DIOXIN LEVELS IN THE IRISH ENVIRONMENT

SECOND ASSESSMENT (SUMMER 2000) BASED ON LEVELS IN COWS' MILK

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An Ghníomhaireacht um Chaomhnú Comhshaoil

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EXECUTIVE SUMMARY

In line with the Agency's intention to maintain surveillance of dioxins and dioxin-like compounds, it was decided to carry out a follow-up survey to the 1995 dioxin cow's milk survey (EPA 1996) in Summer 2000.

"Dioxins" is a collective term for the category of 75 polychlorinated dibenzo-para-dioxins (PCDDs) and 135 polychlorinated dibenzofurans (PCDFs) and arise mainly as unintentional by-products of incomplete combustion and from certain chemical processes. The PCDD and PCDF compounds which are likely to be of toxicological significance are those 17 compounds with chlorine atoms at the 2,3,7 and 8 positions, the most toxic of which is 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD). The toxic responses include dermal effects, immunotoxicity and carcinogenicity, as well as reproductive and developmental toxicity.

Given that the primary mechanism for dioxins entering the food chain is through atmospheric deposition, cow's milk is considered to be a particularly suitable matrix for assessing their presence in the environment since cows tend to graze over relatively large areas and these compounds will, if present, concentrate in the fat content of the milk.

In order to conform to current practice, testing for dioxin-like polychlorinated biphenyls (PCBs) was included in this programme.

Sources of Dioxins

Although PCDDs and PCDFs are not produced intentionally except for research and analysis purposes their formation is often a by-product of many anthropogenic and natural activities. Some significant sources internationally are:

- Incineration of municipal or hazardous waste
- Incineration of medical waste
- Production of steel
- Sinter plants
- Forest fires and other natural fires
- Cement kilns (especially where hazardous waste is co-incinerated)
- Coal fired power plants
- Residential combustion (especially where wood is used)
- Chlorine bleaching of wood pulp
- Backyard burning of household waste and bonfires
- Copper production
- Traffic
- Accidental fires

Results of Survey

Two types of sampling stations were chosen:

Type A	background stations covering the entire country (24 samples)
Type B	potential impact stations in areas of perceived potential risk (13 samples)

The levels for dioxins in milk fat ranged from 0.09 to 0.35 pg I-TEQ/g, using the standard system of International Toxic Equivalents (I-TEQ) for comparing dioxin toxicities of different samples. The mean value for both the A and B sets of samples was 0.20 pg I-TEQ/g milk fat. The overall mean value of 0.20 pg I-TEQ/g milk fat may be compared to a mean value of 0.24 pg I-TEQ/g milk fat for the 1995 survey, corresponding to a reduction of around 16 per cent over the 5 year period. These concentrations were uniformly low by international standards. There were no unusually high values meriting particular attention in any of the type A or type B samples. There was a tendency towards slightly higher values in the East Coast samples but even these levels were low by comparison with similar studies in other countries. Dioxin-like PCBs were analysed in milk for the first time in Ireland. In line with other studies, the PCB levels account for around half the total dioxin figure. The results

were also consistent with a study on dioxin and PCB levels in Irish cheese undertaken by the National Food Centre in 1998-1999.

The results given herein confirm the uniformly low levels of dioxins and dioxin-like PCBs in the Irish environment.

1. INTRODUCTION

Background

In line with the Agency's intention to maintain surveillance of dioxins and dioxin-like compounds, it was decided to carry out a follow-up survey to the 1995 dioxin cow's milk survey (EPA 1996) in Summer 2000. Samples for the second survey were taken as far as possible in the same locations as the 1995 survey although a few additional sampling points were added. In the 2000 survey, 37 samples were taken as opposed to 33 for the first survey. In order to conform to current practice, testing for dioxin-like PCBs was included in this programme.

Dioxins

"Dioxins" is a collective term for the category of 75 polychlorinated dibenzo-para-dioxins (PCDDs) and 135 polychlorinated dibenzofurans (PCDFs). These substances arise mainly as unintentional by-products of incomplete combustion and from certain chemical processes. The toxicity of individual dioxin and dibenzofuran compounds (or congeners) varies considerably. The PCDD and PCDF congeners which are likely to be of toxicological significance are those 17 congeners with chlorine atoms at the 2,3,7 and 8 positions, the most toxic of which is 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD). The toxic responses include dermal effects, immunotoxicity and carcinogenicity, as well as reproductive and developmental toxicity.

Sources of Dioxins

Although PCDDs and PCDFs are not produced intentionally except for research and analysis purposes their formation is often a by-product of many anthropogenic activities. The manufacture of some chlorinated compounds is known to result in the formation of PCDDs and PCDFs as unwanted by-products. They may also be formed in small quantities in a wide range of combustion processes where organic materials and chlorine compounds are burned together. Such sources can include incineration of all types of wastes, metallurgical operations such as smelting and scrap metal recovery furnaces and the burning of fuels such as coal, wood (especially where the wood contains preservatives) and petroleum products. Other sources are believed to be motor vehicle emissions (although as they arise mainly from leaded fuels, this source is expected to decrease) and emissions from accidental and natural fires. Other sources such as illegal or uncontrolled incineration of domestic waste and bonfires (Dyke and Coleman, 1994) are also believed to be significant although obviously are difficult to quantify.

Dioxin emissions from domestic fires are also believed to be a relatively significant source, particularly when wood or domestic waste is used on these fires. Dioxins are also found in paper products arising from the bleaching with chlorine of naturally occurring phenols present in wood pulp and in the manufacture of some chlorinated compounds.

A well known example of an accident involving release of dioxins was the explosion in 1976 at Seveso, Italy where some of the contents of a 2,4,5-trichlorophenol manufacturing plant were released into the atmosphere causing severe local contamination with trichlorophenol and 2,3,7,8- TCDD. Dioxins also attracted particular attention during the Vietnam War where they were found to be present as a contaminant in the defoliant Agent Orange, a mixture of 2,4,5-T and 2,4-D. High levels of dioxins were found in poultry and eggs from Belgium in 1999. The cause of the contamination is thought to be contamination of feeding stuff. Dioxin compounds have no commercial value and have never been intentionally synthesised other than for laboratory use. Monitoring data for dioxins date only from the 1970s as the analytical capabilities for their detection did not exist before then due to the extremely low concentrations at which they were present in the environment. However, there can be little doubt that dioxins from anthropogenic activities have existed at least to some extent since the discovery of fire.

Very little firm information on dioxin emission sources exists for Ireland. In view of this information gap, the Agency has commissioned a 12-month study which started recently, and which will attempt to quantify significant dioxin sources in Ireland.

PCBs

Polychlorinated biphenyls (PCBs) are chlorinated hydrocarbons which were synthesised by direct chlorination of biphenyl but whose production has now been discontinued. Depending on the number and location of the chlorine atom substituents, there are 209 possible PCB congeners. Of these, there are 12 non-ortho and mono-ortho substituted PCBs which show similar toxicological properties to dioxins and are often termed "dioxin-like PCBs".

Unlike dioxins, PCBs have found widespread use in a number of commercial open and closed applications, due to their physical and chemical properties, such as non-flammability, chemical inertia, high boiling points and high dielectric constants. Typical open applications have been their use in pigments, sealants, rubber products and carbonless copy paper. Closed applications have included use of PCBs in hydraulic and heat transfer systems, transformers and capacitors. The production and use of PCBs has been discontinued for some years but because of their persistent qualities they remain in electrical equipment, buildings and the environment.

Dioxins and furans are often found in appreciable quantities in the presence of PCBs.

In conformity with current practice this survey included analysis of these 12 dioxin-like PCBs.

