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Simultaneous determination of planar chlorobiphenyls and polychlorinated dibenzo-p-dioxins and -furans in Dutch milk using isotope dilution and gas chromatography-high-resolution mass spectrometry

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ABSTRACT

A method is described for the simultaneous determination of planar chlorobiphenyls and dioxins in milk using isotope dilution and gas chromatography-high-resolution mass spectrometry (GC-MS). The method is based on gel permeation chromatography, alumina clean-up and carbon chromatography and is highly automated, making a high sample throughput possible. Data on recovery, accuracy and reproducibility of results obtained with quality control samples are presented. Data for both dioxins and planar chlorbiphenyls from the analysis of samples of Dutch milk from several areas in the Netherlands are also presented. Possible interference of the chlorobiphenyls in the determination of the dioxins in the GC-MS method is discussed.

INTRODUCTION

As the knowledge of the toxicological behaviour of organic contaminants increases, so does the need for more sensitive and more accurate methods of analysis. An example is the determination of planar chlorobiphenyls (CBs), *i.e.,* 3,4,3′,4′-tetrachlorobiphenyl (CB 77), 3,4,5,3′,4′-pentachlorobiphenyl (CB 126) and 3,4,5,3′,4′,5′-hexachlorobiphenyl (CB 169) [1]. The toxicities of individual CBs have been reviewed by McFarland and Clarke [2]. The planar (non-o&o-substituted) CBs seem to have much higher toxicity than the non-planar compounds [2–4]. However, naturally occurring levels of these planar compounds in biological samples are much lower than those of the non-planar compounds. Therefore, an extensive clean-up of samples, including separation of planar and non-planar CBs, is necessary.

Methods for the determination of planar CBs in different types of matrices have been reported by several groups [5–8]. The clean-up procedures used are very similar to those used in the determination of dioxins, including the use of carbon chromatography to separate planar and non-planar compounds. The actual determination has been carried out using gas chromatography (GC) with either electron-capture of mass spectrometric (MS) detection [5–8]. The latter is also the method of choice for determining dioxins. Further, the concept of toxicity equivalence factors (TEF) has been developed [9]. However, the TEF values for dioxins [11] have been internationally accepted where as those for PCBs are still the subject of discussion. Therefore, we refer to TEF values for dioxins as I-TEF values. With such close similarities it is obvious to combine the determination of dioxins and planar CBs, which

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may result in more information at approximately the same expense.

In this paper we describe the simultaneous determination of dioxins and planar CBs in the same extract. As the analytical procedures for the separate determination of both planar CBs and dioxins have already been described [10], only relevant modifications are given. Emphasis is on the MS aspects of combining CBs and dioxins in the same MS procedure. Further, a survey study was carried out concerning the dioxin and planar PCB contents of Dutch milk originating from various areas in the Netherlands.

EXPERIMENTAL

The materials and methods used have been described previously [10]. To perform isotope dilution for the determination of the planar chlorobiphenyls ^{13}C-labelled analogues of CB 77, CB 126 and CB ‘169 were purchased from Cambridge Isotope Laboratories (Woburn, MA, USA). All reagents used were of analytical-reagent grade.

Control samples

One quality control sample (QCS 1) was a batch of milk fat originating from an industrial area in the Netherlands. Repeated analysis revealed that this sample contained 2.62 pg of TEQ per gram of fat (2,3,7,8-TCDD toxicity equivalent) originating from the dioxins and *ca.* 4, 25 and 5 pg per gram of fat of CB 77, CB 126 and CB 169, respectively. Using the TEF factors proposed by Van Zorge [9], this corresponds to a total of 2.56 pg TEQ per gram of fat originating from the planar CBs.

The other quality control sample (QCS 2) was milk fat accurately spiked with PCDD/PCDF and planar CBs. Milk fat was first decontaminated using active carbon. Thereafter, amounts of 2 pg per gram per compound of native PCDD/PCDF were added. The octachlorinated compounds were added at a level of 4 pg per gram of fat. The native planar CBs were added at a level of 5 pg/g each for CB 77 and CB 169 and 10 pg/g for CB 126. This resulted in an artificially contaminated batch of milk fat theoretically containing 5.85 pg/g of I-TEQ originating from the dioxins and 1.08 pg/g of TEQ from the planar CBs.

Survey samples

Thirty-nine milk samples from various areas in the Netherlands were collected. Each sample consisted of 1 1 of cows’ milk taken form the pooled milk of the cooperating dairy farms. The samples were stored at -20°C until fat extraction was performed.

Of these samples, seventeen samples were taken from different agricultural areas, five originated from an industrial area, seven were taken from farms near two different municipal waste incinerators and ten from farms that kept their dairy cows on the outlying land of the main rivers of the Netherlands (see Table III).

