Bull. Environm. Contam. Toxicol. 24,634-639 (1980)

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin Levels in Cow's Milk from the Contaminated Area of Seveso, Italy

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Several reports have been published on the accident that occurred near Seveso, Italy, on July 10, 1976, when a chemical cloud containing the extremely toxic compound 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) escaped from ICMESA, a plant owned by La Roche, Switzerland (SEVESO 1976, HAY 1976 , 1977, GARATTINI 1977, ADAMOLI et al. 1978, POCCHIARI 1978, BONACCORSI et al. 1978, ABBRUZZI et al. 1978, REGIONE LOMBARDIA 1979). This paper reports on TCDD levels in cow's milk collected after the accident in the Seveso area.

Cattle breeding (349 head) in this area before the accident consisted mainly of small farms each owning 5 to 10 dairy cattle, which gave about 10 liters of milk each daily, for domestic or local consumption. Cows were fed on fodder (hay, grass, cut up corn), generally harvested around the farm; a limited amount of commercial concentrated feed was also used.

About two weeks after the explosion, consumption of food and animals from polluted zones (called A, B and R, A being the most and R the least contaminated) was prohibited to avoid the risk of people ingesting TCDD from these sources. Cows from zone A were transferred to a special cowshed under sanitary surveillance, while cattle breeding in zones B and R continued under the Regional Veterinary Service control, the cows being fed safe fodder from distant areas. During 1977 and 1978 all cows living in zones B and R were slaughtered.

Milk was collected by the Regional Veterinary Service from cows bred in areas surrounding the chemical plant; usually samples consisted of the pooled milk of the individual cows on each farm. A first set of samples was collected shortly after the accident (July 27 - August 28, 1976) in the contaminated zones and in surrounding areas. From March 1978 on, an intensive programme was started for routinely monitoring TCDD in milk coming from farms outside zone R, where there were, and still are, no restrictions on animal breeding and milk consumption.

EXPERIMENTAL

Milk samples were collected in glass bottles, and kept frozen or at 4°C until analyzed. Samples of 75-100 mL were

0007-4861/80/0024-0634 $01.20

dried using an Edwards Mini-Fast mod.470 lyophilizer. Lyophilization was made from a sample temperature of -30°C to room temperature without external heating, under a pressure of about 100 mtorr for 100 h.

The residue was hydrolyzed at 90°C for 4 h with 20 mL of KOH 10 N and 20 mL of ethanol in a reflux condenser. After cooling, the samples were extracted twice with 25 mL of n-hexane. The combined organic phases were washed by percolation through an Extrelut column (70-230 mesh, Merck, Germany), on which 20 mL of H_2O had been adsorbed. The eluate was then evaporated to dryness under an air stream at 25°C. The residue was chromatographed on a Florisil column (75 mm x 15 mm), with a top layer (5 mm) of Na_2SO_4, prewashed with 30 mL of n-hexane and activated at 170°C for 15 h.

The sample was dissolved in 8 mL of n-hexane and transferred to the top of the column. The column was eluted with 30 mL of n-hexane followed by 20 mL of dichloromethane ; this fraction was collected and evaporated to dryness. The residue was dissolved in 3 mL of n-hexane and transferred onto a column (45 mm x 5 mm) of neutral alumina (70-230 mesh, grade I, Merck, Germany) with a top layer of 5 mm of Na_2SO_4, prewashed with 3 mL of dichloromethane, and activated at 400°C for 4 h. The column was eluted with 6 mL of carbon tetrachloride and then with 4 mL of dichloromethane ; this fraction was evaporated to dryness. The sample was dissolved in 100 µL of diethylene dioxide and 5 µL aliquots were analyzed by gas chromatography-mass fragmentography (GC-MF).

An LKB 2091-051 gas chromatograph-mass spectrometer was used in the electron impact mode; the instrument was equipped with an LKB 2130 computer system for data acquisition and calculation, tuned to monitor the molecular ions of natural TCDD (m/e 320-322) and the molecular ion of ^{37}Cl-TCDD (m/e 328) used as internal standard. The gas chromatographic column was 3% OV 1 on 100-200 mesh Gas Chrom Q packed in a glass column (2 m x 2 mm i.d.).

