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Enzyme vs. Bacterial Electrochemical Sensors for Organophosphorus Pesticides Quantification

Margarita Stoytcheva
Universidad Autónoma de Baja California, Instituto de Ingeniería México

1. Introduction

The worldwide increasing use of organophosphorus (OP) pesticides which are powerful neurotoxins and the resulting environmental and public concerns (CDC, 2005) created a demand for the development of reliable, fast, sensitive, simple and low-costing methods for their quantification, appropriate for on-line and on-site measurements. The conventional chromatographic, spectroscopic and immunoassay techniques for OP compounds determination, despite of their accuracy and sensitivity, are not well suited to these tasks. In contrast, the electrochemical biosensors based methods fulfill all the mentioned requirements. The biosensors are relatively new analytical devices developed taking advantage of the progress in the biotechnology and the material science, in particular, in association with the modern principles of transduction of the chemical information. They represent a variety of chemical sensors, transforming the concentration of the quantified substance into an analytically useful signal (Thévenot et al., 1999).

The electrochemical biosensors provide selective quantitative or semi-quantitative analytical information using a biological recognition element (enzymes, whole cells, organelles or particles, tissues, etc.), in direct spatial contact with an electrochemical transducer, converting the signal produced by the interaction between the bioreceptor and the analyte, into electrical one (Thévenot et al., 1999). A great variety of electrochemical biosensors quantifying the organophosphorus pesticides have been designed over the last decades. This review gives a survey on the state of the art of organophosphorus compounds detection using enzyme- and bacterial-based electrochemical sensors. The survey includes the presentation of the OP pesticides structure and biochemical action, as well as the sources of pollution and the regulatory norms. The current chromatographic and immunoassay methods for OP analysis are briefly discussed. The emerging during the last decades electrochemical biosensors based techniques are presented as their alternative. The analytical performances of the two main types of enzyme-based electrochemical sensors for OP determination (the organophosphorus hydrolase and the acylcholinesterases ones), involving respectively the direct enzyme transformation of the analyte, and the inhibition of the enzyme activity, are summarized.

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The recent trends in the development and in the increasing application of bacterial sensor systems for OP analysis are revised. The advantages and the limitations of the enzyme-based vs. the bacterial electrochemical sensors are discussed.

2. The organophosphorus pesticides

The chemical compounds including stable functional groups that contain the carbon-phosphorus bond or that are organic derivatives of inorganic phosphorus acids are known as organophosphorus (Quin, 2000). Most of them, with the following general structure (Corbett et al., 1984; Eto, 1974; Hassall, 1982):

\[
\text{RO-P-X(OX or SX)}
\]

are highly toxic and are used as chemical warfare agents and pesticides (insecticides, herbicides, fungicides, rodenticides, molluscicides, nematocides, and regulators of vegetal growth, among other).

According to their chemical constitution, the organophosphorus pesticides could be classified into several types (Gupta, 2006). Some representative structures are shown in Fig. 1.

![Fig. 1. Main types of organophosphorus pesticides (R is usually methyl or ethyl group and the leaving group X is aliphatic, homocyclic or heterocyclic one).](https://www.intechopen.com)

The OPs structural variety is reflected in their physicochemical and biological properties (Corbett et al., 1984; Hassall, 1982; WHO, 1986). Data on individual OPs could be found in Dictionary of organophosphorus compounds (Edmundson, 1988), Handbook of pesticide toxicology (Hayes, 1991), The Pesticide manual: A world compendium (Worthing & Hance, 1991), at http://www.pesticideinfo.org/, etc.

The biochemical mode of action of the organophosphorus pesticides primarily involves the inhibition of the acetylcholinesterase occurring throughout the central and peripheral...
nervous system of vertebrates, through phosphorylation of the serine hydroxyl moiety of the enzyme active site, thus preventing the hydrolysis of the neurotransmitter acetylcholine, performed in an analogous manner (Corbett et al., 1984; Fukuto, 1990; Gupta, 2006; Matsumura, 1980), as shown in Fig. 2. The resulting acetylcholine accumulation at the nerve synapses disrupts the nerve impulses propagation.

Slow recovery of the acetylcholinesterase activity could be observed, because of the spontaneous hydrolysis of the phosphorylated enzyme (Fig. 2B). The nucleophilic attack of the phosphorylacetylcholinesterase by some reagents (hydroxylamine, oximes) leads to quicker enzyme reactivation. “Aging” consisting in loss of an alkyl group from an alkoxy group on a phosphoryl residue attached to the active-site serine causes irreversible enzyme inhibition (Fig. 2C).

Fig. 2. A) Acetylcholine enzymatic hydrolysis; B) Acetylcholinesterase inhibition by OP and its reactivation; C) Acetylcholinesterase inhibition by OP and aging. (E-Serine-OH represents the enzyme acetylcholinesterase)

OPs are among the most acutely toxic pesticides. They belong to the toxicity class I (highly toxic) or toxicity class II (moderately toxic), according to the EPA classification. Although less persistent than the organochlorine pesticides, their widespread usage poses risk to man and his environment. Pesticides pollution results from agricultural practices, from industrial waste or discharge, from seepage of buried toxic wastes, and from run-off during spraying (Larson et al., 1997; Majewski & Capel, 1995; Vighi & Funari, 1995). Pesticides production, distribution, use, exposure, environmental levels, and maximum permissible levels in drinking water and food are subject of regulations in accordance with the national and international legislations. The primary involved organizations are the US Environmental Protection Agency (EPA), the EU Commission, the World Health Organization (WHO), the Food and Agricultural Organization of the United Nations (FAO), and the Codex Alimentarius Commission.

