Dioxin in Soil: Bioavailability After Ingestion by Rats and Guinea Pigs

Abstract. *Soil environmentally contaminated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was given by gavage to guinea pigs and rats. The development of a characteristic clinicopathologic syndrome in guinea pigs, the induction of aryl hydrocarbon hydroxylase in rats, and the presence of TCDD in the livers of both species show that TCDD in soil exhibits high biological availability after ingestion.*

2,3,7,8 - Tetrachlorodibenzo - *p* - dioxin (TCDD), a highly toxic compound found as a contaminant in phenoxy acid herbicides, chlorophenols, and other related chemicals (*1*), appears highly persistent in soil (*2*) except when exposed to sunlight (*3*). This compound binds tightly to soil and usually remains on or near the soil surface (*4*). Although the half-life of TCDD on surface soil appears to be < 1 year (*5*), in deeper and more protected soils the half-life may be > 10 years (*6*).

Most exposures of humans to TCDD have been the result of accidents occurring during the manufacture of those chemicals known to be sources of these potential contaminants (*7*). More recently, a different type of dioxin problem has been recognized. Waste oil contaminated with TCDD and other chlorinated dibenzo-*p*-dioxins (CDD's), used to control road dust, served as the source of environmental contamination in Times Beach, Missouri (*8*). A subsequent flood may have distributed the contaminated soil throughout parts of the town. Another example of environmental contamination occurred when TCDD-laced waste oil from the same source was used to suppress dust in horse-riding arenas. This resulted in the deaths of numerous horses and sickness in exposed children (*9*). The contaminated soil was removed from the riding arena and deposited in a rural area some distance away, which is now known as the Minker Stout site.

Although TCDD is nonvolatile and tightly bound in soil, the bioavailability of dioxin in environmentally contaminated soil after either ingestion or cutaneous absorption is not known. Our study was undertaken to determine the extent of bioavailability after ingestion of dioxin-contaminated soil.

The Environmental Protection Agency collected samples of dioxin-contaminated and uncontaminated soils from Times Beach and the Minker Stout site. These samples were shipped to the National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, in stainless steel drums. Samples were allowed to air-dry, were homogenized and sifted through a 6-mm stainless steel wire mesh to remove coarse materials, and then were sifted through a 60-gauge stainless steel mesh sieve to remove finer gravel. The siftings were analyzed for concentrations of TCDD (*10*) and other potentially toxic substances including polychlorinated biphenyls (PCB's), chlorinated dibenzofurans (CDF's), and heavy metals.

We administered samples of these soils to guinea pigs because of their high sensitivity to CDD's (*11*) and to rats because of the ability of CDD's to induce aryl hydrocarbon hydroxylase (AHH) in this species at low doses (*12*). Although AHH is induced by a large number of compounds, AHH induction is one of the most sensitive and well-characterized indicators of TCDD exposure in rats (*12–14*).

Young (1½ weeks) male Hartley guinea pigs were received from the Charles River Breeding Laboratories and allowed to acclimate for 7 days. Each was housed individually in disposable cages with unlimited food and water available. Food was withheld for 24 hours prior to dosing. Each guinea pig was lightly anesthetized with methoxyflurane (Metofane), and groups of six animals each were given by gavage soil samples (Times Beach and Minker Stout) environmentally contaminated with approximately 1, 3, or 10 μg of TCDD per kilogram of body weight; these dosages were based on analyses of soil siftings (60-gauge mesh) from the Times Beach and Minker Stout sites, which indicated concentrations of 770 and 880 parts per billion (ppb) TCDD, respectively. No TCDD, PCB's, or CDF's were detected in the control soil. Individual soil samples were mixed with 5 ml of distilled water. Guinea pigs receiving uncontaminated soil from Times Beach were administered a volume of soil equal to that of the highest amount (3.6 g) of contaminated soil. For comparison, pure TCDD in corn oil was given at either 0, 1, or 3 μg/kg [the reported LD_{50} (dosage lethal to 50 percent of the animals tested) is 2.0 μg/kg (*15*)].