Sample extraction and clean-up

The clean-up procedure has been described previously [10] and only minor adjustments were made here. To combine the elution of the planar CBs with the elution of the dioxins, the carbon column is eluted in the back-flush mode immediately after the solvent change from dichloromethaneecyclohexane (1: 1, v/v) to toluene. To apply isotope dilution to both dioxins and chlorobiphenyls, the fat was not only fortified with 10 pg/g each of the ^{13}C-labelled PCDDs and PCDFs but also with 10 pg/g each of the ^{13}C-labelled planar CBs. As especially CB 77 is much more volatile than the dioxins and the other CBs, 50 μl of dodecane is used as a keeper throughout the clean-up procedure. However, after the carbon chromatography no keeper is used as complete evaporation of the solvent is required. Although the use of isotope dilution also permits automatic correction for evaporation losses, a low recovery causes a high limit of detection. Therefore, evaporation to dryness has to be performed very carefully in order to prevent unnecessary evaporation losses.

Gas chromatography-mass spectrometry

The conditions used have been described previously [10]. Mass spectrometric data acquisition for both dioxins and planar CBs is based on selected ion retrieval (SIR) at mass resolution 10 000. To determine the planar CBs, the number of ions to be monitored had to be extended, resulting in a slightly decreased sensitivity for the tetra- and pentachlorinated dioxin congeners compared with the separate determination of dioxins.

Table I gives an overview of the ions measured within each time window.

TABLE I

SOME ACQUISITION AND IDENTIFICATION PARAMETERS FOR POLYCHLORINATED DIOXINS AND DIBENZOFURANS AND CHLORINATED BIPHENYLS, INCLUDING THEIR ^{13}C-LABELLED ANALOGUES, ACCORDING TO EPA RECOMMENDATIONS FOR DIOXIN ANALYSIS

Compound	Selected ion ratio, A/B[b]	m/z (A)	m/z (B)	Theoretical abundance (A/B)
PCB 77	M/M + 2	289.92	291.92	0.77
[^{13}C]PCB 77	M/M + 2	301.96	303.96	0.77
PCB-126	M/M + 2	323.88	325.88	0.62
TCDF	M/M + 2	303.90	305.90	0.77
TCDD	M/M + 2	319.90	321.89	0.77
[^{13}C]PCB 126	M/M + 2	335.92	337.92	0.62
[^{13}C]TCDF	M/M + 2	315.94	317.94	0.77
[^{13}C]TCDD	M/M + 2	331.94	333.93	0.77
PCB 169	M/M + 2	357.85	359.84	0.51
PeCDF	M + 2/M + 4	339.86	341.86	1.55
PeCDD	M + 2/M + 4	355.85	357.85	1.55
[^{13}C]PCB 169	M/M + 2	369.89	371.88	0.51
[^{13}C]PeCDF	M + 2/M + 4	351.90	353.90	1.55
[^{13}C]PeCDD	M + 2/M + 4	367.89	369.89	1.55
HxCDF	M + 2/M + 4	373.82	375.82	1.24
HxCDD	M + 2/M + 4	389.82	391.81	1.24
[^{13}C]HxCDF	M/M + 2	383.86	385.86	0.51
[^{13}C]HxCDD	M + 2/M + 4	401.86	403.85	1.24
HpCDF	M + 2/M + 4	407.78	409.78	1.03
HpCDD	M + 2/M + 4	423.78	425.77	1.03
[^{13}C]HpCDF	M/M + 2	417.83	419.82	0.44
[^{13}C]HpCDD	M + 2/M + 4	435.82	437.81	1.03
OCDF	M + 2/M + 4	441.74	443.74	0.89
OCDD	M + 2/M + 4	457.74	459.73	0.89
[^{13}C]OCDD	M + 2/M + 4	469.78	471.78	0.89

[a] TCDF = Tetrachlorinated dibenzofuran; TCDD = tetrachlorinated dibenzodioxin; PeCDF = pentachlorinated dibenzofuran; PeCDD = pentachlorinated dibenzodioxin; HxCDF = hexachlorinated dibenzofuran; HxCDD = hexachlorinated dibenzodioxin; HpCDF = heptachlorinated dibenzofuran; HpCDD = heptachlorinated dibenzodioxin; OCDF = octachlorinated dibenzofuran; OCDD = octachlorinated dibenzodioxin.

[b] M = molecular ion, containing ^{35}Cl exclusively.

Calibration was performed using seven calibration standards for the dioxins ranging from 100 fg/μl to 10 pg/μl. For the planar CBs only one calibration standard was used, containing 5 pg/μl for CB 77 and CB 169 and 10 pg/μl for CB 126.

RESULTS AND DISCUSSION

Mass spectrometric aspects

For the simultaneous determination of planar CBs and dioxins to be performed, some ions characteristic of the planar CBs have to be measured in addition to the ions characteristic for the dioxins. Table I gives an overview of the ions measured within each time window. The diagnostic ions for the dioxins are in accordance with EPA recommendations. In comparison with the separate determination of dioxins, measuring a larger number of ions causes some decrease in sensitivity. This decrease, depending on the number of extra ions to be measured, is about 20% and is limited to the time windows in which tetra- and pentachlorinated dioxins elute together with CB 126 and CB 169, respectively. As the limit of determination for the dioxins is sufficiently low, a small decrease in sensitivity can be tolerated.

