Development of a Refined Database of Relative Potency Estimates to Facilitate Better Characterization of Variability and Uncertainty in the Current Mammalian TEFs for PCDDs, PCDFs, and Dioxin-like PCBs

Laurie Haws[1], Mark Harris[2], Steave Su[3], Linda Birnbaum[4], Michael DeVito[5], William Farland[6], Nigel Walker[7], Kevin Connor[8], Annette Santamaria[9], Brent Finley[10]

[1]Exponent, Austin TX
[2]Exponent, Houston TX
[3]Exponent, New York, NY
[4]U.S. Enivironmental Protection Agency, Research Triangle Park, NC
[5]U.S. Environmental Protection Agency, Research Triangle Park, NC
[6]U.S. Environmental Protection Agency, Washington, DC
[7]National Institute of Environmental Health Sciences, Research Triangle Park, NC
[8]Exponent, Natick MA
[9]Exponent, Houston TX
[10]Exponent, Santa Rosa CA

Introduction

The toxic equivalency factor (TEF) approach has been widely accepted as the most feasible and plausible method presently available for evaluating potential health risks associated with exposure to mixtures of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (PCBs)[1,2,3,4,5,6]. In accordance with this approach, the relative potency of each congener is expressed as some fraction of the potency of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The current TEFs for PCDDs, PCDFs, and dioxin-like PCBs were established by the World Health Organization (WHO) following the meeting of an international expert panel in June of 1997[2]. In the course of their review, the WHO expert panel examined data from an extensive body of *in vivo* and *in vitro* studies that had been compiled into a database of relative potency (REP) values by scientists at the Karolinska Institute in Stockholm, Sweden (hereafter referred to as the Karolinska database). As the database was intended to be all-inclusive, data were taken from published manuscripts, manuscripts in press, conference proceedings, theses, dissertations, and unpublished studies. Studies were determined to be suitable for inclusion in the database when the following criteria were met: 1) at least one test congener (PCDD, PCDF, or PCB) and a reference compound (tetrachlorodibenzo-p-dioxin [TCDD] or PCB126) were included in the study or the reference compound (TCDD or PCB126) was from an identical experiment by the same authors; and 2) the relevant endpoint used as the basis for the REP was affected by the test congener, as well as by the reference compound. An effort was also made to include information in the Karolinska database regarding a number of specific study elements. Consensus-based TEF values were established by the WHO expert panel based on

scientific judgment, after consideration of the mammalian data in the Karolinska database and previously published TEFs[2]. Specifically, as mammalian TEFs had been previously established for PCDD/Fs [5,7] and dioxin-like PCBs[8], it was decided by the WHO expert panel that the existing TEFs would remain unchanged unless there was sufficient information to warrant modification[2]. The final TEFs recommended by the WHO expert panel represent order-of-magnitude estimates of potency of each congener relative to the most potent member of this class of compounds, TCDD.

The WHO TEFs are currently used by numerous governmental agencies and others to regulate or otherwise assess health risks associated with exposure to PCDD/Fs and dioxin-like PCBs in foods, consumer products, and environmental media. As has been noted by others, for any given congener, the underlying REP values typically represent a heterogeneous data set, and the range of REPs often spans several orders of magnitude[2,9,10,11,12]. It would therefore be helpful to better understand the degree to which the TEF values contribute to variability and uncertainty in the risk assessment process. As such, the goal of this project was to develop a database that will better characterize the range of REPs, allow for the development and application of quantitative weighting schemes, and facilitate quantitative analyses. This in turn will allow for better characterization of variability and uncertainty inherent in the mammalian TEFs. The development of this database was necessary since the Karolinska database was not intentionally designed or annotated in such a way as to allow for characterization of the variability and uncertainty associated with the current consensus-based TEFs. The analysis reported herein describes our efforts with regard to the development of a refined REP database. We also provide recommendations regarding possible next steps for developing and interpreting a refined REP database for risk assessment purposes.

Methods

An electronic copy of the Karolinska database was obtained from Dr. Fredrik Waern with the Karolinska Institute in Stockholm, Sweden. The Karolinska database contained information on the relative potency of laterally-substituted PCDDs and PCDFs, as well as the dioxin-like PCBs. It is important to note that although the Karolinska database contains REP data for fish, birds, and mammals, the focus of our current effort was to develop a refined database of mammalian REPs as the mammalian data serves as the basis for the TEFs that are ultimately used for human health risk assessment purposes.

The initial phase of this project involved obtaining copies of all of the original *in vivo* and *in vitro* mammalian studies cited in the Karolinska database. Next, a determination was made regarding the specific study elements that were likely to be important metrics of study quality and reliability. The specific study elements that were determined to be important in this context included the following: cell culture system, route of administration, chemical purity, exposure duration, delay between treatment and measurement of response, measurement endpoint, species/strain, tissue type, number of dose levels tested, attainment of a maximal response, method of REP derivation, vehicle, animal age and sex, number of animals per treatment group, controls, and the reference compound included in the study (TCDD vs. PCB-126)[13]. With a few exceptions (i.e., delay between treatment and measurement of effect, attainment of a maximal response, and controls), information concerning these study elements is contained in the original Karolinska database. The Karolinska database was then reviewed to determine whether the information in the database regarding the aforementioned study elements and associated REP values was consistent with that in

each of the original *in vivo* and *in vitro* studies. Information regarding specific study elements was then updated or corrected as necessary in the refined database. The Karolinska database was refined further by eliminating or modifying individual REP values based on decision criteria described herein. Such refinements were determined to be necessary as our goal is to develop a comprehensive database that can be used for the development and application of quantitative weighting schemes, as well as facilitate quantitative analyses.

