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Determination of chlordane in foods by gas chromatography

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Abstract

Chlordane comes under the group of persistent organic pollutants, which, according to the Stockholm Convention, should be completely prohibited or widely limited. Within the Environmental and Health Monitoring Programme the National Institute of Public Health in Prague, Centre for the Hygiene of Food Chains in Brno, is involved in a project, which explores the dietary exposure of the population of the Czech Republic to chemical substances. In order to meet the demands it is necessary to obtain data which would contribute to a comprehensive description of exposure doses of the priority persistent organic pollutants based on the Stockholm Convention.

Hence alpha- and gamma-chlordane and oxychlordane were incorporated into the project in 2002. Chlordane was monitored in food samples of the so-called food basket of the Czech population. After culinary treatment the food samples underwent extraction, were purified (GPC, Florisil) and analysed (GC-ECD). The method for determination of chlordanes in food was developed in our laboratory and was validated. Internal standards were used to determine the recovery of the analytical procedure. The limits of quantification depended on the type of the matrix and ranged between 0.002 and 0.05 $\mu g\,kg^{-1}$. CRM 598 BCR and proficiency testing (FAPAS) are used to assure that the method provides data of required precision and accuracy. The method is accredited by the Czech Accreditation Institute. In the majority of analysed samples the content of chlordane was below the limit of quantification. The highest amount of chlordanes was found in freshwater fish (2.78 $\mu g\,kg^{-1}$), butter and vegetable fat. The results of monitoring are used for estimation of the dietary exposure of the population of the Czech Republic to these substances.

Keywords: Chlordane; Food; Gas chromatography; Analysis; Dietary exposure

1. Introduction

Chlordane belongs to the group of persistent organic pollutants (POPs), the use of which should, under the Stockholm Convention, be either completely prohibited or limited to a large extend (Blaha, 2001a, 2001b; Rice, 2003).

1.1. Chlordane properties

Chlordane is a broad-spectrum contact insecticide that has been applied to agricultural crops including vegetables, maize, other oilseeds, potatoes, sugarcane, sugar beet, fruits, nuts, cotton and jute. It has also been used extensively to eradicate termites. In the Czech Republic chlordane has never been produced or officially registered for use. The reason why it is found in foodstuffs in the Czech Republic is primarily due to the import of the above commodities (Persistent Organic Pollutants, 2003).

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Chlordane is almost insoluble in water and soluble in organic solvents; it is highly stable and semi-volatile. It is easily bound to water sediments and its bioconcentration takes place in the fat of organisms. These chemical properties of chlordane enable its transport to long distances (Persistent Organic Pollutants, 2003).

Technical chlordane is synthesised by Diels–Alder reaction of hexachlorocyclopentadien and cyclopentadien. The resulting compound is chlordene, with 6 atoms of chlorine, which is further chlorinated into technical chlordane. It is a mixture of more than 140 compounds. The main components of technical chlordane are alpha- and gamma-chlordane (8 atoms of chlorine), *cis* and *trans* nonachlor, and heptachlor; the latter is used separately as a pesticide. In the majority of organisms these compounds are metabolised into 2 persistent epoxides, i.e. heptachlorepoxide (with 7 chlorines) and oxychlordane (with 8 chlorines). There are however great differences in the rate of metabolism; for instance fish does not metabolise these compounds as readily as rats (Dearth & Hites, 1991a, 1991b; International Agency for Research (IARC), 2001; Vetter & Schurig, 1997).

Chlordane stimulates the central nervous system. The first symptom of intoxication is a sharper sensitivity to external stimuli. Later on restlessness, tremor, even epileptic spasms are observed. Symptoms of acute poisoning appear 45 min after consumption, although sometimes they appear as late as several hours afterwards. Chlordane damages the parenchyma organs. Cases of fat degeneration of the liver, even necrosis, kidney damage, haemorrhage in the lungs, liver, kidneys, heart muscle and intestine mucous membrane have been reported. Chlordane affects the metabolism and toxicity of a number of simultaneously applied substances by inducing the activity of hepatic microsome enzymes. In the course of metabolic transformation, metabolites which are more toxic than the original compounds, are generated. Analogous to other chlorinated insecticides, these substances are also deposited in the fat tissues of living organisms and are very slowly metabolised and excreted from the body in the stools, urine, and in nursing females also in the milk (Bondy et al., 2003; Campoy et al., 2001; Hirai & Tomokuni, 1991; Marhold, 1986; Vetter, Weichbrodt, Scholz, Luckas, & Oelschlager,; World Health Organization, 1984).

