Available online at www.sciencedirect.com
ScienceDirect
Microchemical Journal 86 (2007) 183 – 188
MICROCHEMICAL JOURNAL
www.elsevier.com/locate/microc

Development and validation of an analytical methodology for the determination of *p*,*p*′-DDT, *p*,*p*′-DDE and *p*,*p*′-DDD in fish oil pills

Juan José Berzas Nevado [a,b], Rosa Carmen Rodríguez Martín-Doimeadiós [a,c], Francisco Javier Guzmán Bernardo [a,c,*], Nuria Rodríguez Fariñas [a,c]

[a] *Department of Analytical Chemistry and Food Technology, University of Castilla-La Mancha, Spain*
[b] *Faculty of Chemical Sciences, Av. Camilo José Cela, 9. E-13071, Ciudad Real, Spain*
[c] *Faculty of Environmental Sciences, Avda. Carlos III, s/n. E-45071, Toledo, Spain*

Received 21 February 2007; accepted 6 March 2007
Available online 16 March 2007

Abstract

An analytical methodology was proposed and validated to be applied to the determination of *p*,*p*′-DDT and its metabolites *p*,*p*′-DDE and *p*,*p*′-DDD in fish oil. The analytical procedure presented in this paper involves a single-step clean up process prior to the analysis. A solution of 1,2,3,4-tetrachloronaphtalene was used as internal standard.

The analytical technique used was gas chromatography coupled to an electron capture detector. Details on the validation process are provided. The limits of detection ranged from 2.6 to 4.7 pg μL^{-1}. The BCR 598 standard reference material (cod liver oil) was used to evaluate the performance of the methodology with satisfactory recoveries for all the compounds.

The analytes were determined in three different fish oil pills sold in Spain as a supplementary vitamin support. The sum of *p*,*p*′-DDT and metabolites was from 13.2 to 51.3 ng g^{-1}, the dominant compound being *p*,*p*′-DDE.
© 2007 Elsevier B.V. All rights reserved.

Keywords: Fish oil; DDT; DDD; DDE; Gas chromatography; Electron capture detector

1. Introduction

Organochlorine pesticides (OCPs) have been of great concern due to their persistent nature and chronic adverse effects on wildlife and humans. Some studies have suggested that these compounds may affect the normal function of the endocrine system and have also been linked to human breast, liver and testicular cancers as well as to low sperm counts in humans [1,2]. Despite the ban and restriction on the usage of OCPs in developed countries during the 1970s and 1980s, some developing countries went on using them for agricultural and public purposes because of the low cost and versatility for various insects [3].

One of the OCPs, *p*,*p*′-dichlorodiphenyltrichloroethane (DDT), is best known for its successful use for malaria eradication soon after it was first synthesized in 1939 [4]. Later on, in the 1960s, it was considered as hazardous and, consequently, banned or restricted in most industrialized countries. DDT and its metabolites, *p*,*p*′-dichlorodiphenyldichloroethylene (DDE) and *p*,*p*′-dichlorodiphenyldichloroethane (DDD), are the archetypes of fat soluble, nonbiodegradable and bioaccumulating compounds. The appearance of DDT and metabolites in human tissues and its effect on wildlife, especially the thinning of eggshells in birds [5] triggered its determination in food [6,7], air, water [8,9] and human milk [10–12]. The ability of DDE, the major and most persistent DDT derivative, to bind to the androgen receptor in male rats has been reported [13].

Exposure to these compounds has been monitored, and regulatory limits are currently mandated. Monitoring indicates that 99.5% of the population has an average of 1.8 to 12.6 pg μL^{-1} of DDT in their serum from nutritional and environmental

* Corresponding author. Department of Analytical Chemistry and Food Technology, University of Castilla-La Mancha, Spain. Tel.: +34 925 26 88 00; fax: +34 925 26 88 40.
E-mail address: fcojavier.guzman@uclm.es (F.J.G. Bernardo).

0026-265X/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.microc.2007.03.001

exposure [14,15]. DDT and DDE concentrate in fatty tissues. Concentrations from 123 to 567 ng g^{-1} lipid were found in adipose tissue [16], whereas the concentrations found in breast milk were from 24 to ng g^{-1} fat [17]. Although DDT can be found in all types of food, it is remarkable that a concentration factor of up to 1000 may occur in fish. The US Food and Drug Administration (FDA) set a tolerance level of 5 μg g^{-1} of DDT in edible portion of fish to avoid detrimental health effects [15,18,19].

