Journal of Chromatography, 643 (1993) 91–106
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2805

Review

Study of polychlorinated dibenzodioxins and furans from municipal waste incinerator emissions in the Netherlands: analytical methods and levels in the environment and human food chain

A. P. J. M. de Jong*, A. K. D. Liem and R. Hoogerbrugge

Laboratory of Organic-Analytical Chemistry, National Institute of Public Health and Environmental Protection, P.O. Box 1, 3720 BA Bilthoven (Netherlands)

ABSTRACT

An overview is given of the methods that have been used in the study of polychlorodibenzo-*p*-dioxins and polychlorodibenzofurans in agriculture and the human food chain in a national survey and monitoring programme, including sampling stategies, sampling in the field and clean-up and analysis in various biological and environmental samples by high-resolution gas chromatography–high resolution mass spectrometry. The quality of data was evaluated as a result of internal quality control protocols and participation in interlaboratory comparison studies. Statistical analysis techniques and modelling were applied in order to compare and relate congener profiles in various matrices and to evaluate levels found in field studies for their use for regulatory purposes.

CONTENTS

* Corresponding author.

0021-9673/93/$24.00

1. INTRODUCTION

Until recently, relatively little was known about the role that disposal waste combustion plays in the contamination of the animal and human food chain by polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs). In 1977, Olie *et al.* [1] identified for the first time these compounds in emissions from municipal incinerators. Soon after this initial report, the presence of PCDD/Fs was demonstrated in flue gas and fly ash of municipal solid waste incinerators (MWIs) in Europe, North America and Japan [2–6], followed by numerous studies on their formation mechanism [7,8] and the relevant parameters in the combustion process. More recent studies have attempted to quantify the emission rates in relation to amounts and composition of the incinerated waste [9]. It was only recently that airborne PCDD/Fs were identified in the food chain of farm animals. In 1987, Rappe *et al.* [10] reported increased levels in milk of cows grazing in the vicinity of incinerators. Two years later, Olie [11] reported elevated levels of PCDD/Fs in cows' milk in the vicinity of a large capacity municipal and hazardous waste incinerator facility in Netherlands. This finding led to a great public health concern about the possible significant contamination of animal feeds in several parts of the country and human foods such as milk, meat and vegetables. These preliminary findings were interpreted that local consumption of such products could easily increase the daily exposure of humans to PCDD/Fs above the tolerable daily intake (TDI). Consequently, PCDD/F levels in cows' milk for human consumption were restricted to a limit of 6 pg of 2,3,7,8-tetrachloro-CDD equivalents (TEQ) per gram of milk fat. This value was based on previous estimates in Germany of human exposure to PCDD/Fs by consumption of foods [12,13], the average milk consumption and a TDI of 240 pg of TEQ per person per day. Regulatory measures near incinerators included either the banning from the market of cows' milk containing PCDD/F levels above the 6-pg limit or the closure of the neighbouring source. In this framework, a national investigation and monitoring programme was initiated, aimed at the identification and quantification of sources, the environmental distribution, the pathway of airborne PCDD/Fs in the farm animal food chain and the exposure of the general Dutch population by consumption of food.

In this paper, attention will be primarily focused on the analytical aspects of PCDD/F in various matrices and the quality of analytical data. In addition, results from our studies on the environmental occurrence of dioxins are discussed.

2. EXPERIMENTAL

2.1. Sampling strategy

Representative sampling is of major importance for obtaining good analytical data in accordance with the objectives of the study or the processes under investigation. Analysis in this study included (1) measurements in emissions from incinerators (conducted by TNO, Delft and Apeldoorn, Netherlands), (2) monitoring and regulatory analysis of cows' milk, (3) study of levels in soil and (4) survey of food stuffs for PCDDs and PCDFs.

2.1.1. Stack gas

Sampling and analysis were carried out by TNO Institute of Environmental Sciences, Delft and Apeldoorn, Netherlands. Stack gas sampling was performed using a dilution-type sampler (Ströhlein) during three consecutive days at each facility. Representativeness of samples was checked by a comparison of other parameters of the combustion process such as the fed load and composition, CO, SO_2, NO_x, HCl, HF, dust concentration and E-filter temperature during the sampling period compared with normal levels at that particular facility.

2.1.2. Cows' milk

Several factors such as emission rates and weather conditions can affect levels in milk [14]. As these factors may vary substantially in field measurements, time compositional samples were obtained at individual dairy farms by mixing subsamples of 25 ml, collected at 2–3-day intervals, over a period of 1 month. These subsamples were taken from the milk containers just before these storage containers were emptied. Criteria for the selection of dairy farms included: (1) a significant part of their pastures should be located inside the expected deposition area (5 × 5 km north-east of the source); (2) their winter forage must be harvested in this region; and (3) supplementary feeding may not be excessive.

2.1.3. Soil

The purpose of soil analysis in this work was twofold: (1) determination of levels in the top layer in order to assess exposure of cows by ingestion of soil and (2) determination of the remaining previous accumulation of PCDD/Fs in soil due to former depositions as a measure for previous emissions. Criteria for the selection of fields were (1) their present and former use (pasture, agricultural), (2) applied mechanical treatment in the past (*e.g.*, tillage) and (3) application of sewage sludge or other products including fertilizers.

At each area, 40 samples were taken diagonally to the depth of interest (0–2, 2–5, 5–10, 10–50 and 50–100 cm below the surface), and cores were combined for each layer separately. Additional parameters determined in soil samples were: water content (at 40 and 105°C), glow losses (550°C) and the total organic carbon content.

2.1.4. Food stuffs

A wide range of lipid-rich food products were investigated in the national food survey [15]. The foods selected for investigation were fats and oils (from the food industry), cows' milk (bottled and cartoned), animal fat, butter and cheese, meat products, nuts, eggs and fish. This selection was based on the relative contribution of each category to the total fat intake by the Dutch population. Data were obtained from a database from the Dutch Food Consumption Study, performed in 1987–88. Samples of (refined) fats and oils were obtained from the food industry and included both vegetable and fish oils. Other samples were randomly collected from food stores and slaughter houses in four different regions in the Netherlands.

2.2. Analysis

Analysis of abiotic and biotic samples followed a similar procedure, including spiking of samples with a mixture of sixteen carbon-13 labelled analogues ($^{13}C_{12}$-labelled standards from Cambridge Isotope Laboratories, Woburn, MA, USA), followed by sample digestion (optional), sample extraction, clean-up and analysis by gas chromatography–mass spectrometry.

2.2.1. Sample pretreatment and extraction

Particulate samples such as fly ash, flue gas and soil were treated with concentrated hydrochloric acid (9%, v/v) to improve the permeability of the surface for extraction. Next, distilled water was added and the resulting solids were extracted for 20 h with toluene in a Soxhlet apparatus.

Fats and oils were dissolved in dichloromethane. Milk samples were mixed with methanol and sodium oxalate prior to extraction with diethyl ether and light petroleum (b.p. 40–60°C). Meat samples were pretreated with anhydrous sodium sulphate (1:10, w/w) and after homogenization refluxed for 16 h with dichloromethane. Adipose fat was heated in an oven and the resulting fat was dissolved in dichloromethane. Butter was first heated in an oven to remove water, dissolved in dichloromethane and dried over anhydrous sodium sulphate. Samples of cheese and nuts were extracted with hexane in a Waring blender and dried over sodium sulphate. Homogenized (boiled) egg and fish samples were

freeze-dried and refluxed for 16 h with dichloromethane.

