ELSEVIER

Journal of Chromatography B, 657 (1994) 103–110

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

Determination of chlorinated phenols and cresols in human urine using solid-phase extraction and gas chromatography

M. Veningerová*, V. Prachar, J. Uhnák, M. Lukácsová, T. Trnovec

Institute of Preventive and Clinical Medicine, Limbová 14, 833 01 Bratislava, Slovak Republic

(First received October 19th, 1993; revised manuscript received February 15th, 1994)

Abstract

A method is described for the isolation, derivatization, separation and determination of chlorinated phenols and cresols in urine. After acid hydrolysis, solid-phase extraction on Separcol SI C_{18} was used. Quantification was based on the internal standard method using 2,6-dibromophenol. Before GC determination the isolated compounds were derivatized with pentafluorobenzyl bromide. The separation of interfering substances followed on an Ekosorb column using elution with dichloromethane–toluene (15:85). The recovery of the method ranged from 72.3 ± 9.9 to $109.9 \pm 6.3\%$ and the limit of determination varied from 0.0005 to 0.002 $\mu g\ ml^{-1}$. Using this method, 52 persons from occupationally and non-occupationally exposed groups were examined for the presence of chlorinated compounds in urine. The levels of chlorinated phenols and cresols were several times higher in the group of occupationally exposed workers, especially for 2,4-dichlorophenol.

1. Introduction

Among the large amounts of xenobiotics polluting the environment, polychlorinated phenols and cresols have been in the limelight in recent years. They enter the environment from industry (production of dyes, disinfectants, pesticides and other polychlorinated aromatic compounds from the paper and wood industry). They are also formed during chlorination of drinking and waste water and by biotic and abiotic transformation of pesticides in agriculture [1]. Photolytic degradation of substituted phenols is connected with the formation of lower chlorinated phenols and chlorinated catechols, dihydrobenzenes, hydroxybiphenyls and chlorophenoxyphenols, known as predioxins. According to the EPA (US Environmental Protection Agency), phenol and its chlorinated, nitrated or alkylated derivatives are classified as priority poollutants [1–7].

The mechanism of the toxic effects of chlorinated phenols and cresols seems to be connected with the chain reaction of their gradual dechlorination in the tissues yielding the corresponding peroxides, which cause inactivation of enzymes and liver dystrophy. Some compounds in this group are suspected of embryotoxicity and teratogenicity [1]. Chlorophenols affect porphyrin metabolism, causing symptomatic liver porphyry. Studies dealing with chronic toxicity of some phenol congeners have confirmed their carcinogenic (hepatocellular tumours, leukaemia) and immunosupressive properties [1,8]. Their elimination from the organism occurs via

* Corresponding author.

0378-4347/94/$07.00
SSDI 0378-4347(94)00153-V

the urine either in the free form or in the form of sulphate and glucuronide conjugates [8–11]. Exposure to chlorophenols in the occupationally and non-occupationally exposed population has been discussed in papers [1,12–15].

In this work, the exposure of occupationally exposed workers in a chemical plant producing pesticides was investigated. The exposure of the general population via the food chain was studied in parallel [16,17].

Many published methods are devoted to the determination of chlorinated phenols and cresols in urine in the form of alkyl, silyl, acetyl or other derivatives. The most frequent methods are GC and HPLC [15–24].

A method for the determination of chlorophenols and cresols in biological materials was developed in this work, based on solid-phase extraction and subsequent determination of pentachlorophenol and the isomers of dichlorophenol (DCP), trichlorophenol (TCP), tetrachlorophenol (TeCP), 2-chloro-5-methylphenol (2-Cl-5-MeP), 4-chloro-3-methylphenol (4-Cl-3-MeP) and 4-chloro-2-methylphenol (4-Cl-2-MeP) by gas chromatography with electron-capture detection (GC–ECD).

