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BIOLOGIC AND TOXIC EFFECTS OF POLYCHLORINATED DIBENZO-*p*-DIOXIN AND DIBENZOFURAN CONGENERS IN THE GUINEA PIG

QUANTITATIVE STRUCTURE–ACTIVITY RELATIONSHIPS

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Abstract—The dose–response effects of 2,3,7,8-tetrachorodibenzo-*p*-dioxin, 1,3,7,8-tetrachlorodibenzo-*p*-dioxin, 1,2,4,7,8-pentachlorodibenzo-*p*-dioxin, 2,3,4,7,8-, 1,2,3,7,9-, and 2,3,4,7,9-pentachlorodibenzofuran on body weight loss and hepatic microsomal aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin *O*-deethylase (EROD) induction were determined in the immature male guinea pig. The ED_{50} values for each compound were measured for the three *in vivo* responses. The quantitative structure–activity relationships clearly illustrated that the most toxic congeners were substituted in the lateral 2, 3, 7 and 8 positions, and removal of a lateral chlorine group substantially reduced the potency of the resulting compound. The most toxic congener in this series was 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in which the *in vivo* ED_{50} values for AHH and EROD induction and body weight loss were 2.8×10^{-10}, 9.3×10^{-11} and 5.6×10^{-9} mol/kg. The structure–activity relationships observed in this study were comparable to those previously reported in rats and rat hepatoma H-4-II E cells in culture. Moreover, there was an excellent linear correlation between *in vivo* $-\log ED_{50}$ values for body weight loss, AHH and EROD induction and the corresponding *in vitro* $-\log EC_{50}$ data for AHH induction in rat hepatoma cells [S. Safe, *Chemosphere* **16**, 791 (1987)].

Halogenated aromatic hydrocarbons such as the polychlorinated biphenyls (PCBs) are industrial compounds which have been widely identified as environmental contaminants in almost every component of the global ecosystem [1–5]. The polychlorinated dibenzofurans (PCDFs) and dibenzo-*p*-dioxins (PCDDs) are industrial or combustion by-products which have also been released into the environment, and relative low levels of these compounds (<parts per billion) have also been identified in diverse environmental matrices including sediment, fish, wildlife, human adipose tissue and milk [6–16]. Invariably, analysis of extracts containing these halogenated aromatic compounds reveals a complex mixture of isomers and congeners from each class.

The most toxic halogenated aryl hydrocarbon, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), has been utilized as a prototype to investigate the mechanism of action of PCDDs and related compounds. Most studies support a mechanism of action which involves the initial interaction of the toxin with a cytosolic aryl hydrocarbon (Ah) receptor protein [17–22]. Structure–activity relationships (SARs) among halogenated aryl hydrocarbons have demonstrated that the most toxic compounds among the PCDDs, PCDFs and PCBs are approximate isostereomers of 2,3,7,8-TCDD and are substituted in at least three of the four lateral positions in the aromatic ring systems (i.e. 2, 3, 7 and 8 positions for PCDDs and PCDFs; 3, 3′, 4, 4′, 5 and 5′ positions for the PCBs) [17–30]. Quantitative SARs for several individual PCB, PCDD and PCDF congeners and mixtures have shown that there is an excellent linear correlation between their *in vitro* $-\log EC_{50}$ values for aryl hydrocarbon hydroxylase (AHH) induction in rat hepatoma H-4-II E cells in culture and their *in vivo* $-\log ED_{50}$ values for several receptor-mediated responses [e.g. body weight loss, thymic atrophy and hepatic microsomal ethoxyresorufin *O*-deethylase (EROD) and AHH induction] in the rat [22, 24–26, 29]. These data suggest that the *in vitro* AHH induction bioassay might serve as a quantitative short-term system for estimating the toxicity (e.g. "2,3,7,8-TCDD equivalents") of halogenated aromatic hydrocarbon pollutant extracts and obviate the need for more expensive congener-specific high resolution gas chromatographic–mass spectrometric (GC–MS) analysis [31]. Moreover, in contrast to GC–MS analysis of PCDDs and PCDFs, the bioassay result can give some indication of the "toxic equivalents" associated with a specific analyte.