Mechanism of Toxicological Action

A broad variety of data primarily on TCDD but also on other members of the class of dioxin-like compounds has shown the importance of the Ah (dioxin) receptor in mediating the biological effects of dioxin. These data have been collected using many experimental models in multiple species and also from studies on human exposure. The precise chain of molecular events by which the ligand-activated receptor elicits these effects is not yet fully understood. However, alterations in key biochemical and cellular functions are expected to form the basis for dioxin toxicity. Pharmacological structure-activity and mouse genetic studies using Ah receptor-deficient animals and cells have demonstrated a key role for the receptor in mediating toxic effects of TCDD.

Toxic Equivalency Factors (TEFs) for Assessing Mixtures of Dioxins and Dioxin-like Compounds

Because real samples containing dioxins are made up of complex mixtures, a system of Toxic Equivalents has been developed in order to address the problem of differing toxicities and environmental behaviour of these substances. This procedure uses a scheme of weighting factors which expresses the toxicity of each individual PCDD and PCDF in terms of an equivalent amount of 2,3,7,8-TCDD. This weighting factor, called a toxic equivalent factor (TEF), is multiplied by the concentration of the individual compounds in a mixture to give a 2,3,7,8-TCDD toxic equivalent, (TEQ) which is the sum of the concentrations of the individual congeners multiplied by their TEFs. A number of different systems for establishing toxic equivalent factors now exist. The NATO/CCMS (North Atlantic Treaty Organisation's Committee on Challenges of Modern Society) I-TEQ system which was used in the EPA 1996 report, defines most of the older data. The newer system devised by the World Health Organisation (WHO) in 1998 also incorporates PCBs. The WHO have also suggested that the TEQ scheme be re-evaluated every five years and that TEFs be reanalysed in the light of any new scientific information. Clearly it is important when comparing data to define correctly the TEQ units and also whether PCBs are being considered. Usually I-TEQ concentrations will be a little lower than WHO-calculated TEQs as some of TEFs have been revised upwards by the WHO. The TEF values for both systems are tabulated in the Appendix (Tab 02 for dioxins and Tab 04 for PCBs). As a general rule, it can be safely assumed that older data will have been calculated according to the I-TEQ system.

In calculating TEQs for compounds which are not found in concentrations above the detection limit the conventional approach is to use one half of the detection level for non-detects. This is a conservative approach to estimating TEQs at trace levels and can result in significantly higher estimates than the use of zero values for non-detects in low level samples. This approach has been used in the calculations below. As not all data consider non-detects it is important to clarify this issue when comparing low level data.

2. NATIONAL DIOXIN SURVEY

Background

It is generally accepted that the principal mechanism of environmental release of dioxins in this country is by low level emission from multiple sources to the atmosphere. It is reasonable to assume, therefore, that the primary mechanism for entering the food chain is through atmospheric deposition. Cow's milk is considered to be a particularly suitable matrix for assessing the presence of dioxins in the environment as cows tend to graze over relatively large areas and these compounds will, if present, concentrate in the fat content of the milk.

This survey was planned as a follow-up to the national survey carried out in 1995. As far as possible the same approach was adopted in terms of time of year and location of samples. However, unlike the 1995 survey, the analyses included the 12 dioxin-like PCBs.

Samples was taken in June and July 2000 when the cows could be expected to be found grazing outdoors. Details are given in Tables 1-4.

Sampling strategy

Two types of sampling stations were chosen:

Type A	background stations covering the entire country (24 samples)
Type B	potential impact stations in areas of perceived potential risk (13 samples)

Type A samples were normally taken from full milk silos in regional dairies. The typical volume of these silos is 30,000 to 50,000 gallons and they are equipped with an agitator to keep the milk mixed. However there were a number of instances where sampling from silos was not possible and the samples were taken instead from road tankers representative of the area to be covered. Type B samples were taken from road tankers representing the "potential impact" areas.

Sampling procedure

Samples were taken in thick walled pyrex glass bottles of one litre capacity which had been washed with detergent and acetone. The sample volume was 800 ml. Duplicate samples were taken with the intention of submitting one sample for analysis and retaining the other sample in the event of a repeat analysis being required.

The samples were taken by EPA personnel while the milk was still in its raw state. The samples were then taken to the nearest EPA regional laboratory where they were frozen at –20° C. Shipment of samples was by overnight courier in ice boxes to the laboratory. (see below)

Analysis

The analysis of very low concentrations of dioxins, such as were found in this survey is exceptionally demanding and no facilities exist in this country. The laboratory chosen for the analyses was the Gesellschaft für Arbeitsplatz und Umweltanalytik (GfA) laboratory in Münster, Germany. This laboratory is very experienced in the analysis of dioxins in milk and other food matrices and has undertaken analyses for clients in many countries. For example, it analysed approximately 500 samples for dioxins from food and feedstuff samples taken across Europe in connection with the 1999 Belgian contamination incident referred to above. The laboratory is fully accredited for the analysis of PCDDs, PCDFs and PCBs in food matrices, including milk.

Analyses were carried out following pre-treatment and extraction using high resolution gas chromatography and high resolution mass spectrometry with ^{13}C-labelled isomers as internal standards. This method is considered to be the most suitable for low-level dioxin measurements.
Further analytical details are contained in the Appendix which contains extracts from the GfA report to the Agency. It is not reproduced here in full for reasons of space and since a number of sections largely overlap with the EPA report. The entire report is available on the EPA website, epa.ie.

Results

The data showing I-TEQs and WHO TEQs are shown in Tables 1-4. The detailed analytical results showing the levels for the individual congeners along with further analytical details comprise 74 pages and are not given here due to space considerations. These are, however, available on the EPA web site.
The fat content was measured separately and TEQs were determined in fat and then back-calculated to give corresponding levels in the original whole milk sample.

Whole milk TEQ levels are reported in Tables 1 and 3 and fat levels in Tables 2 and 4.

Table 1

Survey on the whole milk related PCDD/F and PCB-TEQ values determined in the background samples A 1 - A 25

Sample No.	Milk supply area	Dioxins		PCBs	Dioxins and PCBs
		I-TEQ incl. ½ LOD [a]	WHO-TEQ incl. ½ LOD [a]	WHO-TEQ incl. ½ LOD [a]	Total WHO-TEQ incl. ½ LOD [a]
	Unit	*pg/kg whole milk*	*pg/kg whole milk*	*pg/kg whole milk*	*pg/kg whole milk*
A 1	Mitchelstown Area (T)	4.5	5.2	4.6	9.8
A 2	Dungarvan Catchment (T)	8.3	9.8	6.2	16.0
A 3	Dublin County Catchment (T)	12.4 / 11.5 [b]	14.5 / 13.7 [b]	19.0 / 21.8 [b]	33.5 / 35.5 [b]
A 4	Inch Co. Wexford Area (T)	12.2	14.0	13.9	27.9
A 5	Charleville Area (T)	4.8	5.6	8.3	13.9
A 6	Ballyragget Area (T)	7.7	9.1	7.2	16.3
A 7	Renmore Area	4.9	6.1	7.1	13.2
A 8	Moate Area	8.7	10.1	7.1	17.2
A 9	Tipperary Catchment	7.2	8.4	6.1	14.5
A 10	Nenagh Catchment	6.8	8.1	7.1	15.2
A 11	Cavan/Longford/Leitrim	6.9	8.3	7.3	15.6
A 12	Drinagh Catchment	4.6	5.4	5.8	11.2
A 13	Bandon Area (T)	5.8	6.8	6.9	13.7
A 14	North Kerry Area (T)	4.8	5.7	6.9	12.6
A 15	Sligo Area (T)	5.9	6.9	8.2	15.1
A 16	Roscommon Area(T)	5.4	6.4	6.3	12.7
A 18	Kiltoghert (T)	6.5	7.7	8.0	15.7
A 19	Monaghan Area (T)	8.6	10.0	9.0	19.0
A 20	Louth Catchment (T)	10.9	12.7	13.1	25.8
A 21	Kildare Catchment (T)	8.4	9.6	13.6	23.2
A 22	South Kerry (Cahirciveen)(T)	2.9 / 3.0 [b]	3.4 / 3.6 [b]	4.6 / 4.9 [b]	8.0 / 8.5 [b]
A 23	South Wexford	10.1	11.8	11.4	23.2
A 24	Co.Mayo	5.6	6.7	12.4	19.1
A 25	Co.Donegal	7.5	8.9	6.3	15.2

a TEQ value calculated by including the not detected congeners and taking half of their detection limit (LOD)

b results of duplicate analyses

Sample corresponding to A17 in the 1995 survey was not taken in 2000
(T) Denotes sampling from a road tanker as opposed to a bulk silo