In terms of the hitherto known toxicological properties and existence of exposure limits, alpha-chlordane, gamma-chlordane and oxychlordane, heptachlor and heptachlor epoxide have been recommended for monitoring the human dietary exposure.

Evaluations of the dietary exposure involve estimations of chlordane content as the sum of contents of alpha-chlordane, gamma-chlordane and oxychlordane (Codex Alimentarius; Nakata et al., 2002; Veter et al.).

1.2. Monitoring of chlordane and chlordane-related substances

Unsystematic monitoring of residues of selected chlorinated pesticides and later also of PCBs had been conducted in the Czech Republic as early as the 1970s and 1980s, particularly thanks to the State Veterinary Administration and Public Health Protection Bodies. The reason why this group of substances was included among analytes monitored in the foodstuffs of the so-called food basket within the system of monitoring the health condition of the population of the Czech Republic in relation to the environment, which was initiated by Government Decision 369/1991 and has been implemented on a national scale since 1994 under the guidance of the National Institute of Public Health, was the awareness of the importance of the health issue and the necessity of obtaining integrated information (Cerna & Bencko, 2001).

Within "The Project on Dietary Exposure to Selected Chemical Substances" the National Institute of Public Health in Prague, Centre for the Hygiene of Food Chains in Brno, participates in a project, the objective of which is to describe the dietary exposure of the population of the Czech Republic to chemical substances (Ruprich, 1995). Within the above group of substances, heptachlor and heptachlorepoxide have been monitored in the framework of this project since 1994.

In order to generate data, which would contribute to a complete description of the exposure doses of the priority POPs according to the Stockholm Convention, alpha- and gamma-chlordane and oxychlordane were incorporated into the project in 2002 (Blaha, 2001a, 2001b). Fig. 1 gives the formula of the analytes.

1.3. The possibility of chlordane determination

For the isolation of chlordane from samples of foodstuffs different extraction methods are used, for example liquid–liquid extraction, extraction with hot solvent (Soxtec), extraction with solvent at high speed, solid phase extraction (SPE), solid phase microextraction (SPME), supercritical fluid extraction (SFE), matrix solid phase dispersion (MSPD), semi-permeable membrane diffusion (SPMD), microwave-assisted extraction (MAE), pressurized solvent extraction (PSE) (Baiada-Pereira, Concha-Grana, & Gonzalez-Castro, 2003; Barker, 1998; Bulletin 900B, 1999; Muccio et al., 1997; Jayaraman, Pruell, & McKinney, 2001; Nguyen & Nau, 1996; Page & Lacroix, 1997; Prados-Rosales, García, & Castro, 2003; Tomkins & Barnard, 2002; Wang, Wang, Ma, Wang, & Mo, 2001).

Because chlordane occurs in foodstuffs in trace amounts (about 0.1 $\mu g\,kg^{-1}$), it is necessary to use relatively large amount of the samples for the extraction. Extraction with solvent at high speed is used for the so-

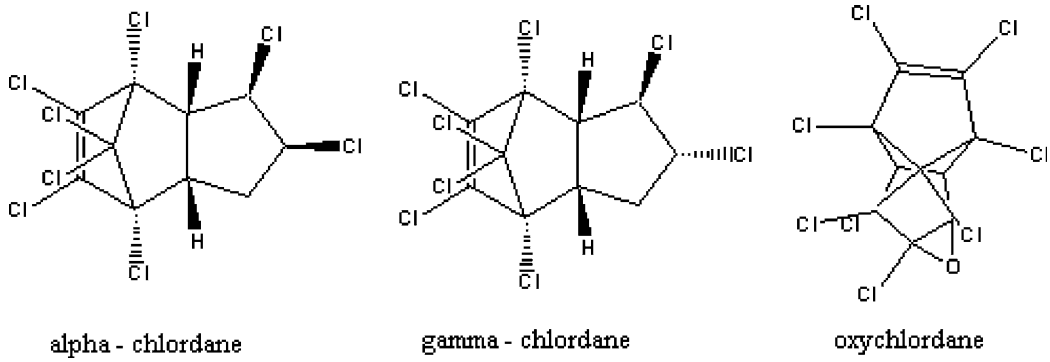

Fig. 1. Chemical formulae of chlordane.

lid samples. Liquid–liquid extraction is used for the liquid samples.

