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# ANALYSIS OF ORGANOCHLORINE PESTICIDES, POLYCHLORINATED BIPHENYLS, DIBENZO-*p*-DIOXINS AND DIBENZOFURANS IN HUMAN MILK BY EXTRACTION WITH THE LIPOPHILIC GEL LIPIDEX® 5000

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## SUMMARY

A method for multicomponent determination of organochlorine contaminants in human milk is described. The lipophilic gel Lipidex® 5000 was used for extraction of lipids and organochlorine compounds. Further purification and separation was achieved by chromatography on partly deactivated aluminium oxide, Lipidex, silica gel and active basic and acidic aluminium oxide. The concentrations of pesticides and polychlorinated biphenyls (PCBs) were determined by electron-capture gas chromatography. Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) were determined by selected-ion monitoring capillary column gas chromatography–mass spectrometry at a resolution of 8000–9000. The levels of 2,2-bis(4-chlorophenyl)-1,1,1-trichloroethane, 1,1-bis(4-chlorophenyl)-2,2-dichloroethene, hexachlorobenzene, $\alpha$-hexachlorocyclohexane, $\beta$-hexachlorocyclohexane, dieldrin, *trans*-nonachlor, oxychlordane and PCBs found by this method agreed with the levels determined using a previous solvent-extraction method. Recovery experiments were performed by addition of pentachlorophenol and certain PCDDs and PCDFs. The average recovery of 0.5–2.0 ng pentachlorophenol per ml milk was 92%. Ten PCDDs and PCDFs reported to occur in milk were added at levels between 0.5 and 50 pg per ml milk. Recoveries of these compounds were on an average 79–91%.

## INTRODUCTION

The first observation of dichlorodiphenyltrichloroethane (DDT) in human milk was made by a spectrophotometric method [1]. Later, the availability of sensitive electron-capture gas chromatography (GC) and mass spectrometry (MS) enabled the detection of many stable, lipophilic pesticides and industrial pollutants in human milk. Levels of the commonly found compounds, DDT and its metabolites, hexachlorobenzene, isomers of hexachlorocyclohexane (HCH), dieldrin,

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*trans*-nonachlor, oxychlordane and polychlorinated biphenyls (PCBs), have been reported from many countries and reviews have been published [2,3]. More recently, camphechlor [4], polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) have also been detected in milk [5–7]. The analytical methods generally involve initial solvent extraction of lipids and contaminants. The subsequent separation of pollutants from endogenous lipids often presents considerable problems. In the analyses of pesticides and PCBs, partitioning between solvents of different polarity, precipitation of lipids by freezing at −70°C, saponification and chromatography using aluminium oxide, silica gel and Florisil, for example, have been used for purification and separation [3,8–10]. The extracts are often treated with concentrated sulphuric acid to remove endogenous compounds. However, this destructive procedure also affects some pesticides, e.g. dieldrin. In the analysis of PCDDs and PCDFs, special techniques have been used with multiple clean-up steps involving different adsorbents [11,12], chemically modified adsorbents [13,14] complemented by high-performance liquid chromatography [6] and gel-permeation chromatography followed by adsorption chromatography [7]. For pentachlorophenol (PCP), which is also present in milk, repeated extractions from acidic and basic media have been used [15]. Depending on their concentrations, the final analysis of organochlorine contaminants is performed by electron-capture GC or GC–MS.

This report describes the use of a lipophilic gel, Lipidex® 5000, for extraction and preliminary purification of organochlorine compounds from human milk. Using different adsorbents for subsequent separation, the method can be applied to multicomponent determination of organochlorine pesticides, PCBs, PCDDs and PCDFs in milk.

## EXPERIMENTAL

### *Materials*

Pooled samples of human milk were supplied from the Mothers' Milk Centre (Stockholm, Sweden). The samples were stored at −20°C.

### *Solvents and reagents*

All solvents were of analytical-reagent grade. Hexane, acetonitrile, chloroform and methylene chloride were redistilled. Pyridine was refluxed with calcium hydride, redistilled and stored over potassium hydroxide. Methanol was left with sodium hydroxide for 24 h and then distilled twice [16]. Water was deionized and purified with a Milli Q® cartridge (Millipore, Bedford, MA, U.S.A.). Formic acid, acetic anhydride and sulphuric acid (Merck, Darmstadt, F.R.G.) were used as supplied. Acidified methanol was prepared by addition of 1 ml of sulphuric acid to 100 ml of methanol. Acetylating reagent was prepared just before use by mixing 1.0 ml of pyridine and 0.4 ml of acetic anhydride.

