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Monitoring water-polluting pesticides in Hungary

Erik Maloschik, András Ernst, Gyöngyvér Hegedűs, Béla Darvas, András Székács *

Plant Protection Institute, Hungarian Academy of Sciences, H-1525 Budapest, P.O. Box 102, Hungary

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Abstract

A 5-year survey of pesticide active ingredients and residues in Hungarian surface water samples was carried out within the framework of a national monitoring program. Based on physicochemical and ecotoxicological properties of currently registered pesticide active ingredients, a range of analytes was selected to cover compounds that potentially contaminate surface waters due to their solubility properties or mode of use. Target analytes thus included acetochlor, atrazine, carbofuran, diazinon, fenoxycarb, metribuzin, phorate, prometryn, terbutryn, and trifluralin. During the sampling campaign these pesticides were monitored in Hungarian surface waters including streams, rivers and lakes. Samples were obtained annually in two runs: before and after pesticide sprayings in spring and early summer. Samples were prepared for analysis by solid-phase extraction and solid-phase microextraction. Target analytes were monitored by gas chromatography – mass spectrometry, using electron impact and chemical ionization techniques. Spatial distribution monitoring of the surface water pollutants indicated two heavily contaminated point sources, as well as a wide range of non-point contamination. One or more pesticide active ingredients above the detection limit of the instrumental method used were measured in 209 samples, giving the result that 59% of the samples collected during the sampling campaign contained pesticide residues.

Keywords: GC–MS; Monitoring; Pesticide; Surface water; Raw drinking water; Acetochlor; Atrazine; Trifluralin

1. Introduction

Newly discovered ecotoxicological problems (e.g. endocrine disrupting effects [1]) related to pesticide residues, as well as the tightening of legal regulations regarding pesticide residues in drinking water (in conjunction with Hungary joining the European Union [2,3]), require analytical methods of increased sensitivity for monitoring pesticide residue levels. Organic micropollutants in surface water may present continuous subacute exposure to humans and wildlife, and consequently monitoring of existing contamination sites for persistent pollutants and newly emerging contamination is essential. Special emphasis has been given to persistent organic pollutants (POPs). In May 2001 Hungary joined the Stockholm POP Treaty, which was established as part of the UN Environmental Program. This treaty stipulates the regulation of 12 compounds and a compound group [4]. Based on Article 9 of the treaty, a National Action Plan has to be put into place to fulfil the commitments of that particular country: national surveys are required to provide information about the incidence of POP compounds in various natural elements. Classical POPs show decade-long half-lives, and are all banned from use. However, persistent pollutants are found among currently used pesticides as well, e.g. the herbicide active ingredient atrazine [5–8]. Although the usage of POPs is prohibited in countries that have signed the Stockholm Convention, pesticides that persist in soil throughout entire vegetation periods continue to present a threat to the environment. Thus, pesticide residue analysis in environmental samples has received increasing attention in the last few decades, resulting in numerous environmental monitoring programs in various countries for a broad range of pesticides [7,9–15].

A common consequence of such persistent pollution is the contamination of surface waters with pesticide residues. This calls for urgent attention in two areas: (a) re-evaluation of environmental persistence and risks of currently registered and applied pesticides, and (b) thorough monitoring of potentially water-contaminating pesticides in surface waters and in natural water

* Corresponding author.
E-mail address: aszek@nki.hu (A. Székács).

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bodies. In order to comply with such monitoring requirements, instrumental, immunoanalytical, as well as bioanalytical methods, are being developed in our environmental analytical and ecotoxicological facilities to detect and quantitatively monitor pesticide active ingredients and residues.

Pesticides are detected in aqueous samples mostly by chromatographic techniques [16–18], e.g., gas chromatography coupled with mass spectrometry (GC–MS) [7,12,19–23], electron capture or nitrogen phosphorus selective detection [16,17], high performance liquid chromatography [16,18,24] and more recently by liquid chromatography coupled with mass spectrometry (LC–MS) [11,25]. Moreover, capillary electrophoresis is also applied for the detection of pesticides [16,21]. Although high sample throughput can be achieved with thin layer chromatography (TLC) or overpressured layer chromatography (OPLC), these techniques have narrower application because of their relatively lower detection capabilities [16,26,27]. In addition bioanalytical methods, e.g., immunoassays/immunosensors [28] and immunochromatographic analyses [29] are also used for detection of pesticides. In our laboratory, the target pesticides are detected partly by GC–MS [30–33], and partly by enzyme-labelled immunosorbent assays (ELISAs) [34–37], while the aqueous toxicity of these substances is measured by the *Daphnia magna* biotest [31,38,39]. In the framework of a 5-year monitoring study, sample preparation methods and a GC–MS analytical protocol have been adapted and optimized for such monitoring purposes, and a nation-wide survey was carried out in Hungary to identify local and non-point pesticide contamination sites in surface waters and raw drinking water. This work has been carried out in close collaboration with the Plant Protection and Soil Conservation Service (PPSCS) of the Hungarian Ministry of Agriculture and Regional Development. Target analytes in GC–MS determinations included currently registered and potentially water-contaminating pesticide active ingredients in (a) herbicides such as acetochlor, atrazine, diazinon, metribuzin, prometryn and terbutryn; (b) insecticides such as carbofuran, fenoxycarb and phorate; and (c) fungicides such as trifluralin (Fig. 1). Physicochemical characteristics and GC–MS spectra of the analytes [40–44] are listed in Table 1.

