ELSEVIER

Food Chemistry 84 (2004) 651–656

Food Chemistry

www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods Section

Screening of food samples for carbamate and organophosphate pesticides using an electrochemical bioassay

Michele Del Carlo[a,*], Marcello Mascini[b], Alessia Pepe[a], Gianfranco Diletti[c], Dario Compagnone[a]

[a]*Department of Food Science, University of Teramo, Via Spagna 1, 64023 Mosciano Stazione (TE), Italy*
[b]*Faculty of Agriculture, University of Teramo, Via Spagna 1, 64023 Mosciano Stazione (TE), Italy*
[c]*Istituto Zooprofilattico dell'Abruzzo e del Molise "G. Caporale", Via Campo Boario, 64100 Teramo, Italy*

Received 18 February 2003; received in revised form 6 July 2003; accepted 1 August 2003

Abstract

In this work we report the application of an electrochemical enzyme inhibition assay, based on screen printed electrodes, for the determination of carbaryl, and parathion methyl in complex food matrices. The calibration curves for carbaryl and parathion methyl showed a comparable working range, respectively 2–90 and 1–100 ng/ml with a I_{50}% at 35 ng/ml, and a coefficient of variation (CV) $\leqslant$10%. The method was applied to food samples showing negligible matrix effect (+1% to −5%). Samples were tested after spiking of two concentration levels: 10 and 30 ng/g of the investigated pesticides. The assay allowed to detect the tested pesticides at 10 ng/ml level in non-purified solvent extract of different complex matrices with an overall assay time of 15 min.

Keywords: Food analysis; Pesticides; Acetylcholinesterase; Screen printed electrodes

1. Introduction

Carbamates and organophosphates pesticides are widely used in agriculture because of their insecticidal activity and their relatively low persistence in the environment (Jury, Winer, Spencer, & Focht, 1987). Their great success in agricultural applications has led to an increase in the production and spread of these pesticides. The Codex Alimentarius Commission of the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) have established maximal residue limits for pesticides in a variety of foods.

In Europe, maximum admissible levels of pesticide residues in foodstuffs of animal or vegetal origin are defined according to the criteria proposed by the European Council (Council Directive 97/41/EC, 1997) and they differ according to pesticide and food. Since carbamates and organophosphates not only inhibit insect acetylcholinesterase but also strongly interfere with neural transmission in other organisms, including humans, they represent a potential hazard for the environment and human health; continuous assessment and monitoring is therefore required (Council Directive 80/778, 1980).

Current analytical methods for pesticides are based on complex laboratory-based instrumental techniques as high-performance liquid chromatography (HPLC) and gas chromatography (GC) (Lacassie et al., 1998; Lacorte & Barceló; 1995; Oliva, Navarro, Barba, & Navarro, 1999). In addition, the determination of pesticides in a variety of food commodities is often complicated by the presence of fats, and multiple extraction-clean-up steps are mandatory prior to conventional analysis (Ahmed, 2001). Among others, solid phase extraction (SPE) (Wells & Yu, 2000), solid phase microextraction (SPME) (Arthur & Pawliszyn, 1990) accelerated solvent extraction (ASE) (Wenzel et al., 1998) are gaining consideration as they own, as a common feature, the reduced use of solvent with respect to classical extraction methods, thus satisfying environmental and health concerns and economical considerations.

* Corresponding author.
E-mail address: delcarlo@agr.unite.it (M. Del Carlo).

0308-8146/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2003.08.006

In food analysis laboratories large number of samples is usually tested, therefore the development of fast, inexpensive, and possibly automated tests are of great interest.

Analytical methods that consent minimal samples pre-treatment, and avoid sample clean-up, are strongly desirable as they are foreseen to reduce both consumables and work time. The use of enzyme inhibition as a mean for pesticide detection is a well established procedure. Biosensors and bioassays, for the detection of anticholinesterase insecticides, has been extensively studied and demonstrated mainly for environmental applications (Andrescu, Barthelmebs, & Marty, 2002; Barceló, Lacorte, & Marty, 1995; Del Carlo, Mascini, Pepe, Compagnone, & Mascini, 2002; Gogol, Evtugyn, Marty, Budnikov, Winter, 2000; Marty, Mionetto, Lacorte, & Barceló 1995; Mascini & Palchetti, 2001; Pogacnik & Franko, 2000).

