Environmental
Contamination
and Toxicology

# Evaluation of Surface Water Pollution with Atrazine, an Endocrine Disruptor Chemical, in Agricultural Areas of Turopolje, Croatia

T. Gojmerac,[1] Z. Ostojić,[2] D. Pauković,[2] J. Pleadin,[1] M. Žurić[1]

[1] Department of Chemistry, Croatian Veterinary Institute, Savska Street 143, 10 000 Zagreb, Croatia
[2] Weed Research Department, Faculty of Agriculture and Forestry, University of Zagreb, Svetošimunska 25, 10 000 Zagreb, Croatia

Corn (*Zea mays*) is the leading culture according to sown area in Croatia. s-Triazines, and atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) in particular, are most widely used in weed control of corn crops. In Croatia, products of atrazine are used in formulations of pure atrazine or in ready mix combination with other herbicides applied in pre-emergence or post-emergence (Maceljski et al. 2005). Due to its chemical properties, atrazine is characterized by high water mobility and environmental persistence (Kramer et al. 2001). Because of these atrazine properties and its longlasting use as a plant-protecting agent in Croatia, residues of this contaminant have been identified in the samples of surface, ground, and drinking waters (Vidaček et al. 1995; Gojmerac et al. 1996). The possible intake of atrazine residues and its dealkylated biodegradation products (de-ethyl atrazine) with drinking water poses a health risk for humans, since the results of previous studies point to the toxic effect of atrazine residues on the reproductive system (Cooper et al. 2000; Gojmerac et al. 2004).

The objective of this study was to evaluate the results of atrazine monitoring in surface water of the corn-production area of Turopolje (west Croatia), with the soil characterized by high hydraulic conductivity.

## MATERIALS AND METHODS

A Transia Plate Triazines kit for rapid triazine (atrazine) detection was provided by Diffchamb S.A. (Lyon, France). Each test kit contains a microtiter plate with 96 wells coated with antitriazine antibodies, negative control, standard solution (0.05 ppb; 0.1 ppb; 0.25 ppb; 1 ppb), conjugated triazine solution, substrate, chromogen, and stop reagent. Atrazine as analytical standard was obtained from Riedel-deHaen AG (Seelze, Germany). Solid-phase extraction cartridges, Supel-clean (packed with silica-octadecyl $C_{18}$), were obtained from Supelco (Bellefonte, USA). All other chemicals were of analytical grade.

Samples of natural surface water (n=90) were taken from six different locations at large waters supply systems near large cities (Zagreb, V. Gorica) from May/June till October 2004 (Figure 1). Atrazine residues were determined by the methods of competitive enzyme immunoassay (EIA) validated for accuracy and precision as analytical method, and reverse phase high performance liquid chromatography (HPLC) with UV spectrometry as a comparison method for possible cross-reactions, with correlation coefficient (R) between the two methods.
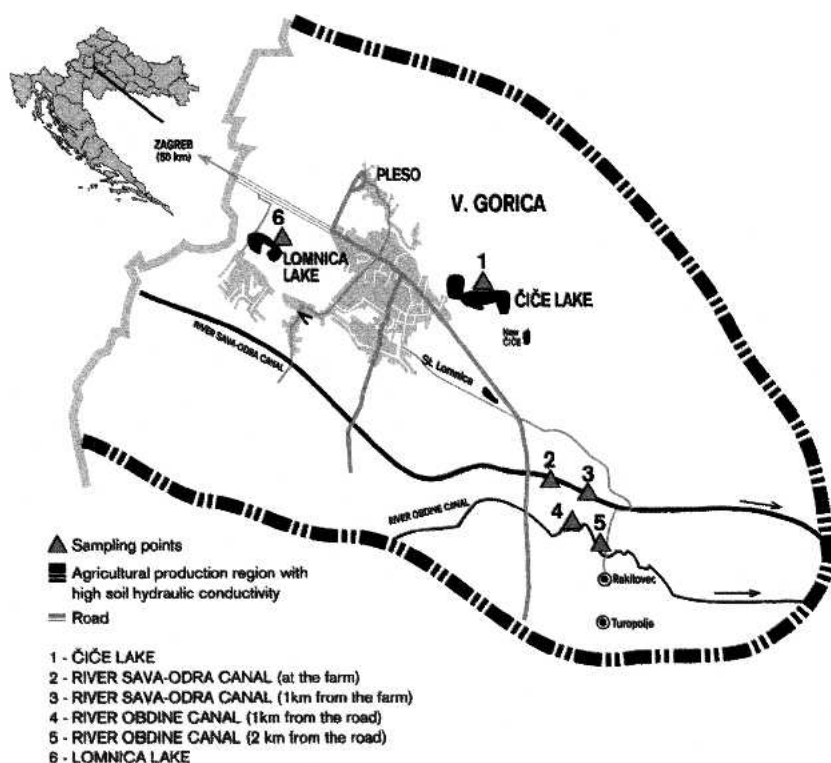
---