Results and Discussion

There are a total of 1,012 mammalian REP values in the original Karolinska database. Of these, 171 values (17%) are qualified as "<" or ">" some specified value, rather than being a specific estimate. The majority of these qualified REP values (94% or 159 REP values) were for the PCB congeners. Further, only 3% (or 12 out of 171 values) of all PCDD/F REP values were qualified, whereas 24% (or 159 out of 171 values) of all PCB REP values were qualified.

Our audit of all of the mammalian data in the Karolinska database identified a substantial number of REP values that either could not be used in a quantitative analysis or were questionable or clearly invalid. Table 1 summarizes the different bases for excluding REP values in the development of a refined database. There were three primary bases for exclusion: 1) values excluded due to procedural errors (e.g., data entry errors, multiple entries of the same REP value published in different studies, etc.); 2) values excluded because they did not meet the original WHO selection criteria (e.g., lack of a reference compound, use of non-mammalian data, lack of a response, etc.); and 3) values excluded for other, more subjective reasons (e.g., values derived from a quantitative structure activity relationship [QSAR] data or a mixtures study, values were derived from unpublished studies that were unobtainable, multiple REPs from a single study that used different assays to measure the same response, etc.). With regard to the latter category, it is possible that some might view a number of the excluded data as being valid and useful, while others might conceive of additional categories for exclusion based on professional judgment.

Table 1: Bases for Removing REP Values

Basis for Omission
REP qualified as ">" or "<" some specified value
Multiplicative entries of the same REP published in different studies
REP omitted in final peer-reviewed publication
REP not valid due to solubility limitations
REP and associated data are actually for another congener
Congener not evaluated in the study
Endpoint not evaluated for the test congener
REP based on replicates in an *in vitro* study
REP based on non-mammalian species
Response for test or reference compound not statistically different from controls
Reference compound not included in study or in identical study from the same laboratory
Multiple REPs from a single study that used different assays to measure the same response (e.g., AHH and EROD)
REP based on QSAR
REP based on mixtures study
REP from an unpublished study that could not be obtained

As indicated in Table 2, our refinement of the Karolinska database resulted in a substantial reduction in the total number of mammalian REPs, with only 58% and 49% of the PCDD/F and PCB REPs remaining following our audit, respectively. Overall, only 52% of all REP values were retained. On a congener-specific basis, 1234678-HpCDF had the greatest percentage of REP values eliminated, with only a single REP value from a single study being retained (Table 3). In addition, 50% or more of the REP values were removed for the following congeners: 123678-HxCDD, TCDF, 12378-PeCDF, 1234789-HpCDF, OCDF, PCB118, PCB123, PCB126, PCB157, and PCB189 (Tables 3 and 4). It is important to consider the number of REP values retained in the context of the number of studies. As is indicated in Tables 3 and 4, there are now several congeners in the refined database that only have a single REP value from a single study (i.e., 123789-HxCDF, 1234678-HpCDF, 1234789-HpCDF). This obviously has the potential to increase the uncertainty inherent in the TEF. In the refined database, the REP range for most PCDD/F congeners was reduced by approximately an order of magnitude, while the REP range for most PCB congeners remained essentially the same.

Table 2
Comparison of the Karolinska and Refined Databases:
Impact on the Total Number of Mammalian REPs

Class of Congeners	Karolinska Database	Refined Database
PCDD/Fs	361	209
PCBs	651	317
Total	1012	526

It is important to note that there were also a significant number of errors and incomplete entries identified for specific study elements described in the Karolinska database (e.g., information concerning purity, number of dose levels, cell culture system, etc.). In addition, the data for a number of studies were preliminary at the time of the WHO expert panel meeting but have since been published in full. In several instances, the REP values and associated study characteristics have been modified in the final publications. Therefore, where appropriate, we corrected and updated the REP values and associated study-element information in the refined database.

