Appendix F

Preliminary Review of Pharmacokinetic Modeling Issues for Estimating Long-Past 2,3,7,8-Tetrachloro-Dibenzo-Dioxin Exposures in New Zealand

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Abstract

This report gives a preliminary review of the New Zealand TCDD toxicokinetic model. Included is

1. A suggestion for a slight revision of the formulae that are being used to estimated % body fat,

2. A new analysis of the Ranch Hand data using alternative formulations of the relationship between TCDD body burden elimination rate and % body fat.

3. A recommendation to revise the formula for estimating TCDD elimination to one based on 1/(body fat fraction) in place of the current % body fat-based formula. This new formula fixes the problem observed earlier that the current model does not give realistic estimates of TCDD elimination for people with very large % body fat.

Introduction

The purpose of this review is to provide some fundamental mechanistic perspectives on the modeling that is in process to estimate past 2,3,7,8-tetrachloro-dibenzo dioxin (TCDD) exposures for members of a New Zealand community (Air and Environmental Sciences Ltd, 2003ab). In particular, this review focuses on the methods for estimating the percentage of body fat, and the relationship between percentage body fat and TCDD elimination.

The current model uses two alternative empirical relationships between body fat content and TCDD elimination. The first is attributed to the 10-year follow-up of the "Ranch Hand" subjects (exposed to TCDD in the course of military service in Vietnam) by Michalek et al., (1996). This takes the form,

"$k(t) = k_o + k_1(F(t) - 25)$

> where k_o is the elimination rate (year^{-1}) for a person with 25% body fat; k_1 is a constant reflecting the change in elimination rate with body fat (year^{-1}); and F(t) is the percentage body fat at year 't' in an individual's life. The model uses the values $k_o = 0.0665$ and $k_1 = -0.00314$ (as reported by Michalek *et al*, 1996) to predict a 2378-TCDD half-life of 10.4 years for a person with 25% body fat.
>
> Pinsky and Lorber (1998) used the same elimination rate formula but derived the values of $k_o = 0.0775$ and $k_1 = -0.00313$. Using these constants, a lower half-life of 8.9 years is calculated for a person with 25% body fat."

A difficulty with these formulae is that they predict impossible negative elimination rates at body fat content levels that are within the range of fat contents that are present in appreciable numbers of people. For the Michalek et al., relationship, negative elimination rates are predicted above about 47% body fat; for the Pinsky and Lorber (1998) estimates, this occurs above a fat content of about 50%.

Figures 1 and 2 show plots of population average fat contents in a large representative sample of U.S. adults, and in New Zealand adults as estimated in the New Zealand model documentation, using different formulae for estimating body fat content from age and body mass index. Table 1 shows the cumulative percentages of U.S. men and women expected to have body fat contents less than the particular values.

The discussion below will first review the different formulae that have been used for assessing body fat content, and recommend a slightly newer formula for use in the New Zealand model. Next, there will be an exploration of the potential use of an alternative mathematical form for the relationship between body fat content and TCDD elimination that avoids the problem of projecting negative elimination rates.

Figure 1

Comparison of New Zealand Model Assumptions for Durenberg (1991) Based Male % Body Fat vs Age with Inferences of Male U.S. % Body Fat for Durenberger (1991) vs Lean (1996) and Knapik (1983) Body Fat Prediction Formulae