The high acute toxicity of the OPs, rapid absorption by the organism, and fast degradation in the environment call for the development of adequate analytical tools for their “in situ” determination. The commonly used methods for OPs quantification include various chromatographic techniques, such as gas-chromatography, gas chromatography with mass spectrometry detection, thin-layer chromatography, and high performance liquid chromatography (Jeannot & Dagnac, 2006; Schlecht & O’Connor, 1994), requiring time-consuming extraction, preconcentration, and clean-up procedures, skilled personnel and expensive laboratory equipment. Immunoassays (Van Emon, 2006) applied for OPs
quantification involve numerous washing steps and long analysis time (one to two hours). Thus, these methods are not suitable for in field determinations and continuous monitoring. Nowadays, the devices of choice for organophosphorus pesticides “in situ” analysis, because of the inexpensive instrumentation, the simple operation procedure and the high sensitivity, are the emerged during the last decades electrochemical biosensors, applicable as well as for real-time and on-line determinations.

3. The electrochemical biosensors for OPs quantification

The electrochemical biosensors for OP pesticides analysis could be classed into two great groups according to the nature of the biological recognition element – enzymes or bacteria.

3.1 Enzyme electrochemical sensors

The function of the acylcholinesterases (acetylcholinesterase or butyrylcholinesterase) and phosphatases (acid or alkaline) electrochemical sensors is based on the ability of the OP compounds to inhibit these enzymes. The quantification is realized measuring the variation of the enzyme activity as a function of the organophosphorus pesticide concentration, applying electrochemical techniques. Thus, according to the transduction mode, the reported biosensors are mainly potentiometric or amperometric.

The potentiometric acylcholinesterase sensors involve the following reaction:

\[
R'-\text{choline} + H_2O \xrightarrow{\text{ChE}} \text{choline} + R'-\text{COOH} \tag{1}
\]

where \(R'\) is an acetyl or butyryl moiety and ChE is the acylcholinesterase.

The pH change of the solution, resulting from the acid release during the enzyme catalyzed hydrolysis of the choline esters is recorded as a sensor response, the latter depending on the cholinesterase activity.

Another potentiometric system is that developed by Ghindilis (Ghindilis et al., 1996), based on mediatorless bioelectrocatalysis:

\[
R'-\text{choline} + H_2O \xrightarrow{\text{ChE}} \text{choline} + R'-\text{COOH} \tag{2}
\]

\[
\text{choline} + 2O_2 + H_2O \xrightarrow{\text{ChO}} \text{betaine} + 2H_2O_2 \tag{3}
\]

\[
H_2O_2 + 2H^+ + 2e^- \xrightarrow{\text{HRP}} 2H_2O \tag{4}
\]

(ChO is the enzyme choline oxidase and HRP is the enzyme peroxidase)

The \(H_2O_2\) electrocatalytical reduction causes a shift in the electrode potential. This tri-enzyme sensor allowed detecting \(2 \times 10^{-13}\) mol L\(^{-1}\) trichlorfon.

The amperometric acylcholinesterase sensors, providing in general faster response, as well as higher sensitivity and accuracy than the potentiometric do, are developed in two directions:

a. First generation ChE amperometric sensors

They exploit the bienzymatic processes described by Eq. 2 and Eq. 3. The current of \(H_2O_2\) oxidation or \(O_2\) reduction, depending on the substrate concentration and the enzyme activity, is recorded as a sensor response. However, since the \(H_2O_2\) oxidation is carried out
at a potential of +0.60 V/SCE, many substances contained in biological liquids and submitted to an oxidation at the same potential (glutathione, ascorbates, urates, etc.) interfere, corrupting the determination. The output signal is influenced by the fluctuations in the oxygen concentration, too.

b. Second generation ChE amperometric sensors
They use synthetic substrates (thiocholine or indoxylacetate esters), transformed upon catalytic hydrolysis in products able to be easily oxidized, as for example:

$$R'\text{-thiocholine} + \text{H}_2\text{O} \xrightarrow{\text{ChE}} \text{thiocholine} + R'\text{COOH} \quad (5)$$

However, $R'$-thiocholine is a subject of a spontaneous non-enzymatic hydrolysis. Although slight, it can produce an increase of the anodic current response. Thiocholine oxidation provoking a passivation of the platinum anodes, because of their interaction with the sulfur containing compounds (Nikol’skaya & Evtuginin, 1992) must be taken into consideration, too.

The process of direct thiocholine oxidation occurring at +0.80 V/SCE at conventional metal and graphite transducers (Martorell et al., 1994; Marty et al., 1992; Marty et al., 1993; Marty et al., 1995; Sužnjević et al., 1985) involves the transfer of one electron from the thiol and a dimerization of the intermediate to disulfide (Evtugyn et al., 1999, Liu et al., 2005). The high potential value however causes the appearance of a high background current, as well as electroactive compounds interferences.

Several types of electrodes providing a sensitive electrochemical detection of enzymatically generated thiocholine at low potential were reported, such as the ones chemically modified with phthalocyanines (Harlbert & Baldwin, 1985; Hart & Hartley, 1994; Skladal, 1991), Prussian blue (Ricci et al., 2004), tetracyanquinodimethane (Kulys & D’Costa, 1991; Martorell et al., 1997) and ferrocene (Evtugyn et al., 1996). However, mediator addition also could provoke interferences.

The alternative route to achieve potential lowering avoiding electrode modification involves acylthiocholine enzymatic hydrolysis (Eq. 5), chemical reduction of the produced thiocholine in solution (Eq. 7), and electrochemical detection of the product of the homogeneous redox reaction (Eq. 8), as suggested by Neufeld (Neufeld et al., 2000) and Ovalle (Ovalle et al., 2009):

$$R'\text{-thiocholine} + 2[\text{Fe(CN)}_6]^{3-} \rightarrow \text{dithio-bis-choline} + 2[\text{Fe(CN)}_6]^{4-} \quad (7)$$

$$[\text{Fe(CN)}_6]^{4-} \rightarrow [\text{Fe(CN)}_6]^{3-} + e^- \quad (8)$$

However, the reported sensitivity of the OPs (chlorofos) determination is lower in comparison to that, attained by direct thiocholine oxidation (Ovalle et al., 2009).

