

Articles

Determination of 25 ED_s in Frog and Fish Tissue by GC-MS (SIM)

Ueon-Sang Shin¹ and Ho-Sang Shin^{2,3,*}

¹Korea Institute of Science and Technology, P.O. Box 131, Cheongryang, Seoul 130-650, Korea

²Department of Environmental Education, Kongju National University, Kongju 314-701, Korea

³RRC NMR, Kongju National University, Kongju 314-701, Korea

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A gas chromatography/mass spectrometric assay method was developed for the simultaneous determination of neutral and basic twenty-five endocrine disruptors (ED_s) in frog and fish. After homogenization and sonication of 5 g of sample, purification was achieved in one step with a solid phase extraction procedure using silica gel-florisil. Elution was performed with 50 mL of acetone : *n*-hexane (1 : 9) solution. The eluate was concentrated to approximately 10 μ L and dissolved with 100 μ L of hexane and analyzed by GC-MS (SIM). The peaks had good chromatographic properties and the extraction of these compounds from sample also gave relatively high recoveries with small variations. Detection limits were 0.1 ng/g for 4-nitrotoluene, benzophenone, hexachlorobenzene, atrazine, malathion, *o,p*-DDD, *o,p*-DDT and permethrin, and 0.2 ng/g for heptachlor epoxide, γ -chlordane, α -chlordane, *p,p'*-DDE, *p,p'*-DDD, cypermethrin and fenvalerate, and 0.3 ng/g for trifluralin, metribuzin, alachlor, dieldrin and *p,p'*-DDT, and 0.5 ng/g for heptachlor, aldrin and parathion, and 0.7 ng/g for endrin, and 0.8 ng/g for nitrofen. The recoveries were between 33 and 109%. The method was used to analyze twenty-five frogs and forty-six fishes samples caught from various regions in Korea. Benzophenone was detected at concentration of up to 17.2 ng/g in frog or fish. Heptachlor, aldrin, γ -chlordane, *p,p'*-DDE, *p,p'*-DDD, endrin and *o,p*-DDD were detected at concentrations of 0.7-12.5 ng/g in frog or fish. Also significant levels of dieldrin (up to 22.5 ng/g) were observed. The developed method may be valuable to be used to the national monitoring project of EDS in biota samples.

