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Accumulation of 2,3,7,8-tetrachlorodibenzo-p-dioxin from soil and nutrient solution by bean and maize plants

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Summary. Maize and bean plants grown on soil polluted with ^{3}H-2,3,7,8-tetrachlorodibenzo-p-dioxin (^{3}H-TCDD) accumulated the toxin in the aerial parts progressively with the time and with the soil contamination. Plants in hydroponic solution polluted with ^{3}H-TCDD accumulated the toxin in the aerial parts in the light and not in the dark; the concentration of TCDD accumulated was not proportional to the surface area of plant organs; the distribution of ^{3}H-TCDD in the leaves suggested that TCDD was translocated through the vessels to the aerial parts by the transpiration stream.

Key words. TCDD; translocation; accumulation; bean and maize plants.

Owing to the fact that in the past years dioxins have sometimes been present in herbicides applied on areas where food plants were growing, some studies have considered the question whether higher plants absorb and accumulate TCDD. The results seem to indicate that TCDD is absorbed by higher plants but only very small amounts are accumulated[3–5].

After the explosion of a reactor in Seveso, in northern Italy, which caused an escape of TCDD, we studied the absorption and translocation of TCDD by plants growing in the contaminated[6,7] area. Dioxin was absorbed by the underground organs of the plants at levels of the same order of magnitude as the TCDD levels found in the soil, and by the aerial parts at a concentration 50% lower. Utilizing the opportunity of performing experiments with high concentrations of TCDD in a confined area of Seveso, we have further studied the uptake, the accumulation and the elimination of TCDD by higher plants using uniformly labeled ^{3}H-TCDD.

Materials and methods. Bean (*Phaseolus vulgaris* L. cv. Borlotto Lingua di Fuoco, Ingegnoli, Milan) and maize (*Zea mays* L. cv. Dekalb XL 640 A, Marchetto, Mestre) plants were used.

Soil cultures. A sandy loam soil (organic matter 15‰, pH 7) was dried, sieved at < 2 mm and fertilized with 90 ppm of N, 140 ppm of P_2O_5, 210 ppm of K_2O. Different amounts of ^{3}H-TCDD (1.54 GBq/µmole) were sprayed onto the soil. The soil was then added with or without peat (20% w/w), put into 25-cm pots and watered with tap water. The addition of peat did not change the pH value. It was found that not less than 90% of the added radioactivity could be extracted from the soil by a methanol:chloroform mixture. The radioactive substance was chromatographed on Silica Gel 60 plates and showed the same R_f as TCDD. Five bean or seven maize plants were grown in a 25-cm pot filled with 7 kg of soil in a greenhouse at 25°C with a 12 h light cycle.

Hydroponic cultures. Plants, grown to the 4th leaf stage in nutrient solution in a greenhouse, were transferred into a thermoregulated (25°C) chamber in a new solution containing 0.2 µg/l ^{3}H-TCDD (1.77 GBq/µmol). The chamber was illuminated with Sylvania fluorescent tubes (F 30 W-T8-GRO); this source has practically no UV irradiation. The chamber was equipped with an air outlet and an air inlet (15 dm^3/h) connected with two pumps regulated to prevent phenomena of under- or overpressure. The outgoing air was forced into a solution of ethyleneglycol:propyleneglycol (50:50 by vol) at 0°C to trap substances which might escape along with the transpired water.

Determination of radioactivity in the plant material. Plant material was ground in a mortar with liquid N_2. Aliquots (about 1 g) of this powder were treated with 0.5 ml of H_2O_2 (35% by weight) for 24 h at 50°C to destroy the photosynthetic pigments and to solubilize the tissues. Radioactivity was measured by liquid scintillation in a Beckman LS 7500 counter. Internal standard addition showed that there was a recovery of radioactivity up to 90%.

