Isomer-specific determination of PCDDs and PCDFs in Flemish cow's milk.

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ABSTRACT

An analytical method has been optimized and validated for isomer-specific determination of polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) in milk. It has subsequently been applied to investigate the occurrence of the pollutants in cow's milk obtained at some typical locations in Flanders, including potential dioxin source areas. Generally, the dioxin concentration levels in Flemish cow's milk appear to be of the same magnitude as in surrounding countries, and do not point out unacceptable health risks to the average consumer.

OBJECTIVES

The research program of which the present study is part primarily aims at the acquisition of know-how and the creation of facilities in Flanders in the field of state-of-the-art dioxin analysis. A further objective is the application of the analytical methodology, well-validated through participation in interlaboratory comparisons, to elucidate occurrence and fate of the contaminants in Flanders in order to enable evaluation of the impact of the pollution levels on man and his environment. For this purpose a small-sized screening program has been set up, thus far addressing mainly sampling and analysis of ambient air[1], soil[2], traffic[3] and waste incinerator emissions. We have included cow's milk as a priority matrix, in view of the absence in Belgium of a laboratory capable of performing a reliable PCDD/PCDF determination in milk, the concerns that rose a few years ago with respect to export of Belgian commercial milk[4], and the important share of milk and its products in the global daily intake of dioxins by man.

DESCRIPTION OF THE ANALYTICAL METHOD

Following the approach adopted internationally, the analytical method focuses on isomer-specific determination of the 'dirty seventeen' PCDDs or PCDFs, i.e., tetra- to octachlorinated congeners that are chloro-substituted on at least the 2-, 3-, 7- and 8- position, allowing calculation of a TEQ-value (toxic equivalent concentration relative to 2,3,7,8-T_4CDD) for each sample using the I-TEF scheme[5].

Briefly, the milk sample (200 g) is spiked with a mixture of 10 or, preferably, 17 ^{13}C-labeled isomers, corresponding to the analytes, either prior to extraction or prior to clean-up, depending on whether the dioxin content has to be determined on a product or fat basis. The fat fraction is isolated by repeated liquid-liquid extraction with diethyl ether and petroleum ether after addition of sodium oxalate and methanol. After evaporation of the combined extracts under a nitrogen stream, the remaining fat is weighed. As an alternative extraction method, a semi-quantitative column extraction has demonstrated its suitability in case of PCDD/PCDF determinations on a product basis. For that purpose milk, partially freeze-dried, is mixed with sodium sulfate and silicagel to result in a dry, free-flowing column packing, from which the fat fraction is eluted subsequently using 2/1 n-hexane/acetone.

To separate PCDDs and PCDFs from fat, the extract is purified by gel permeation chromato-

graphy using a Bio-beads S-X3 column and 1/1 cyclohexane/ethyl acetate as eluting solvent (3 ml/min). The raw fat, dissolved in this solvent, is brought fractionwise (3-4 g fat per run) onto the column via a loop. During the first 90 min of each run the eluate is discarded; the PCDD/PCDF containing fraction is collected for the next 60 min. The combined PCDD/PCDF fractions are reduced under a nitrogen stream to a volume of ca. 2 ml.

The extract is further purified by high pressure liquid chromatography using a Hypercarb S porous graphitized carbon column. Applying 1/1 cyclohexane/dichloromethane as first solvent, the sample, diluted with 5 ml of solvent, is loaded and then a 30 min prefraction is discarded. The PCDDs and PCDFs are subsequently eluted in the backflush mode using toluene during 90 min, after which the eluate is again concentrated under a nitrogen stream to ca. 1 ml.

For the final clean-up step, the sample is transferred onto a column containing 2.5 g of alumina B Super I and a top layer of 2 g of sodium sulfate, using benzene. Prefractions consisting of (i) 15 ml benzene and (ii) 20 ml of 98/2 n-hexane/dichloromethane are discarded. The PCDD/PCDF containing fraction is collected afterwards using 30 ml of 1/1 n-hexane/dichloromethane. After concentration under a nitrogen stream and a solvent exchange for n-nonane, a recovery standard (^{13}C- 1,2,3,4-T_4CDD) is added; the final volume amounts to 25-40 µl.

The gas chromatographic separation is carried out on a HP 5890 Series II GC equipped with a HP 7673 auto-injector and a split/splitless injector operated in the splitless mode (275 °C, 1-2 µl, split time 1 min). A 60 m x 0.25 mm x 0.25 µm DB-5ms fused silica capillary column is mounted, connected to the mass spectrometer via a direct interface (280 °C). Helium is used as carrier gas (inlet pressure 200 kPa). The initial temperature of the oven, 180 °C, is raised after 1 min with a rate of 30 °C/min to 220 °C, and then with a rate of 2 °C/min to the final temperature of 320 °C, which is held constant for a further 2 min. High resolution mass spectrometric detection takes place on a VG Autospec Q instrument by selected ion recording, in which five groups of 10 ions each (1 group per chlorination degree) are consecutively activated. For correction of magnetic drift, 'lock' masses of perfluorokerosine are measured. Electron impact ionization is employed (30 eV, source at 250 °C). The resolution is tuned daily to a value of 10000 at 10% valley depth. Identification and quantification criteria generally meet the requirements stipulated by the US-EPA (in, e.g., Method 8290).

