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***DETERMINATION OF CHLORPYRIFOS IN WATER
USED FOR AGRICULTURAL PRODUCTION***

SUMMARY

The quality of the water for irrigation in conventional, but especially in organic food production is very important. The presence of pesticide residues in water may cause yield reduction and decrease product quality, due to its uptake. Chlorpyrifos is one of the most widely used insecticide in the world and it is detected in surface and groundwaters more frequently than any other organophosphorus insecticide. Also, chlorpyrifos is one of the most important water pollutants (EU Directive 2008/105/EC).

The aim of this study was determination of chlorpyrifos in river and ground water, which are widely used for irrigation in agricultural production. The extraction of chlorpyrifos was performed using ENVI C18 SP disc (47 mm). Prior to the extraction disc was conditioned with 5 ml of acetonitrile/methanol (50/50, v/v) and 5 ml deionized water. Under vacuum 500 ml of river and ground water with added chlorpyrifos standard solutions in concentration 0.01-1.0 µg ml⁻¹, was filtered through the disc. Disc was being dried at 25°C for 1 h. Chlorpyrifos was eluted with 6 ml (2×3 ml) of acetonitrile/methanol (50/50, v/v) mixture and evaporated to dryness. The extract was diluted in 2 ml of acetonitrile/methanol (50/50, v/v). Analysis was performed with a Hewlett-Packard (HP) model 5890 Series II gas chromatograph with EC Ni⁶³ detector. Determination conditions were – t °C of column 190 °C with an increase of 30°C min⁻¹ up to 275°C, t°C of injector 230°C and t°C of detector 300°C. The linearity of the method was evaluated by chromatographing chlorpyrifos solution in concentrations 0.001-1.0 µg ml⁻¹. Correlation coefficient (R²) was 0.995 and limit of detection (LOD) and limit of quantification (LOQ) were 0.004 µg ml⁻¹ and 0.01 µg ml⁻¹, respectively. The average recovery for river water was 87.5% and for groundwater 97.9%.

Key words: chlorpyrifos, river water, groundwater, GC/ECD

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INTRODUCTION

Agriculture is one of the most important users of water resources. Except a direct impact on the amount of available water resources for irrigation, it significantly affects the quality of surface and groundwater. This is reflected, above all, as a result of pesticides and fertilizers application, essential components in contemporary agriculture. Pesticide application can directly or indirectly affect different parts of the environment. In recent decades, more attention is directed to the negative impact of pesticides on water quality. Contamination of surface and groundwater with pesticides may be due to their direct application, discharges of industrial waste water, or leaching from soil treated with pesticides. As well as the erosion of pesticides from agricultural and urban areas, a source of contamination can be their waste and dumps.

The European Union (Council Directive 98/83/EC of 3 November 1998) specifies a limit (MRL) of 0.1 µg/l for individual pesticides in water intended for human consumption, while the total concentration of pesticides should not exceed 0.5 µg/l.

The European Union Framework Directive 2000/60/EC defined the guidelines in protecting and improving the quality of all water resources (rivers, lakes, groundwater, coastal water, etc.). Directive 2008/105/EC is updated by the Directive 2000/60/EC (Dalmacija, 2011) and by Annex X which defines the List of priority substances in the field of water policy. List includes 33 pollutant - 9 are pesticides including chlorpyrifos.

Chlorpyrifos (O, O-diethyl O-3 ,5,6-trichloro-2-pyridyl phosphorothioates) is an organophosphate insecticide (Table 1, Figure 1). It was discovered by The Dow Chemical Company in 1962. Chlorpyrifos was first registered in the United States in 1965. for pests control. It is most commonly used insecticides in agriculture. It is nonsystemic, with contact, inhalation and digestive effects. Chlorpyrifos is the most widely applied organophosphate insecticide. Mode of action of this pesticide is inhibition of the enzyme acetylcholinesterase.