Extraction and measurement of ^{3}H-TCDD from plant material. 5 g of plant material ground in the mortar with liquid N_2 were extracted three times with two volumes of methanol:chloroform (2:1 by vol) and three times with two volumes of chloroform by consecutive centrifugations; the collected supernatants were dried under N_2 by reduced pressure evaporation and resuspended in methanol:chloroform (2:1 by vol) and then an aliquot was chromatographed on Silica Gel 60 plates without fluorescent indicator (Merck, Darmstadt, RFD) with carbontetrachloride-hexane (75:200 by vol). Radioactivity of the material scraped off the chromatogram was measured by liquid scintillation in a Beckman LS 7500 counter. The ^{3}H-TCDD (R_f 0.76) was identified by co-chromatography with cold TCDD and detected by treatment with 10% (w/v) H_2SO_4 in ethanol (10 min at 110°C). The radioactivity of the insoluble material was measured as described in the above section. About 20% and 10% of the radioactivity was not extracted by methanol:chloroform from bean and maize plants respectively. The analysis of methanol:chloroform-soluble material by TLC showed only a radioactive spot corresponding to TCDD. The radioactivity measured was expressed as ng TCDD/kg fresh weight. TCDD was purchased from Carlo Erba, Milan, Italy. Uniformly labeled ^{3}H-TCDD, purified by preparative gas chromatography, was supplied by Kor Isotopes, Cambridge, Mass., USA.

Results and discussion. Bean and maize plants were grown on soil with and without added peat and in the presence of different concentrations of ^{3}H-TCDD. ^{3}H-TCDD present in the aerial parts of bean and maize plants was found to be increased with

Table 1. ^{3}H-TCDD incorporation in the aerials parts of bean and maize plants grown on soil with added peat. The data are the means of three experiments run in triplicate. SE did not exceed 3.8%

^{3}H-TCDD in the soil ng/kg	TCDD ng/kg f.w. Bean plants 7 days old	34 days old	57 days old	Maize plants 17 days old	34 days old	57 days old
12	–	1.13	1.36	0.95	1.62	1.17
29	0.09	1.06	1.37	0.74	1.59	1.48
58	0.27	1.33	1.36	0.80	1.46	2.03
175	0.42	1.14	1.47	0.80	1.69	1.67
417	0.24	2.12	2.95	2.10	2.59	1.90
833	0.81	2.60	3.24	3.10	2.49	1.87
1667	2.40	2.90	5.03	5.36	3.50	2.12
2750	7.20	5.20	4.83	10.02	4.97	3.25

Plants were grown in a greenhouse on soil contaminated with different concentrations of ^{3}H-TCDD (1.54 GBq/µmole) with 20% w/w added peat. Bean plants: 7 days old, 3rd leaf stage; 34 days old, full growth stage; 57 days old, flowering stage. Maize plants: 17 days old, 4th leaf stage; 34 days old, 5th leaf stage; 57 days old, 7th leaf stage.

Table 2. ^{3}H-TCDD incorporation in the aerial parts of bean and maize plats grown on soil without added peat. The data are the means of three experiments run in triplicate. SE did not exceed 3.5%

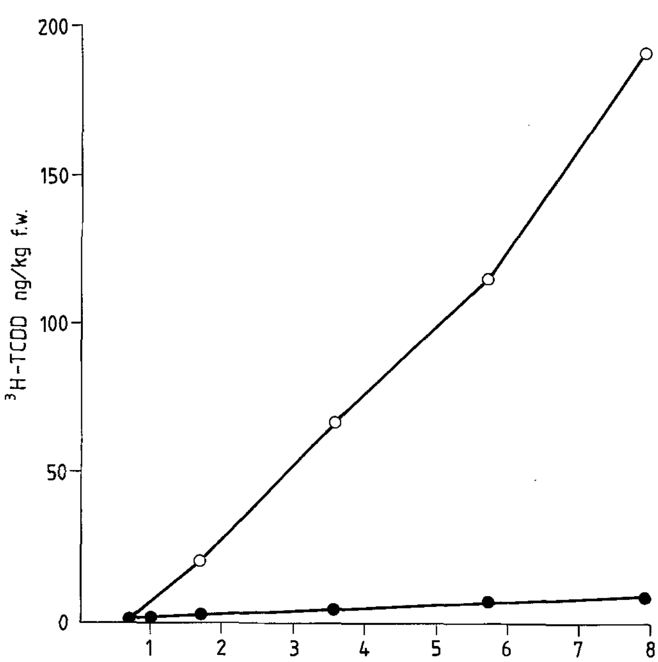
^{3}H-TCDD in the soil ng/kg	TCDD ng/kg f.w. Bean plants 8 days old	77 days old	Maize plants 8 days old	49 days old
14	–	0.67	1.91	4.91
35	–	0.79	2.01	9.13
70	1.28	1.16	3.95	6.23
210	0.75	1.80	3.86	9.91
500	1.52	6.10	4.49	17.92
1000	2.72	12.30	8.62	35.61
2000	7.34	18.00	14.53	25.02
3300	9.51	28.03	33.93	63.07