Few reports on application of acetylcholinesterase based biosensors to food analysis have been reported (Bachmann & Schmid 1997; Palchetti et al., 1997; Skladal, Nunes, Silva, Yamanaka, & Ribeiro, 1997) and to our knowledge no application to animal matrices have been described. In the present paper we describe the application and evaluation of an electrochemical bioassay to samples of animal origin. The assay is based on chemical modification of screen printed electrodes (SPEs) with 7,7,8,8 tetracyanoquinodimethane (Hernandez, Palchetti, & Mascini, 2000) used for the mediated electrochemical detection of acetylcholinesterase activity. Different food commodities were tested, (egg, bovine meat, milk and honey) spiking blank samples with carbaryl and parathion methyl respectively at 10 and 30 ng/g that represent the maximum admissible level.

Considering the large number of samples in the food control laboratory the proposed method appears to be suitable as a screening tool to reduce the samples requiring confirmatory instrumental analysis.

2. Materials and methods

2.1. Reagents and apparatus

Sodium phosphate, potassium chloride, cholinesterase acetyl (AChE) from electric Eel (EC 3.1.1.7, 1070 U/mg protein), acetylthiocholine (ATCh chloride salt), methanol, acetone, hexane, ethyl acetate, ethyl ether, and acetonitrile were obtained from Sigma, Italy. The pesticides carbaryl and parathion methyl were from Riedel-de Haen, (Sigma, Italy). SPE C18 (3 ml) cartridges were obtained by J.T. Baker.

Ultrapure water was obtained with PURELAB pro 20 apparatus (USF).

Screen printed electrodes were produced by Biosensor Laboratory, University of Florence. They were printed as elsewhere described (Cagnini, Palchetti, Mascini, & Turner, 1995). Chemically modified electrodes (CMEs) were obtained by depositing 2 μl of 0.25 mM 7,7,8,8, tetracyanoquinodimethane (TCNQ) solution in perfluorinated ion-exchange resin (Nafion®) on the working electrode surface. Electrochemical experiments were carried out using a PGSTAT12 potentiostat (AUTOLAB Eco Chemie Netherlands) with GPES 4.7 software.

The gas chromatographic analysis was performed by a DANI model 8500 gaschromatograph equipped with a capillary column DB-1 (15 m × 0.25 μm × i.d. 0.25 mm; J&W Scientific, Folsom, CA) and with NPD flame Detector.

The HPLC was a Varian Star 9010 coupled with a spettrofluorimetric detector Varian Star 9070 and a Pickering double derivatisation module with two Gilson pumps and a thermostatic oven. The precolumn was a Pickering cat. 18ECG001 and the column a Pickering carbamate column C 8 cat. 1700-063 (25 cm × 4 mm i.d. × 5 μm).

2.2. Samples collection, extraction and clean-up

The egg, bovine meat, milk and honey samples, used for all the experiments, were checked for the absence of pesticides using the methods indicated by the European Directive 93/256.

In order to evaluate the extraction/clean-up recovery, a blank sample of bovine fat tissue was spiked with 10 ng/g of each pesticide; gas-chromatography and HPLC were performed on the extracts respectively for organophosphates and carbamates analysis.

After pesticides spiking 10 g of samples were incubated for 30 min and homogenised with 25 ml of acetone and 18 ml of hexane for 15 minutes with Ultra-turrax. The whole organic layer was filtered on sodium sulphate anhydrous and 1 ml of the filtrate was vacuum dried. The same procedure was used for milk and egg samples.

The honey samples were dissolved in 10 ml water prior to solvent extraction procedure.

The dried extracts were used without further treatment for electrochemical bioassay analysis after dissolution in 1 ml phosphate buffer.

