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October 2015 | DIOXIN | WWW.RIVKINRADLER.COM

From The Experts

Guest column for the Dioxin Newsletter

By: *Michael E. Ginevan, Ph.D.* and *Deborah K. Watkins, M.S.*

Dioxin Concentration Data: TCDD (and Other Dioxins) vs. TEQ

Dioxin concentrations in both human (e.g., blood, adipose tissue) or environmental (e.g., soil, sediment, fish) samples are usually given as picograms per gram ("pg/gm") or, equivalently, parts per trillion ("ppt") dioxin. In either case, the question arises as to what the word "dioxin" means.

In some situations, such as our studies on Agent Orange exposure (Armitage et al., 2015; Ross et al., 2015; Ross et al., 2015a), dioxin means 2,3,7,8-tetrachlorodibenzo-*p*-dioxin or TCDD, while in others it means dioxin toxicity equivalence or TEQ.

A further complication is that TEQ may be determined by directly measuring "dioxin congeners" using chemical analysis techniques or indirectly using the XDS-CALUX bioassay method (http://www.dioxins.com/pdf/downloads/downloads02.pdf).

In this article, we will show that these distinctions are important in a toxic tort context and that the way in which TEQ is determined is of particular interest.

Dioxin Toxicity

TCDD is the dioxin for which toxic effects are best defined. These effects are thought to be receptor mediated in the sense that TCDD attaches or binds to the aryl-hydrocarbon receptor ("AhR"). This event is assumed to lead to changes in gene expression, cell replication, and programmed cell death (usually termed "apoptosis") (IARC, 2012). These changes have in turn been hypothesized to lead to a diverse collection of health effects, including cancer, diabetes and other endocrine disorders, and birth defects.

We note that although TCDD indisputably causes a variety of effects in laboratory animals, its effects on human health are questionable. For example, it is not clear that TCDD is a human carcinogen (Boffetta et al., 2011). Likewise the causal connection between diabetes and dioxin exposure has been questioned (Goodman et al., 2015; IOM, 2008).

Finally, although there have been claims of widespread birth defects in Vietnam that allegedly resulted from the use of dioxin contaminated herbicides during the Vietnam War, the scientific basis for these claims is tenuous. Moreover, studies at Seveso, which is the only population that has documented, substantial dioxin exposures in pregnant women, have failed to show an excess of birth defects (Mastroiacovo et al., 1988). We also note that because dioxin toxicity is receptor mediated, many scientists believe that it is a threshold toxicant (NRC, 2006). Thus, the health impact of low level exposures may be zero.

WHO TEQ

Nonetheless, the World Health Organization ("WHO") has defined a set of toxicity equivalence factors ("TEFs"; *see* Table 1) that are supposed to convert the concentration of other dioxins as well as chemically similar compounds (called congeners, furans, and PCBs) that also bind to the Ah Receptor to the concentration of an equivalent amount of TCDD.

Table 1: WHO TEF values (Van den Berg et al. 2006, Table 1)

Compound	**WHO 1998 TEF**	**WHO 2005 TEF***
chlorinated dibenzo-p-dioxins		
2,3,7,8-TCDD	1	1
1,2,3,7,8-PeCDD	1	1
1,2,3,4,7,8-HxCDD	0.1	0.1
1,2,3,6,7,8-HxCDD	0.1	0.1
1,2,3,7,8,9-HxCDD	0.1	0.1
1,2,3,4,6,7,8-HpCDD	0.01	0.01
OCDD	0.0001	**0.0003**
chlorinated dibenzofurans		
2,3,7,8-TCDF	0.1	0.1
1,2,3,7,8-PeCDF	0.05	**0.03**
2,3,4,7,8-PeCDF	0.5	**0.3**
1,2,3,4,7,8-HxCDF	0.1	0.1
1,2,3,6,7,8-HxCDF	0.1	0.1
1,2,3,7,8,9-HxCDF	0.1	0.1
2,3,4,6,7,8-HxCDF	0.1	0.1
1,2,3,4,6,7,8-HpCDF	0.01	0.01
1,2,3,4,7,8,9-HpCDF	0.01	0.01
OCDF	0.0001	**0.0003**
non-ortho substituted PCBs		
PCB 77	0.0001	0.0001
PCB 81	0.0001	**0.0003**
PCB 126	0.1	0.1
PCB 169	0.01	**0.03**
mono-ortho substituted PCBs		
PCB 105	0.0001	**0.00003**
PCB 114	0.0005	**0.00003**
PCB 118	0.0001	**0.00003**