GC-MF operating conditions were: oven temperature, 250°C; flash heater, 280°C; separator, 250°C; ion source, 250°C; helium flow, 25 mL min^{-1}; electron energy, 70 eV; trap current, 100 µA; measuring time, 50 msec ion^{-1}; source slit, 0.16 mm; collector slit, 0.25 mm; resolution 400.

Calibration curves were periodically drawn after injecting known amounts of TCDD; a linear response was obtained in the range from 50 to 500 pg injected. The criteria for identification of TCDD were the retention time of the peaks registered m/e 320 and 322 (about 2.5 min) and the isotopic ratio between these ions.

Quantitative determination of TCDD was made by peak height comparison between samples and known amounts of standard TCDD.

Recovery was routinely determined for individual samples by adding known amounts (10-20 ng) of ^{37}Cl-TCDD to each sample before lyophilization. The limit of sensitivity was also calculated for each sample on the basis of recovery and instrumental sensitivity (generally 50 pg), which was determined as the minimum amount coinjected with the sample capable of giving a signal-to-noise ratio better than 3 on m/e 322. The detection limit, calculated on 180 negative samples, averaged 32±19 (S.D.) ng/L of milk and ranged from 10 to 120 ng/L. This variability is due to recovery and background noise variation in the samples.

Control milk used for this study was obtained from the Central Dairy, Milan, Italy.

TCDD standards and contaminated samples were handled with extreme caution to prevent exposure of laboratory personnel.

RESULTS AND DISCUSSION

Table 1 shows the data obtained analyzing the first lot of samples collected between July and August 1976.

TABLE 1

TCDD levels in milk samples collected in July-August 1976

Map number	Date of collection	ng TCDD/L milk
1	28.7.76	76
2	28.7.76	7900
	2.8.76	5100
	10.8.76	2500
3	28.7.76	470
	2.8.76	1600
	10.8.76	500
4	10.8.76	1000
5	29.7.76	120
6	29.7.76	59
7	3.8.76	80
8	3.8.76	94
9	27.7.76	180
	3.8.76	75
10	5.8.76	nd

nd < 40

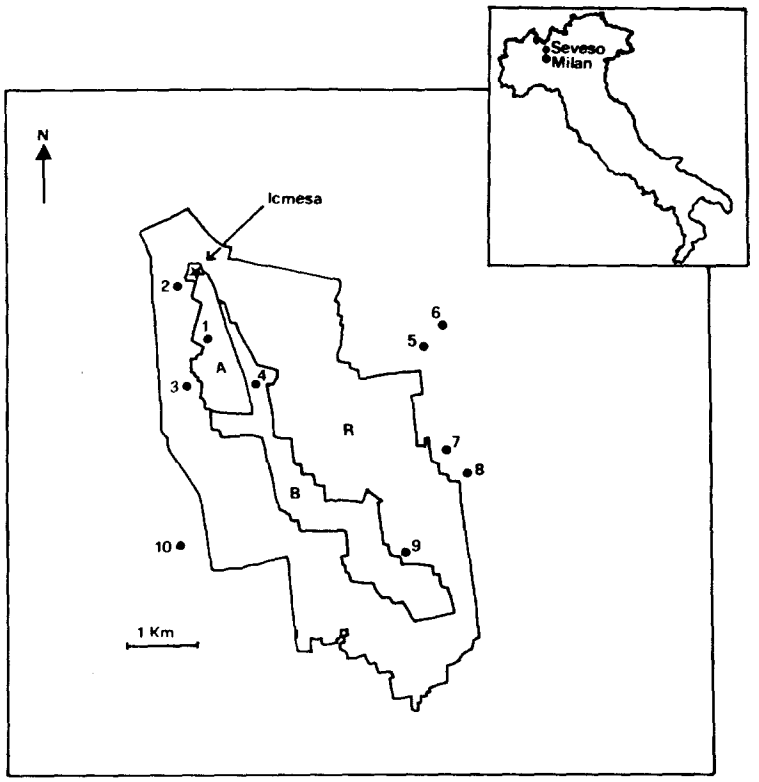

Figure 1: Farms where cow's milk samples were collected for TCDD analysis in 1976 (July-August)