2.2.2. Clean-up

Clean-up methods consisted of consecutive column chromatographic separations on active carbon (Carbosphere activated carbon, 80–100 mesh, surface area 1000 m^2/g, from Chrompack, Middelburg, Netherlands) and alumina (basic, activity super I, from ICN Biomedicals, Eschwege, Germany), initially developed for the determination of PCDDs and PCDFs in milk samples [16]. The method is a modified version of the procedure according to Smith *et al.* [17] and makes use of glass columns filled with active carbon, placed inside a conventional-type reflux unit. This combination allows the rapid and efficient purification of sample extracts with small amounts (30–40 ml) of either dichloromethane or toluene to separate the PCDD/Fs from residual fat and non-planar interferences and to recover the PCDD/Fs by back-elution with toluene. The resulting extracts were then transferred on to a basic alumina column for the separation of PCDD/Fs from residual amounts of other planar compounds. For soil samples, an additional clean-up on multi-layer silica was needed prior to the carbon step [18]. This multi-layer column contained silica impregnated with H_2SO_4, silica impregnated with NaOH and silica impregnated with $AgNO_3$. *n*-Hexane was used as the eluent. Precleaned extracts then followed the standard procedure.

2.2.3. Gas chromatography–mass spectrometry (GC–MS)

2.2.3.1. Gas chromatography. During the programme, several types of columns and conditions were used. In general, GC separations were carried out on an apolar fused-silica capillary column for analysis biotic samples and on a polar column for the analysis of abiotic samples (environmental, fly ash) and aquatic samples.

The usual gas chromatographic conditions were as follows. Non-apolar columns were 50–60 m × 0.25 mm I.D. with a 0.20-μm film thickness, either CP-Sil 5 (Chrompack), HP-Ultra 2 (Hewlett-Packard, Palo Alto, CA, USA), or DB-5 (J&W Scientific, Rancho Cordova, CA, USA). The temperature programme was initially 70°C for 2 min, increased at 25°C/min to 200°C, then at 3°C/min to 300°C and held isothermally for 10 min at 300°C. Polar columns were (A) 50 m × 0.25 mm I.D. with a 0.20-μm film thickness of CP-Sil 88 (Chrompack), the temperature programme being initially temperature 70°C for 2 min, increased at 25°C/min to 200°C, then at 3°C/min to 240°C and held isothermally for 40 min at 240°C and (B) 30 m × 0.25 mm I.D. with a 0.15-μm film thickness of Rtx-2330 (Restek, Bellefonte, PA, USA), the temperature programme being initially temperature 70°C for 2 min, increased at 25°C/min to 200°C, then at 3°C/min to 275°C, and held isothermally for 1 min at 275°C.

In all instances helium was used as the carrier gas at a linear velocity of 30 cm/s. Samples were injected either (initially) by the use of a solid all-glass falling needle injector (Koppen, Best, Netherlands) or using an autosampler (Hewlett-Packard HP 7673A). In the latter instance the injector glass liner (275°C) was filled with deactivated and precleaned glass-wool to prevent severe discrimination. Using polar columns, a piece of non-polar column was connected to the front and back-end of the column for the connection with the injector (275°C) and the mass spectrometer source. The GC–MS interface was maintained at 275°C in all instances.

2.2.3.2. Mass spectrometry. Analyses were carried out on VG 70SQ and VG AutoSpec mass spectrometers (Fisons Instruments, Manchester, UK). Ionization of samples was performed in the electron impact (EI) mode with 30–70-eV electrons. The instruments were operated at increased resolution. The resolving power (RP) was usually between (static) 3000 and 5000 for biotic samples and between 5000 and 10 000 for environmental samples.

Detection was performed by simultaneous recording of the two most abundant ions of the chlorine isotope cluster of molecular ions of analytes, the syringe standard ([$^{13}C_6$]-1,2,3,4-T_4CDD) and the $^{13}C_{12}$-labelled internal standards. The total number of approximately 50 ions, including lock mass ions, were divided over five (apolar column) and six groups, respectively, with 10–15 ions per group. During the analysis, consecutive groups were selected during a certain time interval that matched the congener elution profile. Each group contained a lock mass ion for fine setting of the magnet current in accordance with pre-performed mass callibration of the electrostatic field for each group.

Typical sampling and settling times were 50 and 10 ms and the interchange times between groups were 1 s, resulting in cycle times of less than 1 s.

2.2.3.3. Quantification. Quantification of the analytes was based on the response ratio of analytes and the corresponding internal standards obtained for the unknown sample compared with the ratio for a standard mixture containing known amounts of native PCDD/Fs and the same amount of $^{13}C_{12}$-labelled standards as used in samples. Congeners were identified and quantified when the following criteria were met: (1) signal to noise ratio >3; (2) the ratio between the two isotopic ions monitored should be within 15% of the theoretical value; (3) for native PCDD/Fs having an internal standard, the retention time should be within 1 s of that of the internal standard, the labelled compound eluting earlier; otherwise, the relative retention time must be within 0.1% as determined for standards, and (4) recovery of internal standards used may not exceed 120%.

Other perfomance checks that are regularly included (1) procedural (before a series of samples) and instrument blank (before and within series), (2) check of isomer specificity of GC separation, (3) sensitivity check of MS using the syringe standard and (4) check of MS resolution under dynamic conditions (RP 10 000 only).

3. RESULTS AND DISCUSSION

3.1. Sample preparation

So far, more than 1000 samples have been successfully processed with the Carbosphere methodology, previously described in detail [16]. Most samples were of animal origin (milk, adipose tissue), but when applied to abiotic samples similarly good results were obtained. Carbosphere combines a high efficiency for the separation of planar from non-planar compounds and possesses an extremely low or no affinity for lipids, which allows the processing of large amounts of extracted fat. Moreover, the use of conventional glassware and heating baths to reflux the carbon columns at elevated temperature allows the use of relatively small solvents volumes, which reduces unwanted side-effects of possible impurities in the solvent used. PCDD/Fs are quantitatively recovered from the carbon column by back-refluxing, which typically requires 16 h and 30–40 ml of toluene. In addition, the use of parallel units for each sample reduces the risk of cross contamination, which often occurs with single-loop equipped automated LC or gel permeation chromatographic systems.

3.2. GC–MS

3.2.1. Gas chromatography

High-resolution GC is required for the separation of the large number (210) of compounds of the PCDD/F family. The tetra- to octa-substituted congeners are the most interesting group and include 49 PCDDs and 85 PCDFs. Seventeen of them have the 2,3,7,8-substitution configuration (seven and ten PCDDs and PCDFs, respectively) and are considered to be the most toxic. Generally, analysis is concerned with congener group separation or isomer-specific determination of the toxic congeners, or both. For general survey of sources and environmental distribution, the analysis of total congener groups may be appropriate, whereas risk assessment and toxicological applications need congener-specific determination of the 2,3,7,8-substituted congeners. Total toxicity levels in samples expressed in 2,3,7,8-TCDD equivalents are obtained from the sum of indiviual congeners multiplied by their toxicity factors using the international model for toxicity of PCDD/Fs (i-TEF values) [19].

Non-polar columns (CP-Sil 5, DB-5, HP-Ultra 2 and equivalents) can separate chlorine homologous groups and all toxic congeners from each other but not from all non-toxic congeners. In contrast, polar columns (DB-DIOX, CP-Sil 88, SP 2331, Silar 10C and equivalents) can almost uniquely resolve the toxic congeners in the presence of all other congeners. Incomplete separation is achieved, however, for 2,3,7,8-T_4CDF, 1,2,3,7,8-P_5CDF and 1,2,3,4,7,8-H_6CDF. A not well understood phenomenon that occurs with the use of polar columns is the partial or complete dechlorination of octa-substituted congeners, particularly for OCDF. This occurred both after heated splitless injections and after cold on column injection. In contrast, these compounds travel through the column unaffected when injected with an all-glass solid injector (so-called falling needle injector). The phenomenon of dechlorination is difficult to understand. It can be rationalized that

the process must take takes place inside the stationary phase at the very beginning of the column owing to presence of hot vapours from the injection solvent or possibly by traces of moisture introduced during the injection. This is because dechlorination does not take place either with solvent injections on apolar stationary phases or with solid injections on-column with a non-polar stationary phase. Apolar columns are frequently used in analyses of biological samples from higher terrestrial vertebrates such as farm animals and man. These organisms normally have the ability to metabolize and subsequently excrete the non-2,3,7,8-substituted isomers effectively, which results in a selective accumululation of the planar, toxic isomers. Advantages of the use of apolar columns are that they require shorter analysis times, have longer lifetimes and no decomposition occurs for highly chlorinated PCDD/Fs. Polar columns are required for 2,3,7,8-isomer-specific analysis in samples that may contain non-laterally substituted isomers, such as in environmental samples, fly ash, ambient air and aquatic organisms, and in PCDD/F-contaminated products of different origins.