From the data presented in Table I, possible MS interferences become clear. First, MS interferences only exist when compounds are unresolved by GC. Critical GC-separations, where MS interference also may occur, are the separation between 1,2,3,4-TCDD and 2,3,7,8-TCDD, CB 169 and 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDF and 1,2,3,6,7,8-HxCDF and 1,2,3,4,7,8-HxCDD and 1,2,3,6,7,8-HxCDD. The differences in retention times are all *ca.* 10 s, which is the absolute minimum for GC resolution (Fig. IA-D).

Most of these critical separations already existed in the determination of dioxins without incorporating the planar CBs, as three of the four critical separations concern two dioxin or furan congeners. Incorporating the planar CBs in the method adds only one critical separation, i.e., that of CB 169 and 1,2,3,7,8-PeCDD.

The M^+ and $[M+2]^+$ ions of hexachlorobiphenyl (a.o. CB 169) and the $[M+4]^+$ and $[M+6]^+$ ions of pentachlorodioxin with their respective m/z valves of 357.8517, 359.8415, 357.8517 and 359.8490 are unresolved by MS at mass resolution 10 000. It is worth noting that the $[M+6]^+$ ion of pentachlorodioxin is not a diagnostic ion and is therefore not mentioned in Table I. Nevertheless, it is present and selecting the $[M+2]^+$ ion of hexachlorobiphenyl will give rise to a signal due to the $[M+6]^+$ ion of PeCDD.

As CB 169 is a hexachlorobiphenyl, interference may therefore occur. It is obvious that incomplete removal of other non-planar hexachlorobiphenyls that co-elute with either CB 169 or 1,2,3,7,8-PeCDD will give rise to interference, indicating the

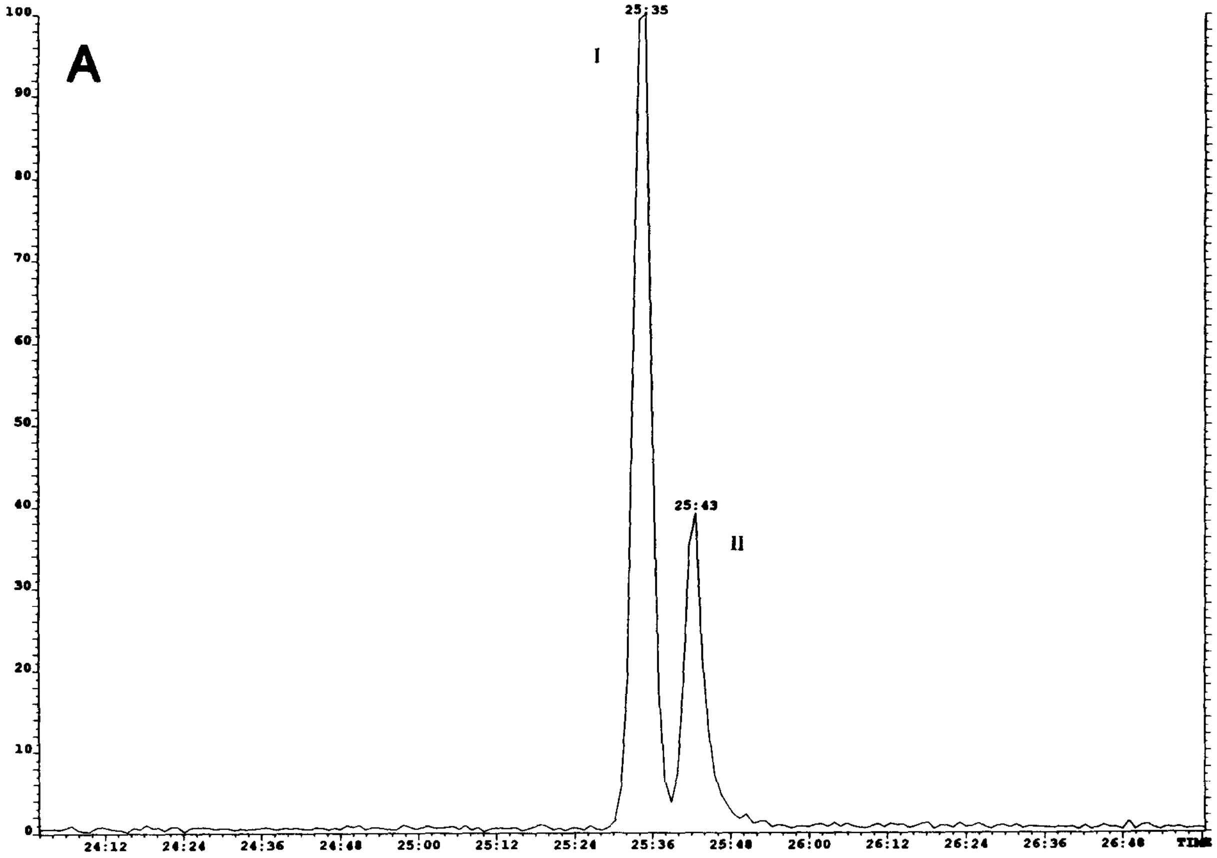

Fig. 1

necessity to separate non-planar from planar compounds. Especially when there is a large difference in concentration between the two compounds, which may easily occur in some types of biological samples, interference can be expected to result in a faulty isotope ratio and high results for the compound subject to interference.

