Bull. Environ. Contam. Toxicol. (1989) 43:683–690

Bulletin of Environmental Contamination and Toxicology

Oral Bioavailability of Aged Polychlorinated Biphenyl Residues Contained in Soil

G. F. Fries, G. S. Marrow, and C. J. Somich

Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland 20705, USA

Bioavailability of toxic chemicals is a potentially important factor in determining the risk associated with soil contamination. Interest in bioavailability of chemicals recently increased because of such incidents as the Missouri (Kimbrough et al., 1984) and Seveso (Fanelli et al., 1982) TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) episodes and the polybrominated biphenyl (PBB) episode in Michigan (Fries and Jacobs, 1986), which involved human and/or food animal exposure to accidentally contaminated soil. Knowledge of bioavailability is also useful in assessing risks associated with waste disposal on land (Fries, 1987).

Poiger and Schlatter (1980) conducted early oral bioavailability work with rats fed laboratory preparations of TCDD-contaminated soil. TCDD adsorbed to soil was only 50% as available as TCDD in corn oil. Bioavailability decreased as the residue on soil aged in short-term experiments. Studies with laboratory animals suggested that the TCDD of Missouri soils contaminated in situ was about half as available as TCDD in corn oil (McConnell et al., 1984; Lucier et al., 1986). Aged PBB residue in soil was 70 to 80% as available as PBB added directly to feed of sheep (Fries, 1985). In contrast to the high bioavailability in normal soils, bioavailabilities of TCDD in soils from several industrial sites were in the 1 to 23% range (Umbreit et al., 1986).

An inherent difficulty in interpreting bioavailability data obtained with environmental samples is that it is not possible to be certain of the extraction efficiency of the system used in the soil analyses. This difficulty can be overcome by using ^{14}C-labelled compounds, but it is difficult to age the soil preparations in the laboratory for months or years that might lapse in a field situation. In this study, it was possible to utilize archival soil samples that had been treated with ^{14}C-labelled polychlorobiphenyls (PCB) eight years previously. The primary

Send reprint requests to G. F. Fries at the above address.

Mention of specific products or suppliers is for identification and does not imply endorsement by the U. S. Department of Agriculture to the exclusion of other suitable products or suppliers.

purpose of this study was to compare the bioavailability of PCB in these soils with the bioavailability of PCB added to normal rat diets or corn oil. The secondary purpose was to compare dietary inclusion with gavage as a method of administration because gavage frequently was used in the previous studies.

MATERIALS AND METHODS

The PCB spiked soils were from a study of PCB uptake and translocation in soybean plants that had been conducted eight years previously (Fries and Marrow, 1981). The soil was Galestown sandy loam that had a 6.7 pH and was composed of 67.3% sand, 22.2% silt, 10.5% clay and 5.2% organic matter. Three spiked soils were prepared by adding 0.25, 1.00, and 0.50 uCi/kg, respectively, of 2,2',5-trichlorobiphenyl (Tr), 2,2',5,5'-tetrachlorobiphenyl (Te) and 2,2',4,5,5'pentachlorobiphenyl (Pe) to separate portions of the base soil. When the 60-day greenhouse experiment ended, the air dried soils that remained after taking samples were stored at -5 C until used in this study. Only fractions passing a 125 um sieve were used.

Each of the three spiked soils was studied in a separate experiment with rats. Treatment groups within an experiment consisted of four male Sprague-Dawley rats (Hilltop Laboratory Animals, Scottsdale, PA) weighing 400 to 500 g. Rats were individually housed in stainless steel metabolism cages in a room maintained at 21 C and 80% relative humidity with a 14/10 hr light/dark cycle. Feed and water were available continuously.

Four experimental treatments were compared with each compound except Tr. In the first treatment, ^{14}C-PCB soil was added to a standard rat diet in meal form (Wayne Lablox) at the rate of 5%. The second treatment was administration by gavage of approximately 1 g/d soil suspended in water. The dosing technique was calibrated each day by delivering the same volume of suspended soil to pans and drying to a constant weight. The third treatment was a control in which ^{14}C-labelled compound in acetone was added to the feed, and the forth treatment was administration by gavage of ^{14}C-labelled compound in corn oil. The groups treated by gavage were offered unspiked feed ad libitum, and treatments were administered under light ether anesthesia to minimize animal distress. The third and forth treatments were omitted from the Tr experiment because labelled compound was no longer available.

