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► **To cite this version:**

Mohamed Amine Berkal, Corinne Nardin. Pesticide biosensors: trends and progresses. *Analytical and Bioanalytical Chemistry*, 2023, 415, pp.5899-5924. 10.1007/s00216-023-04911-4 . hal-04209603

**HAL Id: hal-04209603**

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Submitted on 3 Nov 2023

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# Pesticide biosensors: trends and progresses

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**Keywords:** Pesticides, biosensors, enzyme inhibition-based biosensor, immunosensors, aptasensors.

## Abstract

Pesticides, chemical substances extensively employed in agriculture to optimize crop yields, pose potential risks to human and environmental health. Consequently, regulatory frameworks are in place to restrict pesticide residue concentrations in water intended for human consumption. These regulations are implemented to safeguard consumer safety and mitigate any adverse effects on the environment and public health. Although Gas Chromatography- and Liquid Chromatography- Mass Spectrometry (GC-MS and LC-MS) are highly efficient techniques for pesticides quantification, their use is not suitable for real-time monitoring due to the need for sophisticated laboratory pretreatment of samples prior to analysis. Since they would enable analyte detection with selectivity and sensitivity without sample pretreatment, biosensors appear as a promising alternative. These consist of a bioreceptor allowing for specific recognition of the target and of a detection platform, which translates the biological interaction into a measurable signal. As early detection systems remain urgently needed to

25 promptly alert and act in case of pollution, we review here the biosensors described in the  
26 literature for pesticide detection to advance their development for use on field.

## 27 Introduction

28 Pesticides are essential for agriculture to increase crop yields and to provide suitable food  
29 production levels. Since their launch, they enabled substantial economic benefits and are thus  
30 widely utilized [1]. However, their widespread use concomitantly affects our health [2–5] and  
31 our environment [6, 7]. The International Agency for Research on Cancer (IARC) plays a  
32 crucial role in assessing the impact of pesticides on human health. As a renowned authority in  
33 identifying carcinogenic agents, it provides valuable information to decision-making bodies  
34 responsible for alerting and safeguarding the public from cancer-causing agents found in food,  
35 the environment, and workplaces. Notably, the IARC has classified s-metolachlor, a pesticide  
36 of particular concern in France, as probably carcinogenic. In response, the French Agency for  
37 Food, Environmental and Occupational Health & Safety (ANSES) has taken steps to phase out  
38 its primary use in phytopharmaceuticals [8]. Additionally, the IARC has classified other  
39 pesticides, such as captafol (a fungicide used in seed treatments), chlordane, dichlorvos, and  
40 heptachlorophenol, as probably carcinogenic [9]. These assessments highlight the importance  
41 of rigorous monitoring and control measures to mitigate the potential health risks associated  
42 with these substances.

43 The use of pesticides also leads to the contamination of surface waters [6, 7, 10]. Over the last  
44 20-25 years [11], public concerns about the presence of pesticide residues in food and drinking  
45 water raised, leading to the implementation of strict regulations by legislative authorities in  
46 order to control the quality of consumer products and especially of drinking water. According  
47 to the 98/83/EC EU Directive, specific regulations have been implemented regarding the quality  
48 limits of pesticides active substances and their relevant metabolites in water. The general quality  
49 limit is set at  $0.1 \mu\text{g L}^{-1}$  per individual substance for most pesticides. However, certain highly

50 toxic substances such as aldrin, dieldrin, heptachlor, and heptachlor epoxide are currently  
51 banned, and their quality limit is set at a stricter level of  $0.03 \mu\text{g L}^{-1}$ . Additionally, to address  
52 the potential simultaneous presence of multiple pesticides and relevant metabolites, a  
53 cumulative approach is adopted. The sum of concentrations of all pesticides and relevant  
54 metabolites in water should not exceed  $0.5 \mu\text{g L}^{-1}$ , considering the combined effect of these  
55 substances. In parallel, the EU Directive No 396/2005 ensures the quality of food products by  
56 setting Maximum Residue Limits (MRLs) for pesticides that may be present. These MRLs are  
57 continuously updated to accommodate the introduction of new pesticides on the market,  
58 reflecting the dynamic nature of agricultural practices.

59 These regulations are designed to ensure the safety and quality of water resources, considering  
60 both individual substances and their potential cumulative impact on water quality.

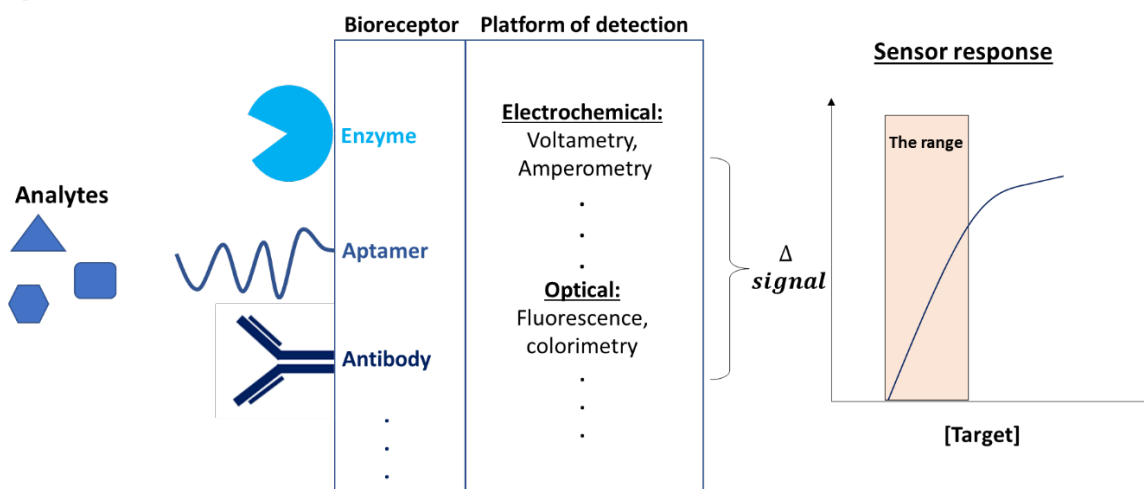
61 To detect pesticides at the concentration limits set by the competent authorities, sophisticated  
62 techniques are required. Gas chromatography and liquid chromatography coupled with mass  
63 spectrometry (GC-MS and LC-MS) are classical techniques used for the sensitive, selective and  
64 reproducible analysis of pesticide residues in water [12–15]. Although these techniques are well  
65 suited for the quantification of pesticides, the samples studied must be pre-treated and then  
66 analyzed in sophisticated laboratories, which renders these techniques unsuitable for survey  
67 analyses of running water that require monitoring at a suitable frequency to detect an eventual  
68 pollution peak in real time and to promptly take an action. In order to meet these requirements,  
69 increasing efforts are devoted to explore alternative measures to detect pesticides with  
70 selectivity and sensitivity. Biosensors appear as elegant tools to replace conventional methods  
71 as they could enable the detection of analytes in real time, minimizing tedious sample  
72 pretreatments. **Table 1** shows a detailed comparison of critical properties between classical  
73 techniques and biosensors employed for the detection of pesticides.

75 **Table 1** Comparison of characteristic parameters between traditional analytical techniques and  
 76 biosensors employed for the detection of pesticides.

Parameters	Traditional Analytical Techniques	Biosensors
Analysis time	Collection of samples, storage, and transport	Limited sample preparation, rapid monitoring
Cost	Sophisticated laboratory detection, high-tech equipment, trained laboratory personnel	Cost-effective, portable and simple in-situ detection
Eco-friendly and reusability	More organic solvent consumption, Not reusable	No organic solvent used, reusable
Sensitivity and selectivity	Highly sensitive and selective	Sensitive and specific
Commercialization	/	Limited commercial applications

77 A biosensor is the combination of a bioreceptor and of a detection platform (**Figure 1**). The  
 78 bioreceptor is a biological molecule that allows the specific recognition of the target, whereas  
 79 detection platform translates the biologic interaction into a measurable, optical, magnetic or  
 80 electrochemical signal [16–18].

Fig. 1



81  
 82 Schematic illustration of the different types of bioreceptors and transducers used in a biosensor.

83 Due to their high sensitivity, electrochemical platforms are extensively used in pesticide  
 84 biosensors [19–21]. In parallel, and due to their ease of use, simple detection techniques, and  
 85 satisfactory sensitivity, optical biosensors are also largely used in biosensors developed for the  
 86 detection of pesticides [20, 22].

87 Different types of biomolecules have been employed in biosensors, mainly antibodies, enzymes  
88 and aptamers [23]. These biomolecules, due to their nature, undergo specific recognition with  
89 their target by different types of interaction, notably electrostatic interactions, hydrogen  
90 bonding, aromatic ring stacking and van der Waals interactions. The bioreceptor ensures the  
91 specificity of the analysis, whereas the sensitivity is ensured by the detection platform.  
92 Biosensors, as point-of-care devices, offer advantages such as rapid detection, user-friendliness,  
93 accuracy, portability, cost-effectiveness and easy on-site detection [24, 25].  
94 This review covers the bioreceptors the most used and investigated for biosensors development  
95 for on-site monitoring of pesticides, namely antibodies, enzymes and aptamers. Aptamers,  
96 which can be considered as chemical antibodies, are synthetic biological molecules that have  
97 been widely investigated in the last decade to develop biosensors for detecting pesticides. In  
98 this context, an in-depth description of the use of aptasensors as tools for pesticide detection is  
99 also reported here.