Table 2

Survey on the milk fat related PCDD/F and PCB-TEQ values determined in the background samples A 1 - A 25

Sample No.	Milk supply area	Dioxins		PCBs	Dioxins and PCBs
		I-TEQ incl. ½ LOD [a]	WHO-TEQ incl. ½ LOD [a]	WHO-TEQ incl. ½ LOD [a]	Total WHO-TEQ incl. ½ LOD [a]
	Unit	*pg/g milk fat*	*pg/g milk fat*	*pg/g milk fat*	*pg/g milk fat*
A1	Mitchelstown Area (T)	0.14	0.16	0.16	0.32
A2	Dungarvan Catchment (T)	0.24	0.28	0.18	0.46
A3	Dublin County Catchment (T)	0.34 / 0.32 [b]	0.40 / 0.38 [b]	0.53 / 0.58 [b]	0.93 / 0.96 [b]
A4	Inch Co. Wexford Area (T)	0.35	0.41	0.37	0.78
A5	Charleville Area (T)	0.13	0.16	0.24	0.40
A6	Ballyragget Area (T)	0.23	0.28	0.23	0.51
A7	Renmore Area	0.14	0.18	0.19	0.37
A8	Moate Area	0.23	0.27	0.28	0.55
A9	Tipperary Catchment	0.21	0.24	0.18	0.42
A10	Nenagh Catchment	0.19	0.23	0.19	0.42
A11	Cavan/Longford/Leitrim	0.20	0.24	0.22	0.46
A12	Drinagh Catchment	0.13	0.15	0.16	0.31
A13	Bandon Area (T)	0.16	0.19	0.19	0.38
A14	North Kerry Area (T)	0.14	0.17	0.21	0.38
A15	Sligo Area (T)	0.17	0.20	0.24	0.44
A16	Roscommon Area(T)	0.14	0.17	0.16	0.33
A18	Kiltoghert (T)	0.18	0.21	0.24	0.45
A19	Monaghan Area (T)	0.25	0.29	0.28	0.57
A20	Louth Catchment (T)	0.32	0.38	0.38	0.76
A21	Kildare Catchment (T)	0.24	0.27	0.37	0.64
A22	South Kerry (Cahirciveen) (T)	0.09 / 0.09 [b]	0.11 / 0.11 [b]	0.13 / 0.16 [b]	0.24 / 0.27 [b]
A23	South Wexford	0.27	0.32	0.32	0.64
A24	Co.Mayo	0.17	0.20	0.38	0.58
A25	Co.Donegal	0.21	0.25	0.17	0.42

a TEQ value calculated by including the not detected congeners and taking half of their detection limit (LOD)

b results of duplicate analyses

Sample corresponding to A17 in the 1995 survey was not taken in 2000

(T) Denotes sampling from a road tanker as opposed to a bulk silo

Table 3

Survey on the whole milk related PCDD/F and PCB-TEQ values determined in the potential impact samples B 1 - B 16

Sample No.[a]	Milk supply area	Dioxins		PCBs	Dioxins and PCBs
		I-TEQ incl. ½ LOD [b]	WHO-TEQ incl. ½ LOD [b]	WHO-TEQ incl. ½ LOD [b]	Total WHO-TEQ incl. ½ LOD [b]
		pg/kg whole milk	*pg/kg whole milk*	*pg/kg whole milk*	*pg/kg whole milk*
B1	Cobh/Great Island	11.0	13.0	15.0	28.0
B2	East Cork Harbour	8.2	10.3	9.2	19.5
B3	Askeaton area	4.3	5.0	6.1	11.1
B4	Tarbert/Glin/ Shanagaolden	2.6	3.2	3.7	6.9
B5	Clarecastle Co.Clare	4.4	5.3	6.3	11.6
B6	Cooraclare Co.Clare	4.6	5.5	7.2	12.7
B7	So. Tipperary	8.6	10.2	7.2	17.4
B8	Mulhuddart, Co.Dublin	9.9	11.3	10.3	21.6
B9	Grannagh, Co.Kilkenny	11.5	13.6	12.7	26.3
B13	Kinsale (Dunderow) Co.Cork	6.2	7.1	6.2	13.3
B14	Ringaskiddy, Co.Cork	4.9	5.7	6.1	11.8
B15	Crossakiel (nr Kells), Co.Meath	10.1	11.7	8.4	20.1
B16	Ballydine, So. Tipperary	7.0	8.1	8.4	16.5

a Samples corresponding to B10, B11 and B12 in the 1995 survey were not taken in 2000

b TEQ value calculated by including the not detected congeners and taking half of their detection limit (LOD)

All "B" samples were taken from road tankers

Table 4

Survey on the milk fat related PCDD/F and PCB-TEQ values determined in the potential impact samples B 1 - B 16

Sample No.[a]	Milk supply area	Dioxins		PCBs	Dioxins and PCBs
		I-TEQ incl. ½ LOD [b]	WHO-TEQ incl. ½ LOD [b]	WHO-TEQ incl. ½ LOD [b]	Total WHO-TEQ incl. ½ LOD [b]
		pg/g milk fat	*pg/g milk fat*	*pg/g milk fat*	*pg/g milk fat*
B1	Cobh/Great Island	0.32	0.38	0.45	0.83
B2	East Cork Harbour	0.23	0.29	0.27	0.56
B3	Askeaton area	0.11	0.13	0.16	0.29
B4	Tarbert/Glin/ Shanagolden	0.09	0.12	0.15	0.27
B5	Clarecastle Co.Clare	0.13	0.15	0.19	0.34
B6	Cooraclare Co.Clare	0.13	0.16	0.22	0.38
B7	So. Tipperary	0.24	0.29	0.21	0.5
B8	Mulhuddart, Co.Dublin	0.29	0.33	0.30	0.63
B9	Grannagh. Co.Kilkenny	0.31	0.36	0.34	0.70
B13	Kinsale (Dunderow) Co. Cork	0.17	0.19	0.18	0.37
B14	Ringaskiddy. Co.Cork	0.13	0.15	0.18	0.33
B15	Crossakiel (nr Kells). Co.Meath	0.28	0.32	0.23	0.55
B16	Ballydine, So. Tipperary	0.17	0.19	0.19	0.38

a Samples corresponding to B10, B11 and B12 in the 1995 survey were not taken in 2000
b TEQ value calculated by including the not detected congeners and taking half of their detection limit (LOD)

All “B” samples were taken from road tankers

Table 5
Summary of Milk Fat Data in pg TEQ/g fat

	"A" Samples				*"B" Samples*				*"A and "B" Samples combined*			
Parameter	**Dioxins**		**PCBs**	**Dioxins & PCBs**	**Dioxins**		**PCBs**	**Dioxins & PCBs**	**Dioxins**		**PCBs**	**Dioxins & PCBs**
	I-TEQ	*WHO-TEQ*	*WHO-TEQ*	*WHO-TEQ*	*I-TEQ*	*WHO-TEQ*	*WHO-TEQ*	*WHO-TEQ*	*I-TEQ*	*WHO-TEQ*	*WHO-TEQ*	*WHO-TEQ*
Mean	0.20	0.24	0.25	0.49	0.20	0.24	0.24	0.48	0.20	0.24	0.25	0.49
Minimum	0.09	0.11	0.15	0.26	0.09	0.12	0.15	0.27	0.09	0.11	0.15	0.26
Maximum	0.35	0.41	0.55	0.95	0.32	0.38	0.45	0.83	0.35	0.41	0.55	0.95
Standard Deviation	0.068	0.08	0.10	0.17	0.085	0.10	0.09	0.18	0.07	0.08	0.09	0.17
Samples	24	24	24	24	13	13	13	13	37	37	37	37

Discussion

Considering the entire of samples (Tables 1 and 3), the reported I-TEQ ranges for dioxins in whole milk are 2.95 to 12.2 pg I-TEQ/kg and 3.5 to 14.1 pg WHO-TEQ/kg. When PCBs are included the ranges are 8.25 to 34.5 WHO-TEQ/kg for dioxins and PCBs.