The spiked feeds were offered and the gavage treatments were administered for five days because of the low concentrations of radioactivity in the soils. Rats then were fed unspiked feed for an additional ten days. Feed intake was measured and summarized at 5-day intervals. Urine and feces were collected daily, stored in refrigerator and composited by 5-day periods. The rats were sacrificed by CO_2 asphyxiation on the 15th day, and samples of omental fat and liver were obtained.

The basic analytical techniques in the study included total

Table 1. Activity levels and distributions in experimental soils.

Chlorobiphenyl	Activity, CPM/g	Extracted, %	Parent, %
2,2′,5-	772	88	>80
2,2′,5,5′-	3145	95	>98
2,2′,4,5,5′-	2026	96	>92

activity by combustion (Packard Tri-Carb Oxidizer) and counting the $^{14}CO_2$ by liquid scintillation (LSC, Beckman LS 6800); extractable activity by soxhlet extraction for 12 hr with a 1:1 acetone:hexane mixture and counting extracts by LSC; and fractionation by high performance liquid chromatography (HPLC, Waters 6000 pumps, 990 detector and a C-18 Novapak column) and eluting with appropriate methanol-water mixtures (85-15 for Tr and Te, and 90-10 for Pe).

The techniques applied to each sample type were:
Soil--Replicate samples were analyzed for total activity, extractable activity, and characterized by fractionation;
Feed--Replicate samples were analyzed for total activity to assure proper preparation and uniform mixing;
Feces-Frozen and pulverized in a blender using dry ice to maintain the frozen state and the analyses of the undried samples were the same as for soil;
Urine-Freeze dried and counted by LSC after combustion because activity was too low for counting directly;
Lipid-Fatty tissue was rendered and expressed lipid was counted directly by LSC;
Liver-Dried with sodium sulfate, extracted with petroleum ether, and the concentrated extracts were counted by LSC.

The data for each compound were analyzed statistically by analysis of variance and significant differences among means were identified by the Newman-Keuls test (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

Most ^{14}C activity in soil was extracted by the acetone-hexane mixture (Table 1). Extraction efficiency increased as chlorination increased, which might be expected because the less chlorinated PCBs are more polar (Shiu and MacKay, 1986) and would adsorb more tightly to soils than more chlorinated PCBs. The HPLC chromatograms of the soil extracts was typified by the chromatogram of the Pe extract shown in Fig. 1. Only single components were found and retention times were identical to retention times of unlabelled standards of known identity. More than 95% of the activity in extracts of all test soils was parent compound but the unextracted activity may have been degradation products formed during the plant experiment or storage.

Although samples and data were collected by 5-day periods, results are presented only for the total experiment because carry over in

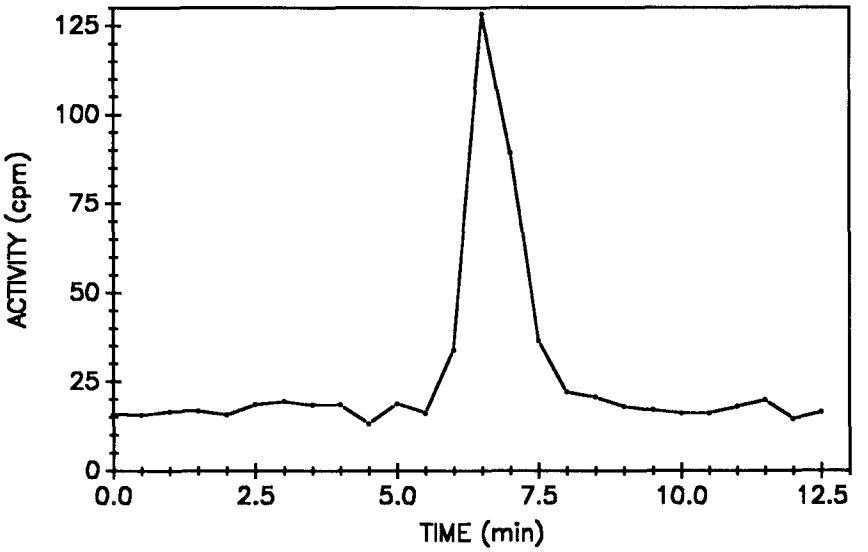

Figure 1. An HPLC chromatogram of the ^{14}C activity of an acetone-hexane extract of soil spiked with 2,2',4,5,5'-chlorobiphenyl. Peak retention time was the same as that of an known standard.