## 100 I. Enzyme-based biosensors

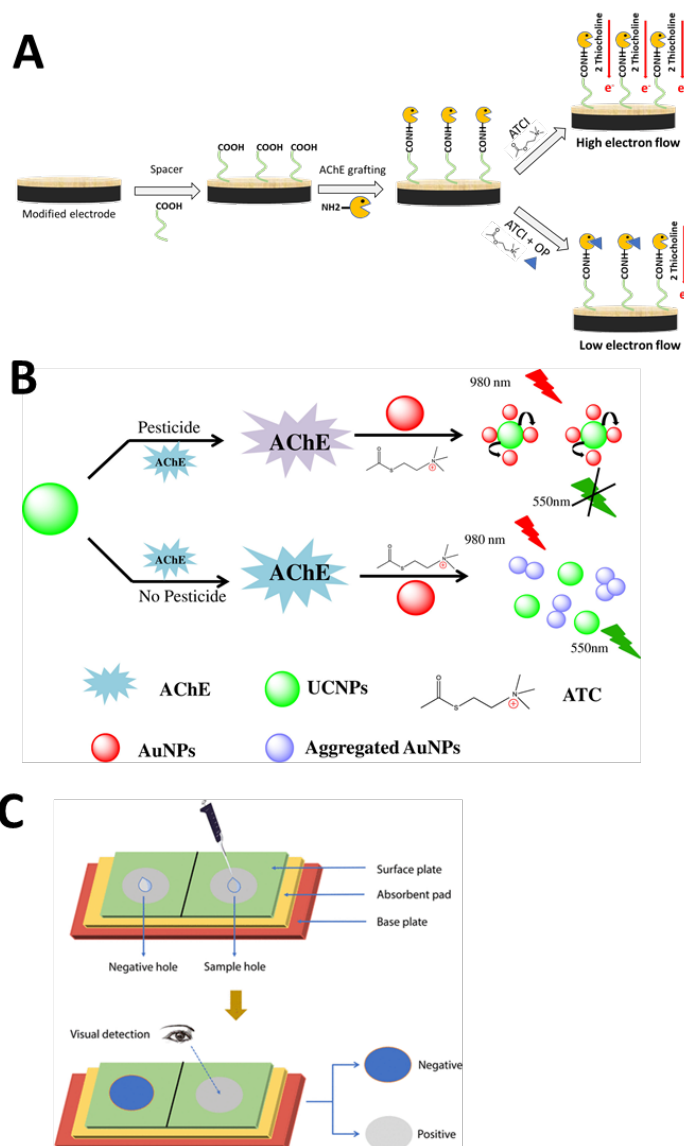
101 Owing to their high selectivity for their substrates to which they bind and drive catalytic  
102 reactions, enzymes are of high potential for biosensors development. They are commonly used  
103 for the detection of organophosphate (OP) and carbamate pesticides that inhibit the activity of  
104 acetylcholinesterase (AChE), an essential enzyme for the functioning of the central nervous  
105 system [26]. The inhibition of AChE by OP and carbamate pesticides takes place via a  
106 phosphorylation mechanism, which blocks serine in the active site through a nucleophilic attack  
107 and production of a serine phosphoester [27]. On the basis of this reaction scheme, the  
108 electrochemical and optical recording of the enzymatic activity reduction enables the detection  
109 of this particular type of pesticide in the matrices to be analyzed. Butyrylcholinesterase (BChE)  
110 [28], tyrosinase [29, 30], alkaline phosphatase [31, 32], peroxidase [33], acid phosphatase [34]  
111 urease [35] and exonuclease I [36] were also used in inhibition-based pesticide biosensors. As

112 described below, several enzyme-based biosensors were reported for pesticides detection, based  
113 on electrochemical and optical transduction [37–39].

#### 114 I.1. Enzymatic inhibition-based electrochemical biosensors

115 Electrochemical detection methods, including cyclic voltammetry, alternating current  
116 voltammetry (ACV), electrochemical impedance spectroscopy (EIS), square wave voltammetry  
117 (SWV), differential pulse voltammetry, photoelectrochemical, and electrochemiluminescence,  
118 enable the rapid and sensitive detection of redox-active target analytes without requiring  
119 complex sample pre-treatment [40]. These electrochemical techniques generate target-induced  
120 signals specifically triggered by the presence of the analyte, allowing for its accurate detection  
121 and quantification. In particular, enzyme inhibition-based electrochemical biosensors have  
122 emerged as a promising approach for pesticide detection (**Figure 2(A)**), enabling real-time and  
123 on-site monitoring of pesticide residues. These biosensors utilize the inhibition of enzymatic  
124 activity to achieve precise and frequency-appropriate detection of pesticides, making them  
125 valuable tools in pesticide analysis and environmental monitoring.

**Fig. 2**



126

127 Comparative schematic diagram of AChE inhibition-based: (A) electrochemical, (B)  
128 fluorometric, and (C) colorimetric biosensors for detection of organophosphorus compounds.

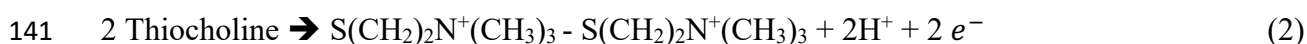
129 (B) Reprinted from [41], with permission from Elsevier. (C) Reprinted from [42], with  
130 permission from American Chemical Society.

### 131 1.1.1. Differential pulse voltammetry

132 Chauhan et al. [37] have selectively detected several organophosphorus pesticides (malathion,  
133 chlorpyrifos, monocrotophos and endosulfan) with a high sensitivity by covalently  
134 immobilizing AChE onto iron oxide nanoparticles ( $\text{Fe}_3\text{O}_4\text{NPs}$ ) and carboxylated multiwalled



135 carbon nanotubes (c-MWCNTs) modified Au electrodes. The acetylcholine (ATCI) is  
136 enzymatically hydrolyzed by AChE into thiocholine that undergoes electrocatalytic oxidative  
137 dimerization at +0.4 V vs. an Ag/AgCl reference electrode and produces a disulphide  
138 compound. The oxidation of thiocholine at the working electrode surface (equations 1 and 2) is  
139 correlated to the activity of AChE.



142 In the presence of pesticides, AChE activity is reduced involving a decrease of thiocholine  
143 production, resulting in a decreased electrical signal which is proportional to pesticides  
144 concentration. Under optimal conditions, the degree of inhibition caused by those pesticides  
145 was found to be directly proportional to their concentrations, within the following ranges: 0.1-  
146 40 nM for malathion, 0.1-50 nM for chlorpyrifos, 1-50 nM for monocrotophos, and 10-100 nM  
147 for endosulfan. The detection limits were determined to be 0.1 nM for malathion and  
148 chlorpyrifos, 1 nM for monocrotophos, and 10 nM for endosulfan.

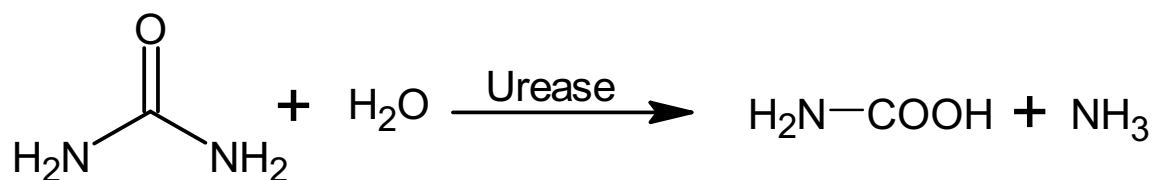
149 Ma et al. [43] have described the fabrication of PtPd@NCS core-shell structured  
150 nanocomposites consisting of a bimetal core (Pt and Pd) encased in an N-doped carbon shell  
151 (NCS). The nanocomposites were prepared using a simple one-pot approach, involving  
152 reduction of metal salt precursors, self-polymerization of dopamine, and co-assembly of  
153 Pluronic F127. The nanocomposites were then used to prepare an AChE-inhibition based  
154 biosensor for detecting the following organophosphate pesticides: malathion, chlorpyrifos and  
155 parathion methyl. The inhibition rate is defined following equation 3 where  $I_0$  represents the  
156 original signal recorded by DPV measurements with the as-prepared  
157 AChE/PtPd@NCS/graphene carbon electrode (GCE) biosensor in phosphate buffer saline  
158 (PBS, pH 7.5) containing ATCI (2.0 mM).  $I_1$  represents the residual signal.

$$159 \text{ Inhibition (\%)} = \frac{I_0 - I_1}{I_0} \times 100\% \quad (3)$$

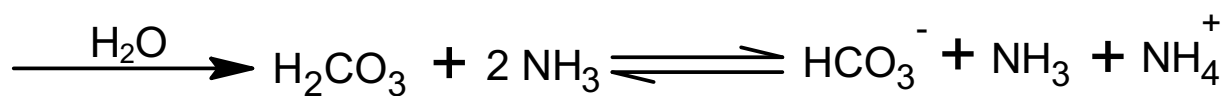
160 The biosensor functions in a linear detection range for each pesticide, at extremely low limits  
 161 of detection in the range of femtomoles to attomoles ( $7.9 \times 10^{-15}$  M,  $7.1 \times 10^{-14}$  M, and  $8.6 \times$   
 162  $10^{-15}$  M for malathion, chlorpyrifos, and parathion-methyl, respectively). These results  
 163 underscore the exceptional sensitivity of the AChE biosensor prepared using PtPd@NCS  
 164 nanocomposites. Recently, nanozyme-based biosensors also show tremendous potential for pesticide  
 165 detection [44]. In their study, Wu et al. [45] presented an innovative electrochemical biosensor for  
 166 detecting organophosphate pesticides using a two-dimensional  $\text{MnO}_2$  nanozyme by DPV measurements.  
 167 The researchers used manganese dioxide nanosheets (MnNS), more specifically two-dimensional (2D)  
 168  $\text{MnO}_2$  sheets, in conjunction with acetylcholinesterase (AChE) to create a homogeneous electrochemical  
 169 biosensor. This novel biosensor demonstrated excellent performance, exhibiting a linear response for  
 170 paraoxon in the 0.1 to 20  $\mu\text{g L}^{-1}$  range, with a LOD of 0.025  $\mu\text{g L}^{-1}$ . Importantly, these results align with  
 171 the maximum residue limit (MRL) requirements established by the European Union (EU).

#### 172 1.1.2. Potentiometry

173 Vaghela et al. [35] have described a potentiometric electrochemical biosensor based on bio-  
 174 nanoconjugate of urease with gold nanoparticles (AuNPs) entrapped in agarose-guar gum to  
 175 detect glyphosate. The action of urease catalyzes the formation of ammonium ions (equation 4)  
 176 that are measured by their selective electrode. In the presence of glyphosate, the urease activity  
 177 is inhibited, leading in a decrease of ammonium ions formation. According to this inhibition  
 178 mechanism, the decrease in potentiometric signal indicates the presence of glyphosate and can  
 179 be used for its detection.



(4)



180

181 According to this mechanism of detection, a linear response with respect to glyphosate  
182 concentration is obtained in the range of 0.5 to 50  $\mu\text{g L}^{-1}$ , which is within the maximum residual  
183 limit established by the World Health Organization (WHO).