For milk fat (Tables 2 and 4), the respective ranges are, 0.09 to 0.35 pg I-TEQ/g and 0.11 to 0.41 pg WHO-TEQ/g. Including PCBs, the ranges are 0.255 to 0.945 WHO-TEQ.

For comparison purposes it is probably more valid to use the milk fat rather than whole milk data due to the varying composition of fat in milk. Using the fat data also facilitates comparisons with other dairy products such as butter and cheese and also with human milk.

A summary of the milk fat data showing a breakdown of the "A" and "B" sampling along with the combined data set is presented in Table 5. Comparing the two groups of samples, the background (type A), and the potential impact (type B), the overall mean values for I-TEQ were identical at 0.20 pg I-TEQ/g. The means for WHO-TEQ for dioxins and WHO-TEQ for dioxins and PCBs combined are also almost identical for the two groups. The highest values of 0.95 pg WHO-TEQ/g for the A group and 0.83 pg WHO-TEQ/g for the B group were subjected to the standard Grubbs test for statistical outliers (95 per cent confidence level). The highest B sample was found not to be an outlier at these confidence levels. The highest A sample at 0.95 pg WHO-TEQ/g was found to be just below the threshold of being a statistical outlier at these levels. Based on these statistical considerations there was a probability of just below 95 per cent that the latter level was outside the expected range of the data. After the A and B data sets were combined (Table 5), which was felt to be justified in view of their evident similarity, the test showed a much lower probability of this sample being an outlier. As the latter test has more statistical validity since combined data set contains more data (37 vs 24), it is probable that the sample is not an outlier and that based on strictly statistical considerations it should not merit particular attention. To eliminate the possibility of measurement error being responsible, both the highest and lowest samples were reanalysed (Tables 1 and 2) and the duplicates were found to agree within acceptable analytical tolerances. (see "Comparison with 1995 Survey" below for further discussion)

3. COMPARISON WITH 1995 SURVEY

Figures 1 and 2 show a comparison of the I-TEQ milk fat data with the 1995 survey. Comparisons incorporating PCBs are not possible, as they were not analysed in the first survey.

The mean value for milk fat in the 2000 survey was 0.20 pg I-TEQ/g compared to a mean value of 0.24 pg I-TEQ/g for the 1995 survey, corresponding to a reduction of around 16 per cent over the 5 year period. It will be readily seen that only sample A3 which was referred to above, showed an appreciably higher result for the 2000 survey compared with the 1995 survey. In all other cases where there was a significant difference in levels, the higher value was recorded in the earlier survey. Since sample A3 was taken from the Dublin area which has enjoyed a considerable population increase over the period, along with the possibility as discussed above that the higher value was not a random occurrence, it is tentatively concluded that the slightly elevated result was probably significant and not a random occurrence. This conclusion is strengthened by the fact that the PCB contribution to the overall TEQ was relatively high; since PCB levels are likely to be particularly influenced by anthropogenic factors, due to their ubiquitous presence in the built environment. Indeed this sample, while showing the highest total Dioxin and PCB TEQ, did not give the highest I-TEQ (Figure 1) which is a further indication of the relatively high PCB content.

It is also noteworthy that the three highest A values for I-TEQ, A3, A4 and A20 were taken from areas along the East Coast. There also appears to be some evidence of particularly low values from the samples taken from Kerry, Clare, Limerick and rural areas in Cork i.e. along the south west Atlantic seaboard. These values were exceptionally low by international standards. Other similarly low results were found in other rural areas in the country which tends to confirm the expectation of particularly low values in such areas.

Notwithstanding the usual difficulties in comparing historical data and bearing in mind that there were only two complete surveys , there is little doubt that the 16 per cent reduction in levels represents a real decrease. This reflects the pattern shown in similar surveys across Europe which have been attributed to a number of regulatory measures and various technological advances. Examples of measures that were taken in Ireland and which may have had an impact on dioxin levels were the virtual abolition of leaded petrol and the shutting down of all hospital incinerators as they were found to be operating incorrectly and were consequently suspected of emitting dioxins.

Figure 1 Comparison with the 1995 survey
A Samples

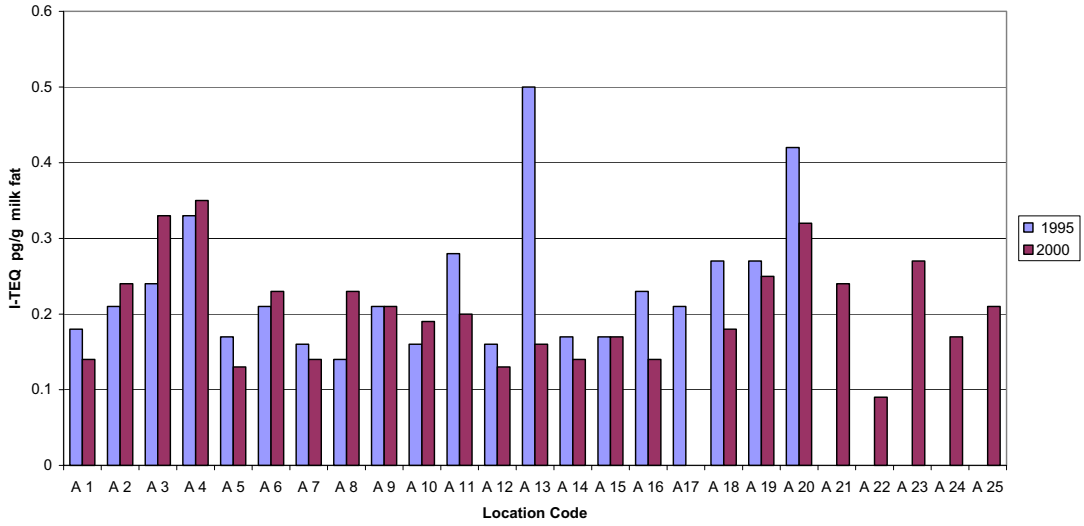
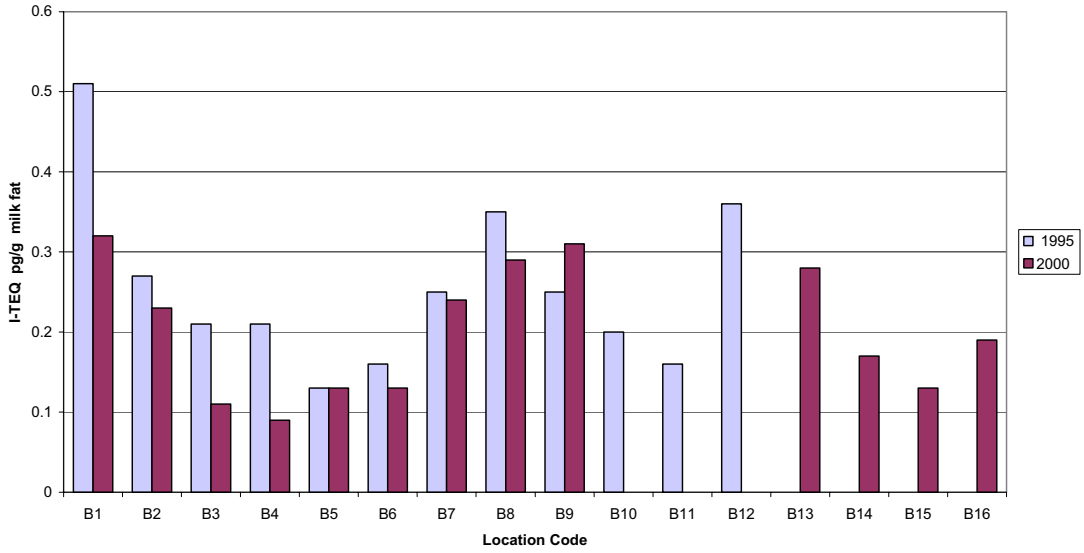