Previous studies have shown that the qualitative SARs for PCBs, PCDDs and PCDFs for Ah receptor binding avidities and several proposed receptor-mediated effects (e.g. keratinization, body weight loss, immunotoxicity, LD_{50}, tumor promotion, reproductive toxicity and AHH induction) are comparable in all species and mammalian cells in culture which have been investigated [17–31]. However, extensive quantitative SARs for these compounds

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have been reported only in cell culture studies and in the rat [22, 24–26, 28–31]. This study investigates the *in vivo* dose–response effects of 2,3,7,8-TCDD and selected PCDD and PCDF congeners in the guinea pig and correlates their *in vivo* $-\log ED_{50}$ data with their $-\log EC_{50}$ values for the induction of AHH in rat hepatoma H-4-II E cells in culture.

MATERIALS AND METHODS

Chemicals and biochemicals. The synthesis and purification of 2,3,7,8-TCDD, 1,3,7,8-TCDD, 1,2,4,7,8-pentachlorodibenzo-*p*-dioxin (PeCDD), 2,3,4,7,8-pentachlorodibenzofuran (PeCDF), 1,2,3,7,8-PeCDF, 2,3,4,7,9-PeCDF and 1,2,3,7,9-PeCDF have been described previously [24–26]. 3-Methylcholanthrene, benzo[*a*]pyrene, NADP and NADPH were purchased from the Sigma Chemical Co., St. Louis, MO. Ethoxyresorufin (>98% purity) was synthesized in this laboratory. 3-Hydroxybenzo[*a*]pyrene was obtained from the Chemical Carcinogen Repository, IIT Research Institute, Chicago, IL.

Animal treatment and isolation of microsomes. Immature male Hartley strain guinea pigs (4 to 5-weeks-old) were purchased from Hilltop, Scottsdale, PA, and acclimatized for 1 week prior to these studies. The PCDD or PCDF congeners were dissolved in corn oil and administered to the guinea pigs by intraperitoneal injection (5 ml corn oil/kg). The doses of each compound administered to the guinea pig were: 2,3,7,8-TCDD (0, 0.01, 0.05, 0.1 and 1.0 μg/kg); 2,3,4,7,8-PeCDF (0, 0.03, 0.3 and 2.89 μg/kg); 1,2,3,7,8-PeCDF (0, 0.02, 2.0 and 20 μg/kg); 2,3,4,7,9-PeCDF (0, 10, 20 and 75 μg/kg); 1,2,4,7,8-PeCDD (0, 1.24, 12.4 and 124 μg/kg); 1,2,3,7,9-PeCDF (0, 50, 200 and 800 μg/kg); and 1,3,7,8-TCDD (0, 11, 110 and 1110 μg/kg). For each set of experiments, three to four animals were used at each dose, and 2,3,7,8-TCDD served as a positive control for maximum induction of the hepatic microsomal monooxygenases. Corn-oil-treated (5 ml/kg) animals served as controls. Body weights were recorded every second day; after 14 days, the animals were killed by asphyxiation and the livers were perfused via the hepatic portal vein with ice-cold saline containing 0.1 mM EDTA, excised, weighed and placed in beakers containing homogenizing buffers. The washed livers were homogenized in 0.25 M sucrose/0.1 M EDTA, and microsomes were isolated by centrifugation at 10,000 *g* and recentrifuging the supernatant fraction at 100,000 *g* as described [24–26, 29]. The microsomal pellet was resuspended in homogenizing buffer and stored at −80° prior to use. The protein concentrations were determined by the method of Lowry *et al.* [32] using bovine serum albumin to standardize the assay, and the liver microsomal fractions typically contained 15–25 mg protein/ml. Hepatic microsomal AHH and EROD were determined as described by Nebert and Gelboin [33] and Pohl and Fouts [34] respectively. The ED_{50} for body weight loss was defined as the dose which causes a 50% decrease in body weight gain compared to the control guinea pigs. The ED_{50} values for AHH and EROD induction were the dose levels which induced 50% of the maximal induction response. The ED_{50} values were determined graphically from the dose–response data as described [24–26, 29]. The *in vitro* EC_{50} values for AHH and EROD induction by the PCDD and PCDF congeners were derived from previous studies [31].