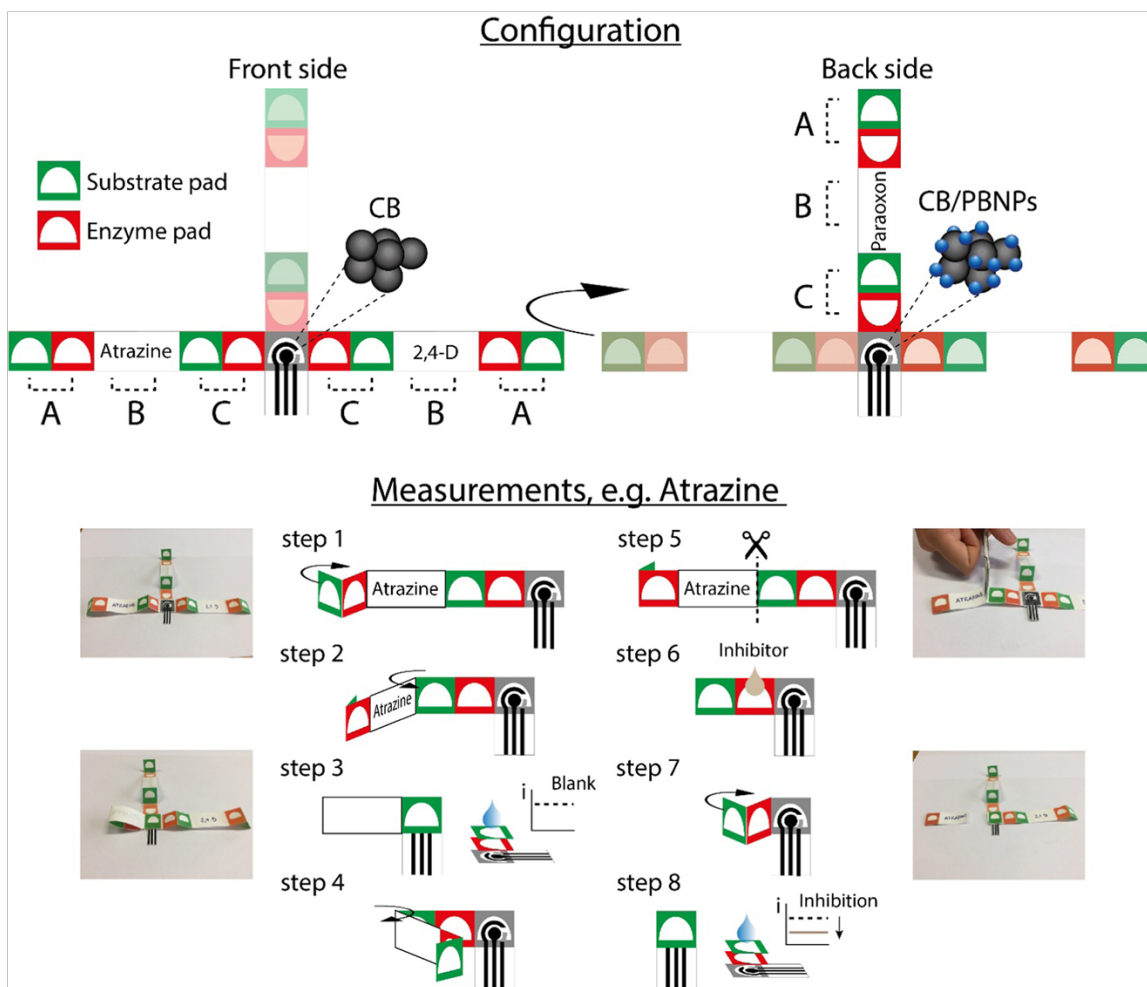
#### 184 1.1.3. Cyclic voltammetry

185 Five pesticides were detected by glutathione-S-transferase (GST) based electrochemical  
186 biosensing: carbendazim, chlorpyrifos, dichlorodiphenyltrichloroethane (DDT), dinocap and  
187 ethion [46]. Cyclic voltammetry was used to detect all five pesticides. GST was immobilized  
188 onto platinum electrodes using a graphene oxide-gelatin matrix. The immobilization of GST  
189 was confirmed through cyclic voltammetry, electrochemical impedance spectroscopy, scanning  
190 electron microscopy and chronoamperometry. The developed GST biosensor has several  
191 advantages over other biosensors: it is cost effective as it is reusable for 8-10 consecutive  
192 measurements, and real-time monitoring was demonstrated when using the  
193 chronoamperometric mode.

#### 194 1.1.4. Chronoamperometry

195 BChE, alkaline phosphatase and tyrosinase were used to develop a novel three-dimensional  
196 origami paper-based device that utilizes enzyme-inhibition to detect several pesticides:  
197 paraxon, 2,4-dichlorophenoxyacetic acid and atrazine [28]. This device combines two office  
198 paper-based screen-printed electrodes with multiple filter paper-based pads for loading  
199 enzymes and their substrates. By folding and unfolding the filter paper-based structure, the  
200 device can analyze pesticides without any need for reagents or sample treatment (**Figure 3**).

Fig. 3



201

202 Schematic illustration of configuration and measurement procedure of the paper-based  
203 platform. Reprinted from [28], with permission from Elsevier.

204 The paper-based platform is advantageous due to its low cost, portability, and ability to analyze  
205 multiple pesticides. However, it is based on enzyme inhibition and may not be suitable for  
206 detecting certain types of pesticides. Zhao et al. [47] reported on an electrochemical sensor  
207 based on AChE inhibition to detect paraoxon. The mentioned biosensor is composed of a screen-  
208 printed carbon electrode (SPCE) modified with electrodeposited gold nanoparticles (AuNPs)  
209 on which MoS<sub>2</sub> nanosheets were immobilized. AChE was immobilized in the last step onto  
210 MoS<sub>2</sub> via a glutaraldehyde crosslinker. Using amperometry under optimized conditions,  
211 paraoxon could be detected at a very low limit of detection (LOD = 0.013 μg L<sup>-1</sup>).

## 212 1.2. Optical biosensors

213 Although enzyme-based biosensors relying on electrochemical detection are highly sensitive  
214 and could be used for real time detection of pesticides, the immobilization of enzymes on the  
215 electrode surface is generally tedious and time consuming. In order to overcome these  
216 difficulties, optical biosensors have been used as an alternative since they are reliable, easy to  
217 use, fast and reveal high sensitivity.

### 218 1.2.1. Fluorescence spectroscopy

219 A fluorometric AChE-based biosensor allowed the rapid, simple and sensitive detection of  
220 parathion-methyl (**Figure 2(B)**) [41]. Gold nanoparticles immobilized on the surface of  
221 NaYF<sub>4</sub>:Yb,Er up-conversion nanoparticles (UCNPs) enable fluorescence resonance energy  
222 transfer (FRET). Thiocholine present in the solution, after hydrolysis of acetylthiocholine by  
223 AChE, interacts with the AuNPs via electrostatic interactions preventing the formation of  
224 AuNPs-UCNP complexes, which leads to an increased fluorescence signal. In the presence of  
225 parathion-methyl, AChE activity is inhibited, thus preventing the production of thiocholine,  
226 which favors the FRET phenomenon, and thus the decrease of fluorescence. The biosensor  
227 developed in this study exhibited high sensitivity and stability, indicating its potential for  
228 detecting organophosphate pesticides in real samples. Moreover, the biosensor preparation  
229 process is less time-consuming compared to grafted enzyme biosensors as the enzyme is not  
230 immobilized onto the nanoparticle surfaces but only adsorbed. This advantageous feature  
231 makes the biosensor a promising tool for practical applications in detecting pesticides.

### 232 1.2.2. Colorimetric assay

233 The rapid and onsite detection of several pesticides remains challenging, like glyphosate, which  
234 is of high polarity, has metal-chelating properties and interfere with organic substances in the  
235 environment. Besides, its similarity with its by-products renders its detection difficult. Enzyme-  
236 based methods along with colorimetric detection reveal several advantages matching with  
237 glyphosate analysis, i. e. simple preparation, rapid detection and ease of results acquisition [48,

238 49]. Luo et al. [42] have reported a novel colorimetric nanozyme sheet for the rapid detection  
239 of glyphosate (**Figure 2(C)**). Physically adsorbed peroxidase enzymes catalyze the oxidation  
240 of chromogenic substrates to produce a color change, the intensity of which is measured by  
241 UV-Visible spectrophotometry. In the presence of glyphosate, peroxidase is inhibited which  
242 results in a decrease of the color intensity.

243 Targeting the same glyphosate analyte, Li Haiyin et al. [50] have reported a peroxidase-mimetic  
244 nanozyme to develop a portable Paper-based Analytical Device (PAD) allowing for its  
245 detection. In this article, the preparation of 2D nanosheet-like  $V_2O_5$  (2D-VONz) with exclusive  
246 peroxidase-mimetic activity under optimal reaction conditions is described. Interestingly, the  
247 activity of 2D-VONz is inhibited by glyphosate, which was then exploited to develop a PAD,  
248 on which, glyphosate reduces the activity of 2D-VONz to prevent the catalytic oxidation of  
249 3,3',5,5'-tetramethylbenzidine, thus contributing to rapid, naked-eye and portable analysis of  
250 glyphosate using a smartphone. Another highly efficient biosensor, based on an enzyme-  
251 mediated dephosphorylation nanozyme, has been developed for the rapid, specific, and  
252 sensitive detection of paraoxon [51]. This biosensor utilizes a novel  $CeO_2@N$ -doped carbon  
253 ( $CeO_2@NC$ ) nanozyme [98]. The mechanism underlying the degradation of phosphotriesters,  
254 catalyzed by  $CeO_2@NC$ , involves  $Ce(IV)/Ce(III)$  species acting as active sites for the  
255 polarization and hydrolysis of phosphoester bonds. Additionally, the N-doped carbon (NC)  
256 material acts as synergistic sites, facilitating the adsorption of the paraoxon substrate and  
257 promoting the hydrolysis process. The characteristic properties of this biosensor are presented  
258 in **Table 2** that shows different types of enzyme-based biosensors developed against pesticides  
259 Different types of enzyme-based biosensors developed against pesticides are described in **Table**  
260 **2**.