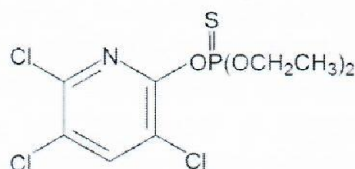


Figure 1. Structural formula of chlorpyrifos

Table 1. Chlorpyrifos

Name	Chlorpyrifos	
IUPAC	O,O-diethyl phosphorothioate	O-3,5,6-trichloro-2-pyridyl
CAS number	2921-88-2	
ECC number	220-864-4	
Molecular formula	C ₉ H ₁₁ Cl ₃ NO ₃ PS	
Molecular weight	350.6 g/mol	

Chlorpyrifos based insecticides are extensively used in agriculture, individually or in combination with other active substances. Pesticides with 480 g/l of chlorpyrifos are used at rates of 1.5 l ha⁻¹ to control harmful insects from different genus - *Aphis*, *Myzus*, *Mamestra*, *Leptinotarsa*. Also, products with the specified amount of chlorpyrifos is applied in cereals, vegetables (except root), sugar beet and tobacco to control wireworms (*Elateridae*) and chafer larvae (*Melolonthinae*) in doses up to 6 - 8 l ha⁻¹, at the same time with sowing, with a necessary incorporation (Janjić and Elezović 2011).

The most important ways of chlorpyrifos degradation in the environment are aerobic and anaerobic metabolism. The most significant metabolite of chlorpyrifos, 3,5,6-trichloro-2-pyridinol (TCP), is a very mobile and persistent in soil when not exposed to light (Tomlin, 2006). The half-life of chlorpyrifos in soil is usually between 60 and 120 days, but can range from 2 weeks to over a year, depending on the soil type, climate, and other conditions (Howard et al. 1991). Because of the high binding capacity for organic matter, chlorpyrifos has a great potential for contamination of soil and sediment.

The concentration and persistence of chlorpyrifos in water depends on the type of formulation. Large increase in chlorpyrifos concentrations occurs when emulsifiable concentrations and wettable powders are released into water. However, as the pesticide adheres to sediments and suspends organic matter, the concentration rapidly declines. The increase in the concentration of insecticide in water is not as rapid as in granules and controlled release formulations, but the resulting concentration persists longer (US EPA 1989).

The contamination of surface water with chlorpyrifos may be due to drift or runoff from areas where chlorpyrifos was applied as a result of contamination of groundwater leaching into deeper soil layers under the influence of precipitation. This is particularly obvious in areas where the chlorpyrifos is applied against soil pests.

Besides the occurrence of chlorpyrifos in drinking water, as the most important problem, control of the presence of his residues in water used for irrigation in agricultural production is very important. This primarily refers to the river water and groundwater, considering that the water and land quality in conventional production, but especially in organic agriculture, is extremely important. The presence of pesticide residues in these matrices may cause yield reduction and decrease product quality (Dragović et al. 2006), due to its uptake. In this way pesticides get into the human food chain.

The work presented here focuses on the extraction of chlorpyrifos from river and groundwater using ENVI™-C18 DSK 47 mm (Supelco, Cat. No. 5-7171, Bellafonte, PA).

MATERIAL AND METHODS

Chemicals and solutions

The analytical standards of chlorpyrifos (99.2%) was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). The solution and extraction solvents,

acetonitrile (ACN) and methanol (HPLC purity) produced by JT Baker (Netherlands) were used.