Key Words : ED_s, Cleanup, Frog, Fish, GC-MS

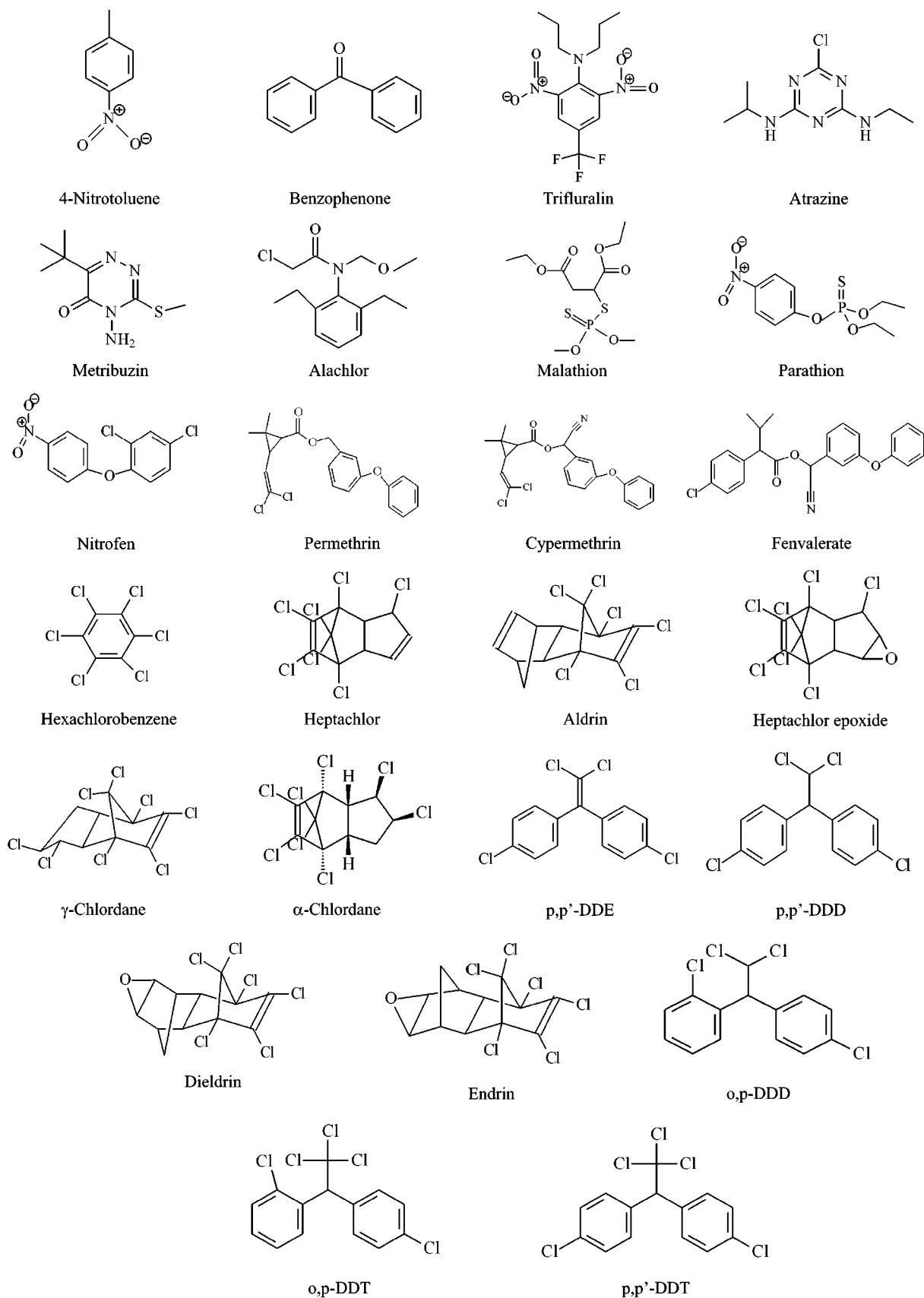
Introduction

Since the early 1950s, abnormalities in production and development as well as disorders suspected of immunodeficiency or brain maldevelopment have been reported in several wildlife species all over the world.¹ Also, a number of abnormalities in the human population have been reported. They include reduced number and deterioration of sperm quality in males, abnormal or delayed development of male reproductive organs such as retained testis and hypospadias, increased incidence of prostate cancer, breast cancer and endometriosis with the associated infertility in female.² A considerable body of evidence indicates that many classes of environmental contaminants, including dioxins, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons and many pesticides have the ability to interfere with normal hormonal activity by mimicking and blocking the action of natural hormones.³⁻⁸ Particularly, the chlorinated pesticides named as persistent organic pollutants (POPs) bioaccumulate in the food chain due to their lipophilicity and persistence, and they have become a major issue of research in order to investigate their ubiquitous environmental occurrence, biochemical and toxic effects, human exposure and health risk assessment.⁹⁻¹³

Since 1999, the Korean Ministry of Environment has monitored ED_s in ecologic samples and dioxins, PCBs, PBBs, alkyl phenols, phthalates, organotins, organochlorinated pesticides, nitrogenated pesticides, 4-nitrotoluene and benzophenone have been selected as target compounds. To quantitatively evaluate the residues of these compounds in the ecosystem, an analytical method at low ng/g level with high precision is prerequisite.

To date, many analytical methods for the determination of EDS in biota have been described.¹⁴⁻²¹ However, the analytical methods published until now exclusively focus on either nitrogenated pesticides,¹⁴⁻¹⁷ chlorinated pesticides¹⁸⁻²¹ or their sub-classes.²²⁻²⁴ Gas chromatographic (GC) methods have been published for the analysis of chlorinated pesticides^{18-21,26} or nitrogenated pesticides.^{14,16-17} Mass spectrometry (MS) has the potential to become a standard analytical tool for detecting these compounds and sensitive analysis is usually accomplished by GC/MS. Although most procedures utilize GC/MS to quantify the analytes, they differ substantially with respect to sample extraction and concentration.

Our aim was to develop analytical method, which allows the simultaneous quantification of several pesticides plus 4-nitrotoluene and benzophenone in frog or fish at the low-ng/g level. The Japanese national monitoring method has

**Figure 1.** Structures of 25 FD_s studied.

generally been used for monitoring of the compounds in Korea, but the method is composed of very complex multi-extraction steps.²⁷ A single method of analysis for these various compounds would have several advantages, such as a shorter overall analysis time, reduced field sampling and cost reduction for the monitoring.

In this paper, we present a new analytical method with SPE/GC-MS for the quantification of regularly monitored neutral and basic EDS in frog and fish. The target compounds (see Fig. 1) can be extracted and cleaned on solid phase sorbent. After concentration of the eluate, the compounds can directly be analyzed by GC-MS.

Experimental Section

Chemicals and reagents. Phenanthrene-d₁₀ (internal standard) and pyrene-d₁₀ (internal standard) were purchased from Aldrich(USA). 4-Nitrotoluene, benzophenone, trifluralin, hexachlorbenzene, atrazine, metribuzin, alachlor, heptachlor,

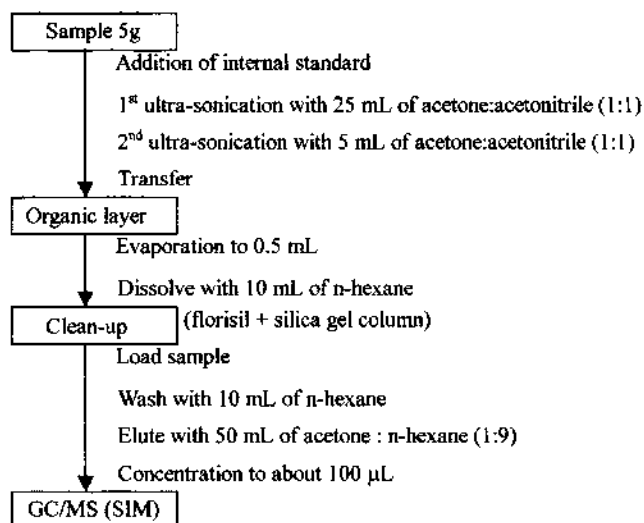


Figure 2. Diagram of whole experiment procedure.

Table 1. GC-MS conditions for the determination of the target compounds

Parameter	Condition			
Column	HP-5MS (Cross-linked 5% phenylmethylsilicon), 30 m × 0.2 mm I.D. × 0.25 µm F.T			
Carrier	He at 0.9 mL/min			
Oven Temp.	50 °C (1 min) → 200 °C → 280 °C (5 min)		5 °C/min	
Split Ratio	1 : 10			
Injector Temp.	250 °C			
Transfer Temp.	280 °C			
Selected Ion Group	Group	Start Time (min)	Compound	Selected Ions, m/z
	1	4.00	4-Nitrotoluene	137, 91
	2	8.50	Benzophenone	105, 182
			Trifluralin	306, 335
	3	10.30	Hexachlorobenzene	284, 286
	4	10.63	Atrazine	200, 215
	5	10.90	Phenanthrene-d ₁₀	188
	6	11.50	Metribuzin	198, 144
	7	12.10	Alachlor	160, 188
			Heptachlor	272, 274
	8	12.60	Malathion	173, 125
	9	13.00	Aldrin	263, 66
			Parathion	291, 109
	10	13.80	Heptachlor epoxide	353, 355
	11	14.30	γ-Chlordane	373, 375
			Pyrene-d ₁₀	212
			α-Chlordane	373, 375
	12	15.30	p,p'-DDE	246, 318
			Dieldrin	79, 263
			p,p'-DDD	235, 237
	13	15.95	Nitrofen	283, 202
			Endrin	263, 81
	14	16.40	o,p'-DDD	235, 237
			o,p'-DDT	235, 237
	15	17.50	p,p'-DDT	235, 354
	16	21.00	Permethrin	183, 163
	17	23.00	Cypermethrin	163, 181
	18	25.00	Fenvalerate	125, 167

