IMPACT OF INTER-CONGENER DIFFERENCES IN TOXICOKINETICS ON ESTIMATION OF CONCENTRATION-BASED TEFS

Aylward LL[1]

[1]Summit Toxicology, LLP, 6343 Carolyn Drive, Falls Church, VA 22044, USA

Introduction

Efforts are underway to evaluate and derive estimates of relative potency for dioxin-like compounds on the basis of tissue concentration metrics rather than administered dose (the "SYSTEQ" project). Such relative potency estimates will be of use in the interpretation of human biomonitoring data for dioxin-like compounds, which provide a more analytically tractable, and potentially, biologically relevant evaluation of exposure than intake estimates. The existing scheme for toxicity equivalency factors (TEFs) is calibrated to observed potency as a function of intake dose *(1)*. Thus, the current TEF system integrates both inter-congener differences in intrinsic potency AND toxicokinetics. That is, congeners with longer elimination half-lives will accumulate to a greater degree for a given administered dose than those with shorter half-lives, providing opportunity for relatively greater internal exposures, and potentially, responses. However, when exposures are assessed on a tissue concentration basis, application of the external dose-based TEFs may exaggerate the actual potency of such compounds. Conversely, response to compounds with shorter relative half-lives on a tissue concentration basis may be under-estimated through use of the external dose-based TEFs.

The evaluation of relative potency across congeners on a tissue concentration basis also requires consideration of the impact of induction of CYP1A2 protein in the liver. The CYP1A2 protein serves as a binding site for several, but not all, of the commonly-evaluated dioxin-like compounds, and the degree of hepatic sequestration among congeners varies widely *(2, 3)*. This results in increasing distribution to the liver of those compounds as dose and CYP1A2 induction increases, to different degrees across congeners. This sequestration may distort estimates of concentration-based relative potency *if* the CYP1A2-bound compound is relatively inactive, or unavailable, for causing dioxin-like responses through binding to the Ah receptor. That is, it may be of interest to examine the "free" vs. total (free plus CYP1A2-bound) compound. Very little data are available to evaluate the hypothesis that the CYP1A2-bound compound is not available to produce dioxin-like responses. However, if this hypothesis is correct, relative potency estimates for a compound based on measures of concentration and responses in hepatic tissue (or mediated through hepatic responses) could be distorted from what is actually relevant for human studies in two ways.

- Because of the strong dose-dependency of the induction of CYP1A2 and hepatic sequestration of dioxin-like compounds, the relationship between hepatic tissue concentration and response may be very different at elevated doses than at environmentally relevant concentrations.
- If a compound is more highly sequestered in the liver than the reference compound, and responses are estimated as being related to total hepatic tissue concentration rather than some estimate of "free" tissue concentration, the resulting relative potency estimate may be an underestimate of the actual tissue-based relative potency for non-hepatic responses. The converse is also true: for compounds not displaying substantial hepatic sequestration, relative potency on a tissue concentration basis could be overestimated compared to the reference compound.

Because toxic responses of most interest in human populations are not generally hepatic responses, this potential distortion may be quite significant.

This analysis provides a preliminary evaluation of data from the series of chronic biossays from the US National Toxicology Program (NTP) on 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD); 2,3,4,7,8-tetrachlorodibenzofuran (4-PeCDF); polychlorinated biphenyl 126 (PCB 126), and a TEQ-equivalent mixture of the three *(4-7)*. In this analyis, hepatic responses are assessed on the basis of various tissue concentration metrics to evaluate relative potencies.