The exploited response-generating reaction in some acylcholinesterase sensors of second generation for OPs quantification is the electrochemical oxidation of the leucoindigo, produced upon enzymatic hydrolysis of indoxylacetate (Kulys, 1989):
The disadvantage of the method consists in the fact that the leucoindigo is exposed to a chemical, as well as to electrochemical oxidation involving $\text{O}_2$, which complicates the formation of the analytical signal (Nikol’skaya & Evtyugin, 1992). The phosphatases inhibition, although reversible (which avoid enzyme reactivation), is rarely applied in the electrochemical biosensors for OPs detection (Danzer & Schwedt, 1996; Mazzei et al., 1996).

The inhibition-based determinations are very sensitive, but indirect. Drawbacks of the method are also the lack of selectivity and the need, in some cases, of enzyme incubation and enzyme reactivation/regeneration. In addition, as shown by Gunaratna (Gunaratna & Wilson, 1990), the cholinesterase is very sensitive to its micro-environment and even small changes provoke significant loss of enzyme activity resulting in decreasing of the sensor sensitivity. An overview of the methods based on enzyme inhibition with emphasis on the non-ideal behavior of the enzyme inhibition-based biosensors and biosensing systems is presented by Luque de Castro (Luque de Castro & Herrera, 2003).

Direct OP pesticides analysis could be achieved applying organophosphorus hydrolase (OPH) electrochemical sensors (Anzai, 2006; Chough et al., 2002; Lei et al., 2007; Mulchandani et al., 2001a; Mulchandani et al., 2001b; Prieto-Simón et al., 2006; Rodriguez-Mozaz et al., 2004; Wang et al., 2003). The enzyme OPH demonstrates substrate specificity toward paraoxon, parathion, coumaphos, diazinon, dursban, methyl parathion, etc, and toward some chemical warfare agents (sarin, soman, tabun, VX, etc.) (Dumas et al., 1990; Munnecke, 1980). The detection of parathion is also possible using parathion hydrolase (PH) (Sacks et al., 2000). The enzymatically catalyzed OP substrates hydrolysis involves pH changes and generates electroactive products:

$$\begin{align*}
\text{RO-P-X} + \text{H}_2\text{O} & \rightarrow \text{RO-P-OH} + \text{HX} \\
(10)
\end{align*}$$

Thus, the detection could be performed in a single step, using potentiometric (pH sensitive) or amperometric transducers (Mulchandani et al., 2001a). OPH-based systems allow the selective determination of the family of the OP compounds, in contrast to the enzyme inhibition based techniques, but the reported detection limit is higher (Mulchandani et al., 2006). An important drawback represents the complex, long-lasting, and expensive procedure for OPH or PH extraction and purification, performed in specialized microbiological laboratories (to note that these enzymes are not commercially available) (Prieto-Simón et al., 2006).

Some reviews summarize the performances of the enzyme electrochemical sensors for OP pesticides determination and the principles of their operation (Andreescu & Marty, 2006; Anzai, 2006; Jaffrezic-Renault, 2001; Mazzei et al., 1996; Mulchandani et al., 2001a; Noguer et al., 1999; Prieto-Simón et al., 2006; Rodriguez-Mozaz et al., 2004; Solé et al., 2003a; Solé et al., 2003b; Tran-Minh, 1985; Turdean et al., 2002). Selected relevant data, demonstrating the sensitivity of the enzyme sensors are given in Table 1 and Table 2.

The commune disadvantages of this group of biosensors are the instability of the response (due to enzyme leaking or deactivation), the observed interferences at high electrode potentials, the passivation of the electrode surface and the short life-time at ambient temperature.
Enzyme vs. Bacterial Electrochemical Sensors for Organophosphorus Pesticides Quantification

| Enzyme      | Target     | LOD       | Reference                  |
|-------------|------------|-----------|----------------------------|
| AChE        | paraoxon   | 0.1 nM    | Tran-Minh et al., 1990     |
| AChE        | malathion  | 1 nM      | Tran-Minh et al., 1990     |
| AChE/BuChE  | paraoxon   | 2.8 ppb   | Skladal, 1991              |
| BuChE       | diazinon   | 2 ppb     | Budnikov & Evtugyn, 1996   |
| BuChE/ChO/HRP | chlorofos | 0.0002 nM | Ghindilis et al., 1996     |
| AChE/BuChE  | paraoxon   | 0.08 ppb  | Skladal et al., 1996       |
| AChE        | paraoxon   | 0.5 ppb   | Noguer et al., 1999        |
| AChE/ChO    | methyl parathion | 0.05 μM  | Lin et al., 2004         |

Table 1. LOD of some acetylcholinesterase sensors for OPs determination (AChE is the acetylcholinesterase and BuChE is the butyrylcholinesterase).

| Enzyme | Target     | LOD       | Reference                  |
|--------|------------|-----------|----------------------------|
| OPH    | paraoxon   | 90 nM     | Mulchandani et al., 1999   |
| OPH    | methyl parathion | 70 nM | Mulchandani et al., 1999 |
| OPH    | paraoxon   | 2 μM      | Mulchandani et al., 2001a  |
| OPH    | methyl parathion | 2 μM | Mulchandani et al., 2001a |
| OPH    | diazinon   | 2 μM      | Mulchandani et al., 2001a  |
| OPH    | parathion  | 15 nM     | Chough et al., 2002        |
| OPH    | paraoxon   | 20 nM     | Chough et al., 2002        |
| OPH    | paraoxon   | 0.4 μM    | Lei et al., 2007           |

Table 2. LOD of some OPH sensors for OPs determination.