First of all chlordane is determined by gas chromatography with electron capture detector (GC-ECD) or gas chromatography with mass spectrometry (GC-MS) (Concha-Grana et al., 2002; Janouskova, Krbuskova, Klimova, Rehurkova, & Ruprich, 2003; Markova, Krbuskova, Antosova, Rehurkova, & Ruprich, 2003; Rood, 1991).

2. Material and methods

The method for chlordanes determination in foods was developed in our laboratory.

2.1. Samples

Chlordane is determined in food samples in the range of the so-called food basket of foodstuffs of the Czech population. The selection is based on the conception of monitoring the dietary exposure (Ruprich, 1995). It involves a total of 108 composite samples collected at 12 places (Fig. 2) representative of the Czech Republic. A list of samples is given in Table 1.

The food samples collected in the market of the Czech Republic were first of all subjected to culinary treatment so that they could be analysed in the same condition as they are consumed. The result of the pre-analytical treatment is a homogenous sample, which is then analysed (Benes et al., 2003; Ruprich, 1997).

2.2. Chemicals and their treatment

Standard solutions of alpha-chlordane (concentration 10 ng μl^{-1}, Dr. Ehrenstorfer), gamma-chlordane (concentration 10 ng μl^{-1}, Dr. Ehrenstorfer) and oxychlordane (concentration 100 ng μl^{-1}, Dr. Ehrenstorfer) are used. Standard solutions of monobrombiphenyl (PBB 1) (concentration 10 ng μl^{-1}, Dr. Ehrenstorfer) and PCB 209 (concentration 10 ng μl^{-1}, Dr. Ehrenstorfer) are used as internal standards. Petroleum ether (p.a., the fraction 40–55 °C) and acetone (p.a., Lachema) are used as solvents. Dichloromethane, diethyl ether and *iso*-octane (Merck) and Florisil (Sigma–Aldrich) are used to clean the samples up. Sodium sulphate (oven heated up to 750 °C for 12 h), a saturated solution of sodium oxalate, saturated solution of sodium chloride and acetonitrile (Merck) are also used in the process.

2.2.1. Florisil activation

For column chromatography Florisil is first of all put into an oven heated to 650 °C for 8 h. Before use Florisil is gradually heated up to 180 °C for 6 h. After cooling in a desiccator, 5 g of re-distilled water are added to 100 g of Florisil. The contents of the flask are shaken several times. After 12 h of standing in darkness the Florisil may be used to prepare the columns for isolation of the studied analytes from the extracts of the composite samples. Florisil activated in this way can be used maximally for 48 h.

2.2.2. Rectification of the solvents

The quality of the solvents for residual analyses of the pesticides is tested and if this analytical quality is satisfactory, the solvents are used directly.

Rectification of petroleum ether and acetone is conducted in an atmospheric, bubble-plate rectification column or in a packed rectification column. The columns are equipped with spiral reflux condensers. The receiver is heated in a water bath. Petroleum ether is a fraction of hydrocarbons from boiling point of 30–65 °C. During rectification, the fraction from 40 to 55 °C is collected. Acetone (p.a.) is rectified in water bath of temperature of 70 °C. Before use, the purity of the rectified solvents is verified by gas chromatography.

2.3. Equipment

The Polytron PT 3000 or PT 3100 homogenisers are used for sample preparation. Gel permeation

