Pogačnik L, Franko M. *PHOTOTHERMAL BIOANALYTICAL METHODS FOR PESTICIDE TOXICITY TESTING*
Arh Hig Rada Toksikol 2003;54:197-205

197

Conference Paper

PHOTOTHERMAL BIOANALYTICAL METHODS FOR PESTICIDE TOXICITY TESTING*

Lea POGAČNIK[1] and Mladen FRANKO[2]

Biotechnical Faculty, University of Ljubljana, Ljubljana[1], Nova Gorica Polytechnic, Nova Gorica[2], Slovenia

This paper describes bioanalytical methods and biosensors which rely on cholinesterase (ChE) inhibition and can be used to detect and test the toxicity of organophosphate (OP) and carbamate pesticides. Particular attention is given to the combined use of these methods and photothermal detection that has recently led to improved sensitivity and increased sample throughput of ChE bioanalytical assay. Following a rapid and simple sample preparation procedure, this assay can detect organophosphate pesticides such as paraoxon in the sub ng/mL concentration range. The AChE bioanalytical method demonstrated different sensitivity to various pesticides, which correlated well with pesticide toxicity expressed as LD_{50} for rats. Similarly, the same pesticide yielded different ChE inhibition in different organisms. This opens the possibility of applying these bioanalytical methods to evaluate the acute toxicity of OP compounds or of environmental samples to particular organisms.

KEY WORDS: *biosensor, carbamates, cholinesterase inhibition, organophosphates, vegetables*

Bioanalytical methods rely on biorecognition capability of specific molecules (examples are enzymes and proteins), which selectively interact with compounds of interest, to produce a measurable analytical signal. They are reliable, rapid and inexpensive tools for assessment of the various physiologically important or toxic compounds in different samples. Their main field of application is in biomedical research and diagnostics, although application in food analysis and environmental research has been on the rise. Generally, the primary objective of bioanalytical methods in environmental analysis is to determine toxic pollutants in environmental samples including potable water and wastewater, sediments, plants, animal tissue and soil. In addition, these measurements can give information on potential toxicity of a particular compound or sample to certain organisms. However, a clear understanding of the relation between the interaction at the molecular level and the physiological effects experienced by an organism is crucial for such application.

For several reasons, detection of pesticides accounts for the greatest number of reports among environmental bioanalytical techniques. First, pesticides typically function by means of interacting with a specific biochemical target either as a substrate (e.g. organophosphate insecticides with organophosphate hydrolase) or as inhibitors (e.g. dithiocarbamate fungicides with aldehyde dehydrogenase; organophosphate or carbamate insecticides with acetylcholinesterase). Both types of pesticide action can be used for bioanalytical purposes. In addition, a wide variety of antibodies have been developed (some of which are commercially available) toward various classes of insecticides, herbicides and fungicides. Examples include triazines, alachlor, aldicarb, 2,4-D and paraquat (1).

Being very specific, the inhibition mechanism between the organophosphate and carbamate pesticides and acetylcholinesterases (AChE, E.C. 3.1.1.7.) or butyrylcholinesterases (BChE, E.C. 3.1.1.8.) has led to the development of several

198

Pogačnik L, Franko M. *PHOTOTHERMAL BIOANALYTICAL METHODS FOR PESTICIDE TOXICITY TESTING*
Arh Hig Rada Toksikol 2003;54:197-205

analytical methods for identification and quantification of such insecticides (2). A detailed review on this subject has been published lately by *Luque de Castro and Herrera* (3). Most of these methods use different kinds of cholinesterases (ChE) (AChE from electric eel, bovine erythrocytes, human erythrocytes or BChE from horse serum or human serum) as biological sensing elements (4). Different recombinant AChEs were applied to various pesticide bioanalytical devices to increase the selectivity as reviewed by *Schulze and co-workers* (5).

In the reaction of cholinesterases with organophosphate and carbamate inhibitors, initially an enzyme-inhibitor complex is formed, which is subsequently converted into an inactive phosphorylated or carbamylated form of enzyme (6). The detection of pesticides is mainly based on direct or indirect determination of enzyme activity before and after the application of the sample. Such biosensors measure *in vitro* enzyme inhibition, which can be extrapolated to *in vivo* enzyme inhibition. Biorecognition-based techniques result in rapid, simple and selective methods for pesticide analysis because they combine the selectivity of enzymatic reactions and operational simplicity.