Materials and methods

Data on liver and adipose tissue concentrations from the NTP bioassays of TCDD, 4-PeCDF, PCB 126, and the TEQ mixture were transcribed by time point and dose level. Data on two hepatic responses were also transcribed: induction of CYP1A1 and incidence of multinucleated hepatocytes. The former response is an early and sensitive marker for AhR activation. The latter response represents a later response that reflects sustained disruption and hepatic pathology in response to TCDD ad related compounds. Dose-response curves on the basis of adminstered dose and hepatic and adipose tissue concentration, both with and without adjustment by current external-dose TEFs, were examined to assess relative potencies and impact of CYP1A2 binding. Adipose tissue concentration was examined as a surrogate of "free" dioxin compound in liver on the basis of the assumption that free liver concentration is that which would have occurred on the basis of liver lipid content alone. In this case, adipose concentration would be a constant multiple of free liver concentration equal to the ratio of lipid content of adipose tissue to that in liver. That is, for a given congener, the free concentration in the liver might be represented as a function of adipose concentration (C_a), fraction of adipose that is lipid ($f_{a,\ lipid}$), and fraction of hepatic tissue that is lipid ($f_{h,\ lipid}$):

$$C_{liver,free} = C_a \frac{f_{h,lipid}}{f_{a,lipid}}$$

Assuming that liver is approximately 4% lipid and adipose tissue in the rat is approximately 85% lipid, this suggests that free, wet-weight liver concentration could be estimated as approximately 5% of adipose concentration across all congeners and dose levels.

Results and discussion

Hepatic sequestration, as reflected by the liver:adipose tissue concentration ratio, varied widely with dose and particularly among congeners (Figure 1). Hepatic EROD activity, which was examined here as a surrogate for CYP1A1 induction, increased with administered dose, liver tissue concentration, and adipose tissue concentration across all congeners (Figure 2). The conventional TEF values did not provide an accurate adjustment of relative potencies on the basis of liver concentration. However, application of the TEFs on an administered dose basis improved the estimates of relative potencies, while application of the TEFs to adipose tissue concentration provided the most coherence among the congener dose-response curves.

Incidence of multinucleated hepatocytes displayed a dose- and time-dependent pattern of response (Figure 3). Based on this, an "area under the curve" approach to dose metrics was used to evaluate relative potencies on the basis of the product of number of weeks and dose as measured by tissue concentration or administered dose (Figure 4). Again, congeners displayed differing relative potencies from those predicted by the external dose TEFs when assessed on the basis of tissue concentrations. However, use of adipose TEQ concentration again provided increased coherence among the dose-response curves compared to use of liver wet weight or TEQ concentration.

These results support the use of adipose tissue concentration as a surrogate for free concentration of compounds in liver. Further evaluations of these dose metrics should be conducted using other available datasets and calculated relative potencies should be evaluated using benchmark dose methods, where possible. A formal analysis presenting estimated free concentration in liver as a function of adipose or serum lipid-adjusted concentration will also be included in future evaluations.

References

1. Van den Berg, M, LS Birnbaum, M Denison, M De Vito, W Farland, M Feeley, H Fiedler, H Hakansson, A Hanberg, L Haws, M Rose, S Safe, D Schrenk, C Tohyama, A Tritscher, J Tuomisto, M Tysklind, N Walker, and RE Peterson, (2006) *Toxicol. Sci.* **93**(2): 223-241.
2. DeVito, MJ, DG Ross, AE Dupuy, Jr., J Ferrario, D McDaniel and LS Birnbaum, (1998) *Toxicol Sci*. **46**(2): 223-34.
3. Diliberto, JJ, DE Burgin and LS Birnbaum, (1999) *Toxicol Appl Pharmacol*. **159**(1): 52-64.
4. National Toxicology Program, (2006a).
5. National Toxicology Program, (2006b).
6. National Toxicology Program, (2006c).
7. National Toxicology Program, (2006d).

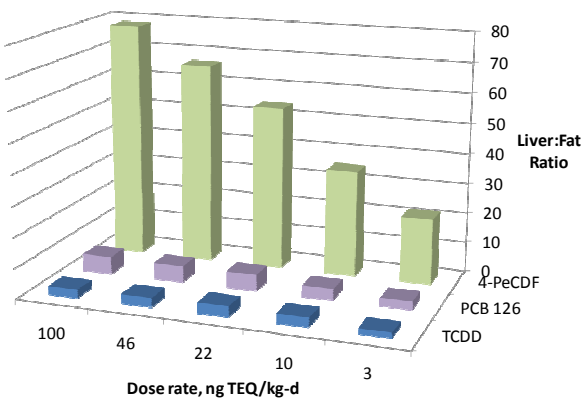

Figure 1: Ratio, liver to adipose concentrations in the NTP bioassays at 14 weeks.

