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The Toxic Equivalents Approach for Fish and Wildlife

Donald E. Tillitt
Columbia Environmental Research Center, Columbia, MO

A NEED FOR THE APPROACH

The use of industrial, agricultural, and commercial products, as well as incineration of the wastes of our society has lead to the release of polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) into the environments around us. PCBs, PCDDs, and PCDFs, as well as other planar halogenated hydrocarbons (PHHs), can persist in the environment, bioaccumulate in top predators of the food web, and result in deleterious effects in exposed fish and wildlife. Mandatory restrictions in use (viz., PCBs) and increased efforts to reduce the unintentional production of other PHHs, such as PCDDs and PCDFs, have largely been successful in controlling the release of PHHs into the environment. Declining concentrations of PHHs in fish and wildlife species have been observed in many areas around the world. For example, the concentrations of PCBs, PCDDs, and PCDFs in most food webs in the Great Lakes have declined precipitously over the past three decades (Baumann and Whittle, 1988; Herbert *et al.*, 1994). Likewise, the concentrations of many of the persistent organochlorine chemicals found in biota of the Baltic Sea have decreased (Bignert *et al.*, 1995). Even with many successful stories of significant reductions in environmental concentrations of persistent PHHs, we still have the need and the requirement to understand if these chemicals are above or below a threshold for adverse, toxic effects in fish and wildlife species. The complex nature of chemical mixtures do not allow simple chemical by chemical regulatory procedures to work as accurately as is needed.

The concept of toxic equivalency factors (TEFs) and toxic equivalents (TEQs) for PHHs was introduced some time ago as the cytosolic receptor, the aryl hydrocarbon receptor (Ah-R) was discovered (Poland and Glover, 1975). The

* Columbia Environmental Research Center, Biological Resources Division, U.S. G.S., Department of Interior, 4200 New Haven Rd., Columbia, MO, USA, 65201

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TEF/TEQ concept for this class of chemicals developed along with our knowledge of the mechanism of action for Ah-R ligands (Safe, 1986; 1990; 1994; 1998). The TEF/TEQ approach allows the integration of a number of exposure variables (chemicals), and as such, has a great deal of intuitive appeal. Fundamental to the TEF/TEQ approach is the assumption of a similar, characterized mechanism of action. The notion that the planar PCBs, PCDDs and PCDFs must bind with the Ah-R prior to eliciting any dioxin-like effects is central to the TEF/TEQ concept. Also central to the TEF/TEQ approach is the idea of additivity. Not only does there need to be an understanding of relative potencies of the individual congeners, but it must be assumed that they all work through an additive model of toxicity to exert their dioxin-specific effects. The requirements for a detailed knowledge of the mechanistic pathway of dioxin-like chemicals and the assumption of complete additivity among responses used in environmental risk assessment of the following dioxin-like chemicals are both the strength and the weakness of the TEF/TEQ approach. Our combined knowledge of: (1) the chemicals which bind and activate the Ah-R; (2) the biochemical and pathological responses which result; (3) the nature and type of symptoms related to dioxin-like toxicity; (4) the critical life stages of greatest sensitivity; and (5) the phylogeny of the Ah-R, are probably greater for PHHs than any other class of environmental pollutants. This combined knowledge has provided the theoretical foundation for the TEF approach. The data generated are extremely rich, and literally thousands of research papers have been written on the responses of vertebrates to dioxin-like chemicals. Yet, there remain some critical gaps in our knowledge that tend to the limit the applicability of the TEF approach.

LIMITATIONS OF THE TEF/TEQ APPROACH

A discussion of limitations of the TEF/TEQ approach has to begin with our true lack of knowledge of the exact biochemical pathways which are altered by PHHs and subsequently lead to the adverse effects on whole organisms. We are well aware of Ah-R as a ligand-activated transcription factor, yet, debate continues regarding which altered gene products are responsible for, or result in toxicity (Schmidt and Bradfield, 1996). Moreover, non-genomic pathways have been hypothesized as being linked to Ah-R mediated toxicity (Matsumura, 1994; Enan and Matsumura, 1995). Additionally, the multitude of carcinogenic, immunologic, neurologic, endocrine, and embryo/fetotoxic endpoints that dioxin-like chemicals induce are immense. We certainly have not sorted out the complex interactions which occur in these biochemical and physiological pathways. As a result, the lack of a clearly defined biochemical and physiological pathway for the toxic effects of Ah-R agonists limits our ability to refine models which might predict responses in various organs, systems, and life stages. Thus, we are limited in our ability to predict relationships among responses and the TEFs based on those responses. We must rely on use of empirically derived relationships between response endpoints (Safe, 1986, 1990), which by their nature offer limited extrapolation to other species.