Recently, we obtained good results with the use of an Rtx 2330 cross-bound polar (90% biscyanopripyl–10% phenylcyanopropyl) stationary phase capillary column. Rapid analyses are facilitated by elevated temperature programming (up to 275°C) with satisfactory isomer-specificity. Fig. 1 shows an example of an isomer specific analysis of a stack gas sample. Concerning dechlorination, Fig. 1 shows that octa-CDD/F (peaks 7 and 17, respectively) travellled through this polar column unaffected, also when injected in the splitless mode, so as far as this problem is concerned there is no need for reanalysis on a second column.

3.2.2. *Mass spectrometry*

High-sensitivity EI sources developed recently for magnetic sector instruments permit improved sensitivity down to the low femtogram range on col-

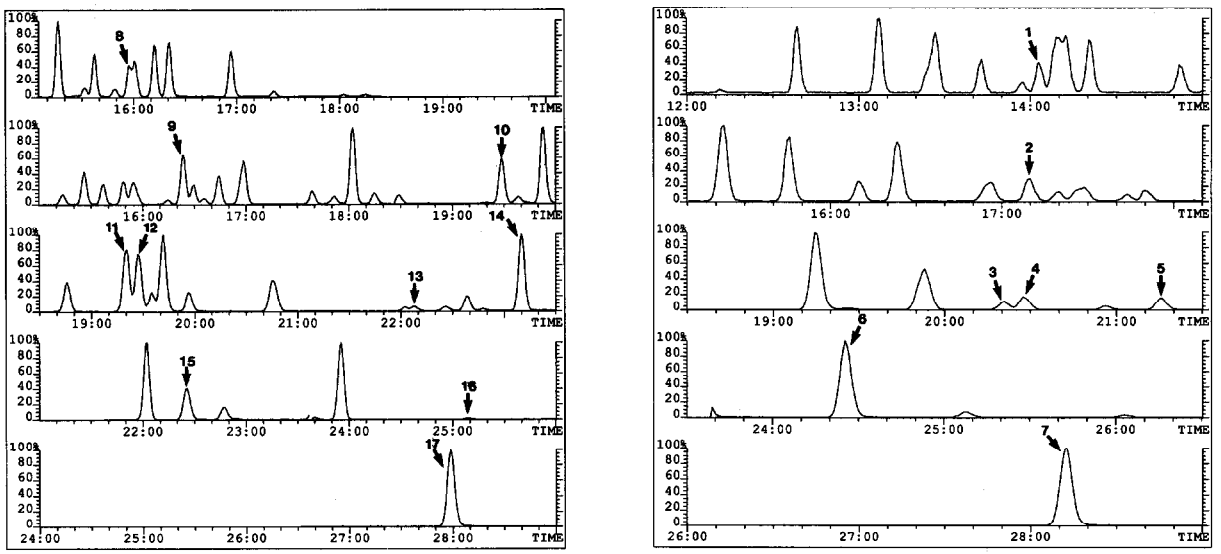

Fig. 1. GC–HRMS analysis of PCDDs (top) and PCDFs (bottom) in a municipal waste incinerator emission sample. Traces are multi-group selected-ion recordings, normalized in each group, of one ion of the molecular mass chlorine isotope cluster of each congener. GC separation was accomplished on a 30-m Rtx-2330 column. Monitoring time windows were selected so that all 2,3,7,8-substituted congeners were measured. Time windows do not match the entire congener group elution region. 1 = 2,3,7,8-T_4CDD; 2 = 1,2,3,7,8-P_5CDD; 3 = 1,2,3,4,7,8-H_6CDD; 4 = 1,2,3,6,7,8-H_6CDD; 5 = 1,2,3,7,8,9-H_6CDD; 6 = 1,2,3,4,6,7,8-H_7CDD; 7 = octa-CDD; 8 = 2,3,7,8-T_4CDF; 9 = 1,2,3,7,8-P_5CDF; 10 = 2,3,4,7,8-P_5CDF; 11 = 1,2,3,4,7,8-H_6CDF; 12 = 1,2,3,6,7,8-H_6CDF; 13 = 1,2,3,7,8,9-H_6CDF; 14 = 2,3,4,7,8,9-H_6CDF; 15 = 1,2,3,4,6,7,8-H_7CDF; 16 = 1,2,3,4,7,8,9-H_7CDF; 17 = octa-CDF. Analysis was performed at a mass resolution of 10 000:1. The calculated TCDD toxic equivalent level in the sample was *ca.* 0.5 ng TEQ/m^3 ind (in normal state dry) (sample size *ca.* 4 m^3 ind). Time in min.

umn with the mass spectrometer operated at increased resolution [high-resolution selected-ion monitoring (HRSIM)]. High-resolution MS (HRMS) [20] and tandem MS (MS–MS) techniques have been shown to be superior to low-resolution MS (LRMS) in trace analysis [21–23]. Although the sensitivity in HRMS and MS–MS are typically a few percent of that of LRMS analysis, the reduced noise levels compensate for this lower sensitivity and superior determination levels may be obtained. MS–MS analysis is considered to be particularly meaningful when using low resolution in the first mass spectrometer or in combination with reduced clean-up. The required resolution in single HRMS depends mainly on the nature and amounts of co-extractants that have passed the clean-up procedure [22]. Moderate resolution in combination with effective clean-up methods will usually be appropriate for the analysis of relatively clean samples such as cows' milk. This is demonstrated in Fig. 2, which compares low (RP 900) and elevated resolution (RP 3000) analyses of TCDD at a level of 0.3 pg/g of milk fat in a cows' milk sample.

3.2.3. *Quality of data*

For generating good analytical data, samples and analysis should meet the following requirements: (1) representative sampling; (2) high sensitivity, selectivity and specificity; and (3) reliable quantification, good reproducibilty and identity confirmation [24]. Sampling strategies in the different studies in this work are described in brief in Section 2.1.

The recovery and accuracy of the method were tested in spiking experiments and in round-robin studies. Determination of compounds added to milk yielded recoveries between 92 and 112% for individual congeners, corresponding to 101% on TEQ basis. In a recent round robin study [25], unknown spikes were determined at *ca.* 80% of the values added, on average. The reliability of determi-