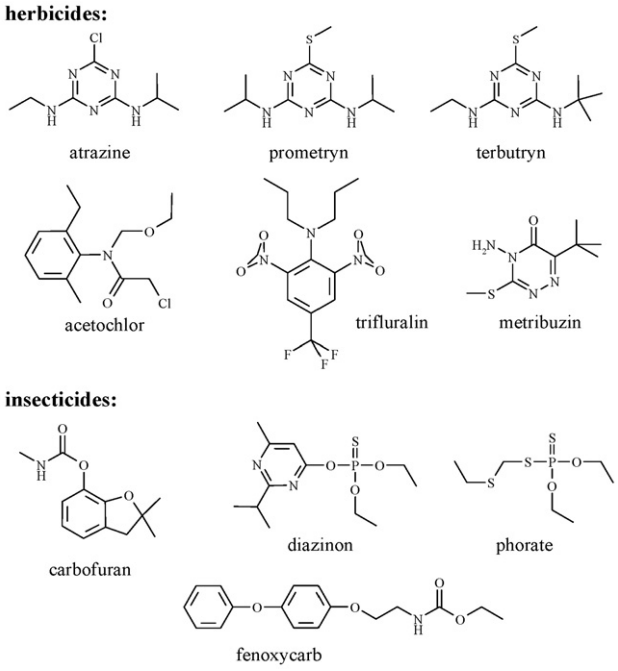

Fig. 1. Structures of the target analytes acetochlor, atrazine, diazinon, metribuzin, prometryn, terbutryn, carbofuran, fenoxycarb, phorate and trifluralin.

2. Materials and methods

2.1. Chemicals

Chemicals were purchased from Aldrich Chemical Co. (Milwaukee, WI) and Sigma Chemical Co. (St. Louis, MO), unless otherwise stated. Analytical standards of the target analyte pesticides were provided by PPSCS from official standard reference materials received from the manufacturers/distributors of acetochlor, atrazine (Nitrokémia Rt., Fűzfőgyártelep, Hungary), carbofuran (Agro-Chemie Kft., Budapest, Hungary), diazinon, fenoxycarb, prometryn (Syngenta Kft., Budapest, Hungary), metribuzin (Bayer Hungária Kft., Budapest, Hungary), phorate (BASF Hungária Kft., Budapest, Hungary), terbutryn (Agrosol Bt., Gödöllő, Hungary) and trifluralin (Budapesti Vegyiművek Rt., Budapest, Hungary). Solvents purchased from Merck KGaA (Darmstadt, Germany) were of analytical grade. CarboPrep-90 (500 mg, 6 ml) and Carbograph (200 mg, 6 ml) columns were purchased from Restek (Bellefonte, PA, USA) and Alltech Associates, Inc. (Deerfield, IL, USA), respectively. Carbowax/divinylbenzene (CW/DVB) solid-phase microextraction fibers and holder assembly were purchased from Supelco (Bellefonte, PA, USA). HPLC grade distilled water was prepared on a MilliQ RG ion-exchanger from Millipore (Bedford, MA, USA). MN (Macherey-Nagel) 640W filter paper was obtained from Reanal Rt. (Budapest, Hungary).

2.2. Instruments

GC–MS analyses were carried out on a Saturn 2000 workstation (Varian Inc., Walnut Creek, CA, USA). It consisted of a Chrompack CP 3800 gas chromatograph and a Saturn 2000R ion-trap detector. The gas chromatograph was equipped with a Varian 1079 split/splitless injector and a CP 8200 autosampler capable of holding 48 vials.

2.3. Sample collection

Water samples for analysis included distilled water, tap water and various surface water samples (water from the River Danube and surface, lake and river water samples collected throughout Hungary). In the scope of a national monitoring program, 603 water samples were collected in total during the 5-year duration of the project: 438 samples between 2000 and 2002, and an additional 165 samples in 2003 and 2004. Surface water sampling was carried out according to the national standard 'MSZ ISO 5667' [45]. Water samples were collected regularly, twice a year, before and after agricultural pesticide applications, during the months of April–May and June–September. Surface water samples (from depths not exceeding 50 cm) were collected by

Table 1
Characteristics, MS molecule fragment ions and GC–MS spectral references of target analytes

Pesticide active ingredient[a]	Formula (molecular weight)	CAS registry number	Molecule ions[b] (*m*/*z*)	NIST ID number [41] (NIST98)[c] {NIST92}[d]	Wiley registry number [42]	Other references
Acetochlor	$C_{14}H_{20}ClNO_2$ (269.771)	[34256-82-1]	223, 174, (162, 146)	245281 (19992)	–	–
Atrazine	$C_8H_{14}ClN_5$ (215.685)	[1912-24-9]	215, 200, (173, 122)	185403 (83166) {26463, 70368–70372}	131694–131700, 423301	[43, p. 256] [44, pp. 170–171]
Carbofuran	$C_{12}H_{15}NO_3$ (221.255)	[1563-66-2]	164, 149, (131, 122)	53743 (72236) {27827, 70570–70573}	45095, 132075, 132076	[43, p. 227]
Diazinon	$C_{12}H_{21}N_2O_3PS$ (304.347)	[333-41-5]	304, 179, (152, 137)	118996 (77073) {48981, 73179–73183}	136419–136424, 93296	[43, p. 310] [44, p. 194]
Fenoxycarb	$C_{17}H_{19}NO_4$ (301.341)	[79127-80-3]	(301, 186), 116, 88,	245267 (52064)	–	–
Metribuzin	$C_8H_{14}N_4OS$ (214.289)	[21087-64-9]	198, (144), 103, (74)	125494 (82576) {26206, 70320}	41841, 131608	[43, p. 254]
Phorate	$C_7H_{17}O_2PS_3$ (260.376)	[298-02-2]	260, (231), 121, (75)	53732 (28981) {43614, 72372–72374}	62283, 135018, 135019	[43, p. 330]
Prometryn	$C_{10}H_{19}N_5S$ (241.358)	[7287-19-6]	241, 226, (199, 184)	59476 (91444) {32110, 71203–71207}	53681, 133150–133153	[43, p. 262] [44, p. 171]
Terbutryn	$C_{10}H_{19}N_5S$ (241.358)	[886-50-0]	241, 226, (185, 170)	245216 (88938) {32111, 71208–71210}	53682, 133154	–
Trifluralin	$C_{13}H_{16}F_3N_3O_4$ (335.282)	[1582-09-8]	306, (290), 264, (206)	125601 (100040) {52267, 73670}	101055, 137219, 137220	[43, p. 159] [44, p. 204]