malathion, aldrin, parathion, heptachlor epoxide, γ -chlordane, α -chlordane, *p,p'*-DDE, dieldrin, *p,p'*-DDD, nitrofen, endrin, *o,p'*-DDD, *o,p'*-DDT, *p,p'*-DDT, permethrin, cypermethrin and fenvalerate were purchased from Sigma (St. Louis, MO, USA). Analytical grade sodium sulfate (Junsei, Japan) was used as reagent, and *n*-hexane, acetone, acetonitrile and methanol (J.T. Baker, USA) were used as solvents. Silica gel and florisil of chromatography grade (Sigma, USA) were activated by overnight heating at 350 °C and 170 °C, respectively, and stored in a desiccator. Water was purified in milli-Q (Millipore Corp., Milford, MA). Fishes and frogs were sampled in seventy-one regions in Korea.

Spiking. Spiked samples were prepared by fortifying fish samples (5 g) with 10-100 μ L of EDS standard solutions at a concentration of 50-5000 ng/mL and with 20 μ L of the solution containing internal standards at a concentration of 5000 ng/mL. The same amount of internal standard solution was added to samples before the extraction.

Extraction procedure. A flow scheme of the whole method including sample homogenization, lipid decomposition, SPE and clean-up before the GC-MS analysis is shown in Figure 2. A 25-mL volume of acetone-acetonitrile (1 : 1, v/v) was added to 5 g of the biota sample. 20 μ L of phenanthrene- d_{10} and pyrene- d_{10} (5000 ng/mL in acetone) were added to the solution. The sample was homogenized in Homogenizer (PowerGen 125, Fisher scientific, USA) and sonicated in an ultrasonic bath (Branson 5210, Branson Ultrasonic Cleaner, USA) for 5 min. The organic phase was transferred to a round flask and a 5-mL volume of acetone-acetonitrile (1 : 1, v/v) was added and the sample was sonicated again. The extract was evaporated to 0.5 mL. The sample was dissolved with 10 mL of *n*-hexane and gradually transferred to a washed and preconditioned (10 mL of *n*-hexane) SPE column. The SPE column consisted of 2.5 g of silica gel, 2.5 g of florisil and 2.0 g of sodium sulfate. The maximum flow rate of sample through the SPE column was 1 mL/min. The SPE column was washed with 10 mL of *n*-hexane at a flow-rate up to 10 mL/min. Then EDS were eluted with 50 mL of acetone-*n*-hexane (1 : 9, v/v) at a flow-rate of 0.5 mL/min. The eluate was dried with anhydrous sodium sulfate, evaporated to approximately 10 μ L and then diluted with 100 μ L of hexane. The solution was transferred into a V-shape auto sampler vial. At appropriate times, 2 μ L sample of the solution was injected into the GC system.

Gas chromatography-mass spectrometry. All mass spectra were obtained with a Agilent 6890/5973 N instrument. The ion source was operated in the electron ionization mode (EI; 70 eV, 230 °C). Full-scan mass spectra (*m/z* 40-800) were recorded for the identification of analytes at high concentration. Confirmation of trace chemicals was completed by two MS characteristic ions, the ratio of two MS characteristic ions and GC-retention times matches to the known standard compounds. The ions selected in this study and the operating parameters of GC-MS are shown in Table I.

Calibration and quantification. Calibration curves for 4-nitrotoluene, benzophenone, atrazine, metribuzin, heptachlor,

malathion, aldrin, *o,p'*-DDD and fenvalerate were established by extraction after adding 0.5, 2.5, 10, 100, 250 and 500 ng of standards and 100 ng of internal standard in 5 g of fish extract. Calibration curves for trifluralin, hexachlorbenzene, alachlor, heptachlor epoxide, γ -chlordane, α -chlordane, *p,p'*-DDD and cypermethrin were established by extraction after adding 2.5, 10, 100, 250 and 500 ng of standards and 100 ng of internal standard in 5 g of fish. Calibration curves for parathion, *p,p'*-DDE, dieldrin, nitrofen, endrin, *o,p'*-DDT, *p,p'*-DDT and permethrin were established by extraction after adding 5.0, 10.0, 100, 250 and 500 ng of standard and 100 ng of internal standard in 5 g of fish. Phenanthrene- d_{10} was used as the internal standard for 4-nitrotoluene, benzophenone, trifluralin, hexachlorbenzene, atrazine, metribuzin, alachlor, heptachlor, malathion, aldrin, parathion, heptachlor epoxide and γ -chlordane. Also pyrene- d_{10} was used as the internal standard for α -chlordane, *p,p'*-DDE, dieldrin, *p,p'*-DDD, nitrofen, endrin, *o,p'*-DDD, *o,p'*-DDT, *p,p'*-DDT, permethrin, cypermethrin and fenvalerate. The ratio of the

