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Ingestion of Soil Contaminated with 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) Alters Hepatic Enzyme Activities in Rats

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Ingestion of Soil Contaminated with 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) Alters Hepatic Enzyme Activities in Rats. LUCIER, G. W., RUMBAUGH, R. C., MCCOY, Z., HASS, R., HARVAN, D., AND ALBRO, P. (1986). *Fundam. Appl. Toxicol.* **6**, 364–371. Female rats were treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in either corn oil or contaminated soil from the Minker site in Missouri. Eight doses ranging from 0.015 to 5 μg TCDD/kg were used in the corn oil group; the range was 0.015 to 5.5 μg TCDD/kg in the TCDD-contaminated soil group. Rats in a third group were given equal amounts of soil uncontaminated with TCDD. No acute toxicity or effects on body weight gain were observed at these doses. In general, equivalent doses of TCDD in corn oil or TCDD in soil produced similar increases in hepatic aryl hydrocarbon hydroxylase activity (AHH) and UDP glucuronyltransferase activity although effects were slightly greater in the TCDD–corn oil groups. In the corn oil groups, the induction of AHH ranged from about 30-fold at the highest dose to twofold at the lowest dose studied. TCDD also caused an increase in cytochrome *P*-450 concentration and a shift in spectral peak from 450 to 448 nm. There was no effect of TCDD on ethylmorphine *N*-demethylase, consistent with previous reports. Liver concentrations of TCDD (mean ± SD) in the 5-μg/kg groups were 40.8 ± 6.3 ppb in the TCDD–corn oil group and 20.3 ± 12.9 ppb in the TCDD-contaminated soil group. Our results suggest that the bioavailability of TCDD in soil in rats is approximately 50%. Therefore, ingestional exposure to TCDD-contaminated soil may constitute a significant health hazard in view of its extremely high toxicity and relatively high bioavailability.

Environmental contamination by halogenated aromatic hydrocarbons, particularly 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), has received widespread attention following industrial accidents in Seveso, Italy, and Nitro, West Virginia. TCDD is an extremely potent and toxic compound that causes a variety of effects in man and animals (Kimbrough, 1980; Poland and Knutson, 1982). These effects include body wasting (McConnell, 1980), thymic atrophy (McConnell *et al.*, 1978), hepatic hyperplasia and hypertrophy (Fowler *et al.*, 1973), and chloracne (Crow, 1970). TCDD is carcinogenic in rats (Kociba *et al.*, 1978). In addition, the chemical causes a variety of biochemical changes in many species, including hepatic porphyria (Goldstein *et al.*, 1973) and microsomal enzyme induction (Lucier *et al.*, 1973; Poland and Glover, 1974; Nebert *et al.*, 1981). In particular, the induction of the cytochrome *P*-450-dependent activity of aryl hydrocarbon hydroxylase (AHH) and UDP glucuronyltransferase (UDPGT) is an early and sensitive indicator of TCDD action (Lucier *et al.*, 1973). Other monooxygenase activities, not associated with the cytochrome *P*-448 gene(s), are relatively unaffected by TCDD treatment (Nebert *et al.*, 1981). Among those enzymes not affected is ethylmorphine *N*-demethylase. Considerable information is available describing the molecular mechanisms by which TCDD and 3-methylcholanthrene (3-MC) increase AHH activity (Poland *et al.*, 1976; Negishi and Nebert, 1981).

Until recent years, the principal route of exposure to TCDD was thought to be occupational. However, it is now known that oil wastes contaminated with TCDD were used

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to control dusts on unpaved roads and in riding arenas (Carter *et al.*, 1975). The soil from one of these arenas was removed to a rural area which subsequently became residential. This area is now known as the Minker site.

When evidence of TCDD contamination at the Minker site was found, people were encouraged to relocate. Although it is known that TCDD is persistent in soil (Isensee and Jones, 1971; Kearney *et al.*, 1973) relatively little is known concerning the bioavailability of TCDD in soil. In one study (Porjen and Schlatter, 1980) 16–24% of an orally administered dose of TCDD in soil was found in the liver. These figures were used by Centers for Disease Control (CDC) to calculate safe exposure levels of TCDD in soil (Kimbrough *et al.*, 1983). A recent study has indicated that TCDD is approximately 25–50% bioavailable when ingested in soil using acute toxicity in guinea pigs and induction of hepatic AHH as biological markers of availability (McConnell *et al.*, 1984). However, this study did not examine other enzyme systems that are responsive or resistant to TCDD actions nor did it provide complete dose–response data or information on biological effects of TCDD when encountered in soil, such as effects on body weight or liver weight. Therefore, the present study compared dose-dependent effects of TCDD in Minker soil or corn oil on a battery of microsomal enzyme systems in rats. Moreover, TCDD concentrations in liver were compared in both treatment groups.