For GC and HPLC analysis an SPE purification of the dried residue was carried out. An SPE C18 cartridge was conditioned with 5 ml of methanol, 5 ml of deionized water and 5 ml of an acetonitrile/water (85% v/v) solution. The dried matter was dissolved in 1.5 ml of ethyl ether and added to the cartridge. The elution was performed using 5 ml of acetonitrile/water solution (85% v/v). The eluted extract was mixed with 25 ml of hexane/ethyl ether solution (1/1 v/v), 75 ml of water and 5 ml of sodium chloride and allowed to separate. The organic layer was removed and evaporated under a cold gentle nitrogen stream. The dried residue was dissolved in 1 ml of hexane and analysed by gas chromatography.

The recovery studies on the spiked samples using the electrochemical bioassay were carried out at two levels, 10 and 30 ng/g, of carbaryl and parathion methyl to match and exceed the legislation requirements.

2.3. Gas chromatographic measurement

To detect organophosphate pesticides the method chosen was GC–NPD.

Analytical conditions were as follow. The injection was made using a split 1:100 injector held at 270 °C. The oven temperatures were: 2 min at 98 °C, from 98 °C to 145 °C at 20 °C/min, 30 sec at 145 °C, from 145 °C to 270 °C at 4.5 °C/ min and finally 30 min at 270 °C for 30 min; carrier gas was He at 2 ml/min. The temperature of the NPD detector was held at 280 °C. The internal standard was chlorpyrifos methyl 100 ng/ml.

2.4. HPLC measurement

Carbamates are quoted as being thermally labile, this property limits the use of GC for their analysis (Delgado, Rubio Barroso, Toledano Fernandez-Tostado, & Polo-Diez, 2001). The HPLC analysis of extracts was performed according to Pelosi et al., (2001). The injection volume was 20 μl, flow-rate was 1 ml/min. The system operated at 42 °C. The mobile phase solvents were water (A) and acetonitrile (B). The elution conditions applied were: 0–35 min 90% of A and 10% of B; 35–45 min 10% of A and 90% of B; 50–60 min 90% of A and 10% of B. The spectrofluorimetric detector was set at 330 and 465 nm.

2.5. Electrochemical procedures

The assay is based on the inhibition of acetylcholinesterase by organophosphate and carbamate pesticides.

The enzyme catalyses the cleavage of acetylthiocholine in acetic acid and thiocholine.

$$\text{Acetylthiocoline} + H_2O \xrightarrow{\text{AChE}} \text{Acetic acid} + \text{Thiocoline}$$

The thiocholine produced in the enzymatic reaction reduces the electrochemical mediator TCNQ; which is re-oxidized at the electrode surface at +0.4 V vs. Ag pseudo reference electrode (Ag-pre) generating a anodic current. Each electrochemical measurement was performed using CME as disposable sensor. The electrochemical procedure consisted of a pre-treatment step to activate the mediator followed by the electrochemical determination of acetylcholinesterase activity.

The pre-treatment was as follows: 100 μl of phosphate buffer pH 7.4, KCl 100 mM, were placed on the CME and two cyclic voltammetry (CV) scans were performed in the range −600 mV/+600 mV vs. Ag-pre, scan rate 100 mV/s; this was followed by three differential pulse voltammetry (DPV) scans in the potential window +250 mV/+750 mV vs. Ag-pre, pulse amplitude 50 mV, pulse width 60 ms, scan rate 25 mV/s.

The electrochemical determination of acetylcholinesterase activity was obtained mixing an enzyme solution (0.4 U/ml) with acetylthiocholine (0.5 mM) in 1 ml of 0.1 M of phosphate buffer with 0.1 M KCl, pH 7.4. The reaction was allowed to proceed for 20 s and a 100 μl aliquot was deposited onto the CME, after 80 seconds a DPV scan was performed using the conditions above described. Following this procedure we obtained a current peak at in the range +350 to +400 mV vs. Ag/AgCl-pre which was exploited as analytical signal.

2.6. Carbaryl and parathion methyl inhibition assays

In this work we choose carbaryl and parathion methyl as model molecules respectively of carbamate and organophosphate pesticides.

Calibration plots for carbaryl and parathion methyl were obtained by the incubation in phosphate buffer with acetylcholinesterase (0.4 U/ml) for 10 min; this solution was then used for the electrochemical enzyme activity measurement as above described. Each measurement was performed in triplicate.

The oxidation current peak obtained by DPV was measured (I_2) and compared with the oxidation current value obtained without pesticide (I_1).

