A PHARMACOKINETIC MODEL TO QUANTIFY LIFETIME DIOXIN EXPOSURE AS A PREDICTOR OF CURRENT SERUM DIOXIN CONCENTRATIONS

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Abstract: The effect of specific food consumption on human serum dioxin levels is often of interest. For this investigation, we consider two methods for modeling serum levels of 2,3,7,8-TCDD as a function of covariates based on quantity of food consumed and yearly lifetime history of consumption: (1) including meals per year and years of consumption as separate variables in the model, and (2) summarizing the lifetime exposure history in terms of pharmacokinetic (PK) estimates of current serum dioxin contribution, given each exposure year's residual concentration based on congener-, age- and body mass index (BMI)-specific half-life values.
Although the PK-based calculation requires greater programming effort, the simplicity of a PK-based variable interpretation is worth the effort.

Introduction: One goal of dioxin exposure studies is to evaluate the effects of lifetime consumption of specific foods on serum dioxin levels. Challenges include (1) designing a questionnaire to collect food consumption over the lifetime, and (2) summarizing the data to evaluate the effects of historical consumption in a regression model setting. Given a questionnaire with yearly responses on food consumption, this paper compares two overall strategies for testing food covariates: (1) including meals per year and years of consumption as separate variables in the model, and (2) summarizing the consumption history in terms of an estimated current serum dioxin contribution, given each year's residual concentration based on congener-, age- and body mass index (BMI)-specific half-life values.

This investigation was carried out using data from the University of Michigan Dioxin Exposure Study (UMDES). In addition to answering an extensive questionnaire, samples of serum, house dust, soil, and vegetation were collected from subjects (and their homes and property) living in Michigan, USA in areas potentially exposed to sources of dioxin-like compounds as well as areas presumably exposed only to background levels of these compounds. Chemical analysis was performed for 29 congeners of dioxin, furan, and dioxin-like PCB compounds.

Methods: The UMDES was carried out in Midland, Saginaw and parts of Bay Counties (potentially exposed areas) and Jackson and Calhoun Counties (control areas) of Michigan, USA. Contamination occurred when the Dow Chemical Company released dioxins into the Tittabawassee River related to historical processes. The river sediment currently has elevated levels of dioxin-like compounds. This contamination has moved into the soil of adjacent areas through flooding, and into the food chain. This region may also have contamination from other sources. One purpose of the UMDES was to investigate potential pathways of dioxin movement into human serum.

The area encompassing Midland/Saginaw/Bay Counties was divided into regions representing the Tittabawassee River Floodplain, Near-floodplain, Plume (near the former Dow Chemical plant incinerator), and all other areas. The populations in each region were sampled using a two-stage probability household sampling design.[1] Eligible subjects were at least 18 years of age, lived in their current residence for at least 5 years, and provided

written informed consent to be administered a detailed exposure questionnaire. Serum samples were collected from subjects who consented and were medically eligible to give blood as defined by the American Red Cross. Chemical analyses were performed by Vista Analytical Laboratory, Inc. (El Dorado Hills, California, USA) for the World Health Organization designated 29 PCDD, PCDF, and dioxin-like PCB congeners using US Environmental Protection Agency (EPA) methods 8290 and 1668.[2,3] Reported for each sample were the concentration in parts per trillion (ppt) on a lipid-adjusted basis, the limit of detection (LOD) and an indicator of whether the sample was below LOD.

The questionnaire asked several hundred questions including demographics, smoking and pregnancy history, occupational exposure, activities in the contaminated area, food consumption, and other questions possibly related to serum levels. For the food consumption section, the ideal information would have been a year-by-year report of amounts eaten of each of dozens of specific foods over the lifetime. Collecting such detailed diet histories was considered to be logistically unfeasible and subject to severe recall bias. Previous studies in survey sampling have shown that diet memory is unreliable beyond approximately five years. As a result, the information we collected included: (1) Food consumption amounts (meals per year) in the previous five years for each of dozens of specific foods, and (2) Specific years over the lifetime in which broad categories of foods were consumed, such as "chicken, turkey, duck or goose". For this investigation, we consider two methods for modeling serum levels of 2,3,7,8-TCDD as a function of covariates from the questionnaire.