2. Experimental

2.1. Chemicals

An SPB-5 fused-silica capillary column and the phenols (Phenols Kit 27, purity 96%) were purchased from Supelco (Gland, Switzerland). A glass capillary column with an Igepal CO 880 + Apiezon L stationary phase was purchased from Expertise and Analytical Service in Environmental Protection, J. Hrivnák (Bratislava, Slovak Republic). Methanol, toluene, hexane and acetone were purchased from Labscan Pestiscan (Dublin, Ireland), hydrochloric acid (25%), sodium hydroxide pellets and potassium carbonate from Merck (Darmstadt, Germany) and pentafluorbenzyl bromide (99%) from Aldrich (Steinheim, Germany). Separcol SI C_{18} (100 mg) solid-phase extraction (SPE) cartridges (the surface area of this adsorbing material is reported to be about 410 $m^2\ g^{-1}$ and the particle size range is 40–100 μm) were purchased from Anaprom (Bratislava, Slovak Republic). Ekosorb was purchased from Kavalier (Votice, Czech Republic).

2.2. Standard solutions

Ethanolic stock standard solutions of 2,4-DCP, 2,4,6-TCP, 2,3,6-TCP, 2,4,5-TCP, 2,3,5,6-TeCP, 2,3,4,5-TeCP, PCP, 2-Cl-5-MeP, 4-Cl-3-MeP, 4-Cl-2-MeP and 2,6-dibromophenol (2,6-DBP) were prepared at concentrations of 100 $\mu g\ ml^{-1}$. From these stock standard solutions, aqueous working standard solutions at concentrations of 1 and 0.005 $\mu g\ ml^{-1}$ were prepared.

2.3. Apparatus

The GC system consisted of a Hewlett-Packard Model 5890A gas chromatograph equipped with an electron-capture detector, Model 3393A integrator and Model 7673 autosampler.

2.4. Separation

A SPB-5 (5% phenyl–methylsilicone) fused-silica capillary column (30 m × 0.25 mm I.D., 0.25-μm film thickness) was used. The column temperature was held at 70°C for 0.5 min, then increased at 30°C min^{-1} to 105°C, which was held for 10 min, then further increased at 4°C min^{-1} to 240°C, the final temperature being held for 3 min. Nitrogen was used as the carrier gas; the column head pressure was 70 kPa. The temperature of electron-capture detector was 300°C. The flow-rate of the make-up gas (nitrogen) was 15 ml min^{-1}, the injector temperature was 250°C and all injections were made in the splitless mode.

2.5. Isolation

To 1 ml of urine sample in a 25-ml round-bottomed flask was added 0.5 ml of concentrated HCl followed by 40 μl of internal standard solution (1.0 $\mu g\ ml^{-1}$ 2,6-DBP). The solution was then refluxed for 0.5 h. After cooling, the

pH was adjusted to 5 with 0.5 *M* KOH. The sample was diluted with 5 ml of water and transferred to the top of the preconditioned Separcol SI C_{18} column. Prior to use, the column was preconditioned with 2.5 ml of methanol and 1.5 ml of water and dried with a nitrogen flow. Elution was carried out with two 500-μl portions of methanol.

2.6. Calibration

Calibration graphs were obtained for each chlorinated phenol using the internal standard method (2,6-DBP). Urine (1 ml) was spiked with 0.005, 0.020, 0.040, 0.060, 0.080, 0.10 and 0.15 μg ml^{-1} of each of the compounds (Fig. 1). These spiked samples were subjected to the same treatment as the test urine samples. Blanks were analysed in the same way.