The $[M + 2]^+$ ion of PeCDD (m/z 355.8546), which is one of the diagnostic ions, and the M^+ ion (m/z 353.8576), are unaffected by the presence of hexachlorobiphenyl. In fact, chosing the M^+ ion of PeCDD instead of $[M + 4]^+$ as the diagnostic ion offers the possibility of avoiding any MS interference due to hexachlorobiphenyls on the PeCDD. There will still be interference of the PeCDD on the hexachlorobiphenyl but, considering the small TEF value of CB 169 (0.005), the effect of slightly higher results for CB 169 is negligible when expressed as the TEQ value of the sample. On the other hand, 1,2,3,7,8-PeCDD has a TEF value of 0.5 and high results for this compound will give rise to a significantly higher I-TEQ value for the sample compared with the non-interfered with result of analysis and should therefore be avoided.

It is worth noting that even when CB 169 is not a target compound, interference of CB 169 on 1,2,3,7,8-PeCDD may still occur when a cleaning procedure is used that is not capable of separating the planar CBs from the dioxins.

Another possible MS interference is caused by the tetrachlorinated dioxins. The ions $[M + 4]^+$ and $[M + 6]^+$ of TCDD interfere with the M^+ and $[M + 2]^+$ ions, respectively, of pentachlorobiphenyl. No interferences of the CB on the TCDD occurs.

However, GC resolution of CB 126 and 2,3,7,8-TCDD is readily achieved. Especially in environmental analysis, other TCDD congeners may be

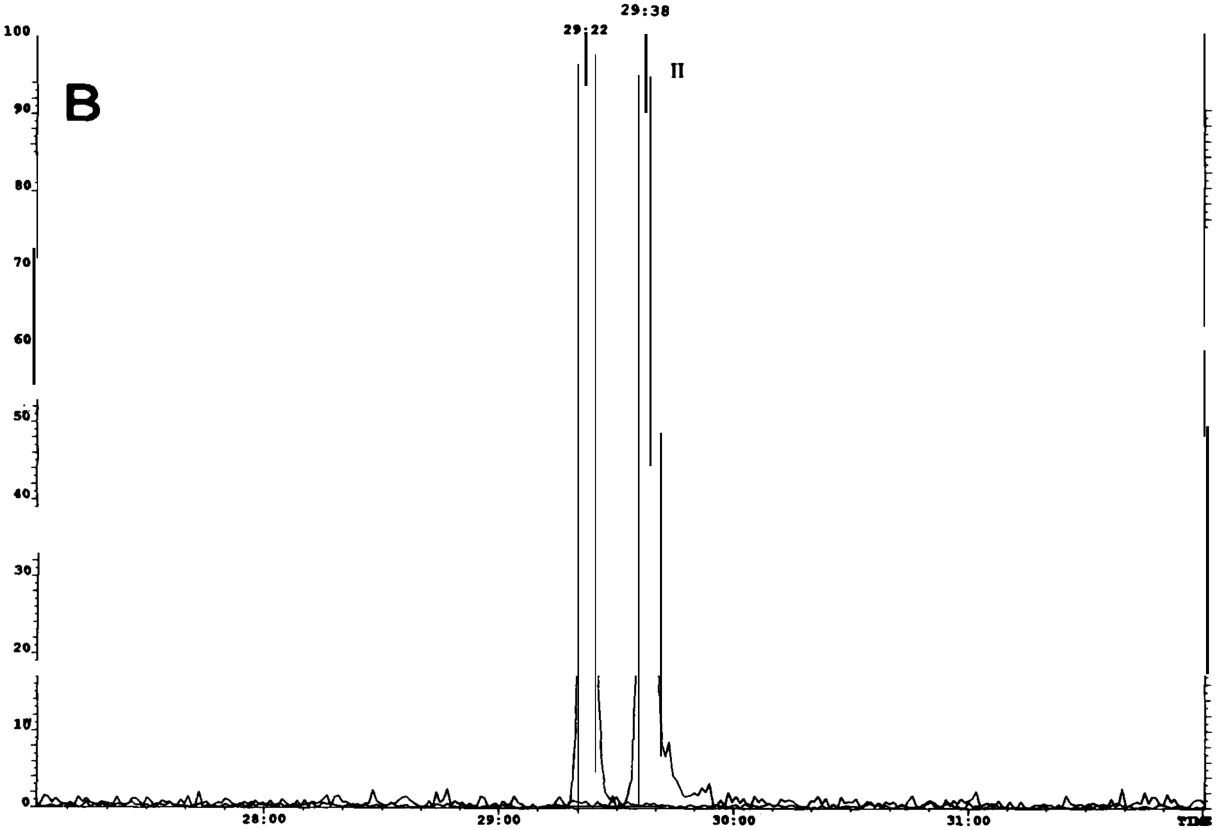

Fig. 1.