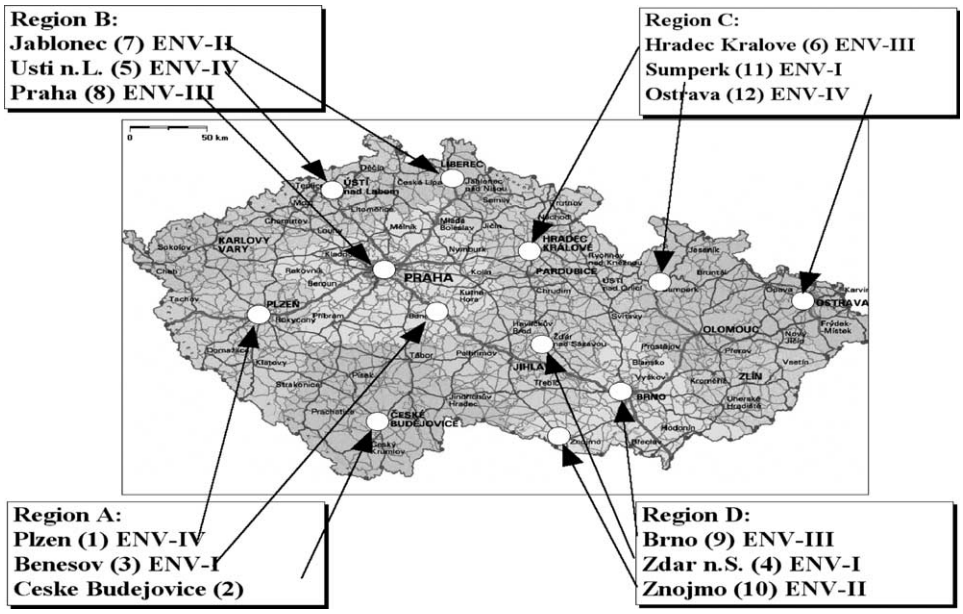

Fig. 2. Places of sample collection (12 places forming 4 regions).

chromatograph from Waters (autosampler 717 plus, HPLC 515 pump) with Envirogel™ GPC clean-up column is used for sample purification. The rotary vacuum evaporator Büchi and water bath Büchi Waterbath B-480 are used. Before use all the necessary laboratory glass is put to muffle furnace heated to 350 °C for 4 h. The Hewlett Packard 5890 gas chromatograph with two electron capture detectors is used for chromatographic determination of chlordane. DB 5 columns (length 30 m, inner diameter 0.25 mm, strength of film thick of stationary phase 0.25 μm – J&W Scientific, USA) and DB 17 (length 30 m, 0.25 mm inner diameter, 0.25 μm strength of film thick of stationary phase – J&W Scientific, USA) are used for the separation.

2.4. Operation procedure

The analytical procedure consists of isolation (extraction) of the analytes from the matrix, the removal of co-extracted compounds of the matrix and of analyses using the GC method. The chart of the entire procedure is shown in Fig. 3. The procedure was optimised as a multiresidual analysis for the determination of polychlorinated biphenyls (PCB) and organochlorine pesticides (OCP).

2.4.1. Solid food samples

The required amount (50–100 g) of the food sample is weighed into a tall beaker and a solution of the internal standard is added (according to the type of sample, from 100 to 500 μl of the PCB 209 and PBB 1 solution, concentration 2000 ng ml^{-1}). A 150 ml mixture of petroleum ether:acetone (ratio 2:1) as solvent is added. The beaker is fastened to the holder of the homogeniser. On starting the homogeniser, anhydrous sodium sulphate is added to the beaker for as long as it makes clusters, which is caused by surplus moisture in the sample. The sample is homogenised for 3 min at 10,000 rpm. The solvent decants through the anhydrous sodium sulphate into a 500 ml Erlenmayer flask. After decantation the sample in the beaker is homogenised once more with 100 ml of solvent mixture. After decantation of the second portion of the extract, the mixture of the sample with sodium sulphate is transferred to the filter through which the sample extracts are filtered. This layer is gradually rinsed in a filtering funnel with 100 ml of petroleum ether. The combined extracts are evaporated on a vacuum rotary evaporator at 40 °C and pressure of 700 mBar. The volume is reduced to dryness.

2.4.2. Liquid samples

The 1000 ml separator funnel is filled with 400 ml of the sample, 100 ml of dichloromethane and the solution of internal standard (from 100 to 500 μl of the PCB 209 and PBB 1 solution, concentration 2000 ng ml^{-1}). The separator funnel is closed with a glass stopper and the content is shaken. After separation of the phases, the bottom dichlormethane phase is transferred through the anhydrous sodium sulphate into a 500 ml flask. This procedure is repeated with another portion of dichlormethane. The combined extracts are evaporated at 40 °C and pressure 700 mBar.