In Europe, regulations are now being strictly enforced with a maximum pesticide concentration of 1 μg/L per pesticide in drinking water. Therefore, development of devices for fast, inexpensive, on-line and field determination of pesticide levels is of great importance (7). This fact has led to the development of a variety of analytical methods for pesticide identification and quantification. The multianalyte assay of pesticides in different samples requires complex and expensive techniques such as gas chromatography (GC) or high performance liquid chromatography (HPLC) in combination with different detection methods. These methods require time-consuming sample preparation with different extraction methods using toxic organic solvents and cannot be done easily outside the laboratory. In addition, these methods are elaborate because they require preconcentration. However, in laboratories where large numbers of samples must be processed rapidly, a method with sufficient sensitivity that can be used for preliminary screening is a good alternative (8). Such possibility was lately offered by enzyme reactors or biosensors and other bioanalytical methods, which have the advantage of reducing time and cost by using immobilised biological compounds, being very selective for the analyte of interest (4). In this first screening step, potentially hazardous samples could be identified by a bioanalytical method and sent for detailed analysis in a laboratory using more expensive and time-consuming analytical methods.

This paper presents recently developed photothermal biosensors constructed with cholinesterases of different origin. Their suitability for the detection of certain frequently used organophosphates and carbamates is elucidated by correlating the results of anticholinesterase (AntiChE) toxicities for different organophosphates and carbamates to the previously determined acute toxicities (from animal studies) of these pesticides (LD$_{50}$). The paper further discusses possible uses of these biosensors for toxicity measurements. Finally, it describes the actual use of the biosensor in the analysis of real vegetable samples, following a simple and rapid sample preparation procedure, and compares the results with values obtained by a well-established standard procedure employing GC-MS detection.

MATERIALS AND METHODS

Detailed description of all materials and procedures used for the preparation and proper operation of photothermal cholinesterase biosensors is given elsewhere (9-12).

Flow-injection analysis (FIA) manifold

The flow-injection analysis manifold (Figure 1) consists of a HPLC pump, two injection valves, a bioanalytical column with immobilised enzyme, and detection unit. The carrier buffer is pumped through the system at 0.5 mL/min. This flow rate was selected as the best compromise between the
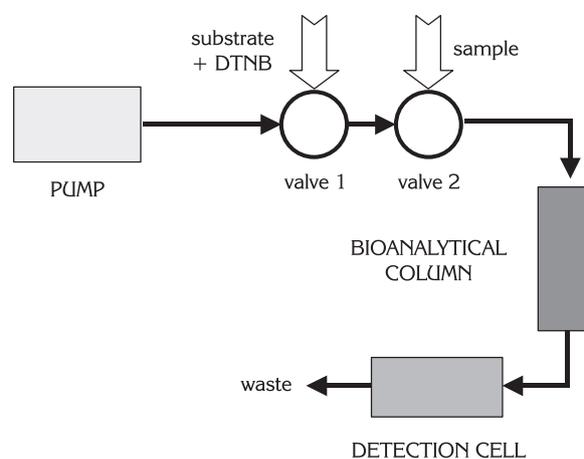
Figure 1 *Experimental set-up for flow-injection analysis*

Pogačnik L, Franko M. *PHOTOTHERMAL BIOANALYTICAL METHODS FOR PESTICIDE TOXICITY TESTING*
Arh Hig Rada Toksikol 2003;54:197-205

199

sensitivity and the duration of measurement, as has been demonstrated experimentally (10). The volume of the substrate and sample injection loops is 20 μL and 1 mL, respectively.

Technical details and operational principles of thermal lens spectrometric (TLS) detection unit (13) have been described elsewhere (14, 16). The optimal detection sensitivity was obtained by using the 488 nm Ar-ion laser line, which provided 60 mW for the excitation of absorbing compound (12).

The enzyme immobilisation

The enzyme was immobilised according to the procedure published elsewhere (17). The controlled-pore glass beads (CPG) with immobilised enzyme were stored at 4 °C in phosphate buffer (pH 6.0). No decrease in the activity of the immobilised enzyme in stock solution was reported after six months of storage. Before use, the immobilised enzyme was packed into a PEEK column (60x2.1 mm i.d.).

Determination of enzyme activity and inhibition

Enzyme activity was determined according to *Ellman and co-workers* (18), and is based on two coupled reactions:

- acetylthiocholine (ASCh) $\xrightarrow{\text{Acetylcholinesterase}}$ acetic acid + thiocholine
- thiocholine + 5,5'-dithio-bis-2-nitrobenzoic acid(DTNB) $\longrightarrow$ 2-nitrobenzoate-5-mercaptothiocholine + 5-thio-2-nitrobenzoate (TNB^{2-})
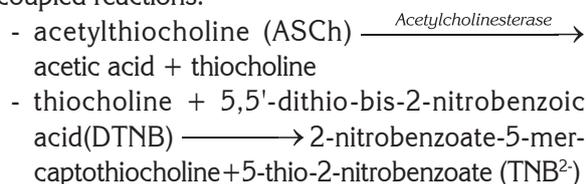
The substrate solution was prepared fresh for each set of experiments and kept on ice to avoid non-enzymatic hydrolysis.
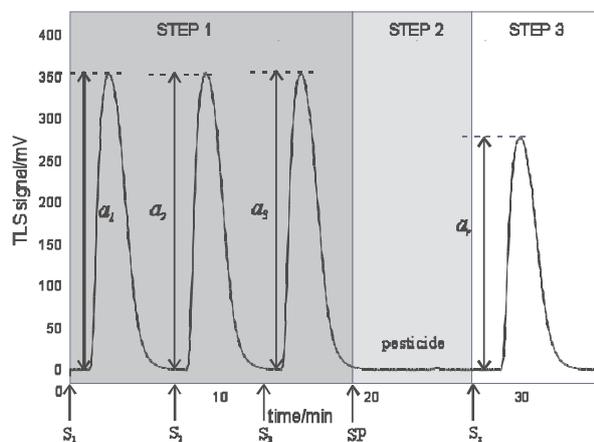
Figure 2 *The evolution of thermal lens spectrometric signals during a three-step determination of pesticide concentration*

The product TNB^{2-} is yellow with the absorption maximum at 410 nm. Enzyme inhibition was determined using an inhibition test that includes the following steps (Figure 2):

1. determination of the average signal corresponding to the initial activity (a_0) of the enzyme in the bioanalytical column by three consecutive injections of the substrate (S$_1$, S$_2$, S$_3$)
2. injection of a sample containing the pesticide (SP)
3. determination of the signal corresponding to the final enzyme activity (a_r) by injection of the substrate (S$_x$) and calculation of the remaining relative enzyme activity (A) according to the formula:

$$A = \frac{a_r}{a_0}$$ [1]

After the inhibition of the enzyme reaches 80 %, the bioanalytical column is to be replaced by a fresh one. The inactive enzyme can then be successfully reactivated with reagent pyridine-2-aldoxime methiodide (2-PAM) (19).

Preparation and analysis of the vegetable sample

Vegetable samples weighing 10-25 g were chopped and meshed in liquid N$_2$ before adding 1 mL of acetone and 9 mL of 0.05 M phosphate buffer (pH 8.0). The mixtures were treated in a stomacher for 15 min. The suspensions were centrifuged (10 min, 2500 rpm) and the supernatants were directly applied onto the bioanalytical column without any extraction or preconcentration step. The extent of enzyme inhibition observed for each sample was used to determine equivalent paraoxon concentration, which corresponds to the concentration of paraoxon in buffer solution causing the inhibition equal to the inhibition by a particular sample. Previously prepared inhibition curves for paraoxon in buffer solutions were used for the calculation of equivalent paraoxon concentrations.

Pesticide concentrations determined by GC-MS were converted into equivalent paraoxon concentrations (c_{eq}) according to the following equation:

$$c_{eq.} = c_x \times \frac{LD_{50(p)}}{LD_{50(x)}}$$ [2],

where $c_{eq.}$ is equivalent paraoxon concentration; c_x is GC-MS determined pesticide concentration; $LD_{50(p)}$ is lethal dose for oral exposure of rats to paraoxon (20,

200

Pogačnik L, Franko M. *PHOTOTHERMAL BIOANALYTICAL METHODS FOR PESTICIDE TOXICITY TESTING*
Arh Hig Rada Toksikol 2003;54:197-205

21); and $LD_{50(x)}$ is lethal dose for oral exposure of rats to a particular pesticide (20, 21).