RESULTS

The dose–response ED_{50} values for body weight loss and hepatic microsomal AHH and EROD induction were determined for the following congeners: 2,3,7,8- and 1,3,7,8-TCDD, 1,2,4,7,8-PeCDD, and 2,3,4,7,8-, 1,2,3,7,8-, 2,3,4,7,9 and 1,2,3,7,9-PeCDF. In addition to the structural diversity of these compounds, none of the PCDD and PCDF congeners contain adjacent unsubstituted carbon atoms; this served to minimize the effects of metabolism over the 2-week duration of this study. The results are summarized in Table 1. In general, the induction of hepatic microsomal AHH and of EROD were more sensitive responses to the PCDD and PCDF congeners than body weight loss, and this was also observed in previous studies in the rat [24–26]. The ED_{50} values for AHH and EROD induction for the most active compound, 2,3,7,8-TCDD, were 2.8×10^{-10} and 9.3×10^{-11} mol/kg respectively; in contrast, 1,3,7,8-TCDD was the least active compound which was >5000 times less potent than

Table 1. Quantitative ED_{50} values for body weight loss and AHH and EROD induction in immature male Hartley strain guinea pigs

PCD/PCDF congener	*In vivo* ED_{50} (mol/kg) values			*In vitro* AHH induction EC_{50} (M) values*
	AHH induction	EROD induction	Body wt loss	
2,3,7,8-TCDD	2.8×10^{-10}	9.3×10^{-11}	5.6×10^{-9}	7.2×10^{-11}
2,3,4,7,8-PeCDF	1.2×10^{-9}	7.1×10^{-10}	1.2×10^{-8}	2.56×10^{-10}
1,2,3,7,8-PeCDF	5.9×10^{-9}	7.0×10^{-9}	5.9×10^{-9}	2.54×10^{-9}
2,3,4,7,9-PeCDF	2.6×10^{-8}	4.4×10^{-8}	4.0×10^{-8}	5.8×10^{-9}
1,2,4,7,8-PeCDD	4.9×10^{-8}	2.1×10^{-8}	6.1×10^{-8}	2.1×10^{-8}
1,2,3,7,9-PeCDF	1.4×10^{-7}	1.2×10^{-7}	1.6×10^{-7}	8.6×10^{-8}
1,3,7,8-TCDD	1.6×10^{-6}	1.4×10^{-6}	8.6×10^{-7}	5.9×10^{-7}

* Taken from Refs, 24–26 and 31.

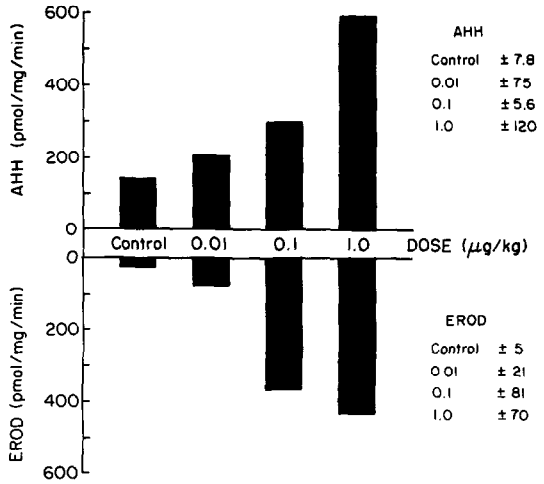

Fig. 1. Dose-response induction of hepatic microsomal AHH and EROD in male guinea pigs by 2,3,7,8-TCDD.