3.2 Bacterial electrochemical sensors
Bacteria-based electrochemical sensors are developed by coupling these microorganisms to electrochemical transducers. Bacteria offer several advantages over the isolated enzymes for biosensor application, as for example: lower cost, because of the elimination of the time-consuming and expensive processes of extraction of the intracellular enzymes and their purification; ability to catalyze sequential reactions involving multiple enzymes; resistance to pH and temperature changes, because of the retention of the enzymes in their natural environment; higher tolerance to toxic substances; enzyme activity recovery in nutrient medium (D’Souza, 1989).

The bacterial electrochemical sensors are less sensitive and less selective than the enzyme ones, and their response time is relatively long, because of the diffusional constraints imposed by the bacterial cell wall. However, these drawbacks could be overcome, by genetic engineering and by cell permeabilizing (D’Souza, 1989) respectively, applying various techniques.

Only few bacterial electrochemical sensors for OP pesticides quantification have been developed until now. They include, as biological recognition element, genetically engineered *Moraxella sp.*, *Pseudomonas putida* or *Escherichia coli* with surface-expressed OPH (Mulchandani et al., 1998; Mulchandani et al., 2001c; Mulchandani et al., 2006; Richins et al., 1997). The detection principle is identical to the described above, when employing the isolated and purified enzyme. Recently, microbial sensors based on Clark dissolved oxygen electrode modified with recombinant p-nitrophenol degrading/oxidizing bacteria endowed
with OPH activity was reported (Lei et al., 2005; Lei et al., 2006). The surface-displayed OPH catalyzes the hydrolysis of OP pesticides with nitrophenyl substituent to release products, metabolized by the bacteria while consuming oxygen. The oxygen consumption is measured and correlated to the OP concentration.

Ley (Lei et al., 2004) reports the construction of a hybrid biosensor for direct determination of OP pesticides using purified OPH for their initial hydrolysis and *Arthrobacter* sp. JS443 for the subsequent oxidation of the released p-nitrophenol to carbon dioxide through electroactive intermediates. The biocatalytic layer is prepared by bacteria and enzyme co-immobilization on a carbon paste electrode. The registered signal is the current of oxidation of the intermediates, function of the OP concentration.

The mentioned microbial and hybrid sensors for direct OP pesticides quantification display long term stability, good reproducibility and accuracy, and relatively short response time. However, the reached LOD is over the OP concentration in environmental samples and higher than that for acylcholinesterases inhibition-based sensors, immunoassays, and gas, liquid and thin layer chromatography (Mulchandani et al., 2006).

Recently, an electrochemical biosensor for OP pesticides trace level concentrations determination was developed and characterized (Stoytcheva et al., 2009). It integrates a hybrid biorecognition element consisting of immobilized *Arthrobacter globiformis* and free acetylcholinesterase (ACh) with a Clark type oxygen probe transducer. The bacteria convert the ACh-generated choline to betaine with oxygen consumption measured as a Clark probe current change. This change, representing the sensor response, correlates to the concentration of the OP pesticides inhibiting the Ach catalyzed acetylcholine hydrolysis to choline.

The conditions for maximal sensor response to choline are optimized according to the methodology of Design of Experiments. The analytical performances of the enzyme substrate determination in a wide concentration range (0.1 μmol dm\(^{-3}\) - 20 μmol dm\(^{-3}\) of acetylcholine) and different ACh activities are established. It is demonstrated that the biosensor ensures reproducible, accurate and reliable chlorofos quantification reaching a LOD of 1 nmol dm\(^{-3}\) and a sensitivity of 0.0252 μA/(p(μmol dm\(^{-3}\)) under optimal experimental conditions.

The biosensor response time is 200 s and the storage stability is \(t_{1,50} = 49\) days for the bacterial membrane at ambient temperature. The device is reusable, the bacterial membrane being not affected by OP. The biosensor was applied to chlorofos determination in contaminated milk.

The proposed approach combines the advantages of the bacterial sensors with those of the cholinesterases inhibition-based ones, namely: stable response and long life-time at ambient temperature, because of the conservation of the enzyme system of the bacteria in its natural environment; reproducible characteristics ensured controlling the bacterial charge and the bacterial activity; high sensitivity. In addition, it provides reliable, free of interferences measurement of the dissolved oxygen reduction current, the polymer membrane of the oxygen probe being permeable only for gases. The biosensor fabrication is simple and cost-effective, enzyme extraction and purification or genetic engineering being avoided.

The biosensor is suitable for general toxicity screening or for determining the concentration of isolated OP pollutants.

Some comparative data are presented in Table 3.
| Microorganism          | Target     | LOD      | Reference                      |
|------------------------|------------|----------|--------------------------------|
| Recombinant *P. coli*  | paraoxon   | 2 μM     | Mulchandani et al., 1998       |
| Recombinant *P. coli*  | methyl parathion | 2 μM     | Mulchandani et al., 1998       |
| Recombinant *P. coli*  | diazinon   | 5 μM     | Mulchandani et al., 1998       |
| Recombinant *Moraxella*| methyl parathion | 1 μM     | Mulchandani et al., 2001c      |
| Recombinant *Moraxella*| paraoxon   | 0.2 μM   | Mulchandani et al., 2001c      |
| Recombinant *P. putida*| paraoxon   | 55 ppb   | Lei et al., 2005               |
| Recombinant *P. putida*| methyl paraoxon | 53 ppb   | Lei et al., 2005               |
| Recombinant *P. putida*| parathion   | 58 ppb   | Lei et al., 2005               |
| Recombinant *P. putida*| fenitrothion | 277 ppb  | Lei et al., 2006               |
| Recombinant *P. putida*| EPN        | 1.6 ppm  | Lei et al., 2006               |
| Recombinant *Moraxella*| paraoxon   | 0.1 μM   | Mulchandani et al, 2006        |
| Arthrobacter globiformis| chlorofos  | 1 nM     | Stoytcheva et al., 2009        |