Figure 2 Comparison with the 1995 survey
B Samples



4. OTHER STUDIES IN MILK AND DAIRY PRODUCTS

Milk Studies In Other Countries

A number of milk studies have been undertaken by the UK Ministry of Agriculture, Fisheries and Food (MAFF). Of particular interest, is a study published in 1997 (MAFF 107) carried out in Northern Ireland both from individual farms and in retail cow's milk. Cows' milk (40 samples) was obtained the Department of Agriculture for Northern Ireland on four occasions - April/May, July and November 1993 and March 1994. Samples were taken prior to retail sale from dairies (20 samples) and from bulk tanks of individual farms (20 samples). The concentrations were within the range 0.74-2.7 (mean 1.2), pg I-TEQ/g milk fat in retail samples from dairies, and 0.84-3.0 (mean 1.2) pg I-TEQ/g fat, in the samples from individual farms. The farms and dairies were selected to provide as wide a coverage of Northern Ireland as possible. Some came from areas which were partially industrialised, but none of the samples came from farms close to specific industrial sites with the potential to release dioxins as there are very few such sites in Northern Ireland.

In England MAFF carried out a survey in 1995 of dioxins and polychlorinated biphenyls (PCBs) in samples of retail cows' milk from 12 locations in England. (MAFF 136) This survey was carried out to determine whether the "levels of dioxins had changed since a comparable survey in 1990 in the light of actions taken by the Environment Agency and others to reduce emissions of dioxins from industry". The concentrations of dioxins found in the survey of retail milk samples taken in summer and winter 1990 were in the approximate range 1.3-3.3 (mean 1.9) pg I-TEQ/g milk fat.

For the MAFF 1995 survey, where samples were taken from comparable locations, and which included PCB analyses, the main findings were:
dioxin concentrations were in the range 0.67-1.4 (mean 1.0) pg I-TEQ/g milk fat, PCB concentrations were in the range 0.75-2.3 (mean 1.8) pg TEQ/g milk fat and the combined dioxin and PCB concentrations were in the range 1.4-3.5 (mean 2.8) pg TEQ/g milk fat. This appears to represent an appreciable reduction in dioxin levels over the 5-year period.

The EU SCOOP database section on dioxins was published in June 2000 (EU SCOOP 2000). It contains a large quantity of data from 10 member states on the occurrence of PCDD/Fs and dioxin-like PCBs in food and human milk and is probably the best general source for recent data from those countries. Other examples are contained in Table 6 which was prepared by GfA.

Care must be exercised in the interpretation of the more recent European milk data as at least some data appear to have been affected by the 1999 Belgian citrus pulp incident.

All of the milk data examined indicate considerably higher levels than in either of the EPA surveys.
They also indicate a trend of reducing dioxin levels across Europe over the past 10 to 15 years.

Other Studies

While the measurement of cow's milk is regarded as probably the best indicator of the presence of dioxins in the environment, it is worth also considering relevant studies in other similar media.

Dioxins in Cheese in Ireland

The National Food Centre, Dublin carried out a survey in 1998-1999 on Dioxins and Coplanar (Dioxin-like) PCBs in 90 samples of cheese, taken from seven dairy companies over a 12 month period (NFC 2000). The range of values for dioxins was from <0.1 – 0.82 pg WHO TEQ/g fat, for coplanar PCBs from <0.1 – 0.54 pg WHO TEQ/g fat and for dioxins and coplanar PCBs combined was from 0.12 – 1.2 pg WHO TEQ/g fat.

These results are broadly comparable with this milk survey.

Table 6[1]

Comparison of the PCDF/D I-TEQ values from Irish cow's milk samples with data from other countries

Country	Period of sampling	Number of samples	Mean value (pg ITE/ g fat)	Range (pg ITE/ g fat)	Remarks	Literature
Ireland	1995	20	0.23	0.13 - 0.36	**background samples**	*a*
Ireland	2000	24	0.20	0.09 - 0.35	**background samples**	*b*
United Kingdom	1995	12	1.0	0.67 - 1.4	retailed cow's milk samples purchased from 12 locations in England (**pooled samples** from each region)	*c*
Northern Ireland	1993 - 1994	40	1.2 1.2	0.74 - 2.7 0.84 - 3.0	20 cow's milk samples **from dairies** and 20 cow's milk samples **from bulk tanks** of individual farms	*d*
Germany, South West	1993 - 1996	538	0.71	n. a.	**cow's milk samples**	*e*
Germany, North West	1994	120	1.02	0.61 - 1.75	cow's milk samples from **30 dairies** in North Rhine-Westphalia	*f*
Germany, North West	1998	111	0.78	0.47 - 1.78	cow's milk samples **from 29 dairies** in North Rhine-Westphalia	*f*
France	1998	148	0.65	0.29 - 1.75	long-life half-skimmed drinking milk from 33 French **dairy establishments**	*g*
United States	1996/1997	26	0.84	0.25 - 2.01	**26 pooled milk samples** from different regions	*h*

n. a. data not available

a Dioxins in the Irish Environment, An assessment based on levels in cow's milk sampled in June 1995, EPA Ireland, Ardcavan, Wexford, 1996

b current study

c Ministry of Agriculture, Fisheries and Food, Food Surveillance Information Sheet No. 107, 1997

d Ministry of Agriculture, Fisheries and Food, Food Surveillance Information Sheet No. 120, 1997

e Malisch, R. Chemosphere 37, 1687 - 1698, 1998

f Fürst, P. ERNO 1, 29 - 35, 2000

g Durand, B.; Dufour, B.; Vindel, E.; Fraisse, D. Chemosphere 41, 865 - 869, 2000

h Lorber, M.N.; Winters, D.L.; Griggs, J.; Cook, R.; Baker, S.; Ferrorio, J.; Byrne, C.; Dupuy, A.; Schaum, I.; A national survey of dioxin-like compounds in the United States milk supply; Organohalogen compounds, Vol. 38, 1998

[1]This table was extracted from the GfA report to the Agency

Dioxin Limits in Milk

While there are currently no limits for milk in this country, it is worth comparing the Irish data with limits in other EU countries. (See Table 7)

It is clear that the levels found in all of the Irish surveys are at least an order of magnitude below any of the above limits. It should be borne in mind, however, that the majority of the above limits probably do not take account of some recent developments in assessing acceptable human intake levels of dioxins in food. A WHO reevaluation in May 1998 of recommended a tolerable daily intake (TDI) for humans in the range **1 - 4 TEQ pg/kg body weight** (bw) for dioxins and dioxin-like PCBs. A very recent opinion of the EU Scientific Committee on Food (SCF 2000) to the European Commission on the risk assessment of dioxins and dioxin-like PCBs in food, after reviewing the most recent studies, including the WHO 1998 reevaluation and the EU SCOOP database established a **temporary tolerable weekly intake (t-TWI) of 7 TEQ pg/kg bw.**

It would appear likely that the intake of milk containing dioxin levels approaching the above national limits would alone cause these WHO and SCF intake guidelines to be exceeded by some consumers (especially children). It might be expected therefore that some the above national limits or guidelines may be revised downwards.

