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Absorption and Translocation of 4-(Trifluoromethyl)chlorobenzene in Soil and Crops

Water containing 1 mg/L 4-(trifluoro[^{14}C]methyl)chlorobenzene (TFCB) was supplied to pot cultures of three grass (*Zea mays* L.; *Festuca rubra* L.; *Lolium multiflorum* L.) and three legume (*Vicia sativa* L.; *Trifolium perenne* L.; *Medicago sativa* L.) species. The chemical was absorbed by soil and subsequently translocated to plant leaves at increasing amounts for maize to ryegrass, clover, alfalfa, red fescue, and vetch. Legumes showed a high capacity of degradation of the contaminant, suggesting their utilization to reclaim soil and water contaminated by TFCB.

4-(Trifluoromethyl)chlorobenzene (TFCB), an intermediate in the synthesis of the herbicide trifluralin (α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine) has been detected in groundwater at a concentration of 1 mg L^{-1} as a result of the incorrect disposal of discharges at a chemical factory in the country north of Vicenza, Italy. The groundwater was used to feed the public aqueduct, and the level of contamination made the water unacceptable for human consumption.

Since the water was used also for irrigation, it is important to determine the mobility of the contaminant in soil and crops. Data on the entry and degradation patterns of certain dinitroaniline herbicides into plants have been described (Parka and Soper, 1977; Probst and Tepe, 1969). However, extrapolation of such data to TFCB was not possible. Therefore, experiments were designed in which water containing ^{14}C-labeled TFCB was applied to pot cultures and the radioactivity was determined in the soil and in the roots, stems, and leaves of the crops.

MATERIALS AND METHODS

Several clay pots (10 × 10 × 14 cm) were prepared, each containing 2 kg of soil. This was representative of the soil found in the contaminated zone, a fluvisol, according to the FAO classification, with the following characteristics: pH 7.2, clay 8.5%, silt 17.9%, sand 73.6%, $CaCO_3$ 2.5%, and organic matter 1.3%.

Three grass (*Zea mays* L.; *Festuca rubra* L.; *Lolium multiflorum* L.) and three legume (*Vicia sativa* L.; *Trifolium perenne* L.; *Medicago sativa* L.) species were sown.

Plants were grown in a controlled environment at 27 °C, 80% humidity, and 14-h light/10-h dark cycle and watered through a hole in the bottom of the pots. After 20 days from sowing, the pots were dipped in a 1-cm layer of water containing 1 mg L^{-1} (trifluoro[^{14}C]methyl)chlorobenzene (TFCB). Since at this concentration loss by volatilization was negligible, at the end of the absorption period no radioactivity was detected in soil and plants of the control pots. The radioactive TFCB prepared by the Radiochemical Centre (Amersham) had 11 μCi/mg specific activity and 92% radiochemical and chemical purity.

Table I. Vertical Distribution of (Trifluoromethyl)chlorobenzene in the Soil after 24-Hour Contact with Water Containing 1 mg L^{-1} Contaminant[a]

soil layer, mm	(trifluoromethyl)-chlorobenzene, mg/kg of soil
0-15 (bottom)	1.32 ± 0.048
15-30	0.65 ± 0.041
30-45	0.31 ± 0.013
45-60	0.28 ± 0.026
60-75	0.26 ± 0.025

[a] Data are the means of three experiments ± standard errors.

Two replications (one pot each) were used in completely randomized design, and the entire study was repeated 3 times.

After 1, 7, 24, 60, and 240 h (see Tables I–IV), the plants were separated from the soil and rapidly washed, and roots, stems, and leaves were sampled. The soil was horizontally cut in 15-mm slices, which were extracted with ethanol to evaluate the absorbed radioactivity.

Plant material was homogenized with an Ultraturrax apparatus, and the homogenate was fractionated into ethanol-soluble and ethanol-insoluble fractions. Radioactivity was evaluated in the soil extract and in each fraction of the plant homogenate by means of a Tricarb spectrometer, with Instagel as the scintillation mixture. The ethanol-soluble fraction was submitted to thin-layer chromatography at −20 °C, using silica gel plates and heptane as the solvent. The low temperature of operation was chosen in order to prevent the volatilization of TFCB. Spots were removed from the plate, after identification by

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Table II. Distribution of ^{14}C-Labeled (Trifluoromethyl)chlorobenzene in Maize Plants after Saturation of the Soil with Water Containing 1 mg L^{-1} Contaminant[a]

plant section	(trifluoromethyl)chlorobenzene, μg/g fresh wt, at absorption time		
	1 h	7 h	24 h
root	0.337 ± 0.0401	0.691 ± 0.0725	0.863 ± 0.0716
stem	0.108 ± 0.0079	0.190 ± 0.0110	0.239 ± 0.0219
leaves	0.001 ± 0.00008	0.0039 ± 0.0028	0.131 ± 0.0090

[a] Data are the means of three experiments ± standard errors.

autoradiography, and radioactivity was assayed as previously indicated.