The TEF/TEQ approach is further limited due to the existence of partial agonists of Ah-R mediated responses. Partial agonists make comparison of responses among individual congeners troublesome because efficacy of the partial agonist is lower than the full agonist (TCDD in this case). Therefore, a comparison of TEFs based on the full response of the dose model is inappropriate. Partial agonists can also lead to antagonism through competition for the Ah-R with full agonists (Gooch et al., 1989). Another limitation of the TEF/TEQ approach is the observance of non-additive interactions (antagonism/synergism) between Ah-R agonist (and non-Ah-R agonists). The fact that the mechanisms of these non-additive interactions are not well understood again limits the use of a strict additive model of toxicity, which is integral to the current TEF/TEQ approach. Interactions of Ah-R and non-Ah-R mechanisms can lead to responses which are specific to cells, organ systems, or a particular species. The TEF/TEQ concept is further limited by our lack of knowledge of the endogenous function and ligand for the Ah-R. Thus, simple activation of the Ah-R complex by endogenous compounds or xenobiotics does not necessarily result in an adverse effect in the organism. The role of the Ah-R and the genes it regulates in the metabolism of natural products is not well characterized with respect to the potential for adverse effects after chronic exposure. All these factors limit the theoretical model and our ability to use the TEF/TEQ approach in the appropriate situations.

Practical information, such as relative species sensitivities, also place constraints on the TEF/TEQ approach, as does our lack of detailed mechanistic information of the biochemical pathways which lead to adverse effects. This lack of data is particularly true for the fish and wildlife species we are trying to protect. The majority of relative potency values for various PHHs toward fish embryo-larval toxicity come from a single species, rainbow trout (Oncorhynchus mykiss) (van den Berg *et al.*, 1998). Limited data from other species indicate that the differences in TEFs among species of fish is not large, but more data are needed. The database for avian TEFs is even more scant, and the TEFs for example for PCDDs/PCDFs are largely derived from CYPIA induction in the chicken embryo (Bosveld *et al.*, 1992). Therefore, there is a clear need for the development of TEFs in a larger set of fish and wildlife species which are focused on the measurement of endpoints that are more readily applied to ecological risk assessment (*i.e.*, growth, development, mortality).

THEORETICAL LIMITATIONS VERSUS PRACTICAL CONSIDERATIONS

The limitations of the TEF/TEQ approach, many of which are related to construction of a theoretical model for dioxin-like toxicity, must be weighed against the practical considerations and benefits that are gained by this approach. Most of the limitations described above are of a theoretical nature, that is, our lack of knowledge in the areas of defined biochemical pathways leading to organismal toxicity, partial agonists, antagonism at biochemical and physiological levels of organization, and the natural function of the receptor, all create uncertainty in the additive model of toxicity that are central to the

TEF/TEQ concept. Ideally, we would understand the crucial steps leading to toxicity and the mechanisms for interaction among congeners of the mixture and construct a model that could be predictive for each. However, neither a complete model nor the validation through testing of combinations and permutations of PHH/non-PHH chemicals is likely to occur soon. Yet, there exists an enormous amount of information that describes the initial events of dioxin-induced toxicity (Ah-R binding) and the adverse toxicological outcomes (immunotoxicity, embryo lethality, etc.). The vast amount of these data suggest that an additive model of toxicity's is appropriate.

The practical benefits derived from the TEF/TEQ approach in ecological risk assessment are greater than the problems associated with not using this approach. The approaches that are currently used in the U.S. to regulate discharges (Clean Water Act) or evaluate clean up requirements at contaminated sites (Comprehensive Environmental Remediation and Liability Act) typically regulate PCBs as "total PCBs" and PCDD/PCDFs by TCDD alone. There are obvious problems with regulation of TCDD alone, since it is present in most situations with other chlorinated dioxins or furans. The estimated exposure to all of the Ah-R active compounds in such a situation is almost certainly an underestimation of the true exposure encountered by the organism (receptor). The problem with regulation of PCBs solely as "total PCBs", without regard to the individual dioxin-like congeners present in the mixture, is the fact that the ratios of PCB congeners are known to be dramatically altered in the environment. This is due to differential fate, transport, and metabolism rates of the individual PCB congeners. The proportion of dioxin-like congeners (planar PCBs) in a mixture of PCBs from the environment is not constant, either temporally or spatially. Therefore, regulation of PCBs as "totals" or "Aroclor" equivalents is critically flawed, because the relative dioxin-like potency is inherently held constant in these methods. It is the dioxin-like potency of PCBs which is often the greatest known or observable risk associated with PCB mixtures (Giesy et al., 1994). The accuracy of predictions made using the TEF/TEQ approach in an environmental risk assessment is expected to be greater than the accuracy of predictions based on TCDD by itself or based on total PCBs. The net result of this greater accuracy is better protection of fish and wildlife and better information upon which to make regulatory decisions.