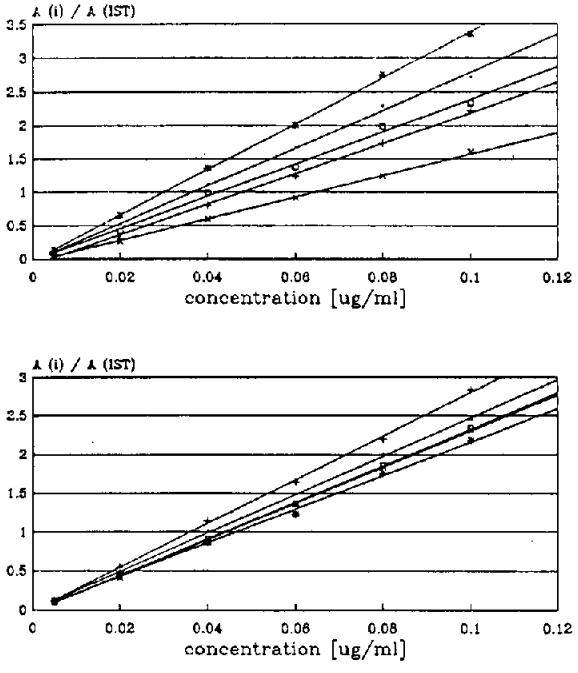

Fig. 1. Calibration graphs for the determination of PFB derivatives of chlorinated phenols and cresols in urine. The internal standard was 2,6-DBP. Top: ● = 2-Cl-5-MeP; + = 4-Cl-3-MeP; * = 4-Cl-2-MeP; □ = 2,4-DCP; × = PCP. Bottom: ● = 2,4,6-TCP; + = 2,3,6-TCP; * = 2,4,5-TCP; □ = 2,3,5,6-TeCP; × = 2,3,4,5-TeCP.

2.7. Derivatization

The eluate was dried under a gentle stream of nitrogen. Subsequently 4 ml of acetone, 30 μl of 30% K_2CO_3 solution and 200 μl of a 1% acetone solution of pentafluorobenzyl bromide (PFBBr) were added. The test-tube was tightly stoppered and heated at 60°C for 3 h in a thermostat [23,24]. After derivatization, the solution was concentrated to 0.5 ml with a gentle stream of nitrogen, 2 ml of hexane were added and the mixture was evaporated to dryness. The residue was dissolved in 1 ml of hexane and this solution was cleaned up on an Ekosorb column [25].

2.8. Column chromatographic clean-up

Ekosorb used for column chromatography (0.125–0.200 mm) was activated for 24 h at 130°C and the activity was adjusted with 5% of water. A 0.5 cm I.D. column was stoppered with precleaned glass-wool, packed with an 8-cm layer (*ca.* 1.3 g) of Ekosorb and washed with 10 ml of 15% dichloromethane in toluene. The sample was then applied to the top of the column and the column was eluted with 30 ml of dichloromethane–toluene (15:85). The eluate was concentrated to 2 ml with a gentle stream of nitrogen and injected with an autosampler into the GC system.

2.9. Sample collection

Urine samples were collected from a group of 28 workers exposed in a chemical plant. Each sample was collected at the end of the work shift after 3 working days. Urine was sampled three or four times from each worker in glass containers cleaned with acetone. The control group consisted of 24 healthy volunteers from the normal population. The samples were immediately frozen. To avoid the contamination of urine samples by chemicals present in the work area, it was decided to collect the samples in a hospital near the chemical plant.

3. Results and discussion

The chlorophenols were analysed either in the free form on a glass capillary column with the stationary phase Igepal CO 880 + Apiezon L, specially deactivated for compounds of acidic character, or after derivatization with PFBBr on an SPB-5 fused-silica capillary column (Fig. 2A and B). In the former instance, the extracts were markedly cleaner and no further clean-up was needed. The chromatographic peaks from the Igepal and Apiezon L columns were symmetrical and the reproducibility was optimum [16]. However, the sensitivity of determination was very low, especially for lower chlorinated congeners, when ECD was used. The limits of determination of free chlorophenols were 0.02 μg ml^{-1} (2,4-DCP), 0.01 μg ml^{-1} (PCP) and 0.008 μg ml^{-1} (isomers of trichlorophenols and tetrachlorophenols). Therefore, the method was not considered adequate for the determination of trace levels of chlorophenols in urine.

Methods for the determination of chlorinated phenols in the form of various derivatives in