[a] Common name.
[b] Characteristic molecule ions. Base ion is underlined, while less important fragments are in parentheses.
[c] Spectra numbers of target compounds in the NIST98 library.
[d] Spectra numbers of target compounds in the NIST92 library.

immersion of a sampling vessel, transferred into clean, 2.5 l volume dark glass bottles sealed with a watertight screw-cap and insulated with Teflon lining, and were transported in cool boxes to the laboratory. Water sampling accuracy was supported by a global positioning system. The sampling strategy increasingly focused on contaminated sites: systematic sampling was carried out at 90 locations nation-wide during the first 3 years of the campaign (2000–2002), and narrowed to contaminated sites in the Balaton region during the subsequent 2 years (2003–2004). Of these surface water samples, 360 were received over a period of 3 years from PPSCS (Hungary). In addition, 41 samples from raw and drinking water from waterworks were also provided by Wedeco Water Treatment and Environmental Technologies Ltd. (Vác, Hungary), 4 groundwater samples by Megaterra Environmental Engineering Office Ltd. (Budapest, Hungary) from a national environmental remediation area, and 198 additional water samples were collected by our research group.

2.4. Sample preparation

Physicochemical characteristics (pH, temperature) of the sampled water were determined on site, while other quality characteristics e.g., total suspended matter (TSM) and evaporation residue (ER) values were measured in the laboratory of PPSCS. The measured pH, TSM and ER values ranged from 7.60 to 8.75, 5 to 120 mg/l, and 48 to 2140 mg/l, respectively. To provide appropriate sample preparation for GC–MS determinations, solid-phase extraction (SPE) and solid-phase microextraction (SPME) methods were applied. Water samples were filtered in a suction filtration apparatus using MN 640W filter paper to remove floating particles, stirred for 1 min, left to settle for 10 min, and then subjected to SPE [46] using graphitized carbon based SPE cartridges. SPE columns (CarboPrep-90, 500 mg, 6 ml) were conditioned on a vacuum suction manifold, applying low eluent flow velocity, with 5 ml of dichloromethane/methanol (8:2), 2 ml of methanol, and 10 ml of distilled water containing 10 mg/ml ascorbic acid. After the conditioning step, 1000 ml of the water sample was passed through the column at a flow rate of 10–15 ml/min. The column was rinsed with 7 ml of distilled water, air-dried for 10 min with suction by vacuum, washed with 1 ml of methanol/distilled water (1:1), and air-dried again. Neutral and alkaline components absorbed into the column were eluted, at a low eluent flow velocity, with 1 ml of methanol and 1 ml of dichloromethane/methanol (8:2). Combined eluates were concentrated to a volume of approximately 0.1 ml under nitrogen gas flow. Then 2 ml of isooctane was added to the concentrated extract, and the solution was evaporated to a final volume of 1 ml. Extract samples were applied for measurement with GC–MS.

In order to evaluate the SPE/GC–MS process, water samples were spiked with standards of the target compounds at concentrations between 0.001 and 25 ng/ml, and subjected to the above SPE protocol and to instrumental analysis. Analytical standards of the active ingredients were added to HPLC grade distilled water (MilliQ) in methanol stock solutions, except for phorate, where stock solution was prepared in acetone. Spike levels included 2- and 5-fold values of the limit of detection (LOD), except for fenoxycarb. Five parallel detections were carried out at these levels for each active ingredient.

In the case of the target analyte trifluralin, with especially low instrumental analytical limit of detection but poor recoveries in

SPE, water samples were subjected to SPME prior to GC–MS [47–49] in order to avoid recovery problems during SPE and to simplify sample preparation. Thus, 4 ml portions of each water sample were directly extracted by SPME using a 65 μm thick CW/DVB fiber. Extraction time was 20 min at room temperature with stirring by means of a magnetic stirrer. For quantification, distilled water was spiked with trifluralin at concentrations between 0.25 and 25 ng/ml, and samples were similarly subjected to SPME and to instrumental analysis.