The above procedure was modified for milk samples. The milk sample is homogenised in the beaker for 2 min at 6000 rpm. After homogenisation, 100 ml of

Table 1
List of samples analysed for chlordane

No.	Name of sample
1	Beef
2	Pork
3	Pork flank
4	Liver
5	Rabbit meat
6	Minced meat
7	Chicken meat
8	Hen meat
9	Turkey meat
10	Poultry offal
11	Canned meat
12	Canned pate
13	Dry salami
14	Cooked salami
15	Cooked salami
16	Frankfurters
17	Sausages
18	Knackwurst
19	Pork ham
20	Head cheese
21	Cooked meat products
22	Liver sausage
23	Poultry specialities
24	Smoked meat
25	Bacon
26	Sea fish
27	Freshwater fish
28	Smoked fish
29	Marinated fish
30	Canned fish
31	Milk
32	Hard cheese
33	Blue and camembert cheese
34	Processed and Fresh cheese
35	Whole milk yogurt
36	Fermented dairy products
37	Cream
38	Ice cream
39	Curd
40	Curd and cream desserts
41	Whipping cream
42	Condensed milk
43	Eggs
44	Mayonnaise
45	Vegetable oil
46	Margarines and vegetable fats
47	Butter
48	Lard
49	Bread
50	Rye bread
51	Wholemeal bread
52	Rolls and french loaf
53	Rye rolls
54	Cakes
55	Biscuits
56	Frozen mixed vegetable
57	Vegetable products
58	Ketchup and mustard
59	Stem vegetables I
60	Stem vegetables II
61	Legumes
62	Onions and garlic
63	Tomatoes
64	Fruiting vegetables
65	Leafy vegetables
66	Forced vegetables
67	Carrots
68	Root vegetables
69	Potatoes
70	Potato products
71	Stewed fruits
72	Fruit products
73	Infant fruit – fruit puree
74	Grapes
75	Apples
76	Peaches and apricots
77	Stone and pome fruits
78	Oranges
79	Subtropical fruits
80	Bananas
81	Small fruits, berries
82	Nuts
83	Chocolate
84	Chocolate confectionery
85	Cream cakes
86	Confectionery (other)
87	Cocoa
88	Wafers
89	Flour and yeast
90	Pasta
91	Rice
92	Cereals (other)
93	Semolina
94	Spices
95	Delicate salads
96	Coffee (infusion)
97	Tea (infusion)
98	Mineral water
99	Table water
100	Nonalcoholic beverages
101	Juice
102	Beer
103	Spirits
104	Wine
105	Syrup
106	Dumplings
107	Packet soups
108	Low-fat yogurt

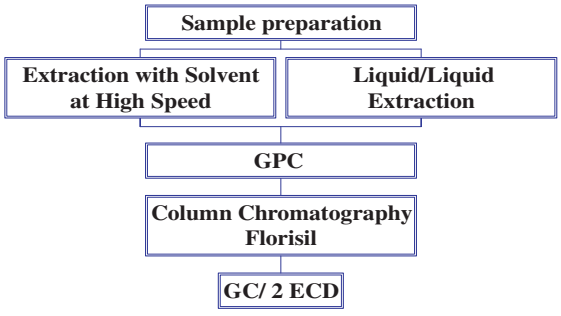

Fig. 3. Chart of the analytical procedure.

the milk sample is weighed. The weighed amount of the milk sample is transferred into a 500 ml separator funnel, and 5 ml of saturated solution of sodium oxalate, 100 ml of acetonitrile, 50 ml of diethyl ether and 100 μl of the solution of internal standard (concentration 2000 ng ml^{-1}) are added. The mixture is intensively shaken for 1 min. During this time precipitation of proteins from the milk sample should take place. Then, 80 ml of petroleum ether is added to the separator funnel (the fraction 30–55 °C) and the mixture is shaken for 1 min. After separation of the phases, the bottom phase is transferred into the other 500 ml separator funnel where further extraction with 80 ml of petroleum ether takes place. The organic phase is transferred into a 1000 ml separator funnel with 300 ml of water and 50 ml of saturated solution of sodium chloride. After the second extraction performed in the above way, the combined phases of organic solvents are shaken for 1 min in a 1000 ml separator funnel with water and saturated solution of sodium chloride. After the separation of the phases, the bottom phase is removed and the organic phase is extracted with 200 ml of water two more times. This repurified organic phase is filtered through anhydrous sodium sulphate and concentrated on a rotary vacuum evaporator at 40 °C and pressure 700 mBar.

Extracts of samples obtained in this way still contain a relatively high amount of co-extracted compounds, fats and dyes and further clean-up of the extracts is therefore necessary.