Statistical Methods: Linear regression, with $\log_{10}$ serum TCDD concentrations as the outcome variable, was performed with two different methods of formulating covariates to evaluate the effects of historical food consumption. This paper compares two overall strategies for testing such covariates: (1) including meals per year and years of consumption as separate variables in the model ('ordinary covariates'), and (2) summarizing the entire consumption history in terms of the estimated of 2005 serum TCDD contribution, given each year's residual concentration based on congener-, age- and body mass index (BMI)-specific half-life values ('PK-based covariates').

More specifically for the 'ordinary covariates', the food consumption variables were asked in the questionnaire as "meals per year" of, e.g., bass, walleye/perch, or eggs. If the consumption variable was very skewed, it was categorized to avoid the assumption of a linear relationship over such a wide range. In addition, the years in which each food item was consumed were recorded as an indicator (Y/N) for each year since birth. However, we grouped years into meaningful intervals in terms of exposure: before 1960, 1960-79, 1980-2005. In each interval, we counted the number of years in which the food was consumed, and considered this value as a continuous variable. For many of the variables, we only considered the last two intervals (starting at 1960) because exposure before 1960 was thought to be minimal, with decades of opportunity for excretion. Thus, for each food item in the questionnaire, the 'ordinary covariates' included the consumption (meals/yr) and the 2 or 3 years-per-interval variables.

The 'PK-based covariates' were developed to integrate food consumption over the lifetime, and account for excretion as well as intake. The quantity of each food consumed in the 5 years prior to interview, as reported in the questionnaire, was assumed to apply for the entire lifetime after multiplying by an age-specific correction factor (e.g., lower consumption among children). These values were applied to the years that the indicated food consumption was reported, and decayed exponentially using elimination half-lives. To summarize, the PK-based calculation required three steps: (1) estimating the amount of the food consumed at previous ages, (2) estimating relative dioxin consumption per meal of the food based on the relative change in TCDD concentration in the U.S. food supply over calendar years (i.e., TCDD levels in the U.S. 'market basket' food rose during the 1970s and early 1980s, and then fell, with somewhat different functions for fish, game, and dairy), and (3) correcting for the metabolic decay in the body between year of intake and 2005 (the year of blood draw for most participants). This factor incorporated the TCDD elimination half-life as a function of age, gender and body fat. These correction factors are described in detail in Jolliet et al.[5]

Results: Over 1300 subjects were interviewed as part of the UMDES study, and 946 provided serum samples. We first illustrate the 'ordinary covariates' (Figure 1a and 1b) and the 'PK-based covariates' (Figure 2) that were

used in the regression models. Figure 1a below gives an example plot of poultry consumption (meals/yr) versus age. For example, eating poultry every day would correspond to 365 meals/yr. Most participants ate poultry less than once a day, but many ate it at least once per week (52 meals/yr). Figure 1b shows a scatterplot of the number of years eating fish between 1960-1979 versus the number of years eating fish between 1980 and 2005. Note the strong patterns as a function of age, and the fact that most people either did not eat fish, or ate fish their whole lives (right-hand bar of points).

Figure 1a. Meals/year of poultry by age

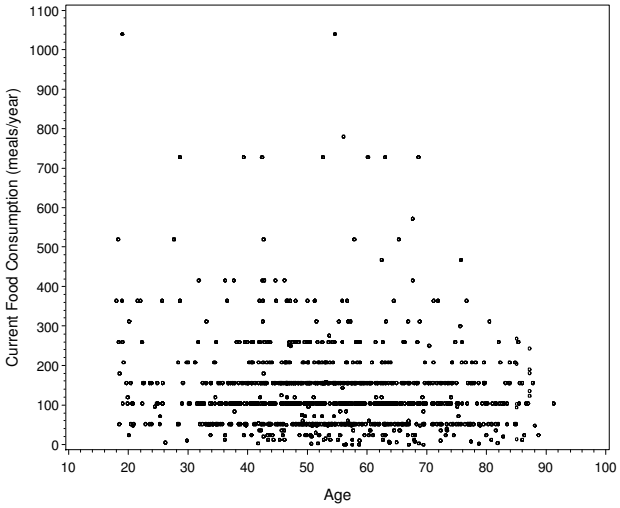

Figure 1b. Years eating fish, 1960-79 vs 1980-2005

Figure 2. PK-based poultry lifetime exposure

Figure 2 plots the PK-based variable for estimated contribution to 2005 serum TCDD concentration versus age. As has been observed in both exposed and background populations, the serum concentration rises with age, reflecting a combination of a cumulating burden over time and high environmental levels in past years.

