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Influence of 2,3,7,8-Tetrachlorodibenzo-*P*-Dioxin on Respiration in a Forest Floor and Soil

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Dioxin, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), one of the most toxic chemicals known, is an impurity arising in the manufacture of the herbicide 2,4,5-T. When 2,4,5-T is used to control unwanted vegetation in forest management, the impurity may find its way into the forest soil. The production, behavior, and significance of TCDD in the environment has become known only in recent years (DAVID 1971). TCDD is low in water solubility (0.2 ppb), immobile in soil, and in laboratory experiments has a half life of 1 year in soil (KEARNEY et al. 1972). Recently, CROSBY and WONG (1977) reported TCDD half-life of only a few hours on vegetation and a few days on soil in the presence of herbicide formulation and sunlight.

Studies have shown that 2,4,5-T at rates registered by EPA (Environmental Protection Agency) has little influence on carbon dioxide production and certain other microbial activities in soil, but the TCDD content was not recognized or was unknown (WOODFORD and SAGAR 1960, BOLLEN 1961, MARTIN 1963). Early production lots of 2,4,5-T contained as much as 30 ppm TCDD, but the manufacturing process has been improved so present commercial formulations contain less than 0.1 ppm TCDD (USDA Forest Service 1978).

Carbon dioxide is evolved from soil and forest floor as microorganisms decompose organic matter. This process is essential to soil fertility and recycling of nutrients in organic residues. In this paper we report the effects of TCDD on CO_2 evolution in an important Pacific Northwest forest floor and soil.

MATERIALS AND METHODS

Bulk samples of forest floor and soil from the Quartz Creek area of the H. J. Andrews Experimental Forest on the foothills of the Cascade Range southeast of Eugene, Oregon, were used. The forest floor (L, F, and H layers)--consisting of needles, twigs, bits of bark and rotten wood, some minor herbaceous residues, and decomposing organic matter--was air-dried, ground, and screened through a 10-mesh sieve. The soil (0-15 cm segment) is a Dystric Cryochrept of volcanic origin, derived almost entirely from deposits of reddish brown bedrock. After it was air-dried, the soil was screened through a 10-mesh sieve, and small gravel (amounting to approximately 10 percent of sample) was discarded.

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About 1 cm of forest floor (20 g) or soil (50 g) (oven-dry basis) were placed in biometer flasks (Bellco Glass Co., No. 2566) (BARTHA and PRAMER 1965) (Table 1).

TABLE 1

Analysis of Quartz Creek Forest Floor and Soil (<10 mesh)

Item[a/]	pH	Water-holding capacity	Ash	Total carbon	Kjeldahl nitrogen	C:N ratio
		percent				
Forest floor	6.2	460	9.23	48.5	2.33	20.8
Soil	5.6	67	90.2	3.54	0.16	22.1

[a/] Forest floor is L, F, and H horizons from a mixed stand of Douglas-fir and red alder. Soil is 0-15 cm segment of Dystric Cryochrept.

The exposed surface area of forest floor or soil in each flask was 60.8 cm^2. Each flask received 0, 2.62×10^{-4}, 2.62×10^{-2}, or 2.62 nanograms (ng) TCDD. Treatments were based on the following surface application rates of 2,4,5-T per ha: 0, 4.48×10^{-3}, 0.448, or 44.8 kg 2,4,5-T containing 0.1 ppm TCDD. This is the equivalent of 13.1×10^{-9}, 13.1×10^{-7}, or 13.1×10^{-5} ppm TCDD in forest floor; 5.2×10^{-9}, 5.2×10^{-7}, or 5.2×10^{-5} ppm in soil. The toxicant was applied in 3 mL of acetone to each sample of forest floor or soil in a Buchner funnel. The mixture was stirred to mix the toxicant and substrate thoroughly while suction was applied to evaporate the acetone. Controls received the same treatment, but the acetone contained no toxicant.

No appreciable degradation of the TCDD could have occurred during the course of the experiment. MATSUMURA and BENEZET (1973) added TCDD to cultures of about 100 strains of microbes with known ability to degrade persistent pesticides. Thin layer chromatography of extracts of the cultures showed only five strains with some ability to degrade TCDD. KEARNEY et al. (1972) found the half-life of TCDD in soil to be 1 year in laboratory conditions similar to the ones we used.