PCB 123	0.0001	**0.00003**
PCB 156	0.0005	**0.00003**
PCB 157	0.0005	**0.00003**
PCB 167	0.00001	**0.00003**
PCB 189	0.0001	**0.00003**

* The numbers in bold indicate the TEF was changed in the 2005 classification.

The basic calculation is simple ("Equation 1"):

$$TEQ = \sum_{i=1}^{N} TEF_i x C_i$$

That is, if one has a collection of concentrations for N furans and/or PCBs, one simply multiplies the concentration of each congener (C_i) by its corresponding TEF (TEF_i) and sums the results.

The resulting is the toxicity equivalence ("TEQ") of the mixture in ppt TCDD.

The process by which these TEQ values were derived is described in some detail in Van den Berg et al. (2006). Two points are relevant here. First the process by which TEQ values are derived is a qualitative, consensus driven, expert opinion process. That is, it lacks quantitative rigor and for many of the compounds that TEQs are given for, it is based on a very modest amount of actual data. Second, the TEQ values are derived for cancer only and are derived almost exclusively from animal data.

Thus, if one encounters an argument based on TEQ values one first should ask which actual compounds were involved in the calculation of the TEQ.

This is of some importance because, although congeners such as OCDD or the various PCBs may have low TEF values, they may be present at relatively high concentrations (OCDD is typically the highest concentration dioxin in both tissue and environmental samples) and, therefore, contribute a substantial amount to the overall TEQ. The problem here is that the low TEF value congeners have rather poorly known toxicity and may in fact not be toxic at all (IARC, 2012).

We note that the most commonly encountered TEQ is based on the first 17 compounds shown in Table 1, but one can encounter TEQs based on all 29 compounds or some other subset, so this is not a hypothetical problem.

CALUX TEQ

TEQs reported as CALUX TEQ are based on the XDS-CALUX bioassay method, developed by Xenobiotic Detection Systems, Inc., and described in some detail on its web site (http://www.dioxins.com/pdf/downloads/downloads02.pdf). Briefly, the method relies on a mouse cell line in which the gene for firefly luciferase (the material that makes fireflies glow) has been placed under the control of the Ah Receptor. The idea is that the more the Ah Receptor is activated, the more brightly the cells will glow. This response is measured photometrically and the result is reported as a bioassay TEQ measurement. Although some authors have reported that it is a useful way of measuring dioxin levels (e.g., Brown et al., 2007), others have questioned its utility for evaluating TEQ in field samples (e.g., Vromman et al., 2012). Our own experience with CALUX in a litigation setting is that is a poor predictor of TEQ. This is based on an analysis of 14 dust samples analyzed by both standard chemical

techniques (gas chromatography/mass spectrometry or GC/MS) and CALUX. Our analysis found that CALUX TEQ was a poor predictor of WHO TEQ.

We believe that the disparity among evaluations of the CALUX bioassay stems from the fact that, although dioxins and related compounds bind to the AhR, many other compounds, unrelated to dioxins also bind to the AhR (Denison et al., 2011). Thus, if one has samples where the only AhR binding compounds are dioxins, furans, and PCBs, then the photometric bioassay results from CALUX may predict WHO TEQ fairly well.

If, on the other hand, one has a set of field samples containing not only dioxins, furans, and PCBs, but also a collection of other compounds that bind to the AhR, then the CALUX bioassay result will be influenced by the other compounds and the ability of CALUX to predict WHO TEQ will be poor.

The important point here is that although CALUX may be a good predictor of WHO TEQ in some cases, there is no guarantee that it will be a good predictor of WHO TEQ *in most cases*.

Our recommendation is that CALUX results should never be taken at face value and that if a large amount of money is at stake, one should have the same samples (or an appropriate subset; we will discuss sampling issues in a future article) analyzed using GC/MS and the WHO TEQ determined using Equation 1 and the 2005 TEF values from Table 1.

