Concerns with the Use of a Toxic Equivalency Factor (TEF) Approach for Risk Assessment of "Dioxin-Like" Compounds

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Introduction

In its reassessment of the potential human health risks from exposure to "dioxin-like" compounds, the U.S. Environmental Protection Agency (US EPA) has concluded that effects ranging from enzyme induction to cancer may be occurring at or within an order of magnitude of current background body burdens (~ 40 to 60 ppt TEQ) of these substances. This conclusion rests heavily on the assumption of additivity and reliance on Toxic Equivalency Factors (TEFs) to quantify the individual contributions to potential toxicity of dioxins other than 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the polychlorinated dibenzofurans (PCDFs), and the dioxin-like polychlorinated biphenyls (PCBs). These non-TCDD substances, present in human lipids at vastly greater concentrations than TCDD itself, contribute about 90% of the background body burden of dioxin-like compounds (in TEQ), so the general population is exposed to a truly complex mixture whose individual constituents exhibit a very broad range of Ah receptor activities. Use of the TEF approach to characterize the various human health risks that might be posed by low level exposure to mixtures of dioxin-like compounds is controversial and lacking in widespread scientific support. Specific concerns with the TEF methodology are outlined below.

Uncertainty Regarding TEF Values

The TEF concept arose from the observation of rank order correlations between the binding affinities of AhR agonists and their respective potencies to induce corresponding AhR-mediated biochemical and toxic responses. For PCDDs and PCDFs, TEF values have only been assigned to the seventeen 2,3,7,8-substituted congeners which appear to be the most persistent and bioaccumulative compounds. A World Health Organization working group has similarly assigned TEFs only for the nine PCB congeners thought to be most toxicologically important and environmentally relevant. Individual TEF values were selected from within the broad range (often spanning multiple orders of magnitude) of possible values derived from studies using a

variety of endpoints in different species, target organs, and cells. While "scientific judgment" was employed in determining these values, it is unlikely that any single TEF value adequately reflects the multiplicity and variability of toxicologically significant findings that have been reported in different species and target organs. Indeed, it may well be that use of TEFs for risk assessment purposes will be scientifically justifiable only if they are constrained to be species- and endpoint-specific.

Evaluation of Additivity Assumption Only at High (e.g., EC50) Doses

The additivity assumption underlying the TEF approach to toxicity assessment for mixtures of AhR agonists has been evaluated quantitatively for a number of *in vitro* and *in vivo* responses including CYP1A1-dependent (AHH and EROD) activities, proliferation and alteration of transformed keratinocytes, maternal thymic atrophy and hepatomegaly, and fetal cleft palate and hydronephrosis. Typically, TEF-derived toxicity estimates for mixtures are compared to toxicity as determined experimentally in the vicinity of EC50s, a response level far greater than that expected from background body burdens. Thus, even if significant departures from additivity are not apparent near EC50s, this does not guarantee that such departures will not occur nearer to typical environmental exposure levels. There is a clear need to evaluate the additivity assumption at doses more relevant to human exposure conditions than are EC50s.

Nonadditivity (Both Synergistic and Antagonistic) is Known to Occur

Nonadditive interactions among various AhR agonists have been demonstrated by several independent research groups. It is now known that PCBs and some PCDFs antagonize AhR-mediated responses including fetal cleft palate, hydronephrosis, immunotoxicity, embryotoxicity, and induction of CYP1A1-dependent activities. Interestingly, certain PCBs markedly enhance TCDD-induced hepatic porphyrin levels in rats, and others cause greater than additive promotional effects in rat hepatic foci. These observations contradict the additivity assumption at high doses near the EC50s at which TEFs have been defined. While nonadditivity, either synergistic or antagonistic, has yet to be demonstrated at dose levels comparable to human background body burdens, the fact that it does appear at high doses and at antagonist/agonist ratios found in many environmental mixtures is cause for concern.

Nonparallel Dose-Responses for Different Endpoints

US EPA's most recent draft of Chapter 8 of its dioxin reassessment, *Dose-Response Modeling for 2,3,7,8-TCDD*[1] includes dose-response analyses for many endpoints ranging from liver enzyme induction to cancer. Flexible mathematical models fit to data for the different endpoints yielded markedly nonparallel dose-response curves. For example, US EPA's analysis of fertility index data for Holtzman Sprague-Dawley rats exposed to TCDD produced an extremely sublinear response (proportional to the 23rd power of dose at low doses), while a similar analysis of hydronephrosis data for C57BL/6N mice produced an extremely supralinear response (proportional to the 1/5th power of dose at low doses). A one order magnitude change in exposure (at low doses) thus produces a differential of about 115 orders of magnitude in the response rates for these two endpoints. Even if the selected set of TEF values were able to reasonably predict these two responses near their respective EC50s, the predictions would thus be

many orders of magnitude discrepant at just 10-fold lower doses. The marked nonparallelism of the various dose-responses for the different AhR-mediated endpoints analyzed by US EPA seriously compromises the applicability of the TEF approach except possibly in the near vicinity of the endpoint-specific EC50s. In other words, to be useful, TEFs may need to be constrained to a specific and fairly narrow range of doses near those used to develop them.

Failure to Consider Natural AhR Agonists

As we noted previously, TEFs have been developed only for the most persistent and bioaccumulative dioxins and furans and a limited number of PCBs. Dietary intake of these AhR agonists amounts to approximately 5 pg TEQ/kg/day for a 60 kg adult. The human diet also contains natural AhR agonists, including indole-3-carbinol, polynuclear aromatic hydrocarbons, and aromatic amines formed during the cooking of foods. On both a mass and TEQ basis, these natural AhR agonists dominate dietary intake despite TEFs far smaller than that of TCDD. For example, dietary intake of PCDDs and PCDFs amounts to about 0.017 - 0.033 ng mass/kg/day (0.002 ng TEQ/kg/day), while the intake of indole-3-carbinol alone is about 12,250 ng mass/kg/day (1.2 ng TEQ/kg/day)[2]. While these natural AhR agonists may not persist and bioaccumulate like the PCDDs, PCDFs, and PCBs, they still contribute significantly to the human background body burden: assuming no bioaccumulation whatsoever, indole-3-carbinol alone contributes about 6 ppt TEQ (lipid adjusted), approximately the same amount contributed by TCDD alone. There is a clear need to consider the natural dioxin-like compounds in any assessment of potential risks posed by AhR agonists.

Summary

At best, the TEF approach may have limited application in risk assessment. Under conditions in which the additivity assumption is justified and parallelism of dose-response curves is demonstrated, it may provide a basis for predicting the potential of a mixture of agonists to activate the Ah receptor and induce subsequent responses. However, it is clear that a given response in an animal model is not necessarily predictive of the same response in other animals, or in humans. Furthermore, a given response is not necessarily predictive of other responses (e.g., thymic atrophy in AhR-positive mice does not predict skin toxicity). Indeed, scientifically defensible application of TEFs may require its restriction to species-, endpoint-, and dose-specific situations. The complexities of the biology that lie downstream from receptor occupancy, particularly, those elements that function as "on/off" switches governing sequential responses, argue against any more than cautious use of the TEF approach for risk assessment purposes, and then, only in clearly defined circumstances.

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