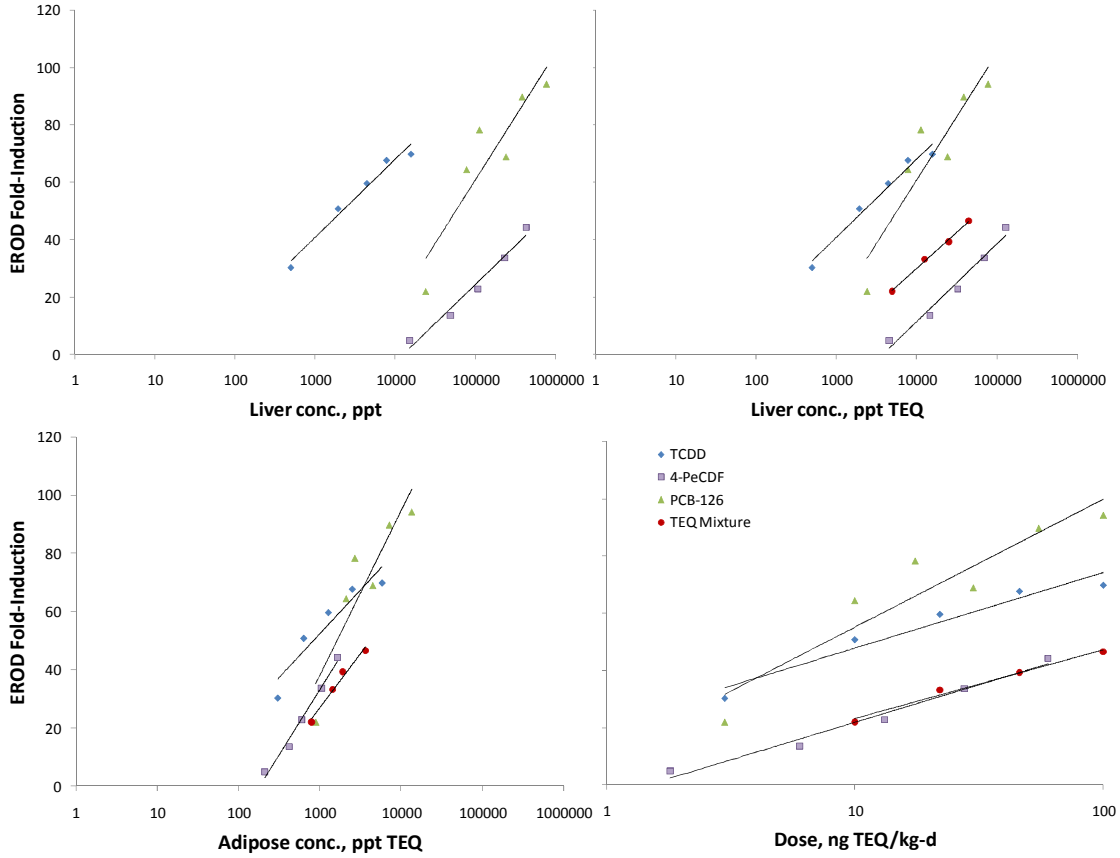

Figure 2: Fold EROD induction as a function of different dose metrics in the NTP bioassays at the 14 week interim sacrifice.