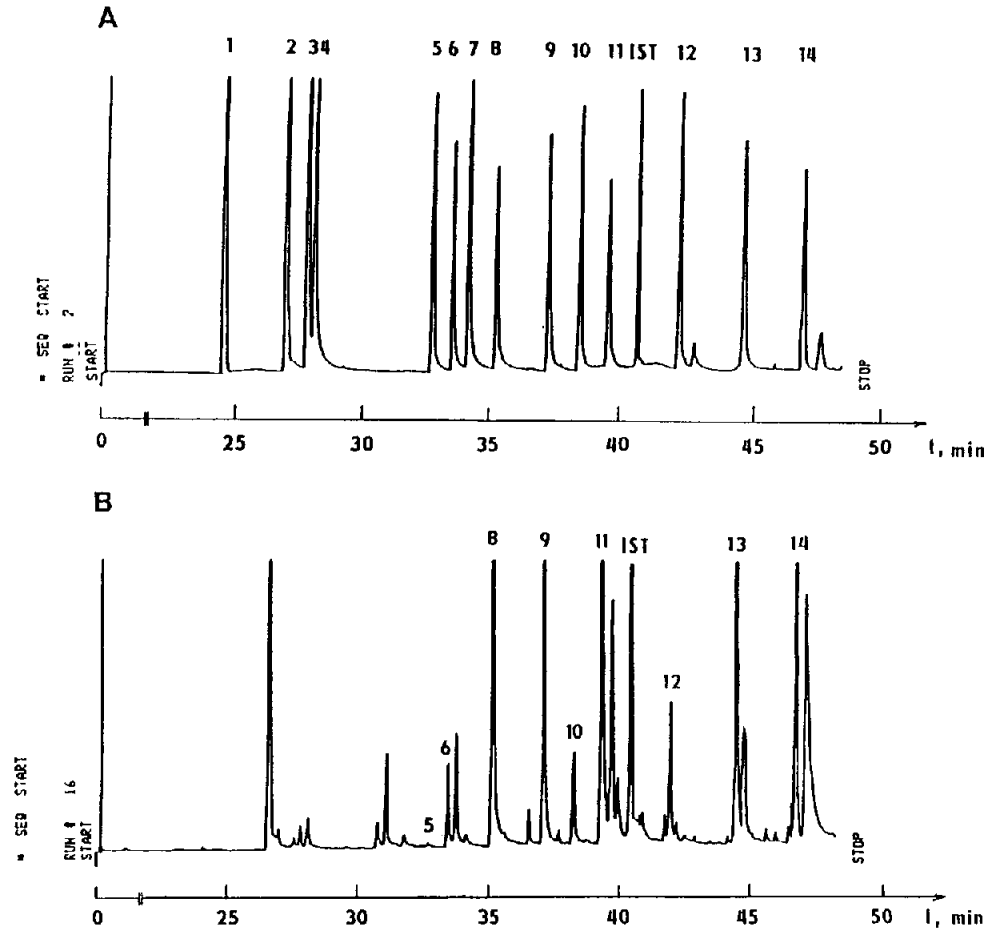

Fig. 2. (A) GC–ECD of urine spiked with PFB derivatives of phenols and cresols resolved on a SPB-5 fused-silica capillary column. Peaks: 1 = Phenol (0.075 ng); 2 = *o*-cresol (0.075 ng); 3 = *m*-cresol (0.075 ng); 4 = *p*-cresol (0.075 ng); 5 = 2-Cl-5-MeP (0.040 ng); 6 = 4-Cl-3-MeP (0.040 ng); 7 = 4-Cl-2-MeP (0.040 ng); 8 = 2,4-DCP (0.040 ng); 9 = 2,4,6-TCP (0.040 ng); 10 = 2,3,6-TCP (0.040 ng); 11 = 2,4,5-TCP (0.040 ng); IST = 2,6-DBP (0.040 ng); 12 = 2,3,5,6-TeCP (0.040 ng); 13 = 2,3,4,5-TeCP (0.040 ng); 14 = PCP (0.040 ng). (B) GC–ECD of a real urine sample. 5 = 2-Cl-5-MeP; 6 = 4-Cl-3-MeP; 8 = 2,4-DCP; 9 = 2,4,6-TCP; 10 = 2,3,6-TCP; 11 = 2,4,5-TCP; IST = 2,6-DBP; 12 = 2,3,5,6-TeCP; 13 = 2,3,4,5-TeCP; 14 = PCP.

several papers [15,23,26,27] were compared. Angerer *et al.* [15] reported the determination of DCP and TCP in urine of the general population. The average recovery of PFB derivatives of DCP and TCP isomers and the detection limits were comparable to our results.

Derivatization with PFBBr resulted in higher sensitivity (Table 1) and good separation was achieved on commercial fused-silica capillary columns. However, it is necessary to remove the interfering compounds on an Ekosorb or a Florisil column using dichloromethane–toluene (15:85) for elution of the derivatized compounds. The coefficient of variation was less than 12% for concentration levels between 0.005 and 0.100 μg ml^{-1} (ten parallel determinations).

