

RESEARCH ARTICLE

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The analyse of chlorobenzenes (CB_s) in the soil environment

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Abstract

Chlorobenzenes (CBs) are a group of substituted benzene compounds with only chlorine and hydrogen atoms on the benzene ring. They are mainly used as solvents, pesticides, fire retardants, degreasers, heat transmitters, chemical intermediates and are released in the environment due to their extensive use. This study presents a developing method for determination of chlorobenzenes in the soil, including extraction techniques, concentration, clean up and injection in GC-ECD system which was equipped with a DB-5 capillary column and an AS 2000 autosampler. The detection limit of the method was calculated based on the chlorobenzene recoveries of the presented analytical method and on the detection limits in GC of each chlorobenzenes. The extraction with ASE technique presented the best results as the recoveries of MCB, 1,2,4-TCB and HCB from soil with were 100,5±2,5, 88,0±3,3 and 88,0±5,0, respectively. The detection limits of chlorobenzenes in the GC-ECD varied from 0,38 pg/μl for HCB to 250,00 pg/μl for MCB. Also, the total recovery of chlorobenzenes was high for HCB (84,13 ± 4,30) and very low for MCB (9,80 ± 1,16). The values of method detection limits of chlorobenzenes in soil were from 0,14 μg/kg soil for HCB to 765,31 μg/kg soil for MCB, whereas other chlorobenzenes had intermediate values. Therefore, by using this determination method, high chlorinated benzenes as HCB and PCB could be detected in very small amounts in soil samples.

Keywords: Chlorobenzenes, method, extraction, detection limit, soil.

1. Introduction

Chlorobenzenes (CBs) are organic compounds with only chlorine and hydrogen atoms on the benzene ring; the number of chlorine atoms can vary from one to six. They are chemically very unreactive and generally stable under environmental conditions. Because of the high electronegativity of the chlorine atom, chlorobenzenes are highly resistant to electrophilic attack, like dechlorination and additional chlorine substitution lowers the compound reactivity [1]. Hydroxylations of chlorobenzenes occur only at high temperature under very alkaline conditions and chlorobenzenes are non-ionised compounds.

The difference between chlorobenzene structures is just the number of chlorine atoms they possess and their substitution place on the benzene ring. This substitution yields 12 compounds, including: monochlorobenzene (MCB), 3 isomeric forms of dichlorobenzene (DCB), 3 isomers of trichlorobenzene (TCB), 3 isomers of tetrachlorobenzene (TeCB), pentachlorobenzene (PCB) and hexachlorobenzene (HCB). MCB, 1,2-

DCB, 1,3-DCB and 1,2,4-TCB are colourless liquids, while all other congeners are white crystalline solids at room temperature. The position of the chlorine atoms leads to different isomers with different properties.

Chlorobenzenes are listed as toxic compounds and their toxicity increase generally with the increase of the number of chlorine atoms in the benzene ring. They are very irritating and react readily with tissues in liver and kidney [8, 9, 20, 23]. The potential health hazard of a xenobiotic compound is a function of its persistence in the environment as well as the toxicity of the chemical class. Hexachlorobenzene is classified as a probable human carcinogen by the US Environmental Protection Agency [2, 3, 9]. Due to their toxicity some chlorobenzenes are ranked in priority list of pollutants [12, 20].

Chlorobenzenes are mainly used as solvents, odorizers, herbicides, insecticides, fungicides, fire retardants, degreasers, heat transmitters and chemical intermediates for the production of several other compounds, such as dyes, and pharmaceutical products [8, 23].

Chlorobenzenes are released in the environment due to their extensive use, the presence in industrial effluents and wastes or by the incineration of wastes which contain organochlorine compounds. They may also enter in the ecosystems due to the biodegradation of chlorinated compounds [8].

Due to the extensive use and long range transport, chlorobenzenes are detected in different environmental compartments. Thus, chlorobenzenes are detected in soil samples of different areas. In uncontaminated soils, chlorobenzene levels were less than 0.4 mg/kg for dichlorobenzene congeners and less than 0.1 mg/kg for other chlorobenzenes [22]. The total chlorobenzene concentrations in soil samples from a former pesticide factory in Germany ranged from 1.5 to 18400 mg/kg [7]. The concentration of HCB in soil samples originated from Leipzig-Halle region were between 0.57 and 3.75 µg/kg dry weight (dw) [13]. In Switzerland, agricultural soil had HCB level of only 0.15-5.0 µg/kg dw [19], while considerably higher values of approximately 40 µg/kg dw are encountered in Italy [11]. Nakata et al. [16] have detected in soil samples a HCB concentration up to 3.2 ng/g dw. Meijer et al., [15] have analysed the HCB contents in nearly 200 background surface soil samples and they found an average value of 680 pg g⁻¹ dw, showing clearly the ubiquitous presence of HCB in the environment.