Table 3
Comparison of the Karolinska and Refined Databases:
Impact on REPs for PCDD/PCDF Congeners

Congener	Karolinska Database		Refined Database		REP Values
	# REPs	# Studies	# REPs	# Studies	Retained
12378PeCDD	52	18	29	14	56%
123478HxCDD	10	6	8	6	80%
123678HxCDD	10	4	5	4	50%
123789HxCDD	6	3	4	3	67%
1234678HpCDD	23	9	20	9	87%
OCDD	15	5	8	3	53%
TCDF	51	20	25	12	49%
12378PeCDF	42	14	21	9	50%
23478PeCDF	74	23	49	19	66%
123478HxCDF	18	6	11	4	61%
123678HxCDF	19	6	12	4	63%
123789HxCDF	1	1	1	1	100%
234678HxCDF	12	4	7	2	58%
1234678HpCDF	5	3	1	1	20%
1234789HpCDF	2	2	1	1	50%
OCDF	21	8	7	4	33%
TOTAL	**361**	**NA**	**209**	**NA**	**58%**

We suggest that some form of a refined REP database ultimately be developed for use in risk assessment applications. The analysis presented here provides one possible methodology for developing such a database; certainly other approaches might be equally valid. At the very least, the obvious data entry errors and multiplicative entries of the same values should be corrected. Values that clearly fall short of established selection criteria or that otherwise appear to be questionable should also be candidates for elimination or some form of diminished weighting. In addition, it is important to be aware of the heterogeneous nature of the REP data, particularly with respect to data quality and relevance. Development of a quantitative, transparent, and reproducible weighting scheme for individual REP values would likely increase consistency in the derivation of the TEF values, facilitate characterization of uncertainty, and could also be used to evaluate new REP data as they become available. The availability of a refined database, like the one that we have described in this paper, will allow for the development and application of quantitative weighting schemes and facilitate quantitative analyses.

Table 4
Comparison of the Karolinska and Refined Databases:
Impact on the REPs for PCB Congeners

Congener	Karolinska Database		Refined Database		REP Values Retained
	# REPs	# Studies	# REPs	# Studies	
PCB77	83	34	57	27	69%
PCB81	13	6	8	5	62%
PCB105	67	22	35	16	52%
PCB114	17	5	10	5	59%
PCB118	55	18	24	14	44%
PCB123	24	5	9	5	37%
PCB126	164	40	62	27	38%
PCB156	91	29	47	24	52%
PCB157	34	10	13	7	38%
PCB167	10	5	6	5	60%
PCB169	73	25	39	18	53%
PCB189	20	6	7	5	35%
TOTAL	**651**	**NA**	**317**	**NA**	**49%**

It might also be useful to develop REP distributions as a supplement to and/or in the derivation of the "point-estimate" TEFs. For example, REP distributions could be used to establish a consistent percentile point-estimate TEF to represent the "central tendency" and "plausible upper bound" for each congener (e.g., the 50^{th} and 90^{th} percentiles of the distribution, respectively). In addition, use of REP distributions in a probabilistic analysis of risk would theoretically allow for a more informed discussion of the variability and uncertainty in the risk estimates

In conclusion, it is worth noting that the WHO has suggested that the TEF approach be reevaluated every 5 years to account for emerging scientific information[2]. Given the findings presented here, as well as the significant number of new studies with relevant REP data that have likely been published since 1997, this may be an appropriate time to undertake such a review.

Acknowledgements

This work was funded in part by Tierra Solutions, Inc. Additional funding was provided by Exponent, Inc. The contents of this paper reflect the opinions and views of the authors and do not represent the official views or policies of NIEHS, NIH, or USEPA. The mention of trade names and commercial products does not constitute endorsement or use recommendation.

References

1. Yrjanheikki E.J. (1992) Toxic Sub. J. 12, 283.
2. van den Berg M., Birnbaum L., Bosveld A.T.C., Brunstrom B., Cook P., Feeley M., Giesy J.P., Hanberg A., Hasegawa R., Kennedy S., Kubiak T., Larsen J.C., van Leeuwen F.X.R., Liem A.K.D., Nolt C., Peterson R.E., Poellinger L., Safe S., Schrenk D., Tillitt D., Tysklind M., Younes M., Waern F., and Zacharewski T. (1998) Environ. Health Perspect. 106(12), 775.
3. Birnbaum, L.S. (1999) Hum. Ecol. Risk Assess. 5, 13.
4. Birnbaum L.S., and DeVito M.J. (1995) Toxicology 105, 391.
5. NATO/CCMS (North Atlantic Treaty Organization/Committee on the Challenges of Modern Society) (1988) Report No. 178, Washington, D.C.
6. Olson J.R., Bellin J.S., and Barnes D.G. (1989) Chemosphere. 18(1-6), 371.
7. U.S. Environmental Protection Agency (1989) EPA/625/3-89/016
8. Ahlborg, U.G., Becking, G.C., Birnbaum, L.S., Brouwer, A., Derks, H.J.G.M., Feeley, M., Golog, G., Hanberg, S., Larsen, J.C., Liem, A.K.D., Safe, S., Schlatter, C., Waern, F., Younes, M., and Yrjanheikki, E. (1994) Chemosphere 28(6), 1049.
9. Finley B.L., Connor K.T., and Scott P.K. (2003) J. Toxicol. Environ. Health. Part A. 66, 533.
10. U.S. Environmental Protection Agency (2000) SAB Review Draft. Chapter 9. EPA/600-P-00/001BE.
11. Haws L., Harris M., Su S., Walker N., Birnbaum L., DeVito M., Farland, W., Connor K., Santamaria A., and Finley B. (2004) Organohalogen Compounds, submitted.
12. Birnbaum L.S., Emond C., and DeVito M.J. (2004) The Toxicologist 78, 362.
13. Connor K., and Finley B. (2003) Organohalogen Compounds 65, 296.