The linear range of the calibration graphs is between 0.005 and 0.1 μg ml^{-1} (Fig. 1). In cases of high concentrations of the analyte compounds in urine, the samples were diluted to the linear range and re-analysed.

Methylation with diazomethane and methyl iodide was the objective of previous studies [16,23]. The advantage of this method was a fairly short time of analysis and a minimum of interfering compounds. However, the use of diazomethane is of toxicological concern and therefore it cannot be recommended for routine use. Methylation with methyl iodide did not result in quantitative conversion of all the compounds analysed, even under optimized conditions.

Therefore, the derivatization with PFBBr in the presence of K_2CO_3 was preferred. Complete evaporation of acetone after the derivatization procedure is essential before the clean-up. The presence of even small traces of acetone can result in the elution of interfering substances during clean-up on the Ekosorb or Florisil column. Complete removal of acetone is achieved by repeated evaporation with hexane.

The method was used for the determination of chlorinated phenols and cresols in a group of 28 workers occupationally exposed in a chemical plant. Table 2 gives the levels of chlorinated phenol and cresol congeners. The highest me-

Table 1
Criteria identifying applicability of the analytical method

Compound	Limit of determination (μg ml^{-1})	Concentration (μg ml^{-1})	Recovery ($n = 10$) (%)
2-Cl-5-MeP	0.0015	0.005	72.3 ± 9.9
		0.100	98.5 ± 3.5
4-Cl-3-MeP	0.0015	0.005	94.9 ± 7.6
		0.100	101.8 ± 3.5
4-Cl-2-MeP	0.0015	0.005	76.2 ± 8.3
		0.100	99.0 ± 3.7
2,4-DCP	0.0005	0.005	107.1 ± 6.2
		0.100	108.8 ± 9.5
2,4,6-TCP	0.0005	0.005	109.2 ± 8.1
		0.100	108.0 ± 9.2
2,3,6-TCP	0.0005	0.005	102.6 ± 6.0
		0.100	103.3 ± 6.5
2,4,5-TCP	0.0005	0.005	84.5 ± 9.2
		0.100	103.0 ± 4.2
2,3,5,6-TeCP	0.0010	0.005	101.4 ± 7.7
		0.100	102.0 ± 1.3
2,3,4,5-TeCP	0.0010	0.005	109.9 ± 6.3
		0.100	101.7 ± 5.6
PCP	0.0020	0.005	106.8 ± 9.8
		0.100	101.7 ± 8.4

Table 2
Chlorinated compounds in urine of occupationally exposed persons

Compounds	Concentration (μg ml^{-1})				P.s. (%)
	Mean	Minimum	Maximum	Median	
2-Cl-5-MeP	0.092	ND	0.728	0.008	75
4-Cl-3-MeP	0.096	ND	0.396	0.022	93
4-Cl-2-MeP	0.042	ND	0.372	0.005	64
2,4-DCP	0.113	ND	0.818	0.017	64
2,4,6-TCP	0.027	ND	0.168	0.009	75
2,3,6-TCP	0.052	ND	0.284	0.017	82
2,4,5-TCP	0.132	ND	1.168	0.038	71
2,3,5,6-TeCP	0.069	ND	0.528	0.039	86
2,3,4,5-TeCP	0.111	ND	0.800	0.031	79
PCP	0.154	ND	1.266	0.074	89

P.s. = percentage of positive samples (levels over limits of determination); ND = not detected (below limit of determination, Table 1); $n = 28$.

dian contents were found for PCP, TeCP and 2,4,5-TCP. Maximum contents were found for PCP and 2,4,5 TCP (1.266 and 1.168 μg ml^{-1}, respectively). A control group of 24 volunteers from the general population was chosen for comparison. The median concentration of chlorophenols in the control group ranged from zero (not detected) to 0.019 μg ml^{-1} (Table 3). Fig. 3 compares the median values of chlorinated phenols and cresols in both groups.

In previous work [16,17,28] we studied the exposure of the general population to chlorophenols via food and water. Further sources of contamination, from wood preservatives, deodorants and repellents, etc, should also be accounted for.