Fish oil is a byproduct of the fish meal manufacturing industry. Concentrated omega-3 fatty acids can be found in fish oil, whose daily ingestion slows down the progression of coronary artery disease [20]. However, as these chlorinated compounds accumulate in the lipid compartment of the fish, the oil extracted from the animal may be contaminated with them. Therefore, it is of special interest to investigate the levels of DDT and its metabolites in fish oil, where these compounds tend to accumulate.

Although the determination of these analytes has been extended to a number of different samples, the papers dealing with fish oil are scarce. In this way, Semb et al. [21] reported the determination of these compounds in cod liver oil using GC coupled to atomic emission detection (AED). In this case, the authors had to increase the injection volume to 5 μL in order to get a limit of detection of 6 ng g^{-1} for DDT without apparent loss of chromatographic resolution. Jacobs et al. [22] reported the levels of DDT, DDD and DDE residues, among other organochlorine compounds, in fish oil dietary supplements using a gas chromatograph (GC) coupled to electron capture detection (ECD) for the analytical determination and mass spectrometry (MS) for confirmation purposes. The results showed a wide range of levels, from not detected up to about 1000 ng g^{-1}, expressed as the sum of DDT, DDE and DDD (∑DDT). Later on, Jacobs et al. [23] investigated the levels of selected persistent organic pollutants, including DDT, DDE and DDD, in fish oils used to supplement the feeds of farmed Atlantic Salmon (*Salmo salar*). The authors used GC-MS operated in electron impact ionisation mode and obtained a detection limit of 0.2 ng g^{-1} lipid for each compound, whereas the recoveries ranged between 72% and 80%. As a result, ∑DDT was 66±87 ng g^{-1} lipid, and DDE was the predominant contributor, whereas the mean ratio DDT to ∑DDT was 0.15±0.07.

In all these cases, the analytical pre-treatment consisted of two separate steps. The first one was the destruction of the lipid by adding concentrated sulphuric acid. The second one was a clean up step by gel permeation (GPC) and/or a mixed silica or alumina column. All these make the process tedious and time consuming. In order to circumvent this, Patel et al. [24] reported the determination of OCPs, including DDT, DDE and DDD, at ng g^{-1} levels in fatty matrices with an only clean up step using GPC. In this work, tandem quadrupole mass spectrometry (GC-MS/MS) was used. Therefore, the authors managed to reduce the analysis time by taking advantage of the selectivity provided by the use of GPC and GC-MS/MS.

Alternatively, our group has focused on the development and validation of a methodology which simplifies the clean up to a single step and shortens the chromatographic separation time.

Then, the aim of this work is to investigate the levels of DDT and its metabolites, DDE and DDD in fish oil pills commercially available in Spain. For this purpose, a full methodology has been validated, including an analytical pre-treatment consisting of a single step in order to reduce the analysis time. Gas chromatography coupled to electron capture detector was used as an analytical tool because of its high sensitivity to the chlorinated compounds.

2. Experimental

2.1. Chemicals and standards

Individual standard solutions of *p*,*p*′-DDT, *p*,*p*′-DDE and *p*,*p*′-DDD at 100 ng μL^{-1} were purchased from Dr. Ehrenstorfer, GmbH (Augsburg, Germany) and kept refrigerated until used.

A stock solution of 1,2,3,4-tetrachloronaphtalene (TCN) (Dr. Ehrenstorfer, GmbH) at 100 pg μL^{-1} was prepared in hexane and kept refrigerated until used as injection standard.

Hexane and sulphuric acid (Merck, Darmstadt, Germany) were analytical grade and pro analysis quality, respectively. Anhydrous sodium sulphate and silica gel 60 (Panreac Química S.A., Barcelona, Spain) were analytical grade.

Nitrogen (Carburos Metálicos S.A., Barcelona, Spain) was Premier X50S quality.