2.5. GC–MS

GC–MS determinations were carried out using electron impact (EI) or chemical ionization (CI) ion sources, detecting total ion count (TIC) in full scan mode or selected ion(s) in selective ion monitoring (SIM) mode. A non-polar capillary column filled with 5% diphenylpolysiloxane and 95% dimethylpolysiloxane, CP-Sil 8 CB (30 m, 0.25 mm, film thickness 0.25 μm) (Chrompack, Middelburg, The Netherlands) was used as a capillary column. The carrier gas was helium 5.0 at a flow rate of 1.0 ml/min. The mode of injection was splitless (0–1.5 min), then the split ratio set to 50. Both isothermal injection (ITI) and temperature-programmed injection (TPI) were applied. During ITI, the temperature at the injection port was set to 230 °C. The injection volume was 1 μl. The corresponding column temperature, following an initial period of 120 °C for 1 min, was increased to 270 °C at 10 °C/min, and kept at 270 °C for 14 min. During TPI, the temperature at the injection port was 60 °C for 0.50 min, raised to 260 °C at 200 °C/min rate, held for 5 min, raised further to 60 °C at 200 °C/min rate, held for 20.00 min. The injection volume was 5 μl. Solvent venting was not applied. The corresponding column temperature, following an initial period of 70 °C for 0.5 min, was increased to 100 °C at 60 °C/min, further increased to 240 °C at 10 °C/min and kept finally at 240 °C for 20 min. During SPME thermodesorption, the injector temperature was held at 250 °C in splitless injection mode. The corresponding column temperature, following an initial period of 80 °C for 1 min, was raised to 300 °C at 20 °C/min. The temperature of the transfer line was adjusted to 270 °C. The mass spectrometer was operated in electron impact (EI) or chemical ionization (CI) mode using methanol as reagent gas with CI storage level of 19.0 *m/z*. The temperature of the ion trap was 150 °C. The maximum ionization time was 2000 μs, the maximum reaction time 40 ms, the ionization level 25 u, the reaction level 40 amu, ejection level 15.0 V, reagent reaction time 9000 μs, target total ion counts (TIC) 5000 counts, scan time 0.60 s/scan, emission current: 10 μA, and mass defect: 40 mmu/100 u. The scan range selected for full-scan data was 45 to 400 amu.

3. Results and discussion

3.1. Limits of detection

Target pesticides (Fig. 1) were selected for the survey based on their physicochemical features, analytical detectability, and based on the results of preceding surveys [15]. In order to detect levels of the pesticide active ingredients monitored it was necessary to evaluate achievable analytical detection levels during instrumental analysis after sample preparation. The analytical sensitivity of the GC–MS process was characterized with minimum detectable amount (MDA) values and corresponding theoretical LODs, while the effect of SPE was evaluated based on recoveries. Spiked standard solutions were used for the quantification of each target analyte. It has to be noted, that a better evaluation of the recovery might be obtained by making standard addition of labelled analytes to real filtered water samples. Theoretical LODs were calculated from MDAs determined in the GC–MS process and recoveries at 2- and 5-fold values of the LODs. Practical LODs were calculated as three times the standard deviation of the lowest measured concentration determined in five replicate analyses of the water sample for each analyte in the overall analytical procedure.

The chosen GC column and temperature program allowed peak retention times (Rt) between 9.4 and 18.2 min during ITI and 6.6 and 21.2 min during TPI. Signal intensity in the instrumental analysis was the most sensitive for trifluralin and the least sensitive for fenoxycarb. Calibration curves for the quantitative determination of the target analytes were established based on peak areas of the target fragment ions in spiked solutions. The selected fragment ions of the target analytes are listed in Table 1. At least two molecule ions were used for quantification of the target analytes: the base ion and one or more other fragment ion(s). The use of multiple molecule ions is essential to evaluate peak purity and to exclude the presence of interfering co-eluted compounds. Under optimized GC–MS parameters MDA was determined for all analyzed active agents and theoretical LOD of the GC–MS procedure was calculated.

Analytical standard concentration series were made in isooctane such that the concentration of the active agent was varied between LOD and its 25-fold level. Analyte concentrations detected at spike levels between 0.5 and 200 ng/ml offered good to excellent regression characteristics and indicated MDAs in the methods ranging from 0.01 to 50 ng in ITI, and from 0.005 to 12.5 ng in TPI protocols. MDAs of the given target compounds in TPI GC–MS were found to be 0.5 ng for acetochlor, 0.5 ng for atrazine, 2.5 ng for carbofuran, 0.05 ng for diazinon, 12.5 ng for fenoxycarb, 2.5 ng for metribuzin, 0.025 ng for phorate, 0.5 ng for prometryn, 0.5 ng for terbutryn, and 0.005 ng for trifluralin. Corresponding theoretical LODs ranged from 0.001 ng/ml (trifluralin) to 2.5 ng/ml (phorate) concentration. The validity of the calibration regression for the target analytes appeared to be adequate for quantitative determination at concentration ranges of the corresponding LOD and its 50-fold level: regression coefficients (r^2) were found to be above 0.991 except for fenoxycarb. In the case of 7 out of 10 compounds, the regression coefficient is over 0.995. Main analytical characteristics (Rt values, theoretical LODs) achieved during GC–MS with ITI or TPI are shown in Table 2. As can be seen, LODs are in all cases equal or better with TPI than under isothermic conditions (1–50 improvement factors). This is explained partly with the increased injection volumes (5 μl instead of 1 μl) due to the rapid temperature elevation in the injector, and partly with the relatively slow heat ramp applied during TPI so that slightly thermolabile compounds are not subjected to overheating, and thus more analyte reaches the column.