2.4.3. Clean-up

During this step the maximal amount of co-extracted compounds of the matrix must be removed with a minimal loss of the analyte. The sample extracts are purified by means of gel permeation chromatography (GPC) and column chromatography with Florisil as stationary phase. A sample is prepared for gel permeation chromatography by dissolving the fat in dichloromethane and filtrating into a 4-ml glass vial. From this vial 3×900 μl of the sample is injected onto the GPC column. The fraction of the sample with the analytes is collected into a 100-ml flask. Dichloromethane is evaporated using a vacuum rotary evaporator at 40 °C and pressure 700 mBar. The following step is purification of the sample by means of column chromatography on Florisil.

Florisil prepared in the above way is poured into an 800 ml beaker and a layer of petroleum ether is poured over it (the fraction 30–55 °C). A roll of glass wool is inserted into the prepared glass columns with a discharge cock, which holds the Florisil column. Petroleum ether is poured into the column (the fraction 30–55 °C). The discharge cock is opened and Florisil in the solvent is gradually added. The column is filled up with Florisil so that the upper edge of the Florisil column reaches about 2 cm below the flask of the column. After filling up, the level of the solvent is reduced to about 1 cm above the upper edge of the Florisil layer. Using a pipette the sample for GPC, dissolved in 5 ml of petroleum ether (the fraction 30–55 °C), is quantitatively transferred to the column; 60 ml of a mixture of petroleum ether (the fraction 30–55 °C):diethylether (ratio 9:1) is used as the elution mixture. The eluent is collected into a 100-ml flask. The solvent is then evaporated to dryness by means of a vacuum rotary evaporator at 40 °C and pressure 700 mBar. After these operations the extract is ready for chromatographic analysis.

After evaporation, 0.8 ml of *iso*-octane is added to a flat-bottom flask with the purified extract. The *iso*-octane is taken from the flask with a graduated pipette, the volume is measured and then it is transferred to the 1.5-ml vial. Chromatographic analysis, which runs in a two-column system, can begin. The splitless method is used. Oven temperature is kept at 90 °C for 2 min and is subsequently increased by 25 °C/min to 220 °C and further 3 °C/min to 250 °C and subsequently 2 °C/min to 270 °C for 12 min. Detection is conducted with two electron-capture detectors (ECD) at 300 °C and argon with methane (90:10, 30 ml min^{-1}) is used as the make-up gas. Hydrogen of ECD purity is used as the carrier gas. An example of the chromatogram is shown in Fig. 4.

2.5. Quality assurance/quality control

The method for the assessment of chlordane is accredited by the Czech Accreditation Institute according CSN EN ISO/IEC 17025 within the framework of multiresidual determination of selected congeners of PCB and selected chlorinated pesticides. This method is validated using software EffiValidation 3.0. The linearity of response of detector is tested by correlation coefficient (critical value 0.99) and QC coefficient (critical value 5.0), which are shown in Table 3. All estimated coefficients are acceptable in term of linearity. CRM 598 BCR is used to verify the accuracy of this method. The estimated recoveries for gamma-chlordane, alpha-chlordane and oxychlordane are 101.2%; 102.9% and 103.3%, respectively. The repeatability of injection (RSD) is found to range from 1% to 3% and retention time ranges from 0.07% to 0.1%. Quality control of the results is conducted by means of testing materials (TM) and certified reference materials (CRM 598 BCR). The results are used for keeping files of the regulation diagrams. As an example the regulation diagram CRM 598 BCR of gamma-chlordane is shown in Fig. 5. The accuracy of the method is verified on a regular basis within the international proficiency testing of the aptitude of the laboratories (FAPAS – UK).