The certified reference material used was BCR 598 (cod liver oil) from the Institute for Reference Materials and Measurements (Geel, Belgium). The certified reference material was used as provided. Cod liver oil pills, “Kromenat” (Kromenat, Madrid, Spain), and fish oil pills, “Verdalia” (Naturland i.c.c., Carros, France) and “Arkocaps” (Arkopharma, Carros, France) were purchased as samples.

2.2. Instrumentation

The separations were performed with a Fisons 8130 gas chromatograph (Carlo Erba Instruments, Milan, Italy) equipped with a ^{63}Ni electron capture detector with a 30 m × 0.25 mm I.D. × 0.25 μm CP-Sil 8 CB (5% phenyl-95% dimethylpolysiloxane) capillary column (Varian Inc.). The chromatographic conditions are described in the Results and Discussion section.

2.3. Sample preparation

Each sample of oil, typically 0.2 to 0.4 g, was accurately weighed in a 5 mL glass vial, dissolved in the least possible volume of hexane and added 50 μL of TCN at 100 pg μL^{-1}. The sample was then loaded into a 35 cm long × 2 cm inner diameter glass column containing different layers of activated silica (baked at 450 °C overnight). From top to bottom, the column was filled with 1 cm of anhydrous Na_2SO_4, 3 cm of sulphuric acid modified activated silica at 44% (w:w), 6 cm of sulphuric acid modified activated silica at 22% (w:w), 2 cm of neuter activated silica and 1 cm of anhydrous Na_2SO_4. This multi-layer silica column was bottomed with glass wool and was washed with 125 mL of hexane prior to use [25].

The samples were then eluted with hexane. The first 15 mL was discarded and the following 75 mL was collected in round bottom flasks. Later on, they were rotary evaporated until a volume of about 1 mL was reached, and finally, they were transferred to conical bottom injection vials and evaporated to dryness under a gentle nitrogen stream.

After all the process, the samples were reconstituted with 50 μL of hexane and analysed by GC-ECD.

3. Results and discussion

3.1. Clean up and fractionation

As stated in the Introduction section, the use of a single step for the clean up and fractionation of the sample, instead of doing so in two separate steps, would result in a decrease in the total analysis time. For this purpose a multi-layer silica column was used. The column was prepared and eluted as described above and proved to be efficient for the removal of the fat.

In order to find out the volume of hexane that was necessary for the elution of the analytes and internal standard from the column, a stock solution (100 pg μL^{-1}) of these compounds was loaded into a mixed silica column prepared and activated as explained above.

The column was then eluted with hexane. Eight fractions of 15 mL each were collected, evaporated to dryness, reconstituted with 50 μL of hexane and injected through the chromatographic system. The results showed that the compounds were present in the fractions #2 to #6. As a consequence, the elution conditions were to discard the first 15 mL and to keep the following 75 mL (5 fractions of 15 mL each) for analysis.

Therefore, the analytical pre-treatment of the samples could be done in a single step and, consequently, a decrease in the analysis time could be reached.

3.2. Capillary gas chromatography-electron capture detection

The GC and ECD conditions were optimised as follows. Nitrogen was used as a carrier gas. The column head pressure was tested from 80 kPa to 150 kPa. A value of 125 kPa was selected as optimum because it provided the best peak efficacy for all the compounds. The selected head pressure produced a gas flow of 1 mL min^{-1} through the column, measured at 250 °C.

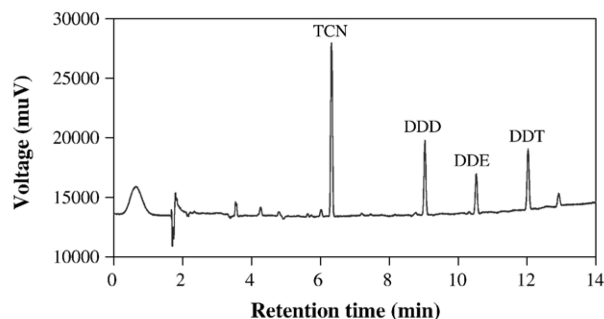

Fig. 1. Chromatogram corresponding to the separation of a standard mixture (83, 40, 84 and 100 pg μL^{-1} of DDE, DDD, DDT and TCN, respectively).