The occurrence of chlorobenzenes is also observed in sediments due to their transport and the affinity to be absorbed into sediment materials, [4, 10, 14, 16, 17]. Thus, determination of chlorobenzenes in soil or sediments is of high importance.

The objective of this study was to develop a method for determination of chlorobenzenes in the soil environment, including extraction techniques, concentration, clean up and injection in GC-ECD system equipped with a DB-5 capillary column and an AS 2000 autosampler. Furthermore, the calculation of the detection limit of the method was based on the chlorobenzene recoveries of the analytical method and on the detection limits in GC of each chlorobenzenes.

2. Materials and Methods

2.1. Chemicals and reagents

Uniformly ¹⁴C-ring-labelled chlorobenzenes: hexachlorobenzene (HCB), pentachlorobenzene (PCB), 1,2,4-trichlorobenzene (TCB) and monochlorobenzene (MCB), purity >98 %, specific radioactivity of 5 mCi/mMol (or 185 MBq/mMol)

were obtained from International Isotope (Munich, Germany).

The non labelled chlorobenzenes – including hexachlorobenzene (HCB), pentachlorobenzene (PCB), monochlorobenzene (MCB) and the isomers of tetrachlorobenzene (TeCB), trichlorobenzene (TCB), dichlorobenzene (DCB), purity >99.5% were purchased from Dr. Ehrenstorfer (Augsburg, Germany). The standard solutions were used for identification and quantitative analysis of chlorobenzenes by GC-ECD.

n-Hexane Picograde® was obtained from Promochem (Wesel, Germany). All other solvents (methanol, acetone and n-hexane) were of analytical grade and were purchased from Merck (Darmstadt, Germany). Sodium sulphate (Na₂SO₄) and sea sand were also obtained from Merck (Darmstadt, Germany).

The scintillation cocktails (Ultima Gold XR; Ultima Flo) were purchased from Packard (Dreieich, Germany).

2.2. Standard solutions

The standard solutions for application were prepared from stock standards of labelled and non labelled chlorobenzene. Different preparation methods were used for the chlorobenzenes.

The ¹⁴C-mono-chlorobenzene standard solution was prepared according to Wang and Jones [21]: ¹⁴C-mono-chlorobenzene was dissolved in hexane, and then mixed with non-labelled MCB in acetone. This mixture was diluted further with distilled water at a rate of 0.06:1:12 (hexane:acetone:water, v/v).

The standard application solutions for the other chlorobenzenes including HCB, PCB and 1,2,4-TCB were prepared by mixing the labelled with unlabelled chlorobenzenes in the required rates and dissolved in n-hexane.

The standard solutions of non labelled chlorobenzenes were prepared by dissolving also in n-hexane in the required concentrations. These standards were used for identification and quantitative analysis of chlorobenzenes by GC-ECD.

The prepared standard solutions were kept in refrigerator until application.

2.3. Extraction, concentration and purification

Three extraction methods (accelerated solvent extraction (ASE), soxhlet extraction, and column extraction) were tested for their extraction efficiency of MCB, 1,2,4-TCB and HCB from soil. For these

tests, the ¹⁴C-chlorobenzene standards were applied prior to the extraction procedure directly to the soil in the ASE cell, the soxhlet thimble, or the glass column.

Accelerated solvent extraction was performed in an ASE-200 (Dionex, Idstein, Germany) with the following parameters:

Temperature:	90°C	Pressure:	10 MPa
Preheat:	0 min	Heat:	6 min
Static:	5 min	Flush:	100 %
Purge:	150 sec	Cycles:	3

Soxhlet extraction was conducted in a soxhlet apparatus for 8 hours at 60°C, the cooler was kept at a temperature of -10°C to minimize the losses during the extraction process.

The column extraction was carried out in a glass column (50x2,5 cm) at the room temperature and atmospheric pressure.