After a single dose of TCDD, the guinea pigs were observed for 30 days. Necropsies were performed on animals that

Table 1. Experimental design and lethality of soil TCDD in guinea pigs; N.D., none detected (detection limit, 100 parts per trillion); S.E.M., standard error of the mean. The TCDD added to corn oil or soil was synthesized by the chemistry branch, National Institute of Environmental Health Sciences; purity, > 99 percent by gas chromatography. It was used to "spike" soil samples at a concentration of 0.8 μg per gram of soil.

Group	*N*	Source	Amount	TCDD (microgram per kilogram of body weight)	Dead/ treated	TCDD content of liver (ppb ± S.E.M.)
1	6	Corn oil	0.1 ml/100 g	0	0/6	N.D.
2	6	TCDD in corn oil	0.1 ml/100 g	1	1/6	1.6 ± 0.2* 4.1†
3	6	TCDD in corn oil	0.1 ml/100 g	3	6/6	13.3 ± 2.3
4	6	Times Beach soil	0.35 g	1.3	0/6	< 1.0
5	5‡	Times Beach soil	1.07 g	3.8	1/5	1.0 ± 0.1* 3.2†
6	5§	Times Beach soil	3.60 g	12.8	5/5	34.3 ± 6.0
7	6	Minker Stout soil	0.26 g	1.1	0/6	< 1.0
8	6	Minker Stout soil	0.80 g	3.3	2/6	1.4 ± 0.3* 2.0 ± 0.1†
9	6	Minker Stout soil	2.67 g	11.0	6/6	25.7 ± 5.2
10	5‖	Times Beach soil, uncontaminated	3.60 g	0	0/5	N.D.
11	6	Times Beach soil, uncontaminated with TCDD added	2.71 g	10	6/6	45.4 ± 8.4

*Animals that were killed at 30 days. †Animals that died before 30 days. ‡One animal died 2 days after dosing (not included). §One animal died at the time of dosing as a result of an error in the anesthesia. ‖One animal died at the time of dosing as a result of an intubation error.

Table 2. Effect of TCDD on aryl hydrocarbon hydroxylase (AHH) in rat liver ($N = 6$) based on the rate of formation of 3-hydroxybenzo[*a*]pyrene (Newman-Keuls multiple range test, one-way analysis of variance). No gavage control = 0.7 ± 0.05 nmole per minute per gram of liver or 37.0 ± 0.9 pmole per minute per milligram of protein. Values are given as the mean ± the standard error of the mean.

TCDD–corn oil			TCDD–soil			Uncontaminated soil	
TCDD–corn oil dose ($\mu g\ kg^{-1}$)	AHH (nmole $min^{-1}\ g^{-1}$)	AHH (pmole $min^{-1}\ mg^{-1}$)	TCDD–soil dose ($\mu g\ kg^{-1}$)	AHH (nmole $min^{-1}\ g^{-1}$)	AHH (pmole $min^{-1}\ mg^{-1}$)	(nmole $min^{-1}\ g^{-1}$)	(pmole $min^{-1}\ mg^{-1}$)
5.0	24.0 ± 1.1	1269.4 ± 41.6	5.5	24.2 ± 1.5	1230.9 ± 48.9	0.2 ± 0.1*†	14.5 ± 2.9*†
1.0	17.5 ± 1.1	980.9 ± 69.7	1.1	9.5 ± 0.5*	499.0 ± 19.1*	0.3 ± 0.1*†	18.2 ± 6.6*†
0.20	6.6 ± 1.0	353.3 ± 41.1	0.22	3.9 ± 0.2*	262.7 ± 27.1*	0.7 ± 0.2*†	34.8 ± 8.2*†
0.40	2.1 ± 0.2	124.4 ± 12.2	0.44	2.6 ± 0.2	142.3 ± 13.6	0.4 ± 0.2*†	22.2 ± 8.7*†
0	0.7 ± 0.1	45.2 ± 3.7	0	0.6 ± 0.1	37.3 ± 4.1	0.7 ± 0.1	39.8 ± 3.7

*$P < 0.05$ versus TCDD–corn oil. †$P < 0.05$ versus TCDD–soil.

died during or were killed at the end of the study, and selected tissues were examined histopathologically. The brain, thymus, spleen, liver, right kidney, and testicle from animals killed at the end of the study were weighed. A portion of liver was frozen for TCDD analysis, which after extraction (*16*) was analyzed as described for soil (*10*).