Table 1
Linearity (n=7), LOD and LOQ of the compounds studied

	DDE	DDD	DDT
Equation [a]	A=0.10+7.05c	A=0.14+5.03c	A=0.04+5.16c
Slope SD [b]	0.24	0.20	0.10
Intercepts SD[b]	0.059	0.048	0.024
r^2	0.9942	0.9924	0.9982
LOD (pg μL^{-1})	2.6	4.3	4.7
LOQ (pg μL^{-1})	8.6	14.4	15.7

[a] Concentration in ng μL^{-1} (c) versus relative peak area (A).
[b] SD, standard deviation.

The temperature injection port was varied from 250 °C up to 300 °C. An increase in peak area was observed as temperature was increasing, but also an increase in base line aberrations. As a consequence, a value of 275 °C was selected as a compromise solution. The selected injection volume, 1 μL (splitless mode) was also selected as a compromise between sensitivity and peak width.

The oven temperature program was as follows. The initial temperature was set at 210 °C for 1 min following injection and then programmed at 3 °C min^{-1} up to 250 °C. This program provided good peak resolutions within less than 14 min.

According to the manufacturer's guidelines, the detector temperature was set at 310 °C, and nitrogen was used as makeup gas at 30 mL min^{-1}.

A separation corresponding to a solution containing 83, 40, 84 and 100 pg μL^{-1} of DDE, DDD, DDT and TCN, respectively is plotted as Fig. 1. This chromatogram shows a satisfactory separation.

3.3. Validation

3.3.1. Limits of detection and of quantification

The instrumental limit of detection (LOD) was estimated in accordance with the baseline noise. The baseline noise was evaluated by recording the detector response over a period about ten times the peak width. The LOD was obtained as the compound concentration that caused a peak with a height of three times the baseline noise level. Likewise, the instrumental limit of quantification (LOQ) was obtained as the compound concentration that caused a peak with a height of ten times the baseline noise level.

Thus, the LODs and LOQs obtained for the analytes under these conditions are presented in Table 1. The instrumental LODs are in the low pg μL^{-1} range, as expected for the ECD.

3.3.2. Linearity

The linearity was checked by triplicate injection of 1 μL of a set of seven standard solutions containing increasing concentrations of the analytes from the limits of quantification up to 545 ng μL^{-1} under the chromatographic conditions described above. The adequate amount of the internal standard solution was added so that its final concentration was 100 pg μL^{-1}. The calibration curves were obtained by plotting the analyte over internal standard peak area versus the analyte concentration.

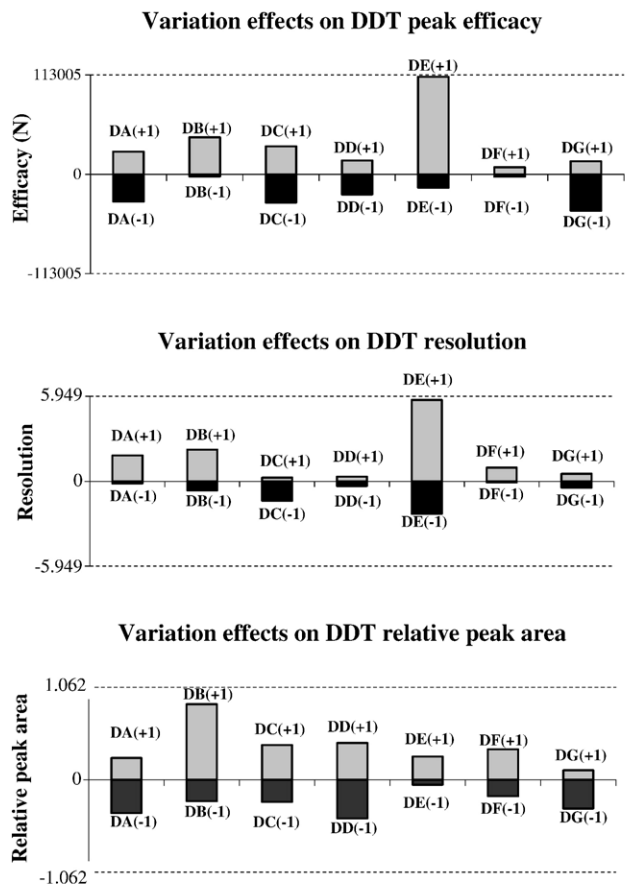

Fig. 2. Variation effects on DDT peak efficacy, resolution and relative peak area of the seven operating factors.