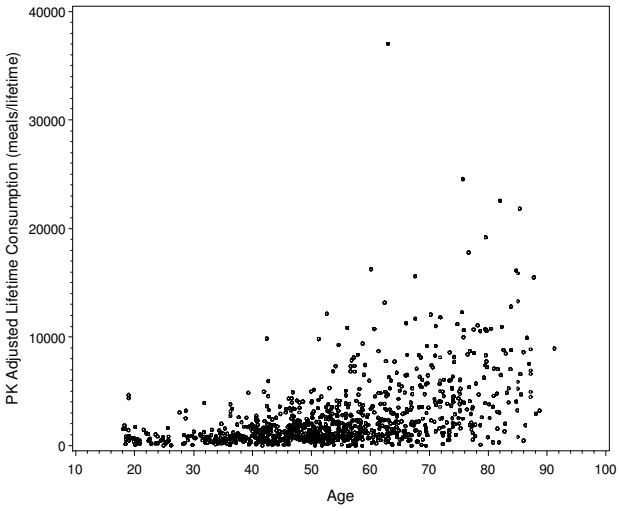

Table 1 gives selected regressions results for meat, game meat, and hunting using 'ordinary' and 'PK-based" covariates. The adjusted R^2 values are very similar in the two models, with the 'ordinary covariates' model having a slightly higher R^2 value. However, the covariate interpretations are somewhat easier in the 'PK-based' model. For example, the ordinary covariates sometimes gave 'wrong signs', e.g., counter-intuitively showing a negative relationship between serum TCDD and eating deer from the Saginaw River. The PK-based covariates did not have as many of these problems, although some did appear for other variables. For example, pan-fish bought and pan-fish caught elsewhere had coefficients of different signs. However, these two variables are likely to be highly collinear.

Table 1. Regression model of serum log_{10} TCDD with 'ordinary' (white) and 'PK-based' covariates (blue),.

	'Ordinary Covariates'		'PK-based Covariates'	
Adjusted R^2 for whole model	**0.70**		**0.68**	
Selected Variables	**Estimate**	**p-value**	**Estimate**	**p-value**
Game meat_1960-79	-0.0107	0.0002		
Deer_from Saginaw River area	-0.1549	0.0024		
Squirrel/rabbit from elsewhere	0.1069	0.0098		
PK _Squirrel/rabbit_Tittabawassee			0.0029	0.0018
PK _Squirrel/rabbit _Other region			0.0018	0.0000
Hunt_Tittabawassee _60_79_c_1	0.3785	0.0005		
Hunt_Tittabawassee _60_79_c_2	-0.0331	0.6671		
Meat_beef,plv_Other region_c_1	0.1017	0.0056		
Meat_beef,plv_Other region_c_2	0.0170	0.7655		
PK_meat_beef,plv_ Saginaw River			0.0000	0.0000
PK_meat_chicken,tdg_StoreBought			0.0000	0.0041
PK_meat_ chicken tdg_ Tittabawassee			0.0002	0.0176

Discussion: The difficulty of interpreting 'ordinary covariates' in the presence of collinearity and competing variables is at least partially solved by the use of 'PK-based covariates'. Although the PK-based calculation requires greater programming effort, the simplicity of a PK model interpretation in terms of lifetime summary serum TCDD level is worthwhile. The assumptions of both model formulations need careful attention.

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References:

1. Lepkowski J, Olson K, Ward B, Ladronka K, Sinibaldi J, Franzblau A, Adriaens P, Gillespie BW, Chang S-C, Chen Q, Demond A, Gwinn D, Hedgemen E, Knutson K, Lee S-Y, Sima C, Swan S, Towey T, Zwica L, Garabrant D. *Organohalogen Comp* 2006.
2. United States Environmental Protection Agency. Method 1668, Revision A: Chlorinated biphenyl congeners in water, soil, sediment, and tissue by HRGC/HRMS. Washington, DC: Office of Water, 19997.
3. United States Environmental Protection Agency. Method 8290: Polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) by high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS). Washington, DC: Office of Solid Waste and Emergency Response, 1994.
4. SAS Institute. SAS/STAT User's Guide Version 9. Cary, NC: SAS Institute Inc., 2004.
5. Jolliet O, Wenger Y, Milbrath M O, Chang C-W, Chen Q, Garabrant D, Towey T, Adriaens P, Gillespie B, Submitted to *Organohalogen Comp 2007*.