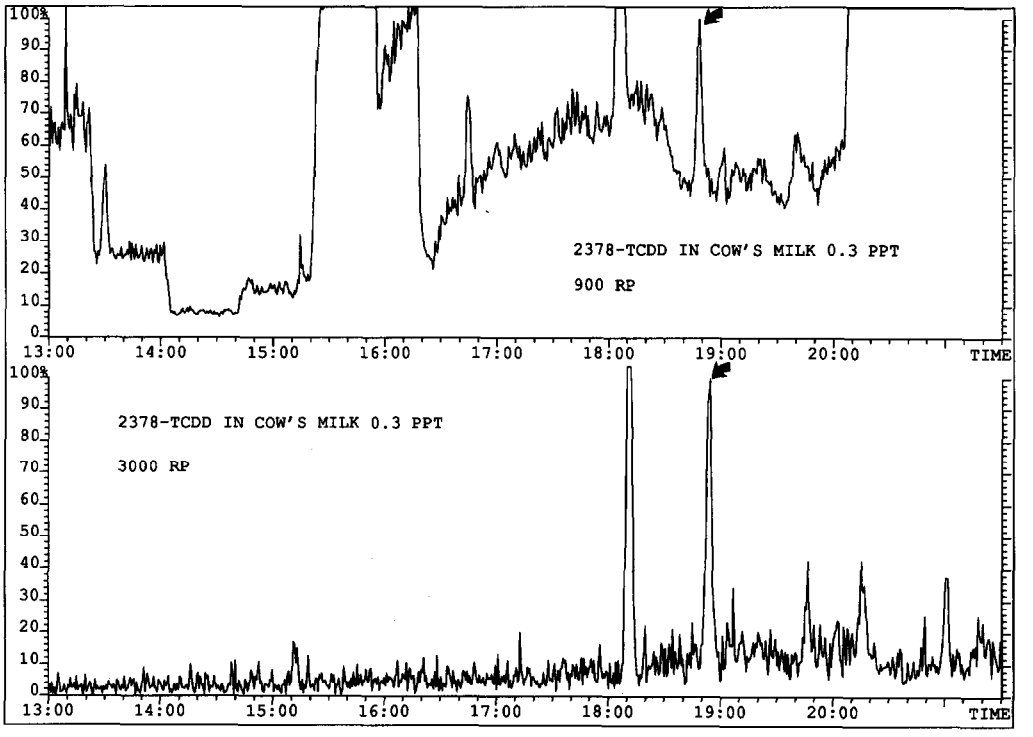

Fig. 2. Comparison of PCDD/F analyses in cows' milk at 900 (top) and 3000 (bottom) resolving power. Shown are the ion trace at m/z 319.8965 of 2,3,7,8-TCDD (arrow) with a level of *ca.* 0.3 pg/g milk fat, corresponding to an amount injected into the GC column of *ca.* 60 fg.

nation was improved by the use of sixteen carbon-13-labelled internal standards (one for each 2,3,7,8-substituted PCDD/F, except OCDF) instead of one in each congener group. By this method temporal sensitivity changes within such groups will be compensated for adequately.

Parallel analysis of control samples is used to verify the between-series reproducibility and the quality of quantitative results. Results of frequent analysis of such a quality control (QC) sample demonstrate a long-term reproducibility of about 7% [relative standard deviation (R.S.D.)] at a level of 3.0 pg TEQ/g milk fat (n = 35; not shown). Additional validation of the method and quality of information was obtained from participation in round-robin studies, such as that recently organised by the WHO/EURO for human milk and blood, cows' milk and fish, the Bureau Communautaire de Référence (BCR) in 1992 for cows' milk and by our laboratory also for cows' milk [26]. The last study showed that analytical results for laboratories having wide experience in PCDD/F analysis were comparable to within 10% on a TEQ basis at levels between 3 and 10 pg TEQ/g milk fat. Within-laboratory reproducibilities on a TEQ basis in this study ranged between 2 and 17%. Limits of determination for individual congeners in cows' milk were on average between 0.1 and 0.5 pg/g milk fat. The determination limit of the TEQ value is dependent on the congener distribution in samples owing to the different TEF values. For example, extreme values for determination limits will correspond to 0.1 and 0.0001 pg TEQ/g milk fat when the only toxic congener present is 2,3,7,8-T_4CDD (TEF = 1) and OCDD (TEF = 0.001), respectively. For normal congener distributions TEQ determination limits in cows' milk were estimated to be *ca.* 0.6 pg TEQ/g milk fat [26]. Normal congener distrubutions in cows' milk in terms of congener TEQ values consist of three major congeners (average% contribution to total TEQ): 2,3,4,7,8-P_5CDF (40 ± 10%), 1,2,3,4,7,8-P_5CDD (25 ± 5%) and 2,3,7,8-T_4CDD (10 ± 5%). The contribution to the total TEQ value of five of the seven hexa-CDD/Fs ranges between 0 and 10% with a sum of about 25 ± 5%. 1,2,3,7,8,9-H_6CDD and 1,2,3,7,8,9-H_6CDF are mostly undetectable and hepta- and octa-DD/Fs are toxicologically minor congeners.

3.3. Results from field measurements

3.3.1. PCDD/F emissions

It has been estimated that the current solid municipal solid waste production amounts over 30 mil-

TABLE 1

SUMMARY OF EMISSION DATA AND CORRESPONDING LEVELS IN COWS' MILK AND SOIL IN THE VICINITY OF THE MAJOR MUNICIPAL WASTE INCINERATORS AND A METAL RECLAMATION PLANT IDENTIFIED IN THE NETHERLANDS

Figures concern the situation in 1989–90.

Source	Capacity ($\cdot 10^6$ kg/year)	PCDD/F			
		Stack gas (ng TEQ/m^3 ind)	Estimated emission (g TEQ/year)	Cows' milk (range, pg TEQ/g fat)	Soil (0–2 cm) (ng TEQ/kg dry matter)
MWI-A	970	53	250	2.8–12.2	18–55
MWI-B	135	240	178	3.1–13.5	13–252
MWI-C	75	100	38	1.0–2.4	3–23[a]
MWI-D	115	31	21	1.6–8.1	NA[b]
MWI-E	75	4	2	3.3–10.0	NA
MWI-F	510	5	14	1.6–3.3	NA
Other MWIs	35–385	8.3–92	1.4–107	NA	NA
MRP	ND[b]	ND	ND	4.0–8.6	NA

[a] 0–5 cm.

[b] ND = Not determined; NA = not analysed.

TABLE 2

RESULTS OF INTERLABORATORY COMPARISON OF ANALYSIS OF PCDD/F COMPOUNDS IN MUNICIPAL INCINERATOR EMISSIONS

The relative composition denotes the mean relative contribution of individual congeners to the total TEQ level per m^3 ind in fly ash, calculated from results for all samples analysed by both laboratories. Crosses in the systematic difference columns indicate which of the congeners are included for a given uncetainity interval for TEQ values. The table also indicates which and the fraction of TEQ of the total TEQ. For example, the systematic difference between the two laboratories was less than 10% for six congeners, which account for 61.3% of the total TEQ, and so on.

Compound	Relative composition (% of TEQ)	Relative standard deviation (%)	Ratio of means, Lab. 1/Lab. 2	Systematic differences between Lab. 1 and Lab. 2 within given ranges (%) of the highest							
				10	20	30	40	50	60	70	80
Dioxins											
2,3,7,8-	2	34	1.09	×	×	×	×	×	×	×	×
1,2,3,7,8-	16.1	22	0.97	×	×	×	×	×	×	×	×
1,2,3,4,7,8-	4.7	40	1.70					×	×	×	×
1,2,3,6,7,8-	4.1	33	1.21		×	×	×	×	×	×	×
1,2,3,7,8,9-	3.5	24	0.99	×	×	×	×	×	×	×	×
1,2,3,4,6,7,8-	2.4	18	1.00	×	×	×	×	×	×	×	×
Octa-	0.3	64	0.85			×	×	×	×	×	×
Furans											
2,3,7,8-	1.3	64	2.79							×	×
1,2,3,7,8-	1.6	46	0.64				×	×	×	×	×
2,3,4,7,8-	29.4	12	0.97		×	×	×	×	×	×	×
1,2,3,4,7,8-	8.4	47	0.71			×	×	×	×	×	×
1,2,3,6,7,8-	7.9	34	1.02		×	×	×	×	×	×	×
1,2,3,7,8,9-	1.3	89	3.82								×
2,3,4,6,7,8-	12.9	45	0.74			×	×	×	×	×	×
1,2,3,4,6,7,8-	3.6	30	1.28			×	×	×	×	×	×
1,2,3,4,7,8,9-	0.3	59	0.82		×	×	×	×	×	×	×
Octa-	0	77	0.63				×	×	×	×	×
TEQ	100	15	0.99	×	×	×	×	×	×	×	×
Number of congeners within the range				6	8	12	14	15	15	16	17
Percentage of TEQ covered				61.3	65.8	91	92.6	97.4	97.4	98.7	100

lion tons ($30 \cdot 10^9$ kg) each year in the Netherlands [27], of which about $3 \cdot 10^9$ kg were combusted (1989) in twelve facilities. Most of these facilities stem from the 1970s and some of them did not comply with the Dutch guidelines (1985) for combustion. Recently, a few facilities have been upgraded for reduced emissions including PCDD/Fs. The capacity of the incinerators varies between $33 \cdot 10^6$ and $970 \cdot 10^6$ kg of waste per year (Table 1). PCDD/F stack gas concentrations ranged between 4 and 240 ng TEQ/m^3 ind (in normal state dry). Anual PCDD/F emissions by individual stationary sources ranged between 2 and 250 g TEQ/year, resulting in an estimated total of about 800 g TEQ/year. In general, the lowest emission rates were found with the modern incinerators. The high range value was found in an old-fashioned incinerator having an improperly functioning electrostatic dust filter (E-filter) at the time of sampling. Extrapolation of emission data to an annual basis must be treated with caution. It has been demonstrated that PCDD/F emissions may vary considerably and in an unpredictable manner [28]. At one facility emission rates differed by a factor of about 15 from one day to another.