Some Other TEQ Issues

As noted above, TEQ is a summary measure of alleged dioxin toxicity. However, one should never allow arguments based only on TEQ to proceed unchallenged.

The first reason for this statement is that a relatively high TEQ may be based on small concentrations of relatively "potent" (high TEF) compounds such as TCDD or larger concentrations of low potency compounds such as the OCDDs. In the first case, one is dealing with the fact that organizations such as IARC and the U.S. Environmental Protection Agency have determined that the compound is a carcinogen, while in the latter, the evidence for carcinogenicity or indeed toxicity of any kind is much more limited.

The second reason one should question TEQ only arguments is that they obscure the actual dioxin/furan/PCB composition of the samples. By composition we mean the relative amounts of different congeners in the samples.

As a concrete example, our work with herbicide/dioxin exposure in Vietnam has focused on TCDD because it is essentially the only toxic congener found in the herbicide 2,4,5-T, which is the only source of dioxin contamination in Agent Orange, the most commonly used herbicide in the Vietnam War. In our work we have seen environmental samples from Vietnam that show high TEQ levels but only about one-half (or less) of the TEQ is due to TCDD.

If the contamination in these samples was from Agent Orange, nearly all the TEQ should be from TCDD. Thus, we can say that these samples contain dioxin contamination from other sources and may be entirely from these other sources.

More generally, dioxin mixtures from different sources usually have different compositions. If the relative amounts of dioxins/furans/PCBs in a sample are different from the relative amounts in the contamination allegedly produced by a company, it is reasonable to argue that the contamination in question was in fact not a result of the company's activities. The topic of compositional analysis and source attribution is a complex one (see, for example, Ginevan, 2007) and will be considered in some detail in a future article.

Although the use of TEFs and the TEQ approach is widespread, its use is not without controversy. The TEF scheme and the TEQ methodology originally presented in 1998 (Van den Berg et al., 1998) was re-evaluated and revised (Van den Berg 2005) to take into account new scientific information.

Conclusion

For the reasons we discuss above, caution should be exercised when using or reviewing studies that employ TEQ measurements to ensure that the conclusions reached accurately represent the methodology employed and the data collected.

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About the Experts:

Dr. Michael E. Ginevan is the President of M.E. Ginevan and Associates, LLC (http://www.ginevan.com/home.html). Dr. Ginevan has more than 35 years of experience in the application of statistics and computer modeling to problems in public health and the environment and in the conduct of environmental, epidemiologic, and risk assessment studies. He is the author of "Statistical Tools for Environmental Quality Measurement" and over 60 other publications in the areas of statistics, computer modeling, epidemiology, and environmental studies.

Dr. Ginevan is a founder and past Secretary of the American Statistical Association ("ASA") Section on Statistics and the Environment and a recipient of its Distinguished Achievement Medal. He is a charter member of the Society for Risk Analysis. Dr. Ginevan has served on numerous review and program committees for the ASA, the U.S. Department of Energy, the U.S. Nuclear Regulatory Commission, the National Institute for Occupational Safety and Health, the National Cancer Institute, and the U.S. Environmental Protection Agency. He also served as a member of the National Academy of Sciences Committee on Health Risks of the Ground Wave Emergency Network. He is currently a member of the Editorial Board of Regulatory Toxicology and Pharmacology and has served as a peer reviewer for many other professional journals.

Deborah Watkins, Proprietor of Watkins Consulting, is an epidemiologist with over 30 years of experience in public health. She formerly was a Research Assistant Professor and Deputy Director of the Division of Occupational Health Studies in the Department of Family Medicine, Georgetown University School of Medicine; she remains a member of the adjunct faculty.

Prior to her work at Georgetown University, Ms. Watkins served concurrently as Director of Legislative Affairs at the Pennsylvania Environmental Council and Director for the Hazardous Waste Management Project at the Pennsylvania Environmental Research Foundation. She was the Executive Director of the Society for Occupational and Environmental Health in Washington, D.C.

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Rivkin Radler LLP
926 RXR Plaza, Uniondale NY 11556
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