(Continued on p. 302)

present that may co-elute with CB 126. In that event interference may occur.

The last planar CB, *i.e.*, CB 77, elutes earlier than the tetrachlorinated dioxins and therefore no interference is expected. In our opinion, it is therefore advantageous to combine the determination of the planar CBs and dioxins. As mentioned before, calibration for the determination of dioxins is performed using as calibration graph consisting of seven different concentration levels whereas calibration for the determination of planar CBs only uses one calibration level. We found that linearity of the calibration graph for the planar CBs is better than or at least equal to that for the dioxins. This is probably due to the better GC properties of the CBs. Hence, when linearity of the dioxins is checked daily, as is common practice in our laboratory, and found to be satisfactory, there is no need to check for calibration linearity for the CBs and single point-calibration can be used without any problems.

Clean-up aspects

To combine the dioxins and the planar CBs in the same extract, the porous graphitized carbon (PGC) column has to be eluted in the back-flush mode immediately after the solvent change from dichloromethane-cyclohexane to toluene. This may alter the elution profile of the CBs and therefore this aspect was investigated. The elution profile shows a shoulder peak for all planar CBs, probably due to switching to the back-flush mode at a relatively high flow-rate. However, carbon chromatography is used for preparative purposes and therefore these shoulder peaks can be tolerated. More serious is the fact that the planar CBs show severe tailing on the PGC column. Collection of the eluate was therefore extended from 15 to 30 min. The total elution time of the column with toluene in the back-flush mode was also extended to 60 min. The planar CBs show much greater tailing than the dioxins, indicating a stronger adherence of these solutes to the sorbent.

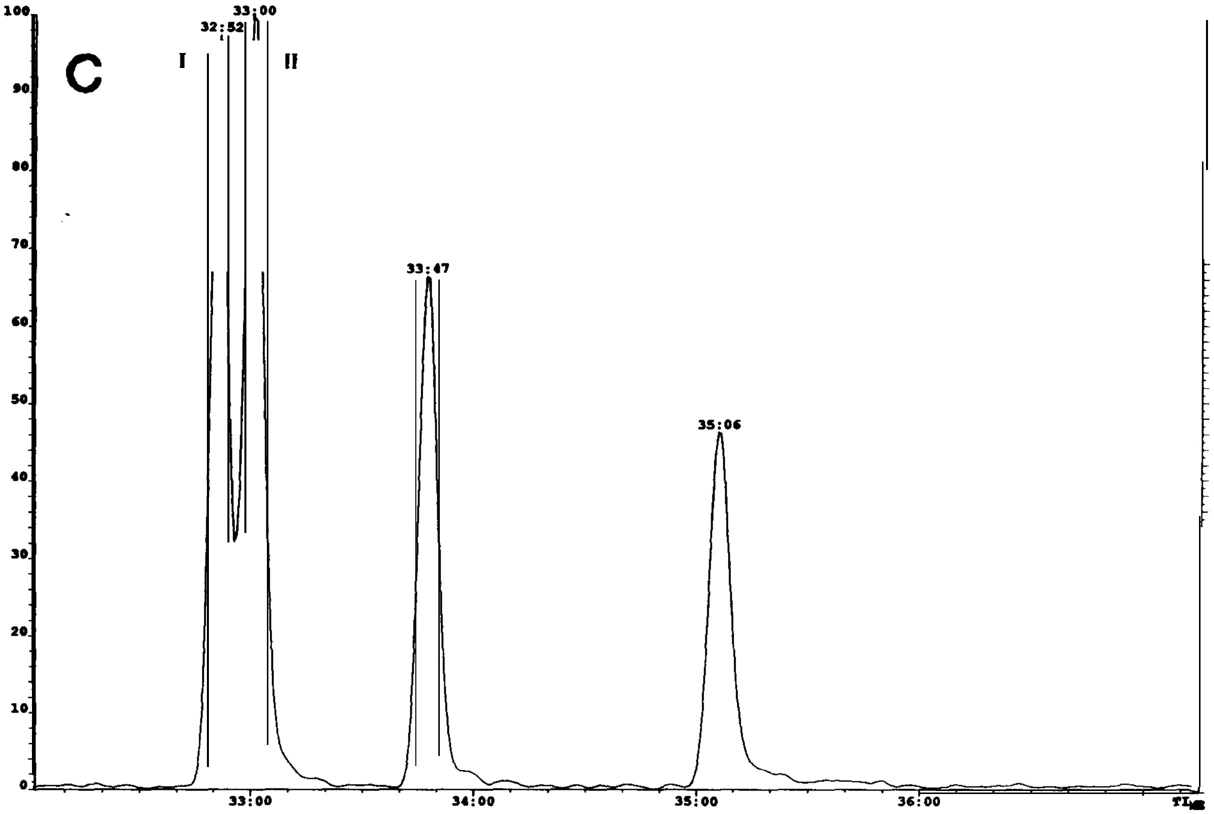

Fig. 1.

