PROMOTION OF PRENEOPLASTIC RAT LIVER FOCI WITH 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN, 1,2,3,4,6,7,8-HEPTACHLORODIBENZO-p-DIOXIN AND A DEFINED MIXTURE OF 49 POLYCHLORINATED DIBENZO-p-DIOXINS (PCDDs)

Schrenk, D.[A], Buchmann, A.[A], Lipp, H.-P.[A], Brunner, H.[B], Sirma, H.[C], Hagenmaier, H.[B], Gebhardt, R.[C] and Bock, K.W.[A]
[A]Institut für Toxikologie, Wilhelmstr.56, D-7400 Tübingen, Germany
[B]Institut für Organische Chemie, Auf der Morgenstelle 18, D-7400 Tübingen, Germany
[C]Physiologisch-chemisches Institut, Hoppe-Seyler-Str.4, D-7400 Tübingen, Germany

Introduction

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) was reported to act as a strong hepatocarcinogen[1] in rodents, probably by promoting the expansion of preneoplastic liver foci[2]. Binding to the cytosolic dioxin receptor may influence growth of hepatocytes[3], an effect probably linked to tumor promotion[4]. The most thoroughly investigated biological effect of TCDD and analogue PCDDs, induction of CYP1A1, allowed the calculation of TCDD equivalents (TE) in rat hepatocytes in primary culture[5]. A broader approach has been chosen to calculate international equivalency (ITE) factors[6] used in risk assessment. In this study the hypothesis was tested that equivalency factors can be used to estimate the promoting potency of 1,2,3,4,6,7,8-heptachlorodibenzo-p-dioxin (HCDD; ITE 0.01; TE 0.02[5]) and of a mixture of 49 PCDD congeners (ITE 0.01; TE 0.01[5]) in rat liver in comparison to TCDD. As a dioxin receptor-mediated effect, induction of hepatic CYP1A activity was analyzed as well.

Methods

M2, a mixture of 49 PCDD congeners was obtained by dechlorination/hydrogenation of octachlorodibenzo-p-dioxin[7] (For composition see ref. 5). For initiation of preneoplastic foci, groups of five animals received N-nitrosomorpholine in the drinking water (80 mg/l) over 25 days, and then were treated with corn oil (controls) or PCDDs by s.c. injection once every 2 weeks at doses of 28, 280, and 2800 ng TCDD/kg; 1,400, 14,000, and 140,000 ng HCDD/kg; and 2,800, 28,000, and 280,000 ng M2/kg over 13 weeks. These doses are equivalent to 2, 20, and 200 ng TCDD/kg x day; 100, 1,000, and 10,000 ng HCDD/kg x day;

and 200, 2,000, and 20,000 ng M2/kg x day. After 13 weeks, livers were analyzed for PCDDs[8], microsomal 7-ethoxyresorufin O-deethylase (EROD) activity, and ATPase-deficient or glutathione S-transferase P (GSTP)-positive preneoplastic foci[9]. Relative focal volume per liver was analyzed stereometrically as described[10].