MATERIAL AND METHODS

Animals

Female Sprague–Dawley rats approximately 60 days of age were purchased from Charles River Laboratories and used for this experiment. The animals were divided into treatment groups of six rats each. All treatment groups were withheld from food beginning the evening prior to treatment (approximately 18 hr) but allowed free access to water. Food and water were provided immediately following treatment. Rats were treated by a single gastric intubation of the test compound with a 16-gauge rodent gavage needle (Popper and Sons, New Hyde Park, N.Y.) fitted to a 3-ml syringe. Rats in each group were killed 6 days after treatment.

Particular care was used to prevent aspiration when gavaging the animals. At autopsy, one animal was found to have a ruptured esophagus secondary to gavage when some test compound lodged in the intrathoracic space. This animal was therefore discarded. No other toxicity was detected as a consequence of treatment in any of the groups.

Preparation and Administration of TCDD

TCDD in corn oil. All rats received 0.2 ml/100 g body wt corn oil containing TCDD. Appropriate dilutions were made from a stock solution of TCDD to yield doses based on body weight.

TCDD in soil. Soil from the Minker site found to be contaminated with TCDD at 880 ppb was used (McConnell *et al.*, 1984). The soil was passed through a 60-gauge sieve prior to use. The amounts of dirt necessary to yield doses based on body weight were calculated using the TCDD concentration given above and assuming body weights of 200 g. The dose groups received an amount of soil ranging from 1.25 g (5.5 μg TCDD/kg) to 0.004 g (0.015 μg TCDD/kg). Soil sufficient to treat six animals was suspended in glass-distilled water. Enough water was added to the soil to give each dose in 2 ml.

Uncontaminated soil. Soil from Times Beach, Missouri, containing less than detectable concentrations of TCDD (<100 ppt), was used. The soil was prepared identically to that used for the TCDD soil group. The amounts of soil given to the rats were also the same as for the TCDD-soil group. Thus, the dose groups received amounts of soil ranging from 1.25 to 0.004 g uncontaminated soil per rat, respectively. The soil was suspended in glass-distilled water and the volumes given each rat were the same as described for the TCDD-soil group. This group provides a control for the effect of soil mass on enzyme activities but it does not give data on effects of soil mass on TCDD bioavailability.

Control groups. Animals received vehicle (corn oil or glass-distilled water) in appropriate volumes only by gavage. There was also a control group that was not gavaged.

Tissue Preparation

Previous studies had demonstrated that the maximal inductive effect of a single TCDD treatment occurs between 3 and 10 days in rats (Lucier *et al.*, 1973). Accordingly, the animals were killed by decapitation 6 days following treatment. The livers were quickly removed and placed in a Petri dish on ice. The livers were cleaned of fat and connective tissue, weighed individually, and minced with scissors. Liver (2 g) was weighed and homogenized in 10 ml buffer containing 0.05 M Tris, pH 7.4, and 1.15%

KCl (KCl-Tris buffer) with a Potter-Elvehjem glass/Teflon homogenizer. The remaining minced liver was stored in glass vials at −70°C. The liver homogenate was centrifuged at 900*g* for 10 min followed by 8500*g* for 20 min. The resulting supernatant was decanted and centrifuged at 105,000*g* for 60 min. The microsomal pellet was washed one time in KCl-Tris buffer and centrifuged again. The washed microsomal pellet was resuspended in an equal volume of KCl–Tris buffer and divided into three equal aliquots. The aliquots were quick frozen in liquid nitrogen and stored at −70°C for approximately 1 week prior to assay of enzyme activity.

Measurement of Microsomal Enzyme Activity

Aryl hydrocarbon hydroxylase. The frozen samples were thawed in cold water and used immediately for enzyme assays. The activity of aryl hydrocarbon hydroxylase was determined by the fluorescence method of Nebert and Gelboin (1968) with 30–75 μg microsomal protein per incubation tube. The reaction was linearly dependent on both time and protein concentration under the reaction conditions employed. The amount of product formed was determined from a standard curve of authentic 3-hydroxybenzo(a)pyrene.