RESULTS AND DISCUSSION

Detection of different organophosphate and carbamate pesticides

The detection of individual organophosphates (paraoxon, diazinon, chlorpyrifos) and carbamates (carbaryl, carbofuran) by the described photothermal biosensor is based on inhibition curves for each pesticide. Each inhibition curve was obtained on a single bioanalytical column. Inhibition curves for bioanalytical columns with immobilised AChE from human erythrocytes are summarised in Figures 3 and 4. Their slopes differ considerably as does the toxicity of used compounds, i.e. their ability to inhibit AChE. The limit of detection (LOD) for each inhibition curve was calculated as a concentration of pesticide yielding the decrease in enzyme activity equal to a three-fold standard deviation of the initial enzyme activity. Among organophosphate pesticides, the lowest LOD (0.2 ng/mL) was determined for paraoxon, which was the most toxic (LD_{50}=5 mg/kg). The least toxic was chlorpyrifos (LD_{50}=135-245 mg/kg) with the detection limit of 4 μg/mL. Among carbamates the most toxic was carbofuran (LD_{50}=8 mg/kg) with a 1 ng/mL LOD, while the LOD of carbaryl (LD_{50}=250-850 mg/kg) was 0.7 μg/mL. The results demonstrate the connection between enzyme inhibition by different pesticides and acute toxicities expressed as LD_{50} (oral, rat). This opens a possibility to use these biosensors in screening for the toxicity of new cholinesterase inhibiting pesticides or other products. The connection was confirmed by further experiments and is discussed in details below.
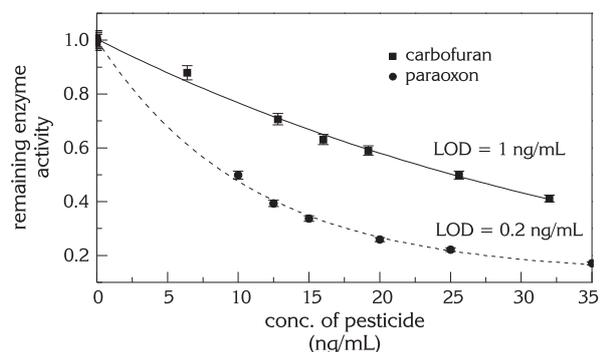
The sensitivity of cholinesterases of various origin

Responses of bioanalytical columns prepared by different ChEs (AChE from bovine erythrocytes, AChE from human erythrocytes, AChE from electric eel or BChE from horse serum) were found considerably different. This was demonstrated for organophosphates (paraoxon, oxydemeton-methyl, triazophos, diazonon) and carbamates (carbofuran, propoxur) frequently applied in agriculture. Their acute toxicities, expressed as LD_{50} (oral, rat), covered the range from very toxic substances (paraoxon, LD_{50}=5 mg/kg) to low toxicity chemicals (diazinon, LD_{50}=300-400 mg/kg). A clear difference was observed between sensitivities of different enzymes to the same pesticide, as illustrated by the results obtained for paraoxon (Figure 5).

To compare the performance of various bioanalytical columns, the LODs were calculated for all combinations of pesticides and enzymes. The LODs

Figure 3 *Inhibition curves for carbofuran and paraoxon showing the remaining enzyme activity as a function of pesticide type and its concentration*

Figure 4 *Inhibition curves for chlorpyrifos, diazinon and carbaryl showing the remaining enzyme activity as a function of pesticide type and its concentration*
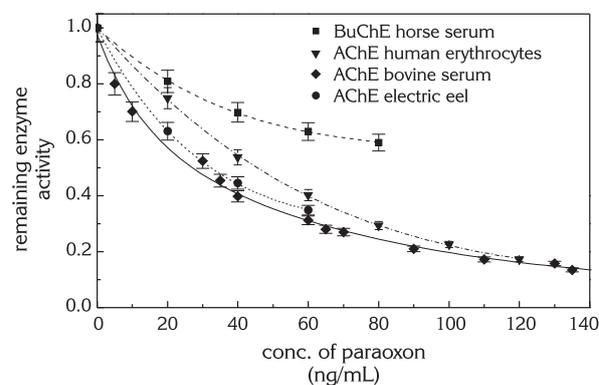
Figure 5 *Comparison of inhibition curves by paraoxon obtained for different cholinesterases.*

Pogačnik L, Franko M. *PHOTOTHERMAL BIOANALYTICAL METHODS FOR PESTICIDE TOXICITY TESTING*
Arh Hig Rada Toksikol 2003;54:197-205

201

represent pesticide concentrations that resulted in a 10 % inhibition of the enzyme, as frequently defined in the literature on biosensors (22, 24).

Relative AntiChE toxicities were calculated from LOD values for all combinations of pesticides and enzymes according to the formula:

$$AntiAChE = \frac{LOD_{paraoxon}}{LOD_x} \qquad [3]$$

The results were compared to the ratio of lethal doses (LD_{50}; oral exposure, rats) for particular pesticide and for paraoxon. The LD_{50} values for rats were selected as reference, since these organisms were the only found to be tested for toxicities of the investigated pesticides. The results are summarised in Table 1. For the three AChEs, the trend of higher AntiChE toxicity can be observed for pesticides with lower LD_{50}. The only exception is oxydemeton-methyl, which shows over 10 times lower AntiChE toxicity compared to the equally toxic propoxur and two to four times lower AntiChE toxicity compared to triazophos, which is about 20 % less toxic as calculated from LD_{50} values. However, the correlation between the LD_{50} and AntiChE toxicity was not found for BChE from horse serum, as it can be observed with carbofuran, propoxur and oxydemeton-methyl. Furthermore, the agreement between relative AntiChE toxicity and LD_{50} ratios is rather poor, except for propoxur, as the values differ by a factor 20 or more. Better agreement was achieved for propoxur and carbofuran, when compared to the less toxic chemicals. This agreement depends on the pesticide as well as on the enzyme used. In almost all cases (except for triazophos), BChE from horse serum produced the lowest relative AntiChE values and had the lowest degree of agreement with LD_{50} ratio. For other enzymes, the agreement with LD_{50} ratios depended on the pesticide detected. The electric eel AChE showed the best agreement for detection of propoxur and human erythrocytes AChE for carbofuran. Most results for relative AntiChE toxicity are lower than pesticide toxicity expressed as its LD_{50} relative to the LD_{50} of paraoxon. The only case where the obtained AntiChE toxicity was higher than the LD_{50} ratio was the combination of propoxur and AChE from human erythrocytes.

In the case of diazinon and triazophos, lower AntiChE values were attributed to the thiono form of the pesticide, which is not a strong inhibitor of AChE. In living organisms, oxidation to oxo-form occurs in the presence of $NADPH_2$ and oxygen. This enhances the inhibition potential of pesticides and their toxicity. It is most likely that similar processes in

Table 1 *Comparison of experimentally determined relative anticholinesterase toxicities and acute toxicities expressed as LD_{50} (oral, rat) for different organophosphates and carbamates. The LD_{50} (oral, rat) for paraoxon is 5 mg/kg.*

PESTICIDE	LD_{50}(mg/kg)[a]	LD_{50p}[b]/LD_{50x}[c] (%)	ENZYME	*Relative AntiChE toxicity (%)*
carbofuran	8	62	AChE electric eel	18±4
			AChE bovine	16±3
			AChE human	40±6
			BChE horse	3.5±0.4
propoxur	50	10	AChE electric eel	8.6±0.9
			AChE bovine	7.7±0.8
			AChE human	17±2
			BChE horse	4.9±0.3
oxydemeton-methyl	50	10	AChE electric eel	0.20±0.02
			AChE bovine	0.23±0.07
			AChE human	0.17±0.01
			BChE horse	0.04±0.01
triazophos	57-59	8.5-8.8	AChE electric eel	0.46±0.05
			AChE bovine	0.51±0.1
			AChE human	0.88±0.06
			BChE horse	0.75±0.03
diazinon	300-400	1-2	AChE electric eel	0.14±0.02
			AChE bovine	0.11±0.02

a lethal doses for oral exposure to pesticide for rats (20, 21)
b lethal dose for oral exposure to paraoxon for rats
c lethal dose for oral exposure to particular pesticide for rats

202

Pogačnik L, Franko M. *PHOTOTHERMAL BIOANALYTICAL METHODS FOR PESTICIDE TOXICITY TESTING*
Arh Hig Rada Toksikol 2003;54:197-205

a living organism also alter the toxicity and increase the AntiChE activity of other pesticides.

It would therefore be unreasonable to expect a perfect agreement between relative AntiChE toxicities and LD_{50} ratios, since LD_{50} is determined from studies of live rats and not of isolated enzymes.

Furthermore, LD_{50} for different organisms does not correspond to values obtained in rats, which is suggested by different sensitivities of various ChEs to selected pesticides. However, there are no data on the susceptibility of isolated rat AChE to different pesticides that could be used to compare LD_{50} ratios and relative AntiChE toxicities for the same species.

Analysis of vegetable samples

Even though most AChE-biosensors described so far are limited to analysis based on aqueous solutions because they may be susceptible to matrix effect (25), the photothermal AChE biosensor showed good results in determining total organophosphate and carbamate pesticides concentrations in vegetable samples (onion, iceberg lettuce and lettuce). Filtered liquid fractions of homogenised vegetable samples were injected directly onto the bioanalytical column. A blank sample was prepared from uncontaminated lettuce and analysed in the same manner.

The increase in the initial enzyme activity after the application of blank sample on the biosensor was similar to the matrix effect observed in fruit juices (about 5 % of the initial enzyme activity) (9). AChE inhibitions due to the presence of pesticides were 13, 33 and 26 % for onion, iceberg lettuce and lettuce, respectively (Figure 6). The detected enzyme inhibitions were used to evaluate equivalent paraoxon concentrations according to the calibration curves for paraoxon in buffer solution.
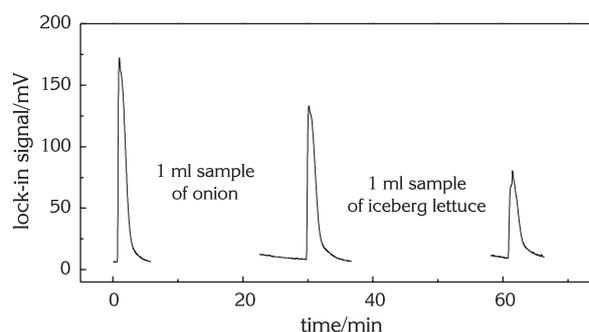
Figure 6 *Thermal lens spectrometric signals obtained before and after injection of pesticides containing vegetables*

The same samples were later analysed using GC-MS to determine actual pesticides and their concentrations.