the gastro-intestinal tract between the first and second 5-day periods made interpretation by periods difficult. Most of the ^{14}C dose was recovered in feces (Table 2). Recovery of activity in feces tended to decrease with increasing chlorination, being greatest with Tr and least with Pe. Neither the matrix nor the method of administration had an effect on recovery of activity infeces except with Tr. Soil gavage yielded a significantly lower recovery with Tr but there was no suggestion of a similar result with the other compounds. It is possible that a dosing error occurred with the Tr gavage group because use of this technique with soil suspensions was more prone to error than other methods of administration.

Originally, it was contemplated that levels of metabolite excretion in urine would be an important criteria for evaluating the relative bioavailability of the different treatments. The amounts of activity excreted in urine, however, were too small to be more than suggestive (Table 2). Recovery of ^{14}C in urine increased with decreasing chlorination, which is expected because ease of metabolism is related to the number adjacent nonchlorinated carbon atoms (Matthews et al., 1978). There is an indication that less activity was excreted in urine of the soil groups in the Te experiment but the differences were not significant. Urine activity in the Pe experiment was too low for reliable analyses.

Another criteria for evaluating bioavailability is the relative retention of activity in body tissues. Counts of activity in liver for all compounds was too near background for reliable quantitation and results are not presented. The relative

Table 2. Recovery of ^{14}C in feces, urine and body fat in the three experiments.[a]

Component	Soil Diet	Soil Gavage	Normal Diet	Corn Oil Gavage	Standard Error
2,2′,5-Trichlorobiphenyl					
Extracted Fecal Fractions					
Parent, %	17.9	21.7			
Metabolites, %	47.8	35.6			
Unextracted, %	25.0	25.3			
Total Feces, %	92.7[b]	82.6[c]			2.0
Urine, %	6.5	5.6			1.2
Body Fat, %/g	0.14[b]	0.24[c]			0.02
Bioavailability, %	82.1	78.3			
2,2′,5,5′-Tetrachlorobiphenyl					
Extracted Fecal Fractions					
Parent, %	20.5	11.5	8.7	5.4	
Metabolites, %	46.3	57.8	60.9	60.7	
Unextracted, %	17.4	13.8	14.4	15.6	
Total Feces, %	84.2	83.1	84.0	81.7	2.1
Urine, %	2.5[b]	2.3[b]	2.9[b]	4.0[c]	0.2
Body Fat, %/g	0.16[b]	0.13[b]	0.20[c]	0.25[c]	0.01
Bioavailability, %	79.5	88.5	91.3	94.6	
2,2′,4,5,5′-Pentachlorobiphenyl					
Extracted Fecal Fractions					
Parent, %	33.2	21.9	13.9	12.9	
Metabolites, %	31.6	40.4	49.7	47.1	
Unextracted, %	15.5	16.7	11.1	14.8	
Total Feces, %	80.3	79.0	74.7	74.8	2.8
Urine, %	0.9	1.1	1.0	0.7	0.2
Body Fat, %/g	0.42[bc]	0.28[b]	0.62[c]	0.45[bc]	0.06
Bioavailability, %	66.8	78.1	86.1	81.1	

[a] Bioavailability in this table is the percentage of the administered compound that did not appear as parent in the feces. Analyses of feces fractions were on a composite basis and were not analysed statistically.

[bc] Values in a row with different superscripts differ ($P<0.05$).