A stronger interaction of the planar CBs than the dioxins with the carbon sorbent is unexpected, as the dioxins are perfectly planar compounds whereas CBs are only planar or approximately planar owing to steric hindrance of the attached chlorine atoms.

In order to improve the peak shape of the planar CBs on he PGC column we used several eluents, including benzene and tetrahydrofuran–toluene mixtures, but tailing was not removed or decreased. This tailing may aiso be the cause of the contaminated blanks that were encountered. Blanks do not contain significant amounts of dioxins but they may contain planar CBs. Especially CB 77 is present in appreciable and widely varying amounts up to about 1 pg/μl. CB 126 and CB 169 are present in much lower concentrations. In terms of TEQ value, the influence of the presence of CB 77 is almost negligible as the proposed TEF value for this component and its natural occurence are only small. Further, CB 126, with its high occurrence and proposed TEF value, contributes more than 90% of the PCB TEQ value of a sample. For this reason and because the actual contamination of sample extracts with CB 77 not originating from the sample cannot be estimated properly, results of analysis are not corrected for blank values.

Quality control samples

The quality control procedure and the performance of the method for the determination of dioxins has been described previously [10]. Therefore, attention is focused on the quality control (QC) aspects for the determination of planar CBs. As described under Experimental, two QC samples are used; QCS1 is a naturally contaminated milk fat and QCS2 is a decontaminated and subsequently

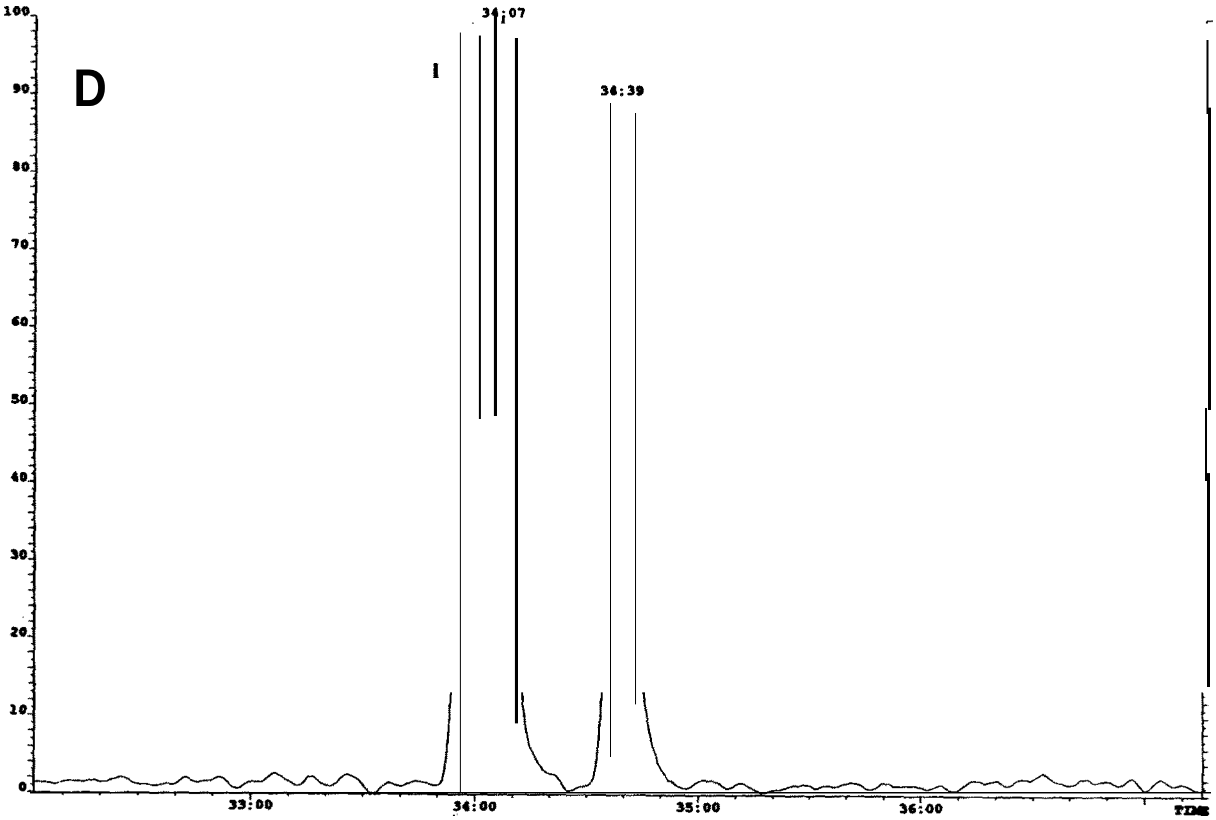

Fig. 1. Parts of chromatograms showing critical separations in the determination of planar CBs and dioxins. (A) (I) 1,2,3,4-TCDD and (II) 2,3,7,8-TCDD; (B) (I) PCB 169 and (II) 1,2,3,7,8-PeCDD; (C) (I) 1,2,3,4,7,8-HxCDF and (II) 1,2,3,6,7,8-HxCDF; (D) (I) 1,2,3,4,7,8-HxCDD and (II) 1,2,3,6,7,8-HxCDD.