Pesticides used for standard solutions were of at least 99% purity (K & K Labs., New York, NY, U.S.A.; Vesicol Chemical, Chicago, IL, U.S.A.; Riedel-de Haën, Seelze-Hannover, F.R.G.). Pentachlorophenyl acetate was prepared according to Rudling [17]. Clophen A50 (Bayer, Leverkusen, F.R.G.) was used as a standard

for determination of PCBs [10]. PCDDs and PCDFs (Cambridge Isotope Labs., Wobum, MA, U.S.A.) were of at least 98% purity and the following compounds were used: [$^{13}C_{12}$]2,3,7,8-tetrachlorodibenzo-*p*-dioxin ($^{13}$C-TCDD), 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (PeCDD), 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin (HxCDD), 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (HpCDD), [$^{13}C_{12}$]octachlorodibenzo-*p*-dioxin ($^{13}$C-OCDD), 2,3,7,8-tetrachlorodibenzofuran (TCDF), 2,3,4,7,8-pentachlorodibenzofuran (PeCDF), 1,2,3,6,7,8-hexachlorodibenzofuran (HxCDF), 1,2,3,4,6,7,8-heptachlorodibenzofuran (HpCDF) and octachlorodibenzofuran (OCDF). Decachlorobiphenyl (DCB) was used as an internal standard for volume correction in the determination of PCDDs and PCDFs, $\alpha$-HCH was used for the same purpose in the determination of PCP and 4,4′-dichlorobiphenyl (4,4′-PCB) for the other compounds. Aroclor 5460 (Monsanto, St. Louis, MO, U.S.A.) and Nibren-Wachs D130 (Bayer) were used to study possible interferences by polychlorinated terphenyls (PCTs) and polychlorinated naphthalenes (PCNs), respectively.

*Column chromatography*

Chromatography was performed in glass columns with a PTFE stopcock. The columns had a sintered glass disc (1 and 2 cm I.D.) or a plug of cotton wool (0.4 cm I.D.) at the end. The chemically pure cotton was washed with hexane and diethyl ether on a Büchner funnel and dried. The solvents used for chromatography were stored over anhydrous sodium sulphate.

Lipidex 5000 (Packard Instrument, Downers Grove, IL, U.S.A.) was washed with 20 and 50% aqueous ethanol and ethanol at 70°C [18] and stored in methanol at 4°C. Immediately before use, it was washed with methanol on a Büchner funnel, and the solvent was removed by continued suction. Aluminium oxide 90 (activity grade II–III, Merck) was activated at 800°C for 4 h and partly deactivated with 5% of water. Basic and acidic aluminium oxide (Woelm, Eschwege, F.R.G.) were heated at 450°C overnight and allowed to cool at 130°C. Silica gel 60, 70–230 mesh (Merck), was heated for at least 48 h at 130°C and stored at this temperature. Anhydrous sodium sulphate (Merck) was dried at 450°C overnight and stored in a desiccator with silica gel.

*Gas chromatography and gas chromatography–mass spectrometry*

GC was performed using a Pye Unicam GCV instrument with a $^{63}$Ni electron-capture detector. Glass columns (2 m×2 mm I.D.) were packed with a mixture (32:68, w/w) of 3% silicone GE SF-96 and 6% DC QF-1 on Chromosorb W HP, 100–120 mesh, and used for analyses of the fraction containing PCBs. Fused-silica capillary columns (25 m×0.32 mm I.D.) were coated with a 0.25-$\mu$m layer of cross-linked OV-1701 (Orion, Helsinki, Finland) and used with an all-glass falling-needle injection system for analyses of other contaminants.

Selected-ion monitoring GC–MS was performed on a VG 7070E instrument (VG Analytical, Manchester, U.K.) equipped with a VG 11-250 data system. Fused-silica capillary columns (25 m×0.32 mm I.D.) coated with a 0.25-$\mu$m layer of cross-linked methyl silicone (Quadrex, New Haven, CT, U.S.A.) or unicoat methyl silicone (KSV, Helsinki, Finland) were used isothermally at 150°C for 1