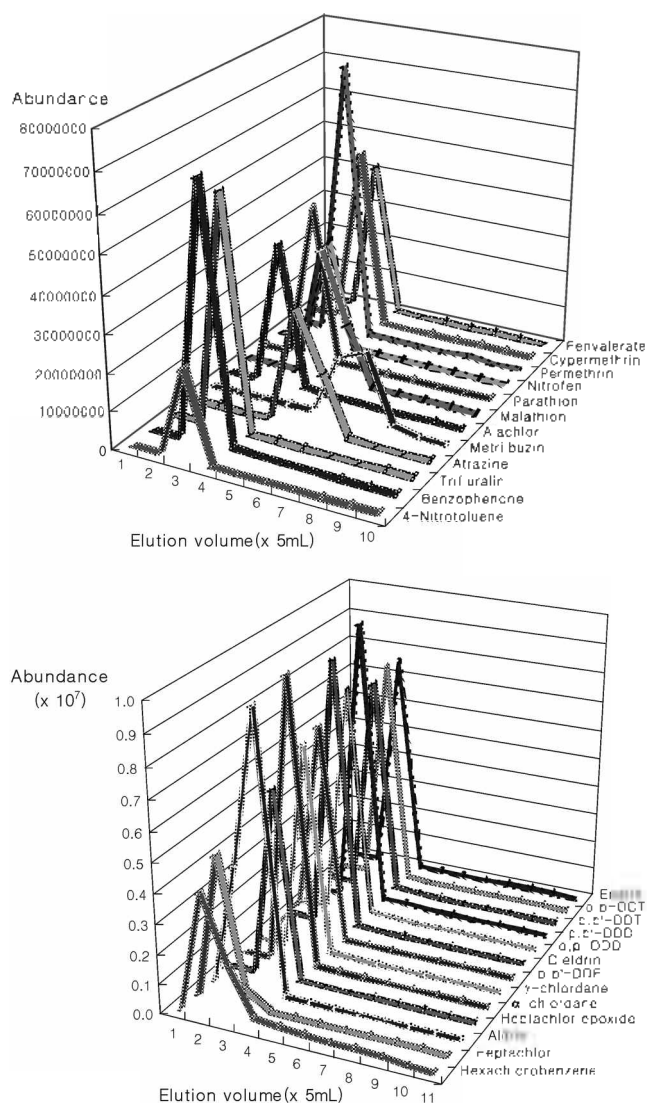


Figure 3. Elution patterns of neutral and basic twenty-five EDS through the adsorbents.

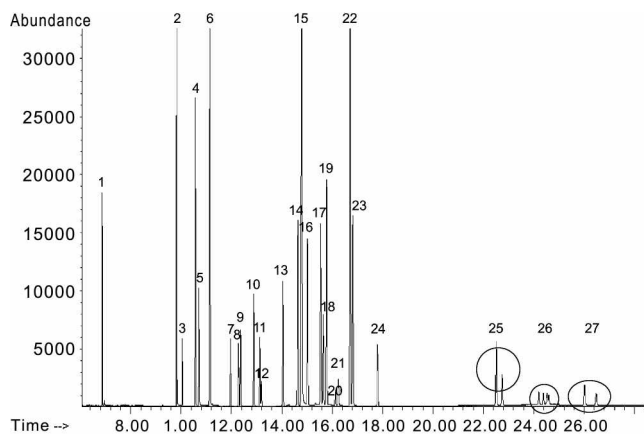


Figure 4. Chromatogram of 20 ng/mL of standards. 1: 4-nitrotoluene, 2: benzophenone, 3: trifluralin, 4: hexachlorbenzene, 5: atrazine, 6: phenanthrene- d_{10} (ISTD), 7: metribuzin, 8: alachlor, 9: heptachlor, 10: malathion, 11: aldrin, 12: parathion, 13: heptachlor epoxide, 14: γ -chlordane, 15: pyrene- d_{10} (ISTD), 16: α -chlordane, 17: p,p' -DDE, 18: dieldrin, 19: p,p' -DDD, 20: nitrofen, 21: endrin, 22: o,p' -DDD, 23: o,p' -DDT, 24: p,p' -DDT, 25: permethrin (2 isomers), 26: cypermethrin (4 isomers), 27: fenvalerate (2 isomers).

peak area of standard to that of internal standard was used in the quantification of the compound.

Results and Discussion

Extraction and clean up. The optimization of the whole procedure resulted in quantitative isolation, purification and concentration of neutral and basic compounds from biota samples. A mixture of acetone-acetonitrile (1 : 1) was very efficient for precipitation of lipid and as a solvent for ultrasonication. Good recoveries and purification were achieved in one step with a solid phase extraction procedure using the silica gel-florisil and the elution solvent of acetone-*n*-hexane (1 : 9, v/v). Elution patterns of neutral and basic twenty-five EDS through the adsorbents are shown in Figure 3. Most of EDS were eluted with 50 mL of acetone-*n*-hexane (1 : 9, v/v) at a flow-rate of 0.5 mL/min.

Identification of EDS in samples. In this study, nine EDS were identified in frog or fish. The chromatograms of standards and the extract from sample spiked are shown in