Table 7
Summary of Dioxin Limits for Cow's Milk in EU Countries

Country	Limit Value	Comment
Belgium	5 pg TCDD/g fat	
Germany	5 pg I-TEQ/g fat	Non Statutory Limit. A desirable target for milk and dairy products has also been set at 0.9 pg I-TEQ/g fat.
France	5 pg I-TEQ/g fat	An objective has also been set of achieving less than 1 pg I-TEQ/g fat
Netherlands	6 pg I-TEQ/g fat	
United Kingdom	20 pg TEQ/g fat	To include dioxin-like PCBs

5. CONCLUSIONS

1. All levels recorded in this survey were considerably below those taken from a random selection of similar studies in other EU countries. While assessment of consumer exposure to dioxins through the consumption of milk was not the object of this survey, the levels were at least an order of magnitude below limits set in other countries.

2. The reduction of c 16 per cent in I-TEQ values compared with the 1995 study is in line with similar reductions in environmental levels across Europe.

3. There is some evidence of slightly higher values from samples taken along the East coast with correspondingly lower values from rural samples in other areas. This is hardly surprising in view of the population distribution. However, undue significance should not be attached to this as levels are still very low by international standards.

4. Dioxin-like PCBs were analysed in milk for the first time in Ireland. In line with other studies, the PCB levels account for around half the total dioxin figure.

References

1. EPA 1996. Dioxins in the Irish Environment, An Assessment Based on Levels in Cow's Milk. EPA Wexford.

2. Dyke P. and Coleman P., Dioxin Emissions from Bonfires, Bonfire Night 1994, AEA Technology. http://www.residua.com/previous/WB47-Bonfire.html, 1994

3. MAFF 107. Ministry of Agriculture, Fisheries and Food, Food Surveillance Information Sheet No. 107, 1997.

4. MAFF 136. Ministry of Agriculture, Fisheries and Food, Food Surveillance Information Sheet No. 136, 1997.

5. EU SCOOP 2000. Assessment of Dietary Intake of Dioxins and Related PCBs by the Population of EU Member States. European Commission, http://europa.eu.int/comm/dgs/health_consumer/library/pub/pub08_en.pdf, June 2000.

6. SCF 2000. EU Scientific Committee on Food, Opinion of the SCF on the Risk Assessment of Dioxins and Dioxin-like PCBs in Food. European Commission, http://europa.eu.int/comm/food/fs/sc/scf/out78_en.pdf, November 2000.

7. NFC 2000. Dioxins and Coplanar PCBs in Dairy Products. The National Food Centre, Dublin, 2000.

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1. European Dioxin Inventory, North Rhine-Westphalia State Environment Agency on behalf of the European Commission, DG XI, http://europa.eu.int/comm/environment/dioxin/download.htm, 1997

2. Compilation of EU Dioxin exposure and health data, AEA Technology, produced for the European Commission, DG Environment and UK Department of the Environment, Transport and the Regions, http://europa.eu.int/comm/environment/dioxin/download.htm, 1999.

APPENDIX

Extract from GfA report[a]

[a]The GfA report is not reproduced in full since a number of sections largely overlap with the EPA report. The entire report is available on the EPA website, epa.ie. The detailed congener analyses referred to the GfA report are also available on the EPA website.

1 Subject of Investigations

On behalf of the Environmental Protection Agency of Ireland (EPA), 37 Irish cow's milk samples were analysed for Polychlorinated Dibenzo(p)dioxins (PCDDs), Dibenzofurans (PCDFs) and Polychlorinated Biphenyls (PCBs) by GfA. All milk samples were provided by the Irish Environmental Protection Agency. As specified by EPA, one part of the samples was collected at so-called background stations and a second part at potential impact stations.

The milk analyses included the determination of the seventeen PCDF/D and the twelve dioxin-like PCB congeners[1] for which toxicity equivalent factors (TEFs) relative to 2,3,7,8-Tetrachlorodibenzo(p)dioxin were specified by the World Health Organisation (WHO)[2]. For quality control, three blank samples were analysed parallel to the milk sample analyses. Two duplicate PCDF/D and PCB determinations were also included in the QC program.

On the basis of the PCDF/D and PCB concentrations determined analytically, Toxic Equivalents (TEQs) were calculated for both classes of compounds by using the WHO TEFs[1] and the so-called I-TEFs[3] (for dioxins only). In the case of the WHO TEFs total PCDF/D and PCB TEQs could be reported. The analytical results and the TEQs were related to the whole milk and to the fat content of each cow's milk sample. Finally, the TEQ values determined for the Irish cow's milk samples were summarised and the background values compared to data from other countries.

2 Materials and methods

2.1 Sample Materials

After sampling in Ireland, portions of 400 ml of each milk sample were frozen in 500 ml bottles and shipped to GfA in cooling boxes. On arrival at GfA on 26th of July 2000, the samples were still frozen and immediately transferred into a deep freezer (-20°C), where they were stored until start of the analyses. The designation of the cow's milk samples can be seen from table 1.

[1] congeners = single PCDF/D isomers

[2] TEFs proposed by the WHO European Centre for Environment and Health (WHO-ECEH) in colaboration with the International Program on Chemical Safety (IPCS) in 1998

[3] So-called international TEFs according to the NATO/CCMS model established in 1989

2.2 Analytical methods

2.2.1 Fat extraction

The PCDF/Ds and PCBs are extracted from milk by means of fat extraction. The contaminants are then separated from the fat and other interfering components and finally analysed by gas chromatography/mass spectrometry (GC/MS).

For fat extraction, 250 g of each cow's milk sample were extracted in a separatory funnel by shaking with 15 ml of a potassium oxalate solution, 250 ml of ethanol, 125 ml diethyl ether and 200 ml of pentane. After phase separation, the aqueous layer was separated and extracted again three times with 100 ml of pentane in each case. The combined organic layers were washed two times with 200 ml of 2% sodium sulfate solution, dried by anhydrous sodium sulfate and filtrated. After addition of the internal $^{13}C_{12}$-labeled PCDF/D and PCB standards for quantification (see below), the solvents were evaporated by means of a rotary evaporator and the remaining fat determined gravimetrically.

2.2.2 Analysis of cow's milk samples for PCDF/Ds

The PCDF/D analysis was performed according to the DIN EN 45001 accredited method GfA PA_156/97 1997-07. Each analysis included the determination of the seventeen PCDF/D congeners with 2,3,7,8-chlorosubstitution.

For PCDF/D analysis, seventeen $^{13}C_{12}$-labeled PCDF/D congeners[1] were added to the fat extract of each milk sample as internal standards. As can be seen from table 2, for each native PCDF/D congener to be determined, the corresponding $^{13}C_{12}$-labeled PCDF/D standard was added (isotope dilution). For the separation of the PCDF/Ds from the milk fat, the total fat extract was dissolved in hexane and then percolated through a mixed silica and an alumina column by means of different solvents (hexane, benzene and methylene chloride).

Part of the fraction containing the contaminants was used for the PCDF/D analysis and the other part for the determination of the PCBs, which was done separately.

The PCDF/D fraction was further cleaned-up by liquid chromatography using a Florisil column and two solvents (hexane and methylene chloride). Prior to the instrumental analysis, another PCDD standard ($^{13}C_6$-1,2,3,4-TetraCDD) was added to the PCDF/D fraction to determine the recovery of the $^{13}C_{12}$-labeled internal PCDF/D standards through the clean-up.

For the PCDF/D determination, a capillary gas chromatograph (HRGC, HP 5890) equipped with a PTV injector and connected to a high resolution mass spectrometer (HRMS, VG-AutoSpec) was used. The operation parameters of the instruments are listed in table 3. Before starting an analysis sequence, a HRMS tune was performed to adjust the instrumental performance (at least once per analysis day, including mass axis calibration, adjustment of mass resolution and sensitivity). The instrument sensitivity was then checked by means of native PCDF/D standards. A mixture of the seventeen $^{13}C_{12}$-labelled standards mentioned above (see table 2) and of the corresponding seventeen native standards was always injected to determine the relative retention times[4] and the relative response factors[5] for identification and quantification. During sample analysis, the stability of the mass focus was assured by means of perfluorokerosene lock masses.