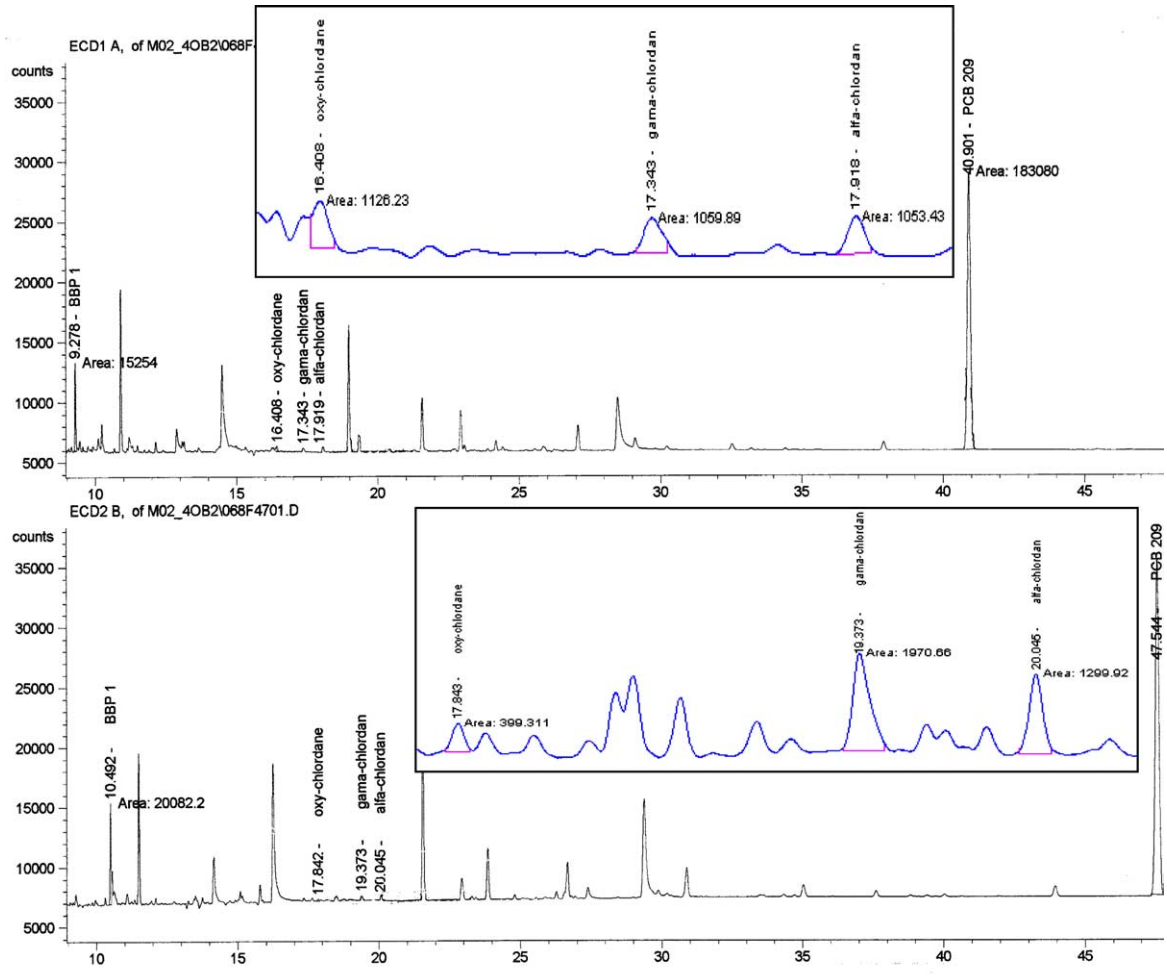

Fig. 4. Chromatogram of a butter sample.

3. Results and discussion

Analytes, which were isolated from the sample, were separated on the two capillary columns. The columns are connected parallel to each other. Different elution times of analytes on the capillary columns DB5 and DB17 are used to confirm the identity of the analytes. On the column DB5 oxychlordane is eluted with heptachloroepoxide B. These analytes were separated on the column DB17. On the column DB17 alpha-chlordane is eluted with endosulphane I. These analytes were separated on the column DB5. Retention times were dependant on the atmospheric pressure. Therefore it was necessary to perform recalibration. The peak area is used for quantification of the analytes.

The optimised and validated method of assessing chlordane in foodstuffs within the framework of the multiresidual method for PCB and OCP assessment provides results of satisfactory accuracy and precision. The method was used to analyse 108 food samples of different matrices. The calculated limits of quantification ranged from 0.002 to 0.05 $\mu g\,kg^{-1}$ and were dependant on the type of the matrix. The method can be used for monitoring chlordane in the framework of dietary exposure monitoring. The highest concentrations of the sum of chlordane were found in freshwater fish, meat products and in fats. Nonfat samples are less contaminated with this group of chlordanes; 76% of the chlordane levels were below the limit of quantification. The concentrations of chlordane in the most heavily chlordane exposed samples are shown in Table 2. Detected concentrations of chlordane in foodstuffs