### 261 1.2.3. Chemiluminescence Assay

262 Chang et al. [52] introduced a highly sensitive nanozyme chemiluminescence-based (CL)  
263 biosensor for the specific detection of glyphosate. The biosensor utilizes a porous hydroxy

264 zirconium oxide nanozyme (ZrOX-OH) obtained through a straightforward alkali solution  
 265 treatment of  $\text{UIO}_{66}\cdot\text{ZrO}_x\text{-OH}$  which demonstrates remarkable phosphatase-like activity,  
 266 enabling the dephosphorylation of 3-(2'-spiroadamantyl)-4-methoxy-4-(3'-  
 267 phosphoryloxyphenyl)-1,2-dioxetane (AMPPD), which results in the generation of an intense  
 268 CL signals. Notably, the phosphatase-like  $\text{ZrO}_x\text{-OH}$  exhibited a distinctive response to  
 269 glyphosate due to the unique interaction between the carboxyl group of glyphosates and the  
 270 surface hydroxyl group, leading to the development of a CL biosensor for the direct and  
 271 selective detection of glyphosate without the need for bio-enzymes.

272 **Table 2** Enzyme-based biosensors developed for pesticides detection.

Pesticide	Detection technique	Enzyme used	LOD	Validation with real samples	Reference
Malathion, chlorpyrifos, monocrotophos and endosulfan	DPV	AChE	33 ng L <sup>-1</sup> , 35 ng L <sup>-1</sup> , 223.2 ng L <sup>-1</sup> , and 4 µg L <sup>-1</sup>	Milk	Chauhan and Pundir (2011) [37]
Glyphosate	Potentiometric assay	Urease	0.5 mg L <sup>-1</sup>	Tap water	Vaghela et al (2018) [35]
Carbendazim, chlorpyrifos, dichlorodiphenyltri chloroethane (DDT), dinocap, and ethion	CA and CV	Glutathione-S-transferase	2 µg L <sup>-1</sup> , 60 µg L <sup>-1</sup> , 40 µg L <sup>-1</sup> , 50 µg L <sup>-1</sup> , and 100 µg L <sup>-1</sup>	Potatos	Borah et al (2018) [46]
Chlorpyrifos	High open circuit voltage (photoelectrochemical enzymatic fuel cell)	AChE	0.012 µg L <sup>-1</sup>	Not mentioned (PB)	Gai et al (2018) [39]
Omethoate	DPV	AChE	0.36 ng L <sup>-1</sup>	Cabbage and cucumber	Ma et al (2018) [38]
Paraoxon	DPV	2D MnO <sub>2</sub> sheets/AChE nanozyme	25 ng L <sup>-1</sup>	Pakchoi	Wu et al. [45]
Paraoxon	CA	AChE	13 ng L <sup>-1</sup>	Apple and pakchoi	Zhao et al (2017) [47]

Paraoxon, 2,4-dichlorophenoxyacetic acid, and atrazine	CA	BChE, alkaline phosphatase, and tyrosinase	2 $\mu\text{g L}^{-1}$ , 50 $\mu\text{g L}^{-1}$ , and 10 $\mu\text{g L}^{-1}$	River water	Arduini et al (2019) [28]
Malathion	CV and EIS	AChE	0.39 $\mu\text{g L}^{-1}$	Cabbage and carrots	Li et al (2020) [53]
Nitrofen	DPV	Candida Rugosa Lipase	7.38 $\mu\text{g L}^{-1}$	Apricot	Cheng et al (2021) [54]
Ethyl paraoxon	CV	AChE-ChOx	0.46 $\text{ng L}^{-1}$	Pakchoi, cabbage and lettuce	Yang et al (2021) [55]
Paraoxon	DPV	AChE	1.7 $\mu\text{g L}^{-1}$	Apple and Eggplant	Li et al (2020) [56]
Chlorpyrifos	DPV	AChE	20 $\text{ng L}^{-1}$	Cabbage and spinach	Wang et al (2016)[57]
Chlorpyrifos	CV	AChE	50 $\text{ng L}^{-1}$	Cabbage, rape and lettuce	Chen et al (2017) [58]
Malathion and methyl parathion	CV	AChE	0.31 $\text{pg L}^{-1}$ and 0.188 $\text{pg L}^{-1}$	Tap water and Chinese cabbage	Jian et al (2019) [59]
Fenthion	CV and EIS	AChE	100 $\mu\text{g L}^{-1}$	Cabbage juice	Cui et al (2019) [60]
Phosmet	CV and EIS	Tribolium castaneum acetylcholinesterase	1.14 $\mu\text{g L}^{-1}$	Wheat flour	Bial et al (2021) [61]
11 organophosphorus and methomyl	DPV and EIS	AChE	Organophosphorus: 19 – 77 $\text{ng L}^{-1}$ Methomyl: 81 $\text{ng L}^{-1}$	Trichlorfon and dichlorvos: Apple and cabbage	Zhao et al (2021) [62]
Paraxon	CV	AChE	1.4 $\mu\text{g L}^{-1}$	Vegetable leaves	Jia et al (2020) [63]
Paraxon	CV	AChE	4 $\mu\text{g L}^{-1}$	Chinese chives and cabbage	Chen et al (2020) [64]
Paraoxon, dichlorvos, malathion and triazophos	Fluorescence	AChE and ChOx	0.44 $\text{pg L}^{-1}$ , 16.6 $\text{pg L}^{-1}$ , 76 $\mu\text{g L}^{-1}$ and 3 $\text{ng L}^{-1}$	ND	Korram et al (2020) [65]
Pirimicarb, dichlorvos and carbaryl	Fluorescence	AChE	50 $\mu\text{g L}^{-1}$ , 10 $\mu\text{g L}^{-1}$ and 10 $\mu\text{g L}^{-1}$	Pirimicarb: Lettuce, choy and rice	Apilux et al (2017)[66]



Ethylparathion	Fluorescence	AChE	0.7 ng L <sup>-1</sup>	Tap water, soil water, apple and orange juice	Sharma et al (2021) [67]
Parathionmethyl, monocrotophos and dimethoate	Fluorescence	AChE	0.67 ng L <sup>-1</sup> , 23 ng L <sup>-1</sup> and 67 ng L <sup>-1</sup>	Apple, cucumber and capsicum	Long et al (2015) [41]
Carbaryl, ethodan, endosulfan and acephate	Colorimetry and fluorescence	AChE	0.4 to 3 µg L <sup>-1</sup>	River water	Luo et al (2018) [68]
Glyphosate	Fluorescence	Exonuclease I	/	Drinking water	Berkal et al (2023) [36]
Glyphosate	Colorimetry	peroxidase-mimetic nanozyme/ 2D-VONz	/	/	Li Haiyin et al. (2023) [50]
Paraxon	Colorimetry	Phosphatases-mimetic nanozyme/CeO <sub>2</sub> @NC	0.82 mg L <sup>-1</sup>	Garlic chives	Gai et al. (2022) [51]
Glyphosate	Chemiluminiscence	Phosphatases-mimetic nanozyme /ZrO <sub>x</sub> -OH	55.8 mg L <sup>-1</sup>	Cabbage juice	Chang et al. [52] (2023)

273 Upon reviewing **Table 2**, notable disparities in the Limit of Detection (LOD) become evident  
274 among biosensors utilizing the same bioreceptor and targeting the identical pesticide. This  
275 variability predominantly stems from the choice of the detection technique employed. Certain  
276 detection methods prove more accurate to detect pesticides than others. Furthermore, additional  
277 factors, such as the composition of the analyzed matrix, whether it is a simple medium like  
278 water or a more complex one like food or vegetables, also contribute significantly to the  
279 observed variation in LOD values.

## 280 I. Immunosensors

281 Immunosensors are characterized by the directed and highly selective interaction between a  
282 ligand, or antigen (Ag), and its antibody (Ab), immobilized on the transducer surface [69–72].  
283 Once the equilibrium is reached, the ratio of bound-to-free antigen is quantitatively related to  
284 the global amount of ligand. Unlike AChE inhibition based biosensors, immunosensors have

285 the advantage of being specific [73]. Several immunosensors have been developed for  
286 pesticides detection, based essentially on optical and electrochemical transduction methods.  
287 Given the relatively small size of pesticides, competitive assay schemes are frequently utilized  
288 for analyzing pesticides with immunosensors [74]. Based on the detection principle, these  
289 immunosensors can be classified into two main categories, namely optical and electrochemical  
290 immunosensors. In contrast, electrochemical immunosensors utilize changes in current or  
291 potential to detect pesticides, offering several advantages, such as rapid detection, portability,  
292 and low cost.

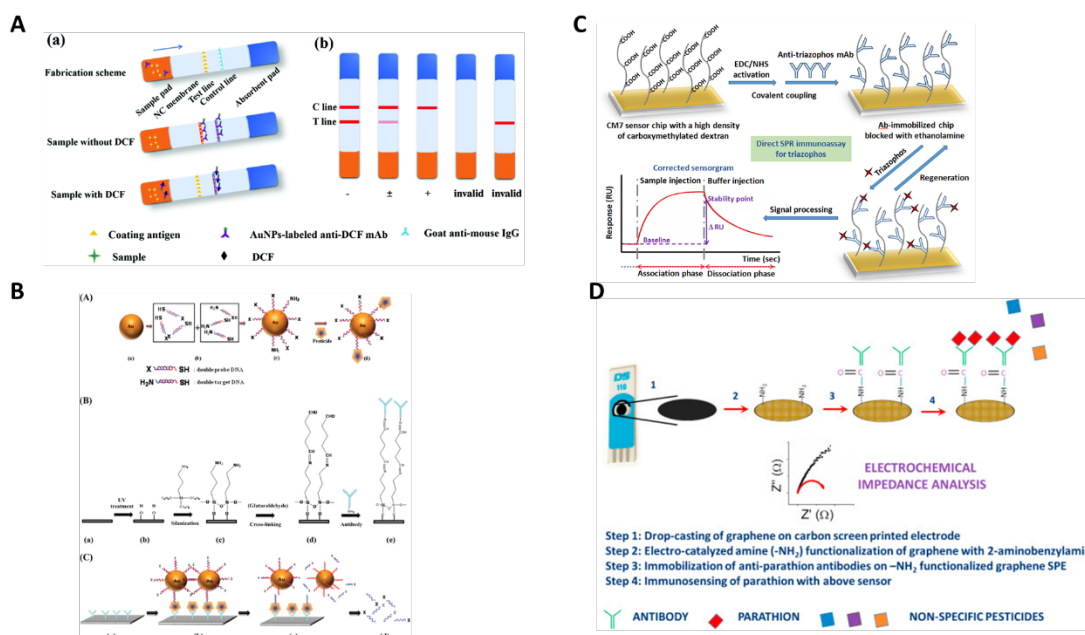
### 293 1.1. Optical immunosensors

294 Optical immunosensors rely on the specific binding interactions between an antibody or antigen  
295 and its corresponding analyte, resulting in a detectable signal change that can be measured using  
296 optical techniques such as absorbance, fluorescence, or surface plasmon resonance. These  
297 immunosensors offer several benefits, including high sensitivity, selectivity, real-time  
298 detection, and the ability to be easily miniaturized for use in portable devices.