Table 3. LOD of some bacterial electrochemical sensors for OPs determination

4. Conclusion

Despite the still limited application of the electrochemical biosensors for OPs quantification in real samples, their analytical potential is obvious. Thus, current efforts are axed on biosensors’ performance improvement, development of compact and portable or disposable devices for in-field analysis and their commercialization. Promising opportunities offer the nanomaterials transducers modification, permitting the sensitive OPs monitoring at low electrode potential (Periasamy et al., 2009) and the genetic engineering of the biological recognition elements leading to selectivity increase (Campás et al., 2009).

5. References

Andreeescu, S. & Marty, J.-L. (2006). Twenty years research in cholinesterase biosensors: from basic research to practical applications. Biomol. Eng., 23, 1, (March, 2006) 1-15, ISSN: 13890344

Anzai, J. (2006). Use of biosensors for detecting organophosphorus agents. *Yakugaku Zasshi*, 126, 12, (December, 2006) 1301-1308, ISSN: 0031-6903, EISSN: 1347-5231

Aprea, C.; Colosio, C.; Mammone, T.; Minoia, C. & Maroni, M. (2002). Biological monitoring of pesticide exposure: a review of analytical methods. *J. Chromatogr. B*, 769, 2, (April 2002) 191–219, ISSN: 1570-0232

Budnikov, H. V. & Evtugyn, G. A. (1996). Electrochemical biosensors for inhibitor determination: selectivity and sensitivity control. *Electroanalysis*, 8, 8-9, (August-September, 1996) 817-820, ISSN: 1040-0397, ESSN: 1521-4109

Campás, M; Prieto-Simón, B. & Marty J.-L. (2009). A review of the use of genetically engineered enzymes in electrochemical biosensors. *Seminars in Cell & Developmental Biology*, 20, 1, (February 2009) 3-9, ISSN: 1084-9521

CDC, Third national report on human exposure to environmental chemicals (2005). Centers for Disease Control and Prevention (CDC), Atlanta

Chough, S. H.; Mulchandani, A.; Mulchandani, P.; Chen, W.; Wang, J. & Rogers, K. R. (2002). Organophosphorus hydrolase-based amperometric sensor: modulation of
sensitivity and substrate selectivity. *Electroanalysis*, 14, 4, (February, 2002) 273-276, ISSN: 1040-0397, EISSN: 1521-4109

Corbett, J. R.; Wright, K. & Baille, A. C. (1984). *The biochemical mode of action of pesticides*, 2nd ed., Academic press, ISBN 0-12-187860-0, ISBN-13: 978-0-12-187860-3, London

Danzer, T. & Schwedt, G. (1996). Chemometric methods for the development of a biosensor system and the evaluation of inhibition studies with solutions and mixtures of pesticides and heavy metals. Part I. Development of an enzyme electrode system for pesticides and heavy metal screening using selected chemometric methods. *Anal. Chim. Acta*, 318, 3, (January, 1996) 275-296, ISSN: 0003-2670

D’Souza, S., F. (1989). Immobilized cells: techniques and applications. *Indian J. Microbiol.*, 29, 2, (June, 1989) 83-117, ISSN: 0046-8991, EISSN: 0973-7715

Dumas, D. P.; Durst, H. D.; Landis, W. G.; Rausche, F. M. & Wild, J. R. (1990). Inactivation of organophosphorus nerve agents by the phosphotriesterase from *Pseudomonas diminuta*. *Arch. Biochem. Biophys.*, 227, 1, (February, 1990) 155-159, ISSN: 0003-9861

Edmundson, R. S. (1988). *Dictionary of organophosphorus compounds*, Chapman & Hall, ISBN 10: 0-412-25790-4, ISBN-13: 978-0-412-25790-2, London

Eto, M. (1974). *Organophosphorus pesticides: organic and biological chemistry*, CRS Press, ISBN-10: 0-87819-023-6, ISBN-13: 978-0-87819-023-2, Cleveland

Evtugyn, G.; Budnikov, H.; Galymetdinov, Yu. & Suntsov E. (1996). Amperometric determination of thiocholine esters in the presence of butyrylcholinesterase. *Zh. Anal. Khim.,* 51, 4, 391-393, ISSN: 0044-4502

Evtugyn, G.; Ivanov, A.; Gogol, E.; Marty, J.-L. & Budnikov, H. (1999). Amperometric flow-through biosensor for the determination of cholinesterase inhibitors. *Anal. Chim. Acta*, 385, 1-3, (April, 1999) 13-21, ISSN: 0003-2670

Fukuto, R. (1990). Mechanism of action of organophosphorus and carbamate insecticides. *Environmental Health Perspectives*, 87, (July 1990) 245-254, ISSN: 00916765, EISSN 15529924

Ghindilis, A.; Morzunova, H.; Barmin, A. & Kurochkin, I. (1996). Potentiometric biosensors for cholinesterase inhibitor analysis based on mediatorless bioelectrocatalysis. *Biosens. Bioelectr.*, 11, 9, 873-880, ISSN: 0956-5663

Gunaratna, C. & Wilson, G. (1990). Optimization of multienzyme flow reactors for determination of acetylcholine, *Anal. Chem.*, 62, 4, (February, 1990) 402-407, ISSN: 0003-2700, EISSN: 1520-6882

