DIOXIN CONCENTRATIONS IN FOODS PRODUCED IN SEVESO AREA.

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Introduction

In July 1976, a certain amount of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) was spread over a large area surrounding Seveso owing to the explosion of a reactor of the ICMESA factory. The public authorities decided to divide the polluted area in three risk zones corresponding to decreasing levels of contamination. Zone A (2,3,7,8-TCDD concentration > 50 $\mu g/m^2$ soil), after having moved the inhabitants to new houses, was cleaned up by collection and disposal of the contaminated soil, and converted to a park (Bosco delle Querce). Zones B and R, affected by lower contamination (2,3,7,8-TCDD concentration 5-50 $\mu g/m^2$ and < 5 $\mu g/m^2$ respectively), were reclaimed by mixing the most contaminated soil with uncontaminated underlying layers, in order to decrease the 2,3,7,8-TCDD concentrations down to safety levels (1), and owing to the long-life of 2,3,7,8-TCDD (2), low but measurable soil and air residues should be still present in the area. Moreover Seveso is located in a heavy urbanised and industrialised area where significant levels of air polychlorodibenzodioxins (PCDD) and polychlorodibenzofurans (PCDF) have been observed (3) leading probably to significant atmospheric deposition on vegetation.

It is generally agreed that the human diet represents the main route of exposure to PCDD/PCDF. This route is believed to be responsible for over 90% of the total daily intake (4,5). In the Seveso area several inhabitants have orchards where they grow vegetables or breed small animals, especially rabbits. The purpose of this investigation was to quantify the levels of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/F) in vegetables and rabbit livers, were these lipophilic compounds accumulate.

Materials and methods

Vegetables were washed carefully and old cabbage leaves were discarded. Prepared vegetables and rabbit livers were homogenised, weighted, lyophilised, and weighted again. Lipids were extracted for 12 h in a Soxhlet apparatus, after the addition of the standard solution (a mixture of 15 2,3,7,8-Cl substituted dibenzodioxins and dibenzofurans, 1 ng each component). The extraction was performed with hexane:acetone (9:1). The samples were purified by gel filtration (Bio Beads S-X3, 200-400 mesh, Biorad), Extrelut column (Merck) loaded with concentrated sulphuric acid, and aluminium oxide column. Instrumental analysis was carried out with a DANI 6500 VG 70-250 gas chromatograph-mass spectrometer (HRGC-MS). Two different capillary columns were used. The first was a Chrompack capillary column CP sil 88, 50 m x 0.25 mm, film thickness 0.25 µm. Temperature programme: 100 °C for 2 min, 30 °C/min until 190 °C, then 5 °C/min until 240 °C, maintained for 30 min. Column pressure 180 kPA; injector temperature: from 100 °C to 240 °C by programmed temperature vaporisation (PTV), splitless mode. The second column was a SGE BPX5 column 50 m x 0.20 mm, film thickness 0.25 µm, Temperature programme: 160 °C for 1 min, 2.5 °C/min until 300 °C for 30 min. Column pressure 240 kPA; injector temperature: from 100 °C to 280 °C by programmed temperature vaporisation (PTV), splitless mode. The HRGC-MS was employed in selected ion recording (SIR) mode (6). The methodology described above was validated through interlaboratory studies.

Results and discussion

Vegetable are considered to contribute only for 10 % to the daily intake of PCDD and PCDF (4,5). Nevertheless they are the first link in the food chain and therefore a full-risk assessment is necessary, moreover the typical Italian diet is rich in vegetables, and orchard owners consume a lot of their own products. The vegetables chosen were zucchini, cucumbers, and cabbages. Zucchini were selected because it has been demonstrated that they can absorb PCDD and PCDF from the soil (7). Cucumbers belong to the cucurbitaceae family too where other species can absorb from the soil very lipophilic polychlorinated compounds (8,9). There is also indication that cabbages can contain about 0.2 pg I-TEq/g_{ww}, probably because they have very large and waxy leaves and are collected after many months (10).

Table 1. PCDD/PCDF Contamination of Vegetables in the Seveso Area

sample	vegetable pg I-TEq/g dry weight	vegetable 2,3,7,8-TCDD %	soil pg I-TEq/g	soil 2,3,7,8-TCDD %	veget/ soil
Zucchini, R30	5.61	71	19	80	0.30
Zucchini, M20	4.51	44	11	60	0.41
Zucchini, M19	4.46	60	41	60	0.11
Zucchini a, F18	1.67	62	10	38	0.17
Zucchini, L8	3.49	49	21	52	0.17
Zucchini b, F18	2.19	76	10	38	0.22
Cucumber, R30	0.72	83	19	80	0.04
Cucumber, F18	0.88	62	10	38	0.09
Cabbage, Seveso R	0.64	96			
Cabbage, Seveso A	1.11	95			

The level of PCDD and PCDF contamination of the samples we have collected is reported in Table 1 in comparison with the data of the soil.