Our results compare well with those presented by Angerer *et al.* [15], who found that median levels of 2,4-DCP, 2,4,6-TCP and 2,4,5-TCP in the common population ranged from 0.001 to 0.004 μg ml^{-1} in urine. Kleinman *et al.* [29], and Fenske *et al.* [30] determined the ranges of TeCP

Table 3
Chlorinated compounds in urine of non-occupationally exposed persons

Compounds	Concentration (μg ml^{-1})				P.s. (%)
	Mean	Minimum	Maximum	Median	
2-Cl-5-MeP	0.020	ND	0.176	0.007	60
4-Cl-3-MeP	0.026	ND	0.506	ND	10
4-Cl-2-MeP	0.017	ND	0.092	0.006	85
2,4-DCP	0.001	ND	0.012	ND	15
2,4,6-TCP	0.008	ND	0.106	ND	45
2,3,6-TCP	0.028	ND	0.116	0.017	80
2,4,5-TCP	0.058	ND	0.380	ND	45
2,3,5,6-TeCP	0.021	ND	0.161	ND	25
2,3,4,5-TeCP	0.034	ND	0.268	ND	47
PCP	0.051	ND	0.300	0.019	85

P.s. = percentage of positive samples (levels over limits of determination); ND = not detected (below limit of determination, Table 1); $n = 24$.

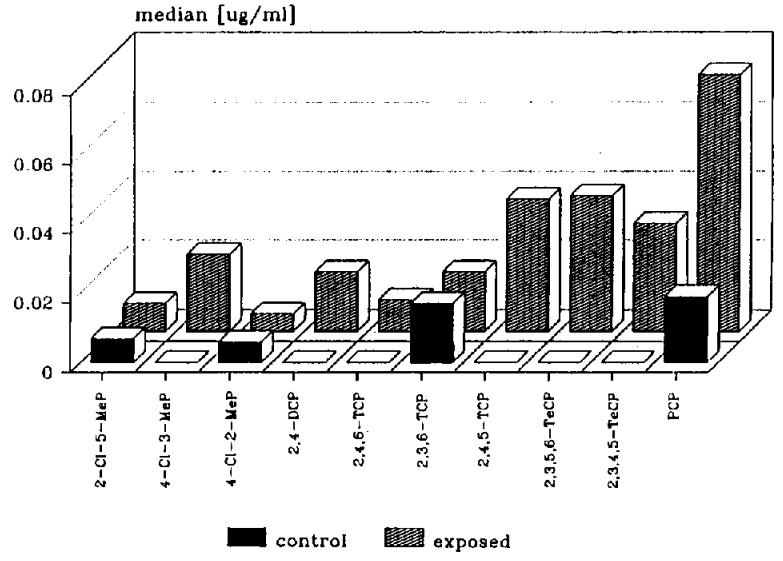

Fig. 3. Comparison of chlorinated compounds in urine of occupationally and non-occupationally exposed persons (median levels).

and PCP concentrations in exposed workers from a lumber-mill. Plant air concentrations of TeCP ranged from 0.8 to 12.2 $\mu g\ m^{-3}$, whereas PCP was not detected (the limit of detection was 0.5 $\mu g\ m^{-3}$). It was estimated that dermal exposure accounted for 95% of the dose taken up by exposed workers. Average concentrations in the urine of exposed workers were from 0.031 to 0.497 $\mu g\ ml^{-1}$ (TeCP) and from 0.057 to 0.103 $\mu g\ ml^{-1}$ (PCP), and the corresponding values in the control group were from 0.006 to 0.029 $\mu g\ ml^{-1}$ (TeCP) and from 0.029 to 0.039 $\mu g\ ml^{-1}$ (PCP).

4. Conclusion

The proposed method solves the problem of the isolation of chlorinated phenols and cresols from urine after acid hydrolysis of conjugates, using SPE on Separcol SI C_{18} columns and elution with methanol, followed by GC–ECD. Derivatization with PFBBr was found to be the most suitable method of determination, increasing the sensitivity by several orders of magnitude. Nevertheless, for higher concentration levels the GC of free phenols on a specially deactivated capillary column is acceptable.