Table 2
The highest levels of the sum of chlordane (alpha-chlordane, gamma-chlordane, oxychlordane) in 2002

Name of sample	Concentration ($\mu g\,kg^{-1}$)
Freshwater fish	2.78 ± 0.07
Butter	2.3 ± 0.11
Margarines and vegetable fats	1.32 ± 0.04
Delicate salads	0.94 ± 0.02
Frankfurters	0.74 ± 0.07
Marinated fish	0.57 ± 0.01

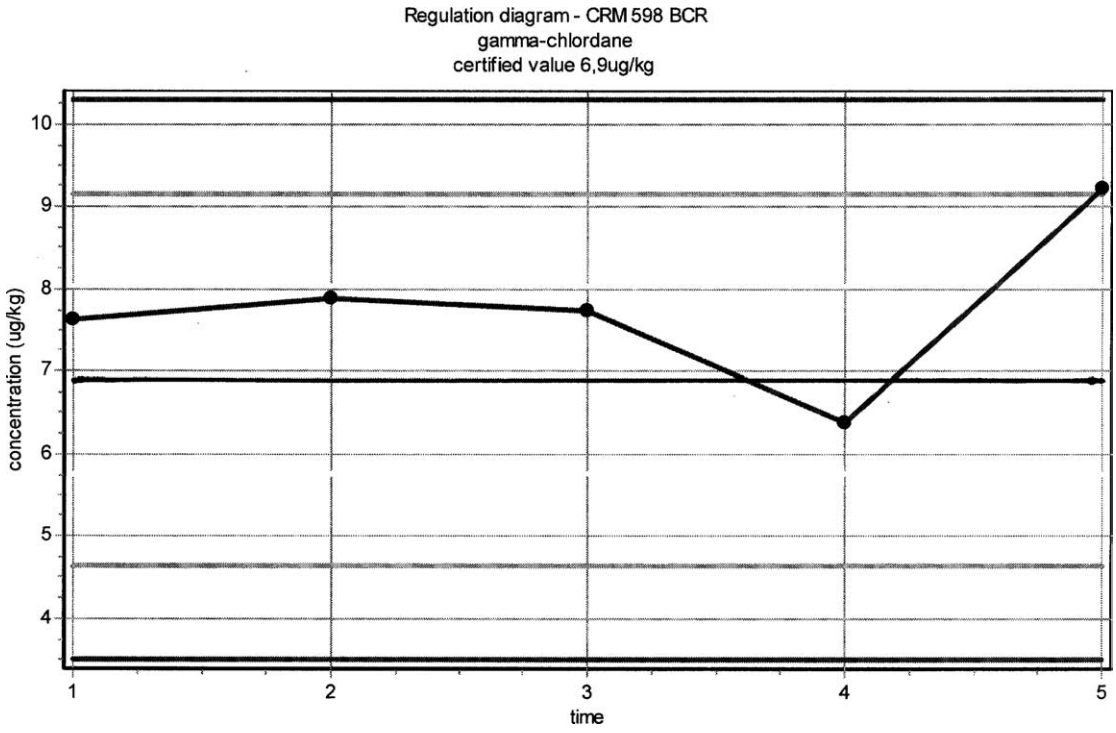

Fig. 5. Regulation diagram for CRM 598 BCR of gamma-chlordane.

Table 3
The linearity of response of detector is tested by correlation coefficient and QC coefficient

HP 5890	Correlation coefficient		QC coefficient	
Analyte	Column DB 5	Column DB 17	Column DB 5	Column DB 17
Oxychlordane	0.99858	0.99877	4.57	4.29
Gamma-chlordane	0.99972	0.99949	2.65	3.49
Alpha-chlordane	0.99883	0.99967	4.90	2.31

are used for estimations of dietary exposures to chlordane.

4. Conclusion

In the course of applications of this method within the framework of monitoring the dietary exposure it was discovered that the content of chlordane in foodstuffs is relatively low. The found results expressed as the dietary exposure for the average Czech population (considering food consumption and the culinary factor) did not even reach one per cent of the acceptable daily intake (ADI). The ADI value was set by FAO/WHO in 1986 as 0.0005 mg kg^{-1} b.w/day (Codex Alimentarius).

The method is applicable not only for the determination of chlordane, but also for that of chlorinated pesticides and polychlorinated biphenyls in various types of food samples.

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