A mixture of hexane/acetone (3:1 v/v) was used as extraction solvent in each extraction method. The extraction efficiency experiments were conducted in three replicates. For determination of radioactivity in liquid samples, aliquots of the sample were mixed with a scintillation cocktail (Ultima Gold XR) and measured in a liquid scintillation counter (Tricarb 1900 TR, Packard, Dreieich, Germany).

The extracts were reduced to a volume of 3-4 ml in a Kuderna-Danish apparatus at a temperature of 70°C. The concentrated extracts were cleaned up by passing them through SPE columns containing 2 g of Florisil (Varian, Darmstadt, Germany). The SPE columns were conditioned with 15-20 ml of n-hexane Picograde®, prior to the application of the samples. n-Hexane Picograde® was used for elution and the first 15 ml were collected in a graduated vial for further analysis.

2.4. Identification and quantification of chlorobenzenes

The analysis of the chlorobenzenes was carried out on a GC -ECD system (Trace GC, 2000 Series,

Table 1. The extraction recoveries of ¹⁴C-chlorobenzenes from soil for the tested extraction methods (n=3, ±SD).

Chlorobenzenes	Recoveries (%) ± SD		
	ASE extraction	Soxhlet extraction	Column extraction
¹⁴ C-HCB	100,5±2,5	100,4±13,9	90,3±6,9
¹⁴ C-TCB	88,0±3,3	92,9±14,5	86,8±7,6
¹⁴ C-MCB	88,0±5,0	71,4±3,9	86,2±6

The accelerant solvent extraction (ASE) technique can be recommended for the extraction of chlorobenzenes from the soil samples, since the extraction efficiencies were satisfactory and furthermore the deviation standards were lower and

ThermoQuest, Egelsbach, Germany) which was equipped with a DB-5 capillary column (30 m length, 0.32 mm ID and 0.25µm film thickness, J&W Scientific, U.S.A) and an AS 2000 autosampler. On the DB-5 column all chlorobenzenes could be separated with the exception of 1,2,3,5- and 1,2,4,5-TeCB, which were evaluated together. The GC-ECD operated in the following conditions:

Temperature program: 60°C for 2 min,
5 °C/min to 190°C,
20 °C/min to 280°C,
and hold for 7 min.

Injector temperature: 240°C

Detector temperature: 290°C.

Helium was used as carrier gas and nitrogen as make-up gas. The injection volume was 1 µl in the splitless mode (75 sec).

The chlorobenzenes were identified by comparing their retention times with reference substances. For this purpose, a mixture of non labelled chlorobenzenes standards was injected in GC-ECD.

3. Results and Discissions

The obtained results of extraction recoveries for the extraction methods (ASE extraction, Soxhlet extraction and Column extraction) are presented in the Table 1. Higher recoveries were observed for HCB in the three extraction methods, this could be due to the stability and low volatility of this compound [5]. The lowest recoveries were obtained for MCB, DCBs and TCBs, because these compounds present higher volatility [6, 18], therefore higher losses during the extraction procedure.

The ASE extraction method presents the lowest standard deviation (SD), as it is an automated technique when the extraction parameters are regularly the same from one to the other replicate.

consume less time and solvents than the other extraction techniques.

The qualification of the chlorobenzenes was attained by comparing their retention times with

chlorobenzenes standards that were injected in GC-ECD under the mentioned conditions.

The figure 1 shows the obtained chromatogram from the injection of chlorobenzene standards in GC-

ECD and the injected concentrations are presented in the Table 2.

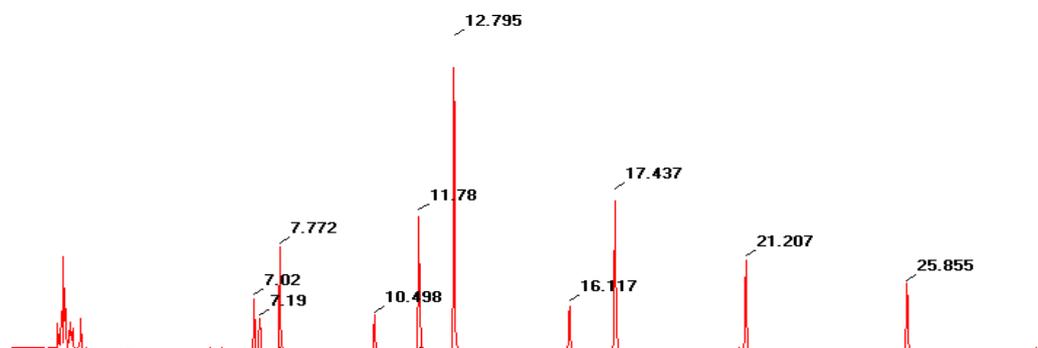


Figure 1. The GC chromatogram of chlorobenzenes standards.