The clinicopathologic effects in guinea pigs exposed to either TCDD in corn oil or in contaminated soil were comparable. These animals either failed to gain weight or lost weight and died in a severe state of cachexia from 5 to 21 days after exposure (Table 1). The calculated (Spearman-Karber method) LD_{50} was 1.75 (95 percent confidence limits, 1.26 to 2.24) μg/kg for TCDD in corn oil, 7.15 (4.90 to 9.40) μg/kg for Times Beach soil, and 5.50 (3.45 to 7.55) μg/kg for the Minker Stout site soil.

The guinea pigs that died exhibited a severe loss of body fat, markedly reduced thymus and testicle size, hemorrhage of the adrenal gland, and, on occasion, prolapsed rectum. The only organs in surviving animals that showed a treatment-related weight effect were the thymus (reduced) and the liver (slightly increased). Histopathologic changes in animals exposed to TCDD in corn oil and to TCDD in contaminated soil were similar and were characteristic of exposure to CDD's (*15*). These lesions included atrophy (involution) of the cortex of the thymus and periarticular lymphoid sheath in the spleen, hemorrhage and atrophy (zona glomerulosa) of the adrenal gland, atrophy of the bone marrow, testicular atrophy, and urothelial hyperplasia of the renal pelvis and urinary bladder. No adverse effects were observed in animals exposed to uncontaminated soil.

Analysis of the TCDD content of the livers of the exposed guinea pigs revealed a clear dose-response relation (Table 1). However, the amounts of TCDD found in the livers of guinea pigs that survived were lower than those of equally exposed animals that died. Factors that may be responsible for this effect include the absence of body fat in guinea pigs that died or additional time for metabolism and excretion in the animals that survived (*17*). If TCDD initially concentrates in the adipose tissue in a manner analogous to that for tetrachlorodibenzofuran (TCDF) (*18*), the mobilization of fat as toxicity develops may lead to increased accumulation in the liver.

Groups of six female Sprague-Dawley rats each were exposed to the same soil samples (Minker Stout site) used in the guinea pig study. In this experiment we measured hepatic enzyme activities dependent on cytochrome P-450 (*19*) and total cytochrome P-450 concentrations (*19*) 6 days after a single dose (by gavage) of TCDD in either corn oil or soil. Doses of 0, 0.04, 0.20, 1.0, and 5.0 μg of TCDD in corn oil per kilogram of body weight were used. Amounts of soil giving similar doses of TCDD were calculated based on the soil contamination figure of 880 ppb. The control group received an amount of uncontaminated Times Beach soil equal to the highest volume of contaminated soil.

After the rats were killed, the livers were removed and microsomes were prepared and washed by differential centrifugation and quick-frozen in liquid nitrogen. Subsequently, the microsomes were thawed and used immediately for enzyme assays. Portions of fresh minced liver were frozen and used to determine TCDD concentrations.

Hepatic microsomal AHH was the best indicator of TCDD action (Table 2). A maximum 40-fold induction was seen at a dosage of 5 μg/kg. Effects on cytochrome P-450 were also observed (data not shown), but this parameter was not as sensitive as AHH; the maximum induction of cytochrome P-450 was twofold in both 5.0- and 1.0-μg/kg treatment groups. After dioxin treatment, a shift occurred in the absorption maxima for the CO difference spectrum from 450 to 448 nm, confirming that TCDD has induction characteristics similar to those of 3-methylcholanthrene (*12*). This characteristic shift in binding spectra occurred in rats exposed either to TCDD in corn oil or to TCDD-contaminated soil. No consistent effects on ethylmorphine *N*-demethylase were observed. In general, TCDD in soil was nearly as potent an inducer of AHH as pure TCDD in corn oil. Even the lowest dose of TCDD studied (0.044 μg/kg or about 10 mg of soil per rat) induced about four times as much hepatic AHH activity as uncontaminated soil. Other parameters such as body weight, liver weight, and liver protein concentration were apparently unaffected by TCDD in corn oil or in soil. The liver concentrations of TCDD (mean ± standard deviation) in the highest dose 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.

