized US population. Using analytical guidelines for these data provided by the National Center for Health Statistics and the NHANES Program, we analyzed TCDD and TEQ concentrations in people who were 20 to 45 years of age in 2001-2002 (to compare against the similarly aged group in the 1970-1987 NHATS survey) or were born between 1936 and 1954 (47 to 66 years of age, i.e., of the same birth cohort as included in the study by Kang et al. 1990) using Stata 9.0 (Stata Corporation, College Station, TX).

Finally, to assess margin of exposure in young adults in the US based on current and robust data, the sampling results from the University of Michigan Dioxin Exposure Study (UMDES, *6*) reference population aged 18 to 29, including all 29 dioxin, furan, and PCB contributors to TEQ, was used. The detection limits in this study were substantially more sensitive than those in the NHANES study, allowing a reduction of the uncertainty associated with treatment of non-detectable concentrations in the TEQ sum.

Dose-response characterization

The general approach used was to assess a quantitative relationship between the endpoint of interest and measured serum lipid adjusted TEQ concentrations, and then to estimate a dose associated with a consistent benchmark response level across studies. For data sets addressing continuous variables, the benchmark response level was set at 10% extra risk of exceeding the "normal" range (as discussed, for example, in *7*). In this case the limits of the normal range are identified as the 2.5^{th} and 97.5^{th} percentiles in the general population, which corresponds to the typical delineation of clinical reference ranges. For data sets addressing quantal endpoints, the benchmark response level was likewise set to 10% extra risk of the event. These benchmark doses (BMD_{10}s) can then be used as the basis of an assessment of margin of exposure and changes in the margin of exposure over time in the general US population.

Three endpoints, each one based on data from a different study, were selected for this exploratory analysis. The selection of these studies or endpoints does not represent a conclusion that a causal association between the exposure and the response has been established. Endpoints were selected to be carried forward for quantitative analysis based on the biological relevance and plausibility of the endpoint examined, previous interest in the study and population, and as examples of various types of data that are found in the epidemiological literature. All analyses were conducted using the WHO 1998 TEF values, because the response data sets examined generally used this system, and the raw data from those studies were not available for reanalysis using the newer TEF values.

CYP1A2 activity. Lambert et al. (2006) followed a group of people highly exposed to PCBs and PCDFs due to accidental ingestion of contaminated rice oil in Taiwan, the Yucheng cohort (*8*). A total of 174 Yucheng and 134 control subjects were studied in an effort to determine the effectiveness of using induction of the cytochrome P450 1 (CYP1) family of enzymes as a biomarker of exposure and effect in that cohort. Because CYP1A2 activity cannot be measured directly in humans, the caffeine breath test (CBT), a marker for CYP1A2 activity, was conducted. Total serum lipid-adjusted dioxin TEQ was calculated based on the 1998 WHO TEFs (*2*). Lambert et al. (2006) presented a linear regression of % of ^{13}C-labeled caffeine dose metabolized in an hour versus ppt serum TEQ. A BMD_{10} for CYP1A2 induction was developed using this regression together with reported information on CBT variation among the controls.

Developmental defects of tooth enamel. Alaluusua et al. (2004) examined developmental defects of tooth enamel among subjects who were < 5 years old (representing the age window during which development of permanent teeth occurs) at the time of exposure to TCDD following the explosion of a trichlorophenol production reactor in Seveso, Italy in 1976 (*9)*. Dental examinations conducted twenty-five years after the accident were reported for 36 individuals who lived within the "ABR" zone—the area exposed during the accident—and 39 individuals who lived outside the ABR zone. The authors reported TCDD levels (but not levels of other dioxin congeners) for those subjects based on serum that had been collected and frozen in 1976, and presented a categorical analysis by level of measured serum lipid TCDD in four exposure categories: non-ABR zone (background exposure), 31-226 ng/kg TCDD, 238-592 ng/kg TCDD, and 700-26,000 ng/kg TCDD. No individual measurements of other TEQ-contributing congeners were made in the Seveso serum samples and no serum TCDD or TEQ measurements were available for the reference individuals. Therefore, we used the concentrations of 17 PCDD/F compounds and nine TEQ-contributing PCB congeners measured in pooled serum samples for children aged 1-12 collected during the 1970s period from outside the Seveso area as reported in (*10)*. We assumed that Seveso children had similar serum levels of these non-TCDD congeners at the time of the accident and therefore estimated non-TCDD TEQ concentrations for Seveso residents as well as average total TEQ for the non-ABR reference individuals based on the data from (*10*). Because exposures are likely to be lognormally distributed, we took the anti-log of the average of the log of the minimum and maximum of each categorical exposure range to represent a central estimate of exposure within each category. Benchmark dose modeling was conducted to estimate the serum lipid TEQ concentration corresponding to a 10 percent extra risk of dental defects using USEPA Benchmark Dose Software (version 1.4.1b with a variety of dichotomous

models, with slopes restricted to be non-negative in order to allow for the possibility of non-zero defect levels at zero exposure).