Ethylmorphine N-demethylase. The activity of ethylmorphine *N*-demethylase was determined by the spectrophotometric measurement of formaldehyde production by the Nash reaction as described previously with 1.5–2 mg microsomal protein per reaction vessel (Werringloer, 1978). The reaction included both NADPH and NADPH-generating system and was linearly dependent on time and protein concentration under the conditions used.

Cytochrome P-450. The concentration of cytochrome *P*-450 was determined from the carbon monoxide-difference spectrum of dithionite-reduced microsomes with an Aminco DW-2 spectrophotometer according to the method of Omura and Sato (1964). The concentrations were calculated based on a millimolar extinction of coefficient of 91.

UDP glucuronyltransferase. Glucuronidation of *p*-nitrophenol was determined spectrophotometrically using 0.9 mM *p*-nitrophenol and Triton X-100 activated microsomes and 1.7 mM UDPGA in a final volume of 2.0 ml (Lucier *et al.*, 1971). Reaction time was 10 min and substrate disappearance was quantified at 405 nm.

Measurement of Liver TCDD Content

For TCDD analysis, liver samples were weighed and spiked with an internal standard (^{13}C-TCDD) dissolved in *n*-butanol. The livers were extracted with chloroform: methanol as described previously (Albro and Corbett, 1977). After concentration by rotary evaporation at 35°C, the extracted material was dissolved in methylene chloride: methanol, 1:1 (v/v), and separated into aliphatic and aromatic fractions on LH-20 Sephadex. The aromatic fraction was chromatographed on type A-540 basic alumina, and the fraction containing TCDD (20% methylene chloride) rechromatographed on acidic alumina (Merck). The final 20% methylene chloride fraction was dried under nitrogen for analysis by high resolution gas chromatography–mass spectrometry using capillary gas chromatography for sample introduction (McConnell *et al.*, 1984). Exact mass measurements were made on the *M* and *M* + 2 ions of TCDD and on the ^{13}C-labeled TCDD that was used as the internal standard. The mass measurements were made by selected ion monitoring at 10,000 resolving power (Harvan *et al.*, 1982). Since these samples contained relatively high concentrations of TCDD, calibration curves were prepared for ranges from 1 to 1000 ppb, with a lower detection limit of approximately 100 ppt, based on the 1% aliquots that were analyzed.

Other Methods

Protein concentration was measured by the Lowry method. Statistical significance was determined by a one-way analysis of variance coupled with the Newman–Keuls multiple range test (Zivin and Bartho, 1970). Significance was tested only at the 5% level.

RESULTS

These experiments were designed so that the doses of TCDD administered did not produce significant effects on the rate of body weight gain during the course of the study. The average weight gain was 15–30 g during the 6 days that followed TCDD administration and no consistent differences were seen between the two routes of TCDD exposure in soil or corn oil (data not presented). There were small but statistically insignificant increases in the liver weight to body weight ratios. Microsomal protein content on a per gram liver basis also showed slight increases in the TCDD-treated groups. There was no evidence of hepatomegaly in any dose group and in general the animals did not exhibit symptoms of acute TCDD poisoning. Accordingly, we were satisfied that our investigations on the bioavailability of TCDD in soil would not be confounded by acute toxicity.

The primary marker of bioavailability of TCDD in soil was induction of AHH. Dose–

response curves for TCDD in corn oil or TCDD in contaminated soil are presented in Fig. 1. These data indicate that AHH activity is induced by approximately 30-fold at the higher doses. Significant induction of AHH was detected by a dose as low as 40 ng/kg in groups exposed to either TCDD in soil or TCDD in corn oil. In general, increases in enzyme activity were dose dependent but no consistent differences were observed between dose groups. It was not possible to attain a TCDD dose that gave maximum induction after a single dose in the TCDD–soil group because of the volume of soil that would have to be intubated (2.5 g for a 10 μg/kg dose). In the TCDD–corn oil group maximum induction occurred at 10 μg/kg and activity was 1400 pmol product/min/mg microsomal protein in this group (data not shown). Assuming a similar induction maximum for the TCDD–soil group, induction by the TCDD–soil exposure was at least 60% that of the TCDD–corn oil group as determined by evaluation of data in Fig. 1.

Data presented in Table 1 compare the magnitude of induction at equivalent doses and reveals that the TCDD–corn oil to TCDD–soil induction ratio (derived by dividing the magnitude of AHH induction observed in the TCDD–soil group into the magnitude of induction observed in the TCDD–corn oil group) were similar. Therefore by this parameter, bioavailability of TCDD in ingested soil was nearly as great as the bioavailability when TCDD was administered in corn oil. It is important to note that the data in Table 1 are derived from comparisons with the zero TCDD dose groups and AHH activity was 37 and 45 pmol/min/mg protein in the TCDD–soil and TCDD–corn oil groups, respectively.