Gupta, R. C. (Ed.) (2005). *Toxicology of organophosphate & carbamate compounds*, 1st ed., Elsevier Academic Press ISBN-10: 0-12-088523-9, ISBN-13: 978-0-12-088523-7, London

Hayes, W. J. (1991). *Handbook of pesticide toxicology*, Academic Press, ISBN-10: 0-12-334160-4, ISBN-13: 978-0-12-334160-4, San Diego

Harlbert, M. & Baldwin, R. (1985). Electroanalytic and analytical response of cobalt phthalocyanine containing carbon paste electrodes toward sulphhydryl compounds. *Anal. Chem.*, 57, 3, (March, 1985) 591-595, ISSN: 0003-2700, EISSN: 1520-6882

Hart, J. & Hartley, I. (1994). Voltammetric and amperometric studies of thiocholine at a screen-printed carbon electrode chemically modified with cobalt phthalocyanine: studies towards a pesticide sensor. *Analyst*, 119, 2, 259-265, ISSN: 0003-2654

Hassall, K. A. (1982). *The chemistry of pesticides. Their metabolism, mode of action and uses in crop protection*, Verlag Chemie, ISBN-10: 3527259694, ISBN-13: 9783527259694, Weinheim, Deerfield Beach, Florida, Basel, 1982
Jaffrezic-Renault, N. (2001). New trends in biosensors for organophosphorus pesticides. *Sensors*, 1, 2, (July, 2001) 60-64, ISSN: 1424-8220

Jeannot, R. & Dagnac, T. (2006). In: *Chromatographic analysis of the environmental*. 3rd edition, Nollet L. (Ed.), 841-889, CRC Press, Boca Raton, London, New York

Kulys, J. (1989). Amperometric enzyme electrodes in analytical chemistry, *Frez. J. Anal. Chem.*, 335, 1, (January 1989) 86-91, ISSN: 0937-0633; EISSN: 1432-1130

Kulys, J. & D’Costa, E. J. (1991). Printed amperometric sensor based on TCNQ and cholinesterase. *Biosens. Bioelectron.*, 6, 2, 109-115, ISSN: 0956-5663

 Larson, S. J.; Capel, P. D. & Majewski, M. S. (1997). *Pesticides in surface waters: distribution, trends, and governing factors*, CRC Press, ISBN-10: 1-57504-006-9, ISBN-13: 978-1-57504-006-6

Lee, H. S.; Kim, Y. A.; Chao, Y. A. & Lee, Y. T. (2002). Oxidation of organophosphorus pesticides for the sensitive detection by a cholinesterase-based biosensor. *Chemosphere*, 46, 4, (January, 2002) 571-576, ISSN: 0045-6535

Lei, Y.; Mulchandani, P.; Chen, W.; Wang J. & Mulchandani, A. (2004). Whole cell-enzyme hybrid amperometric biosensor for direct determination of organophosphorous nerve agents with p-nitrophenyl substituent. *Biotechnol. Bioeng.*, 85, 7, (March, 2004) 706-713, ISSN: 0006-3592, EISSN: 1097-0290.

Lei, Y.; Mulchandani, P.; Chen, W. & Mulchandani, A. (2005). Direct determination of p-nitrophenyl substituent organophosphorus nerve agents using a recombinant *Pseudomonas putida* JS444-modified Clark oxygen electrode. *J. Agric. Food Chem.*, 53, 3, (February, 2005) 524-527, ISSN: 0021-8561, EISSN: 1520-5118

Lei, Y.; Mulchandani, P.;  Chen, W. & Mulchandani, A. (2006). Biosensor for direct determination of fenitrothion and EPN using recombinant *Pseudomonas putida* JS444 with surface expressed organophosphorus hydrolase. 1. Modified Clark oxygen electrode. *Sensors* 6, 4, (April, 2006) 466-472, ISSN: 1424-8220

Lei, C., Valenta, M., Sapiralli, K. P. & Ackerman, E. J. (2007). Biosensing paraoxon in simulated environmental samples by immobilized organophosphorus hydrolase in functionalized mesoporous silica. *J. Environ. Qual.*, 36, 1, (January-February, 2007) 233-238, ISSN: 0047-2425, EISSN: 1537-2537

Lin, Y. H., Lu, F. & Wang, J. (2004). Disposable carbon nanotube modified screen-printed biosensor for amperometric detection of organophosphorous pesticides and nerve agents. *Electroanalysis*, 16, 1-2, (January, 2004) 145-149, ISSN: 1040-0397, EISSN: 1521-4109

Liu, G.; Riechers, S.; Mellen, M. & Lin, Y. (2005). Sensitive electrochemical detection of enzymatically generated thiocholine at carbon nanotube modified glassy carbon electrode. *Electrochem. Commun.*, 7, 11, (November, 2005) 1163-1169, ISSN: 1388-2481

Luque de Castro M. D. & Herrera, M. C. (2003). Enzyme inhibition-based biosensors and biosensing systems: questionable analytical devices. *Biosens. Bioelectron.*, 18, 2-3, (March, 2003) 279-294, ISSN: 0956-5663

Majewski M. S. & Capel, P. D. (1995). *Pesticides in the atmosphere: distribution, trends, and governing factors*, CRC Press, ISBN-10: 1-57504-004-2, ISBN-13: 978-1-57504-004-2

Martorell, D.; Céspedes, F.; Martínez-Fábregas, E. & Alegret, S. (1994). Amperometric determination of pesticides using a biosensor based on a polishesable graphie-epoxy biocomposite. *Anal. Chim. Acta*, 290, 3, (May, 1994) 343-348, ISSN: 0003-2670
Martorell, D.; Céspedes, F.; Martínez-Fàbregas, E. & Alegret, S. (1997). Determination of organophosphorus and carbamate pesticides using a biosensor based on a polishable, 7,7,8,8-tetracyanoquino – di methane - modified, graphite - epoxy biocomposite. *Anal. Chim. Acta*, 337, 3, (January, 1997) 305-313, ISSN: 0003-2670