References

[1] *Chlorophenols Other Than Pentachlorophenol (Environmental Health Criteria*, 93), WHO, Geneva, 1989.
[2] J.O. Levin and C.A. Nilsson, *Chemosphere*, 7 (1977) 443.
[3] U.G. Ahlborg and T.M. Thunberg, *CRC Crit. Rev. Toxicol.*, 7 (1980) 1.
[4] T.P. Kupilien and L. Lindroos, *Am. Ind. Hyg. Assoc. J.*, 46 (1985) 34.
[5] J.H. Exon, G.M. Henningsen and C.A. Osborne, *J. Toxicol. Environ. Health*, 14 (1984) 723.
[6] N. Kinae, T. Hashizume, T. Makita, I. Tomita, I. Kimura and H. Kanamori, *Water Res.*, 15 (1981) 17.
[7] D.G. Crosby, K.I. Beynon, P.A. Greve, F. Korte, G.G. Still and J.W. Vouk, *Pure Appl. Chem.*, 53 (1981) 1051.
[8] L. Hardell, *Scand. J. Work Environ. Health*, 7 (1981) 119.
[9] S.M. Somani, T. Smart and A. Khalique, *J. Toxicol. Environ. Health*, 13 (1984) 787.
[10] D. Freitag, H. Geyer, A. Kraus, R. Viswanathan, D. Kotzias, A. Attar, W. Klein and F. Korte, *Ecotoxicol. Environ. Saf.*, 6 (1982) 60.
[11] M.G. Ott, B.B. Holder and R.D. Olson, *J. Occup. Med.*, 22 (1980) 47.
[12] L. Jirasek, J. Kalensky, K. Kubec, J. Pazderova and E. Lukas, *Cesk. Dermatol.*, 49 (1974) 145.
[13] T.D. Sterling, L.D. Stoffman, D.A. Sterling and G. Mate, *Int. J. Health Serv.*, 12 (1982) 559.
[14] *Pentachlorophenol (Environamental Health Criteria*, 71), WHO, Geneva, 1978.
[15] J. Angerer, B. Heinzow, K.H. Schaller, D. Weltle and G. Lehnert, *Fresenius' J. Anal. Chem.*, 342 (1992) 443.

[16] M. Veningerová, J. Uhnák, V. Prachar, K. Farkašová and K. Biliková, *Final Report No. 05-535-863-05-01*, Institute of Preventive and Clinical Medicine, Bratislava, Slovak Republic, 1990.
[17] M. Veningerová, V. Prachar, J. Uhnák and J. Kovačičová, *Z. Lebensm.-Unters.-Forsch.*, in press.
[18] H.B. Lee, Y.D. Stakker and A.S. Chau, *J. Assoc. Off. Anal. Chem.*, 69 (1986) 557.
[19] L. Renberg, *Gas Chromatographic Determination of Chlorophenols in Environmental Samples*, National Swedish Environmental Protection Board, Stockholm, 1981.
[20] O. Bunto, J.C. Olucha and F. Borrull, *Chromatographia*, 32 (1991) 566.
[21] C. de Ruiter, J.W. Brinkman, R.W. Frei, H. Lingeman and U.A.Th. Brinkman, *Analyst*, 115 (1990) 1033.
[22] S. Yamauchi, *J. Chromatogr.*, 635 (1993) 61.
[23] J. Hajšlová, V. Kocourek, I. Zemanová, F. Pudil and J. Davídek, *J. Chromatogr.*, 489 (1988) 88.
[24] B.H. Lee and A.S.Y. Chau, *J. Assoc. Off. Anal. Chem.*, 67 (1984) 1086.
[25] K. Chmel and V. Gajdušková, *J. Chromatogr.*, 552 (1991) 449.
[26] H.B. Lee and A.S.Y. Chau, *J. Assoc. Off. Chem.*, 66 (1983) 1029.
[27] K. Abrahamsson and T.M. Xie, *J. Chromatogr.*, 279 (1983) 199.
[28] M. Veningerová, V. Prachar and J. Uhnák, *Fresenius' Environ. Bull.*, 2 (1993) 386.
[29] G.D. Kleinman, S.W. Horstman, D.A. Kalman and D. Stanzel, *Am. Ind. Hyg. Assoc. J.*, 47 (1986) 731.
[30] R.A. Fenske, S.W. Horstman and R.K. Bentley, *Appl. Ind. Hyg.*, 2 (1987) 143.