Levels of Polychlorinated Dibenzo(p)dioxins, Dibenzofurans and Dioxin-like PCBs in Milk, Milk Products and Eggs from West European Countries

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Introduction

With the Council Regulation EC/2375/2001[1] the EU Commission set maximum levels for polychlorinated Dibenzo(p) dioxins (PCDDs) and polychlorinated Dibenzofurans (PCDFs) in various foodstuffs. These maximum levels have been entered into force on July 1, 2002. In this Regulation the Commission, moreover, announced to review section 5 of Annex I for the first time until end of 2004 in particular with the a view to the inclusion of dioxin-like PCBs in the levels to be set. It is true that the Commission did not keep this but the revision is expected for 2005 instead.

While for dioxin contents in foodstuffs a substantial database has been collected[2] meanwhile, dioxin-like PCBs for the determination of a total TEQ have only been increasingly included during the last two/three years. Following the lack of legal regulations especially routine controls of foodstuffs by producers, converters and trade have often only been made for dioxins in the past. Partly only indicator PCBs have been included in the analyses in order to exclude possible PCB contaminations[3] or to check the compliance with legal regulations[4]. In recent times this has changed because of the forthcoming inclusion also of dioxin-like PCBs (dl-PCBs) into the maximum levels.

For many years the GfA has routinely performed analyses in food and feed for dioxins and dioxin-like PCBs [e.g. 5, 6, 7] on behalf of producers, converters, trade or supervising authorities from most various EU member states. This paper presents mean values and ranges of dioxin, dl-PCB and total TEQ values from 138 milk / milk product and 45 egg samples which were analysed as part of routine controls. As these samples have been taken during the last two years, they reflect quite a current contamination level.

Methods and Materials

The 138 milk and milk product samples are composed of 107 milk, 17 cheese, 11 butter and 3 yoghurt samples. Just like the 45 egg samples, these specimen were taken from mid 2003 to the beginning of 2005. The customers who ordered these examinations mainly come from West European countries. In both cases the sample collectives developed randomly but nevertheless a certain degree of representativity can be attributed to them.

The fresh samples were freeze-dried (Christ, Beta 1-8 Freeze-dryer) and further homogenised by means of grinding. A fat extraction of about 20 g of the dried and grinded sample material was done by means of Accelerated Solvent Extraction (ASE) using an ASE 300 instrument of Dionex Corp., Sunnyvale, CA, USA. The fat fraction finally was determined gravimetrically after evaporation of the solvents.

All PCDD/F and PCB analyses were performed by HRGC/HRMS. Each analysis included the determination of the seventeen PCDD/F congeners with 2,3,7,8-chlorosubstitution and the 12 dioxin-like PCB congeners for which toxic equivalency factors (TEF) were established by a working group of the WHO[8]. Prior to the fat extraction an isotope-labelled dioxin and a PCB surrogate standard were added to the sample material in order to control the extraction efficiency. For 16 native PCDD/F and each PCB congener to be quantified, the corresponding $^{13}C_{12}$-labelled compound was added to the fat extract as internal standard prior to the defatting and the subsequent chromatographic clean-up. The recoveries of the internal standards through the fat separation and all clean-up steps were determined by means of further ^{13}C-labelled internal PCDD and PCB standards added to the PCDD/F and the PCB fraction before GC/MS analysis. All the ^{13}C-labelled standards were from Cambridge Isotope Labs, Endover, USA.

A Power-Prep workstation (Fluid Management Systems, FMS) for automated clean-up was mainly used the for milk,

milk product and egg sample analyses. For some of the samples a conventional clean-up was applied. Both, the PCDD/F and PCB analyses were performed on a HP 5890 HRGC connected to a VG AutoSpec HRMS (mass resolution > 9000). A 60 m DB-5 MS capillary column was used for the gas chromatographic PCDD/F separation and a 25 m HT-5 column for the PCB analyses. The limit of quantification (LOQ) was in the range of 0.14 pg/g fat for the dioxin TEQ and of 0.07 pg/g fat for the PCB TEQ. Thus, a LOQ of around 0.2 pg/g fat resulted for the total dioxin and PCB TEQ. Expanded uncertainties were in the range of 12 % for PCDD/F TEQs and around 13% for PCB TEQs. The uncertainties were calculated on the basis of the "Guide to the expression of Uncertainty in Measurement (GUM)" and the EURACHEM/CITAC Guide "Quantifying Uncertainty in Analytical Measurement (QUAM)". The expanded uncertainties calculated were based on a coverage factor of 2 and give a level of confidence of 95 %

The analytical methodology used for the milk, milk product and egg sample analysis is in compliance with the requirements for the HRGC/HRMS confirmatory analysis of food for PCDD/Fs and PCBs as laid down by the EU Directive 2002/69. The methods are further accredited according to DIN EN ISO/IEC 17025:2000. Further details of the analytical procedures are reported in references 5 and 9.