The percent of inhibition ($I\%$) was obtained according to the following formula:

$$I\% = 100(I_1 - I_2)/I_1. \quad (1)$$

2.7. Acetylcholinesterase inhibition measurements using food samples

The electrochemical bioassay was used to analyse the blank egg, bovine meat, milk and honey samples and the same matrices spiked at two levels (10 and 30 ng/g).

To evaluate the effect of the matrices on both the assay operation and CME performance, the blank sample responses were compared with a blank buffer solution.

Samples were extracted and the dried residues were dissolved in 1 ml of 0.1 M of phosphate buffer with 0.1 M KCl, pH 7.4. The enzyme inhibition was evaluated as described for standard solutions.

Once the negligible effect of the matrix was demonstrated, the same protocol was used for the spiked samples.

A quantitative evaluation of each pesticide was obtained using the corresponding calibration plot.

3. Results and discussion

3.1. Extraction recovery

Samples were analysed using the extraction procedure described in the experimental section both with GC–NPD and HPLC analysis. All the matrices gave no analytically detectable peaks at elution times obtained for the pesticide standards. Therefore they were used as blank samples for both recovery experiment and the bioassay.

The extraction recoveries (Table 1), using bovine fat tissue spiked with 10 ng/g of carbaryl and parathion methyl were respectively 106 and 89%, with acceptable repeatability ($<10\%$, $n=5$). Results showed that the extraction method was suitable for a quantitative extraction of residues from complex food samples.

3.2. Calibration curves of pesticides

In Figs. 1 and 2 the calibration curves of carbaryl and parathion methyl are reported and the analytical features are compared with relevant literature data in Table 3.

The detection limits were calculated using the value $I_{LOD}\% = 2\times$SD blank in the calibration curve equation of each pesticide. The calculated $I_{50}\%$ were 35 ng/ml both for carbaryl and parathion methyl (Table 2).

Table 1
Extraction recovery from bovine fat tissue

	Expected (ng/g)	Obtained (ng/g)	Recovery (%)
Carbaryl[a]	10.0	10.6	106
Parathion methyl[b]	10.0	8.9	89

[a] HPLC.
[b] GC–NPD.

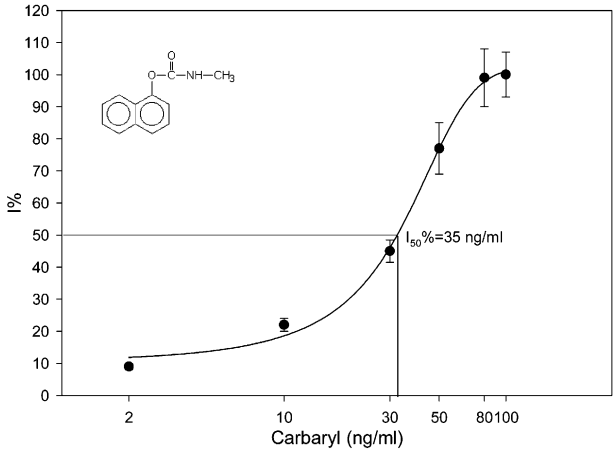

Fig. 1. Calibration plot obtained with standard solutions of carbaryl, values are the mean of three replicates.

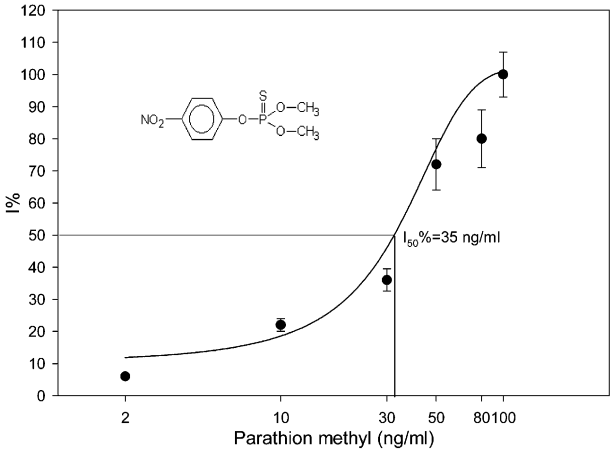

Fig. 2. Calibration plot obtained with standard solutions of parathion methyl, values are the mean of three replicates.