The equations and determination coefficients are summarized in Table 1. In all cases, the calibration curves showed an excellent linear relationship between relative concentrations and areas. Likewise, the intercepts were found not different from zero according to the Student's test "*t*" ($P=0.05$).

3.3.3. Precision

The repeatability of the chromatographic method was assessed by running nine replicates of a standard solution containing 200 ng μL^{-1} of each compound. The results showed that the relative standard deviation of the areas for each and every compound was under 4.4% in all cases.

The reproducibility over time of the chromatographic method was evaluated by separating nine replicates of a stock solution prepared as above, in two consecutive days and comparing the standard deviations (below 5% in both days for each compound) of the peak areas of each compound. For this purpose, the Snedecor test "*F*" was used and, as a result, no significant differences between the series for both days were found ($P=0.05$, $n=8$).

Likewise, the results obtained for the retention times were satisfactory as well, in terms of both repeatability and reproducibility.

Table 2
Recoveries obtained for the analyses of the solutions and the standard reference material BCR 598 (cod liver oil)

Sample	Added (ng μL^{-1})			Recovery (%)		
	DDE	DDD	DDT	DDE	DDD	DDT
Solution 1	FS	211	225	115.1±5.2	89.7±4.3	94.8±3.3
Solution 2	22	22	22	103.6±6.1	99.5±1.1	104.5±3.7
Solution 3	67	65	67	108.7±5.8	88.3±6.1	100.7±3.7
	Certified (ng g^{-1})			Recovery (%)		
	DDE	DDD	DDT	DDE	DDD	DDT
BCR 598	610.0	400.0	179.0	108.4±5.8	89.2±6.4	93.4±7.5

Table 3
Analysis of real samples (mean±standard deviation) ($n=3$)

	DDE	DDD	DDT	∑DDT	DDT/∑DDT
Verdalia	9.23±0.78	2.38±0.42	1.61±0.21	13.2±1.0	0.122±0.018
Kromenat	28.5±1.2	15.6±2.5	7.24±0.96	51.3±2.9	0.141±0.020
Arkocaps	16.1±1.9	5.66±0.30	3.87±0.45	25.6±1.3	0.151±0.088

Concentrations are in ng g^{-1}.

3.3.4. Robustness

The aim of a robustness test is to identify possible sources of error when changes occur in the specified method conditions [26]. In this work, we evaluated if small changes in the main chromatographic parameters (factors) had a significant influence on relative peak area, peak efficacy and resolution. For this purpose, fractional factorial designs developed by Plackett and Burman [27], based on balanced incomplete blocks, were used. The factors and levels selected in our case were the following:

Factor A Column head pressure ($115_{(-1)}$, $125_{(0)}$, $135_{(+1)}$) (kPa).
Factor B Time of the splitless step ($0.75_{(-1)}$, $1.00_{(0)}$, $1.25_{(+1)}$) (min).
Factor C Temperature for the splitless step ($205_{(-1)}$, $210_{(0)}$, $215_{(+1)}$) (°C)
Factor D Injector temperature ($270_{(-1)}$, $275_{(0)}$, $280_{(+1)}$) (°C)
Factor E Detector temperature ($300_{(-1)}$, $310_{(0)}$, $320_{(+1)}$) (°C)
Factor F Injected volume ($0.5_{(-1)}$, $1.0_{(0)}$, $1.5_{(+1)}$) (μL)
Factor G Final oven temperature ($240_{(-1)}$, $250_{(0)}$, $260_{(+1)}$) (°C)

The optimum value is labelled as (0), whereas the values labelled as (−1) and (+1) are the maximum variation in the value of the factor. The average and standard errors (DA, DB, DC,...) were calculated using the procedures described by Youden and Steiner [28].

The robustness was determined in our case by triplicate injection of solutions of DDE, DDD, and DDT at 266 ng μL^{-1} and TCN at 100 pg μL^{-1} as internal standard. The effects of each factor on resolution, efficacy, and relative peak area were calculated. The efficacy was expressed in terms of the number of theoretical plates (N), where the peak width at half height was considered.