Plants were grown in a greenhouse on soil contaminated with different concentrations of ^{3}H-TCDD (1.54 GBg/µmole) without added peat. Bean plants: 8 days old, 3rd leaf stage; 77 days old, flowering stage. Maize plants: 8 days old, 2nd leaf stage; 49 days old, 5th leaf stage.

Table 3. ^{3}H-TCDD incorporation in the 2nd leaf of bean plants and 3rd leaf of maize plants grown on soil without added peat. The data are the means of three experiments run in triplicate. S E did not exceed 4.3%

^{3}H-TCDD in the soil ng/kg	TCDD ng/kg f.w. Bean plants	Maize plants
14	3.81	12.10
35	4.49	12.00
70	3.40	9.62
210	3.66	11.12
500	9.01	9.02
1000	10.80	12.45
2000	20.60	26.94

Plants were grown for 27 days: bean plants, full growth; maize plants, 4th leaf stage.



^{3}H-TCDD incorporation in the 3rd leaf of a maize plant grown in continuous light (○—○) or dark (●—●) in a nutrient solution containing ^{3}H-TCDD. Maize plants were grown in a nutrient solution in a greenhouse to the 5th leaf stage before being placed in the treatment chamber. ^{3}H-TCDD (1.77 GBq/µmole) concentration was 0.2 µg/l. The results of a representative experiment are shown. Each value is the mean of two determinations performed on two samples of five leaf discs (5 mm diameter) from five plants.

the increase in the concentration of the toxin in the soil (tables 1 and 2). Peat decreased the amount of the toxin accumulated (compare table 1 with table 2). The pollution increased with time in the aerial parts of both plants when they were grown at all the different ^{3}H-TCDD concentrations in the two soil conditions, except when they were grown at high ^{3}H-TCDD concentration in soil with added peat. In these conditions the pollution decreased with time, more evidently in maize than in bean. This result might be due to the peat, immobilizing ^{3}H-TCDD and thus decreasing the level of pollutant readily available for absorption, so that with time the supply of ^{3}H-TCDD from the soil is limited; the ^{3}H-TCDD accumulated in the aerial parts might be eliminated through transpiration.

The levels of the toxin were also measured in the same leaf (the 2nd one for bean and the 3rd one for maize) of plants grown on soil with increasing ^{3}H-TCDD content without added peat (table 3). Leaf pollution was the same at low ^{3}H-TCDD concentrations in the soil and increased progressively at higher ones. The same amount accumulated for lower ^{3}H-TCDD concentrations in the soil might be due to a balance between the continuous supply of a low amount of ^{3}H-TCDD to the leaves, its binding to higher affinity sites of leaf material and the amount of ^{3}H-TCDD lost by transpiration. The increasing concentrations accumulated in the leaves for higher ^{3}H-TCDD concentrations in the soil might be due to a continuous supply of increasing amounts of ^{3}H-TCDD to the leaves which was not balanced by its low and constant loss by transpiration. This hypothesis is in agreement with the data of tables 1 and 2 where there is a constant value for plant pollution mainly when peat is mixed with soil, and limits the availability of the toxin.