TABLE II

MEAN RESULTS OF ANALYSIS OF PLANAR CHLORINATED BIPHENYLS FROM REPEATED ANALYSIS OF QC SAMPLES

	QCS1			QCS2			
	Recovery (%)	Mean” (pg/g)	C.V. (%)	Recovery (%)	Mean^b (pg/g)	C.V. (%)	Accuracy (%)
CB 77	48	4.4	13.1	43	6.5	6.4	131
CB 126	50	25.9	10.3	46	10.6	5.9	110
CB 169	39	5.3	28.9	43	4.1	6.9	95
TEQ		2.56	5.2		1.24	2.8	111

[a] n = 8.
[b] n = 11.

spiked sample of milk fat of exactly known content. In Table II data from repeated analysis of both QC samples are presented. Fig. 2 gives the QC charts for the determination of the TEQ value due to planar CBs.

The accuracy for QCS1 cannot be estimated because, being a naturally contaminated and not a spiked sample, its true value is unknown. Nevertheless, the results are reproducible with relative standard deviations (R.S.D.) of 5–10%. The amount of CB 77 may be high owing to high blanks. For true samples, CB 126 seems to make by far the largest contribution to the TEQ value. Not only is its proposed TEF the largest of the planar CBs, but the levels of occurrence are also the highest. In QCS1 about 95% of the PCB TEQ value is due to CB 126.

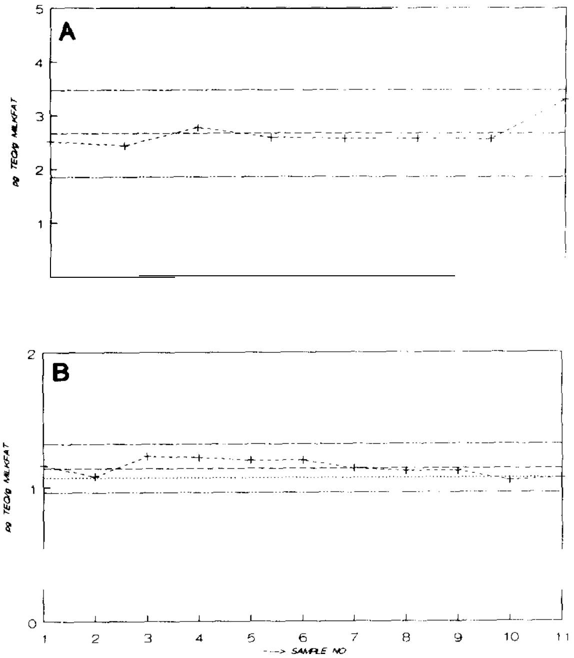

Fig. 2. Quality control charts based on data resulting from the determination of planar CBs in (A) quality control sample 1 and (B) quality control sample 2 (see text). + (- - -) = Sample; ——— = mean; -_ _ = +3 S.D.; —·—·— = −3 S.D.; ····· = theoretical.

QCS2 was spiked with 5 pg/g each of CB 77 and CB 169 and 10 pg/g of CB 126. It is clear from Table III that the results for CB 77 are systematically high. The results for CB 126 and CB 169 are, in our opinion, fairly good concerning both accuracy and reproducibility. Results for the QC samples concerning the determination of dioxins are comparable to the previously presented data [10].

Table II is based on only 8–11 determinations of the QC samples. As with the QC programme for the determination of dioxins, the number of QC data will increase as more samples are analysed. Nevertheless, the initial results are encouraging although the problems with contaminated blanks have still to be solved. The amount of the main component, CB 126, can be determined accurately and consequently so can the PCB TEQ value.

Survey samples

Table III gives the mean results of the analysis for milk samples collected from several areas in the Netherlands. The TEF values used for the PCBs are 0.01, 0.1 and 0.005 for CB 77, CB 126 and CB 169

TABLE III

ORIGIN OF THE SURVEY SAMPLES AND MEAN RESULTS OF ANALYSIS FOR DIOXINS AND PLANAR CHLORINATED BIPHENYLS

Origin	*n*	Dioxins I-TEQ (pg/g)	Amount (pg/g) CB 77	CB 126	CB 169	PCBs TEQ (pg/g)
Agricultural area						
Friesland	4	0.8	3.3	11.6	2.0	1.1
Woerden	5	1.8	2.8	18.0	2.1	1.9
Achterhoek	3	1.6	3.2	18.8	2.8	1.9
Betuwe	5	1.5	6.1	11.7	2.1	1.9
Industrial area						
Rijnmond	5	2.1	3.3	***20.5***	4.0	2.1
Near municipal waste incinerator						
Duiven	2	3.6	2.8	17.9	3.8	1.8
Rijnmond	5	7.1	11.4	42.9	10.2	4.5
Outlying land of main rivers						
Roer/Meuse	5	1.5	11.9	29.1	4.0	3.1
Rhine/Yssel/Waal	5	3.0	4.9	30.8	4.3	3.2

respectively [9]. Fig. 3 gives a graphical representation of the 39 individual data. The samples are grouped together according to the type of area where they were collected.