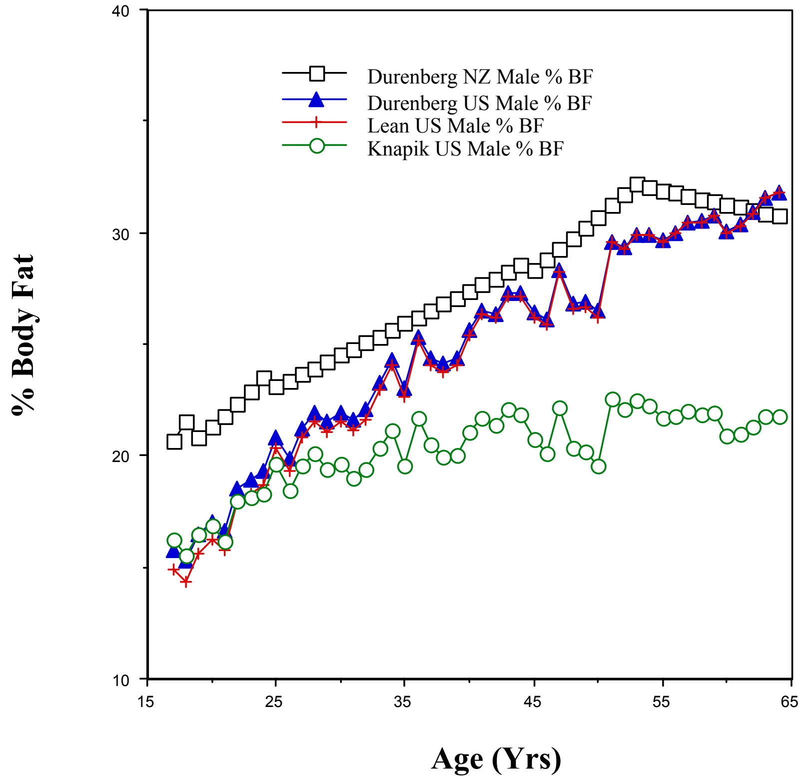

Figure 2

Comparison of New Zealand Model Assumptions for Durenberg (1991) Based Female % Body Fat vs Age with Inferences of Female U.S. % Body Fat for Durenberger (1991) vs Lean (1996) Body Fat Prediction Formulae

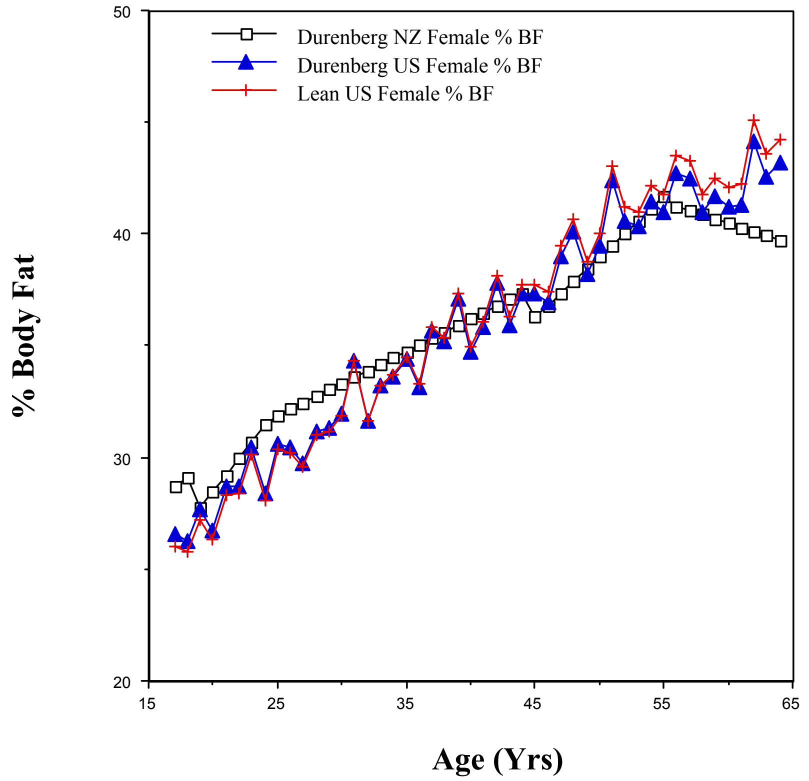

Table 1
Population Distribution of % Body Fat in Representative Samples of U.S. Males and Females Based on the Formulae of Lean et al. (1996) and the Body Mass Index Data from the National Health and Nutrition Survey III

% Body Fat	Cumulative % US Males Age 18-64 (N = 6133)	Cumulative % US Females Age 18-64 (N = 7084)
10	0.38	
15	5.9	
20	20.6	0.34
25	44.4	5.7
30	68.5	19.8
35	85.2	37.8
40	93.8	57.2
45	97.0	74.5
50	98.5	86.4
55	99.2	93.7
60	99.6	96.9

Formulae for Estimating Individual % Body Fat from Body Mass Index Data, Age, and Sex

As can be seen in Figures 1 and 2 above, several different formulae have been used to estimate % body fat in the context of modeling TCDD elimination relationships. The first of these—by Knapik et al. (1983), was used in the original analyses of the Ranch Hand data by the Michalek and Tripathathi group. Their most recent paper assessing TCDD elimination (Michalek and Tripathy, 1999), covering the 15 year follow-up of the Ranch Hand observations, continues with this older relationship:

% Body Fat = 1.264*BMI – 13.305

This formula was probably a natural choice for use when the Ranch Hand study started. From the title of the Knapik paper, it appears that it was derived from observations of relatively young people entering the U.S. military. There was no need for a treatment of sex because all the Ranch Hand study participants were male. However, the lack of a term for age effects has the potential to distort relationships in a longitudinal study lasting a few decades. The effect of predictions using this formula on expected average body fat content in U.S. males can be seen in Figure 1, in comparison with predictions using the formulae of Durenberg et al. (1991) and Lean et al. (1996), which do include age terms.

The formula that is now used to estimate % body fat in the New Zealand model are taken from Durenberg et al. (1991):

(1.2 x BMI) + (0.23 x age) - (10.8 x sex) - 5.4 (where "sex" for males = 1, females = 0)

It can be seen that in this formula the same coefficient is used for both sexes for the relationships between BMI and age. The only difference in predictions between males and females comes from the larger negative constant term used for males (-16.2%) compared to –5.4% for females.

A more recent paper that includes Durenberg as a coauthor (Lean et al., 1996) is based on underwater weighing observations of 63 men and 84 women (age range 16.8-65.4) and separate analysis of the data for the two sexes, resulting in:

% Body Fat (males) = 1.33*BMI + 0.236*age – 20.2

% Body Fat (females) = 1.21*BMI + 0.262*age – 6.7

As it happens, these newer formulae do not seem to make an appreciable difference in population mean body fat content predictions for U.S. adults* (particularly men) (Figures 1 and 2 above). Nevertheless, it seems preferable to utilize these formulae from the more recent paper with the apparent separate treatment of data for the two sexes.

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* However distributions in expected fat contents for various percentiles of the population have not yet been examined. Therefore the formulae may have some greater influence on the assessed variability in fat content within populations.

Mechanistic Perspective for Modeling the Influence of Differences in Body Fat for the Elimination of Poorly Metabolized Lipophilic Compounds

TCDD is eliminated from the body in part via the gastrointestinal tract, and probably in part via liver metabolism. A third pathway is also possible, but has not been quantitatively assessed as far as is known to the author. That is, via exfoliation of the outer layers of skin.

An earlier analysis of the gastrointestinal elimination of TCDD was done based on data of Rhode et al. (1999) in six volunteer subjects indicated that the rate of elimination via the gastrointestinal tract appeared somewhat smaller in subjects with greater estimated body fat content (Figure 3). Overall, however the elimination rate calculated from these data suggests a value that at most appears to correspond to half the total elimination rate observed in the Ranch Hand veterans.

How and why should one expect that the size of the fat compartment would influence the rate at which lipophilic compounds are eliminated from the body—either via feces or via liver metabolism or by some third pathway? Essentially we should expect elimination to be smaller in individuals with more fat because the pathways to elimination both depend on the redistribution of the TCDD from fat to other compartments (liver or gut contents, respectively). The basic notion is that the TCDD in the fat should be sequestered and not subject to direct elimination either physically or chemically. Therefore, the larger the storehouse of (presumed inert) fat, the smaller the proportion of total body TCDD that should be contained in the relatively small compartments where elimination takes place (gut contents, liver, and possibly epidermis).

Two mathematical formulations have been explored to represent this. The first is to express the elimination of TCDD from the fat as a "clearance"—the mass of fat whose TCDD contents are effectively removed per year. The second is to model the elimination rate in relation to the reciprocal of the body fat fraction:

$$K_{elimination} = B_0 + B_1 * [1/(.01 * \%\ \text{Body Fat})]$$

To test out the application of these ideas, a preliminary set of regression models was done utilizing the Ranch Hand data, which were kindly made available for this analysis. The regression treatment here is much less sophisticated than that used for the original Ranch Hand study of Michalek and Tripathy (1999), in that the present analysis does not imply the iterative statistical weighting approaches deemed by the authors to be helpful. The data processing was done in the following steps:

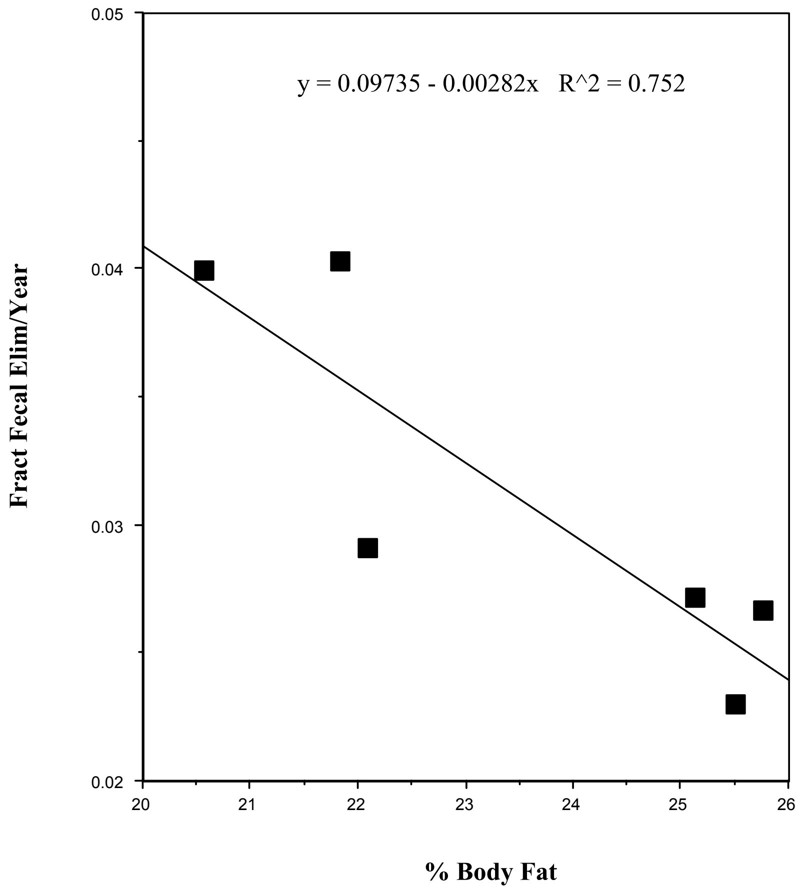
1. Data points with readings below 10 ppt were not used in the analysis.

2. A "background" level of 4 ppt was subtracted from all data points.

3. 37 subjects were excluded who experienced more than a 10 ppt increase in lipid-corrected serum TCDD levels in adjacent readings. This was to avoid including subjects who experienced unusual TCDD exposures not related to their original Vietnam exposures, and the associated complications of large negative "clearance" estimates in some cases.

Figure 3

Data of Rohde et al. (1999) on the Relationship of Fecal 2,3,7,8-TCDD Clearance and Estimated % Body Fat



Detailed data and calculations are provided in an Excel workbook accompanying this report.

Tables 2 and 3 below give an overview of the data points remaining after these exclusions. Overall, data for 307 out of the original 343 subjects survived the screening. (It should be noted that because of additional exclusion, the analysis of the 15 year follow-up of the Ranch Hand data by the original authors was limited to only 97 of the original 343 veterans.)

Table 2
Individual Mean Elimination Rates Based on Total TCDD Body Burdens for Ranch Hand Participants with Various Numbers of Valid (over 10 ppt serum) Readings in Sequential Measurements

Number of Data Points	Individual Mean Ln(Fract Body Burden Lost Per Year)	Stdev	N	Std error	Implied T1/2 (yrs)
3	0.0724	0.0314	110	0.0030	9.6
2	0.0743	0.0425	98	0.0043	9.3
1	0.0696	0.0741	99	0.0074	10.0
total	0.0721	0.0518	307	0.0030	9.6

Table 3
Individual Mean Lipid Clearance Rates for Ranch Hand Participants with Various Numbers of Valid (over 10 ppt serum) Readings in Sequential Measurements

Number of Data Points	Individual Mean g lipid cleared per year	Stdev	N	Std error
3	1420	630	110	60
2	1157	826	98	83
1	965	1150	99	116
total	1190	904	307	52

Tables 4, 5, and 6 show the results of the regression analysis. In all cases the points were weighted according to the number data points included in the averages for each participant (a weight of 3 for those with 3 valid readings, 2 for those with 2 valid readings, and 1 for those with 1 valid reading). Also, in all cases regressions were run with and without an age parameter. In no case was the age parameter statistically significantly different from zero, and therefore these results are not presented. The central estimates of the regression coefficients (the B's in the equations similar to the one shown on the first page of this section) are given in the column labeled "estimate". The standard error and other statistics follow in the subsequent columns.