The degree of intestinal absorption of TCDD depends on the vehicle used. Studies of rats given TCDD in the diet reported 50 to 60 percent absorption (*20*). A mixture of TCDD in acetone and corn oil given to rats by gavage resulted in 86 percent absorption (*21*). The absorption in rats of TCDD artificially added to soil has been estimated to be approximately 50 percent as efficient as the absorption of TCDD in ethanol, with the efficiency decreasing as the time of TCDD-soil contact increases (*22*). A recent study on the bioavailability of TCDD in rabbits after ingestion of TCDD in soil from a site in Seveso, Italy, indicated that the absorption of TCDD bound in soil was 68 percent less than that of TCDD in solvent (*23*).

Because the soils used in our studies could contain other chemicals that produce the same spectrum of toxic effects as TCDD and could also induce hepatic AHH, we analyzed the contaminated soil from the Minker Stout and Times

Beach sites for dibenzofurans and PCB's. The concentration of total TCDF in the soil was 40 to 80 ppb and that of PCB's was 3 to 4 ppm. 2,3,7,8-TCDF is approximately one-fifth as efficacious as 2,3,7,8-TCDD in inducing AHH (*24*). As the concentration of total TCDF in the contaminated soil is one-tenth that of TCDD, TCDF should account for less than 2 percent of the observed inductive effect. Although the PCB concentration is five times that of TCDD, the most potent PCB is 1/100 as active as TCDD in inducing AHH (*14, 25*), and this congener is not routinely detected in commercial PCB mixtures. By our estimation, PCB's could account for no more than 0.2 percent of the inductive effect of contaminated soil. However, the possibility exists that other contaminants present in the soil might potentiate the action of TCDD and could be, in part, responsible for the toxic and enzyme induction effects observed in these studies.

From the foregoing it is readily apparent that TCDD in soil is biologically available in two animal species, as measured in terms of a clinicopathologic syndrome in guinea pigs, hepatic enzyme induction in rats, and uptake of TCDD in the livers in both species. Although one has difficulty arriving at an exact percentage for bioavailability, the absorption of TCDD from soil appears to be highly efficient in the guinea pig and rat models. The guinea pig study provides only a crude estimate of bioavailability. The importance of this model lies in its ability to respond to CDD's with a characteristic clinicopathologic syndrome that was duplicated in this study. The induction of AHH in rats is a more quantifiable measure of bioavailability. On the basis of the high bioavailability detected in these two animal species, it seems clear that TCDD-contaminated soil presents a potential hazard to humans if ingested.

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Rat Transforming Growth Factor Type 1: Structure and Relation to Epidermal Growth Factor

Abstract. *The complete amino acid sequence of rat transforming growth factor type 1 has been determined. This growth factor, obtained from retrovirus-transformed fibroblasts, is structurally and functionally related to mouse epidermal growth factor and human urogastrone. Production of this polypeptide by various neoplastic cells might contribute to the continued expression of the transformed phenotype.*

Retrovirus-transformed cells and certain human tumor cells produce transforming growth factor type 1 (TGF-1) (*1*). In contrast, control cells or cells infected with transformation-defective viral mutants (*2–4*) do not produce and release detectable levels of TFG-1. The factor has been purified from serum-free medium conditioned by human melanoma cells (*5*) and by retrovirus-transformed rat and mouse fibroblasts (*6*). The amino-terminal sequences of human, rat, and mouse TGF-1 (hTGF-1, rTGF-1, and mTGF-1, respectively) have been described (*6*). Here we report the complete amino acid sequence of rTGF-1 and compare its biological properties with those of mouse epidermal growth factor (mEGF).

Purification of rTGF-1 from serum-free medium conditioned by Fischer rat embryo fibroblast line C110 (*7*), a subclone of Fischer rat embryo fibroblast line 3A nonproductively transformed by Snyder-Theilen feline sarcoma virus, was monitored in a radioreceptor assay based on receptor cross-reactivity with mEGF (*6*). The amino-terminal sequence of residues 1 to 43, with the exception of residues 26, 30, 36, 40, and 42, was determined by automated Edman degradation of *S*-carboxamidomethylated rTGF-1 in a gas-liquid solid-phase microsequencer (Fig. 1) (*6, 8*). The remainder