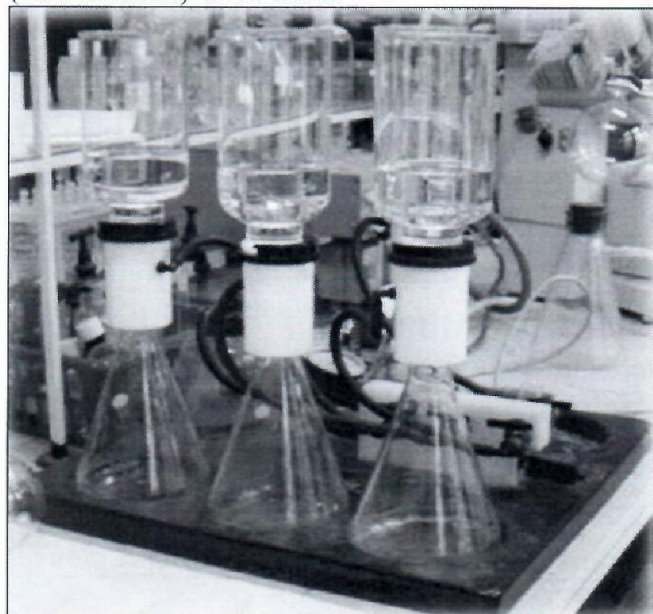


Figure 2. SP disc extraction

The extraction of chlorpyrifos from water was performed using ENVI™-C18 DSK 47 mm (Supelco, Cat. No. 5-7171, Bellafonte, PA) and glass apparatus for solid-phase (SP) extraction for disc of 47 mm (Figure 2). The standard solution of chlorpyrifos was prepared in mixture of ACN/methanol (50/50, v/v). Working solutions ($0.001 \mu\text{g ml}^{-1}$ - $1 \mu\text{g ml}^{-1}$) were prepared by the appropriate dilution of the standard solution, also in a mixture of ACN/methanol (50/50, v/v). All the solutions

were protected from light and kept in a refrigerator at 5°C until being used.

For the recovery test (was used) model solution – 500 ml of river water and groundwater enriched with 1 ml of chlorpyrifos solution in concentration of $0.01 \mu\text{g ml}^{-1}$, $0.1 \mu\text{g ml}^{-1}$ and $1 \mu\text{g ml}^{-1}$ was used.

SP extraction. Previously, samples of river water and groundwater used in the study were tested for the presence of chlorpyrifos. Prior to the extraction disc was conditioned with 5 ml of mixture ACN/methanol (50/50, v/v), followed by 5 ml of ultrapure water, respectively, at a rate of 2 ml min^{-1} . Then under vacuum model solution was filtered through the disc, at rate of 10 ml min^{-1} . After the disc was dried under vacuum 1 h at 25°C , chlorpyrifos from the disk were eluated with 6 ml ($2 \times 3 \text{ ml}$) of mixture of ACN/methanol (50/50, v/v) and evaporated to dryness. The extract was dissolved in 2 ml of ACN/methanol (50/50), ultrasonically homogenized and analyzed by GC/ECD. Injecting volume was $2 \mu\text{l}$. The quantification was performed using external standard method. The calibration solutions were analyzed before and after each water samples.

GC/ECD analysis. Content of chlorpyrifos was determined using a Hewlett-Packard model 5890 Series II gas chromatograph equipped with Supelco column 24048 (SPBTM-5, $30\text{m} \times 0,32 \text{ mm id}$, film tickness 0.25 mm) and an electron capture detector Ni^{63} (ECD). Carrier gas flow rate was 1 ml min^{-1} . The spliless injection was done.

Definition of chromatographic conditions was performed by determining the following chromatographic parameters - linearity, repeatability of the peak areas, limit of detection (LOD) and limit of quantification (LOQ).

Linearity of detector response was determined by injecting standard of chlorpyrifos at concentrations of 0.001 to 1 $\mu\text{g ml}^{-1}$. Standard solution for calibration curve was defined as the dependence of the peak concentration and expressed the correlation coefficient (R^2). Repeatability was determined by injecting the chlorpyrifos standard solution (0.1 $\mu\text{g ml}^{-1}$) five times and tested by calculating the variation coefficient. The limit of detection (LOD) was calculated using the formula: $\text{LOD} = 3 \times \text{Sa}/a$, where the Sa is standard deviation and a is the slope of the calibration curve. The limit of quantification (LOQ) was calculated from the expression $\text{LOQ} = 10 \times \text{Sa}/a$.

RESULTS AND DISCUSSION

Optimization of GC/ECD determination. Table 2 shows the temperature program for determination of chlorpyrifos by GC/ECD. All analyses were performed with three replicates. Figure 3 shows the GC/ECD chromatogram of chlorpyrifos.