Marty, J.-L.; Mionetto, N. & Rouillon, R. (1992). Entrained enzymes in photocrosslinkable gel for enzyme electrodes. *Anal. Lett.*, 25, 8, 1389-1398, ISSN: 0003-2719, EISSN: 1532-236X

Marty, J.-L.; Mionetto, N.; Noguer, T.; Ortega, F. & Roux, C. (1993). Enzyme sensors for the detection of pesticides. *Biosens. Bioelectron.*, 8, 6, 273-280, ISSN: 0956-5663

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. *Anal. Chim. Acta*, 311, 3, (August, 1995) 265-271, ISSN: 0003-2670

Matsumura, F. (1980). *Toxicology of insecticides*, Plenum Press, ISBN-10: 0-306-30787-1, ISBN-13: 978-0-306-30787-4, New York

Mazzei, F.; Botrè, F. & Botrè, C. (1996). Acid phosphatase/glucose oxidase-based biosensors for the determination of pesticides. *Anal. Chim. Acta*, 336, 1-3, (December, 1996) 67-75, ISSN: 0003-2670

Mulchandani, A.; Mulchandani, P.; Kaneva, I. & Chen, W. (1998). Biosensor for direct determination of organophosphate nerve agents using recombinant *Escherichia coli* with surface-expressed organophosphorus hydrolase. 1. Potentiometric microbial electrode. *Anal. Chem.*, 70, 19, (October, 1998) 4140-4145, ISSN: 0003-2700, EISSN: 1520-6882

Mulchandani, A.; Mulchandani, P.; Chen, W.; Wang, J. & Chen, L. (1999). Amperometric thick-film strip electrodes for monitoring organophosphate nerve agents based on immobilized organophosphorus hydrolase. Anal. Chem., 71, 11, (June, 1999) 2246-2249, ISSN: 0003-2700, EISSN: 1520-6882

Mulchandani, A.; Chen, W.; Mulchandani, P.; Wang, J. & Rogers, K. R. (2001a). Biosensors for direct determination of organophosphate pesticides. *Biosens. Bioelectron.*, 16, 4-5, (June, 2001) 225-230, ISSN: 0956-5663

Mulchandani, P.; Chen, W. & Mulchandani, A. (2001b). Flow injection amperometric enzyme biosensor for direct determination of organophosphate nerve agents. *Environ. Sci. Technol.*, 35, 12, (June, 2001) 2562-2565, ISSN: 0013-936X, EISSN: 1520-5851

Mulchandani, P.; Chen, W.; Mulchandani, A.; Wang, J. & Chen, L. (2001c). Amperometric microbial biosensor for direct determination of organophosphate pesticides using recombinant microorganism with surface expressed organophosphorus hydrolase. *Biosens. Bioelectron.*, 16, 7-8, (September, 2001) 433-437, ISSN: 0956-5663

Mulchandani, P.; Chen, W. & Mulchandani, A. (2006). Microbial biosensor for direct determination of nitrophenyl-substituted organophosphate nerve agents using genetically engineered Moraxella sp. *Anal. Chim. Acta*, 568, 1-2, (May, 2006) 217-221, ISSN: 0003-2670

Munnecke, D. M. (1980). Enzymatic detoxification of waste organophosphate pesticides. *J. Agric. Food Chem.*, 28, 1, (January, 1980) 105-111, ISSN: 0021-8561, EISSN: 1520-5118

Nikol’skaya, E. B. & Evtyugin, G. A. (1992). Cholinesterases application in analytical chemistry. *Zh. Anal. Khim.*, 47, 8, 1358-1378, ISSN: 1061-9348, EISSN: 1608-3199

www.intechopen.com
Enzyme vs. Bacterial Electrochemical Sensors for Organophosphorus Pesticides Quantification

Neufeld, T; Eshkenazi, I; Cohen, E. & Rishpon, J. (2000). A micro flow injection electrochemical biosensor for organophosphorus pesticides. *Biosens. Bioelectr.*, 15, 5-6, (August, 2000) 323-329, ISSN: 0956-5663

Noguer, T.; Leba, B.; Jeanty, G. & Marty, J.-L. (1999). Biosensors based on enzyme inhibition: Detection of organophosphorus and carbamate insecticides and dithiocarbamate fungicides. *Field Anal. Chem. Technol.*, 3, 3, 171-178, ISSN: 1086-900X, EI ISSN: 1520-6521

Ovalle, M.; Stoytcheva, M.; Zlatev, R. & Valdez, B. (2009). Electrochemical study of rat brain acetylcholinesterase inhibition by chlorofos: kinetic aspects and analytical applications. *Electrochimica acta*, DOI 10.1016/j.electacta.2009.09.008, (in press), ISSN: 0013-4686

Periasamy, A. P.; Umasankar Y. & Chen S.-M. (2009). Nanomaterials-acetylcholinesterase enzyme matrices for organophosphorus pesticides electrochemical sensors: a review. *Sensors*, 2009, 9, (September, 2009) 4034-4055; ISSN 1424-8220

Prieto-Simón, B.; Campàs, M.; Andreeescu, S. & Marty, J.-L. (2006). Trends in flow-based biosensing systems for pesticide assessment. *Sensors*, 6, 10, (October, 2006) 1161-1186, ISSN: 1424-8220

Quin, L. D. (2000). *A guide to organophosphorus chemistry*, Wiley-Interscience, ISBN-10: 0-471-31824-8, ISBN-13: 978-0-471-31824-8