Table 2
Analytical characteristics of the target analytes in SPE/GC–MS method

Pesticide active ingredient	Retention time (min)		Limit of detection (LOD) (ng/ml)		LOD improvement factor	Water solubility [40] (μg/ml)	Maximum contaminant level (MCL) in water (ng/ml)
	Injector		Injector				
	Isothermic	Temperature-programmed	Isothermic	Temperature-programmed			
Trifluralin	9.414	10.855	0.01	0.001	10	0.221	0.05
Phorate	9.912	10.293	0.01	0.005	2	50	0.05
Carbofuran	10.424	6.616	5	0.5	10	320	5.00
Atrazine	10.570	11.929	0.1	0.1	1	33	1.00
Diazinon	10.912	12.264	0.5	0.05	10	60	0.10
Acetochlor	12.017	13.240	0.1	0.1	1	223	0.25
Metribuzin	12.122	13.339	0.5	0.5	1	1050	2.00
Prometryn	12.412	13.675	0.1	0.1	1	33	2.00
Terbutryn	12.732	13.878	5.0	0.1	50	22	2.00
Fenoxycarb	18.195	21.156	50	2.5	20	7.9	–

For the estimation of SPE, recoveries were determined. Recoveries at spike levels corresponding to 2-fold LOD ranged between 92.9% and 102.3%, and to 5-fold LOD ranged between 84.1% and 109.1% for atrazine, carbofuran, acetochlor, prometryn, terbutryn and fenoxycarb, but were modest (30–45%) for diazinon and metribuzin at both two levels. Relative standard deviations (RSD) for recoveries were typically in the 9.5–19.9% range for the lower spiked level and in the 8.2–15.9% range for the higher level. Rather poor recoveries (below 10%) were reached with phorate and trifluralin. Low recoveries of these analytes were explained partly with poor performance of the SPE sample preparation process and partly with analyte volatility. An especially high contrast between theoretical and practical LODs was seen in the case of trifluralin, where SPE did not permit analyte detection at low levels (0.01–0.001 ng/ml) in the high sensitivity range of the GC–MS process. Using the approach of practical LOD, however, this analyte was readily detectable in water at concentrations between 0.05 and 1 ng/ml. In the case of phorate, low recoveries are explained mainly with high volatility of the compound resulting in analyte loss during solvent exchange (from methanol to isooctane). Phorate levels found later in real samples were therefore only regarded as approximate. Nonetheless, as seen from Table 2, the GC–MS method appears to be applicable at or below the maximum contaminant level (MCL) for eight target analytes. Moreover, for 5 active agents the GC–MS method can detect appropriate concentration according to the EU drinking water thresholds (0.1 ng/ml).

Poor recoveries of trifluralin in SPE were attempted to overcome using alternative SPME sample preparation for this analyte (see Sections 2.4 and 2.5). The chosen GC column and temperature program allowed a peak Rt of 7.27 min. Signals detected at spike levels of 0, 0.25 and 0.50 ng/ml did not differ significantly from zero, indicating a practical LOD of the SPME/GC–MS method of approximately 1 ng/ml. The calibration offered good regression characteristics ($r^2 = 0.994$) between 0.5 and 25 ng/ml, suitable as a standard curve for SPME/GC–MS detection from distilled water samples. Trifuralin content detected in parallel by SPE and SPME in a single surface water sample was practically identical.

3.2. Chemical ionization

As seen above, TPI appeared to be expedient for analytical LODs in the GC–MS protocol. Nonetheless, it resulted in significantly increased Rt for fenoxycarb relative to the ITI protocol and corresponding column conditions (from 18.2 min to 20.3 min). This was not a major disadvantage in our case, as not a single water sample, out of 345, was found to contain this analyte. Metribuzin was difficult to measure together with acetochlor under column conditions applied in the TPI protocol; the peaks of these two analytes appeared in close proximity to each other, and the signal of acetochlor even at low concentrations repressed the metribuzin peak, due to both analytes having identical fragments of small relative mass that are still important in intensity in the mass spectra (e.g., *m/z* 45, 57, 103, 115, 144). This problem was due to a lack of phase selectivity or too fast temperature programming, and was overcome when the EI fragmentation method was replaced with the milder CI fragmentation resulting in fewer, larger molecule fragments. During this less destructive ionization, the analyte molecule is predominantly converted without fragmentation into its molecule ion resulting in (1) a stronger base signal and (2) direct indication of the molecular mass of the target analyte in the mass spectrum. Similar improvements due to CI were seen for target analytes atrazine and acetochlor. As seen on Fig. 2, mainly a single M+1 molecule was formed in CI of atrazine (*m/z* 216), while several fragments were formed in EI (*m/z* 215, 200, 173). This allowed better detection of atrazine in the presence of (and relative to) other contaminants. Milder fragmentation also allowed better parallel detection of and differentiation between various triazine herbicides (atrazine, prometryn, terbutryn), as identical fragments were not formed during less destructive CI fragmentation, in contrast to EI. In the case of acetochlor, definite fragmentation (cleavage of an ethoxy group) was also seen in CI. Consequently, the concentration of this analyte was calculated not from the parent molecule ion, but from that of the deethoxylated fragment. The advantage of CI over EI was seen here as well: more severe fragmentation appeared in EI, while CI resulted in a single and therefore easier to determine fragment. An additional advantage of the CI method, besides improving