Table 2. GC/ECD conditions for chlorpyrifos determinations

	Temperature
Inlet column temperature	190 °C
Rate	30 °C/min
Final column temperature	275 °C
Injector	230 °C
EC Detector	300 °C

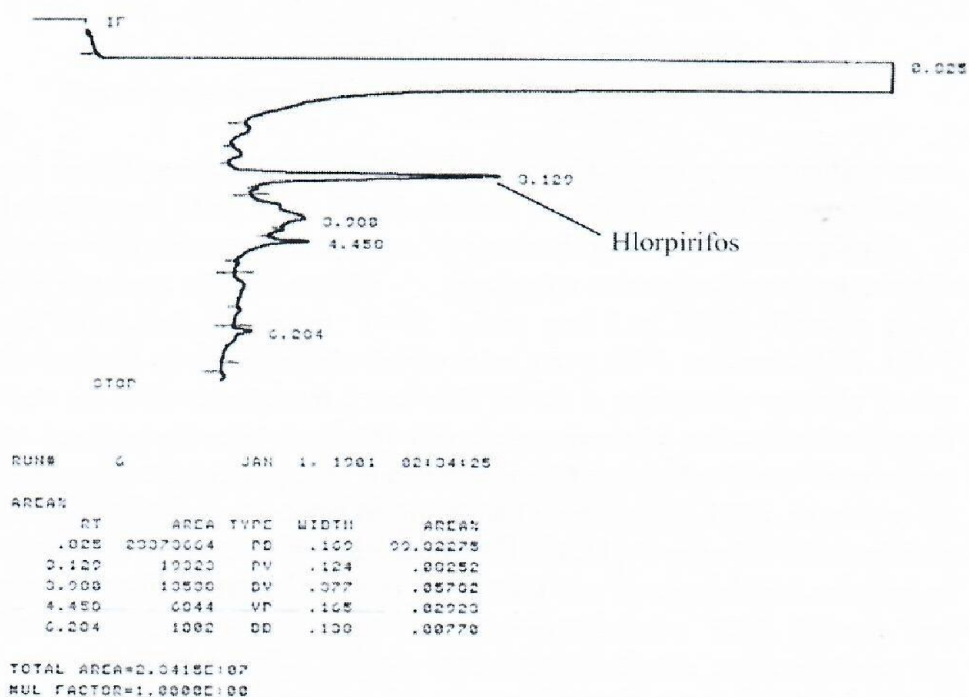


Figure 3. GC/ECD chromatogram of chlorpyrifos

Using the selected conditions the linearity of the calibration curve was evaluated at a concentration range between 0.001 to 1 $\mu\text{g ml}^{-1}$ using five calibration solutions prepared in mixture of ACN/methanol. A standard calibration curve of chlorpyrifos was done by plotting analyte concentrations against peak areas. The corresponding standard curve equation was $y = 2183x - 97$, while correlation coefficient (R^2) was 0.995 (Figure 4). The obtained value of variation coefficient ($CV=2.62\%$) indicating that high reproducibility was achieved.