VALIDATION OF THE ANALYTICAL METHOD

As final part of the validation study, the laboratory participated in an international interlaboratory comparison organized by the Community Bureau of Reference (BCR) of the CEC. This implied the analysis of five replicates from each of a naturally contaminated freeze-dried milk (ca. 3 pg/g fat, as TEQ), a moderately enriched one (ca. 9 pg/g fat, as TEQ), a highly enriched one (ca. 13 pg/g fat, as TEQ) and a standard solution to trace calibration errors. For the latter, which contained the 'dirty dozen' (tetra- to hexachlorinated) congeners at a level between 0.4 and 24 µg/l and in similar proportions as in natural milk, an accuracy of 93-107% vs. the target values could be demonstrated for all congeners measured.

From the analysis of the enriched milk samples, in which the typical congener profile of milk was preserved, the accuracy of the complete analytical procedure could be estimated. For the three main congeners in milk - being 2,3,4,7,8-P_5CDF, 1,2,3,7,8-P_5CDD and 2,3,7,8-T_4CDD -, which together represent about 75% of the total TEQ value, the accuracy of the procedure, averaged over both spiking levels, amounted to 88-97% vs. the target values. On a TEQ basis, the accuracy averaged 91.4% vs. the target value and 102.9% vs. the calculated value using the interlaboratory means from the laboratories selected after technical evaluation. For the naturally contaminated freeze-dried milk, our optimized procedure led to a TEQ-value of 0.92 ± 0.05 pg/g milk powder, compared to an interlaboratory mean of means of 0.81 ± 0.13 pg/g milk powder.

The coefficient of variation of the complete procedure amounted to 5.5% on a TEQ-basis at the background level and down to 4.3% on a TEQ-basis for enriched milk. For individual congeners (at a concentration level of at least 2 pg/g fat) the average coefficient of variation was 11.9%. For most of the hepta- and octachlorinated PCDDs and PCDFs, a nonnegligible procedure

blank was measured. This tends to be a common problem to many laboratories. Our current research aims at tracing and reducing these blank values, as well as at further improving the recovery of the internal standards (40-80%) and the detection limit, estimated to be about 0.6 pg/g fat, as TEQ, using a 1 µl injection out of a 40 µl final extract.

PCDDs AND PCDFs IN FLEMISH COW'S MILK

In collaboration with the National Dairy Office, cow's milk was sampled at seven, geographically spread, locations in Flanders, Belgium, being:

- Mol: a rural location, further characterized by a coal-fired power station and some nuclear industry at 1-4 km E from the sampling site
- Moerkerke: a rural location, the nearest known point source being a large municipal waste incinerator (MWI, 175000 ton/yr) at about 10 km W from the sampling site
- Berendrecht: a location in the harbour area near Antwerpen, with (petro)chemical industry extending 2-15 km SE to SW from the sampling site, and the city of Antwerpen at 15 km SE
- Zelzate: a location at the canal Gent-Terneuzen, with metallurgical and chemical industry and a highway at 1-15 km SW from the sampling site and the city of Gent at 20 km SW
- Ham: a location characterized by a highway and chemical industry (production of vinyl chloride, incineration of chlorinated waste) at 2-7 km SE to W from the sampling site
- Vilvoorde: a location at about 15 km NE from the city centre of Brussel, characterized in addition by a coal-fired power station at 1 km W and industry extending 1-7 km SW-W, including a MWI at about 6 km SW from the sampling site
- Menen: a location characterized by a MWI (40000 ton/yr) at 3 km SW from the sampling site; in a local milk sample collected in December 1991 and analyzed at another laboratory on request of the National Dairy Office and the Ministry of Public Health a significant dioxin contamination (27.3 pg/g fat, as TEQ) had been detected[6].

The first six of the above locations were also selected for air and soil sampling, the results of which have been reported elsewhere[1,2]. The cow's milk was collected in September 1992 by taking a single raw milk subsample from the collection tank at a local farm; all samples were kept frozen (-20 °C) until the analysis for PCDDs and PCDFs.

