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Seasonal Atrazine Contamination of Drinking Water in Pig-Breeding Farm Surroundings in Agricultural and Industrial Areas of Croatia

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Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) a s-triazine herbicide, has been widely used in Croatian agriculture.Due to atrazine extensive use and its biodegradation in nature within at least one year (Klassen and Kadoum 1979),atrazine residues are found in ground,surface,drain and drinking water (Vidacek et al. 1994; Gojmerac et al. 1994).Groundwater downgradient from atrazine treated fields may show seasonal concentration peaks which could exceed the safe level (Wehtje et al. l983). Therefore,the use of atrazine includes permanent control of its residues in water,particularly in relation to its use as a herbicidal chemical and groundwater contamination(Graham 1991)).Furthermore,the presence of atrazine in the environment and its possible ingestion *via* the water,food and feed chain,may present a risk for the animal and human health.

The analysis of atrazine residues in soil can be performed by either calorimetry or high performance liquid chromatography (HPLC) (Vickrey et al. 1980), and in water, soil and food by immunoassay in comparison with HPLC or gas chromatography/mass spectrometry (GS-MS) (Bushway et al. 1988;Bushway et al. 1989;Bushway et al. 1992;Thurman et al. 1990).We describe the use of enzyme-linked immunosorbent assay (ELISA) for one-year seasonal monitoring of atrazine residues in drinking water from two differently situated pig-breeding farms (agricultural and industrial areas) in Croatia. Results obtained by ELISA were compared to those produced by HPLC.

MATERIALS AND METHODS

Thirty-two and 20 samples of drinking water were collected from the *Dubravica* pig-breeding farm (an agricultural area,about 50 km far from Zagreb) and from the *Sljeme-Sesvete* pig-breeding farm (an industrial area near the city of Zagreb),i.e. from two different locations,a piggery and breeding center,respectively.

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Sampling periods were the same days in April,May,September and October 1993.
For immunoassay, the samples were collected into 50-ml glass vials without any previous preparation.For HPLC analysis, the solid-phase extraction procedure for atrazine in 250 ml of each water sample was used(Brooks et al. 1989).A two-ml eluate from C-18 Sep-Pak cartridges (Waters,Milford,USA) was evaporated to dryness under nitrogen,and HPLC was performed after the addition of 0.4 ml of HPLC mobile phase(Bushway et al. 1992) for the analysis of the remaining atrazine residue.ELISA and solid-phase extraction were immediately performed. The eluents from Sep-Paks were stored frozen at -20°C until analysis. The samples of water previously verified to be free of triazine at 0.05 µg/L(ELISA) were spiked at 0.5 µg/L atrazine. These samples were used as controls in transportation,handling and analysis.
Atrazine was obtained from *Herbos Chemical Industries* (Sisak,Croatia).Technical grade atrazine was purified by recrystallization from ethanol-water. All solvents were HPLC grade (*Merck*,Darmstadt,Germany).
For determination of atrazine residues in the samples of drinking water during the spring,late summer and early autumn of 1993,ELISA plate kits were obtained from *Transia* (Lyon,France) .Each kit consisted of a plate with 96 antibody-coated wells,0.1, 0.25 and 1 µg/L atrazine standard solution, negative control,triazine enzyme conjugate solution,enzyme substrate solution, chromogen solution and stop solution.Protocol for each kit was followed according to the manufacturer's instructions.A set of standards (0.05,0.1,0.25,0.5 and 1 µg/L) were run at the beginning of each analysis with the means used to prepare the standard curve.Atrazine standard solution at 0.05 and 0.5 µg/L were prepared by dilutions from 0.1 and 1 µg/L atrazine standards,respectively,according to the manufacturer's instructions.Other equipment included a microplate reader (*Boehring* ELISA ProcessorII,Marburg,Germany),orbital mini-shaker (*B.Braun*,Melsungen,Germany) and digital multi -channel micropipettes (*Nichiryo*,Tokyo,Japan)
HPLC was used to verify the immunoassay results.Stock solution of atrazine (20 mg/100 mL) was prepared by dissolving atrazine in methanol. Working standards of atrazine (0.1, 0.5, 1, 2 and 4 µg/L) were prepared by serial dilution of the stock solution and intermediate standard solutions, using distilled water to make up to the volume.HPLC was performed using a *Rheodyne* injector with 25 µl loop,a *Perkin Elmer* LC 250 pump, *Perkin Elmer* LC-95 UV/visible spectrophotometer and model 1020 Personal Integrator. The column was LiChrosorb RP-18, 200 x 4,6 mm(*Hewlett-Packard*). Mobile phase was methanol: acetonitrile: water (40: 20: 40 v/v) at a flow rate of 1.2 mL/min.Detection was at 224 nm.

RESULTS AND DISCUSSION

Results of the triazine analysis in the samples of drinking water from the *Dubravica* and *Sljeme* pig-breeding farms,presented in Tables 1 and 2, are expressed as the amounts of atrazine detected, since the HPLC method was found to be specific for atrazine. Atrazine was found in 75.0 % of the non-spiked samples of drinking water collected from the *Dubravica* pig-breeding farm(Table 1). During early spring (April),the concentration of atrazine varied from 0.93 to 2.3 µg/L (ELISA) and from 0.79 to 2.1 µg/L (HPLC).

In a previous study, the greatest amounts of atrazine in water samples collected from the river Sava in Croatia, ranging between 0.6 and 1.7 µg/L, were found in July and August,and decreased rapidly in the autumn (Hrlec 1990). In our study, during the late summer and autumn (September and October), the concentration of atrazine were lower and varied from 0.15 to 0.28 µg/L (ELISA) or from 0.11 to 0.23 µg/L(HPLC).Five samples were found to be triazine-positive by ELISA,but atrazine-negative by HPLC.