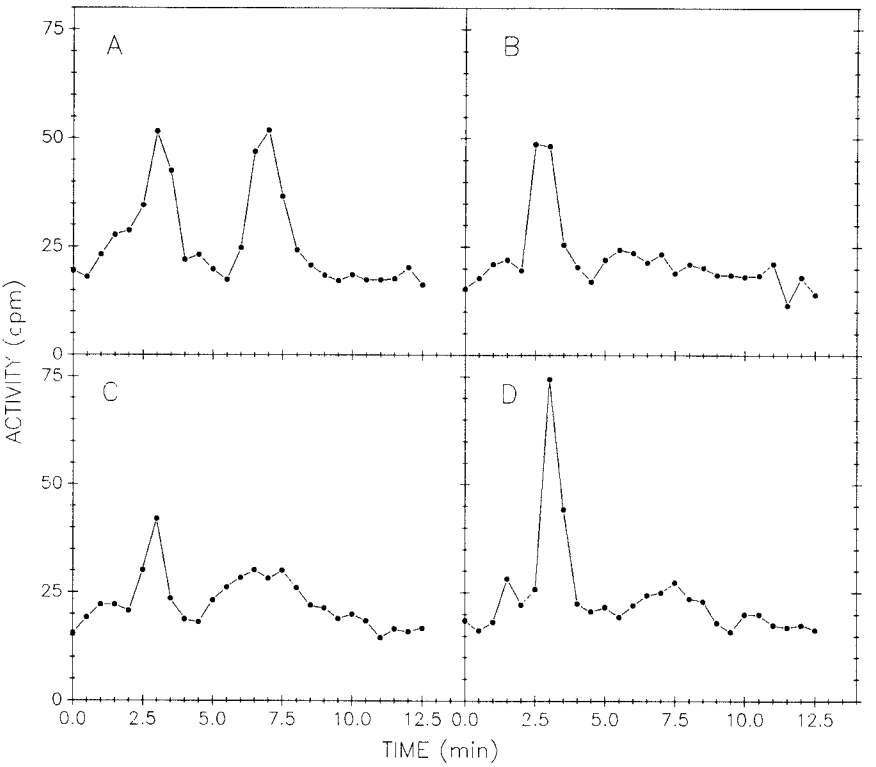

Figure 2. HPLC chromatograms of the ^{14}C activity of acetone-hexane extracts of feces of the rats administered 2,2',4,5,5'-chlorobiphenyl (Pe). A is Pe in soil in the diet, B is Pe in normal diet, C is Pe in soil by gavage and D is Pe in corn oil by gavage. Retention time of the parent Pe is 7.0 min.

concentrations of ^{14}C in body fat of the soil groups in the Te experiment were lower than concentrations in the other groups (Table 2). Since it is reasonable to assume that concentrations in fat, as well as amounts excreted in urine, are correlated with absorption, the results with Te indicate that adsorption to soil reduced bioavailability. In the Pe experiment, however, the results were not so clear because the concentration of the corn oil group was not as high, relatively, as in the Te experiment. Concentrations in body fat increased with increased chlorination, which reflects the inverse relationship of chlorination and metabolism (Matthews et al., 1978).

The activity in feces was characterized in samples composited by treatment groups to determine if it was unabsorbed parent compound, or if it was activity derived from compounds that had been adsorbed, metabolized and excreted through bile to feces. The unextracted activity in feces ranged from 11 to 25% (Table 2), which was considerably greater than the 4 to 12% unextracted activity in the soils (Table 1). There were no consistent differences in fractions of unextracted activity in feces due to the

presence or absence of soil, or the method of administration. Among compounds, Tr had the greatest fraction of unextracted activity but there was little difference between Te and Pe. The acetone-hexane extracts were concentrated and fractionated by HPLC. Chromatograms of the extracts for the treatments in the Pe experiment, which are representative of chromatograms of the other compounds, are presented in Fig. 2. As noted above, the soil extracts contained only parent material as illustrated in Fig. 1. Feces from animals fed soil in the diet (Fig. 2A) and soil administered by gavage (Fig. 2C) had significant amounts of parent compound. In contrast, fecal activities of the normal diet (Fig. 2B) and corn oil (Fig. 2D) groups were mainly metabolites. The quantities of extracted parent and metabolite compounds are presented in Table 2. Quantities of parent compounds in feces were in the range of 20 to 30% of dose when administered with soil and only 5 to 15% when added to the diet directly or when dissolved in corn oil.