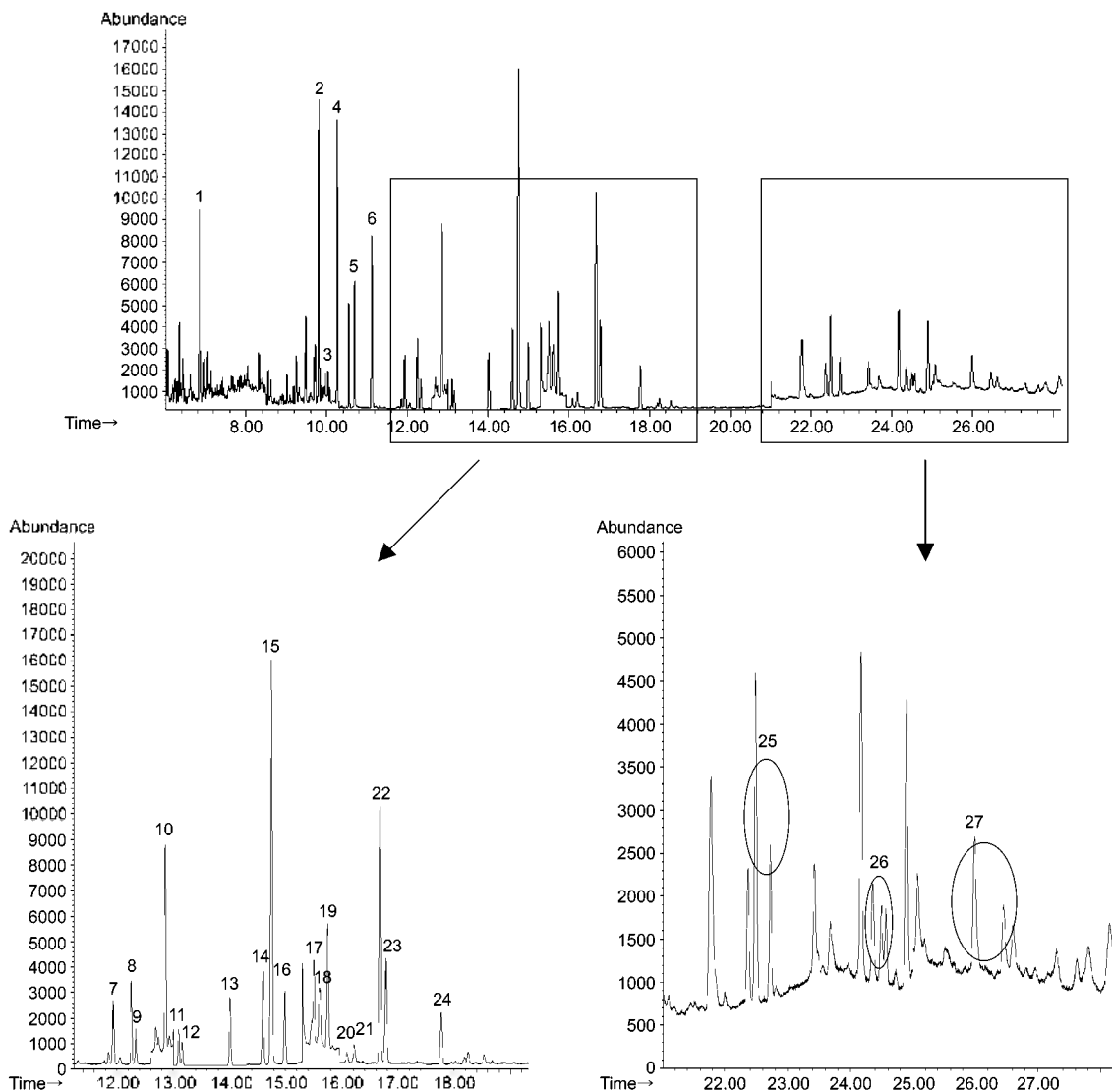


Figure 5. Chromatogram of extract of sample spiked with 1.0 ng/g of standards.

Table 2. Linearity and detection limits of the target compounds

Compounds	Detection range (ng/g)	No of points	Linear equation ^a	Linearity	Method detection limit (ng/g)
4-Nitrotoluene	0.1–100	7	$Y = 0.0036X + 0.0047$	0.9969	0.1
Benzophenone	0.1–50	6	$Y = 0.0408X + 0.0748$	0.9964	0.1
Trifluralin	0.5–100	6	$Y = 0.0092X - 0.0026$	0.9999	0.3
Hexachlorobenzene	0.5–100	6	$Y = 0.0061X + 0.0102$	0.9996	0.1
Atrazine	0.1–50	6	$Y = 0.0151X - 0.0105$	0.9987	0.1
Metribuzin	0.1–50	6	$Y = 0.0133X + 0.0159$	0.9990	0.3
Alachlor	0.5–50	5	$Y = 0.0101X + 0.0056$	0.9979	0.3
Heptachlor	0.1–100	7	$Y = 0.0092X + 0.0051$	0.9997	0.5
Malathion	0.1–50	6	$Y = 0.0155X - 0.0003$	0.9985	0.1
Aldrin	0.1–100	7	$Y = 0.0037X + 0.0007$	0.9996	0.5
Parathion	1.0–50	5	$Y = 0.0082X - 0.0033$	0.9987	0.5
Heptachlor epoxide	0.5–100	6	$Y = 0.0064X + 0.0007$	0.9999	0.2
γ -Chlordane	0.5–100	6	$Y = 0.0065X + 0.0042$	0.9996	0.2
α -Chlordane	0.5–100	6	$Y = 0.0088X + 0.0006$	0.9995	0.2
<i>p,p'</i> -DDE	1.0–100	6	$Y = 0.0139X + 0.0019$	0.9996	0.2
Dieldrin	1.0–100	6	$Y = 0.0065X + 0.0070$	0.9997	0.3
<i>p,p'</i> -DDD	0.5–100	6	$Y = 0.0261X + 0.0097$	0.9998	0.2
Nitrofen	1.0–50	5	$Y = 0.0051X + 0.0014$	0.9997	0.8
Endrin	1.0–100	6	$Y = 0.0031X + 0.0007$	0.9993	0.7
<i>o,p</i> -DDD	0.1–100	7	$Y = 0.0213X + 0.0082$	0.9999	0.1
<i>o,p</i> -DDT	1.0–100	6	$Y = 0.0049X + 0.0111$	0.9955	0.1
<i>p,p'</i> -DDT	1.0–100	6	$Y = 0.0080X + 0.0106$	0.9977	0.3
Permethrin	1.0–100	6	$Y = 0.0217X + 0.0062$	0.9990	0.1
Cypermethrin	0.5–50	5	$Y = 0.0053X + 0.0104$	0.9982	0.2
Fenvalerate	0.1–50	6	$Y = 0.0034X + 0.0046$	0.9987	0.2

^aX – the analyte concentration (ng/g); y – the peak area ratio of the analyte to internal standard.