The results of these analyses are summarized in Table I. The dioxin content (expressed as pg/g fat, as TEQ) of the samples from Mol, Moerkerke, Berendrecht, Ham and Vilvoorde was found clearly below 6 pg/g fat (as TEQ), which is the tolerance limit referred to in The Netherlands. As could be expected from the origin of these samples, the concentrations generally appear slightly higher than typical background contamination levels in West European milk; in the Netherlands, few years ago a background level of 0.7-2.5 pg/g fat (as TEQ) was determined[7],

LOCATION	SUM 17 PCDD/F (pg/g fat)	TEQ (pg/g fat, as TEQ)
Mol	29.2	3.9
Moerkerke	20.2	2.4
Berendrecht	20.1	2.7
Zelzate	39.4	5.1
Ham	27.3	3.7
Vilvoorde	27.8	3.1
Menen	97.1	12.6

Table I: Dioxin concentration in cow's milk from some typical locations in Flanders.

whereas a recent large-scale investigation in Northrhine-Westfalia, Germany, yielded a mean background value for consumer milk of 1.4 pg/g fat (as TEQ), with a range from 0.8 to 2.6 (about 120 samples)[8]. With regard to Belgium, recently analytical data were disclosed of eight consumer milk samples, which were taken in May 1991 from tank trucks collecting and combining the milk produced in a large region and which were analyzed on request of the National Dairy Office and the Ministry of Public Health; they were found to contain 1.1-3.1 pg/g fat (as TEQ) of PCDD/-PCDF, with a mean value of 2.1 pg/g fat (as TEQ)[6].

In the milk sample from the farm in Zelzate, a dioxin concentration significantly higher than normal background levels was observed, though still not exceeding the Dutch tolerance limit. The results of top-soil analyses at the various locations selected for the screening program confirm that the Zelzate area may be somewhat more heavily polluted with dioxins than the other areas[2]. The pollution degree in industrialized areas, as apparent from the present study, agrees well with recently reported figures from Germany (1.9-6.5 pg/g fat, as TEQ, for 9 samples, with an average of 4.5)[9].

Finally the sample taken close to the municipal waste incinerator of Menen contained 12.6 pg/g fat (as TEQ), less than half the amount detected in the sample collected earlier[6]. Both in Belgium and in other countries, there is plenty of evidence that some municipal waste incinerators constitute a source of dioxin contamination for cattle grazing under the plume. Thus far the National Dairy Office and the Ministry of Public Health have had analyzed cow's milk (1 or 2 samples) from the surroundings of 12 Flemish MWIs; near 7 of them, the dioxin burden in cow's milk was found to be below 6 pg/g fat (as TEQ), whereas near 4 others, it ranged between 6 and 9 pg/g fat (as TEQ)[6]. With exception of the earliest sample from Menen, the dioxin contamination of local cow's milk near the Flemish MWIs does not differ markedly from that near several Dutch installations, which have been subjected to an extensive monitoring program since the past few years; near some of these a temporary ban on consumption of the local cow's milk was issued, and in certain cases the MWIs meanwhile have been closed down. Near the country's largest MWI in the Lickebaert area, e.g., almost monthly analyses of milk from four farms in the period 1990-1991 yielded an average PCDD/PCDF content (as TEQ) for each farm between 6.3 ± 0.9 and 7.7 ± 2.7 pg/g fat, individual values varying between 4.3 and 13.7 pg/g fat (as TEQ)[10]. The Dutch studies also revealed a rather large variability of the dioxin content of cow's milk, which should be due to differences in, e.g., emissions, weather conditions and/or animal feeding, with a tendency of more elevated concentrations in the period October - December.

In summary, the dioxin analyses on Flemish cow's milk reported here, together with those carried out earlier[4,6], in our opinion do not indicate unacceptable health risks to the average consumer. A more systematic inventoring of, and monitoring near, dioxin point sources such as the MWI in Menen nevertheless seems desirable, in order to obtain a clearer picture of the possible threat to the local public health.

REFERENCES

1 Wevers M. et al., Extended abstract submitted to Dioxin'93
2 Van Cleuvenbergen R. et al., Extended abstract submitted to Dioxin'93
3 Wevers M. et al., Extended abstract Dioxin'92 (Organohalogen Compounds vol 9), 1992: 321-324
4 de Jong A.P.J.M. et al., Report no. 730501002, RIVM Bilthoven - NL (Ed.), 1990 (in Dutch)
5 NATO-CCMS (Ed.) Report Number 176, 1988
6 Belgische Kamer van Volksvertegenwoordigers, Vragen en Antwoorden (GZ 1992-1993), 37, 1992: 2763-2764 (in Dutch and French)
7 Liem A.K.D. et al., Organohalogen Compounds vol 1, Eco-informa Bayreuth - FRG (Ed.), 1990: 567-570
8 Fürst P. et al., Chemosphere 25, 1992: 1039-1048
9 Beck H. et al., Chemosphere 21, 1990: 789-798
10 Kootstra P.R. et al., Report no. 730501045, RIVM Bilthoven - NL (Ed.), 1992 (in Dutch).