2.2.3 Analysis of cow's milk for PCBs

The PCB analyses were performed by a combination of the DIN EN 45001 accredited methods GfA PA_06/97 1997-06 and GfA PA_154/97 1997-07. The analyses covered the determination of the twelve dioxin-like PCB congeners for which TEFs were established by the WHO in 1998 (see table 4).

Like for the dioxin analysis, for each native PCB congener to be determined the corresponding $^{13}C_{12}$-labelled PCB was added as internal standard to the fat extract (isotope dilution). After fat separation by means of the mixed silica/alumina column a small amount of nonane (keeper) was added to the extract portion which was used for PCB analysis (see chapter 2.2.2). After solvent evaporation the PCB fraction was further cleaned-up by liquid chromatography.

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4 Retention time relative to the corresponding $^{13}C_{12}$-labelled internal standard

5 Response factor relative to the internal $^{13}C_{12}$-labelled standard which was used for the quantification of the native DF/Ds

For PCB detection, a capillary gas chromatograph (HRGC, HP 5890) equipped with a PTV injector and connected to a high resolution mass spectrometer (HRMS, VG-AutoSpec) was used also. The operation parameters of the instruments used for PCB analyses are listed in table 5. The procedures for the instrument tuning, the determination of relative retention times and response factors, and the lock mass check were basically the same as described for the PCDF/Ds, however, adjusted to the PCB determination. The twelve $^{13}C_{12}$-labelled internal PCB standards used for the identification and quantitative determination of native PCB congeners are listed in table 4.

2.2.4 Calculation of toxic equivalents (TEQs)

Dioxin-like compounds are often found in complex mixtures. For risk assessment purposes, a toxicity equivalent procedure was developed to describe the cumulative toxicity of such mixtures. This involves assigning individual toxic equivalency factors (TEFs) to single compounds. TEFs are estimates of the toxicity of a compound relative to the toxicity of a reference compound. Such a scheme was first established for PCDF/Ds with 2,3,7,8 Tetrachlorodibenzo(p)dioxin (2,3,7,8-TCDD) as reference compound. TCDD is the most toxic member of this class of compounds. A TEF of 1 is assigned to TCDD, while the other congeners with 2,3,7,8-chlorosubstitution as a rule have lower TEFs. Calculating the toxic equivalency (TEQ) of a PCDF/D mixture involves multiplying the concentration of individual congeners by their respective TEF. The sum of the TEQ concentrations for the individual congeners is the TEQ concentration of the mixture. Meanwhile not only PCDF/D but also twelve dioxin-like PCB congeners are included in the TCDD TEQ system (see table 4).
Until recently, the so-called international TEF scheme (I-TEF scheme), developed by NATO/CCMS in 1988[2,6] was mainly used for PCDF/D risk assessment. The I-TEF scheme covered the 7 PCDDs and 10 PCDFs with 2,3,7,8-chlorosubstitution only. In 1997 a working group of the WHO re-evaluated the previously established TEFs and further proposed a new set of TCDD TEFs for dioxin-like PCBs. The newer WHO TE-factors for PCBs can be seen from table 04. With respect to the TES for PCDF/Ds two changes were made:

- for 1,2,3,7,8-PentaCDD, the WHO-TEF is 1 instead of 0,5 within the I-TEF scheme.
- for octal and octave, the WHO-TEF is 0.0001 instead of 0.001.

A comparison of the I-TES and the WHO TES for human risk assessment and mammals can be seen from table 2. The TES for the dioxin-like PCBs are shown in table 4.
For the milk samples analysed here, both, the I-TEQs and the newer WHO-TEQs were calculated. By addition of the WHO-TEQs for the seventeen PCDF/Ds and the twelve WHO-PCBs, total WHO-TEQs were determined.

2.2.5 Internal quality control

The recoveries of $^{13}C_{12}$-labelled internal PCDF/D and PCB standards through the fat separation and extract clean-up are listed for each analysis in the tables of results in the annex. For those homologue groups where more than one $^{13}C_{12}$-labelled internal standard was used, the recovery of only one of the internal standards is reported as example. Basically, the recoveries of the other standards of the respective homologue group are similar. The internal PCDF/D and PCB standards for which the recoveries are reported are indicated in table 2 and 4.
As can be seen from the tables 1 to 37 of the annex, recoveries above 60 % were achieved for the internal $^{13}C_{12}$-labelled Tetra- to HeptaCDF/D standards in most cases. For the $^{13}C_{12}$-labelled OctaCDF and OctaCDD standard, recovery rates above 40 % were determined for almost all analyses.
For the internal $^{13}C_{12}$-labelled PCB standards the recoveries mainly were between 50 and 100% (see tables 38 to 74 of the annex). Lower recoveries between 30% and 50% were found in some cases, especially for the higher chlorinated Hexa- and HeptaCBs.

[6] North Atlantik Treaty Organization, Committe on the challenges of Modern Society, Report No. 176, NATO, Brussels, 1988

The recovery rates are sufficient and acceptable with respect to the fact that for all PCDF/D and PCB congeners to be determined, the corresponding $^{13}C_{12}$-labeled compound is present during all extract clean-up steps (isotope dilution). Losses of one of the native PCDF/D or PCB compounds during the fat separation and clean-up are compensated by comparable losses of the corresponding $^{13}C_{12}$-labeled internal standard which is used for quantification.

Within the PCDF/D and PCB analyses of the milk samples, three method blanks were determined for each parameter. The blanks were analysed within the indicated sets of milk sample analyses. Each method blank covered all steps of the analyses, starting with the solvents used for fat extraction and running through all steps of the clean-up. The results of the blank analyses are presented in the table 75 to 80 of the annex. To make the results of the method blanks directly comparable to the sample results, the blank were referred to a hypothetical sample weight of 250 g milk and to a mean fat content of 3,6 %.

As can be seen from the PCDF/D tables 75 to 77 of the annex, only 2,3,7,8-TetraCDF, HeptaCDD and OctaCDD were detected at levels slightly above the detection limits. Expressed in TEQ-values, blanks between 0,001 and 0,004 pg TEQ/g fat were found. Compared to the PCDF/D TEQ-values determined for the cow's milk samples, the blank values are below 5 %. Since such contributions are in the range of the analytical precision, no blank corrections were made for the PCDF/Ds.

In the case of the PCB analyses the method blanks showed WHO-TEQs between 0,002 and 0,030 pg/g fat. The latter value could cause a contribution of up to 19 % to the PCB TEQ-values determined for the cow's milk sample. Therefore the PCB data of the milk samples were corrected for blanks. The corrections were made on the basis of absolute blank concentrations of the different PCB congeners in the milk samples.

Two duplicate analyses for PCDF/Ds and PCBs were also included in the QS measures of the study. For this purpose one of the cow's milk samples with the highest PCDF/D and PCB content and one of the milk samples showing the lowest concentrations were re-analysed. The results of the repeated analyses of these samples are presented in the tables 3, 21, 40 and 58 of the annex. The results of the duplicate PCDF/D and PCB analyses showed good agreement. With respect to the TEQ-values, the differences between the single PCDF/D and PCB analyses of a sample were below 15 %. This lays well within the expected analytical precision (up to ± 20 %).

