Enzyme vs. Bacterial Electrochemical Sensors for Organophosphorus Pesticides Quantification

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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.
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):

$$\begin{align*}
    & \text{O(S)} \\
    & \text{RO-P-X(OX or SX)} \\
    & \text{OR}
\end{align*}$$

are highly toxic and are used as chemical warfare agents and pesticides (insecticides, herbicides, fungicides, rodenticides, moluscocides, 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.


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 phosphorylacetylestherase 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).

A) E-Serine-OH + acetylcholine $\rightarrow$ E-Serine-OAc + choline $\rightarrow$ E-Serine-OH + Ac

Enzyme acetylation                      Enzyme recovery

B) E-Serine-OH + (RO)$_2$POX $\rightarrow$ E-Serine-O-P-(OR)$_2$ $\rightarrow$ E-Serine-OH + (RO)$_2$POX

Enzyme phosphorylation                  Slow enzyme recovery

C) E-Serine-OH + (RO)$_2$POX $\rightarrow$ E-Serine-O-P-(OR)$_2$ $\rightarrow$ E-Serine-O-P-OH

Enzyme phosphorylation                  Aging                                     Irreversibly inhibited enzyme

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:

$$R'\text{-choline} + H_2O \xrightarrow{\text{ChE}} \text{choline} + R'\text{-COOH}$$  \hspace{1cm} (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:

$$R'\text{-choline} + H_2O \xrightarrow{\text{ChE}} \text{choline} + R'\text{-COOH}$$  \hspace{1cm} (2)

$$\text{choline} + 2O_2 + H_2O \xrightarrow{\text{ChO}} \text{betaine} + 2H_2O_2$$  \hspace{1cm} (3)

$$H_2O_2 + 2H^+ + 2e^- \xrightarrow{\text{HRP}} 2H_2O$$  \hspace{1cm} (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 $2\times10^{-13}$ 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:

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

\[
\text{thiocholine} \rightarrow \text{dithio-bis-choline} + 2\text{H}^+ + 2\text{e}^- \quad (6)
\]

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 & Evtyugin, 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 phtalocyanines (Harlbert & Baldwin, 1985; Hart & Hartley, 1994; Skladal, 1991), Prussian blue (Ricci et al., 2004), tetracyanoquinodimethane (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):

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

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

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 lost 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:

\[
\text{RO-P-X} + \text{H}_2\text{O} \xrightarrow{\text{OPH}} \text{RO-P-OH} + \text{HX}
\]

(10)

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 (Andreeescu & 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

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

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

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Target</th>
<th>LOD</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPH</td>
<td>paraoxon</td>
<td>90 nM</td>
<td>Mulchandani et al., 1999</td>
</tr>
<tr>
<td>OPH</td>
<td>methyl parathion</td>
<td>70 nM</td>
<td>Mulchandani et al., 1999</td>
</tr>
<tr>
<td>OPH</td>
<td>paraoxon</td>
<td>2 μM</td>
<td>Mulchandani et al., 2001a</td>
</tr>
<tr>
<td>OPH</td>
<td>methyl parathion</td>
<td>2 μM</td>
<td>Mulchandani et al., 2001a</td>
</tr>
<tr>
<td>OPH</td>
<td>diazinon</td>
<td>2 μM</td>
<td>Mulchandani et al., 2001a</td>
</tr>
<tr>
<td>OPH</td>
<td>parathion</td>
<td>15 nM</td>
<td>Chough et al., 2002</td>
</tr>
<tr>
<td>OPH</td>
<td>paraoxon</td>
<td>20 nM</td>
<td>Chough et al., 2002</td>
</tr>
<tr>
<td>OPH</td>
<td>paraoxon</td>
<td>0.4 μM</td>
<td>Lei et al., 2007</td>
</tr>
</tbody>
</table>

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 difusional 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(mol dm⁻³) under optimal experimental conditions.

The biosensor response time is 200 s and the storage stability is tL50 = 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.
### Table 3. LOD of some bacterial electrochemical sensors for OPs determination

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Target</th>
<th>LOD</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recombinant <em>P. coli</em></td>
<td>paraoxon</td>
<td>2 μM</td>
<td>Mulchandani et al., 1998</td>
</tr>
<tr>
<td>Recombinant <em>P. coli</em></td>
<td>methyl parathion</td>
<td>2 μM</td>
<td>Mulchandani et al., 1998</td>
</tr>
<tr>
<td>Recombinant <em>P. coli</em></td>
<td>diazinon</td>
<td>5 μM</td>
<td>Mulchandani et al., 1998</td>
</tr>
<tr>
<td>Recombinant <em>Moraxella</em></td>
<td>methyl parathion</td>
<td>1 μM</td>
<td>Mulchandani et al., 2001c</td>
</tr>
<tr>
<td>Recombinant <em>Moraxella</em></td>
<td>paraoxon</td>
<td>0.2 μM</td>
<td>Mulchandani et al., 2001c</td>
</tr>
<tr>
<td>Recombinant <em>P. putida</em></td>
<td>paraoxon</td>
<td>55 ppb</td>
<td>Lei et al., 2005</td>
</tr>
<tr>
<td>Recombinant <em>P. putida</em></td>
<td>methyl paraoxon</td>
<td>53 ppb</td>
<td>Lei et al., 2005</td>
</tr>
<tr>
<td>Recombinant <em>P. putida</em></td>
<td>parathion</td>
<td>58 ppb</td>
<td>Lei et al., 2005</td>
</tr>
<tr>
<td>Recombinant <em>P. putida</em></td>
<td>fenitrothion</td>
<td>277 ppb</td>
<td>Lei et al., 2006</td>
</tr>
<tr>
<td>Recombinant <em>P. putida</em></td>
<td>EPN</td>
<td>1.6 ppm</td>
<td>Lei et al., 2006</td>
</tr>
<tr>
<td>Recombinant <em>Moraxella</em></td>
<td>paraoxon</td>
<td>0.1 μM</td>
<td>Mulchandani et al, 2006</td>
</tr>
<tr>
<td><em>Arthrobacter globiformis</em></td>
<td>chlorofos</td>
<td>1 nM</td>
<td>Stoytcheva et al., 2009</td>
</tr>
</tbody>
</table>

### 4. Conclusion

Despite of 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 (Campàs et al., 2009).

### 5. References


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


The use of intelligent sensors have revolutionized the way in which we gather data from the world around us, how we extract useful information from that data, and the manner in which we use the newly obtained information for various operations and decision making. This book is an attempt to highlight the current research in the field of Intelligent and Biosensors, thereby describing state-of-the-art techniques in the field and emerging new technologies, also showcasing some examples and applications.

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