#### 299 1.1.1. Colorimetric immunosensors

300 Colorimetric immunoassays have several advantages over conventional methods for pesticides  
301 analysis, such as rapid detection, ease of use, suitability for high-throughput analysis with  
302 limited technical requirements [75]. Liu and coworkers [76] have developed a colorimetric  
303 immunochromatographic strip (ICA) using a monoclonal antibody (mAb) for the detection of  
304 dicofol (**Figure 4(A)**), an organo-insecticide widely spread on vegetables, fruits, teas,  
305 ornamental plants, and field crops [77]. The ICA displayed high sensitivity with a limit of  
306 detection of 50  $\mu\text{g L}^{-1}$  with both apple and cucumber, and the cross-reactivity test revealed a  
307 good specificity for dicofol. These results were consistent with LC-MS and immunocapture  
308 ELISA, suggesting that the ICA method is reliable and practical for detecting dicofol in fruits  
309 and vegetables.

Fig. 4



310

311 (A) Schematic of the ICA strip (a) and interpretation of the test results (b). Reprinted from [76],  
 312 with permission from Royal Society of Chemistry. (B) Novel immunoassay containing  
 313 glyphosate-double DNA-gold nanoparticles based on competitive inhibition reaction.  
 314 Reprinted (adapted) from [78], with permission from American Chemical Society. (C)  
 315 Preparation of mAb-immobilized sensor chip for direct detection of triazophos and real-time  
 316 SPR sensorgram for association and dissociation of the immunocomplex. Reprinted (adapted)  
 317 from [79], with permission from Elsevier. (D) Schematic of the graphene-based screen-printed  
 318 immunosensor for parathion. Reprinted (adapted) from [80], with permission from Elsevier.

### 319 1.1.2. Fluorescence-based immunosensors

320 Gonzalez-Martinez and coworkers [81] have developed a fluorescent immunosensor based on  
 321 an immunocomplex capture assay protocol to detect glyphosate. The described immunosensor  
 322 is fully automated and performs on-line analyte derivatization prior to the assay. It utilizes a  
 323 highly selective anti-glyphosate serum, a glyphosate peroxidase enzyme tracer, and fluorescent  
 324 detection system for high sensitivity and accuracy. Its specific and sensitive detection reaches  
 325 a limit of detection of 0.021  $\mu\text{g L}^{-1}$ , which is lower than the concentration limit set by the  
 326 European council directive [82]. Lee et al. [78] have also described a new method for detecting  
 327 glyphosate using Co-B/SiO<sub>2</sub>/dye nanoparticles in a water-in-oil microemulsion (**Figure 4(B)**).  
 328 The nanoparticles have been surface-modified to enhance their detection limit. The approach  
 329 utilized fluorescence magnetic nanoparticles (FMP) and a glyphosate antibody. With this

330 method, a limit of detection of  $45.6 \text{ ng L}^{-1}$  was reached, with a linear correlation in the range of  
331  $0.169 \text{ } \mu\text{g L}^{-1} - 1.69 \text{ mg L}^{-1}$ .

### 332 1.1.3. Reflectometric interference spectroscopy immunosensors

333 Reflectometric interference spectroscopy presents several advantages over classical optical  
334 techniques such as simple instrumentation, no-need for optical alignment and low cost of the  
335 sensing element. Koukouvinos et al. [83] have developed a white light reflectance spectroscopy  
336 (WLRS) based immunosensor for the fast, real time and label-free simultaneous detection of  
337 chlorpyrifos, imazalil and thiabendazole pesticides in drinking water and wine samples. The  
338 biosensor allowed the entire detection of those pesticides within 10 min. The accuracy of the  
339 measurements was evaluated through recovery experiments and comparison of the results with  
340 validated liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS).  
341 Recovery values ranged from 86 to 116%, and the results were in good agreement with LC-  
342 MS/MS.

### 343 1.1.4. Surface Plasmon Resonance-(SPR) based immunosensors

344 SPR is an optical detection platform widely used for the detection of large molecules, with  
345 which the high mass of the analyte and the use of a sandwich immunoassay format provide a  
346 high signal and thus the desired sensitivity [84, 85]. In contrast and due to the low change of  
347 refractive index induced by the binding of these analytes to the sensor surface, small molecular  
348 compounds such as pesticides present at low concentrations are very difficult to be directly  
349 detected by traditional SPR immunosensors [86]. Recent improvements in SPR devices,  
350 including lower-noise valves and improved microfluidics with more efficient vacuum pumps,  
351 have reduced the overall noise of these systems and thus improved sensitivity and reliability  
352 [87, 88]. Owing to these improvements, SPR immunosensors could be employed for pesticide  
353 monitoring. Guo et al. [79] have described an SPR immunosensor for the detection of an  
354 organophosphate pesticide, triazophos (**Figure 4(C)**). They immobilized two anti-triazophos  
355 monoclonal antibodies (mAbs) on the sensor chip, and characterized them by SPR-based kinetic

356 analysis. The mAb, characterized by a relatively slow dissociation rate, could be used for the  
357 development of a non-competitive SPR-immunosensor for direct monitoring of triazophos  
358 residue in environmental and agricultural samples.

### 359 1.2. Electrochemical immunosensors:

360 Electrochemical immunosensors are interesting alternatives to classical chromatographic  
361 methods for pesticide analysis. Owing to their ability to detect specific targets with high  
362 sensitivity, they are promising tools for rapid and on-site analysis of pesticides [89–91].

#### 363 1.2.1. Cyclic voltammetry

364 Talan et al. [92] have described a highly sensitive fluorine doped tin-oxide (FTO) based  
365 electrochemical immunosensor to detect chlorpyrifos using gold nanoparticles (AuNPs) and  
366 anti-chlorpyrifos antibodies (Chl-Ab). The developed biosensor allowed, via cyclic  
367 voltammetry measurements, the successful detection of chlorpyrifos with very -high sensitivity  
368 ranging from 1 fM to 1  $\mu$ M with a LOD of 10 fM.

#### 369 1.2.2. Chronoamperometric assay

370 Bettazzi et al. [93] developed an electrochemical competitive immunoassay that utilizes  
371 antibody-modified magnetic particles. The assay works by employing a Horseradish Peroxidase  
372 (HRP)-conjugated glyphosate tracer and anti-glyphosate IgG-modified magnetic beads (MBs)  
373 for detection. A screen-printed electrochemical cell has been used to detect glyphosate. The  
374 calibration curve demonstrated a linear concentration range of 0 – 10000 ng L<sup>-1</sup> with a LOD of  
375 5 ng L<sup>-1</sup> and LOQ of 30 ng L<sup>-1</sup> which is well below the EU legislative recommendations.  
376 Furthermore, the developed biosensor showed a good applicability for the analysis of real  
377 samples, namely spiked beer samples.

#### 378 1.2.3. Impedance spectroscopy

379 A graphene-based immunosensor was developed and used to detect parathion [80] (**Figure**  
380 **4(D)**). The process involved modifying screen-printed carbon electrodes with graphene sheets  
381 and their functionalization with 2-aminobenzyl amine before bio-interfacing with anti-  
382 parathion antibodies. The biosensor demonstrated a broad linear range of detection (0.1–1000

383 ng L<sup>-1</sup>) and a very low limit of detection (52 pg L<sup>-1</sup>) with high selectivity towards parathion.  
 384 The biosensor was also successfully used to detect parathion in real samples such as tomato and  
 385 carrot and was cross-calibrated against high performance liquid chromatography (HPLC) to  
 386 confirm its viability. **Table 3** provides a summary of the various types of immunosensors  
 387 developed for the detection of pesticides.

388 **Table 3** Immunosensors developed against pesticides

Pesticide	Detection technique	LOD	Validity for real samples	Reference
Parathion, methyl-parathion, and fenitrothion	ELISA	2.5 µg L <sup>-1</sup> for parathion and methyl-parathion 5 µg L <sup>-1</sup> for fenitrothion	Tap water	Jiao et al. (2018) [94]
Glyphosate	Fluorometric assay	0.021 µg L <sup>-1</sup>	Water and soil	Gonzalez-Martinez et al. (2005) [81]
Glyphosate	Fluorometric assay	10 µg L <sup>-1</sup>	Not mentioned (PBS buffer)	Lee et al. (2010) [78]
Acetochlor and fenpropathrin	Colorimetry	0.6 µg L <sup>-1</sup> and 0.24 µg L <sup>-1</sup>	Corn, apple and cabbage	Cheng et al (2019) [95]
Glyphosate	Chronoamperometric assay	5 ng L <sup>-1</sup>	Commercial beer	Betazzi et al. (2018) [93]
Chlorpyrifos	DPV and CV	3.5 pg L <sup>-1</sup>	Apple, cabbage and pomegranate	Talan et al. (2018) [92]
Thiazophos	SPR	96 ng L <sup>-1</sup>	Environmental water	Guo et al. (2018) [79]
Chlorpyrifos, amazalil, and thiabendazol	WLRS	0.6 µg L <sup>-1</sup> for chlorpyrifos and imazalil 0.8 µg L <sup>-1</sup> for thiabendazol	Drinking water and wine	Koukouvinos et al. (2017) [83]
Chlorpyrifos	EIS	70 pg L <sup>-1</sup>	Chinese cabbage and lettuce	Hou et al (2020) [96]
Malathion	DPV	0.33 pg L <sup>-1</sup>	Lettuce	Kaur et al (2021) [97]
Endosulfan and paraoxon	CV and SWV	0.05 µg L <sup>-1</sup> and 2 µg L <sup>-1</sup>	Environmental water	Liu et al (2014) [98]
Paraquat	Amperometry	1.4 µg L <sup>-1</sup>	Potatos	Valera et al (2014) [99]
Parathion	EIS	52 pg L <sup>-1</sup>	Tomato and carrot	Mehta et al (2016) [80]

## 389 II. Aptasensors

390 The development of the Systematic Evolution of Ligands by Exponential enrichment (SELEX)  
 391 technique has led to the discovery of aptamers, oligonucleotide sequences composed of  
 392 nucleotide bases (Adenine, Thymine, Cytosine, Guanine), capable of complexing a target with  
 393 very high affinity and specificity. Aptamers offer many advantages over antibodies (**Table 4**)  
 394 and are specific to a wide variety of targets: ions [100–102], proteins [103], small molecules

395 [104–106], and cells [107, 108]. These characteristics make aptamers excellent candidates to  
 396 replace antibodies in various fields, more particularly environmental analysis that requires  
 397 stable and robust sensors.