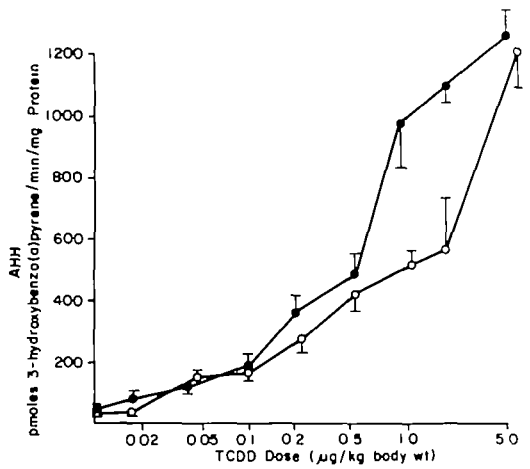

FIG. 1. Dose response for TCDD induction of hepatic AHH as a consequence of exposure to TCDD in corn oil or TCDD in contaminated soil. Enzyme activity measured 6 days after exposure and values are in means ± SE with $n = 6$. ●, TCDD-corn oil; ○, TCDD-soil.

TABLE 1

MAGNITUDE OF INDUCTION OF AHH BY TCDD IN CONTAMINATED SOIL OR CORN OIL COMPARED TO RESPECTIVE CONTROL VALUES

Dose (μg/kg)	Corn oil	Contaminated soil	Corn oil/contaminated soil
0.015	1.8[a]	1.0	1.8
0.040[b]	2.8[a]	3.4[a]	0.8
0.1	3.8[a]	4.3[a]	0.9
0.2[b]	7.9[a]	6.4[a]	1.2
0.5	10.8[a]	11.1[a]	0.9
1.0[b]	21.8[a]	12.3[a]	1.7
2.0	24.4[a]	14.9[a]	1.5
5.0[b]	28.2[a]	29.9[a]	0.9

[a] Values derived from data in Fig. 1 and significantly different than control values at least at $p < 0.05$.

[b] Values for induction of AHH in some contaminated soil groups were corrected for the 10% higher dose of TCDD in these groups compared to corresponding corn oil groups. This was done by assuming a linear relationship between dose and induction.

Ethylmorphine *N*-demethylase activity is a *P*-450-dependent system which is not TCDD inducible and in fact exhibits decreased activity at moderate TCDD doses (Lucier *et al.*, 1973). Our results demonstrate that this marker is generally unaffected by TCDD exposure either in corn oil or soil, although at the highest dose decreased enzyme activity was observed (Table 2).

Total cytochrome *P*-450 concentrations are also increased following TCDD exposures but increases are less than those observed for AHH activity and increases were generally greater

TABLE 2

EFFECT OF TCDD IN CONTAMINATED SOIL OR CORN OIL ON HEPATIC ETHYLMORPHINE *N*-DEMETHYLASE AND TOTAL CYTOCHROME *P*-450

Dose (μg/kg)	Ethylmorphine *N*-demethylase[a]	Cytochrome *P*-450[a]
Corn oil		
0	41.2 ± 4.1	0.59 ± 0.01
0.015		0.54 ± 0.04
0.040	42.8 ± 4.8	0.61 ± 0.02
0.1		0.62 ± 0.04
0.2	39.1 ± 2.3	0.75 ± 0.02
0.5		0.78 ± 0.13
1.0	34.4 ± 1.8	0.94 ± 0.05[b]
2.0		1.16 ± 0.12[b]
5.0	29.8 ± 1.9	1.18 ± 0.06[b]
Contaminated soil		
0	33.0 ± 2.5	0.49 ± 0.03
0.015		0.63 ± 0.08
0.044	59.7 ± 2.9[b]	0.61 ± 0.02
0.1		0.56 ± 0.06
0.22	38.8 ± 3.7	0.63 ± 0.05
0.5		0.82 ± 0.04[b]
1.1	34.5 ± 3.2	0.67 ± 0.27
2.0		0.82 ± 0.11[b]
5.5	29.3 ± 3.3	1.09 ± 0.06[b]

[a] Values are mean ± SE (n = 6). Ethylmorphine *N*-demethylase activity in nmol formaldehyde formed/min/mg microsomal protein. Cytochrome *P*-450 values are in nmol *P*-450/mg microsomal protein.