The effects of these factors on the efficacy, resolution and peak area of DDT are shown in Fig. 2, as an example. In this particular case, the values of the variations of the seven factors

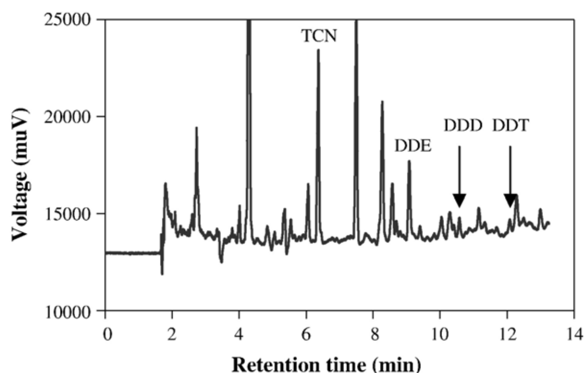

Fig. 3. Chromatogram corresponding to a commercial fish oil pill sample (“Verdalia”).

on the efficacy were always within the range calculated using the Youden–Steiner statistical model, which means that the method is robust in terms of efficacy. Likewise, similar results were obtained for resolution, efficacy and relative peak areas, for DDD and DDE.

3.3.5. Recovery studies

Three solutions containing the analytes at different concentrations were prepared by diluting the stock solutions and analysed under the conditions explained above. The obtained recoveries were from 88.3% to 115.1%.

Also, a standard reference material (SRM), the BCR 598 of cod liver oil was used for recovery studies. In this case, 0.010 g of SRM was accurately weighed and conducted through the described procedure and the recoveries obtained were from 89.2% to 108.4%, which are similar to those obtained for the solutions prepared previously.

The figures of this studies are summarized in Table 2.

3.4. Applications

The developed analytical procedure was applied to the determination of DDT in fish liver oil pills. These pills are sold without prescription as a vitamin supplement. The samples were analysed by triplicate as explained in “sample preparation” and the results are summarized in Table 3 in terms of both total content, ∑DDT, and concentration of each analyte separately.

As an example, a chromatogram corresponding to one of the samples, “Verdalia” pill, is plotted as Fig. 3.

The highest total content was found in “Kromenat”. According to the label, this is a cod liver oil, whereas the other two are salmon oil, which implies not only from liver. In terms of individual analytes, all of them are also more concentrated in “Kromenat” than in the other two samples. These findings back the evidence that these compounds accumulate more in fatty and/or adipose animal tissues, like liver.

The chemical pattern is dominated by DDE in all the samples, whereas DDT was the least concentrated. This is in agreement with the data reported by Jacobs et al. for fish oil [23]. Additionally, owing to the fact that DDE is a metabolite of DDT, the low DDT/DDE ratio, from 0.17 up to 0.25, indicates that the exposure to DDT of the animals that the analysed oil comes from, is far behind in time.

Moreover, as can be seen in Table 3, the DDT/∑DDT ratio is very similar in the three cases studied, and it is also comparable to data reported by Jacobs et al. [23], who estimated that the average value for this ratio was 0.15. This value was also found for the DDT/∑DDT ratio in the reference cod liver oil mentioned above.

Another issue to be covered is the dosing. The manufacturers recommend to take from 2 to 4 pills a day, which means a DDT daily intake ranging from 1.2 to 11 ng a day. Therefore, since the acceptable daily intake for DDT for human beings is estimated to be 20.0 ng g^{-1} body weight [29], we can state that the found concentrations are not expected to produce toxic effects if the pills are taken following the manufacturer’s recommended dosing.

4. Conclusions

An analytical methodology was successfully validated for the determination of DDT, DDE and DDD in fish oil pills involving an only step for sample preparation prior to the GC-ECD determination. As a result, the methodology was proved to be sensitive and selective enough as well as simple and time saving in comparison to the ones existing up to present.

The levels of DDT, DDE and DDD in fish oil pills sold in Spain were investigated and discussed. The results showed that the levels of the analytes were higher in fish liver oil than in fish oil and that the chemical pattern was dominated by DDE, the major metabolite of DDT, in all cases. However, the presence of these compounds should pose no risk for human health, as long as the pills are consumed as recommended.

These results were consistent with the reported previously in literature in terms of both concentrations and chemical pattern.