398 **Table 4** Elevating the Distinctive Attributes of Aptamers over Antibodies: A Comparative  
 399 Analysis [109].

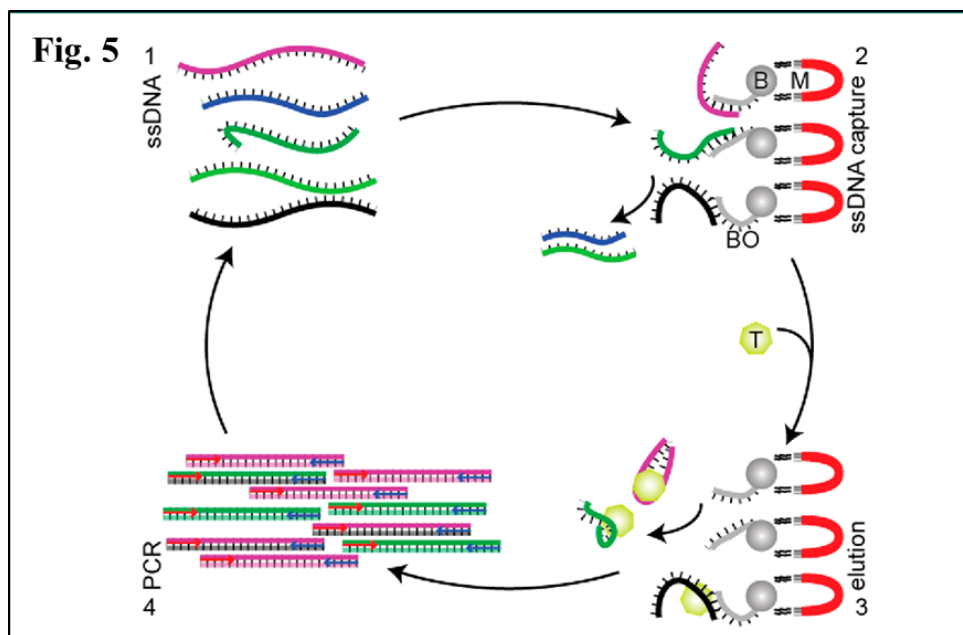
Properties	Aptamers	Antibodies
Stability	Withstand repeated rounds of denaturation/renaturation. Temperature resistant: stable at room temperature. Long shelf life (several years). Can be lyophilized. Degradable by nucleases. Resistant to proteases.	Easily denatured. Temperature sensitive and require refrigeration to avoid denaturation. Limited shelf life. Must be refrigerated for storage and transport. Degradable by proteases. Resistant to nucleases.
Synthesis	In vitro SELEX takes only 2–8 weeks No batch-to-batch variation Cheap to synthesize	Produced in vivo More than 6 months Batch-to-batch variations Laborious and expensive
Target potential	From ions and small molecules to whole cells and live animals	Targets must cause a strong immune response for antibodies to be produced
Size	Small molecules	Relatively large by comparison
Modifiability	Aptamers can readily and easily be modified without affinity loss	Modifications often lead to reduced activity
Affinity	High and increased in multivalent aptamers	Dependent on the number of epitopes on the antigen.
Specificity	Single point mutations identifiable	Different antibodies might bind the same antigen
Tissue uptake/kidney filtration	Fast	Slow

400 Conventional SELEX methods are however not compatible with small molecules because of  
 401 the immobilization step that is not only complicated [110], but leaves weak functional groups  
 402 exposed to the oligonucleotides thus reducing the chance of selecting aptamers. In 2005,  
 403 Stoltenburg et al. reported a method for selecting fluorophore-labeled aptamers that involves  
 404 the grafting of oligonucleotides onto magnetic beads (FluMag-SELEX) [111], which  
 405 subsequently furthered Capture-SELEX, a method suitable for small molecule aptamers  
 406 selection. Unlike conventional SELEX, the immobilization in Capture-SELEX is done with the  
 407 oligonucleotides and not with the target molecules, which allows the preservation of all  
 408 functional groups of these molecules thus increasing the chance of selecting aptamers. The  
 409 operating principle of the Capture-SELEX can be summarized in the following steps (**Figure**  
 410 **5**):

- 411 1. Immobilization: the capture oligonucleotide (DNA complementary to the library) is  
 412 immobilized on the solid matrix (magnetic beads for example).
- 413 2. Hybridization: the oligonucleotide library is hybridized with the immobilized capture  
 414 oligonucleotides.
- 415 3. Incubation: the target is incubated with the oligonucleotide library.

- 416 4. Elution: the sequences with an affinity towards the target will detach from the capture  
417 oligonucleotide and go into solution.  
418 5. Amplification: the recovered sequences are enriched by Polymerase Chain Reaction  
419 (PCR) for the next round.

420 Similar to conventional SELEX, candidate aptamers are sequenced and cloned at the end of  
421 the Capture-SELEX (6 to 20 rounds) to identify the aptamers that have the desired  
422 properties.



423  
424 Schematic illustration of Capture-SELEX using magnetic beads. Reprinted from [112], with  
425 permission from American Chemical Society.

## 426 II.1. Optical aptasensors

427 Optical aptasensors have become a well-established technology in the field of bioanalysis,  
428 utilizing aptamers as recognition elements to directly target and identify substances. These  
429 sensors utilize various light sources, including fluorescence, colorimetry, SERS, etc., and  
430 convert signals in the form of UV, visible, and IR radiation into different data formats.  
431 Compared to other detection methods, optical aptasensors offer several advantages, such as low  
432 cost, repeatable use, high specificity, simple sample preparation, minimal interference, and high

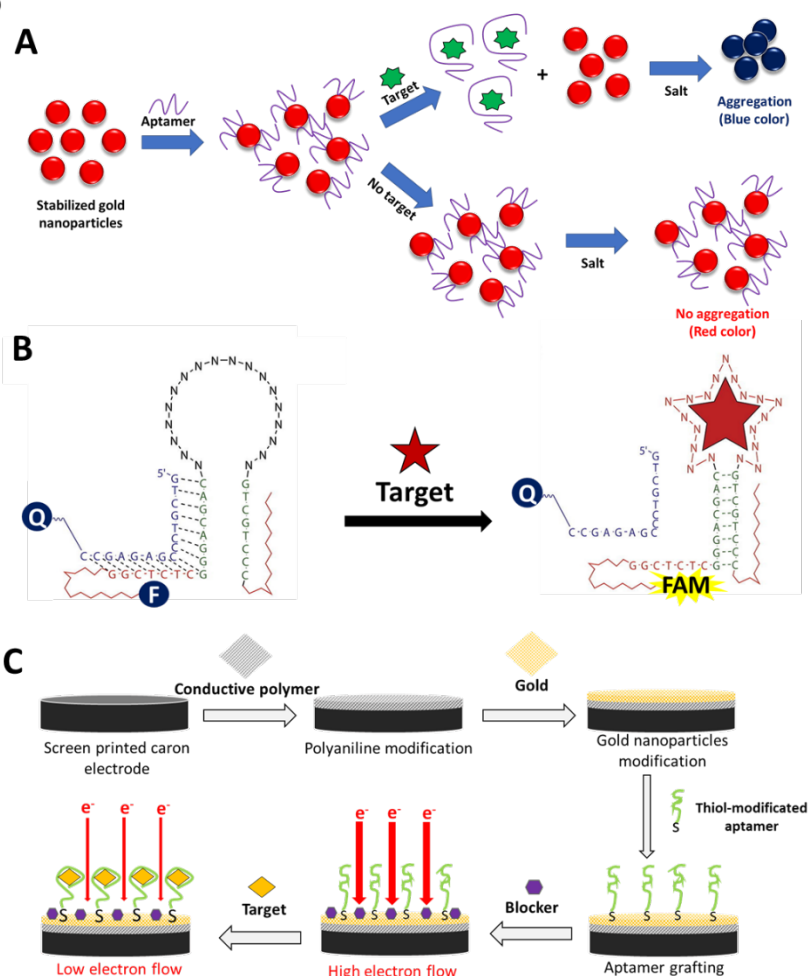


433 accuracy. These characteristics make optical aptasensors particularly suitable for on-site  
434 detection applications [113].

### 435 II.1.1. Colorimetric aptasensors

436 The primary benefit of colorimetric sensing is its straightforwardness and ease of  
437 implementation. Colorimetric aptasensors have been extensively employed for detecting  
438 pesticides in real samples due to the convenience of visual observation. However, a significant  
439 challenge in designing colorimetric aptasensors relies on converting the response change into a  
440 noticeable color change. Gold (Au) and silver (Ag) nanoparticles are the most commonly used  
441 probes in colorimetric assays, due to their ability to enhance surface plasmon resonance and  
442 thus produce robust signals as monitored by colorimetry (**Figure 6(A)**) [114, 115].

**Fig. 6**



443 Comparative schematic diagram of (A) colorimetric aptasensor, (B) FRET aptasensor and (C)  
444 electrochemical aptasensor. (B) Reprinted from [116], with permission from ScienceDirect.  
445

## 446 II.1.2. Fluorescence-based aptasensors

447 Fluorescence-based aptasensors rely on the correlation between changes in fluorescence  
448 intensity and the concentration of the target upon aptamer binding. Fluorescence changes are  
449 typically achieved through the introduction of a fluorescence quenching or fluorescence  
450 resonance energy transfer agent (FRET, **Figure 6(B)**). FRET occurs between two fluorophores  
451 at an appropriate distance and can utilize various materials as fluorescent donors, including  
452 dyes, quantum dots, metal nanoparticles, carbon dots, and carbon nanomaterials.  
453 Nanomaterials, such as graphene oxide, gold nanoparticles, carbon nanotubes, and  
454 nanocomposites, are also used as well-known fluorescence quenchers [117].