*Correspondence to:* T. Gojmerac

**Figure 1.** Location of sampling points.

For competitive EIA, an ELx800 absorbance microplate reader (Bio-Tek, Vermont, USA) was used. Reverse phase HPLC was performed by use of Rheodyne injector with a 25-μl loop, Perkin Elmer LC 250 pump, Perkin Elmer LC-95 UV/Vis spectrophotometer, and model 1020 PE Nelson. The column (150x3.9 mm ID) was Nova-Pak $C_{18}$, 4 μm. Detection was done at 224 nm using methanol-water (60:40 v/v) as a mobile phase.

Water samples were collected into 500-mL glass vials without any preparation for EIA, which was performed according to the manufacturer's instructions. Intra- and interassay reproducibility was assessed by testing drinking water samples previously verified as being free from triazines, spiked at 100, 500 and 1000 ppt of atrazine, as five singlicates per assay over 3 consecutive days. The accuracy of EIA was assessed by analyzing five water samples individually fortified with atrazine at 100, 500 and 1000 ppt. HPLC was used to verify the results of the EIA. The solid phase extraction procedure, preparation of the stock solution of atrazine, working standards of atrazine, separation and detection by HPLC, and detection at 224 nm were performed as previously described (Brooks et al. 1989; Gojmerac et al. 1996). Correlation between the methods of atrazine EIA and conventional HPLC was

evaluated by linear regression analysis of all positive samples defined by atrazine concentration >0.1 ng/mL.

## RESULTS AND DISCUSSION

The calibration curve of atrazine standard is shown in Figure 2. EIA precision expressed as coefficients of variation (%CV) of intra- and interassay is illustrated in Table 2, and accuracy as percentage of sample recovery in Table 1. Correlation between atrazine surface water concentration as determined by EIA and HPLC is presented in Figure 3. Seasonal atrazine concentration (ng/mL) in the samples of surface water from six sites in Turopolje, west Croatia, was determined by EIA (Figure 4).

In this study, EIA proved sensitive enough (the least detectable dose was 0.05 ng/mL), which is consistent with literature data (Mouvet et al. 1995), detecting atrazine concentration of 0.1 ng/mL as a maximum admissible concentration established by both EU (Directive 98/83/EC) and Republic of Croatia guideline for drinking water (Official Gazette of the Republic of Croatia, 2004). EIA validation revealed very good recovery (recovery range 93%-99%) (Table 1), whereas coefficients of variation (% CV) for within- and between-day values (intra- and interassay) were $\leq 6.1$ % and $\leq 6.8$ %, respectively, showed acceptable variation between individual measurements in all samples to which 100, 500 or 1000 ppt atrazine were added (Table 2). These results were also consistent with literature data (Kramer et al. 2001). The high correlation coefficient (R=0.975) between EIA and HPLC results pointed to good accordance of the two methods, suggesting the absence of immunoactive metabolites in water samples that could interfere with atrazine residues as determined by EIA. Thus, all atrazine positive water samples according to the threshold value of >0.1 ng/mL were correctly evaluated. However, the findings of residual atrazine concentration below 0.1 ng/mL (0.05-0.08 ng/mL) determined by EIA in water samples taken from lakes without tributary streams (sites 1 and 2) during both springtime and autumn were not submitted to HPLC validation and thus could not be correctly evaluated.