Figure 1 shows the sites where these samples were collected. The highest TCDD levels were found in samples collected from farms close to the chemical plant. When more than one sample was collected at different times from the same farm, TCDD levels seem to decrease with time. Positive samples were also found outside the boundary of zone R.

The very high levels found (up to 7 ppb) show that human exposure to TCDD must have occurred after the accident through the consumption of dairy products. In fact, several days passed before the real nature of the contamination was revealed to local health authorities, so that sanitary counter-measures and restrictions on food consumption could be imposed.

During the intensive monitoring programme that started in March, 1978, more than two hundred samples were analyzed in farms outside the boundary of zone R. No TCDD was detected in these samples, with the exception of those collected from one

farm (six cows), whose location is shown in figure 2. It was found that part of the fodder given to these cows had been harvested in zone R. The first pooled sample (April, 1978) showed 18 ng TCDD/L of milk; subsequent samples (May, 1978) taken from individual cows showed levels ranging between 20 and 32 ng/L. These cows were thereafter fed fodder purchased in uncontaminated areas, and after a few months TCDD became undetectable.

Data from the monitoring programme, which is still in progress, suggest that so far TCDD is not accumulating to measurable levels in cow's milk outside the contaminated zones.

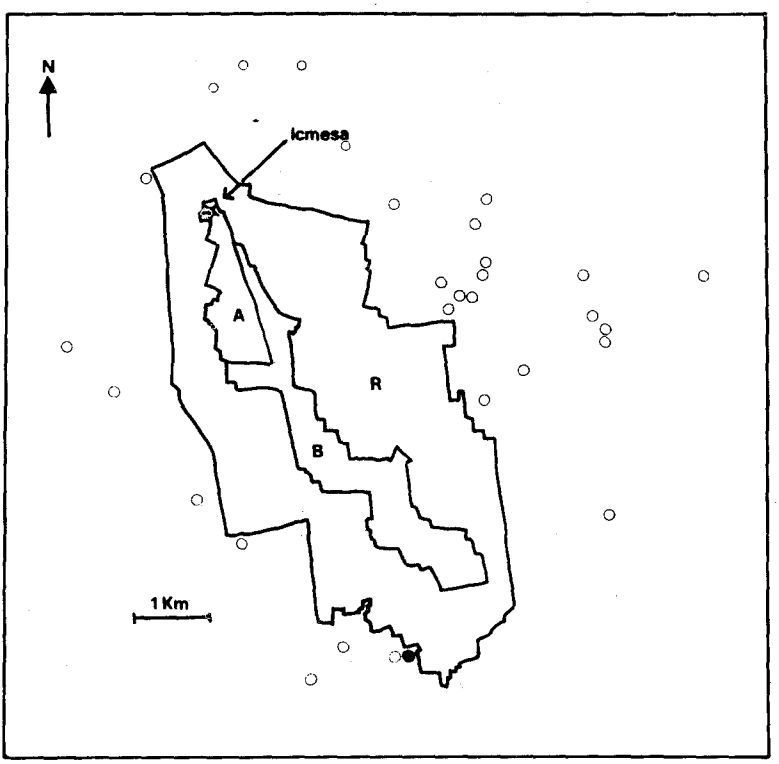

Figure 2: Farms where cow's milk samples were collected for TCDD analysis in 1978-1979:
- o farms where negative samples were found
- • farms where positive samples were found

ACKNOWLEDGEMENTS

The Regione Lombardia, Ufficio Speciale di Seveso, is gratefully acknowledged for providing financial support for this project.

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