Table 2. The concentration of chlorobenzene standards injected in GC-ECD

Nr.	Chlorobenzenes	Injected concentrations (pg/μl)
1.	1,3-DCB	434.0
2.	1,4-DCB	608.0
3.	1,2-DCB	1116.0
4.	1,3,5-TCB	118.5
5.	1,2,4-TCB	248.8
6.	1,2,3-TCB	301.0
7.	1,2,4,5-TeCB	53.0
8.	1,2,3,4-TeCB	92.0
9.	PCB	21.4
10.	HCB	12.4

The retention time of chlorobenzenes as can be observed in the Figure 1, varied from low to high chlorinated benzenes. DCBs and TCBs can be detected first and then TeCBs, PCB and HCB as high chlorinated benzenes which are characterized by volatilization in higher temperature.

The quantification was performed by using linear calibration curves ($r^2=0,99$) of the individual chlorobenzenes. In the Figure 2 are presented the calibration curves of HCB and 1,3,5-TCB. It should be mentioned that the samples were diluted in the cases of quantifications of HCB and the other dechlorination products when the concentrations were above linear range of calibration curves.

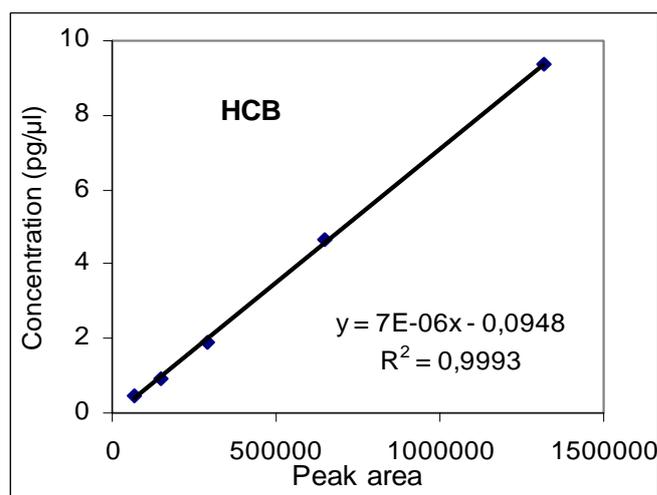
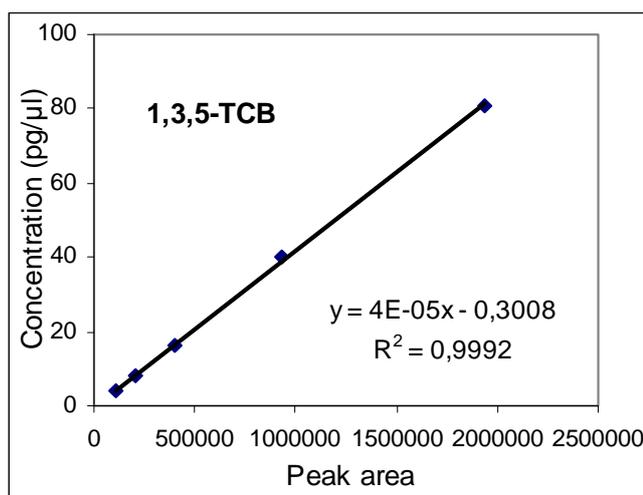


Figure 2. Calibration curves of 1,3,5-TCB and HCB for their quantification in the soil samples.

3.1. Chlorobenzenes recoveries and detection limits

The total recoveries of all chlorobenzenes were tested in the preliminary tests for the presented analytical method. Standard solutions of chlorobenzenes with known concentrations were applied to the soil sample and followed the same procedure as describe above including soil sample preparation, ASE extraction, liquid-liquid separation filtration, concentration, clean up and injection in GC-ECD.