T a b l e s

Tab. 02: $^{13}C_{12}$-labelled internal standards used for quantification of the PCDF/Ds and Toxicity Equivalent Factors (TEFs) used for calculation of TE-values (TEQs)

PCDF/D parameter to be determined	Internal $^{13}C_{12}$-labelled standard used for quantification	I-TEF (1989)	WHO-TEF (1998)
PCDF congeners			
2,3,7,8-TetraCDF	$^{13}C_{12}$-2,3,7,8-Tetrachlorodibenzofuran [a]	0.1	0.1
1,2,3,7,8-PentaCDF	$^{13}C_{12}$-1,2,3,7,8-Pentachlorodibenzofuran	0.05	0.05
2,3,4,7,8-PentaCDF	$^{13}C_{12}$-2,3,4,7,8-Pentachlorodibenzofuran [a]	0.5	0.5
1,2,3,4,7,8-HexaCDF	$^{13}C_{12}$-1,2,3,4,7,8-Hexachlorodibenzofuran [a]	0.1	0.1
1,2,3,6,7,8-HexaCDF	$^{13}C_{12}$-1,2,3,6,7,8-Hexachlorodibenzofuran	0.1	0.1
2,3,4,6,7,8-HexaCDF	$^{13}C_{12}$-2,3,4,6,7,8-Hexachlorodibenzofuran	0.1	0.1
1,2,3,7,8,9-HexaCDF	$^{13}C_{12}$-1,2,3,7,8,9-Hexachlorodibenzofuran	0.1	0.1
1,2,3,4,6,7,8-HeptaCDF	$^{13}C_{12}$-1,2,3,4,6,7,8-Heptachlorodibenzofuran [a]	0.01	0.01
1,2,3,4,7,8,9-HeptaCDF	$^{13}C_{12}$-1,2,3,4,7,8,9-Heptachlorodibenzofuran	0.01	0.01
OctaCDF	$^{13}C_{12}$-1,2,3,4,6,7,8,9-Octachlorodibenzofuran [a]	0.001	0.0001
PCDD congeners			
2,3,7,8-TetraCDD	$^{13}C_{12}$-2,3,7,8-Tetrachlorodibenzo-p-dioxin [a]	1.0	1.0
1,2,3,7,8-PentaCDD	$^{13}C_{12}$-1,2,3,7,8-Pentachlorodibenzo-p-dioxin [a]	0.5	1.0
1,2,3,4,7,8-HexaCDD	$^{13}C_{12}$-1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin	0.1	0.1
1,2,3,6,7,8-HexaCDD	$^{13}C_{12}$-1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin [a]	0.1	0.1
1,2,3,7,8,9-HexaCDD	$^{13}C_{12}$-1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin	0.1	0.1
1,2,3,4,6,7,8-HeptaCDD	$^{13}C_{12}$-1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin [a]	0.01	0.01
OctaCDD	$^{13}C_{12}$-1,2,3,4,6,7,8,9-Octachlorodibenzo-p-dioxin [a]	0.001	0.0001

a $^{13}C_{12}$-labelled internal standard for which the recovery through the extract clean-up is reported in the tables of results

Tab. 03: HRGC/HRMS operation parameters for the PCDF/D analysis of cow's milk samples

Capillary gas chromatograph, Hewlett Packard 5890	
Injection:	PTV injection, 4 µl injection volume, solvent: toluene
GC-Column:	DB-5, 60 m x 0,25 mm ID, film thickness: 0,25 µm
Carrier gas:	Helium
Temperature program:	3 min 80 °C isotherm, at 40 °C/min up to 200 °C, 0.5 min at 200 °C isotherm, at 2 °C/min up to 270 °C at 5 °C/min to 320 °C, 10 min 320 °C isotherm
High resolution mass spectrometer, VG-AutoSpec	
Transfer line:	320 °C
Source temperature:	300 °C
Ionization:	EI positive, electron energy: 34 eV
Mass reference:	PFK (Perfluorokerosene)
Mass resolution:	≥ 8000
Mode:	SIM, monitoring of several masses of the molecular ion duster, setting of time windows

Tab. 04: $^{13}C_{12}$-labelled internal standards used for quantification of the WHO-PCBs and Toxicity Equivalent Factors (TEFs) used for calculation of TE-values (TEQ)

PCB congeners		**Internal $^{13}C_{12}$-labelled standard used for quantification**	**WHO-TEF (1998)**
Chlorosubstitution Pattern	IUPAC Number		
3,4,4‘,5-Tetrachlorobiphenyl	PCB 81	$^{13}C_{12}$-3,4,4‘,5-Tetrachlorobiphenyl ($^{13}C_{12}$-PCB 81)	0.0001
3,3‘,4,4‘-Tetrachlorobiphenyl	PCB 77	$^{13}C_{12}$-3,3‘,4,4‘-Tetrachlorobiphenyl ($^{13}C_{12}$-PCB 77) [a]	0.0001
2‘,3,4,4‘,5-Pentachlorobiphenyl	PCB 123 [b]	$^{13}C_{12}$-2‘,3,4,4‘,5-Pentachlorobiphenyl ($^{13}C_{12}$-PCB 123)	0.0001
2,3‘,4,4‘,5-Pentachlorobiphenyl	PCB 118	$^{13}C_{12}$-2,3‘,4,4‘,5-Pentachlorobiphenyl ($^{13}C_{12}$-PCB 118)	0.0001
2,3,4,4‘,5-Pentachlorobiphenyl	PCB 114	$^{13}C_{12}$-2,3,4,4‘,5-Pentachlorobiphenyl ($^{13}C_{12}$-PCB 114)	0.0005
2,3,3‘,4,4‘-Pentachlorobiphenyl	PCB 105	$^{13}C_{12}$-2,3,3‘,4,4‘-Pentachlorobiphenyl ($^{13}C_{12}$-PCB 105)	0.0001
3,3‘,4,4‘,5,-Pentachlorobiphenyl	PCB 126	$^{13}C_{12}$-3,3‘,4,4‘,5,-Pentachlorobiphenyl ($^{13}C_{12}$-PCB 126) [a]	0.1
2,3‘,4,4‘,5,5‘-Hexachlorobiphenyl	PCB 167	$^{13}C_{12}$-2,3‘,4,4‘,5,5‘-Hexachlorobiphenyl ($^{13}C_{12}$-PCB 167)	0.00001
2,3,3‘,4,4‘,5-Hexachlorobiphenyl	PCB 156	$^{13}C_{12}$-2,3,3‘,4,4‘,5-Hexachlorobiphenyl ($^{13}C_{12}$-PCB 156)	0.0005
2,3,3‘,4,4‘,5‘-Hexachlorobiphenyl	PCB 157	$^{13}C_{12}$-2,3,3‘,4,4‘,5‘-Hexachlorobiphenyl ($^{13}C_{12}$-PCB 157)	0.0005
3,3‘,4,4‘,5,5‘-Hexachlorobiphenyl	PCB 169	$^{13}C_{12}$-3,3‘,4,4‘,5,5‘-Hexachlorobiphenyl ($^{13}C_{12}$-PCB 169) [a]	0.01
2,3,3‘,4,4‘,5,5‘-Heptachlorobiphenyl	PCB 189	$^{13}C_{12}$-2,3,3‘,4,4‘,5,5‘-Heptachlorobiphenyl ($^{13}C_{12}$-PCB 189)[a]	0.0001

a $^{13}C_{12}$-labelled internal standard for which the recovery through the extract clean-up is reported in the tables of results in the annex

b coelutes with PCB 106

Tab. 05: HRGC/HRMS operation parameters for the PCB analysis of cow's milk samples

Capillary gas chromatograph, Hewlett Packard 5890	
Injection:	PTV injection, 2 µl injection volume, solvent: methyl acetate / hexane
GC-Column:	HT-5, 25 m x 0,22 mm ID, film thickness: 0,10 µm
Carrier gas:	Helium
Temperature program:	4 min 100 °C isotherm, at 30 °C/min up to 143 °C, 1 min at 143 °C isotherm, at 1.5 °C/min up to 200 °C at 5 °C/min to 280 °C, 9 min 280 °C isotherm
High resolution mass spectrometer, VG-AutoSpec	
Transfer line:	320 °C
Source temperature:	300 °C
Ionization:	EI positive, electron energy: 34 eV
Mass reference:	PFK (Perfluorokerosene)
Mass resolution:	≥ 8000
Mode:	SIM, monitoring of several masses of the molecular ion duster, setting of time windows