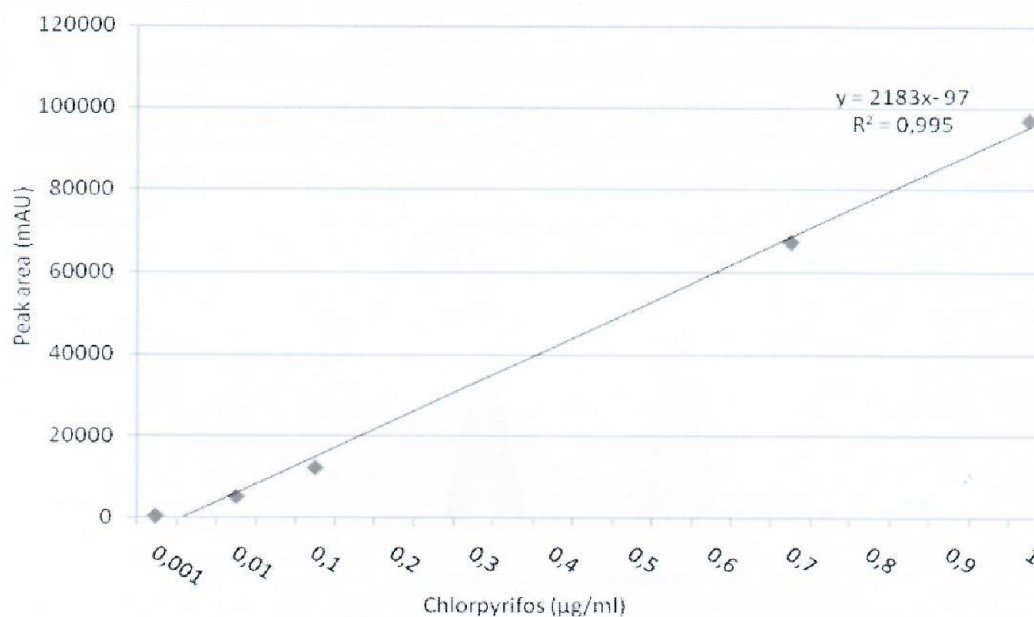


Figure 4. Assessment of peak area from chlorpyrifos content

Based on the results the limit of detection and limit of quantification were calculated. LOD and LOQ were 0.004 $\mu\text{g ml}^{-1}$ and 0.01 $\mu\text{g ml}^{-1}$, respectively. These data are within the accepted range for pesticide residue determination.

One of the most applied methods of pesticides extraction from water is the liquid-liquid extraction (Blanchet, 1979; Glaze and Lin 1984; Thielen et al. 1987). This method of extraction is the basis of most EPA methods (U.S. EPA, 1983). Given the absence of such a method, which is reflected primarily in the use of large amounts of solvents, many researchers have turned to methods such as solid-phase extraction (SPE). For the extraction of pesticides from water using mostly C18 solid phase in the form of columns (Kolpin et al. 1996; Louter et al. 1992; Psathaki et al. 1994). This study used a C18 solid phase in the form of disc. Activation and conditioning of the disk is carried out by applying a mixture of ACN/methanol (50/50, v/v). Otherwise, the hydrophobic C18 ligands are entangled and reduce the active surface interaction with the analytes from the water. Failure model solution through the disk, chlorpyrifos remains "trapped" on the disk. In this way the maximum possible loss of the target analytes will reduce. Chlorpyrifos elution from disc was done with a small amount of solvent.

Table 3 shows the average value of the recovery of chlorpyrifos for river and groundwater. Results represent the mean of three replicates performed under the same conditions.

Table 3. Results of chlorpyrifos extraction from water

	Concentration of chlorpyrifos		
	0.01 $\mu\text{g ml}^{-1}$	0.1 $\mu\text{g ml}^{-1}$	1 $\mu\text{g ml}^{-1}$
River water	84.1 \pm 1.2	88.2 \pm 1.0	90.3 \pm 0.8
Groundwater	95.9 \pm 2.3	97.9 \pm 1.5	99.7 \pm 0.4

Determination of the recovery was done with relatively low concentrations of chlorpyrifos - 0.01 $\mu\text{g ml}^{-1}$, 0.1 $\mu\text{g ml}^{-1}$ and 1 $\mu\text{g ml}^{-1}$. The lowest value of chlorpyrifos used for the recovery for both types of water is 10 times lower than the EU MRL. However, even that, value of recovery for 0.01 $\mu\text{g ml}^{-1}$ was above 80 %.