Thyroid hormone concentrations in infants. Koopman-Esseboom et al. (1994) examined thyroid hormone levels in 78 two-week-old infants from the general population in Rotterdam between 1990 and 1992 and related them to TEQ in their mothers' milk (which was considered to represent lipid-adjusted TEQ of the mother and therefore a marker for *in utero* exposure levels) (*11*). The mother-infant pairs were divided into two exposure groups: low (maternal milk TEQ less than or equal to the median, 72.43 pg TEQ/g lipid) and high (maternal milk TEQ > 72.43 pg TEQ/g lipid), and the mean and standard deviation of infant free thyroxine (FT4) concentrations were reported for each group. Based on the reported mean, median, and standard deviation of maternal TEQ concentration, and assuming an overall lognormal distribution of TEQ concentrations in maternal milk, we estimated the mean milk TEQ concentrations in the lower and upper exposure groups and assumed a simple linear relationship between maternal milk TEQ and infant FT4 concentrations. The effect of TEQ on mean FT4 is calculated by the regression line between the "high" and "low" exposure groups. Maternal milk lipid-adjusted TEQ concentrations were assumed to reflect maternal serum TEQ concentrations, an assumption that appears to be approximately correct, although some differential partitioning between breast and serum lipids occurs for higher chlorinated congeners (*12*).

Characterization of changes in margin of exposure

The margin of exposure in a population is a unitless ratio between the point of departure and the estimate of dose or exposure in that population:

$$MOE = \frac{POD}{Dose}$$

For this effort, both the POD and current exposure estimates are presented in terms of lipid-adjusted TEQ concentration, and an estimate of the current MOE, as well as discussion of change in MOE from the 1970s era, are presented.

Results and Discussion

Table 1 presents the median and 95th percentile PCDD/F TEQ concentrations in persons from the NHATS (1970s) and NHANES (2001-2002) surveys. Concentrations have declined approximately 70% among persons born between 1936 and 1954, and young adults in the NHANES (2001-2002) survey showed concentrations approximately 5-fold lower than persons of similar age in the 1970s. PCB contributors to TEQ were not measured in the NHATS (1970s) survey, but other data sets suggest a similar magnitude of decline in PCB concentrations over the same time period (*13*, *14*).

Table 2 presents estimated benchmark dose concentrations for each of the data sets examined and margin of exposure (MOE) estimates based on the UMDES serum lipid concentration data for young adults (ages 18 to 29). These MOE estimates can be considered in the context of a typical uncertainty factor analysis used in deriving tolerable daily intakes. Because both the dose-response assessment and the exposure assessments for this evaluation are based upon biologically relevant internal dose metrics (circulating serum TEQ concentration), uncertainty factors typically applied for interspecies extrapolation and intra-species toxicokinetic differences would likely not be necessary or applicable to the identification of a target minimum MOE. As a result, target MOEs for analyses based on benchmark dose assessments of human data could be in the range of 3 to 30, depending upon judgments regarding the adversity of the response modeled and the need for additional uncertainty factors to account for interindividual sensitivity differences.

The approach demonstrated here can be applied within a framework that includes a weight-of-evidence evaluation for each endpoint of interest to provide an MOE assessment of current environmental exposures to dioxins.

Table 1: Median and 95th percentile of measured lipid-adjusted PCDD/F TEQ concentrations from sampling conducted in the US in the 1970s and from 2001 to 2005.

		WHO_{1998} TEQ, PCDD/F	
	Sampling year(s)	**Median**	**95th %ile**
Birth cohort, 1936-1954			
Kang et al. NHATS	1971-1982	60.2	151.1
NHANES	2001-2002	17.2	44.5
Adults aged 20-45			
Kang et al. NHATS	1971-1982	60.2	151.1
NHANES, ages 20-45	2001-2002	11.7	26.7

Table 2: Summary of modeled BMD_{10} values for three endpoints and estimated MOEs at the median and upper bound of current lipid-adjusted TEQ concentrations in young adults of reproductive age in the US for three endpoints based on example data sets.

Endpoint	**BMD_{10} ng TEQ/kg lipid**	**MOE[a]**	
		At median US TEQ (9.2 ppt)	**At 95%ile US TEQ (13.3 ppt)**
CYP1A2 activity	340	35	25
Dental defects	450-1300[b]	50-140	30-95
Neonatal FT4 changes	70	8	5

[a] Compared to current median and 95%ile exposures as estimated by UMDES reference population aged 18-29, 29 PCDD/F and PCB congeners, 1998 WHO TEQ. Median: 9.2 ppt. 95%ile: 13.3 ppt.
[b] Range of BMD_{10} estimates from different benchmark dose models.

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