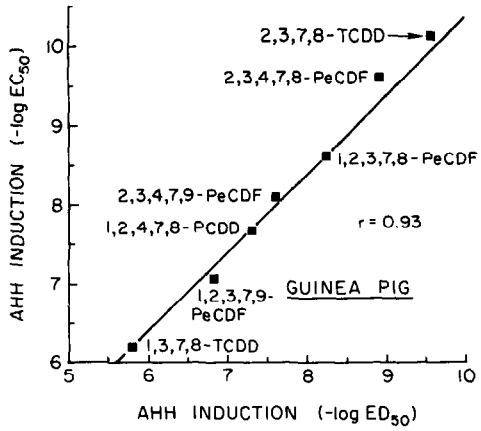

Fig. 3. Plot of the *in vitro* $-\log EC_{50}$ (AHH induction) vs the *in vivo* $-\log ED_{50}$ values for hepatic microsomal AHH induction for several PCDD and PCDF congeners in male guinea pigs.

2,3,7,8-TCDD as an inducer of hepatic microsomal monooxygenases. Figure 1 summarizes the dose–response induction of hepatic microsomal AHH and EROD in the guinea pig. The results showed that the induced levels of these enzymes (i.e. <600 pmol metabolite formed/mg protein/min) and the fold-inducibility were significantly lower than comparable values reported in the rat [24–26, 29]. SARs for both monooxygenase enzyme induction and body weight loss demonstrate that the most active compounds contained four lateral substituents (i.e. 2,3,7,8-TCDD, 1,2,3,7,8-PCDF, 2,3,4,7,8-PeCDF) and the removal of one of the lateral chlorine groups markedly decreased activity (i.e. 1,3,7,8-TCDD, 1,2,4,7,8-PeCDD, 1,2,3,7,9-PeCDF and 2,3,4,7,9-PeCDF (Figs. 2 and 3). These relationships are comparable to those reported for PCDDs and PCDFs in the rat and rat hepatoma H-4-II E cells in culture [31].

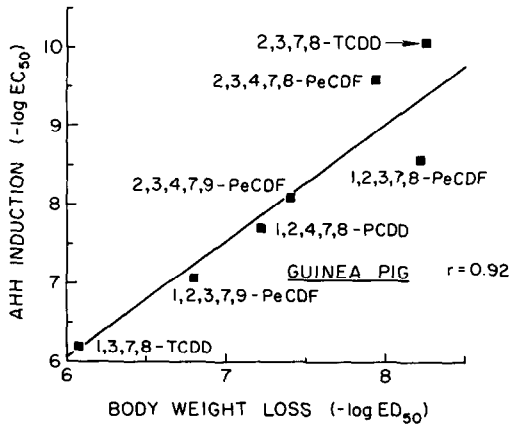

Fig. 2. Plot of the *in vitro* $-\log EC_{50}$ (AHH induction) vs the *in vivo* $-\log ED_{50}$ values for body weight loss for several PCDD and PCDF congeners in male guinea pigs.