455 To create efficient aptasensors with high sensitivity, high throughput, and multiplexing  
456 capabilities, various combinations of nanomaterials are employed as fluorescent donors and  
457 receptors in sensing assays. Fluorescence detection relies on switching the fluorescence signals  
458 on and off to indicate the presence of analytes [118, 119]. In most cases, fluorescence signals  
459 are in the "signal-off" state until the target is present and the blocking of signals is lifted, leading  
460 to a "signal-on" response. Fluorescence signals can be amplified using a variety of  
461 nanomaterials such as gold and silver nanoparticles, known for their superior performance [120,  
462 121]. Su et al. proposed a method for detecting carbendazim (CBZ) in water using a fluorescent  
463 aptasensor [122]. The aptasensor used a CBZ-specific aptamer as a sensing probe, gold  
464 nanoparticles (AuNPs) and Rhodamine B (RhoB) as an indicator. In the absence of CBZ, the  
465 aptamer wrapped around the AuNPs and kept them dispersed in a solution. However, in the  
466 presence of CBZ, the aptamer forms a complex with CBZ, leaving the AuNPs which aggregate  
467 in a NaCl solution. The concentration of CBZ was determined by measuring the fluorescence  
468 intensity. The method had a wide linear range from 2.33 to 800 nM and a LOD of 2.33 nM. The  
469 fluorescent aptasensor showed potential for use in detecting CBZ in aquatic environments.

### 470 II.1.3. Luminescence-based aptasensors

471 Common fluorescence techniques that rely on organic dyes are vulnerable to interference from  
472 naturally occurring fluorescent substances in the environment [123]. Time-resolved emission  
473 spectroscopy (TRES) is a useful method for discriminating between fluorophores with similar  
474 emission spectra but varying decay times. TRES records long-lived phosphorescence once the  
475 initial short-lived fluorescence background has faded [124–127]. Therefore, luminescent  
476 transition metal complexes, such as iridium(III) complexes, have become preferred for TRES  
477 due to their excellent optical characteristics and long-lived phosphorescence [128]. Chen et al.  
478 [129] have reported on a luminescent G-quadruplex-derived aptasensor for the monitoring of  
479 glyphosate using a phosphorescent iridium(III)-based probe. The developed platform displayed  
480 excellent sensitivity with good selectivity for glyphosate with a limit of detection of  $4.46 \mu\text{g L}^{-1}$   
481  $^1$  in Tris-buffer, and  $13.4 \mu\text{g L}^{-1}$  in Tris-buffer containing soybean extract to simulate a complex  
482 environment.

### 483 II.1.4. Phosphorescence aptasensors

484 Persistent luminescence nanorods (PLNRs) have become a subject of extensive research owing  
485 to their unique afterglow or phosphorescence characteristics, which unlike fluorophores allow  
486 for reduced or even eliminated background luminescence. These nanorods exhibit persistent  
487 luminescence, which refers to the emission of light after the cessation of the excitation source.  
488 Wang et al. have developed an aptamer-based colorimetric-phosphorescence assay for the  
489 detection of isocarbophos [130]. The colorimetric assay used AuNPs that were aggregated by  
490 competitive binding of the aptamer between isocarbophos and AuNPs at high salt  
491 concentration. The addition of persistent luminescence nanorods (PLNRs) to the system  
492 resulted in phosphorescence that was sensitive to the concentration of isocarbophos due to the  
493 inner filter effect between PLNRs and AuNPs. The assay showed good linearity within the  
494 range of  $50\text{-}500 \mu\text{g L}^{-1}$  and  $5\text{-}160 \mu\text{g L}^{-1}$ , with a limit of detection of  $7.1 \mu\text{g L}^{-1}$  and  $0.54 \mu\text{g L}^{-1}$   
495 in colorimetry and phosphorescence mode, respectively. The method was successfully

496 demonstrated for food analysis with the detection of isocarbophos residues in vegetables,  
497 showing both sensitivity and selectivity.

#### 498 II.1.5. SERS-based aptasensors

499 Surface-Enhanced Raman spectroscopy (SERS) is a powerful spectroscopic technique that  
500 leverages plasmonic metal nanostructures for the detection of trace-level chemicals with high  
501 sensitivity and selectivity [131]. SERS has been widely adopted in analytical chemistry [132],  
502 biochemical analysis [133], and environmental sensing [134, 135], including the detection of  
503 harmful agrochemicals, such as pesticides [136–138]. However, the lack of selectivity when  
504 the target molecule is mixed with other molecules in a complex matrix and susceptibility to  
505 interference from various ambient influences can limit SERS detection techniques. Therefore,  
506 sample pretreatment methods, such as purification and extraction, are critical for examining  
507 environmental samples. The use of aptamers, specific biomolecules that can enhance the  
508 selectivity of target analysis when combined with SERS, can address these limitations.  
509 Recently, Kamkrua et al. [139] developed a SERS aptasensor using gold nanoparticles to detect  
510 parquat, a commonly used herbicide. The platform used AuNPs-SERS substrates modified with  
511 a thiol-modified aptamer as bioreceptor to selectively bind to parquat molecules. The aptamer-  
512 modified SERS substrate showed improved sensitivity and selectivity compared to SERS  
513 substrates without aptamer modifications, with a limit of detection of  $0.10 \pm 0.03 \mu\text{M}$ . The  
514 platform was evaluated using natural water samples and demonstrated good stability against  
515 interferences from other similar herbicides and insecticides present along with parquat in water.

#### 516 II.2. Electrochemical aptasensors

517 Electrochemical aptasensors produce an electrochemical signal upon the capture of a particular  
518 target substance, achieved through the use of aptamers. The design of electrochemical  
519 aptasensors involves three crucial steps, including the utilization of nanomaterials to amplify  
520 the signal, binding of the aptamer to the sensor surface, and the detection process itself (**Figure**

521 **6(C)**). Covalent bond formation is the most commonly employed method for aptamer  
522 immobilization onto the surface in these types of sensors [140]. Numerous electrochemical  
523 aptasensors have been reported for the identification of proteins, which are large molecules  
524 producing significant variation in electrochemical signal upon binding to the aptamer, thereby  
525 facilitating their detection. However, detecting pesticides which are small molecules requires a  
526 notable change in aptamer conformation to enable their detection by electrochemical platforms.  
527 To overcome this challenge, multiple approaches have been adopted, one of which is the use of  
528 oligonucleotide switching structures generated through the Capture-SELEX method that has  
529 proven to be more suitable for detecting pesticides due to the aptamers selection process: a  
530 significant conformational change occurs upon binding to the target [141]. This conformational  
531 change leads to an improved ability to detect and quantify small molecule pesticides using  
532 electrochemical aptasensors. In **Table 5**, various aptasensors that were developed for the  
533 detection of pesticides have been listed.

**Table 5** Recent advances in aptasensors developed against pesticides.

Target	Detection technique	Aptamer sequence	LOD	Dynamic range	Validity for real samples	Reference
Acetamiprid	EIS	Acetamiprid: 5'-(SH)-(CH <sub>2</sub> ) <sub>6</sub> - TGTAATTTGTCTGCAGCGGTTCTTGATCGCTGA CACCATATTATGAAGA-[FITC]-3'	Acetamiprid: 8.9 ng L <sup>-1</sup>	ND	Buffer	Madianos et al (2018) [142]
Atrazine		Atrazine: 5'-(SH)-(CH <sub>2</sub> ) <sub>6</sub> - TACTGTTTGCCTGGCGGATTTAGCCAGTCAGT G-[FITC]-3'	Atrazine: 1.3 ng L <sup>-1</sup>			
Acetamiprid	EIS	Acetamiprid: 5'-(SH)-(CH <sub>2</sub> ) <sub>6</sub> - TGTAATTTGTCTGCAGCGGTTCTTGATCGCTGA CACCATATTATGAAGA-[Flc]-3'	Acetamiprid: 0.2 ng L <sup>-1</sup>	Acetamiprid: 2.2 ng L <sup>-1</sup> - 22.2 µg L <sup>-1</sup>	Tap water	Madianos et al (2018) [143]
Atrazine		Atrazine: 5'-(SH)-(CH <sub>2</sub> ) <sub>6</sub> - TACTGTTTGCCTGGCGGATTTAGCCAGTCAGT G-[Flc]-3'	Atrazine: 2.1 ng L <sup>-1</sup>	Atrazine: 21.5 ng L <sup>-1</sup> - 215.7 µg L <sup>-1</sup>		
Atrazine	Photoelectrochemical assay	5'-SH-(CH <sub>2</sub> ) <sub>6</sub> -TGT-ACC-GTC-TGA-GCG-ATT- CGT-ACG-AAC-GGCTTT-GTA-CTG-TTT-GCA- CTG-GCG-GAT-TTA-GCC-AGT-CAG-TGT-TAA- GGA-GTG-C-3'	0.21 ng L <sup>-1</sup>	0.5 ng L <sup>-1</sup> - 0.11 µg L <sup>-1</sup>	Lake water, agricultural wastewater and sewage water	Fan et al (2021) [144]
Carbofuran	DPV	5'- CACCTGGGGGAGTATTGCGGAGGAAAGAGAAC ACTGGGGCAGATATGGGCCAGCAGGTC- (CH <sub>2</sub> ) <sub>3</sub> -SH-3'	14.8 µg L <sup>-1</sup>	44 ng L <sup>-1</sup> -11 µg L <sup>-1</sup>	Vegetables and fruits	Li et al (2018) [145]
Chlorpyrifos	DPV	5'- CCTGCCACGCTCCGCAAGCTTAGGGTTACGCCT GCAGCGATTCTTGATCGCGCTGCTGGTAATCCT TCTTTAAGCTTGGCACCCGCATCGT-3'	70 ng L <sup>-1</sup>	0.1 µg L <sup>-1</sup> - 150 µg L <sup>-1</sup>	Apple and celery cabbage	Xu et al (2018) [146]