Another way to gauge the utility of the TEF/TEQ approach is by the uncertainties associated with this approach as compared to other components of the ecological risk assessment framework. Limitations of the theoretical TEF/TEQ model and practical data add to the uncertainties of this approach, as described above. The uncertainty associated with the application of TEFs to an untested endpoint, or application of TEFs to untested mixtures of compounds can be significant. However, when compared to the other uncertainties encountered in ecological risk assessment, those uncertainties asssociated with additivity do not appear to be great. It is not uncommon for risk assessment methods to have uncertainty factors of two to ten associated with each extrapolation of the data between species, endpoints, exposure routes/regimes or for protection of sensitive individuals within a population (USEPA, 1992; Suter

et al., 1993). The level of uncertainty in the TEF/TEQ model is thought to be less than an order of magnitude when the appropriate TEFs are applied (van den Berg *et al.*, 1998). There are many examples that indicate less than an order of magnitude can be expected (van den Berg *et al.*, 1998), particularly in applications with fish and wildlife. It is currently a lack of knowledge rather than a lack of confidence in the TEF/TEQ model which creates uncertainty and limits this approach in ecological risk assessment.

APPLICATION TO FISH AND WILDLIFE

Ecological risk assessments conducted to protect fish and wildlife and, in particular fish and avian wildlife species, are unique within the TEF/TEQ approach. They are unique in the sense that management is conducted at the level of the population rather than the individual. Key organismal endpoints used in population dynamics models are natality (*i.e.*, embryo lethality) rates and early life stage growth rates (USEPA, 1992; Suter *et al.*, 1993). These are the most common and consistently measured endpoints in toxicity studies of PHHs in fish and birds, and often form the basis for determination of the TEF (van den Berg *et al.*, 1998). As such, the uncertainty of extrapolation among endpoints is reduced in the application of the TEF/TEQ approach for fish and avian species. The derivation of TEFs in fish and avian wildlife species often includes delivery of a precise dose of the compound to an egg. The exact dose of the PHH is known, or measured; thus the implications of metabolic differences among species are reduced.

Validation of the TEF/TEQ approach as an additive model is an on-going effort. Studies that have examined the validity of an additive model of toxicity for PCDDs, PCDFs, and PCBs in fish and wildlife species range from isobolographic studies of two Ah-R agonists (and/or antagonists) to the testing of complex environmental mixtures. Additive effects of Ah-R agonists and non-Ah-R compounds have generally been found in rainbow trout and lake trout embryos when two component mixtures were tested following the isobolographic method (Zabel *et al.*, 1995a,b; Hornung *et al.*, 1996). The deviations from strict additivity were less than a factor of two in the LD50 values. Brominated analogs of 2,3,7,8-TCDD and other dioxin, furan and biphenyl congeners also have shown additive interactions (Hornung *et al.*, 1996). Although synergistic interactions have been observed for AHH induction in rainbow trout (Janz and Metcalfe, 1991), most studies of early life stage mortality have resulted in additive toxicity.

Additivity appears to be the general case when synthetic or complex environmental mixtures of chemicals have been tested in fish. Walker *et al.* (1996) tested a synthetic mixture of Ah-R agonists and non-Ah-R compounds in rainbow trout to determine if the toxicity followed a simple additive model. Additionally, graded doses of an organic extract made from Lake Michigan lake trout, injected into eggs of hatchery reared rainbow trout (Wright and Tillitt, 1999) and lake trout (Tillitt and Wright, 1997), produced symptoms of dioxin-like toxicity (*i.e.*, yolk-sac edema, craniofacial deformities, and hemorrhaging)

in a dose-related fashion in both species. The TEF/TEQ approach for quantification of the doses suggested additive toxicity in both of these species.

The concept of TEF/TEQs and an additive model of toxicity is supported by studies of embryotoxicity in birds as well. The toxicity of an environmentally derived mixture of chemicals, including dioxin-like chemicals, was tested in chickens due to their known sensitivity (Powell *et al.*, 1997b). The predicted LD50 of this mixture was 144 pg TEQs/g of egg, which is remarkably close to the actual LD50 of 150 pg TCDD/g of egg (Powell *et al.*, 1996; Henshel *et al.*, 1997). Additionally, in support of the TEQ approach, these authors found that application of the PCB 126 TEF for embryolethality in chickens (Powell *et al.*, 1996) successfully predicted the LD50 for TCDD in double crested cormorant embryos (Powell *et al.*, 1997a; 1998).

Additivity is most prevalent toxic interaction among Ah-R agonists and non-Ah-R compounds in species of fish and wildlife. The studies of chemical pairs or complex mixtures of organochlorine chemicals found in the environment have largely shown additive interactions for embryo lethality. Some non-additive responses have been observed when endpoints other than mortality have been measured. However, ecological risk assessments to protect the integrity of fish and wildlife populations are most often conducted using organismal level responses and in particular mortality. Thus, the use of the TEF/TEQ approach to address the significance of potential or existing exposure of fish and wildlife species to dioxin-like chemicals is supported by the current status of our scientific information. It would be scientifically unsound to disregard this approach and not use it in ecological risk assessments for fish and wildlife species. Clearly, additional studies are needed to more precisely understand the limitations of application of the TEF/TEQ approach. Yet, current documentation in fish and wildlife species indicates that the uncertainties associated with not using such an approach are greater than the uncertainties of using it.

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