Even 50% of the samples of drinking water collected from the *Sljeme* pig-breeding farm were atrazine-positive(Table 2).Throughout the four-month monitoring period, the concentration of atrazine varied from 0.15 to 0.29 µg/L (ELISA) or from 0.12 to 0.23 µg/L(HPLC).It is evident that in spring,late summer and autumn,the concentrations of atrazine were quite similar and very low.Seven samples were found to be triazine-positive by ELISA,but atrazine-negative by HPLC.The detection limit of HPLC was 0.1 µg/L.GeneralIy,the HPLC results were slightly lower than those obtained by ELISA,and this could be the reason for no atrazine detected below 0.1 µg/L,as compared to ELISA.However,the most likely reason for atrazine-negative samples by HPLC was that these compounds were other triazines or dealkylated metabolites of atrazine that cross-react with the atrazine antibody(Bushway et al. 1988;Bushway et al. 1992).Most triazine-positive samples contained atrazine.Linear regression was performed for the atrazine-positive samples,including spiked samples of drinking water from both farms,to compare the two techniques.The regression equation was:Y= - 0.035 + 0.906 X,standard error = 0.021 µg/L, R=0.986, where Y and X were the concentrations obtained by HPLC(µg/L) and ELISA (µg/L),respectively.Our results showed a very good correlation of the ELISA method with the conventional HPLC analysis of spiked or nonspiked samples of drinking water for atrazine.This correlation was consistent with the results reported elsewhere(Bushway et al. 1992;Muldoon and Nelson 1994).

In our study, 65.4 % of the samples of drinking water from both pig-breeding farms were atrazine-positive.The concentration of atrazine exceeded the maximal tolerable concentration of 0.1 µg/L(analyzed by HPLC) set by the European Communities for single pesticides in water intended for human consumption (Council of the European Communities 1980) and the respective by-law of the Republic of Croatia (Official Gazette of the Republic of Croatia,1994), pointing to the need for regular analytical control of the sources of drinking water for this contaminant. For this purpose, ELISA has several advantages (simple, usually more sensitive,less expensive) as a screening method (Bushway et al. 1988; Bushway et al.1992; Muldoon and Nelson 1994; Wittmann and Hock 1993).

Table 1. Seasonal atrazine concentration in drinking water from the *Dubravica* pig-breeding farm[1]

Month 1993	Atrazine concentration (μg/L)	
	ELISA[2]	HPLC[3]
April	1.23[4]	1.02[5]
	0.98	0.79
	1.31	1.00
	1.12	0.93
	0.93	1.10
	2.3	2.0
	2.2	2.0
	2.3	2.1
	0.58*	0.40*
	1.15**	0.95**
May	0.85	0.70
	0.90	1.10
	0.63	0.50
	1.01	0.85
	0.82	0.71
	0.80	0.84
	0.71	0.63
	0.85	0.80
	0.61*	0.53*
	1.10**	0.91**
September	0.22	0.12
	0.31	0.20
	0.13	ND
	0.09	ND
	0.15	0.12
	ND	ND
	0.18	0.11
	ND	ND
	0.55*	0.43*
	0.97**	0.81**
October	0.28	0.18
	0.12	ND
	0.21	0.23
	ND	ND
	0.11	ND
	0.19	0.22
	0.12	ND
	0.18	0.14
	0.59*	0.48*
	1.15**	0.92**

[1] *Dubravica* pig-breeding farm, 50 km from the city of Zagreb (agricultural area)

[2] enzyme-linked immunoassay (ELISA)

[3] high performance liqid chromatography (HPLC)

[4] means of two samples from different locations (piggery and breeding center)

[5] ND, not detectable at a detection limit of 0.05 μg/L for ELISA and 0.1 μg/L for HPLC

* samples spiked at 0.5 μg/L of atrazine

** samples spiked at 1 μg/L of atrazine

Table 2. Seasonal atrazine concentration in drinking water from the *Sljeme* pig- breeding farm[1]

Month 1993	Atrazine concentration (µg/L)	
	ELISA[2]	HPLC[3]
April	0.27[4]	0.20
	0.10	ND
	0.18	0.13
	0.29	0.21
	0.19	ND
May	ND	ND
	0.09	ND
	0.09	ND
	0.20	0.13
	0.19	0.12
September	0.09	ND
	0.29	0.20
	0.31	0.23
	0.16	ND
	0.23	0.14
October	0.26	0.17
	ND	ND
	0.08	ND
	0.15	0.12
	ND	ND

[1] *Sljeme* pig-breeding farm (industrial estate of the city of Zagreb)

The area from which the samples of drinking water were obtained, is a source of drinking water for the *Dubravica* pig-breeding farm,which is surrounded by individual agricultural lots whose owners use atrazine as a herbicidal chemical.The maximal concentration of atrazine in the *Dubravica* drinking water (2.3 µg/L by ELISA or 2.1 µg/L by HPLC) collected during April after a two-week rainy period,was eight- to ten-fold higher than that determined for the same period at the *Sljeme* pig-breeding farm (0.29 µg/L by ELISA or 0.21 µg/L by HPLC) in the Zagreb industrial estate.Although it was impossible to grasp all relevant aspects of the relationship between crop protection chemicals (herbicides) and water contamination (Graham 1991),the results obtained in this study appear to suggest that the possible mechanism of water contamination was soil leaching of the contaminant (atrazine) after its usual field application.

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