The parallel tests were conducted with labelled MCB and HCB to compare the obtained results in concentration calculation from GC analysis with the measuring of the radioactivity. The total recovery for each chlorobenzene (Table 3) was calculated based on the initial applied concentration and the finned concentration from GC analysis. The obtained results were corrected according to the respective recovery of chlorobenzenes.

The detection limits of chlorobenzenes in the GC-ECD were also estimated (Table 4). The prepared standard solutions of each chlorobenzene in different

concentrations were injected in GC-ECD at least in four replicates. Linear regression between concentration and peak area of respective chlorobenzene was used for estimation. Furthermore, the detection limits of chlorobenzenes were calculated by using a software programme StatCal version 3.3.

Table 3. The total recoveries of chlorobenzenes

Nr.	Chlorobenzenes	Recoveries (% of applied amount, \pm SD)
1.	MCB	9,80 \pm 1,16
2.	1,3-DCB	20,52 \pm 2,41
3.	1,4-DCB	19,62 \pm 2,91
4.	1,2-DCB	25,30 \pm 2,75
5.	1,3,5-TCB	26,48 \pm 1,39
6.	1,2,4-TCB	36,63 \pm 2,01
7.	1,2,3-TCB	40,36 \pm 3,61
8.	1,2,4,5- & 1,2,3,5-TeCB	59,46 \pm 2,14
9.	1,2,3,4-TeCB	64,36 \pm 1,68
10.	PCB	81,93 \pm 3,70
11.	HCB	84,13 \pm 4,30

Table 4. Detection limits of chlorobenzenes in GC-ECD.

Nr.	Chlorobenzenes	Injected concentrations (pg/ μ l)					GC-detection limits (pg/ μ l)
1.	MCB	7380	3690	736	368	184	250.00
2.	1,3-DCB	77.30	38.65	15.46	7.73	3.87	4.97
3.	1,4-DCB	174.40	87.20	34.88	17.44	8.72	7.79
4.	1,2-DCB	130.50	65.25	26.10	13.05	6.53	3.64
5.	1,3,5-TCB	16.12	8.06	4.03	1.61	0.81	1.91
6.	1,2,4-TCB	14.62	7.31	3.66	1.46	0.73	1.54
7.	1,2,3-TCB	14.60	7.30	3.65	1.46	0.73	2.19
8.	1,2,4,5- & 1,2,3,5-TeCB	6.96	3.48	1.74	0.70	0.35	0.99
9.	1,2,3,4-TeCB	6.12	3.06	1.53	0.61	0.31	0.75
10.	PCB	3.16	1.58	0.79	0.32	0.16	0.64
11.	HCB	1.87	0.94	0.47	0.19	0.09	0.38

Table 5. Method detection limits of chlorobenzenes

Nr.	Chlorobenzenes	Method detection limits (μ g/kg soil, d.w.)
1.	MCB	765,31
2.	1,3-DCB	7,26
3.	1,4-DCB	11,91
4.	1,2-DCB	4,32
5.	1,3,5-TCB	2,16
6.	1,2,4-TCB	1,26
7.	1,2,3-TCB	1,63
8.	1,2,4,5- & 1,2,3,5-TeCB	0,50
9.	1,2,3,4-TeCB	0,35
10.	PCB	0,23
11.	HCB	0,14

The detection limit of the presented method was calculated based on the chlorobenzene recoveries of the presented analytical method and on the detection limits in GC of each chlorobenzenes. The results from these calculations are presented in the Table 5.

4. Conclusions

The accelerant solvent extraction (ASE) technique can be used for the extraction of chlorobenzenes from the soil samples, since the extraction efficiencies were satisfactory and furthermore the deviation standards were lower and consume less time and solvents than the other extraction techniques.

The presented method can be used for the determination of chlorobenzenes (CBs) in soil samples. Furthermore, high chlorinated benzenes as HCB and PCB could be detected by using this method in very small amounts in soil samples.