The preparations were placed in their respective biometer flasks, and sufficient water was added to adjust water content to 50 percent of water-holding capacity. Each flask was connected to an oxygen supply regulated to a pressure of 50 mm water. Oxygen consumed by microbial activity was thus replaced;

therefore, CO_2 production would not be retarded by a diminishing oxygen level.

The apparatus was incubated at 28°C in a dark growth chamber. At 7, 14, 21, and 28 days the CO_2 absorbent (2 *M* NaOH) was removed and replaced with fresh absorbent. The absorbed CO_2 was precipitated by adding 10 mL of 1 *M* $BaCl_2$ and 1 *M* NH_4Cl solution to the absorbent solution which was heated to 70°C. When cool, the precipitate was collected on preweighed filter discs, washed with CO_2-free H_2O and 50 percent ethanol, dried at 105°C, and weighed to determine periodic and total CO_2 efflux.

The study was arranged as a completely randomized split plot experiment with three replications. The rates of chemical application were the whole plot treatment; time periods after application were the split plot treatments. Experiments with forest floor and soil were run at different times.

RESULTS AND DISCUSSION

TCDD had no effect on CO_2 evolution from forest floor (Table 2). Analysis of variance shows no significant difference ($P>0.05$) in the levels of CO_2 produced per week among flasks receiving different levels of TCDD. There was also no difference ($P>0.05$) in CO_2 output from forest floor among weeks, which means the rate of carbon metabolism was relatively constant over the 4-week study period.

TABLE 2

Mean CO_2 Evolution[a] from Quartz Creek Forest Floor[b] and Soil[c]

TCDD (ng/flask)	Substrate	Weeks after treatment 1	2	3	4	Total (mg C as CO_2)
		---mg C as CO_2/week--				
0	Forest floor	61.7	60.3	61.3	60.9	244.2
2.62×10^{-4}	Forest floor	60.6	61.0	59.3	59.2	240.1
2.62×10^{-2}	Forest floor	61.3	61.2	61.4	61.0	244.9
2.62	Forest floor	60.5	59.7	60.9	61.0	242.1
0	Soil	28.6	22.2	11.1	15.9	77.8
2.62×10^{-4}	Soil	32.8	29.2	25.1	27.4	114.5
2.62×10^{-2}	Soil	28.7	23.9	30.4	22.1	105.1
2.62	Soil	30.1	27.3	27.8	13.0	98.2

[a] Mean of 3 replications.

[b] 20 g, oven-dry basis, incubated at 28°C with moisture adjusted to 50 percent of water-holding capacity.

[c] 50 g, oven-dry basic, incubated at 28°C with moisture adjusted to 50 percent of water-holding capacity.

TCDD stimulated CO_2 evolution from the soil (Table 2). Analysis of variance shows stimulation was significant and was best described by a quadratic function ($P < 0.01$). CO_2 production decreased linearly with time ($P < 0.01$) after application, but there was no significant interaction ($P > 0.05$) between level of TCDD and time after application. The lack of interaction means the effects of different levels of TCDD were consistent among time periods (1, 2, 3, or 4 weeks) after application.

The lack of declining CO_2 evolution from forest floor with time in this study is unusual. The reason could be that the content of only moderately decomposable organic material in the forest floor is so high that its rate of oxidation would be nearly constant over the course of the study. In the soil, on the other hand, the total organic matter was low and although likely to be less decomposable, the mild stimulating effect could appear and decrease during the incubation.

That the stimulation decreased as the toxicant level increased follows HEUPPE's principle (HEUPPE and JORDAN 1899) "that a substance which in definite concentration will kill protoplasm, will inhibit development in smaller amounts, and in still greater dilution act as a stimulant." Levels of TCDD greater than used in our experiment would probably have caused decreased CO_2 production. The levels of TCDD we tested, however, extend well beyond the levels expected to occur in the forest from the use of herbicides containing TCDD.

We tested only a single forest floor and soil with TCDD, but in other studies we have found relatively little difference in the response of microbial populations to toxicants among soils and forest floors (BOLLEN 1961, BOLLEN et al. 1974, 1977). In view of the lack of effect of TCDD on CO_2 evolution from the forest floor and the small magnitude of the stimulating effect on the soil in this test, we believe that the TCDD will not directly alter soil microbial populations, carbon metabolism, or recycling of nutrients in forest ecosystems sprayed with pesticides containing TCDD at normal rates of application. Alteration of the composition and density of the vegetative community from the use of herbicides is more likely to influence soil microbes and their activities than is the direct toxic action of herbicides, their carriers or contaminants.

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