The results of this monitoring indicated the concentrations of residual atrazine in surface water samples from sites 2, 3, 4 and 5 to exceed the maximal allowed concentration (0.1 ng/mL) in drinking water in May and June (0.27, 0.18, 0.95 and 1.3 ng/mL, and 0.20, 0.80, 0.75 and 1.2 ng/mL, respectively), and in those from sites 4 and 5 in October (0.30 and 1.0 ng/mL, respectively). The highest concentration of atrazine residues was measured at site 5, persisting throughout the period of measurement. Previous reports have suggested atrazine to be run off to surface waters from cultivated ground, with highest concentration for up to 2 months of application, thereafter gradually declining over year (Steen et al. 2001; Acosta et al. 2004). However, our results pointed to surface water contamination with atrazine also in the autumn (sites 4 and 5), indicating residual atrazine persistence due to its physicochemical properties, which is in turn directly related to the longstanding use of atrazine in plant-protecting agents. Although a very high precipitation level was recorded in the autumn, and a lower level of atrazine should have therefore been expected in surface waters as the result of atrazine concentration dilution (Du Preez et al. 2005), the constant presence of atrazine residues through the autumn (October), i.e. months after its application, recorded in the present study, may pose a serious threat of both potential contamination of underground waters and for the
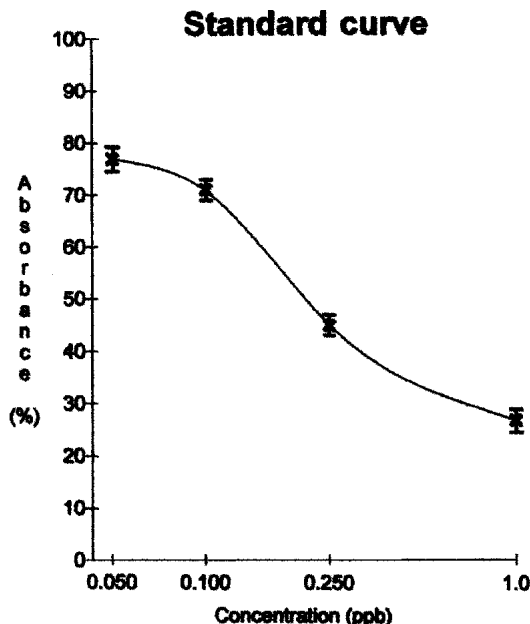
**Figure 2.** Atrazine (ppb; mean±S.D.; n=10) calibration curve obtained by the competitive enzyme immunoassay.

aquatic environment (Solomon et al. 1998; Giddings and Hall 1998). Contamination of underground waters could be explained by possible atrazine infiltration through the soil, favored by the hydrogeologic properties of the study area, since the atrazine positive sites are located adjacently to the Zagreb-Velika Gorica quartal homogeneous gravel unconfined aquifier with a high filtration coefficient favoring undisturbed transport of contaminants such as atrazine (Mayer 1995).

There are few reports on the presence of atrazine residues in surface waters at locations comparable to those sampled in the present study. The latest study of continuous systematic monitoring of the herbicide atrazine in surface waters in Croatia also points to surface water contamination with atrazine recorded in other investigations (Ostojić et al. 2004).

Based on the results of the present study, the measured concentrations of atrazine exceeding the maximal allowed concentrations indicate the need of systematic monitoring for this contaminant in both surface waters and underground waters at particular locations selected according to the above mentioned hydrogeologic soil properties, thus to allow for protective measures to prevent the possible adverse effects to be taken on time.

**Table 1.** Accuracy of the atrazine enzyme immunoassay (EIA).

| atrazine added (ppt) | Atrazine recovered[a] | | | |
|---|---|---|---|---|
| | mean[b] (ppt) | n | SD (ppt) | recovery (%) |
| 100 | 108 | 15 | 4.5 | 93 |
| 500 | 510 | 15 | 2.2 | 98 |
| 1000 | 1010 | 15 | 6.9 | 99 |

[a] Five water samples were individually fortified at the 100, 500 and 1000 ppt and assayed by EIA.
[b] Each concentration represents the mean of three immunoassay determinations for each sample.