Table 1: Mean, minimum and maximum values for dioxin, dioxin-like PCB and total TEQ values as determined in milk/milk product and egg samples from various West European countries (analysed between mid 2003 and 2005)

Food	Number of Samples	Dioxins pg WHO-TEQ/g fat			Dioxin-like PCBs pg WHO-TEQ/g fat			Total TEQ pg WHO-TEQ/g fat		
		Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Milk & Milk Products	138	0.354	0.110	1.33	0.622	0.082	2.36	0.976	0.192	2.85
Eggs	45	0.374	0.136	1.20	0.316	0.087	4.68	0.690	0.260	5.62

Table 2: Percentage of dioxin and dioxin-like PCB TEQ on total TEQ for milk/milk product and egg samples

Food	Number of Samples	Dioxin TEQ %			Dioxin-like PCB TEQ %		
		Mean	Min	Max	Mean	Min	Max
Milk & Milk Products	138	38.7	17.1	65.5	61.3	34.5	82.9
Eggs	45	60.0	16.7	85.7	40.0	14.3	83.3

Results and Discussion

Table 1 presents the mean, minimum and maximum values for the dioxin, dioxin-like PCB and total TEQs of the 138 milk and milk product samples and of the 45 egg samples. No distinction was made between eggs from caged and free range hens. All TEQs are reported as levels in fat (pg WHO-TEQ/g fat). Only upper bound values were included in calculation of the means.

The dioxin content of the milk and milk product samples were between 0.11 and 1.33 pg WHO-TEQ/g fat with a mean of 0.35 pg WHO TEQ/g fat. The mean of the dioxin-like PCB TEQs was higher, showing a value of 0.62 pg WHO-TEQ/g fat. The range of values was between 0.08 and 2.36 pg PCB WHO-TEQ /g fat. Consequently, the mean of the total WHO-TEQ showed a value of 0.98 pg WHO-TEQ/g fat. Although on average the PCB TEQ is significantly higher than the dioxin TEQ, the situation may be different for individual milk/milk product samples. As can be seen from Table 2, the proportion of the dioxin-like PCB TEQ to the total TEQ ranges from 34% to 83% which means that in some cases the dioxins show a higher contribution to the total TEQ than the dioxin-like PCBs.

The mean of the dioxin TEQs of the 45 egg samples shows a similar level as found for the milk/milk products (0.37 pg TEQ/g fat compared to 0.35 pg TEQ/g fat). However, the mean of the dioxin-like PCB TEQ of the eggs is lower (0.32 pg TEQ/g fat compared to 0.62 pg TEQ/g fat for milk/milk products). This implies that a lower mean of 0.69 pg WHO-TEQ/g fat for the total TEQ in the eggs results in comparison to the milk/milk products. The contribution of the dioxin-like PCBs to the total TEQ is 40% on average but the variation between individual samples is obvious. The

PCB contribution of the eggs ranges from 14% to 83% for the egg samples.

The EC limit value for the maximum PCDD/F level of cow's milk, milk products and eggs is 3 pg WHO-TEQ/g fat[1]. The recommended EC action level which would entail measures to identify and reduce the source of contamination when exceeded, is 2 pg WHO-TEQ/g fat.[10]. If you have a look at the range of PCDD/F TEQ values of the 138 milk/milk product and 45 egg samples analysed here (see Table 1), it can be seen that all samples were below the maximum levels and even below the action levels for dioxins in milk and eggs. With respect to the dioxin-like PCBs, the highest TEQ values were in the range of the dioxin action level in case of milk/milk products and above the dioxin limit value in case of the eggs.

The mean TEQ values determined for the food samples analysed here are by factor 1.7 to 2.7 lower than the mean values of 152 milk/milk product samples and 68 egg samples from the years 1997 to 2003 analysed by the EU[2, 10]. Although the sample collectives analysed here developed randomly, a certain representativity can be attributed to them for samples from West European countries. And as the samples have been taken from mid 2003 to the beginning of 2005, they represent quite a current contamination level. The fact that the mean TEQ values are lower than those of the EU samples from the anticipating years indicates that the PCDD/F and PCB background contamination of these foodstuffs decreases further.

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