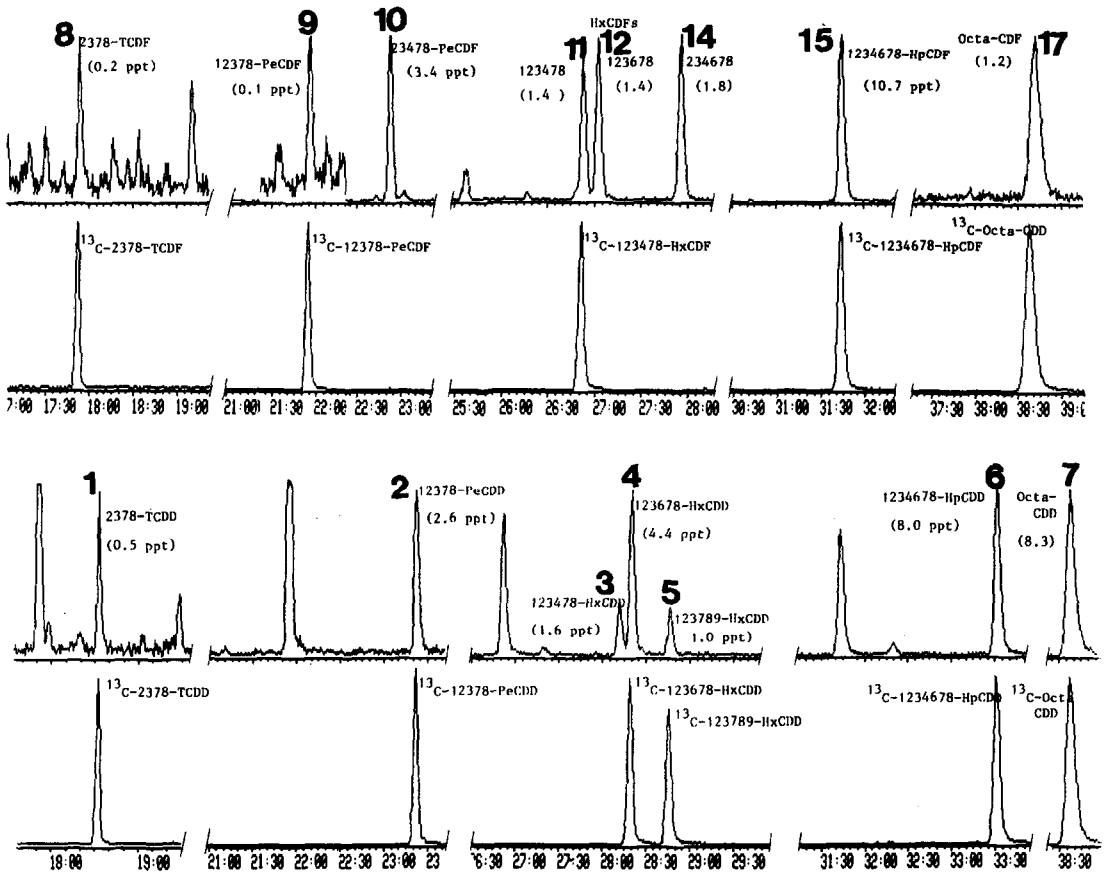

Fig. 3. Example of GC–MS analysis of 2,3,7,8-substituted PCDFs (top) and PCDDs (bottom) in cows' milk. Traces were obtained by multi-group (five) selected-ion recordings of the most abundant ions of the molecular chlorine cluster of native PCDD/Fs (top) and carbon-13-labelled internal standards (bottom). GC separation was carried out on a 50-m non-polar column (HP-Ultra 2). Peaks and levels (in parentheses in pg/g milk fat) of individual congeners were identified and detemined as follows: 1 = 2,3,7,8-T_4CDD (0.5); 2 = 1,2,3,7,8-P_5CDD (2.6); 3 = 1,2,3,4,7,8-H_6CDD (1.6); 4 = 1,2,3,6,7,8-H_6CDD (24.4); 5 = 1,2,3,7,8,9-H_6CDD (1.0); 6 = 1,2,3,4,6,7,8-H_7CDD (8.0); 7 = octa-CDD (8.3); 8 = 2,3,7,8-T_4CDF (0.2); 9 = 1,2,3,7,8-P_5CDF (0.1); 10 = 2,3,4,7,8-P_5CDF (3.4); 11 = 1,2,3,4,7,8-H_6CDF (1.4); 12 = 1,2,3,6,7,8-H_6CDF (1.4); 14 = 2,3,4,7,8,9-H_6CDF (1.8); 15 = 1,2,3,4,6,7,8-H_7CDF (10.7); 17 = octa-CDF (1.2).

In the course of the survey programme, an interlaboratory comparison was performed, involving clean-up and analysis of ten stack gas samples. The results are summarized in Table 2. It can be seen that systematic differences were within 20–30% for most of the individual congeners, resulting in an overall standard deviation of 15% on a TEQ basis. Large differences were found for 2,3,7,8-T_4CDF and 1,2,3,7,8,9-H_6CDF. For the latter, differences between the two laboratories could be ascribed to an insufficient separation between 1,2,3,7,8,9-H_6CDF and 1,2,3,4,6,7,8-H_7CDF by one laboratory, causing interferences with the molecular ion chlorine cluster of the hexa-congener (M) by a relatively low-abundant M-HCl fragment ion cluster of the otherwise prominent hepta-congener in stack gases.

3.3.2. Regulatory analysis of farm animal samples

The main objectives of the cows' milk survey were (1) to identify areas where increased levels in milk could occur and (2) to determine the area boundaries where levels exceed the limit of 6 pg TEQ/g milk fat. Such an investigation is challenged by the availability of representative samples. As an example, levels in milk may vary considerably depending on the actual and earlier exposure of animals to PCDD/F emissions in conjunction with (1) fluctuating emissions and associated deposition rates depending on meteorological conditions and half-lives of PCDD/Fs deposited on grass, (2) the pharmokinetics of PCDD/F in the lactating cow, (3) the movement of herds across the polluted area and (4) animal feeding and management systems [29–31].

During the course of the programme, several hundred biological samples were analysed for PCDD/Fs, including cows' milk and sheep milk, meat and other tissue samples from cows, sheep and horses. Fig. 3 shows a typical example of the determination of PCDDs and PCDFs in a cows' milk sample containing fifteen detectable (> 0.1 pg/g fat) 2,3,7,8-substituted congeners. The TEQ level was determined as 4.9 ± 0.3 pg/g milk fat. In most milk samples, *i.e.*, in background and in MWI exposed cows, 2,3,4,7,8-P_5CDF, 1,2,3,7,8-P_5CDD and 2,3,7,8 T_4CDD were the most prominent compounds when expressed in toxicity terms. These isomers represent typically 40 ± 10, 25 ± 5 and 10 ± 5%, respectively, of the total TEQ level in normal milk. The chromatogram shows that a near-baseline separation on a non-polar column was obtained for all toxic congeners.