All measurements were performed in triplicate, the coefficient of variation was $\leqslant 10\%$ in the whole working range. The analytical features of the assay were comparable with literature results obtained using similar approaches as detailed in Table 2 (Kumaran & Tranh-Minh, 1992; Skladal et al., 1997).

The calibration plots obtained for the tested pesticides suggested that the electrochemical bioassay was suitable as a 'watchdog' in food monitoring. In fact the detection limit obtained for all the tested standards permit to discriminate between contaminated and safe commodities according to the European legislation which sets 10 ng/g the action level for carbaryl and parathion methyl in such matrices.

3.3. Spiked samples

In Table 4 the influence of each matrix (blank samples) on the assay response is reported. $A_M\% = 100{*}I_1/I_m$ (Activity in Matrix) represents the enzyme activity as affected by the matrix (I_m) with respect to that in buffer (I_1). The matrix effect ranged from -1% (egg; $A_M\% = 99\%$) to $+5\%$ (honey; $A_M\% = 105\%$). The mean matrix effect for each sample is significatively lower than the standard deviation of the measurement, therefore the influence of the matrix on the analysis is negligible.

This is a key feature of the assay with respect to other analytical methods where cumbersome clean-up steps are required.

Typical analytical DPV responses, respectively for a blank, 10, 50 and 90 ng/g carbaryl spiked bovine meat samples, are reported in Fig. 3. Similar response profiles were obtained for the other samples with both pesticides. The inhibition ($I\%$) obtained from samples respectively spiked with 10 and 30 ng/g of each investigated pesticide were calculated according to Eq. (1). All measurements were carried out in triplicate, $7 < CV\% < 10$.

Table 2
Analytical features of the calibrations obtained with the inhibition assay

	LOD (ng/ml)	I 50% (ng/ml)	Curve fitting: sigmoidal, three parameters equation	Working range (ng/ml)	R^2
Carbaryl	2	35	$y = 102.80/(1 + \exp(-(x - 33.57)/15.33))$	2–90	0.997
Parathion methyl	1	35	$y = 96.88/(1 + \exp(-(x - 34.55)/18.69))$	1–100	0.957

Table 3
Comparison of analytical results with relevant literature data

	LOD (ng/ml)	Working range (ng/ml)	Incubation time (min)	EC technique/Enz. I mm. (Y/N)	Ref.
Carbaryl	0.1	0.01–2500	10	DPP/CA (Y)	Skladal et al. (1997)
	2	2–90	10	DPV (N)	This work
Parathion methyl	15.0	N.A.	N.A.	Potentiometry (Y)	Kumaran and Tranh-Minh (1992)
	1	1–100	10	DPV (N)	This work

Table 4
Effect of the different matrices on the bioassay results

	$A_M\% = 100^*I_1/I_m$	S.D. ($n = 3$)
Egg	99	±6
Bovine	104	±8
Milk	101	±4
Honey	105	±4

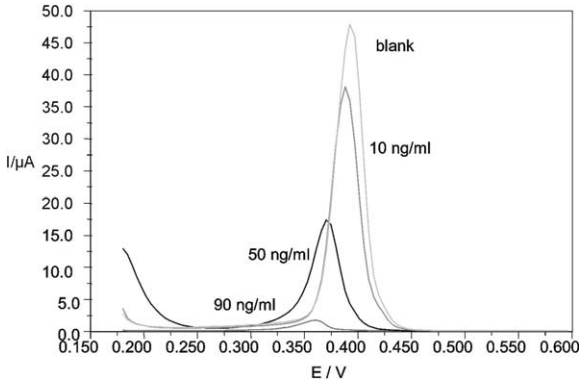

Fig. 3. Electrochemical signals obtained in the electrochemical bioassay using parathion methyl standards.

The pesticide recovery, calculated as the mean of two repeated measurements (Table 5), obtained using the $I\%$ value in the calibration equation of the corresponding pesticide, shows that no false negative samples were detected by the assay as well as no false positives. These were crucial aspects for the applicability of the assay as a screening method.