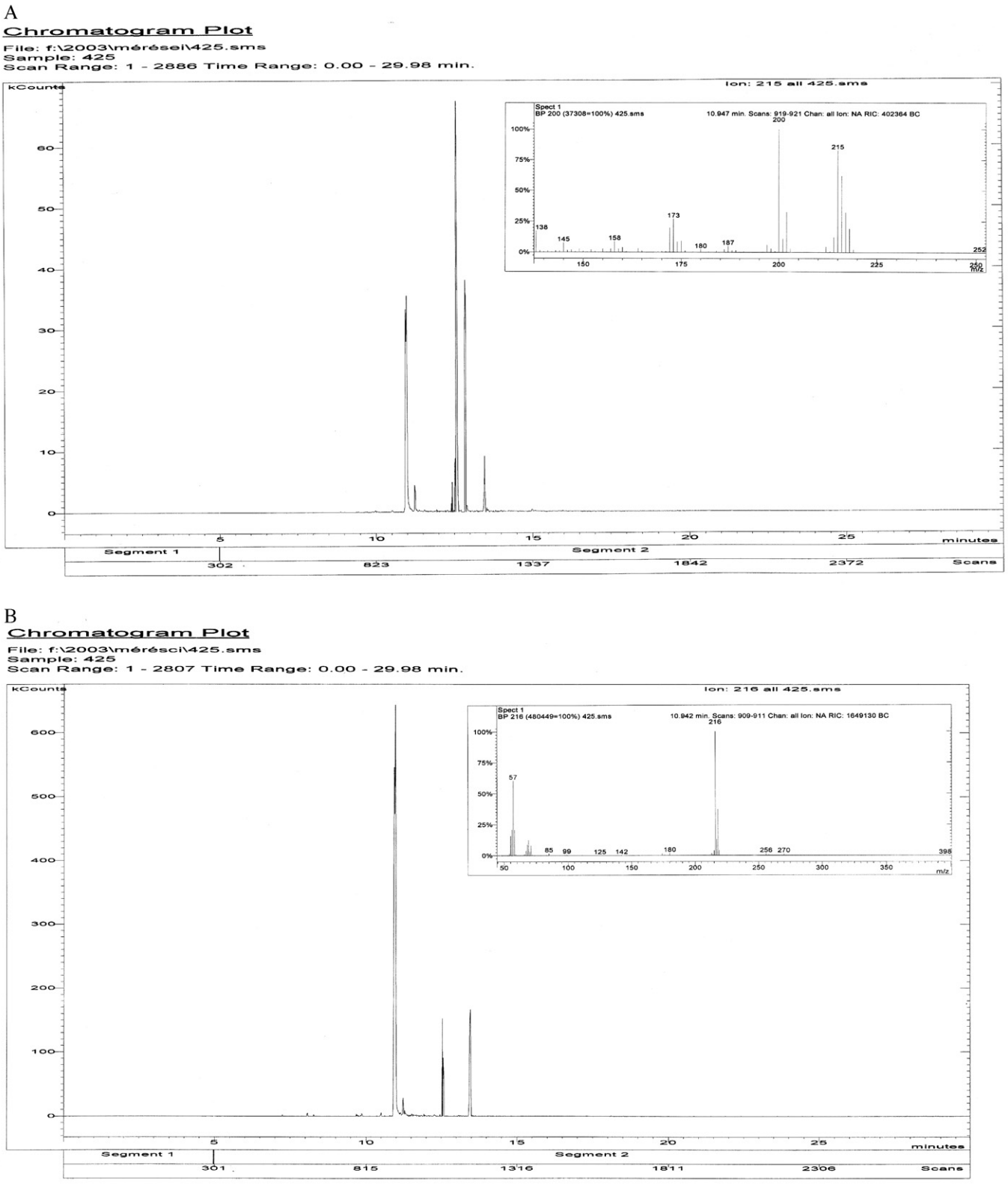

Fig. 2. GC–MS chromatograms of a contaminated water sample by EI (above) and CI (below). The peak of atrazine appears on the molecule ion scan at Rt of 10.947 min and at 10.942 min in the EI and CI spectra, respectively. It is apparent that the atrazine signal intensity relative to that of contaminants has greatly improved in CI due to less destructive fragmentation. The corresponding mass spectra pertaining to atrazine are depicted in the inserts.

detection sensitivity, is that certain ubiquiter contaminants (e.g. phthalate plasticizers) caused less interference. In several samples, dibutyl phthalate emerged both as a real and as an "artefact" contaminant. As an example of this, when using the *m*/*z* 223 ion alone for quantification of acetochlor during EI, it was identical to that of dibutyl phthalate, and therefore, the

EI protocol may have resulted in an overestimation of the acetochlor content.

3.3. Quality control

The accuracy and reliability of the analytical determinations were assured by the use of spiked standard solutions for analytical quality control. Thus, analytical standards of all analytes were used both alone and in combination, in laboratory fortified blank samples for the determination of the recovery of the SPE process; three analytes (acetochlor, atrazine and terbutryn) were applied routinely at a concentration of 0.5 ng/ml in fortified water samples to control the performance of the analytical process during the monitoring campaign in each year of the project. Quality control data obtained with atrazine are depicted on Fig. 3. The average error for the determination of acetochlor, atrazine and terbutryn was 6.3%, −2.1% and −5.1%, respectively. Upper and lower warning levels (UWL, LWL, defined as levels differing from the mean by two times its standard deviation) and upper and lower control levels (UCL, LCL, defined as levels differing from the mean by three times its standard deviation) were determined for each analyte. Thus, LWL for acetochlor, atrazine and terbutryn were found to be 0.407, 0.424 and 0.397 ng/ml, respectively.