Table 3. Recoveries of the target compounds from fish ($n = 3$)

Compound	Recovery (%), Mean (ng/g) ± SD (RSD%)	
	2.0 ng/g	20.0 ng/g
4-Nitrotoluene	107.7 ± 8.1 (7.5%)	105.5 ± 9.4 (8.9%)
Benzophenone	103.8 ± 10.4 (10.0%)	101.9 ± 5.6 (5.5%)
Trifluralin	85.8 ± 8.1 (9.4%)	96.7 ± 11.7 (12.1%)
Hexachlorobenzene	47.4 ± 4.7 (7.1%)	32.9 ± 0.8 (2.3%)
Atrazine	94.4 ± 4.3 (4.5%)	77.8 ± 7.5 (9.6%)
Metribuzin	86.5 ± 5.6 (6.4%)	68.8 ± 1.5 (2.2%)
Alachlor	99.6 ± 6.4 (6.4%)	82.9 ± 5.7 (6.8%)
Heptachlor	96.2 ± 7.5 (7.6%)	65.8 ± 4.4 (6.7%)
Malathion	101.9 ± 1.3 (1.2%)	87.6 ± 8.2 (9.3%)
Aldrin	71.7 ± 5.4 (7.6%)	84.3 ± 9.2 (10.9%)
Parathion	103.6 ± 3.0 (2.9%)	96.8 ± 4.9 (5.0%)
Heptachlor epoxide	71.7 ± 5.4 (7.6%)	63.5 ± 6.6 (9.7%)
γ -Chlordane	91.5 ± 5.8 (6.3%)	88.0 ± 9.3 (10.6%)
α -Chlordane	75.8 ± 5.7 (7.6%)	88.6 ± 7.5 (8.4%)
<i>p,p'</i> -DDE	93.8 ± 5.9 (6.3%)	92.8 ± 8.0 (8.6%)
Dieldrin	91.0 ± 9.1 (9.9%)	102.7 ± 4.1 (4.0%)
<i>p,p'</i> -DDD	89.3 ± 3.7 (4.1%)	60.2 ± 8.9 (14.7%)
Nitrofen	93.2 ± 2.0 (2.2%)	82.2 ± 6.9 (8.4%)
Endrin	78.0 ± 6.5 (8.3%)	100.3 ± 4.4 (4.4%)
<i>o,p</i> -DDD	104.6 ± 4.2 (4.0%)	94.9 ± 10.7 (11.3%)
<i>o,p</i> -DDT	83.9 ± 5.3 (4.8%)	108.7 ± 5.3 (4.8%)
<i>p,p'</i> -DDT	99.0 ± 5.2 (5.3%)	107.6 ± 4.9 (4.6%)
Permethrin	88.0 ± 9.9 (11.3%)	79.2 ± 4.4 (5.5%)
Cypermethrin	88.9 ± 8.1 (9.1%)	76.6 ± 4.2 (5.5%)
Fenvalerate	101.6 ± 5.0 (4.9%)	97.1 ± 4.5 (4.6%)

Figure 4-5. Separation of the target compounds and internal standard from the background was very good.

Linearity and detection limits. Examination of a typical standard curve was found by computing a regression line of peak area ratios for 4-nitrotoluene, benzophenone, trifluralin, hexachlorobenzene, atrazine, metribuzin, alachlor, heptachlor, malathion, aldrin, parathion, heptachlor epoxide, γ -chlordane, α -chlordane, *p,p'*-DDE, dieldrin, *p,p'*-DDD, nitrofen, endrin, *o,p*-DDD, *o,p*-DDT, *p,p'*-DDT, permethrin, cypermethrin and fenvalerate to internal standard on concentration using a least-squares fit demonstrated a linear relationship with correlation coefficients being greater than 0.9955 (Table 2).

Detection limits were 0.1 ng/g for 4-nitrotoluene, benzophenone, hexachlorobenzene, atrazine, malathion, *o,p*-DDD, *o,p*-DDT, permethrin and 0.2 ng/g for heptachlor epoxide, γ -chlordane, α -chlordane, *p,p'*-DDE, *p,p'*-DDD, cypermethrin, fenvalerate and 0.3 ng/g for trifluralin, metribuzin, alachlor, dieldrin, *p,p'*-DDT and 0.5 ng/g for heptachlor, aldrin, parathion and 0.7 ng/g for endrin and 0.8 ng/g for nitrofen based upon an assayed sample of 5 g (Table 2). Detection limits were defined by a minimum signal-to-noise ratio of 3 and coefficients of variation for replicate determinations ($n = 5$) of 15% or less of the extract of sample.