TABLE I

IONS SELECTED IN THE GC–MS DETERMINATION OF PCDDs AND PCDFs

| Compound | Ions, *m/z* |
|---|---|
| TCDD | 319.8965 ($M^+$), 321.8936 |
| [$^{13}$C]TCDD | 331.9367 ($M^+$), 333.9338 |
| PeCDD | 353.8576 ($M^+$), 355.8546 |
| HxCDD | 387.8186 ($M^+$), 389.8156, 391.8127 |
| HpCDD | 423.7767 ($[M+2]^+$), 425.7737 |
| OCDD | 457.7377 ($[M+2]^+$), 459.7347 |
| [$^{13}$C]OCDD | 469.7779 ($[M+2]^+$), 471.7749 |
| TCDF | 303.9016 ($M^+$), 305.8986 |
| PeCDF | 337.8626 ($M^+$), 339.8597 |
| HxCDF | 371.8237 ($M^+$), 373.8207, 375.8178 |
| HpCDF | 407.7817 ($[M+2]^+$), 409.7788 |
| OCDF | 441.7428 ($[M+2]^+$), 443.7398 |
| DCB | 423.7508 ($[M-Cl_2]^+$) |

min and then programmed at 10°C/min to 270°C. The column outlet extended into the ion source, which was at 250°C. An all-glass falling-needle injector was used with an injection heater at 250°C. Helium was the carrier gas. Samples were ionized by electron impact at 70 eV. The resolution was 8000–9000 at an accelerating voltage of 6 kV. The ions monitored are given in Table I. For each *m/z* value the dwell-time was 100 ms and the delay-time 20 ms. Three injections were made of each sample for analysis of TCDD, PeCDD–OCDD and TCDF–OCDF, respectively. The compounds were monitored in groups determined by the number of chlorine atoms in the molecule. The added standard of DCB was monitored in the same group as HpCDD or HpCDF. For each group, one ion from the column bleeding was selected as the lock mass. Thus, at most five *m/z* values were monitored in one group.

*Analytical procedures*

A flow scheme of the method is shown in Fig. 1.

*Extraction*

A 10-ml sample of human milk was weighed into a flask with a PTFE-lined screw cap and mixed with 10 ml of formic acid and 5.0 g of Lipidex 5000. The mixture was shaken at 35°C for 2.5 h and then transferred to a glass column (2 cm I.D.). The solvent was drained and the gel was washed with 40 ml of 30% methanol (fraction 1) followed by 40 ml of 50% methanol at a flow-rate of 2 ml/min (fraction 2). The chlorinated compounds and part of the lipids were eluted with 75 ml of acetonitrile (fraction 3), and remaining lipids with 60 ml of chloroform–methanol–hexane (1:1:1, v/v/v) (fraction 4). Fractions 3 and 4 were taken to dryness under reduced pressure at 35°C, and the residues were dried to constant weight in a desiccator with silica gel. The sum of the residues of fraction 3 and 4 was used for calculation of the fat content.

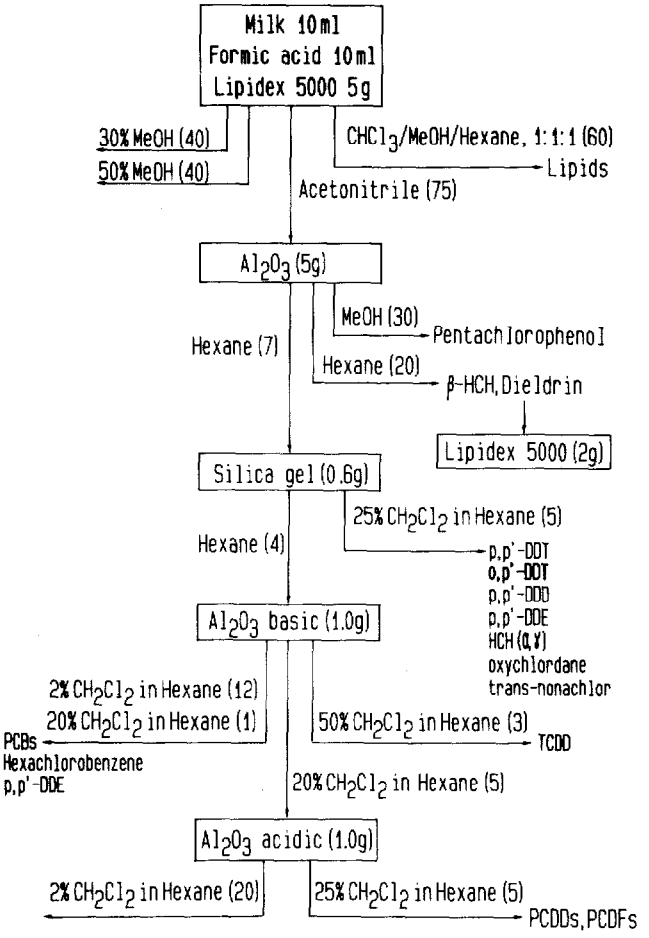


**Fig. 1. Schematic outline of the method. Numbers in parenthesis after solvents refer to millilitres of solvent used for elution.**