The equivalent paraoxon concentrations for pesticides found in the samples were calculated from LD_{50} data for oral exposure of rats to particular pesticide and paraoxon (20, 21) according to equation [2]. GC-MS results and values for equivalent paraoxon concentrations determined by a biosensor (Table 2), demonstrated a good agreement in iceberg lettuce and onion. The best agreement was observed in onion, which contained carbofuran and propamocarb. The equivalent paraoxon concentration for iceberg lettuce was 15 % higher when analysed by a biosensor than by GC-MS, but was still within the range of experimental error. The least agreement was achieved in lettuce containing parathion-ethyl. The equivalent paraoxon concentration determined by the photothermal biosensor was five times lower than the actual concentration of the pesticide found in the sample. This discrepancy was attributed to insufficient oxidation of the compound (the parent compound parathion-ethyl does not inhibit ChE, whereas the

Table 2 *Comparison of pesticide concentrations in vegetable samples determined by the bioanalytical flow-injection analysis - thermal lens spectrometry and by GC-MS*

SAMPLE	FIA-TLS enzyme	inhibition (%)	paraoxon equivalent mg/kg	GC-MS pesticides in the sample	paraoxon equivalent mg/kg	EU regulation (mg/kg)
Onion	BChE horse serum	13.1±0.4	0.013±0.005	0.02 mg/kg carbofuran	0.012	0.3 carbofuran
				0.11 mg/kg propamocarb		Propamocarb not allowed
Iceberg lettuce	BChE horse serum	33±1	0.021±0.005	0.18 mg/kg oxydemeton-methyl	0.018	0.5 oxydemeton-methyl
Lettuce	AChE electric eel	25.8±0.8	0.009±0.005	0.05 mg/kg parathion-ethyl	0.05	0.05 parathion-ethyl

Pogačnik L, Franko M. *PHOTOTHERMAL BIOANALYTICAL METHODS FOR PESTICIDE TOXICITY TESTING*
Arh Hig Rada Toksikol 2003;54:197-205

203

oxidised form paraoxon-ethyl is a strong inhibitor of the enzyme).

It is important to emphasize that all pesticide concentrations determined in samples were well below the levels permitted by the EU regulations. This confirms adequate sensitivity of the photothermal AChE biosensor for the detection of pesticides in samples, and its suitability for simple, rapid and accurate identification of samples that meet quality standards on organophoshate and carbamate pesticides. The same applies to samples which should be further analysed by GC-MS or other technique, when the concentrations of organophosphates and carbamates are close to or already above maximum values.

CONCLUSIONS AND FUTURE OUTLOOK

The sensitive photothermal cholinesterase FIA system offers the possibility to screen for the presence of organophosphate and carbamate pesticides in different samples and can provide an alternative to toxicity assays. Our study has shown that, in addition to differences in sensitivity for various pesticides (paraoxon, oxydemeton-methyl, triazophos, diazinon, carbofuran, propoxur, chlorpyrifos, carbaryl), FIA systems with bioanalytical columns constructed from enzymes (AChEs and BChE) of different origin (electric eel, human erythrocytes, bovine erythrocytes, horse serum) show different sensitivities to the same pesticide.

When appropriate cholinesterase is used, relative AntiChE toxicities for different pesticides show a general trend of increase with the increasing acute toxicities expressed as LD_{50} (oral, rat), suggesting the possibility of using such FIA system as a screening test to evaluate the toxicity of different compounds or environmental samples to a particular organism.

Chemical toxicity testing with specific enzymes for bioanalytical sensing and the described FIA system should provide more accurate information on the toxicity to a particular organism than LD_{50} values obtained on rats, since the susceptibility of cholinesterases to pesticide inhibition in various organisms differs considerably. This is even more important when the toxicity of chemicals to humans is studied.

This study shows that the differences in the susceptibility of different organisms to cholinesterase-inhibiting pesticides is easily determined by the FIA system based on isolated cholinesterase from a particular organism, rather than on experimental animals. The use of laboratory animals could therefore be avoided in such tests.