Results from analyses of cows' milk and soil are summarized in Table 1. In general, the levels in cows' milk correlate fairly well with estimated emission rates from the nearby source. Interesting data were obtained after the closure of a facility. The TEQ levels in cows' milk declined with a half-life of about 40–50 days, which is comparable to the half-

TABLE 3

COMPARISON PF PCDD/F DETERMINATION IN SOIL IN A ROW OF 0–8 km IN THE PREVALENT DIRECTION OF MUNICIPAL WASTE INCINERATOR EMISSIONS AND DEPOSITIONS WITH PREDICTED VALUES USING MODEL CALCULATIONS FOR PARTICLE DEPOSITIONS FROM A STATIONARY SOURCE

Levels expressed in ng TEQ/dm^3 were measured in the top layer of pastures the surfaces of which have not been mechanically treated for several years.

Location	Distance to source (km)	PCDD/F (ng TEQ/dm^3)		
		Measured	Predicted	Predicted/measured
1	0.9	27.1	20.9	0.77
2	2.4	22.1	13.7	0.62
3	2.7	16.8	12.1	0.72
4	3	15.1	11.0	0.73
5	4.6	9.2	7.7	0.84
6	5.7	6.7	6.9	1.03
7	8.1	3.2	4.8	1.51

life found from the study with lactating cows orally administered carbon-13 labelled PCDD/Fs. Near-steady-state levels after 6 months differed slightly for the various dairy farms, all being higher than the background level of 0.8–2.5 pg TEQ/g milk fat. Reasons for these differences may be the different initial values and probably also because of the variations in PCDD/F levels in soil (see below). Other interesting data were obtained from analyses of cheese from earlier years (Table 3). Although the cheese samples have an incident character (produced from milk of one or two days) and therefore are not necessarily representative, then analyses may be a valuable means for obtaining information on contaminations in earlier years.

Fig. 4 shows two year time courses of PCDD/F levels in milk from dairy farms in the deposition area of an MWI with high PCDD/F emissions (MWI-A in Table 1). It is seen that the levels fluctuate considerably. Apparently, the levels tend to increase in the autumn. The reason for this is difficult to give, but it might be that during this season the lower growth dilution rates [14] and higher wet deposition rates lead to higher concentrations on grass. In contrast, weathering processes such as increased leaf wash-off by increased wet precipitation would counteract such increased grass contamination.

3.3.2.1. Pattern comparison and modelling. Principal component analysis (PCA) [32,33] was found to be a very useful technique for the recognition of related samples from their PCDD/F patterns. The data set used contained several hundred cows' milk samples ranging from background locations and dairy farms in the neighbourhood of MWIs and a metal reclamation plant. Briefly, data treatment consisted of scaling to the most abundant congener in individual samples followed by the determination of the principal components of the entire set. In the present application, the first two principal components (PC-1 and PC-2) represented 43.7 and 17.0% of the total variance in the dataset, respectively. Fig. 5 shows the projection of the sample pattern on PC-1 and PC-2. Clustering of samples having similar patterns using 95% confidence intervals yielded a distinct separation between cows' milk samples from different locations. From this it may be con-

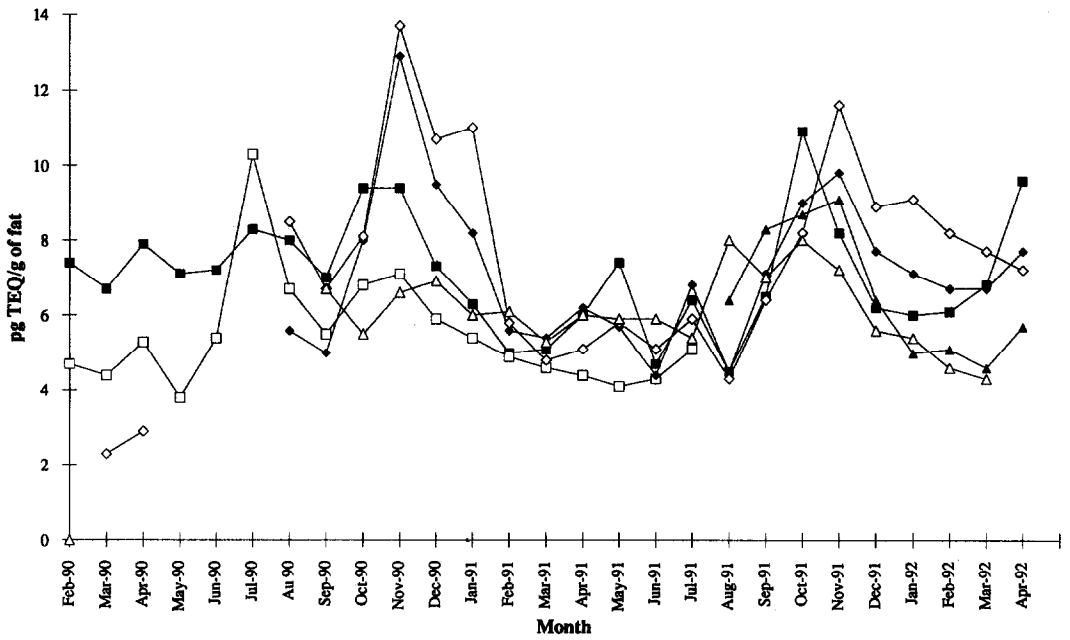

Fig. 4. Time courses for a period of 2 years for measured dioxin levels (in pg TEQ/g milk fat) in cows' milk from dairy farms in the vicinity of a municipal waste incinerator (MWI-A in Table 1).

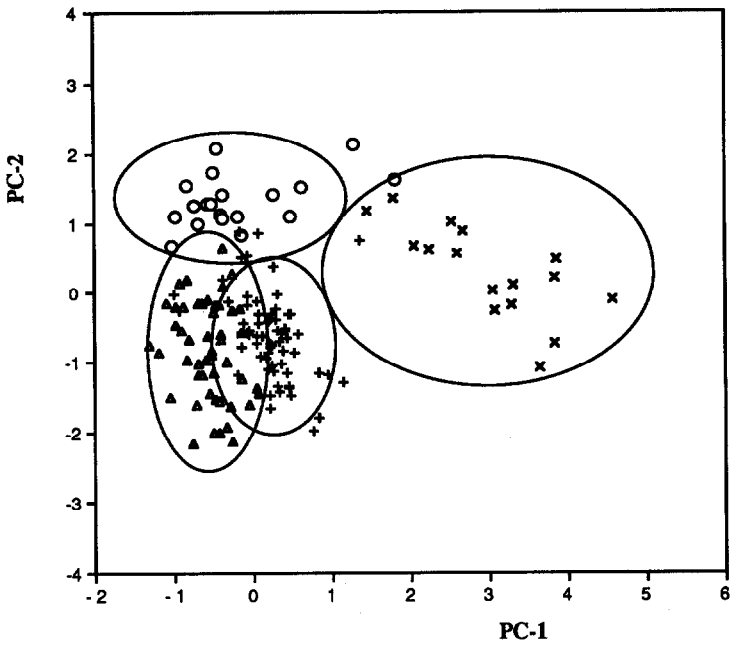

Fig. 5. Result of principal component analysis (PCA) of PCDD/F pattern in cows' milk in the vicinity of municipal waste incinerators (MWI) and a metal reclamation plant (MRP). Shown are the sample projections on the first and second principal components (PC-1 and PC-2). Ellipses are the 95% confidence contours for identified sample subgroupings. From Liem *et al.* [33]. + = MWI-A; △ = MWI-B; ○ = MWI-C; × = MRP.

cluded that the patterns are consistently related to the exposure pattern. A most striking example was the difference between cows exposed to MWI and metal reclamation emissions; the latter were found to contain relatively more dibenzofurans [34]. Analysis of the principle components showed that PC-1 was related to the relative fractions of PCDDs and PCDFs in samples and PC-2 to the chlorination level of both PCDDs and PCDFs, respectively.