*Purification and separation*

*Aluminium oxide (partly deactivated).* A column (1 cm I.D.) was packed with 5.0 g of aluminium oxide (partly deactivated with 5% water) and washed with 10 ml of hexane. The material in fraction 3 from the extraction was quantitatively transferred with small volumes of hexane to the column with the stopcock closed. The excess of hexane was evaporated with a gentle stream of nitrogen. The column was eluted with 7.0 ml of hexane (fraction A1). This fraction contained PCBs, PCDDs, PCDFs, *p,p*-DDT, *o,p'*-DDT, 1,1-bis(4-chlorophenyl)-2,2-dichloroethane (*p,p'*-DDD), 1,1-bis(4-chlorophenyl)-2,2-dichloroethene (*p,p'*-DDE), hexachlorobenzene, $\alpha$-HCH, lindane, *trans*-nonachlor and oxychlordane. Another 20 ml of hexane (fraction A2) eluted $\beta$-HCH and dieldrin. PCP was eluted with 10 ml of acidified methanol and 20 ml of methanol (fraction A3).

*Silica gel.* PCBs, PCDDs and PCDFs were separated from most of the pesticides by chromatography on silica gel. Activated silica gel, 0.6 g, was transferred

to a column (0.4 cm I.D.) containing 2 ml of hexane. The column was equilibrated for 20 min and washed with 15 ml of hexane. Fraction A1 from the aluminium oxide column was evaporated to ca. 50 μl and transferred with three 50-μl portions of hexane to the silica gel column, which was then eluted with 4.0 ml of hexane (fraction S1). This fraction contained PCBs, PCDDs, PCDFs, hexachlorobenzene and some *p,p'*-DDE. An additional 5.0 ml of 25% methylene chloride in hexane (fraction S2) eluted α-HCH, lindane, oxychlordane, *trans*-nonachlor, *p,p'*-DDE (remaining part), *p,p'*-DDD, *o,p'*-DDT and *p,p'*-DDT. After addition of 1.0 ml of internal standard (0.15 μg 4,4'-PCB per ml hexane) the fraction was analysed by GC.

*Aluminium oxide, basic, activated.* Basic aluminium oxide (1 g) was dry-packed in a column (0.4 cm I.D.) followed by a 0.3-cm layer of anhydrous sodium sulphate. Fraction S1 was gently evaporated to 50 μl and quantitatively transferred to the column. The PCBs, hexachlorobenzene and *p,p'*-DDE were eluted with 12 ml of 2% methylene chloride and 1 ml of 20% methylene chloride in hexane (fraction Ab1). This fraction was concentrated to appropriate volume and analysed for PCBs, hexachlorobenzene and *p,p'*-DDE by GC (packed column). PCDDs (except TCDD) and PCDFs were eluted with 5.0 ml of 20% methylene chloride in hexane (fraction Ab2) and TCDD with 3 ml of 50% methylene chloride in hexane (fraction Ab3). Fraction Ab3 was concentrated (not do dryness) under a gentle stream of nitrogen, and 50 μl of internal standard (1 pg DCB per μl hexane) were added. After further concentration the fraction was analysed by GC–MS.

*Aluminium oxide, acidic, activated.* Acidic aluminium oxide (1 g) was dry-packed in a glass column (0.4 cm I.D.), and a 0.3-cm layer of anhydrous sodium sulphate was added to the top. Fraction Ab2 from the basic aluminium oxide column, containing PCDDs and PCDFs, was concentrated to ca. 50 μl and quantitatively transferred to the column. A first eluate of 20 ml of 2% methylene chloride in hexane was discarded. The subsequent eluate of 5.0 ml of 25% methylene chloride (fraction AS2) contained PCDDs and PCDFs. After addition of 100 μl of internal standard (1 pg DCB per μl hexane) and appropriate concentration, the fraction was analysed by GC–MS.