Additional testing with various enzymes, some of which still need to be isolated, and *in vivo* toxicity studies on corresponding organisms is however needed to definitely confirm the photothermal bioanalytical FIA method as a suitable approach for testing organophosphate and carbamate pesticides, and environmental sample toxicity to various animals and eventually humans.

It has been shown that photothermal biosensors can detect pesticides in vegetable samples without time-consuming and environmentally unfriendly extraction and preconcentration. Contaminated samples of onion, iceberg lettuce and lettuce produced positive results for organophosphate and carbamate pesticides. Additional GC-MS analyses confirmed the presence of various organophosphates (parathion-ethyl, oxydemeton-methyl) and carbamates (carbofuran, propamocarb) in concentrations below the regulatory limits set by the EU. A satisfactory agreement between the results obtained by the bioanalytical FIA system and GC-MS analysis was found when the samples contained oxo-organophosphates or carbamates (carbofuran, propamocarb, oxydemeton-methyl). This agreement can be improved for sulphur derivatives such as parathion-ethyl by adding a weak oxidising agent before analysing the sample.

The photothermal AChE biosensor is a new screening tool for the presence of organophosphate and carbamate pesticides. The flow-injection experimental manifold enables quick analysis of different samples. No additional incubation time between the enzyme and pesticide is required to achieve reasonably low limits of detection. This lowers the cost and the time of analysis considerably. It can therefore be used as a reliable high-throughput screening test when the presence of organophosphate and carbamate pesticides is suspected in samples. Samples with high AntiChE toxicity can be further analysed by standard chromatographic methods to determine particular pesticides present in the sample.

At present, the major drawbacks of the described method are the need of two separate measurements to obtain information on enzyme inhibition and the limited number of available excitation lines (457.9-

204

Pogačnik L, Franko M. *PHOTOTHERMAL BIOANALYTICAL METHODS FOR PESTICIDE TOXICITY TESTING*
Arh Hig Rada Toksikol 2003;54:197-205

514.5 nm). This is true not only for the Ar-ion laser, as very few lasers are available in the spectral range below 450 nm. However, rapid progress in laser technology has brought new solid-state lasers to the market, which are characterised by a shorter emission wavelength, small size, and robustness. This is an important breakthrough for the future development of robust and portable instruments for field measurements.