Atrazine	EIS	5' -TGT ACC GTC TGA GCG ATT CGT ACG AAC GGC TTT GTA CTG TTT GCA CTG GCG GAT TTA GCC AGT CAG TGT TAA GGA GTG C-3'	0.67 ng L <sup>-1</sup>	1 ng L <sup>-1</sup> -50 µg L <sup>-1</sup>	River and tap water	Zhu et al (2021) [147]
Atrazine	Photoelectrochemical assay	5'-TGT-ACC-GTC-TGA-GCG-ATT-CGT-ACG-AAC-GGC-TTT-GTA-CTG-TTT-GCA-CTG-GCG-GAT-TTA-GCC-AGT-CAG-TGT-TAA-GGA-GTG-C-3'	2.6 pg L <sup>-1</sup>	10.7 pg L <sup>-1</sup> –64.7 ng L <sup>-1</sup>	Water	Sun et al (2019) [148]
Atrazine	DPV	5'-SH-(CH <sub>2</sub> ) <sub>6</sub> -TGT-ACC-GTC-TGA-GCG-ATT-CGT-ACG-AAC-GGCTTT-GTA-CTG-TTT-GCA-CTG-GCG-GAT-TTA-GCC-AGT-CAG-TGT-TAAGGA-GTG-C-3'	21.5 pg L <sup>-1</sup>	53.9 pg L <sup>-1</sup> –53.7 ng L <sup>-1</sup>	Lake and river water	Fan et al (2019) [149]
Atrazine	Linear Sweep Voltametry (LSV)	5'-HS-(CH <sub>2</sub> ) <sub>6</sub> -TGT-ACC-GTC-TGA-GCG-ATT-CGT-ACG-AAC-GGCTTT-GTA-CTG-TTT-GCA-CTG-GCG-GAT-TTA-GCC-AGT-CAG-TGT-TAAGGA-GTG-C-3'	1.6 µg L <sup>-1</sup>	ND	Environmental water	Wang et al (2020) [150]
Atrazine	Adsorption spectroscopy (ATR-SEIRAS)	5'-HS-(CH <sub>2</sub> ) <sub>6</sub> -TGT-ACC-GTC-TGA-GCG-ATT-CGT-ACG-AAC-GGC-TTT-GTA-CTG-TTT-GCA-CTG-GCG-GAT-TTA-GCC-AGT-CAG-TGT-TAA-GGA-GT <sub>7</sub> G-C-3'	0.23 µg L <sup>-1</sup>	ND	ND	Sun et al (2021) [151]
Atrazine	Ultrafiltration system	5'-/FAM/-TACTGTTTGC ACTGGCGGATTTAGCCAGTCAGTG-3'	ND	ND	ND	Romero-Reyes and Heemstra (2021) [152]
Atrazine	Fluorescence	5'-TTT-TTT-TTT-TTA-CTG-TTT-GCA-CTG-GCG-GAT-TTA-GCC-AGT-CAG-TG-3'	2 ng L <sup>-1</sup>	ND	River simple	Yao et al (2021) [153]
Atrazine	SERS	5'-(SH)-TACTG TTTGC ACTGG CGGAT TTAGC CAGTC AGTG-3'	0.14 µg L <sup>-1</sup>	0.22 µg L <sup>-1</sup> –10.7 µg L <sup>-1</sup>	Cherry tomato and grape	Wei et al (2020) [154]
Carbendazim	Fluorescence	5'-CGACACAGCGGAGGCCACCCGCCACCCAGCCCCTGCAGCTCCTGTACCTGTGTGTGTG-3'	0.44 µg L <sup>-1</sup>	0.44 µg L <sup>-1</sup> –0.15 mg L <sup>-1</sup>	Water	Su et al (2020) [122]
Chloramphenicol	Colorimetry	5'-ACTTCAGTGAGTTGTCCCACGGTCGGCGAGTCGGTGGTAG-Biotin-3' and antibody	0.19 µg L <sup>-1</sup> in milk 0.23 µg L <sup>-1</sup> in serum	ND	Milk and mouse serum	Abnous et al (2016) [155]

Chlorpyrifos	Colorimetry	5'- CCTGCCACGCTCCGCAAGCTTAGGGTTACGCCT GCAGCGATTCTGATCGCGCTGCTGGTAATCCTT CTTTAAGCTTGGCACCCGCATCGT-3'	11.3 mg L <sup>-1</sup>	10 mg L <sup>-1</sup> - 200 mg L <sup>-1</sup>	River water	Weerathunge et al (2019) [156]
Chlorpyrifos and diazinon and	Fluorescence	Chlorpyrifos aptamer 5'- CCTGCCACGCTCCGCAAGCTTAGGGTTACGCCT GCAGCGATTCTTGTATCGCGCTGCTGGTAATCCT TCTTTAAGCTTGGCACCCGCATCGT-3'	0.73 ng L <sup>-1</sup> and 6.7 ng L <sup>-1</sup>	ND	12 vegetables and fruits	Cheng et al (2018) [157]
Diazinon	Fluorescence	5'-NH <sub>2</sub> -C <sub>6</sub> - ATCCGTCACACCTGCTCTAATATAGAGGTATTG CTCTTGGACAAGGTACAGGGATGGTGTGGCT CCCGTAT-3'	23 ng L <sup>-1</sup>	0.05 µg L <sup>-1</sup> - 500 µg L <sup>-1</sup>	Tea, apple and tap water	Ron et al (2020) [158]
Glyphosate	Fluorescence	5'-CGC ATT CAG GAT TGC ATG ATT GCC AAA AAA AAA A-NH <sub>2</sub> -3'	10 µg L <sup>-1</sup>	10 µg L <sup>-1</sup> - 100 mg L <sup>-1</sup>	PBS buffer	Lee et al (2010) [78]
Glyphosate	SERS	5'-TGC TAG ACG ATA TTC GTC CAT CCG AGC CCG TGG CGG GTC TTA GGA CTC TGC GGG CTT CGC GGC GCT GTC AGA CTG AAT ATG TCA-3'	0.34 ng L <sup>-1</sup>	0.51 ng L <sup>-1</sup> - 11.8 ng L <sup>-1</sup>	Soil	Liu et al (2021) [159]
Glyphosate	Luminiscence	5'- TGCTAGACGATATTCGTCCATCCGAGCCCGTGG CGGGCTTTAGGACTCTGCGGGCTTCGCGGCGCT GTCAGACTGAATATGTCA-3'	4.5 µg L <sup>-1</sup> in buffer 13.4 µg L <sup>-1</sup> in buffer containing soybean extract	8.5 µg L <sup>-1</sup> - 50.7 µg L <sup>-1</sup>	Buffer containing soybean extract	Chen et al (2020) [129]
Glyphosate, malathion and trichlorfon	Fluorescence	5'-6-FAM- AGCTTGCTGCAGCGATTCTTGATCGCCACAGA GCT-3'	88.8 ng L <sup>-1</sup> , 195.37 ng L <sup>-1</sup> and 72.2 ng L <sup>-1</sup> ,	0.1 µg L <sup>-1</sup> -10 mg L <sup>-1</sup>	Lettuce and carrot	Jiang et al (2020) [160]
Isocarbophos	Fluorescence	5'- ATTCTTGATCGCCACGGTCTGGAAAAAGAGAA GTGGGTAGGGCGGGTTGG-3'	2.9 µg L <sup>-1</sup>	2.9 µg L <sup>-1</sup> - 0.14 mg L <sup>-1</sup>	Chinese cabbage and apple	Li et al (2018) [161]



Isocarbophos	Phosphorescence and colorimetric assays	5'-AGCTTGCTGCAGCGATTCTTGATCGCCACAGAGCT-3'	Phosphorescence: 0.54 $\mu\text{g L}^{-1}$ Colorimetry: 7.1 $\mu\text{g L}^{-1}$	Phosphorescence: 5-160 $\mu\text{g L}^{-1}$ Colorimetry: 50-500 $\mu\text{g L}^{-1}$	Chinese cabbage, brassica rape and lettuce	Wang et al (2019) [130]
Isocarbophos and omethoate	Colorimetry	5'-AAG CTT TTT TGA CTG ACT GCA GCG ATT CTT GAT CGC CAC GGT CTG GAA AAA GAG-3'	0.47 $\mu\text{g L}^{-1}$ and 0.35 $\mu\text{g L}^{-1}$	50-1000 $\mu\text{g L}^{-1}$ and 100-500 $\mu\text{g L}^{-1}$	Buffer	Liu et al (2020) [162]
Malathion	Colorimetry	5'-ATCCGTCACACCTGCTCTTATACACAATTGTTT TTCTCTTAACTTCTTGACTGCTGGTGTTGGCTCCCGTAT-3'	0.33 $\text{ng L}^{-1}$	1.65 $\text{ng L}^{-1}$ – 3.3 $\mu\text{g L}^{-1}$	Human serum	Abnous et al (2018) [163]
Malathion	Fluorescence	5'-ATCCGTCACACCTGCTCTTATACACAATTGTTT TTCTCTTAACTTCTTGACTGCTGGTGTTGGCTCCCGTAT-3'	0.74 $\text{ng L}^{-1}$	ND	12 vegetables and fruits	Cheng et al (2018) [157]

## Conclusion and perspectives

Biosensors have emerged as a promising technology for the detection of pesticides, offering rapid, sensitive, and reliable results. Recent advances in biosensor design, and especially the use of biorecognition elements such as antibodies, enzymes and recently aptamers, have enabled the development of highly sensitive and specific biosensors that can detect a wide range of pesticides with high accuracy.

Despite the numerous advantages of biosensors, several challenges remain to be addressed to unlock their full potential. One key challenge lies in the selectivity of immunosensors and enzyme-based biosensors, which often encounter issues such as cross-interactions among antibodies and a lack of specificity of many enzymes employed in biosensors which are inhibited by organophosphate pesticides, limiting their effectiveness.

Furthermore, biosensors deployed in complex sample matrices face hurdles due to interference from various substances. Mitigating these effects requires the development of effective strategies. Moreover, the limited commercial applications of biosensors call for efforts to scale up production, reduce costs, and comply with regulatory requirements.

To foster wider adoption and successful integration of biosensor technologies, standardized protocols for their development, validation, and manufacturing are essential. Establishing these protocols will ensure consistent performance and facilitate comparability of results across different platforms and laboratories.

Looking to the future, biosensors are expected to play an increasingly important role in the detection and monitoring of pesticides, particularly in the context of environmental and food safety. The integration of biosensors with other technologies, such as smartphone apps and cloud computing, is also expected to further enhance the capabilities of biosensors and expand their applications.

In conclusion, biosensors represent a promising technology for the detection of pesticides, and further research and development in this area is essential to address the challenges and unlock the full potential of this technology for environmental and food safety.

### **Declarations**

No conflicts of interests/Competing interests to declare

### **Acknowledgment and funding information**

We acknowledge grants from the Université de Pau et de Pays de l'Adour through its project Energy and Environment Solutions supported by the Agence National pour la recherche (ANR OPE-2018-0020) and the Communauté d'agglomération de Pau Béarn Pyrénées (CDAPBP, OPE-2020-0032) and from the Région Nouvelle Aquitaine (CONV-2019-0227).

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