*Lipidex 5000.* Hexane (10 ml) was added to 2 g of moist Lipidex 5000, and the mixture was transferred to a glass column (1 cm I.D.). Fraction A2 from the initial aluminium oxide column, containing dieldrin and β-HCH, was evaporated to 1 ml and transferred quantitatively with hexane to a 3-ml centrifuge tube. The solvent was gently evaporated with nitrogen to ca. 50 μl, and the residue was transferred quantitatively with small portions of hexane to the column. The first eluate of 6 ml of hexane (fraction L1) contained impurities and was discarded. The second fraction of 3.0 ml of hexane (fraction L2) contained dieldrin. Then, 4 ml of 50% methylene chloride in hexane were transferred to and passed through the column and the eluate (fraction L3) was discarded. An additional 12 ml of 50% methylene chloride in hexane eluted β-HCH (fraction L4). After appropriate concentration and addition of internal standard, fractions L2 and L4 were analysed by GC. If fraction L4 was not sufficiently clean for GC it was shaken for 30 s with an equal volume of concentrated sulphuric acid and centrifuged. The

Lipidex column was restored to the original volume by elution with hexane. After equilibration in hexane overnight it could be reused.

*Pentachlorophenol.* Fraction A3 from the initial aluminium oxide column was evaporated to ca. 1 ml. The residue was quantitatively transferred to a 15-ml tube using hexane to a total volume of 4 ml. A 1-ml volume of internal standard (6 ng $\alpha$-HCH per ml hexane) was added, and the mixture was shaken for 1 min. The tube was placed in an ice-bath, 5 ml of 90% sulphuric acid were added, and the tube was turned up and down six times. After centrifugation, the hexane layer was transferred to a 5-ml tube and concentrated to 1 ml, 150 $\mu$l of acetylating reagent were added, and the tube was placed in an ultrasonic bath for 15 min. The mixture was washed with 4 ml of 0.01 *M* potassium carbonate and centrifuged. The hexane phase was concentrated to ca. 0.1 ml and transferred to a silica gel (0.6 g) column prepared as described above. The sample was rinsed with 0.5 ml of hexane, and an eluate of 4 ml of 25% methylene chloride in hexane was collected. The fraction was concentrated and analysed by GC on the capillary column.

## RESULTS

Human milk samples that had previously been analysed with respect to organochlorine pesticides and PCBs were analysed by the present method. The previous method involved extraction with diethyl ether–light petroleum followed by partitioning between hexane and dimethyl formamide (containing 8% water) and chromatography on aluminium oxide and silica gel [10]. PCBs were analysed by GC using a packed column and Clophen A50 as a standard to get the same conditions as in earlier investigations [10,19]. Congruent levels of pesticides and PCBs were obtained by the two methods (Table II). The agreement in values for fat content was also good (Table III).

Recovery experiments were performed by addition of 50–100 $\mu$l of a solution containing different amounts of PCP and a solution containing certain PCDDs and PCDFs. Fortified and non-fortified samples were run in parallel. The average recovery of 0.5–2.0 ng PCP per ml milk was 92% (range 82–101%, $n=8$). The average recoveries of PCDDs and PCDFs were 79–91% (range 60–104%) (Table IV).

The selected-ion chromatograms obtained in analyses of PCDDs and PCDFs in pooled milk samples indicated absence of major interfering compounds (Figs. 2 and 3). Isotope ratios were within 10% of the calculated values. Possible interferences in the analyses of PCDDs and PCDFs by camphechlor, PCBs, PCTs (Aroclor 5460) and PCNs (Nibren-Wachs D130) were studied by taking these substances through the analytical system. Selected samples were analysed by monitoring the *m/z* values of molecular ions of PCBs and polychlorinated diphenyl ethers. Interferences by these substances were not found at the retention times of the specific PCDDs and PCDFs analysed.