With this large number of milk data we have developed a model to predict TEQ levels in milk near stationary sources [35]. The main parameters in the model are the source characteristics and emissions, weather conditions and the geographical location of pastures relative to the source.

The model uses the assumption of a constant background deposition level (A) and a variable influence by the source, the weather and other variables:

$$\mathrm{TEQ}(r,\phi)_{ij} = A + B_i \frac{f(\phi - \phi_j)r}{(r + 3R)^4}$$

where R = distance between maximum deposition and source, r = estimated distance between the (centre of) pasture(s) and the source, ϕ = direction of the centre of pastures of a dairy farm relative to the source and ϕ_j = direction of maximum deposition in period j. The variable f describes the average weather conditions in a period of 1 month, *i.e.*, the direction of deposition . The distribution of the dependent deposition is given by

$f(\phi - \phi_j) = c + cos^2(\phi - \phi_j)$ when $\cos(\phi - \phi_j) > 0$ and $f(\phi - \phi_j) = c$ when $\cos(\phi - \phi_j) < 0$

The term B_i in the model represents the source parameters, such as the emission rate, stack height and other parameters for a particular source relative to the so-called standard source.

Parameters in the model were estimated by minimizing the sum of differences between the calculated and measured TEQ values in a training set (109 samples with TEQ levels between 0.7 and 13.5 pg TEQ/g milk fat). The following values were obtained:

Background TEQ level in milk:
$A = 1.6 \pm 0.3$ pg/TEQ g milk fat
Distance of maximum deposition:
$R = 1.6 \pm 0.2$ and 0.3 ± 0.1 km for MWIs and RMPs, respectively
Angular parameter:
$c = 0.6 \pm 0.2$

These parameter values will probably be closely related to the local conditions and may not be valid for other countries with different weather profiles, landscapes, etc. Fig. 6 shows the predicted distribution of TEQ levels in milk in the vicinity of MWI-B (Table 1). The model was found to be useful for (i) the determination of area boundaries in which TEQ levels could exceed certain limit values and (ii) for the selection of sampling sites.

The model has also been used to estimate of the uncertainty interval for the TEQ value in milk samples. Variations depending on different variables in the contamination pathway were estimated to be of the order of 1.3 pg/g milk fat. This means that the representativeness of milk samples for a certain area may be approximated within this range. Combination with the analytical uncertainty of 10% (result of an intercomparison study) yields an overall uncertainty interval of *ca.* 1.5 pg TEQ/g at a level of 6 pg TEQ/g milk fat.

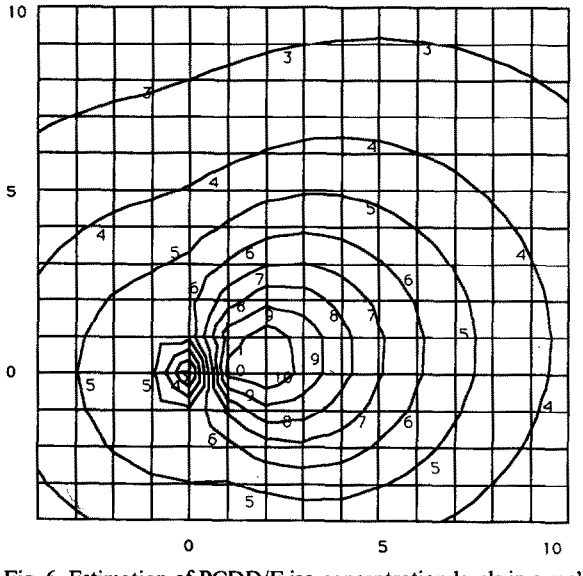

Fig. 6. Estimation of PCDD/F iso-concentration levels in cows' milk (in pg TEQ/g of milk fat) in the vicinity of MWI-B for october 1989 according to an emprical model (see text). Distances in km. The source is situated in the origin (0,0). Reprinted from Hoogerbrugge *et al.* [35].

3.3.3. Environmental analysis

Environmental analysis in this study was applied mainly to soil samples. The origin of PCDD/Fs in soil may vary greatly. They may originate from the former use of herbicides containing PCDD/Fs, spills of pentachlorophenols, airborne depositions and others. Soil analysis was primarily directed to determinations in top layer levels, as these PCDD/Fs may cause an additional exposure of cows by soil ingestion. Deposition-related steady-state levels in top soil layers will be dependent on the dilution caused by mixing to various depths and the persistence of PCDD/Fs in soil. Leaching and volatilization are not considered to be important factors affecting the movement and dissipation of PCDD/Fs in soil [36]. A second objective of soil analysis was to use the accumulated PCDD/F mass in soils as an indicative measure of former emissions. Data were used together with estimates of depositions and deposition profiles near sources in attempts to differentiate between the relative importance of wet and dry depositions near stationary sources [37].

Table 3 shows results of PCDD/F determinations in soil in a row between 0 and 8 km from an MSW and one compared with calculated values. The differences between the measured and predicted values may be due to lower background depositions than expected (assumption: 4 ng/m^2 · year) and a possible underestimation of wet deposition rates in the model used [37]. The congener patterns in the top layers were consistent with that found in fly-ash. In the lower layers (< 10 cm) the hepta- and octa-PCDDs were the most prominent congeners, a pattern similar to that for pentachlorophenol.

3.3.4. Dietary intake of PCDDs and PCDFs

In order to assess the dietary intake of PCDDs and PCDFs by the Dutch population, 63 different food products were analysed [15]. A detailed description of the study design, including sampling strategy, analytical methods, levels and the statistical model used to calculate dietary intakes, will be published elsewhere. The results are summarised in Table 4, expressing the relative contributions of dif-

TABLE 4

RELATIVE CONTRIBUTION OF PCDD/F IN FOODS TO THE AVERAGE DAILY INTAKE OF TOXIC EQUIVALENTS (TEQ) IN THE NETHERLANDS

Category	Food	Contribution to daily TEQ intake (%)
Dairy foods	Milk	23.3
	Cheese	14.5
	Butter	7.1
Meat	Beef	7.1
	Horse and lamb	0.5
	Pork	5.8
Poultry	Eggs	4.5
	Meat	2.0
Micellaneous	Sliced cold meat	9.0
	Oils and nuts	1.1
	Industrial fats	21.8
Fish	Fish	3.4

ferent food categories to the total dietary intake of PCDDs and PCDFs by the average Dutch person during a lifetime of 70 years. It can be seen that the consumption of milk, butter, cheese and associated bovine fats accounts for almost half of the total exposure by foods. Another 20% of the total dietary intake appeared to be associated with the consumption of fats in food products from the food industry (so called hidden fat). A median daily intake by food was calculated on 35–70 pg TEQ for children below 20 years and on 70 pg TEQ for adults (20–70 years). These intake figures compare well with previous reports from the UK [38], Germany [12,13] and Canada [39], in which intakes ranged between 92 and 203 pg TEQ per person per day. This may again illustrate the diffuse global distribution of persistent compounds such as PCDDs and PCDFs responsible for general contamination of the human food chain.

4. CONCLUSIONS

Chromatographic separation and mass spectrometric detection techniques currently used in analyses for PCDD/Fs are capable of the determination of ultra-trace levels in different environmental and biological samples. Difficulties still exist with the unique determination of some 2,3,7,8-substituted congeners in complex samples, which may lead to overestimation to some extent of reported TEQ values in risk assesment studies, particularly for environmental and fly-ash samples. Problems with the determination of OCDF and OCDD on polar stationary phases due to dechlorination were solved by the use of a new type slightly modified polar stationary phase, which avoids the need for costly and laborious re-analysis of environmental samples on a non-polar column. Mass spectrometric resolutions of 3000–5000 were found to be adequate for analyses of most biological samples. For environmental samples slightly higher resolutions of 5000–10 000 were sometimes found to be beneficial, depending on the nature and concentration of co-extractants.