It should be noted that the presented samples are not characteristic of the whole of the Netherlands. These samples were taken from selected areas to obtain impression of the order of magnitude and

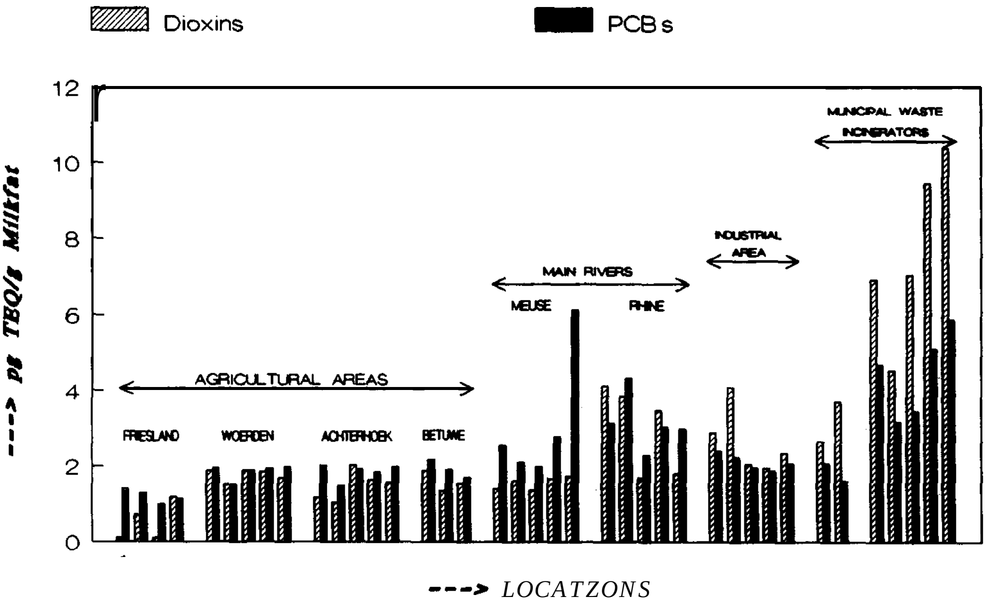

Fig. 3. Results of the determination of planar CBs and dioxins in Dutch cows' milk from several areas in the Netherlands, expressed as pg TEQ and pg I-TEQ, respectively, per gram of milk fat.

the relationship between CB and dioxin contamination in different types of areas.

As expected, the samples from agricultural areas are the least contaminated, with minimum contamination in Friesland, a relatively remote area, whereas samples from the surroundings of municipal waste incinerators are most contaminated. For the samples originating from agricultural areas the contamination with dioxins and CBs expressed as TEQ value generally seems to be of the same order of magnitude with slightly higher PCB TEQ values. The contamination with CBs in industrial areas is only slightly higher but significantly more dioxins were found. The reverse is true for the outlying land of the river Roer and Meuse; the dioxin levels are approximately the same as in agricultural areas but the CB levels are higher, probably because these rivers flow through a mining area where CBs might have been used as hydraulic fluids in the past. The river Rhine and its side rivers show both higher dioxin and higher CB levels.

The results for the samples taken near municipal waste incinerators need some extra comment. One of the incinerators is relatively old-fashioned and is located in an industrial area. Both CBs and dioxins show high levels of contamination compared with the agricultural areas. Especially the dioxin contamination is higher by a factor of about five; CB contamination is higher by a factor of about two. Compared with the industrial area these factors are about three and two, respectively. The other incinerator is located in an agricultural area and samples from the surroundings do not show higher CB contamination, the dioxin levels being higher by about a factor of two. This indicates, as expected, that CB contamination is a more general problem whereas dioxin contamination is mainly caused by local sources of pollution.

CONCLUSIONS

Combination of the determination of planar CBs with that of dioxins has been shown to be possible without introducing serious difficulties. One minor drawback is the small decrease in sensitivity for the tetra- and pentachlorinated dioxins. The clean-up procedure gives reproducible and accurate results for CB 126 and CB 169. Blanks contaminated with CB 77 are obtained, causing systematically high results for the determination of this compound. When expressed as a PCB TEQ value, the impact of this error is negligible. A survey study on 39 selected milk samples from several areas in the Netherlands gave good results, showing that dioxin contamination is mainly caused by local sources whereas CBs are thought to be evenly distributed throughout the environment.

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