TABLE II

LEVELS OF ORGANOCHLORINE CONTAMINANTS IN HUMAN MILK ANALYSED BY A PREVIOUS AND THE PRESENT METHOD

| Compound | Sample | Previous method* (mg/kg of fat) | Present method (mg/kg of fat) | | $n$** |
|---|---|---|---|---|---|
| | | | Average | Range | |
| *p,p'*-DDT | A | 0.16 | 0.15 | 0.131–0.158 | 3 |
| | B | 0.11 | 0.095 | 0.092–0.100 | 4 |
| | C | 0.093 | 0.088 | 0.084–0.095 | 4 |
| *p,p'*-DDE | A | 1.07 | 1.10 | 1.07 –1.13 | 2 |
| | B | 1.09 | 0.99 | 0.97 –1.00 | 3 |
| | C | 0.99 | 0.94 | 0.92 –0.99 | 4 |
| $\alpha$-HCH | A | 0.006 | 0.006 | 0.005–0.006 | 2 |
| | B | 0.003 | 0.003 | 0.003–0.003 | 3 |
| | C | 0.006 | 0.006 | 0.006–0.006 | 4 |
| $\beta$-HCH | A | 0.10 | 0.10 | 0.095–0.104 | 4 |
| | B | 0.085 | 0.086 | 0.078–0.090 | 3 |
| Oxychlordane | C | 0.009 | 0.010 | 0.009–0.012 | 4 |
| *trans*-Nonachlor | A | 0.009 | 0.009 | 0.007–0.010 | 3 |
| | B | 0.014 | 0.015 | 0.014–0.017 | 4 |
| | C | 0.009 | 0.012 | 0.012–0.012 | 4 |
| Dieldrin | A | 0.022 | 0.024 | 0.022–0.026 | 4 |
| | B | 0.021 | 0.022 | 0.017–0.025 | 4 |
| | C | 0.015 | 0.016 | 0.015–0.016 | 4 |
| Hexachlorobenzene | A | 0.13 | 0.12 | 0.114–0.116 | 3 |
| | B | 0.11 | 0.097 | 0.085–0.110 | 3 |
| | C | 0.086 | 0.070 | 0.065–0.078 | 4 |
| PCBs | A | 0.76 | 0.80 | 0.76 –0.82 | 4 |
| | B | 0.82 | 0.79 | 0.75 –0.83 | 4 |
| | C | 0.86 | 0.90 | 0.83 –0.93 | 4 |

*Ref. 10; one analysis only.
**Number of analyses by present method.

TABLE III

CONCENTRATION OF LIPIDS IN MILK SAMPLES ANALYSED BY A PREVIOUS AND THE PRESENT METHOD

| Sample | Previous method ($n=1$) | Present method ($n=4$) (weight %) | |
|---|---|---|---|
| | | Average | Range |
| A | 3.18 | 3.04 | 2.97–3.17 |
| B | 2.93 | 2.93 | 2.90–2.94 |
| C | 3.24 | 3.30 | 3.22–3.37 |

TABLE IV

RECOVERIES OF PCDDs AND PCDFs ADDED TO MILK SAMPLES

| Compound | Amount added (pg/ml of milk) | Number of samples | Recovery (%) | |
|---|---|---|---|---|
| | | | Average | Range |
| [$^{13}$C]TCDD | 0.5 | 8 | 91 | 64-100 |
| PeCDD | 0.5- 1.0 | 8 | 88 | 73-100 |
| HxCDD | 2.5- 5.0 | 10 | 86 | 60- 97 |
| HpCDD | 2.5- 5.0 | 10 | 79 | 63- 95 |
| [$^{13}$C]OCDD | 10.0-50.0 | 10 | 91 | 78-100 |
| TCDF | 0.5 | 6 | 90 | 74- 98 |
| PeCDF | 2.5- 5.0 | 8 | 90 | 64-100 |
| HxCDF | 1.0- 5.0 | 8 | 83 | 65-104 |
| HpCDF | 1.0 | 6 | 81 | 68- 98 |
| OCDF | 0.8 | 7 | 88 | 75-100 |

## DISCUSSION

One aim of this study was to obtain a method for analysis of the most common pesticides, PCBs, PCDDs and PCDFs, in a single milk sample. This causes special problems, as pesticides and PCBs are present in much higher concentrations than PCDDs and PCDFs and may interfere with the analysis of these compounds. It also demands precautions to prevent the decomposition of certain compounds. Neutral methods for analysis of TCDD in bovine milk [11] and PCDDs and PCDFs in liver samples [20] have been published, but analyses of pesticides and PCBs were not included. Usually more destructive methods are used, i.e. saponification with hot alkali [12] and purification by extraction with concentrated sulphuric acid and chromatography on adsorbents impregnated with strong acids or bases [13,14]. Hot alkali destroys not only DDT but also OCDD and OCDF [20], and sulphuric acid decomposes dieldrin. This makes these procedures unsuitable for multicomponent analyses.

Another aim was to simplify the preliminary extraction and purification procedure. Lipidex 1000 was previously shown to be an efficient sorbent of lipids and [$^{14}$C]*p,p'*-DDT from cow's milk, and the pesticide could be eluted separated from 90% of the lipids [21]. When applied to human milk this method was not sufficiently effective. Lipidex 1000 is a hydroxyalkylated derivative of Sephadex LH-20 with a hydroxyalkyl group content of 10 weight%. The less polar Lipidex 5000, which has a hydroxyalkyl group content of 50%, was found to be more suitable in the present study. Extraction with a solid sorbent has advantages compared with solvent partitioning as no emulsions are formed and passage of the sample through a column bed replaces repeated extraction and centrifugation steps. By performing the extraction at 35°C, aggregation of the gel and lipids was avoided and complete incorporation of lipids and pollutants into the gel was achieved.