Recovery. Test samples at 2.0 and 20.0 ng/g were prepared and the analyte recoveries were calculated. The recoveries of the test compounds except hexachlorobenzene were more than 60% as shown in Table 3. These recoveries

Table 4. Precision and accuracy of target compounds in fish ($n = 5$)

Compound	Amount spiked (ng/g)	Results	
		Calculated conc. (ng/g)	Mean \pm SD (RSD%)
4-Nitrotoluene	2.0	2.1, 1.9, 1.8, 2.1, 2.1	2.0 \pm 0.2 (7.9%)
	20.0	20.6, 19.8, 19.9, 20.1, 19.1	19.9 \pm 0.5 (2.7%)
Benzophenone	2.0	1.9, 1.6, 1.8, 1.9, 1.9	1.9 \pm 0.1 (7.2%)
	20.0	19.7, 21.4, 19.2, 19.1, 19.3	19.8 \pm 0.9 (4.7%)
Trifluralin	2.0	2.2, 2.1, 2.0, 2.1, 2.0	2.0 \pm 0.1 (4.3%)
	20.0	20.6, 17.7, 22.0, 20.1, 20.9	20.3 \pm 1.6 (7.9%)
Hexachlorobenzene	2.0	2.0, 1.8, 2.0, 1.9, 1.9	1.90 \pm 0.1 (4.7%)
	20.0	20.0, 19.9, 19.0, 21.4, 18.5	19.8 \pm 1.1 (5.6%)
Atrazine	2.0	1.9, 1.9, 1.9, 2.1, 1.9	1.9 \pm 0.1 (5.3%)
	20.0	19.1, 20.5, 19.3, 19.8, 19.3	19.6 \pm 0.6 (2.9%)
Metribuzin	2.0	2.0, 2.1, 2.0, 1.8, 2.1	2.0 \pm 0.1 (5.3%)
	20.0	21.1, 18.5, 19.5, 20.1, 21.0	20.0 \pm 1.1 (5.5%)
Alachlor	2.0	1.7, 2.1, 1.9, 2.2, 1.9	2.0 \pm 0.2 (9.3%)
	20.0	21.7, 18.1, 19.3, 20.0, 18.9	19.6 \pm 1.4 (7.0%)
Heptachlor	2.0	2.0, 1.9, 1.8, 1.9, 2.0	1.9 \pm 0.1 (5.0%)
	20.0	20.3, 19.3, 20.4, 22.4, 22.3	20.9 \pm 1.3 (6.4%)
Malathion	2.0	1.8, 1.7, 1.8, 1.7, 1.8	1.9 \pm 0.1 (2.4%)
	20.0	19.5, 20.3, 19.2, 19.6, 19.6	19.5 \pm 1.2 (5.9%)
Aldrin	2.0	2.0, 1.9, 1.8, 1.9, 1.9	1.9 \pm 0.1 (4.5%)
	20.0	21.0, 19.1, 20.8, 20.0, 18.4	19.9 \pm 1.1 (5.6%)
Parathion	2.0	2.0, 2.0, 1.8, 2.1, 1.8	1.9 \pm 0.1 (5.4%)
	20.0	20.3, 19.8, 17.5, 20.4, 19.6	19.5 \pm 1.2 (5.9%)
Heptachlor epoxide	2.0	1.7, 1.7, 1.6, 2.0, 1.9	1.8 \pm 0.2 (9.8%)
	20.0	20.8, 20.1, 23.2, 20.6, 19.4	20.8 \pm 1.4 (6.9%)
γ -Chlordane	2.0	2.0, 1.8, 1.7, 1.9, 1.9	1.9 \pm 0.1 (6.8%)
	20.0	20.2, 20.4, 20.2, 21.8, 20.9	20.7 \pm 0.7 (3.3%)
α -Chlordane	2.0	1.9, 1.6, 1.9, 1.6, 1.7	1.7 \pm 0.1 (7.3%)
	20.0	21.6, 20.4, 21.3, 19.6, 21.8	20.9 \pm 0.9 (4.4%)
<i>p,p'</i> -DDE	2.0	1.8, 1.7, 1.8, 1.7, 1.8	1.8 \pm 0.0 (2.4%)
	20.0	21.6, 21.2, 17.9, 19.6, 21.0	20.3 \pm 1.5 (7.4%)
Dieldrin	2.0	2.0, 1.9, 1.6, 1.9, 2.0	1.9 \pm 0.2 (10.2%)
	20.0	20.6, 21.9, 19.5, 19.1, 23.7	21.0 \pm 1.9 (8.9%)
<i>p,p'</i> -DDD	2.0	1.8, 1.7, 1.5, 1.8, 1.9	1.7 \pm 0.2 (10.5%)
	20.0	21.2, 19.1, 20.3, 21.7, 18.6	20.2 \pm 1.3 (6.6%)
Nitrofen	2.0	2.0, 1.9, 2.0, 2.0, 1.9	2.0 \pm 0.1 (3.0%)
	20.0	19.0, 18.2, 21.2, 20.3, 20.0	19.7 \pm 1.2 (6.0%)
Endrin	2.0	2.0, 1.6, 2.0, 1.9, 2.0	1.9 \pm 0.2 (9.8%)
	20.0	20.6, 20.2, 21.5, 20.2, 21.6	20.8 \pm 0.7 (3.3%)
<i>o,p'</i> -DDD	2.0	1.9, 1.6, 1.7, 1.8, 1.8	1.8 \pm 0.1 (7.3%)
	20.0	21.6, 21.3, 20.8, 21.3, 18.8	20.7 \pm 1.1 (5.3%)
<i>o,p'</i> -DDT	2.0	1.8, 1.6, 1.6, 1.8, 1.5	1.6 \pm 0.1 (9.4%)
	20.0	19.7, 19.2, 21.3, 19.4, 22.2	20.3 \pm 1.1 (6.5%)
<i>p,p'</i> -DDT	2.0	1.9, 1.6, 1.6, 1.7, 1.6	1.7 \pm 0.1 (4.3%)
	20.0	19.4, 19.5, 20.2, 18.9, 21.4	19.9 \pm 0.9 (4.8%)
Permethrin	2.0	1.8, 1.8, 1.7, 2.1, 2.0	1.9 \pm 0.2 (9.3%)
	20.0	20.3, 19.0, 20.2, 20.8, 19.1	19.9 \pm 0.8 (4.0%)
Cypermethrin	2.0	1.8, 2.0, 1.6, 2.1, 1.8	1.9 \pm 0.2 (10.6%)
	20.0	21.0, 19.5, 21.6, 19.1, 20.9	20.4 \pm 1.1 (5.3%)
Fenvalerate	2.0	2.1, 1.9, 1.8, 1.7, 1.7	1.8 \pm 0.2 (8.9%)
	20.0	19.9, 21.3, 20.2, 23.2, 20.5	21.0 \pm 1.3 (6.3%)

X = mean value (ng/g); SD = standard deviation; RSD = relative standard deviation (%)

were very good considering the amount of sample and the low concentration of analytes. The compounds such as hexachlorobenzene showed low recoveries due to their

volatility.