3.4. Monitoring of surface waters

According to a countrywide state of affairs report on surface water monitoring in Hungary [15], the rate of 8 pesticide active ingredients or their residues out of 21 screened exceeded the 0.1 μg/l limit concentration permitted by the EU directives. Based on these findings, a 3-year countrywide systematic surface water sampling campaign was launched in 2000, followed by additional 2-year sampling at identified contaminated sites. In total, 386 water samples were collected in the capital (Budapest and vicinity) and 16 counties of Hungary. Sampling locations for surface water included agricultural, rural, urban and recreational areas (55 sampling sites) as well as an area for environmental remediation (4 sampling sites); and water catchment areas (31 sampling sites) for surface and raw drinking water. Sample collection was continued in contaminated areas identified during the first 3-year period, mainly in the area of Lake Balaton (83 sampling sites), but samples were also collected from other national recreation areas (19 sampling sites) and water catchment areas (6 sampling sites). Collected water samples were prepared and analyzed through the above-mentioned optimized GC–MS procedure, and the obtained chromatograms were qualitatively and quantitatively evaluated.

Detected pesticide contamination levels were ranked, graphically summarized by geographical location, and correlated with plant protection technologies used in the sampling region; contamination sources were estimated, and recommendations of necessary steps to eliminate the contamination in outstanding point sources of contamination and to evaluate the effects of these given contamination were formulated. The areal distribution of contaminated sites and contamination levels found in the first 3 years of the survey are depicted in Fig. 4. Based on data gathered during the analysis of 438 samples during the first 3-year campaign, contamination levels have been ranked into three classes: (1) strongly contaminated sites (>10,000 ng/ml), 2.2% of all sampling locations; (2) contaminated sites (1000–10,000 ng/ml), 15.6% of all sampling locations; and (3) slightly contaminated sites (<1000 ng/ml), 35.2% of all sampling locations. Locations in the first group (2 sites) are considered clearly point sources of

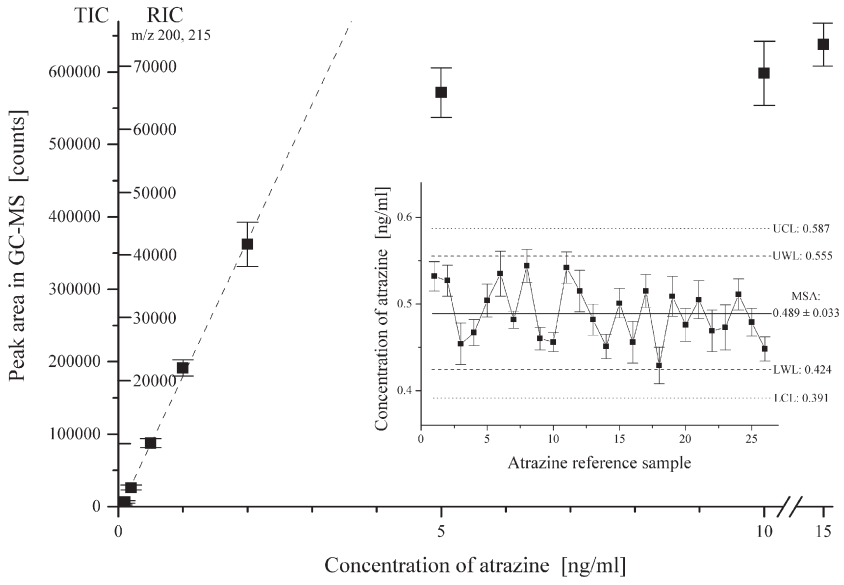

Fig. 3. External standard calibration curve for atrazine in the TPI GC–MS mode. Determinations were carried out in triplicates using spiked concentrations of atrazine of 15, 10, 5, 2, 1, 0.5, 0.2, 0.1, 0.05, 0.02, 0.01 and 0 ng/ml. Rt values ranged between 11.891 and 11.964 min (11.91±0.02 min), and the time slice for calculation of peak areas was 11.841–12.129 min. Peak areas were integrated from total ion chromatograms (TIC) and reconstructed ion chromatograms (RIC) based on selected molecule ions (*m/z* 200 and 215). Regression (r^2=0.99) was fit to concentrations 0.05 to 2 ng/ml. Analytical data quality control chart obtained with standard solutions of atrazine at a nominal concentration of 0.5 ng/ml is depicted in the insert. The mean of spiked analytes (MSA), upper and lower warning levels (UWL, LWL) and upper and lower control levels (UCL, LCL) are indicated in the chart in ng/ml unit.

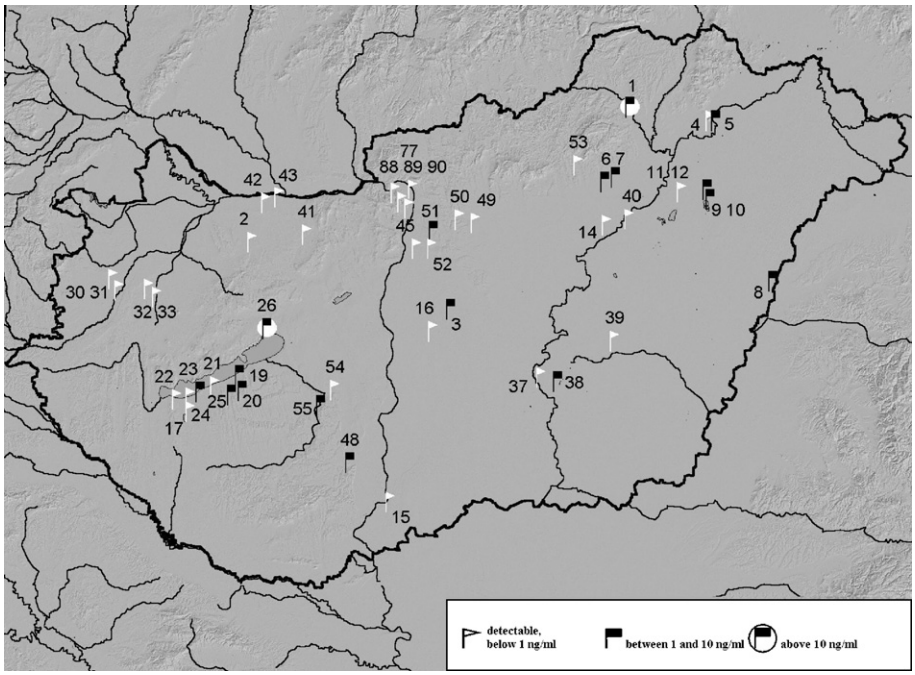

Fig. 4. Areal distribution of contaminated sites in Hungary at three contamination levels during the monitoring period of 2000–2002. Identified site contamination may indicate pollution by several pesticides.