Addition of formic acid to the milk was essential for quantitative extraction of lipids and organochlorine contaminants in the chylomicrons. Formic acid has previously been used in solvent extractions of insecticides from plasma [22,23].

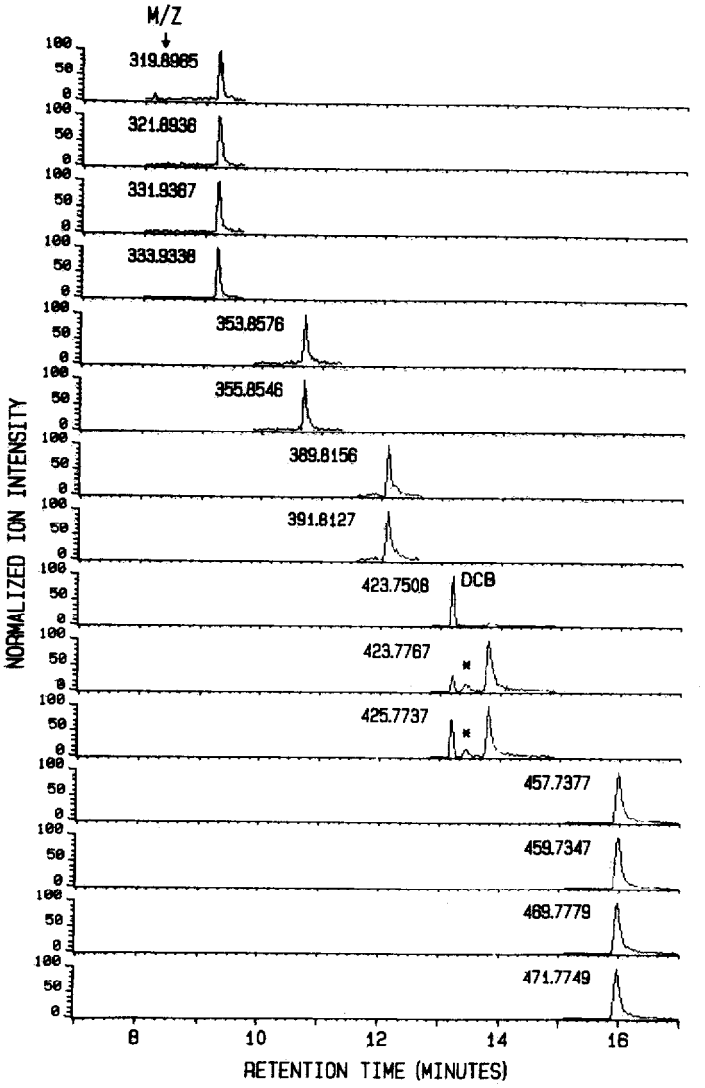


Fig. 2. Selected-ion monitoring of PCDDs in human milk from 1972. For ions monitored see Table I. Peaks labelled with an asterisk are not due to PCDD. DCB was added before injection.

A comparative study showed that recoveries of DDT and HCH isomers were higher in the presence than in the absence of formic acid [24]. The acid was assumed to decrease binding of the chlorinated contaminants to the plasma proteins [23]. In the analysis of milk it is possible that formic acid disrupts the chylomicrons, thus facilitating the extraction of organochlorine compounds in the lipid core (cf. ref. 25).

The Lipidex column could be eluted with up to 50% aqueous methanol to remove polar components. To include all the organochlorine compounds studied, the column was eluted with 75 ml of acetonitrile, which also released 25–50% of the lipids. The latter had to be removed, which was done with partly deactivated aluminium oxide. Most of the organochlorine contaminants were eluted from this