The method shows an excellent correlation between expected and obtained data in some cases (e.g. parathion methyl determination at 10 ng/g level in all matrices) and a severe overestimation in some other samples (e.g. carbaryl at 10 and 30 ng/g in egg and bovine meat). The average recovery, calculated as the mean of the values obtained for the four matrices, corrected for the extraction recovery (106 and 89% for carbaryl and parathion methyl, respectively), was 125% at 10 ng/g level and 121% at 30 ng/g level for carbaryl whereas for parathion methyl was 100% at 10 ng/g level and 129% at 30 ng/g level. The results showed that the assay can operate at the limit of concentration proposed by the European Legislation (10 ng/g). These results indicate that the proposed assay may serve as a prescreening tool in food analysis laboratory.

Table 5
Recovery data obtained with the spiked samples

Expected value (ng/g)	Egg (ng/g)	Bovine meat (ng/g)	Milk (ng/g)	Honey (ng/g)
Carbaryl				
0	0	0	0	0
10	15	15	10	10
30	40	35	30	40
Parathion methyl				
0	0	0	0	0
10	10	10	10	10
30	35	40	40	40

4. Conclusions

The ability of this bioassay with cheap and disposable electrochemical detection for carbamate and organophosphate pesticides in complex matrices like food was demonstrated. The analytical features of the assay for carbaryl and parathion methyl (i.e. LOD and $I_{50}\%$) allowed to detect these pesticides in spiked samples at two concentration levels: 10 and 30 ng/g, moreover the assay showed no false negative samples at this level. The lower concentration level (10 ng/g) is the level set by the

European legislation as limit value. The analysis in food matrices was performed with a simplified extraction procedure with acceptable precision. The unequivocal decrease of the electrochemical signal observed for the two concentration considered (10 and 30 ng/g) demonstrates the capability of this system to work as screening assay.

To our best knowledge this is a unique example of study on the effect of animal origin food samples on the performance of such an analytical method. This work shows that the extraction procedure can be greatly simplified with respect to instrumental analysis and extraction protocols.

Acknowledgements

This work was carried out with financial support from Italian Ministry of Health (IZS AM 011/2000).

References

Ahmed, F. E. (2001). Analyses of pesticides and their metabolites in foods and drinks. *Trends in Analytical Chemistry*, *20*(11), 649–661.

Andrescu, S., Barthelmebs, L., & Marty, J. L. (2002). Immobilization of acetylcholinesterase on screen-printed electrodes: comparative study between three immobilization methods and applications to the detection of organophosphorus insecticides. *Analytica Chimica Acta*, *464*, 171–180.

Arthur, C., & Pawliszyn, J. (1990). Solid phase microextraction with thermal desorption fused silica optical fibres. *Analytical Chemistry*, *62*, 2145–2148.

Bachmann, T. T., & Schmid, R. D. (1999). A disposable multielectrode biosensor for rapid simultaneous detection of the insecticides paraoxon and carbofuran at high resolution. *Analytica Chimica Acta*, *401*(1–2), 95–103.

Barceló, D., Lacorte, S., & Marty, J. L. (1995). Validation of an enzymatic biosensor with liquid chromatography for pesticide monitoring. *Trends in Analytical Chemistry*, *14*, 334–340.

Cagnini, A., Palchetti, I., Mascini, M., & Turner, A. P. F. (1995). Ruthenized screen-printed choline oxidase-based biosensors for measurement of anticholinesterase activity. *Mikrochimica Acta*, *121*, 155–166.

Council Directive 97/41/EC of 25 June 1997 amending Directives 76/895/EEC, 86/362/EEC, 86/363/EEC and 90/642/EEC.

Council Directive 80/778/EEC, L229 (30.08.1980).

Del Carlo, M., Mascini, M., Compagnone, D., Pepe, A., & Mascini, M. (2002). Electrochemical bioassay for the determination of chlorpyriphos methyl in vine samples. *Journal of Agriculture and Food Chemistry*, *50* (25) 7206-7210,.

Delgado, M. J., Rubio Barroso, S., Toledano Fernandez-Tostado, G., & Polo-Diez, L. M. (2001). Stability studies of carbamate pesticides and analysis by gas chromatography with flame ionization and nitrogen-phosphorus detection. *Journal of Chromatography A*, *921*, 287–296.