**Table 2.** Precision of the atrazine immunoassay (EIA).

| | fortified sample concentration[a] (ppt) | | |
|---|---|---|---|
| | **100** | **500** | **1000** |
| replicates | 5 | 5 | 5 |
| days | 3 | 3 | 3 |
| N | 15 | 15 | 15 |
| mean | 107 | 522 | 976 |
| % CV (within assay) | 5.8 | 3.3 | 6.1 |
| % CV (between assay) | 5.6 | 4.1 | 6.8 |

[a] Atrazine-fortified control water samples were each assayed in five single determinations over 3 days.

y = 0.6767x - 0.024
R² = 0.9511

HPLC (atrazine ng/ml)
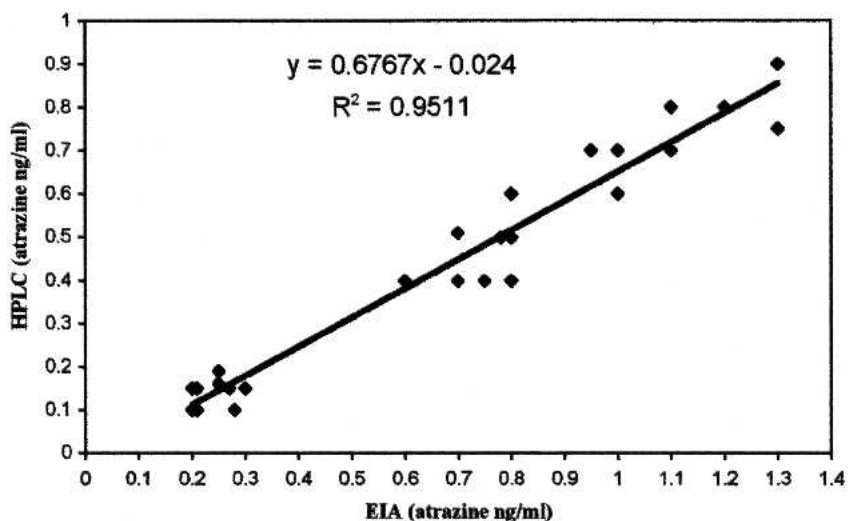
EIA (atrazine ng/ml)

**Figure 3.** Correlation between the atrazine concentration measured by high performance liquid chromatography (HPLC) and those determined by competitive enzyme immunoassay (EIA) for the 25 natural water samples; R = correlation coefficient.
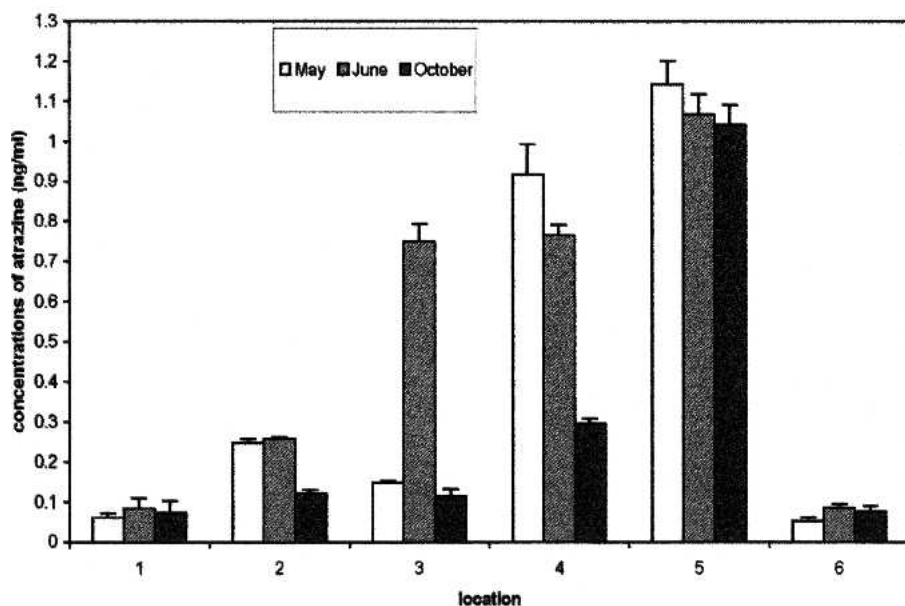
☐ May ☒ June ■ October

concentrations of atrazine (ng/ml)

location

**Figure 4.** Seasonal atrazine concentration (mean ± S.D.) in surface water samples from different sampling sites *, in Turopolje, west-Croatia, determined by enzyme immunoassay.