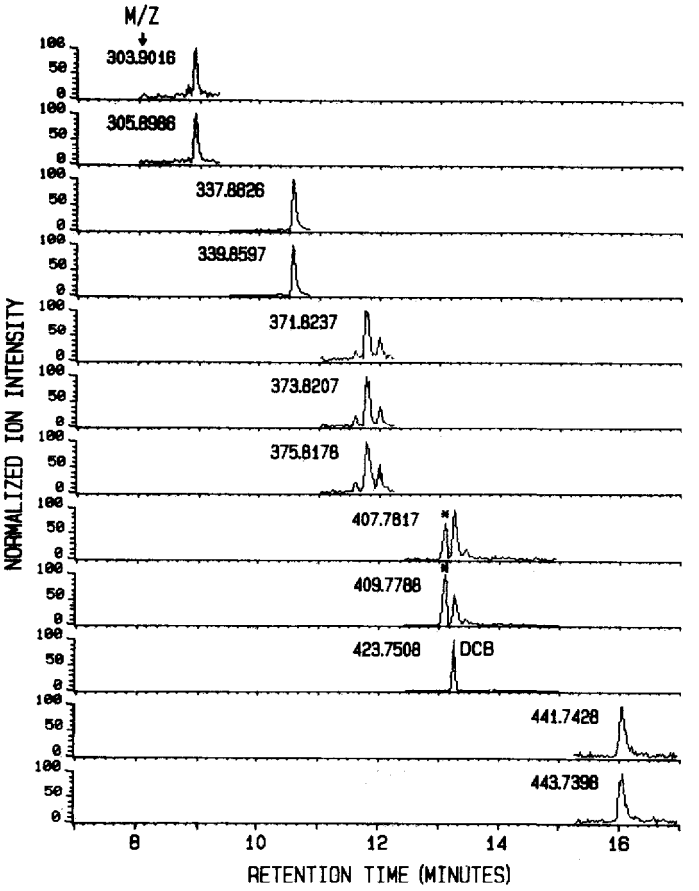


Fig. 3. Selected-ion monitoring of PCDFs in human milk from 1972. For ions monitored see Table I. Peaks labelled with an asterisk are not due to PCDF. DCB was added before injection.

column with the first 7 ml of hexane (A1) and this fraction was subjected to chromatography on silica gel and activated basic and acidic aluminium oxide for further separation of the compounds. The second fraction from aluminium oxide (A2) was not clean enough for GC analysis. Dieldrin and $\beta$-HCH were therefore analysed after separation on Lipidex 5000 in a straight-phase system. The third fraction from aluminium oxide (A3) contained PCP and lipids. The analysis of PCP was performed immediately after elution to prevent losses. A gentle extraction with 90% sulphuric acid removed the impurities without affecting PCP. The over-all blank value, using 10 ml of water instead of milk, corresponded to 0.03–0.06 ng of PCP per ml.

Previous methods for analysis of PCDDs and PCDFs have included several clean-up steps with a variety of sorbents. An early method for analysis of TCDD in 150-ml samples of milk utilized solvent partitioning and purification on magnesia-Celite 545, aluminium oxide and Florisil [11]. In another method, 20–30 g of milk were refluxed with potassium hydroxide and subjected to solvent partitioning and chromatography on Florisil and aluminium oxide [12]. Later meth-

ods for determination of several congeners utilized the more elaborate systems described by Lamparski and Nestrick [26], which included series of adsorbents (silica gel and aluminium oxide) and silica gel impregnated with solutions of sulphuric acid, sodium hydroxide and silver nitrate. Final clean-up was achieved by high-performance liquid chromatography in two systems [6,13]. For environmental samples, Smith et al. [14] used activated carbon, silica gel, aluminium oxide and silica gels prepared with caesium hydroxide, potassium hydroxide and sulphuric acid. The principles of this method have been used by Rappe et al. [27]. Gel permeation on Bio-Beads and chromatography on Florisil have also been used [7].

In the present method, only common commercially available adsorbents have been used. The small amount of milk, 10 ml compared with 120 g and 150 ml used in the published investigations [6,7], permitted the use of small columns for separation. Consequently, small volumes of solvents were needed for fractionation, and impurities from solvents were kept to a minimum in the final concentrate. The PCDDs and PCDFs were separated from PCBs with highly activated aluminium oxide. The separation of PCDDs from PCBs with basic and acidic aluminium oxide was studied by Albro et al. [20]. They activated the sorbents at 130°C and dry-packed the column. In the present investigation a higher temperature, 450°C, was used for activation, which permitted a separation of TCDD from the other PCDDs and PCDFs on basic aluminium oxide. In this way the whole fraction containing TCDD could be used in the GS–MS analysis, which lowered the detection limit of this toxicologically important compound. A similar separation of TCDD from other PCDDs and PCDFs has recently been achieved with Woelms aluminium oxide B Super I, and was used for the determination of TCDD in PCP and 2,4-dichlorophenol [28]. In our experience the degree of activation is critical and differences between batches necessitate careful control of elution volumes.

The method described in this paper has been used to analyse samples of milk collected during the period 1972–1985. The results of this study will be reported separately.

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