Bioavailability is neither defined nor measured consistently in the literature. In this paper, bioavailability is defined as the fraction of an administered compound that is absorbed by an animal where it may be metabolized, stored or excreted. It was not possible in these experiments to distinguish between parent compound in feces that had not been absorbed (unavailable) and that which had been absorbed and recycled to the gastro-intestinal tract (available). But, the compounds used in these studies are metabolized readily by rats, and it can be assumed that most of the parent compound recovered from the feces had not been adsorbed. In view of the finding that almost all of the original activity in soil had been extractable parent compound (Table 1), it is reasonable to assume that the unextractable activity in feces is a metabolite. Thus, all activity except the parent compound extracted from feces can be considered bioavailable.

Bioavailability in the Te and Pe experiments was greatest when the test compounds were administered in corn oil by gavage (Table 2). The disappearance of 85 to 95% of the parent compounds agree well with published absorption values for PCB in corn oil (Matthews et al., 1978). Bioavailability was reduced by only 3 to 4 percentage points when compounds were added to feed with a solvent, but it was reduced by 10 to 20 percentage points when compounds were contained in soil. Although not consistent among all compounds, administration by gavage appeared to yield higher bioavailability values than administration by adding soils to diets. This conclusion is supported by the lower recoveries of parent in feces and the higher concentrations of activity in body fat. The gavage treatments did cause some animal distress as evidenced by lower feed consumption during treatment. Thus, the lower level of feed residues that can bind compounds in the gastrointestinal tracts of the gavaged animals may have enhanced absorption.

The desire to use well-aged residues in these experiments brought limitations in analytical precision and sensitivity because the specific activity of the labelled soil was lower than that needed

for optimum analyses. In a practice, however, bioavailability reductions of 10 to 20 percentage points, although interesting, are not great enough to warrant consideration when assessing or managing risks associated with environmental contamination. It is recognized, however, that this study, in common with most bioavailability studies, involved only one soil type, one animal species and one class of compounds. Further effort will be required to determine the potential importance of these variables.

REFERENCES

Fanelli R, Chiabrando C, Bonaccorsi, A (1982) TCDD contamination in the Seveso incident. Drug Metab Rev 13:407-422

Fries GF (1985) Bioavailability of soil-borne polybrominated biphenyls ingested by farm animals. J Toxicol Environ Health 16:565-579

Fries GF (1987) Assessment of potential residues in foods derived from animals exposed to TCDD-contaminated soil. Chemosphere 16:2123-2128

Fries GF, Jacobs LW (1986) Evaluation of residual polybrominated biphenyl contamination present on Michigan farms in 1978. Res Rep 477, Mich State Univ Agric Exp Sta, East Lansing, Michigan

Fries GF, Marrow, GS (1981) Chlorobiphenyl movement from soil to soybean plants. J Agric Food Chem 29:757-759

Kimbrough RD, Falk H, Stehr P, Fries G (1984) Health implications of 2,3,7,8-itetrachlorodibenzodioxin (TCDD) contamination of residential soil. J Toxicol Environ Health 14:47-93

Lucier GW, Rumbaugh RC, McCoy Z, Hass R, Harvan D, Albro P (1986) Ingestion of soil contaminated with 2,3,7,8-tetrachloro-p-dioxin (TCDD) alters hepatic enzyme activities in rats. Fundam Appl Toxicol 6:364-371

Matthews H, Fries G, Gardner A, Gartoff L, Goldstein J, Ku Y, Moore J (1978) Metabolism and biochemical toxicology of PCBs and PBBs. Environ Health Perspect 24:147-155

McConnell EE, Lucier GW, Rumbaugh RC, Albro PW, Harvan DJ, Hass JR, Harris MW (1984) Dioxin in soil: Bioavailability after ingestion by rats and guinea pigs. Science 223:1077-1079

Poiger H, Schlatter C (1980) Influence of solvents and adsorbents on dermal and intestinal absorption of TCDD. Food Cosmet Toxicol 18:477-481

Shiu WY, Mackay D (1986) A critical review of aqueous solubilities, vapor pressures, Henry's law constants, and octanol-water partition coefficients of the polychlorinated biphenyls. J Phys Chem Ref Data 15:911-929

Snedecor GW, Cochran WG (1967) Statistical methods. Iowa State University Press, Ames, Iowa

Umbreit TH, Hesse EJ, Gallo MA (1986) Bioavailability of dioxin in soil from a 2,4,5-T manufacturing site. Science 232:497-499

Received February 22, 1989; accepted May 18, 1989.