DISCUSSION

Previous studies have demonstrated that the guinea pig is the most sensitive species to the lethal effects of 2,3,7,8-TCDD with LD_{50} values reported to be 0.6 or 2.1 μg/kg [23, 35]. However, very few studies have investigated the effects of 2,3,7,8-TCDD on other highly characteristic Ah receptor-mediated responses such as body weight loss and hepatic microsomal AHH and EROD induction. The results summarized in Table 1 confirm that the susceptibility of guinea pigs to 2,3,7,8-TCDD-mediated lethality is paralleled by their responsiveness to monooxygenase enzyme induction and body weight loss. The ED_{50} for body weight loss was 5.6×10^{-9} mol/kg (1.8 μg/kg) which was similar to the LD_{50} value. The ED_{50} values for the induction of hepatic microsomal AHH and EROD were 2.8×10^{-10} mol/kg (0.09 μg/kg) and 9.3×10^{-11} mol/kg (0.03 μg/kg), respectively, and confirm that the monooxygenase induction response is one of the most sensitive indicators of 2,3,7,8-TCDD exposure. The rat, which is less sensitive than the guinea pig to the lethal effects of 2,3,7,8-TCDD, is also less responsive than the guinea pig to the induction and toxic effects of this compound [26]. The ED_{50} values for body weight loss and hepatic microsomal AHH and EROD induction in immature male Wistar rats are 5×10^{-8}, 4×10^{-9} and 3×10^{-9} mol/kg, respectively [26], and these values are >ten times higher than the corresponding guinea pig ED_{50} values. Moreover, a comparison of the ED_{50} values for body weight loss and AHH and EROD induction for the remaining PCDD and PCDF congeners used in this study demonstrates that the guinea pig is generally more sensitive than the rat to the effects of these compounds [24–26]. However, it is apparent that comparable SARs were observed for both sets of compounds in the rat and guinea pig, and these results support a role for the Ah receptor protein in mediating these responses. The guinea pig and mink are two animal species that are highly susceptible to the toxic effects of 2,3,7,8-TCDD and related

compounds [18, 22, 23, 26, 35, 36] and yet exhibit relatively low inducibility of hepatic microsomal AHH and EROD. The reasons for the differences in toxic susceptibility and inducibility are unknown; however, it is possible that benzo[*a*]-pyrene and ethoxyresorufin are poor substrates for the induced cytochrome P-450 isozymes.

Previous studies in our laboratory have shown that for several PCB, PCDF and PCDD congeners and reconstituted mixtures their *in vitro* $-\log EC_{50}$ values for AHH and EROD induction in rat hepatoma H-4-II E cells and their *in vivo* $-\log ED_{50}$ values for body weight loss, thymic atrophy and hepatic microsomal AHH and EROD induction in rats [24–26, 29, 31] are linearly related ($r > 0.90$). Figures 2 and 3 illustrate plots of the *in vitro* $-\log EC_{50}$ AHH induction values versus the *in vivo* $-\log ED_{50}$ values for body weight loss and AHH induction in the guinea pig, and it is apparent that the two sets of data are linearly and highly, correlated ($r > 0.92$ for the correlations shown in Figs. 2–4). These results clearly demonstrate the utility of the *in vitro* AHH induction bioassay for predicting the toxicity of PCDDs and PCDFs in both the rat and guinea pig and supports the use of this procedure for determining the "toxic equivalent" of halogenated aromatic compounds or mixtures [31].

Although the SARs for PCDDs and PCDFs are comparable in most mammalian systems [17–31], with the exception of extensive studies in the rat [24–26, 29], only a limited number of individual congeners have been investigated in other animal species. It is apparent from this report that the SARs for PCDDs and PCDFs are comparable in the male rat and guinea pig, and this conclusion is strengthened by the excellent linear correlation between the quantitative SAR data from these two animal species as shown in Fig. 4. These SARs provide additional support for the role of the Ah receptor in mediating the biologic and toxic responses elicited by PCDDs, PCDFs and related toxic halogenated aryl hydrocarbons.

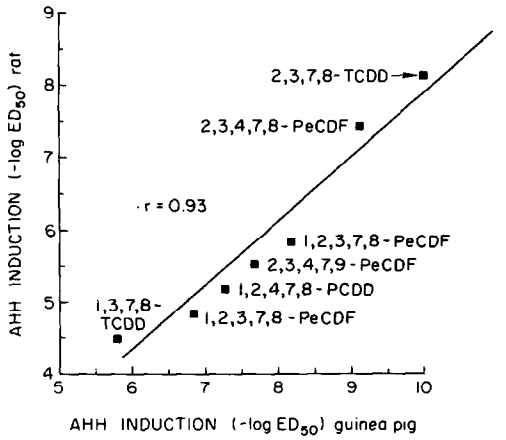

Fig. 4. Plot of the *in vivo* $-\log ED_{50}$ values for hepatic microsomal AHH induction for several PCDD and PCDF congeners in male guinea pigs and rats (rat data from Ref. 31).

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