Acknowledgements

The authors wish to thank José Antonio Castro Osma and Ester Pulido Martín for their kind contribution to this work.

References

[1] D.L. Davies, H.L. Barlow, Sci. Am. (October 1995) 144.
[2] P. Cocco, A. Blair, P. Congia, G. Saba, C. Flore, M.R. Ecca, C. Palmas, Arch. Environ. Health 52 (1997) 299.
[3] S. Tanabe, F. Gondaira, A.N. Subramanian, A. Ramesh, D. Mohan, P.L. Kumaran, Agric. Food Chem. 38 (1990) 899.
[4] WHO (World Health Organization), Environmental Health Criteria 83: DDT and its Derivatives, Environmental Aspects, World Health Organization, Geneva, Switzerland, 1989.
[5] USEPA (US Environmental Protection Agency), DDT: A Review of Scientific and Economic Aspects of the Decision to Ban its Use as a Pesticide, EPA, Washington, DC., U.S.A, 1975.
[6] K. Kannan, S. Tanabe, J.P. Giesy, R. Tatsukawa, Rev. Environ. Contam. Toxicol. 152 (1997) 1.
[7] R. Doong, C. Lee, Analyst 124 (1999) 1287.
[8] H. Iwata, S. Tanabe, N. Sakai, A. Nishimura, Environ. Pollut. 85 (1994) 15.
[9] A. Aydin, T. Yurdin, Water Air Soil Pollut. 111 (1999) 385.
[10] S. Tanabe, H. Iwata, R. Tasukawa, Sci. Total Environ. 154 (1994) 163.

[11] A. Schecter, P. Fürt, C. Fürt, O. Päpke, M. Ball, L.C. Dai, H.T. Quynh, N.T.N. Phoung, A. Beim, B. Blasov, V. Chongchet, J.D. Constable, K. Charles, Chemosphere 23 (1991) 1903.
[12] J.O. Okonkwo, L. Kampita, D.D. Chingakule, Bull. Environ. Contam. Toxicol. 63 (1999) 243.
[13] W.R. Keice, R.C. Stone, S.C. Law, L. Earl-Gray, J.A. Kemppainen, E.M. Wilson, Nature 375 (1995) 581.
[14] H. Mussalo-Rauhamaa, Sci. Total Environ. 103 (1991) 159.
[15] M.P. Longnecker, W.J. Rogan, G. Lucier, Annu. Rev. Public Health 18 (1997) 211.
[16] Smeds, P. Saukko, Chemosphere 44 (2001) 1463.
[17] G. Schade, B. Heinzow, Sci. Total Environ. 215 (1998) 31.
[18] K.S. Schafer, S.E. Kegley, J. Epidemiol. Community Health 56 (2002) 813.
[19] Colosio, M. Tiramani, M. Maroni, Neurotoxicology 24 (2003) 577.
[20] C. von Schacky, P. Angerer, W. Kothny, K. Thiesen, H. Mudra, Ann. Intern. Med. 130 (1999) 554–562.
[21] S.I. Semb, E.M. Brevik, S. Pedersen-Bjergaard, Chemosphere 36 (1998) 213.
[22] M.A. Jacobs, D. Santillo, P.A. Johnston, C.L. Wyatt, M.C. French, Chemosphere 37 (1998) 1709.
[23] M.A. Jacobs, A. Covaci, P. Schepens, Environ. Sci. Technol. 36 (2002) 2797.
[24] K. Patel, R.J. Fussell, M. Hetmanski, D.M. Goodall, J.K. Brendan, J. Chromatogr., A 1068 (2005) 289.
[25] F.J. Guzmán Bernardo, M.A. Fernández, M.J. González, Chemosphere 61 (2005) 398.
[26] M. Mulholland, J. Waterhouse, J. Chromatogr. 395 (1987) 539.
[27] R.L. Plackett, J.P. Burman, Biometrika 33 (1946) 305.
[28] W.J. Youden, E.H. Steiner, Statistical manual of the AOAC. In: AOAC International (Ed.). Washington D.C., U.S.A., (1975), pp. 33–41.
[29] L. Ritter, K.R. Solomon, J. Forget, Stockholm Convention on Persistent Organic Pollutants, 1995.