RESULTS AND DISCUSSION

The absorption and exchange capacity of the soil strongly reduced the transfer of TFCB by the water moving upward through the soil (Table I). The quantity of water absorbed after 24 h was 118 mL/kg of soil. At this time the contaminant accumulated in the 0–15-mm soil layer was about 10 times that present in the irrigation water. The concentration of TFCB decreased with each successive upper layer. After 48-h absorption the concentration of TFCB in the first layer (bottom) had not significantly changed (1.32 mg/kg): this value can be considered to represent the steady-state condition of the soil after prolonged water absorption.

Preliminary information on the entry and movement of TFCB into plants was obtained from a short-term experiment with maize (Table II).

The contaminant reached the aerial part of the plant within 24 h from the saturation of the soil with the contaminated water. The ratio between TFCB concentration in leaves and in roots was 1:337, 1:18, and 1:7, respectively, at 1, 7, and 24 h from the beginning of the absorption. This indicated rapid translocation of the contaminant to the leaves. The main interest shifted to the accumulation of TFCB in the leaves of different species and the possible degradation of the contaminant by plant metabolism.

The concentration of TFCB in the leaves continued to increase from 60 to 240 h. The magnitude of the increases was 50, 30, 10, 5, 4, and 3, respectively, for vetch, red fescue, clover, alfalfa, ryegrass, and maize (Table III). A sharp difference was shown by the species relative to the fate of TFCB, as in the case of trifluralin (Golab et al., 1967).

The radioactivity from the [^{14}C] TFCB was distributed between the ethanol-soluble and ethanol-insoluble compounds in a different ratio: 11:1 to 19:1 for grasses; 1:1 to 1:2 for legumes (Table IV).

The thin-layer chromatography of the soluble fraction showed an overlapping pattern with respect to the original compound supplied to plants. Both patterns were characterized by a radioactive spot with R_f 0.25 accounting for 90% of the total radioactivity and corresponding to the TFCB. The two secondary spots with R_f 0.80 and 0.92 contained the remaining 10% radioactivity and were impurities corresponding to intermediates of the TFCB synthesis carried out by the Amersham Radiochemical Centre. Since the ratio between the radioactivity of impurities and that of TFCB was the same in the nutrient solution and in the plant extract, the absorption and degradation patterns of the impurities and TFCB were similar.

Table III. (Trifluoromethyl)chlorobenzene (TFCB) Content of Leaves of Different Plants after 60 and 240 Hours from Saturation of the Soil with Water Containing 1 mg L^{-1} Contaminant[a]

plant	TFCB, μg/g fresh wt, at absorption time		fresh wt of plants, g/pot
	60 h	240 h	
maize	0.210 ± 0.0113	0.64 ± 0.039	40.6 ± 4.79
red fescue	0.081 ± 0.0088	2.67 ± 0.138	11.3 ± 0.64
ryegrass	0.164 ± 0.0154	0.61 ± 0.048	12.1 ± 0.74
vetch	0.278 ± 0.0169	13.61 ± 0.721	21.1 ± 2.04
clover	0.385 ± 0.0285	3.59 ± 0.290	10.7 ± 0.92
alfalfa	0.212 ± 0.0812	1.97 ± 0.116	13.8 ± 1.11

[a] Data are the means of three experiments ± standard errors.

Table IV. Percent Distribution of Ethanol-Soluble and Ethanol-Insoluble Radioactivity in Plant Leaves after 10 Days from Saturation of the Soil with Water Containing (Trifluoro[^{14}C]methyl)chlorobenzene[a]

plant	% ^{14}C radioactivity	
	ethanol soluble	ethanol insoluble
maize	91.4 ± 5.11	8.6 ± 0.70
red fescue	95.0 ± 7.50	5.0 ± 0.45
ryegrass	93.0 ± 7.63	7.0 ± 1.04
vetch	44.3 ± 2.70	55.7 ± 7.46
clover	46.9 ± 3.33	53.1 ± 5.15
alfalfa	34.0 ± 2.14	66.0 ± 6.67

[a] Data are the means of three experiments ± standard errors.

The high fraction of ethanol-insoluble radioactivity in legumes suggests the presence of a mechanism of degradation of TFCB in these species. Since the radioactivity of ethanol-soluble metabolites was negligible, the rate of TFCB degradation must be lower than that of incorporation of its intermediates into the ethanol-insoluble compounds. This behavior offers the basis for the tentative use for decontamination of water and soil from TFCB. Since the TFCB in the soil of each pot was 2.6 mg, the contaminant removed from the soil by the different species at 240-h absorption was 0.99, 1.16, 0.28, 11.0, 1.47, and 1.04% of that present in the soil, respectively, for maize, red fescue, ryegrass, vetch, clover, and alfalfa in the quantity per pot shown in Table III.

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