EC 93/256 Commission decision of 14 April 1993. *Official Journal of the European Communities*, L118.

Gogol, E. V., Evtugyn, G. A., Marty, J. L., Budnikov, H. C., & Winter, V. G. (2000). Amperometric biosensors based on nafion coated screen-printed electrodes for the determination of cholinesterase inhibitors. *Talanta*, *53*, 2 379-389.

Hernandez, S., Palchetti, I., & Mascini, M. (2000). Determination of anticholinesterase activity for pesticide monitoring using a thiocoline sensor. *International Journal of Environmental Analytical Chemistry*, *54*, 213–222.

Jury, W. A., Winer, A. M., Spencer, W. F., & Focht, D. D. (1987). Transport and transformations of organic chemicals in the soil-air-water ecosystem. *Review of Environmental Contaminants Toxicology*, *99*, 119–164.

Kumaran, S., & Tranh-Minh, C. (1992). Determination of organophosphorus and carbamate insecticides by flow injection analysis. *Analytical Biochemistry*, 187–194.

Lacassie, E., Dreyfuss, M. F., Daguet, J. L., Vignaud, M., Marquet, P., & Lach, G. (1998). Multiresidue determination of pesticides in apples and pears by gas chromatography-mass spectrometry. *Journal of Chromatography A*, *805*, 319–326.

Lacorte, S., & Barceló, D. (1995). Determination of organophosphorus pesticides and their transformation products in river waters by automated on-line solid-phase extraction followed by thermospray liquid chromatography-mass spectrometry. *Journal of Chromatography A*, *712*, 103–112.

Oliva, J., Navarro, S., Barba, A., & Navarro, G. (1999). Determination of chlorpyrifos, penconazole, fenarimol, vinclozolin and metalaxyl in grapes, must and wine by on-line microextraction and gas chromatography. *Journal of Chromatography A*, *833*(1), 43–51.

Marty, J. L., Mionetto, N., Lacorte, S., & Barceló, D. (1995). Validation of an enzymatic biosensor with various liquid chromatographic techniques for determining organophosphorus pesticides and carbaryl in freeze-dried waters. *Analytica Chimica Acta*, *311*, 265–271.

Mascini, M., & Palchetti, I. (2001). Electrochemical biosensors for evaluation of contaminants in food. *Arhivza Higijenu Rada I Toksikologiju*, *52*(1), 49–59.

Palchetti, I., Cagnini, A., Del Carlo, M., Coppi, C., Mascini, M., & Turner, A. P. F. (1997). Determination of anticholinesterase pesticides detection in real samples using a disposable biosensor. *Analytica Chimica Acta*, *337*, 315–321.

Palchetti, I., Cagnini, A., Mascini, M., & Turner, A. P. F. (1999). Characterisation of screen-printed electrodes for detection of heavy metals. *Mikrochimica Acta*, *131*, 65–73.

Pelosi, P., Stefanelli, P., Attard Barbini, D., Generali, T., Amendola, G., Girolimetti, S., Vanni, F. and Di Muccio, A. (2002) *Methods for organochlorine, organophosphorus, pyrethroid and carbamate pesticide residues in foods of animal origin*. (ISS internal procedure cod. ISS-ToA-RRA 2002-01).

Pogacnik, L., & Franko, M. (2001). Optimisation of FIA system for detection of organophosphorus and carbamate pesticides based on cholinesterase inhibition. *Talanta*, *54*, 631–641.

Skladal, P., Nunes, G. S., Yamanaka, H., & Ribeiro, M. L. (1997). Detection of carbamate pesticides in vegetable samples using cholinesterase-based biosensors. *Electroanalysis*, *9*(14), 1083–1087.

Wells, M. J. M., & Yu, L. Z. (2000). Solid-phase extraction of acidic herbicides. *Journal of Chromatography A*, *885*, 67.

Wenzel, K.-D., Hubert, A., Manz, M., Weißflog, L., Engewald, W., & Schüürmann, G. (1998). Accelerated solvent extraction of semivolatile organic compounds from biomonitoring samples of pine needles and mosses. *Analytical Chemistry*, *70*, 4827–4835.