In all the vegetables analysed the most abundant measured 2,3,7,8 isomers were hepta and octacloro dioxin indicating that atmospheric deposition plays here an important role in determining levels in vegetables. All the vegetables presented variable amounts of the other 15 2,3,7,8-substituted isomers analysed. Average soil concentrations, where vegetable samples were collected, showed an important relative presence of the ICMESA accident residual 2,3,7,8 TCDD ranging from 38 to 80 % of total Teq. concentrations. Vegetables total TEqs ranged from 0.64 to 5.61 pg I-TEq/g dry weight, with 2,3,7,8 TCDD levels accounting for most of the Teq burden (44 - 96 %). These data suggest that 2,3,7,8 TCDD in soil could have an effect on vegetables levels however no significant relationship appeared to be present between soil and vegetables levels. Zucchini were more contaminated than the other species. Hülster et al. (7) have studied the soil-plant transfer of PCDD and PCDF in the cucumber family demonstrating that the uptake is at least 2 orders of magnitude higher than in other families. They have also shown that for zucchini root uptake and subsequent translocation to the shoots and into the fruits are the main contamination pathway. Wuthe at al (11) examined the PCDD/PCDF levels in blood in people living in Germany in highly contaminated areas and concluded that the consumption of certain vegetables, e. g. zucchini, could be an important source of intake in humans. In our samples, zucchini contamination, expressed as dry weight, ranged from 1.67 to 5.61 pg I-TEq/g_{dw}, corresponding to 0.076-0.310 pg I-TEq/g on wet weight. Although these values are not very high, taking into account that a normal portion can correspond to 200 g of fresh vegetable, a meal could contain at least 15-60 pg I-TEq/g. The admissible daily intake (ADI) of these compounds is 70 pg/die for a person of 70 Kg, and with daily diet containing two vegetable portions the ADI is easily exceeded.

Meat is considered one of the main human exposure sources (4,5). It was decided to breed some rabbits and to feed them for 3-4 months mostly with grass cut in four areas selected for their specific contamination. The locations were a) in Bosco delle Querce, very close to the highway Milano-Meda (SU) and b) on the boundary of the hillock that covers the basin which contains the most contaminated soil and the debris of the knocked out houses (CO), c) in the Seveso intersection of the just cited highway at the North of the Bosco delle Querce (SV), and d) in Meda over another basin (CM) which contains some of the machines used during the decontamination of zone A and B. The aim was to distinguish the contamination coming from the ICMESA accident and from air deposition. The amount of PCDD/PCDF are reported in Table 2. In rabbits PCDD/F accumulate mostly in liver (12) and this organ was extracted for the analysis. The control was bought in a supermarket in Milan. Rabbit livers contained variable amounts of each of the 17 PCDD/PCDF 2,3,7,8 substituted isomers. 2,3,7,8 TCDD was present in amounts ranging from 0.13 pg/g wet liver tissue (control) and 0.49 - 3.52 pg/g for samples coming from the experimental zone.The control contains 1.85 pg I-TEq/g_{ww}, only 7 % of contamination derives from 2,3,7,8-TCDD, and OCDD is the most abundant isomer. The samples can be divided in two groups. Rabbits CO and CM were fed with grass cut over basins which contain materials and debris of the ICMESA accident. They are the most contaminated (27-31 pg I-TEq/g_{ww}), even if the 2,3,7,8-TCDD contribution to the total I-TEq is relatively small, whereas 2,3,4,7,8-PeCDF, is the prevalent isomer. Rabbits SV and SU were fed with grass that should be contaminated mostly by deposition of traffic pollution. They are less contaminated (5-13 pg I-TEq/g_{ww}), 1,2,3,4,,6,7-HeCDD and OCDD are prevalent, and the contribution of 2,3,7,8-TCDD to the total I-TEq/g_{ww} is higher. In all cases the contamination should derive mostly from air deposition.

Table 2. PCDD/PCDF contamination of rabbit liver in Seveso area (pg/g wet liver)

sample	total TCDD Eq	% 2,3,4,7,8-PeCDF	% 2,3,7,8-TCDD	% others
SV1	6.475	50.5	37.5	12
SV2	8.676	34.7	40.6	24.7
SV3	13.043	59.1	20.7	20.2
CO1	30.878	84.2	7.0	8.8
CO2	30.786	91.2	1.6	7.2
CM1	27.156	85.1	5.4	9.5
SU1	5.029	57.3	13.0	29.7
SU2	9.874	58.3	28.3	13.4
control	1.853	60.6	7.2	32.2

As for the evaluation of the human risk assessment, the consumption of one liver (average weight 200 g) containing 20-30 I-TEq /g_{ww} corresponds to 4000-6000 pg TEq. The ADI value of 70 pg/die for a body weight of 70 kg, is largely exceeded consuming only one liver, indicating clearly that small animal consumption should be ruled by the public authority.

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