[b] Significantly different than control values at least at $p < 0.05$.

in the TCDD–corn oil groups (Table 2). Concomitant with the induction of cytochrome *P*-450 there was a clear shift in the spectral absorbance maximum from 450 to 448 nm at the highest doses of TCDD (data not shown). This spectral shift was less evident with lower doses and not present at all in vehicle-treated controls. TCDD, as expected, increased AHH activity and decreased ethylmorphine *N*-demethylase activity based on cytochrome *P*-450 concentrations. AHH values for the high-dose groups in nanomoles per minute per milligram cytochrome *P*-450 were 1.05 (TCDD in corn oil) and 1.14 (TCDD in soil) compared to control values of 0.08 (Table 3). Ethylmorphine *N*-demethylase activity (nmoles/min/mg cytochrome *P*-450) were decreased by a maximal threefold for both treatment groups.

UDP glucuronyltransferase is not *P*-450 dependent but is a sensitive marker of TCDD-mediated inductive actions (Lucier *et al.*, 1973). In the current study, this microsomal enzyme system was induced by TCDD in a dose-dependent manner although TCDD in corn oil was approximately twice as efficacious as TCDD in soil (Fig. 2). Induction was sixfold in the highest dose groups.

Results from the group of animals which received doses of uncontaminated soil equal in volume to those of the TCDD–soil group revealed no significant differences at any soil volume given. There was no body or liver weight change nor was there any change in enzyme activities tested or in the concentration of cytochrome *P*-450.

Liver concentrations of TCDD 6 days after treatment in the two higher dose groups are given in Table 4. These data reveal that TCDD concentrations were 40.8 ppb in the 5-μg/kg corn oil group and 20.3 ppb in the 5.5-μg/kg contaminated soil group. In the 1- and 1.1-μg groups values were 7.6 and 1.8, respectively.

DISCUSSION

Although TCDD appears to be rather persistent in soil (Isense and Jones, 1975; Kearney

TABLE 3

AHH AND ETHYLMORPHINE-*N*-DEMETHYLASE ACTIVITIES BASED ON CYTOCHROME *P*-450 CONCENTRATIONS

Dose (μg/kg)	AHH per *P*-450[a]	Ethylmorphine *N*-demethylase[a] per *P*-450
Corn oil		
0	0.08	69.8
5.0	1.05	25.3
Contaminated soil		
0	0.08	67.4
5.5	1.14	26.6

[a] Values in nmol 3-hydroxy benzo(a)pyrene formed/min/mg protein/nmol cytochrome *P*-450 or nmol formaldehyde formed/min/mg protein/nmol cytochrome *P*-450.

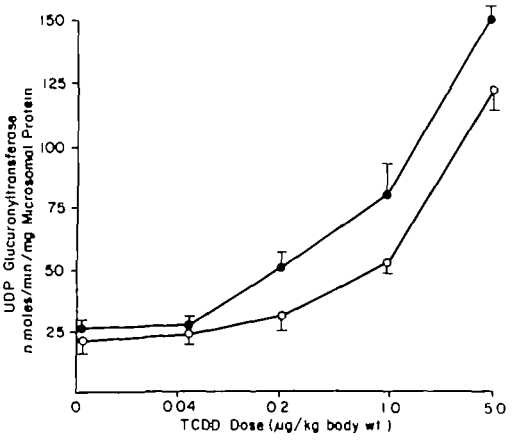

FIG. 2. Dose response for TCDD induction of hepatic UDP glucuronyltransferase as a consequence of exposure to TCDD in corn oil or TCDD in contaminated soil. Enzyme activity measured 6 days after exposure and values are in mean ± SE with $n = 6$. ●, TCDD-corn oil; ○, TCDD-soil.

et al., 1973) very little is known concerning the absorption of TCDD through the gastrointestinal tract when the medium of exposure is soil. However, Porjen and Schlatter (1980) reported that 16–24% of the administered dose of TCDD was absorbed after 24 hr when the compound was presented in an aqueous suspension of TCDD-spiked soil. Federal regulatory documents addressing a "safe" level of TCDD exposure now use the figure of 10–30% absorption from soil as a basis for determining acceptable levels of TCDD contamination in soil (Kimbrough *et al.*, 1983). McConnell *et al.*, (1984) demonstrated the bioavailability of TCDD in contaminated soil by using acute toxicity in guinea pigs and induction of AHH in rats as markers. They concluded that TCDD was bioavailable when ingested in contaminated soil. However, more precise conclusions were not made because of limited dose–response data and the use of only one biochemical marker.