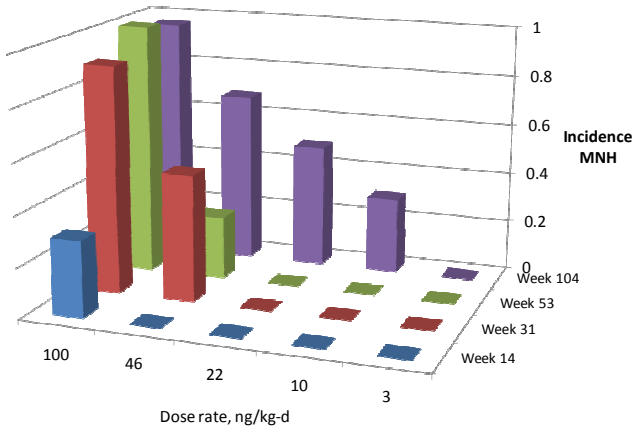

Figure 3: Pattern of incidence of multinucleated hepatocytes in the NTP bioassay of TCDD with dose and time.

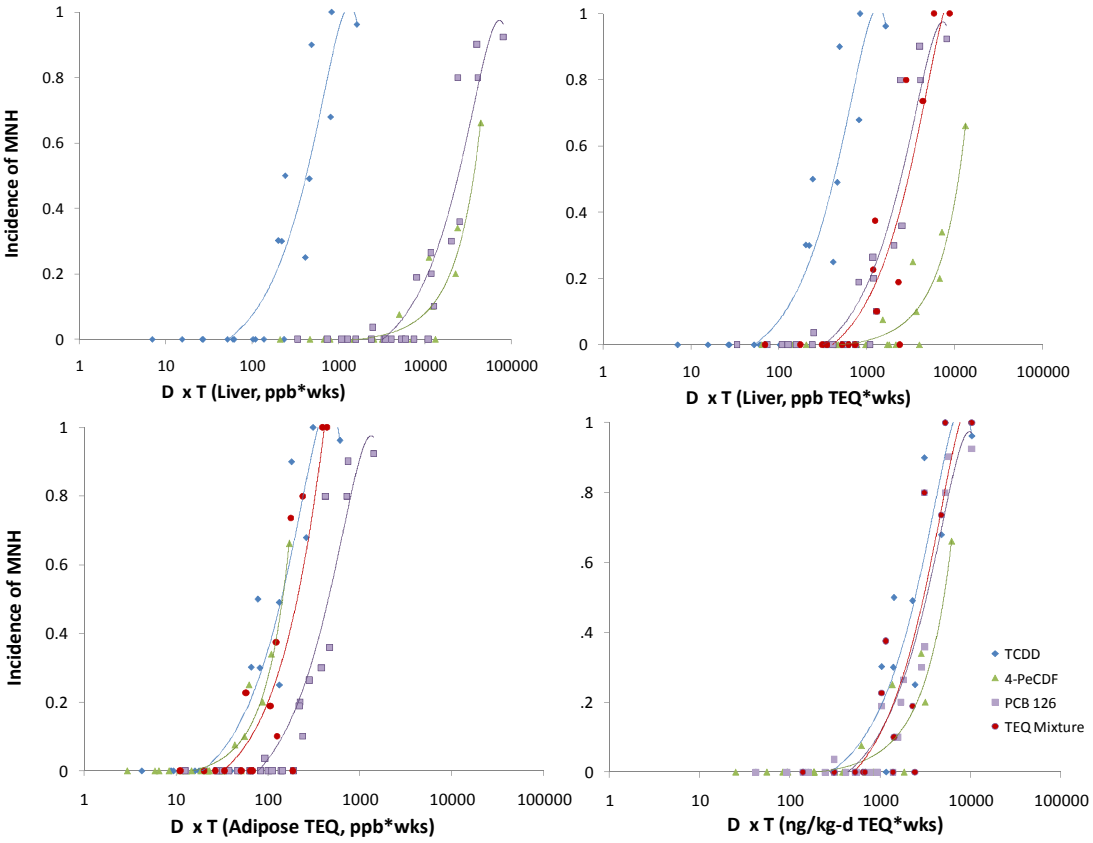

Figure 4: Incidence of multinucleated hepatocytes as a function of dose x time for several dose metrics.