contamination not of agricultural but industrial origin. Sites in the second group, mostly in the Balaton and Northern Hungary regions (14 sites) as well as in the third group (32 sites), display a rather even distribution with presumably non-point sources of contamination, presenting a continuous environmental stress factor. Thus, determinations indicated pesticide residues in 53.3% of all sampling locations, which is a rather large proportion relative to previous expectations. An even more severe factor was that contamination levels showed an annual variation during the sampling period, being the worst in the third year. In 2002, over 90% of the collected surface water samples contained detectable levels of one or more pesticide active ingredients in the following order by active ingredients: diazinon 64.8%, atrazine 44.4%, acetochlor 30.6%, prometryn 17.6%, terbutryn 2.8%, while carbofuran, metribuzin, phorate and fenoxycarb have not been found in any water sample. Trifluralin contamination was seen on a single occasion out of 386 surface water samples analyzed. This sample, collected from the Eastern Main Irrigation Canal (Keleti-főcsatorna) in Hungary, was found to contain 1.94 ng/ml trifluralin, detected both by SPE and SPME/GC–MS.

As seen from these distribution patterns, the two most abundant pesticide contaminants from the list of pesticides monitored are acetochlor and atrazine. Detected pollution in some cases did not correlate strongly with actual pesticide application (diazinon). Contamination by acetochlor reached an alarming 2–3 ng/ml level, and atrazine contamination of agricultural origin were not much below that. At two locations, levels of both acetochlor and atrazine were far above that attributable to agricultural origin; the sporadically detected 16–47 ng/ml pesticide level in surface waters raise serious concerns with regard to environmental and human toxicology.

Based on these findings, sampling focused on contaminated sites of the Balaton region during the last 2 years of the project. Lake Balaton is the largest lake in Central Europe and is fed by the River Zala, 31 other constant streams and 20 perennial watercourses. The water of the lake, originally without an outlet, is occasionally released into the Sió Canal, which runs into the River Danube. Sampling covered the main watercourses and an industrial area in the north-eastern region of the lake.

Sampling in the Balaton region focused partly on the industrialized north-eastern (Balatonfűzfő) region with its pesticide production and formulation facilities, and on areas less exposed to agricultural and industrial contamination, and of higher importance for tourism. Strongly contaminated sites (26 locations) were sampled more frequently (up to four times a year) in this period. Contamination levels in the surface water samples from the pesticide production area of Balatonfűzfő were found to be alarmingly high (0.2–10 ng/ml), mostly by atrazine and acetochlor, though diazinon, prometryn and terbutryn were also detected, albeit at a lower level (0.1–0.4 ng/ml). Sampling was carried out in the May–September term, and contamination levels at the sampling sites displayed, with the exception of certain locations with persistent contamination, a moderately or strongly decreasing trend. A particular concern is represented by the water paths flowing from the industrial plant into artificial ponds, basins, and then into the Séd Stream and from there into the lake. In some cases these waterways contained atrazine and/or acetochlor at concentrations of 2–6 ng/ml, or even over 10 ng/ml.

In addition to the strongly contaminated north-eastern Balaton region, samples were taken at 14 locations from Lake Velence (and its water catchment area), and at 11 locations near the capital and in the Great Hungarian Plain. Atrazine and acetochlor were the most abundant pesticide contaminants in these regions as well, yet at much lower levels (below 1 ng/ml).

Polluting pesticides were detected in contaminated samples both by EI and CI GC–MS methods. Detected atrazine and

acetochlor levels by the EI and CI protocol showed a good concurrence.

3.5. Monitoring of raw drinking waters

In the above, mostly 0.1–1 ng/ml contamination levels of surface waters are of ecotoxicological concern as a permanent environmental load. On the other hand, this concern is moderated by the fact that this level is only slightly higher than the 0.1 ng/ml level stipulated by the European Union water regulatory level [2,3]. Drinking and raw drinking water samples collected over the duration of the project displayed a significantly lower level of contamination than surface waters: contamination with the target analytes at or below 0.1 ng/ml was detected only in 21.7% of all sampling locations (10 sites) and in 19.7% of all samples tested (14 samples). Nonetheless, an alarming fact was revealed when the levels of acetochlor were monitored in the Danube. Although the level of this analyte was found at about the 0.1 ng/ml concentration limit even in the river water, this concentration was detected in the sampling rounds in 2000 and 2002 in the raw drinking water and drinking water samples in the sampling area. This may indicate that the contaminant can pass into the bank-filtered waterwells, and may emerge at practically unchanged concentrations in raw drinking water. In the case of heavier atrazine and acetochlor contamination, this may present a direct threat to the drinking water supplies, as further purification technologies (chlorination, ozonization) may not decompose these organic micropollutants with sufficient efficacy.

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