First, Table 4 shows the clearance model, in which the dependent variable is the g of fat that was cleared per year of the time intervals between adjacent TCDD measurements. (For this purpose, the g of fat cleared was the decrease in the µg of TCDD in the total body burden between the two data points divided by the concentration of TCDD in serum lipid.) Overall, this parameter was found to have considerably more intrinsic variability than the elimination rate parameter used for the other models.

Table 4
Results of Regression Analysis Using G of Fat Cleared of TCDD per Year as the Dependent Variable

Response:	Individual Mean g lipid cleared/year
Summary of Fit	
RSquare	0.0458
RSquare Adj	0.0426
Root Mean Square Error	1134
Mean of Response	1266
Observations (or Sum Wgts)	625

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	422.5	225.1	1.88	0.0615
Mean % BF	29.61	7.74	3.82	0.0002

Table 5 and 6 on the next page both show regressions with the Dioxin body burden elimination rate (as used in the New Zealand TCDD toxicokinetic model) as the dependent variable. Table 5 shows results of using the current model structure (model 1) where the independent variable is % Body Fat (calculated using the Lean et al., 1996 formula). Table 6, by contrast, uses 1/Body Fat Fraction as the independent variable. Statistically, the % Body Fat variable explains slightly more of the variance than the mechanistically preferred 1/Body Fat Fraction variable (R squared value of 0.086 vs 0.076).

Table 7 compares the predictions of the two fitted models for elimination rates as a function of % body fat. Here the advantage of the 1/body fat fraction relationship is clearly apparent. Whereas the model based on % Body Fat goes to very high values above 50-60% body fat, the model derived using the 1/Body Fat Fraction independent variable is well-behaved throughout the entire range of likely body fat contents. It is recommended that, pending further and possibly more sophisticated regression modeling of the Ranch Hand and other data, the model results represented by Tables 6 and 7 be used for interim estimation of TCDD loss in the residents of the exposed New Zealand community.

Table 5
Results of Regression Analysis Using Elimination Rate Constant as the Dependent Variable and % Body Fat as the Independent Variable

Response:	Individual Mean Ln(Fract Body Burden lost/yr)
Summary of Fit	
RSquare	0.0858
RSquare Adj	0.0828
Root Mean Square Error	0.0603
Mean of Response	0.0725
Observations (or Sum Wgts)	625

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.1353	0.0120	11.3	<.0001
Mean % Body Fat	-0.00220	0.00041	-5.35	<.0001

Table 6
Results of Regression Analysis Using Elimination Rate Constant as the Dependent Variable and 1/Body Fat Fraction as the Independent Variable

Response:	Individual Mean Ln(Fract Body B
Summary of Fit	
RSquare	0.0756
RSquare Adj	0.0725
Root Mean Square Error	0.0606
Mean of Response	0.0725
Observations (or Sum Wgts)	625

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.0209	0.0106	1.97	0.0494
1/Body Fat Fraction	0.01403	0.00281	4.99	<.0001

Table 7
Results of Regression Analysis Using Elimination Rate Constant as the Dependent Variable and 1/Body Fat Fraction as the Independent Variable

% Body Fat	1/(Body Fat Fraction)	Kelim Prediction from % Body Fat Model	T1/2 (yrs) from % Body Fat Model	Kelim Prediction from 1/(Body Fat Fraction) Model	T1/2 (yrs) Prediction from 1/(Body Fat Fraction) Model
10	10.00	0.113	6.12	0.161	4.30
15	6.67	0.102	6.78	0.114	6.05
20	5.00	0.091	7.60	0.091	7.61
25	4.00	0.080	8.65	0.077	8.99
30	3.33	0.069	10.02	0.068	10.24
35	2.86	0.058	11.92	0.061	11.36
40	2.50	0.047	14.71	0.056	12.37
45	2.22	0.036	19.19	0.052	13.30
50	2.00	0.025	27.61	0.049	14.14
55	1.82	0.014	49.20	0.046	14.92
60	1.67	0.003	225.51	0.044	15.64
65	1.54	-0.008	not meaningful	0.043	16.30
70	1.43	-0.019	not meaningful	0.041	16.91

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