Extensive studies of emissions of municipal and some other combustion processes have yielded a much better insight into the behaviour and distribution in the environment of PCDD/Fs and the subsequent contamination of farm animals and the human food chain.

5. ACKNOWLEDGEMENTS

We thank our co-workers, A. C. den Boer, G. R. Groenemeijer, R. S. den Hartog, W. C. Hijman, S. H. M. A. Linders and J. A. Marsman, for their excellent and skilful work on the analysis of large numbers of samples. We also thank K. Olie of the University of Amsterdam, J. de Koning, J. P. Boers and E. de Leer of TNO, J. A. van Zorge of the Directorate General for Environmental Protection and colleagues H. J. G. M. Derks, J. A. van Jaarsveld, P. R. Kootstra, M. Olling, A. A. Sein, W. Slob, R. M. C. Theelen, E. G. van der Velde and many others who have contributed to this work.

REFERENCES

1 K. Olie, P. L. Vermeulen and O. Hutzinger, *Chemosphere*, 6 (1977) 455.
2 H.-R. Buser, H. P. Bosshard and C. Rappe, *Chemosphere*, 7 (1978) 165.
3 G. A. Eiceman, R. E. Clement and F. W. Karasek, *Anal. Chem.*, 51 (1979) 2343.
4 A. Cavallano, L. Luciani, G. Ceroni. I. Rocchi, G. Ivernizzi and A. Gorni, *Chemosphere*, 11 (1982) 859.
5 C. Chiu, R. S. Thomas, J. Lockwood, K. Li and R. C. C. Rao, *Chemosphere*, 12 (1983) 607.

6 T. O. Tiernan, M. L. Taylor, J. H. Garrnett, G. F. van Ness, J. G. Solch, D. A. Dies and D. J. Wagel, *Chemosphere*, 12 (1983) 595.
7 L. Stieglitz, G. Zwick, J. Beck, W. Roth and H. Vogg, *Chemosphere*, 18 (1989) 1219.
8 F. W. Karasek and L. C. Dickson, *Science*, 237 (1987) 754.
9 J. Theisen, W. Funcke, E. Balfanz and J. König, *Chemosphere*, 19 (1989) 423.
10 C. Rappe, M. Nygren, G. Lindström, H.-R. Buser, O. Blaser and C. Wüthrich, *Environ. Sci. Technol.*, 21 (1987) 964.
11 K. Olie, personal communication.
12 H. Beck, K. Eckart, W. Mathar and R. Wittkowski, *Chemosphere*, 18 (1989) 417.
13 P. Fürst, C. Fürst and W. Goebel, *Chemosphere*, 20 (1990) 787.
14 G. F. Fries and D. J. Paustenbach, *J. Toxicol. Environ. Health*, 29 (1990) 1.
15 A. K. D. Liem, A. P. J. M. de Jong, R. M. C. Theelen, J. H. van Wijnen, P. C. Beijen, H. A. van der Schee, H. A. M. G. Vaessen, G. Kleter and J. A. van Zorge, *11th International Symposium on Chlorinated Dioxins and Related Compounds, DIOXIN'91, Research Triangle Park, North Carolina, USA, September 23–27, 1991, Book of Abstracts*, p. 365.
16 A. K. D. Liem, A. P. J. M. de Jong, J. A. Marsman, A. C. den Boer, G. S. Groenemeijer, R. S. den Hartog, G. A. L. de Korte, R. Hoogerbrugge, P. R. Kootstra and H. A. van 't Klooster, *Chemosphere*, 20 (1990) 843.
17 L. M. Smith, D. L. Stalling and J. C. Johnson, *Anal. Chem.*, 56 (1984) 1830.
18 R. C. C. Wegman, J. Freudenthal, G. A. L. de Korte, G. S. Groenemeijer and J. Japenga, *Chemosphere*, 15 (1986) 1107.
19 J. A. van Zorge, J. H. van Wijnen, R. M. C. Theelen, K. Olie and M. van de Berg, *Chemosphere*, 19 (1989) 1881.
20 R. E. Clement, B. Bobbie and V. Taguchi, *Chemosphere*, 15 (1986) 1147.
21 Y. Tondeur, W. N. Niederhut, J. E. Campana and S. R. Missler, *Biomed. Mass Spectrom.*, 14 (1987) 449.
22 A. P. J. M. de Jong, A. K. D. Liem, A. C. den Boer, E. v.d. Heeft, J. A. Marsman, G. van de Werken and R. C. C. Wegman, *Chemosphere*, 19 (1989) 59.
23 C. M. Meyer, P. W. O'Keefe, R. G. Briggs and D. R. Hieker, *Biomed. Environm. Mass Spectrom.*, 13 (1986) 47.
24 C. Rappe, *Chemosphere*, 18 (1989) 17.
25 E. J. Yrjänheikki (Editor), *Levels of PCBs, PCDDs and PCDFs in Human Milk and Blood. Second Round of Quality Control Studies (Environment and Health in Europe*, 37), FADL, Copenhagen, 1991.
26 A. P. J. M. de Jong, A. Dross, P. Fürst, G. Lindström, P. Päpke and J. R. Startin, *Fresenius' J. Anal. Chem.*, 345 (1993) 72.
27 H. J. Bremmer, *Report no. 790501014*, National Institute of Public Health and Environmental Protection, Bilthoven, 1991.
28 S. Marklund, *Thesis*, University of Umeå, Umeå, 1990.
29 M. S. Mclachlan, H. Thoma, M. Reissinger and O. Hutzinger, *Chemosphere*, 20 (1990) 1013.
30 D. Firestone, M. Clower, Jr., A. P. Borsetti, R. H. Teske and P. E. Long, *J. Agric. Food. Chem.*, 27 (1979) 1171.
31 M. Olling, H. J. G. M. Derks, P. L. M. Berende, A. K. D Liem and A. P. J. M. de Jong, *Chemosphere*, 23 (1991) 1377.
32 D. C. Massart, G. B. M. Vandeginste, S. N. Deming, Y. Michotte and C. Kaufman, *Chemometrics: a Textbook*, Elsevier, Amsterdam, 1988.
33 A. K. D. Liem, R. Hoogerbrugge, P. R. Kootstra, E. G. van der Velde and A. P. J. M. de Jong, *Chemosphere*, 23 (1991) 1675.
34 W. Christmann, D. Kasiske, K. D. Klöppel, H. Partscht and W. Rotard, *Chemosphere*, 19 (1989) 387.
35 R. Hoogerbrugge, A. P. J. M. de Jong, A. K. D. Liem, P. R. Kootstra and H. A. van 't Klooster, in P. Henschel (Editor), *Water Pollution Research Report 26, Commission of the European Communities*, Guyot, Brussels, 1990, p. 90.
36 D. R. Jackson, M. H. Grota, S. W. Rust, J. S. Warmer, M. F. Arthur and F. L. Deroos, *Report No. 600/9-85-013, US Environmental Protection Agency*, Washington, DC, 1985.
37 J. A. van Jaarsveld and M. A. A. Schutter, in *12th International Symposium on Dioxins and Related Compounds, Dioxin 92, University of Tampere, Tampere, Finland, 24–28 August, 1992 (Organohalogen Compounds*, Vol. 8), Finnish Institute of Occupational Health, Helsinki, 1992, p. 299.
38 Ministry of Agriculture, Fisheries and Food, *Dioxins in Food. The Thirty-First Report of the Steering Group on Chemical Aspects of Food Surveillance, (Food Surveillance Paper*, No. 31), HM Stationary Office, 1992.
39 B. Birmingham, B. Thorpe, R. Frank, R. Clement, H. Tosine, G. Fleming, J. Ashman, J. Wheeler, B. D. Ripley and J. J. Ryan, *Chemosphere*, 19 (1989) 507.