The possibility that the presence of TCDD in plants may be linked to a contamination with TCDD vaporized from soil and plants has been considered. Recently, a model has been developed to describe the vaporization and diffusion through a column of soil, and a good agreement has been found between predicted and measured TCDD concentrations based on samples taken at the Time Beach site in 1983[8]. Bean or maize plants were grown in different pots (10 cm apart) containing contaminated (3300 ng TCDD/kg) and uncontaminated soil respectively at the 3rd leaf stage and at the 4th leaf stage. The aerial parts of plants grown on uncontaminated soil contained about 5% of the radioactivity accumulated in the aerial parts of plants grown on contaminated soil. The pollution, expressed as TCDD concentration, was respectively 0.12 ng/kg fresh weight for bean plants and 0.54 ng/kg fresh weight for maize plants. This suggests a contamination with TCDD vaporized from soil or/and from other plants.

In the experiments in hydroponic culture when maize and bean plants were transferred into contaminated nutrient solution almost 90% of ^{3}H-TCDD administered was very soon (in about 1 h) found in the plant roots. ^{3}H-TCDD was only adsorbed on the root surface: in fact, the same result was obtained if an equal number of roots, killed by freezing, was transferred into the labeled nutrient solution. Table 4 shows the amount (ng/kg f.w.) of ^{3}H-TCDD present in the aerial parts of bean and maize plants grown under continuous light. The amount of ^{3}H-TCDD accumulated was progressive with the age of the internodes and leaves of the bean plants and it was about the same for different leaves of the maize plants. This was in agreement with the different anatomical location of the vessels in bean (Dicotyledon) and in maize (Monocotyledon) plants. ^{3}H-TCDD accumulated in the aerial parts of bean plants was not proportional to the surface area of the different organs examinated: it was higher in the bean internodes than in the corresponding leaves, excluding the possibility that it might be due to contamination by vaporized TCDD from the nutrient solution. ^{3}H-TCDD, measured at different times in discs (5 mm diameter) from the 3rd leaf of maize plants, was accumulated in the light and not in the dark (fig). Under the conditions used, the transpirational flow was 0.6 g/dm^2/h in the light and 0.04 g/dm^2/h in the dark. These results suggested that TCDD was translocated through the vessels along with the transpirational flow. This hypothesis is also supported by results indicating that fusicoccin, a substance capable of opening the stomata in the dark and thus increasing the transpirational flow, also increases the accumulation of ^{3}H-TCDD in the leaves in the dark.

In the experiments using nutrient solution some radioactivity was found in the transpired substances from bean and maize plants (600–700 cpm/ml and 1200–2000 cpm/ml of transpired water, respectively). This radioactivity was less than 1% in ^{3}H-TCDD; the remnant accounted for one or more volatile substances, not extractable by hexane and not adsorbable either on norit or on silicic acid. The radioactive volatile substance(s) might be $^{3}H_2O$; under our biological conditions it is not possible to recognize whether the tritiated water is a metabolic product or the consequence of a transfer reaction; however, the tritium atoms in the uniformly labelled TCDD molecule are known to be firmly bound[9]. Moreover, in these same experiments, the material extracted by methanol:chloroform from bean plants showed on TLC a spot with R_f 0.11. The nature of this more polar compound is at the moment unknown. A substance more polar than TCDD has previously been found as a microbial metabolite and it was identified as 1-hydroxy-2,3,7,8,tetra-chlorodibenzo-p-dioxin[10]. These last data seem to suggest that TCDD might be metabolized by plants. We cannot exclude the possibility that the different radioactive substances found might have been present, as contaminants, in the ^{3}H-TCDD used, or derived from microbial degradation, and that they were just concentrated by the plants after absorption. We can rule out a degradation by UV light[11] because in the experiments in the plexiglas chamber the source of light used had practically no UV irradiation.

Table 4. ^{3}H-TCDD incorporation in the aerial parts of bean (leaf and internode) and maize (leaf) plants from a nutrient solution containing ^{3}H-TCDD. The results of one representative experiment are shown

Leaf or internode	^{3}H-TCDD ng/kg f.w. Bean Leaf	Internode	Maize Leaf
1st	–	51	–
2nd	13.6	18.3	52.4
3rd	7.5	16.6	68.4
4th	0.9	17.9	45.2
5th	–	6.1	76.1

Plants were grown in the nutrient solution to the 4th leaf stage, then transferred into a new nutrient solution polluted with 0.2 µg/l ^{3}H-TCDD (1.77 GBq/µmole) in continuous light for 72 h.

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