Precision and accuracy. The range and standard deviation values for the precision and accuracy are given in Table 4.

For five independent determinations at 2.0 and 20.0 ng/g, the coefficient of variation was less than 11%.

Application. We analyzed the target compounds in frog and fish. Samples had been collected from seventy-one regions in Korea. We detected 9 analytes ED₅ in frogs and fishes by GC-MS (SIM). Benzophenone was observed up to 17.2 ng/g. Other compounds were not detected in the samples.

Conclusions

The peaks have good chromatographic properties and offer very sensitive response for the EI-MS (SIM). Analytical procedure of target compounds with a range of method detection limits of 0.1-0.8 ng/g was established. The detection limits from this study were lower than or similar to those obtained by procedures described before [9-29].

For the national monitoring project of ED₅ in ecology, the developed method may be valuable in understanding the contamination level of biota. The simple SPE/GC-MS-SIM procedure can reduce the analysis time and the sampling amount to 1/3, therefore be used to the national monitoring project of EDS.

References

- Bonfanti, M.; Magagnotti, C.; Galli, A.; Bagnati, R.; Moret, M.; Gariboldi, P.; Fanelli, R.; Airoldi, L. *Cancer Res.* **1990**, *50*, 6870.
- Vom Saal, F. S.; Cooke, P. S.; Buchanan, D. I.; Palanza, P.; Thayer, K. A.; Nagel, S. C.; Parmigiani, S.; Welshons, W. V. *Toxicol. Ind. Health* **1998**, *14*, 239.
- Folies, P. G.; Akerkar, S. A.; Carmeila, S. G.; Kagan, M.; Stoner, G. D.; Resau, J. II.; Hecht, S. S. *Chem. Res. Toxicol.* **1991**, *4*, 364.
- Saha, M.; Abushama, A.; Giede, R. W. *J. Chromatogr. A* **1995**, *712*, 345.
- Climie, I. J. G.; Hutson, D. H.; Stoydin, G. *Xenobiotica* **1981**, *11*, 391.
- Vanhoutte, K.; Van Dongen, W.; Esmans, E. L.; Van den Eeckhout, E.; Van Onckelen, H. *Eur. Mass Spectrom.* **1996**, *2*, 81.
- Report on Hormone-like Effects Exogenous Substances*. Japan Chemical Industry Association: 1997; p 11.
- McLachlan, J. A. *Environ. Health Perspect* **1993**, *101*, 386.
- Adrian, C.; Carmen, H.; Paul, S. *Sci. Total Environ.* **2001**, *280*, 143.
- Analytical Chemistry of PCBs*, 2nd Ed.; Erickson, M.D., Ed.; CRC Press: Boca Raton, FL, 1997.
- Cecilia, S. E.; Erland, B. *J. Chromatogr. A* **2000**, *902*, 227.
- Nakata, H.; Kannan, K.; Jing, L.; Thomas, N.; Tanabe, S.; Giesy, J. P. *USA. Environ. Pollut.* **1998**, *103*, 45.
- Karlsson, H.; Muir, D. C. G.; Teixeira, C. F.; Burniston, D. A.; Strachan, W. M. J.; Hecky, R. E.; Mwita, J.; Bootsma, H. A.; Grift, N. P.; Kidd, K. A.; Rosenberg, B. *Environ. Sci. Technol.* **2000**, *34*, 4490.
- Triantafyllos, A. A.; Dimitra, G. II. *J. Chromatogr. A* **1995**, *707*, 283.
- Sandra, B.; Mira, P.; Marija, K. M. *J. Chromatogr. A* **1998**, *823*, 3.
- Yrieix, C.; Gonzalez, C.; Deroux, J. M.; Lacoste, C.; Leybros, J. *Wat. Res.* **1996**, *30*, 1791.
- Albanis, T. A.; Hela, D. G.; Sakellarides, T. M.; Konstantinou, I. K. *J. Chromatogr. A* **1998**, *823*, 59.
- Deelstra, H.; Power, J. L.; Kenner, C. T. *Bull. Environ. Contam. Toxicol.* **1976**, *15*, 277.
- Trim, A. J.; Brown, P. M.; Bunyan, P. J.; Odam, E. M.; Stanley, P. I. *Analyst* **1983**, *108*, 33.
- Munn, M. D.; Gruber, S. J. *USA. Environ. Toxicol. Chem.* **1997**, *16*, 1877.
- United State Geological Survey* (USGS), Fact Sheet FS-027-97; 1995.
- Food and Drug Administration*, Pesticide Analytical Manual I; 1999.
- Carter, D. S.; Lydy, M. J.; Crawford, C. G. *US. Geological Survey-Water Resources Investigations Report 94-4024*; 1972-1992.
- Nowell, I. II.; Resck, E. A. *US. Geological Survey Open File Report 94-44*; 1994.
- Irani, M.; Madhuban, G. *J. Chromatogr. A* **1996**, *754*, 33.
- René van der Hoff, G.; Piet van Zoonen, *J. Chromatogr. A* **1999**, *843*, 301.
- National Monitoring Method of EDS*; Japanese Environmental Protection Agency; 1999.