RELATIVE POTENCY FACTORS FOR ENZYME INDUCTION BY DIOXIN-LIKE COMPOUNDS IN CHRONIC RODENT CARCINOGENICITY STUDIES

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Introduction

When considering the risk of human exposure to dioxins, it is well established that exposure is to complex mixtures of dioxin-like compounds rather than to the specific individual congeners most often used in laboratory studies[1,2]. To assess the risk of these mixtures the Toxic Equivalency Factor (TEF) approach has been developed to characterize the toxicity of mixtures of dioxin-like compounds [3,4]. TEFs have been developed for persistent compounds that bind to the Ah receptor, and result in dioxin-like effects. Summation of the mass of each congener in a mixture, after adjustment for its potency relative to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the most potent congener, gives an estimation of the total Toxic Equivalents (TEQ) in the mixture. Currently polychlorinated dibenzodioxins, polychlorinated dibenzofurans and non-ortho and mono-ortho polychlorinated biphenyls are included in this TEF scheme[3]. While TEFs were developed as an interim approach to facilitate exposure assessment and hazard identification, there has been an increasing use of this scheme to assess cancer dose-response relationships in exposed human populations [5]. The development of TEFs for dioxin-like compounds has primarily been based upon a variety of endpoints including preneoplastic liver lesions, immunotoxicity, and dioxin-regulated responses that reflect the activation of the aryl hydrocarbon receptor (AhR).

Currently cancer risk is a driving force in the establishment of guidelines for allowable human exposures to dioxins. While TEFs have been shown to be predictive for the effects of mixtures on endpoints such as tumor promotion, immunotoxicity and enzyme induction, the validity of current TEFs for predicting cancer risk is not known. To test the validity of the TEF approach for the prediction of cancer risk, multiple two-year life-time rat studies are currently being conducted by the National Toxicology Program to evaluate the chronic toxicity and carcinogenicity of dioxin-like compounds and mixtures of these compounds[6]. Although these studies are currently ongoing, interim necropsies have been conducted at earlier time points for the analysis of multiple non-cancer dioxin-regulated responses, prior to the development of tumors. The focus of this paper is to evaluate the relative potencies for induction of cytochrome P450 1A1 enzyme activity in the livers of rats taken from these interim necropsies.

Materials and Methods

Female Harlan Sprague-Dawley rats were treated by oral gavage five times per week with either 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (3, 10, 22, 46 or 100ng/kg/day), 3,3',4,4',5-pentachlorobiphenyl (PCB126)(10, 30, 100, 175, 300, 550 or 1000 ng/kg/day) or a "TEF mixture" consisting of an equal TEQ ratio of TCDD:PCB126:2,3,4,7,8-pentachlorodibenzofuran (PCDF) (10, 22, 46, or 100 ngTEQ/kg/day) for up to 52 weeks. Animals were sacrificed 24 hours after the last dose. Control animals received corn oil alone. Group sizes were 50 animals per chemical group per dose for the 104 week carcinogenicity studies, and 8-10 animals per dose, per chemical for interim necropsies carried out after 13, 30, and 52 weeks of treatment for which enzyme activity was assessed. Cytochrome P450 1A1(CYP1A1) associated 7–ethoxyresorufin–O-deethylase (EROD) activity was determined in microsomal proteins, isolated from frozen liver tissue according to established procedures. Dose-dependent changes in mean EROD activity were modeled using a sigmoidal Hill function[7] $y=E_0+(E_{max}*x^n/(ED_{50}^n+x^n))$, where y = activity, x = daily dose, E_0 = activity at zero dose, E_{max}= the maximum increase in activity above E_0, ED_{50} = daily dose at 50% of the maximum response, n = Hill coefficient (shape parameter). Relative potencies (REP) were calculated at the ED_x where REP = ED_xTCDD/ED_xTest compound.

Results and Discussion

Chronic exposure to TCDD, PCB 126 and the "TEF Mixture" led to a dose-dependent increase in hepatic CYP1A1-associated EROD activity. The absolute level of the maximally induced EROD activity varied between the time points of necropsy and also between the different individual studies. As illustrated in Figure 1, in many cases the predicted maximal activity, based on the Hill model fits, was not achieved within the dose ranges used in the studies. In addition there was substantial variation between different chemicals. Since it is known that EROD activity diminishes as a result of tissue freezing and storage, some of these differences may be reflective of both methodological considerations in addition to potential differences in efficacies of the different compounds/mixtures. For the calculation of relative potencies, the dose-response curves for induction of EROD activity were modeled using simple Hill kinetics and the 50% effective dose was calculated (Table I). At the ED_{50} the relative potency for induction of EROD activity by PCB126 ranged only two-fold (0.07-0.15) and was similar to the current TEF value of 0.1 [3] (Table II). The relative potency of the "TEF mixture", whose design was based on current TEF values varied almost 4-fold (0.32-1.24) and was similar to the expected value of 1.0 (Table II). These data confirm the validity of the TEF methodology under chronic steady state conditions for assessing the potency of mixtures, for activation of a known AHR-inducible response such as induction of CYP1A1 activity.

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Table I. Dose-response model predictions of the half-maximal effective dose (ED_{50}) (ng/kg/day) .

	Duration of exposure		
Compound	13 weeks	30 weeks	52 weeks
TCDD	4.5	8.2	6.0
PCB126	61.4	45.6	41.1
"TEF Mixture"	14.1	6.6	10.8

Table II. Relative potencies based on the half-maximal effective dose.

	Duration of exposure		
Compound	13 weeks	30 weeks	52 weeks
PCB126	0.07	0.18	0.15
"TEF Mixture"	0.32	1.24	0.56

Figure 1. Dose dependent induction of EROD activity by TCDD, PCB126 and the "TEF" mixture. Hill model fits are shown for TCDD (closed circles) PCB126 (open circles) or the TEF mixture (open triangles). For the "TEF mixture" dose units are ngTEQ/kg/day.