5. References

- Adrian L, Görisch H.: **Microbial transformation of chlorinated benzenes under anaerobic conditions**. Res. Microbiol, 2002, 153: 131-137.
- ATSDR: **Hexachlorobenzene. Public Health Statement: Agency for Toxic Substances and Disease Registry**, Division Toxicology, Atlanta, GA, 1999.
- ATSDR: **Toxicological Profile for Hexachlorobenzene**. ATSDR Public Health Statement: U.S. Department of Health and Human Services, 2002.
- Beurskens J E M, Dekker C G C, Jonkhoff J, Pompstra L.: **Microbial dechlorination of hexachlorobenzene in a sedimentation area of Rhine River**. Biogeochemistry, 1993, 19: 61-81.
- Brahushi F, Dörfler U, Schroll R, Munch J.C.: **Stimulation of reductive dechlorination of hexachlorobenzene in soil by inducing the native microbial activity**. Chemosphere, 2004, 55: 1477-1484.
- Brahushi F, Dörfler U, Schroll R, Feicht E, Munch J C.: **Environmental behavior of monochlorobenzene in an arable soil**. Fres. Environ. Bull, 2002, 11: 599-604.
- Feidieker D, Kampfer P, Dott W.: **Microbiological and chemical evaluation of a site contaminated with chlorinated aromatic compounds and hexachlorocyclohexanes**. FEMS Microbiology Ecology, 1994, 15: 265-278.
- IPCS: **Chlorobenzenes other than hexachlorobenzene**. Geneva, World Health Organization, International Programme on Chemical Safety. Environmental Health Criteria 128, 1991.
- IPCS: **Hexachlorobenzene**. Geneva, World Health Organization, International Programme on Chemical Safety. Environmental Health Criteria 195, 1997.
- Lee C.-L, Song H.-J, Fang M.-D.: **Pollution topography of chlorobenzenes and hexachlorobutadiene in sediment along the Kaohsiung coast, Taiwan- a comparison of two consecutive years' survey with statistical interpretation**. Chemosphere, 2005, 58: 1503-1516.
- Leoni V, D'Arca S.U.: **Experimental data and critical review of the occurrence of hexachlorobenzene in the Italian environment**. Sci. Total Environ, 1976, 253-272.
- Lerche D, van de Plassche E, Schwegler Balk, A.: **Selecting chemicals substances fro the UN-UNE POP Protocol**. Chemosphere, 2002, 47: 617-630.
- Manz M, Wenzel K.-D, Dietze U, Schüürmann G.: **Persistent organic pollutants in agricultural soils of central Germany**. Sci. Total Environ, 2001, 277: 187-198
- Masunaga S, Yonezawa Y, Urushigawa Y.: **The distribution of chlorobenzenes in the bottom sediments of Ise Bay**. Water Res, 1991, 25: 275-288.
- Meijer S N, Ockenden W A, Sweetman A, Breivik K, Grimalt J O, Jones K C.: **Global distribution and budget of PCBs and HCB in background surface soil: implications for sources and environmental processes**. Environ. Sci. Technol, 2003, 667-672.
- Nakata H, Hirakawa Y, Kawazoe M, Nakabo T, Arizono K, Abe S-I, Kitano T, Shimada H, Watanabe I, Li W, Ding X.: **Concentration and composition of organochlorine contaminants in sediment, soils, crustaceans, fishes and birds collected from Lake Tai, Hangzhou Bay and Shanghai city region, China**. Env. Pollution , 2005, 133: 415-429.
- Oliver B G, Nicol K D.: **Chlorobenzenes in sediments, water, and selected fish from Lakes Superior, Huron, Erie and Ontario**. Environ. Sci. Technol, 1982, 16: 532-536.
- Schroll R, Brahushi F, Dörfler U, Kühn S, Fekete J, Munch J.C.: **Biominalisation of 1,2,4-trichlorobenzene in soils by an adapted microbial population**. Environmental Pollution, 2004, 127: 395-401.
- Streit B.: **Lexikon Ökotoxikologie**. Weinheim, New York; VCH-Verlag, 1994.
- UNEP: **Final act of the conference of plenipotentiaries on the Stockholm convention on persistent organic pollutants**. Document: UNEP/POPS/CONF/4. Available from: <http://irptc.unep.ch/pops>, 2001.
- Wang M.-J. and Jones C J.: **Behavior and fate of chlorobenzenes in spiked and sewage sludge-amended soil**. Environ. Sci. Technol, 1994, 28: 1843-1852.

22. Wang M.-J, McGrath S P, Jones KC.: **Chlorobenzenes in field soil with a history of multiple sewage sludge application.** Environ. Sci. Technol, 1995, 29: 356-362.
23. WHO: **Chlorobenzenes other than hexachlorobenzene:** Environmental aspects. World Health Organization, Geneva, 55p, 2004.