*1-Lake Čiče (n=15); 2-River Sava-Odra Canal (at a farm) (n=15); 3-River Sava-Odra Canal (1 km from the farm) (n=15); 4-River Obdine Canal (1 km from the road) (n=15); 5-River Obdine Canal (2 km from the road) (n=15) and 6-Lomnica Lake (n=15).

# REFERENCES

Acosta EJ, Steffensen MB, Tichy SE, Simanek EE (2004) Removal of atrazine from water using covalent sequestration. J Agric Food Chem 52: 545-549

Brooks MW, Jenkins J, Jimanez M, Quinn T, Clark JM (1989) Rapid method for the determination of alachlor, atrazine and metolachlor in groundwater by solid-phase extraction. Analyst 114: 405-406

Cooper RL, Stoker TE, Tyrey L, Goldman JM, McElroy WK (2000) Atrazine disrupts the hypothalamic control of pituitary-ovarian function. Toxicol Sci 53: 297-307

Croatian government (2004) By-law on health rightness of drinking water. Official Gazette Republic of Croatia 182: 8476-8486

Du Preez LH, Van Rensburg PJJ, Jooste AM, Carr JA, Giesy JP, Gross TS, Kendall RJ, Smith EE, Van der Kraak G, Solomon KR (2005) Seasonal exposures to triazine and other pesticides in surface water in the western Highveld com-production region in South Africa. Environ Pollut 135: 131-141

Giddings JM, Hall LW Jr.(1998) The aquatic ecotoxicology of triazine herbicides. In: Ballantine LG, McFarland JE, Hackett DS (eds). Triazine herbicide risk assessment. Amer Chem Soc, Washington, DC, pp 347-356

Gojmerac T, Kartal B, Bilandžić N, Roić B, Rajković-Janje R,(1996) Seasonal atrazine contamination of drinking water in pig-breeding farm surroundings in agricultural and industrial areas of Croatia. Bull Environ Contam Toxicol 56: 225-230.

Gojmerac T, Koršić M, Pleadin J, Žurić M, Rajković-Janje R.(2004) Serum luteinizing hormone response to administration of gonadotrophin-releasing hormone to atrazine-treated gilts. Vet Human Toxicol 46: 245-247.

Kramer K, Lepschy J, Hock B (2001) Long-term monitoring of atrazine contamination in soil by ELISA. J Assoc Off Anal Chem 84: 150-155.

Maceljski M, Hrlec G, Ostojić Z, Cvjetković B, Barić K (2005) Plant protection products in Croatia. Plant Prot Bull 2-3: 65-211.

Mayer B (1995) Ground and surface water in the lowland forest « Turopoljski lug» during period from 1989-1993. Radovi 40: 47-73.

Mouvet C, Broussard S, Jeannot R, Maciag C, Abuknesha R, Ismail G (1995) Validation of commercially avilable ELISA micrototer plates for triazines in water samples. Anal Chim Acta 311: 331-339.

Ostojić Z, Barić K, Galzina N, Ostojić Ž, Širac S (2004) Study of permanent monitoring of herbicide atrazine in surface water in Croatia. In: Državna uprava za vode (ed) Study of atrazine, Faculty of Agriculture, University of Zagreb, Croatia, pp.31-34.

Solomon KR, Chappel MJ (1998) Triazine herbicides: Ecological risk assessment in surface water. In: Ballantine LG, McFarland JE, Hackett DS (eds). Triazine herbicides risk assessment. American Chem Soc, Washington, DC, pp. 357-368.

Steen RJCA, Van der Vaart J, Hiep M, Van Hattum B. Cofino WP, Brinkman UATh (2001) Gross fluxes and estuarine behaviour of pesticides in the Scheldt Estuary. Environ Pollut 115: 65-79.

Vidaček Ž, Drevenkar V, Prpić M, Raspor B, Uremović K, Dadić Ž (1995) Quality of surface and ground water in Croatia. Hrvatske vode 3: 215-235.