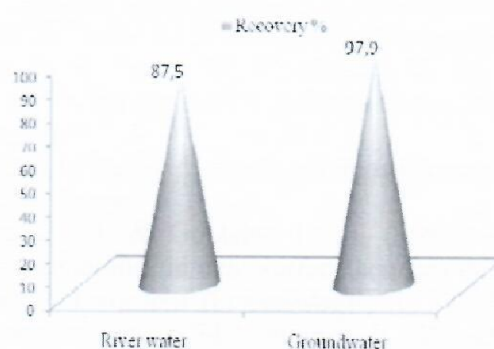


Figure 5. Average value of the recovery

The mean recoveries for river water were in the range 84 % and 90 % with relative standard deviations (RSDs) in the range of 0.8 - 1.2 % for three fortification levels. Some loss in recoveries was noted probably because of the matrix influence. Average value of the recovery for groundwater were between 96 % - 100 %, with RSD of 0.4 - 2.3 %, also for three fortification levels (Figure 5).

CONCLUSIONS

In this work SP extraction of chlorpyrifos from water samples using ENVI C18 SP disc (47 mm) was developed. The recoveries of the method ranged between 84 % and 90 % for river water and 96 % - 100 % for groundwater. LOD and LOQ were 0.004 $\mu\text{g ml}^{-1}$ and 0.01 $\mu\text{g ml}^{-1}$, respectively. High values of the recovery suggest the possible use of this method for chlorpyrifos extraction from different types of water, especially bearing in mind that the described method does not use large amounts of toxic organic solvents.

The results show that the extraction and analysis procedures are efficient for determination of chlorpyrifos in water samples, considering that the EU MRL for individual pesticide in water for human consumption is 0.1 $\mu\text{g l}^{-1}$.

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ODREĐIVANJE HLORPIRIFOSA U VODI SA PRIMENOM U POLJOPRIVREDNOJ PROIZVODNJI

SAŽETAK

Kvalitet vode za navodnjavanje u konvencionalnoj, a posebno u organskoj proizvodnji od izuzetnog je značaja. Prisustvo ostataka pesticida u vodi može doprineti smanjenju visine prinosa gajenih biljaka i umanjiti kvalitet dobijenog proizvoda. Jedan od najintenzivnije primenjivanih insekticida u svetu je hlорpirifos. Prema EU Direktivi 2008/105/EC hlорpirifos je jedan od prioriternih polutanata vode i odnosu na druge organofosfate, najčešće je detektovan u površinskim i podzemnim vodama.

U ovom istraživanju ispitana je mogućnost određivanja hlорpirifosa u rečnoj i podzemnoj vodi, koje se u poljoprivrednoj proizvodnji intenzivno koriste. Izdvajanje hlорpirifosa iz vode izvedeno je primenom ENVI C18 SP-diska (47 mm). Ekstrakciji je prethodilo kondicioniranje diska sa 5 ml mešavine acetonitril/metanol (50/50, v/v) i 5 ml dejonizovane vode. Kroz disk je pod vakumom zatim propušteno 500 ml rečne, odnosno podzemne vode, obogaćene standardnim rastvorom hlорpirifosa u koncentracijama 0,01-0,1 µg/ml. Disk se na temperaturi od 25°C sušio 1h. Hlorpirifos je sa diska eluiran sa 6 ml mešavine acetonitril/metanol (50/50, v/v), u porcijama 2x3 ml i uparen do suvog. Suvi ostatak je rastvoren u 2 ml acetonitril/metanol (50/50, v/v) i hromatografisan. Analiza je izvedena gasnim hromatografom Hewlett-Packard (HP) model 5890 Series II sa EC Ni⁶³ detektorom. Uslovi određivanja hlорpirifosa - t°C kolone 190°C, sa porastom od 30°C min do 275°C, t°C injektora 230°C i t°C detektora 300°C.

Linearnost odgovora detektora određena je injektovanjem rastvora standarda hlорpirifosa u koncentracijama 0,001-1,0 µg/ml. Dobijen je koeficijent korelacije (R²) od 0,995%. Limit detekcije (LOD) i limit kvantifikacije (LOQ) za hlорpirifos iznose 0,004 µg/l i 0,01 µg/l, respektivno. Primenjenom metodom ostvaren je prosečan prinos za rečnu vodu od 87,5% i za podzemnu vodu od 97,9%.

Ključne riječi: hlорpirifos, rečna voda, podzemna voda, GC/ECD