REFERENCES

1. Rogers KR, Mascini M. Biosensors for field analytical monitoring. Field Anal Chem Technol 1998;2:317-31.
2. Kumaran S, Tran-Minh C. Determination of organophosphorous and carbamate insecticides by flow-injection analysis. Anal Biochem 1992;200:187-94.
3. Luque de Castro MD, Herrera MC. Enzyme inhibition-based biosensors and biosensing systems: questionable analytical devices. Biosens Bioelectron 2003;18:279-94.
4. Nunes S, Skladal P, Yamanaka H, Barcelo D. Determination of carbamate residues in crop samples by cholinesterase-based biosensors and chromatographic techniques. Anal Chim Acta 1998;362:59-68.
5. Schulze H, Vorlova S, Villate F, Bachmann TT, Schmid RD. Design of acetylcholinesterases for biosensor application. Biosens Bioelectron 2003;18:201-9.
6. Eto M. Organophosphorous pesticides: Organic and biological chemistry. Boca Raton (FL): CRC; 1974. p. 123.
7. Dzydevich SV, Shulga AA, Soldatkin AP, Hendji AMN, Jaffrezic-Renault N, Martelet C. Conductometric biosensors based on cholinesterases for sensitive detection of pesticides. Electroanalysis, 1994;6:752-8.
8. Leon-Gonzalez ME, Townshend A. Flow-injection determination of paraoxon by inhibition of immobilized acetylcholinesterase. Anal Chim Acta 1990;236:267-72.
9. Pogačnik L, Franko M. Determination of organophosphate and carbamate pesticides in spiked samples of tap water and fruit juices by a biosensor with photothermal detection. Biosens Bioelectr 1999;14:569-78.
10. Pogačnik L, Franko M. Optimisation of FIA system for detection of organophosphorous and carbamate pesticides based on cholinesterase inhibition. Talanta 2001;54:631-41.
11. Pogačnik L, Franko M. Validation of different commercially available cholinesterases for pesticide toxicity test. Ann di Chim 2002;92:93-101.
12. Pogačnik L, Franko M. Detection of organophosphate and carbamate pesticides in vegetable samples by a photothermal biosensor. Biosens Bioelectr 2003;18:1-9.
13. Šikovec M, Novič M, Hudnik V, Franko M. On-line thermal lens spectrometric detection of Cr(III) and Cr(VI) after separation by ion chromatography. J Chromatogr A 1995;706:121-6.
14. Bialkowski SE In: Winefordner JD, editor. Chemical Analysis 134. New York (NY): John Wiley & Sons, Inc; 1996.
15. Dovichi NJ, Harris JM Laser-induced thermal lens effect for calorimetric trace analysis. Anal Chem 1979;51:728-31.
16. Franko M, Tran CD. Analytical thermal lens instrumentation. Rev. Sci. Instrum 1996;67:1-18.
17. Leon-Gonzalez MF, Townshend A. Determination of organophosphorous and carbamate pesticide standards by liquid chromatography with detection by inhibition of immobilized acetylcholinesterase. J Chromatogr 1991;539:47-54.
18. Ellman GL, Courtney KD, Andres V, Featherstone RM. A new rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961;7:88-95.
19. Kumaran S, Tran-Minh C. Determination of organophosphorous and carbamate insecticides by flow-injection analysis. Anal Biochem 1992;200:187-94.
20. Ternifi V. Priročnik o toksikoloških lastnostih pesticidov v Republiki Sloveniji [(Handbook of pesticide toxicological properties in the Republic of Slovenia), in Slovenian]. Ljubljana: Ministry of Health of the Republic of Slovenia; 1998. p. 144-434.
21. Tomlin CDS, editor. The Pesticide Manual. 11th ed. Farnham: British Crop Protection Council; 1997.
22. Marty JL, Mionetto N, Lacorte S, Barcelo D. Validation of an enzymatic biosensor with various liquid-chromatographic techniques for determining organophosphorous pesticides and carbaryl in freeze-dried waters. Anal Chim Acta 1995;311:265-71.
23. Abad JM, Pariente F, Hernandez L, Abruña HD, Lorenzo E. Determination of organophosphorous and carbamate pesticides using a piezoelectric biosensor. Anal Chem 1998;70:2848-55.
24. Koglin EN, Williams LR. Testing, performance validation and quality assurance quality-control of field-portable instrumentation. Trac Trend Anal Chem 1994;13:294-9.
25. Schulze H, Scherbaum E, Anastassiades M, Vorlova S, Schmid RD, Bachmann TT. Development, validation, and application of an acetylcholinesterase-biosensor test for the direct detection of insecticide residues in infant food. Biosens Bioelectron 2002;17:1095-105.

Pogačnik L, Franko M. *PHOTOTHERMAL BIOANALYTICAL METHODS FOR PESTICIDE TOXICITY TESTING*
Arh Hig Rada Toksikol 2003;54:197-205

205

Sažetak

FOTOTERMIČKE BIOANALITIČKE METODE ZA TESTIRANJE TOKSIČNOSTI PESTICIDA

U ovom se radu razrađuje koncepcija bioanalitičkih metoda i biosenzora na primjeru inhibicije kolinesteraza. Ovi bi se postupci mogli primijeniti za testiranje toksičnosti organofosfornih i karbamatnih pesticida. Kombiniranom primjenom ove koncepcije i fototermalne detekcije postiglo se povećanje osjetljivosti detekcije i broja analiziranih uzoraka. Autori su analizirali uzorke povrća na prisutnost organofosfornih spojeva paraoksona, diazinona i klorpirifosa te monometilnih karbamata karbarila i karbofurana. Autori su utvrdili da i nakon primijenjene jednostavne i brze pripreme mogu detektirati koncentracije npr. paraoksona ispod 1 ng/ml. Pokazalo se da je detekcija pesticida u povrću (luk, salata) s pomoću fototermalnog biosenzora moguća i bez ekstrakcijskih i prekoncentracijskih postupaka, što znatno ubrzava i olakšava analize. Bioanalitička metoda s acetilkolinesterazom pokazala je različitu osjetljivost prema pojedinim pesticidima i to je bilo u skladu s poznatim podacima o akutnoj toksičnosti izraženoj oralnim LD_{50} vrijednostima za štakore. Stoga autori smatraju da bi primjena ove metode mogla nadopuniti ili nadomjestiti upotrebu laboratorijskih životinja u testiranju toksičnosti antikolinesteraznih pesticida. Autori su također utvrdili različitosti u interakciji istog insekticida i acetilkolinesteraze različitih organizama.

KLJUČNE RIJEČI: *biosenzor, inhibicija kolinesteraze, karbamati, organofosforni spojevi, povrće*

REQUESTS FOR REPRINTS:

Lea Pogačnik, Ph. D.
University of Ljubljana, Biotechnical Faculty
Department of Food Science and Technology
Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia
E-mail: *lea.pogacnik@bf.uni-lj.si*