Our study demonstrates that TCDD contaminated soil is a remarkably effective inducer of hepatic AHH and UDP glucuronyltransferase. By these standards, TCDD-contaminated soil is nearly as effective as pure TCDD dissolved in corn oil. Effects on cytochrome *P*-450 (*P*-448) concentration were also observed but these were not as dramatic as those on AHH. Accompanying the TCDD-induced AHH activities in both corn oil and soil was a shift in the absorbance maxima for the carbon monoxide difference spectrum from 450 to 448 nm. This shift was dose dependent, being reduced in size with the lower doses of TCDD or soil, and is consistent with previous induction studies (Nebert *et al.*, 1981; Poland and Glover, 1974). The lowest dose of TCDD in soil which caused a significant increase in hepatic AHH activity when compared to an equal volume of uncontaminated soil was 0.044 μg/kg. This induction required administration of only a single dose of 10 mg contaminated soil per rat.

The source of the TCDD-contaminated soil used in this experiment was the so-called Minker site which contains soil that had previously been sprayed with waste oil that contained a wide variety of hydrocarbon contaminants. Therefore, it is possible that the soil

TABLE 4
TCDD CONCENTRATIONS IN LIVER[a]

TCDD–Corn oil		TCDD–Soil		Uncontaminated soil
Dose	Concentration	Dose	Concentration	Concentration
5 μg/kg	40.8 ± 6.3	5.5 μg/kg	20.3 ± 12.9	ND
1 μg/kg	7.6 ± 2.5	1.1 μg/kg	1.8 ± 0.3	ND

[a] Concentrations given in ppb (mean ± SD) derived from livers of six rats in each group; ND = not detectable (<100 ppt).

might contain other chemicals that produce the same spectrum of toxic effects as TCDD and which also induce AHH. Because of this concern, we analyzed the contaminated soil from the Minker site for dibenzofurans and polychlorinated biphenyls (PCBs). The concentration of total tetrachlorodibenzofurans (TCDF) in soil was 40–80 ppb and the concentration of PCBs was 3–4 ppm (Hass, unpublished data). 2,3,7,8-TCDF is approximately one-fifth as effective as 2,3,7,8-TCDD in induction of AHH (Poland *et al.*, 1976; Bradlaw and Casterline, 1979). Because the concentration of total TCDF is roughly one-tenth that of TCDD in our soil sample, TCDF would be expected to account for no more than 2% of the observed AHH induction even if all the TCDF in soil was the 2,3,7,8-congener. Although the soil PCB concentration is fivefold higher than TCDD, the more potent PCBs found in commercial mixtures (Goldstein, 1980) are only 0.001 times as effective inducers of AHH as TCDD. Therefore, the PCBs could account for no more than 0.2% of the observed inductive effect. Nevertheless, the possibility remains that unknown pollutants present in the soil could be, in part, responsible for the toxic effects observed in our studies. Alternatively, the PCBs, TCDFs, and TCDD could produce greater than additive effects when administered together. These possibilities are made more credible by our finding that induced AHH activities were similar for the 5-μg/kg TCDD–corn oil group and 5.5-μg/kg TCDD–soil group whereas liver concentrations of TCDD were twice as high in the TCDD–corn oil group. If AHH induction is a linear function of TCDD concentration, these data suggest that at high doses only one-half the inductive effect of contaminated soil is attributable to TCDD acting alone. Moreover, liver TCDD concentrations in the 1-μg/kg groups were four times higher in animals receiving TCDD–corn oil than in TCDD–soil although AHH activities were only twice as high in the TCDD–corn oil group.

Our results demonstrate that TCDD in soil can be biologically available in the rat. While it is not appropriate to generalize from a single study, in our experiment the availability of TCDD following ingestion of contaminated soil appears to be approximately 50%. Therefore, our results also suggest that TCDD-contaminated soil may present a health hazard if ingested because of its extremely high toxicity and relatively high bioavailability. It is known that children and even some adults ingest significant quantities of soil from dirty hands, working and playing outside, etc. However, further studies will now be required to determine if the high degree of bioavailability of TCDD, which we have demonstrated in rats, is also present in humans and other species. Furthermore, studies are needed to ascertain whether TCDD-contaminated soil in locations other than Missouri is bioavailable to the same extent reported in our study. In fact, a recent abstract by Umbreit *et al.* (1985) suggests that TCDD-contaminated soil from a New Jersey manufacturing site is not bioavailable.

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