Ricci, F.; Arduini, F.; Amine, A.; Moscone, D. & Palleschi, G. (2004). Characterisation of Prussian blue modified screen-printed electrodes for thiol detection. *J. Electroanal. Chem.*, 563, 2, (March, 2004) 229-237, ISSN: 0022-0728

Richins, R.; Kaneva, I.; Mulchandani, A. & Chen, W. (1997). Biodegradation of organophosphorus pesticides by surface-expressed organophosphorus hydrolase. *Nature Biotechnol.*, 15, 10, (October, 1997) 984-987, ISSN: 1087-0156, EI ISSN: 1546-1696

Rodriguez-Mozaz, S.; Marco, M.-P.; Lopez de Alda M. J. & Barceló, D. (2004). Biosensors for environmental applications: future development trends. *Pure Appl. Chem.*, 76, 4, 723-752, ISSN: 0033-4545, EI ISSN: 1365-3075

Sacks, V.; Eshkenazi, I.; Neufeld, T.; Dosoretz, C. & Rishpon, J. (2000). Immobilized parathion hydrolase: An amperometric sensor for parathion. *Anal. Chem.*, 72, 9, (May, 2000) 2055-2058, ISSN: 0003-2700, EI ISSN: 1520-6882

Schlecht, P. C. & O’Connor, P. F., (Eds.) (1994). *NIOSH manual of analytical methods*, 4th ed., DHHS (NIOSH) Publication 94-113

Skladal, P. (1991). Determination of organophosphate and carbamate pesticides using a cobalt phthalocyanine-modified carbon paste electrode and a cholinesterase enzyme membrane. *Anal. Chim. Acta*, 252, 1-2, (November, 1991) 11, ISSN: 0003-2670

Skladal, P.; Fiala, M. & Krejci, J. (1996). Detection of pesticides in the environment using biosensors based on cholinesterases. *Intern. J. Environ. Anal. Chem.*, 65, 1-4, 139-148, ISSN: 0306-7319

Solé, S.; Merkoçi, A. & Alegret, S. (2003a). Determination of toxic substances based on enzyme inhibition. Part I. Electrochemical biosensors for the determination of pesticides using batch procedures. *Crit. Rev. Anal. Chem.*, 33, 2, 89-126, ISSN: 1040-8347, EI ISSN: 1547-6510

www.intechopen.com
Solé, S.; Merkoçi, A. & Alegret, S. (2003b). Determination of toxic substances based on enzyme inhibition. Part I. Electrochemical biosensors for the determination of pesticides using flow procedures. *Crit. Rev. Anal. Chem.*, 33, 2, 127-143, ISSN: 1040-8347, EISSN: 1547-6510

Stoytcheva, M.; Zlatev, R.; Velkova, Z.; Valdez, B.; Ovalle, M. & Petkov, L. (2009). Hybrid electrochemical biosensor for organophosphorus pesticides quantification. Electrochimica Acta, 54, 6, (February, 2009) 1721-1727, ISSN: 0013-4686

Sužnjević, D. Ž.; Veselinović, D. S.; Vukelić, N. S.; Pavlović, D. Ž. & Nikolić, A. V. (1985). Investigation of the system butyrylthiocholineiodide-butyrocholinesterase by cyclovoltammetry and chronopotentiometry using inert working electrodes. *J. Serb. Chem. Soc.*, 50, 2, 83-88, ISSN: 0352-5139

Thévenot, D. R.; Tóth, K.; Durst, R. A. & Wilson, G. S. (1999). Electrochemical biosensors: recommended definitions and classification. *Pure Appl. Chem.*, 71, 12, 2333-2348, ISSN: 0033-4545, EISSN: 1365-3075

Tran-Minh, C. (1985). Immobilized enzyme probes for determining inhibitors. *Ion-Selective Electrode Rev.*, 7, 41-75, ISBN-10: 0-08-033201-3, ISBN-13: 978-0-08-033201-7

Tran-Minh, C.; Pandey, P. C. & Kumaran, S. (1990). Studies on acetylcholine sensor and its analytical application based on the inhibition of cholinesterase. *Biosens. Bioelectron.*, 5, 6, 461-471, ISSN: 0956-5663

Turdean, G.; Popescu, I. C. & Oniciu, L. (2002). Biocapteurs ampérométriques a cholinestérases pour la détermination des pesticides organophosphorés. *Can. J. Chem.*, 80, (March, 2002) 315-331, ISSN: 1480-3291

Van Emon, J. M., (Ed.) (2006). *Immunoassay and other bioanalytical techniques*, CRC Press, ISBN-10: 0-8493-3942-1, ISBN-13: 978-0-8493-3942-4, Boca Raton, London, New York

Vighi, M. & Funari, E. (1995). *Pesticide risk in groundwater*, CRC Press, ISBN-10: 0-87371-439-3, ISBN-13: 978-0-87371-439-6

Wang, J.; Krause, R.; Block, K.; Musameh, M.; Mulchandani, A. & Schöning, M. J. (2003). Flow injection amperometric detection of OP nerve agents based on an organophosphorus–hydrolase biosensor detector. *Biosens. Bioelectron.*, 18, 2-3, (March, 2003) 255-260, ISSN: 0956-5663

WHO/IPCS. (1986). *Organophosphorus insecticides: a general introduction* (Environmental health criteria Series No 63), ISBN-10: 92-4-154263-2, ISBN-13: 978-92-4-154263-0, Geneva

Worthing, C. R. & Hance, R. J. (Eds). (1991). *The pesticide manual: A world compendium*, 9th ed., British Crop Protection, ISBN-10: 0948404426, ISBN-13: 9780948404429, Surrey UK
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