# Beyond TEFs: Mixtures of dioxins and non-dioxins

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD, dioxin) is the prototype for a family of chemicals which are structurally related, have a common mechanism of action, and induce a common spectrum of biological responses[1,2]. Many of these chemicals are highly persistent and bioaccumulate in the environment and up the food chain. This family of chemicals includes the polyhalogenated dibenzo-p-dioxins, dibenzofurans, biphenyls, naphthalenes, diphenyl ethers, and others. Because binding to the Ah receptor is necessary, if not sufficient, for the dioxin-like biological effects, these compounds must be able to assume a planar configuration and have lateral halogen substitution. Chlorine, bromine, or mixed chloro-bromo substitution can meet these requirements. The number and position of halogenation also determines the biological persistence.

TCDD is never found in isolation in the environment or in biological tissues. Because many of the related compounds have similar biological properties, it is important to consider the sum total of the biological activity present in a complex mixture in order to estimate the potential for risk. In order to meet this risk assessment and regulatory need, toxic equivalency factors (TEFs) have been developed[3-6]. TEF values are consensus values based on all the available data and scientific judgment[7-9]. They represent a relative potency ranking scheme, with the relative potency of each chemical being compared to that of TCDD. Since the mechanistic basis is binding to the Ah receptor, this common mechanism of action requires parallel dose/response curves for all congeners for any given response. However, the dose/response curves for different responses need not be parallel. Structure/binding as well as structure/activity relationships have been used to develop the consensus TEF values, which are order of magnitude estimates of relative potency. The TEF values are NOT based solely on ligand binding, nor are they based only on enzyme induction, either *in vivo* or *in vitro*. A broad array of both *in vivo* and *in vitro* responses have been evaluated in the determination of consensus TEF values, ranging from keratinization of skin cells in culture, through developmental toxicity, immunotoxicity, and tumor promotion.

Recent studies from our laboratory have involved subchronic studies in both rats and mice to individual dioxins, furans, and PCBs, as well as complex mixtures resembling concentration ratios observed in food samples[9-19]. By conducting long term studies, the relative potency values (REPs) determined in these studies were able to correct for any pharmacokinetic differences between the different congeners, which have often caused confusion in the past in the determination of TEF values[20]. REPs were determined for the induction of CYP1A1 and CYP1A2 activity in liver and CYP1A1 activity in lung and skin in female mice. These values were compared on the basis of administered dose as well as tissue concentration. For several of the congeners (TCDF, 1,2,3,7,8-PeCDF, 2,3,4,7,8-PeCDF, PCB #77), the REP based on administered dose was approximately 10X different from that based on dose in a given tissue[21]. However, these differences did not have a major impact on the TEQ of the mixture. The REPs determined for enzyme induction did an excellent job of predicting immune suppression of the complex mixture[17]. They also predicted the decreases in hepatic retinoids in both the mice[15] and rats[16], as well as predicting enzyme induction (both CYPs , all

3 tissues) in the rat[19]. However, while the REPs for enzyme induction predicted the induction of hepatic porphyrins by all the PCDDs, PCDFs, and coplanar PCBs in the mouse, they drastically underpredicted the porphyrogenic response to the mono-ortho PCBs[14]. Similarly, the TEQ based on the REPs for enzyme induction also underpredicted the hepatic and urinary porphyrin accumulation in the "food" mixture[22]. Addition of PCB 153 to this mixture further exacerbated this synergism. Likewise, synergistic interactions appear to be involved in the decrease in circulating T4 levels in rats when the "food" mixture, containing both dioxins, furans, coplanar and monortho-coplanar PCBs are used[18]. This may in part reflect the greater than additive increase in UDP-glucuronosyl transferase activity when both dioxin-like and nondioxin-like compounds are present in a mixture. It is important to stress that in both of these examples of synergistic effects, mechanisms in addition to activation of the Ah receptor are involved. There is no evidence for anything other than strict dose additivity if the response mechanism involves activation of the Ah receptor only.

Likewise, antagonistic effects may also involve multiple mechanisms. Alterations in enzyme induction have been suggested to involve changes in target tissue distribution[23]. Recently, our laboratory has demonstrated that the ability of PCB 153 to antagonize the TCDD-induced immunosuppression is a functional, not receptor-mediated, antagonism[24]. High doses of PCB 153 enhance the primary antibody response. However, this increase in "set-point'" of the immune system can still be blocked by dioxin. These effects also involve very high exposure concentrations and environmentally unrealistic concentration ratios of the chemicals in question. It is important to note that non-additive interactions are often both concentration and concentration ratio dependent, as well as response specific.

Partial agonists, such as rapidly metabolized, non-halogenated compounds, do not fit the basic requirements of the dioxin-TEF scheme. However, they do bind to the Ah receptor with reasonable affinity. At very high concentrations, they would have the ability to compete with the persistent ligands. However, it is important to note that physiology has evolved to be able to recognize the difference between a pulsatile signal, such as from rapidly metabolized compounds which involve daily exposure, and a continuous signal. There is little evidence from studies in which the pharamcokinetic properties of these chemicals are taken into consideration that the rapidly metabolized, nonpersistent compounds are having significant impact on the Ah-mediated responses from persistent dioxins[25].

However, it is imperative that the real-world issues of environmental mixtures begin to be addressed. Not only is TCDD never present alone, but the dioxins, furans, and PCBs are also not present in a vacuum. They are often in the presence of other ligands for the Ah receptor, albeit non-persistent and partial agonists, such as PAHs, flavenoids, etc. There are also other persistent organics present, as well as the nondioxin-like PCBs, such as the chlorinated pesticides. In addition, there is co-exposure to metals, volatiles, and gases. If there are common phenotypes brought about by multiple mechanisms, is there not an obvious potential for response synergism or antagonism? A key issue is the dose levels at which interactions occur. Are they environmentally relevant? We are just beginning to recognize the need to address these exceedingly complicated issues.

(This abstract does not reflect EPA policy.)

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