Results

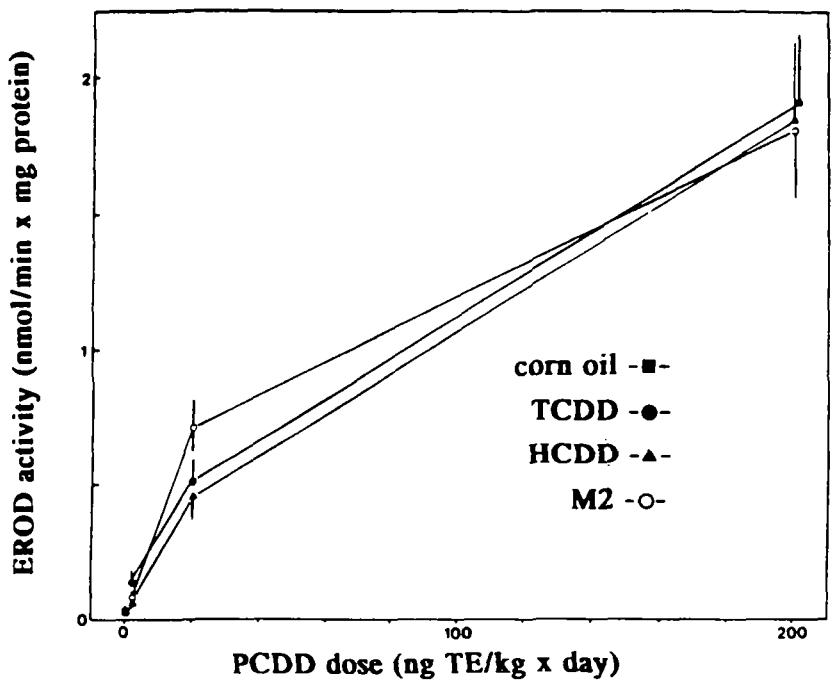

Fig. 1: Effect of treatment with PCDDs or corn oil on EROD activity in liver microsomes from female Wistar rats. Animals initially received N-nitrosomorpholine and were subsequently treated over 13 weeks. Data represent means ± SD from groups of 5 animals.

Based on the dose administered, equivalent induction of hepatic CYP1A activity was obtained with TCDD, HCDD and M2 (Fig. 1). However, based on tissue levels (Tab. 1) the potencies of HCDD and M2 were overestimated 2-5fold at higher doses (not shown). Analysis of the relative focal volume (RFV) of preneoplastic lesions (Tab. 1) was hampered by a high degree of inter-individual variability. However, the following conclusions may be drawn from analysis of mean RFV values: i) 2 and 20 ng TCDD/kg x day resulted in a slight increase in mean RFV; at 200 ng a more pronounced effect was seen. ii) For the highest dose of M2 the promoting efficacy was apparently lower than equivalent to TCDD.iii) With HCDD an increased RFV was only seen at a dosage level of 10,000 ng/kg x day.

Tab. 1. **Effect of PCDD levels and relative focal volumes of preneoplastic foci in liver from rats according to Fig. 1.**

Treatment	dose (ng/kg x day)	liver level (ng ITE[a]/g)	RFV (%) ATPase	RFV (%) GSTP
Control	-	0.1±0.1[b]	0.19[c] (0.01-0.52)	0.88 (0.04-2.19)
TCDD	2	0.5±0.1	0.43 (0.02-1.16)	0.93 (0.12-2.95)
	20	3.0±0.1	0.36 (0.06-0.95)	1.63 (0.41-3.04)
	200	23.1±4.1	1.83 (0.40-4.90)	2.31 (0.54-6.76)
HCDD	100	0.27±0.1	0.28 (0.08-0.49)	0.41 (0.08-0.82)
	1,000	3.2±1.3	0.13 (0.06-0.19)	0.21 (0.01-0.38)
	10,000	35.0±4.8	1.12 (0.23-2.58)	1.36 (0.38-2.41)
M2	200	0.8±0.1	0.24 (0.05-0.47)	0.55 (0.12-0.83)
	2,000	8.0±0.9	0.66 (0.05-2.19)	1.17 (0.04-3.74)
	20,000	93.0±7.0	0.75 (0.09-1.41)	0.82 (0.04-1.87)

[a]According to ref. 6.
[b]Mean±SD from 5 animals.
[c]Mean and range (in parentheses) from 5 animals (liver sections).

Discussion

The use of ITE factors is very common in toxicological practice to estimate the risk of human exposure towards PCDD mixtures. The hypothesis was tested whether equivalency factors obtained from induction studies in hepatocyte cultures are useful to predict the tumor promoting potency of HCDD and a mixture (M2) of 49 PCDD congeners in comparison to TCDD. Dioxin-receptor mediated induction of CYP1A was also determined. The **inducing** potencies of HCDD or M2 were equivalent based on dosage levels but were overestimated 2-5fold based on liver levels (ng ITE or ng TE per g). This discrepancy may be due to toxicokinetic differences which lead to an accumulation of higher-chlorinated congeners. Lower inducing potencies of M2 and HCDD, based on actual liver levels, may reflect an overestimation of these congeners.

Drawbacks in the analysis of the **promoting** effects were a high inter-individual variability and our incomplete knowledge on the toxicokinetics of PCDD congeners. However, suggestive evidence is provided that the promoting potency of M2 was approximatly equivalent to TCDD, except for the highest dose of M2. With HCDD, increased RFV of preneoplastic foci was obtained at 10,000 but not at 100 or 1,000 ng/kg x day, while liver levels were in the expected range. Thus, the use of equivalency factors apparently leads to an overestimation of the promoting potency of HCDD at lower doses.
In conclusion our findings suggest, that TCDD equivalency factors may be useful as rough estimates for the tumor promoting potency of PCDD mixtures. For HCDD, and probably for other highly-chlorinated congeners, current ITE factors may overestimate the actual risk.

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