ISSN 0036-0244, Russian Journal of Physical Chemistry A, 2023, Vol. 97, No. 12, pp. 2894–2902. © Pleiades Publishing, Ltd., 2023.

BIOPHYSICAL CHEMISTRY AND PHYSICAL AND CHEMICAL BIOLOGY

Oxidation Pretreatment to Improve the Sensitivity of Acetylcholinesterase-Based Detection of Thioorganophosphates

M. B. Čolović[a],*, D. Z. Krstić[b], and V. M. Vasić[a]

[a] *University of Belgrade, "Vinča" Institute of Nuclear Sciences-National Institute of the Republic of Serbia, P.O. Box 522, Belgrade, 11351 Serbia*

[b] *University of Belgrade, Faculty of Medicine, Institute of Medical Chemistry, Belgrade, 11000 Serbia*

* *e-mail: colovicm@vin.bg.ac.rs*

Received May 8, 2023; revised May 8, 2023; accepted June 27, 2023

Abstract—Bioassays based on the determination of acetylcholinesterase (AChE) activity are used for the detection and neurotoxicity evaluation of organophosphate insecticides. However, AChE bioassays are convenient for oxo and iso forms, whereas the limits of detection (LODs) of thio forms, being used as commercial insecticide preparations are significantly higher. In this study various malathion concentrations (1×10^{-9}–5×10^{-4} mol/L) were treated with the oxidizing agent, *N*-bromosuccinimide (NBS) in concentration ratios: 1 : 1, 1 : 2, 1 : 10, and 1 : 20 to find efficient oxidation resulting in as possible as lower LODs of the used assays based on electric eel AChE (20 min-preincubation) and immobilized AChE incorporated in flow-injection analysis (FIA) system. Malathion–NBS ratio of 1 : 10 was found as the most efficient and resulted in a decrease of LOD about 100 times for both AChE bioassays. In the case of free AChE the obtained LOD values after the NBS-induced oxidation of thioorganophosphates (1 : 10), malathion, diazinon, and chlorpyrifos were as follows: 1.0×10^{-8}, 1.3×10^{-8}, and 1.0×10^{-8} mol/L, respectively. In addition, LOD values for the FIA-AChE system involving a pre-step with NBS induced the following LOD values: 7.2×10^{-7}, 1.3×10^{-6}, and 1.8×10^{-7} for malathion, diazinon, and chlorpyrifos, respectively. Furthermore, IC_{50} values of the corresponding oxo forms were found to be similar to those for the studied thioorganophosphates, which indicates a potential stoichiometric conversion of the thio to oxo forms under the established oxidation conditions.

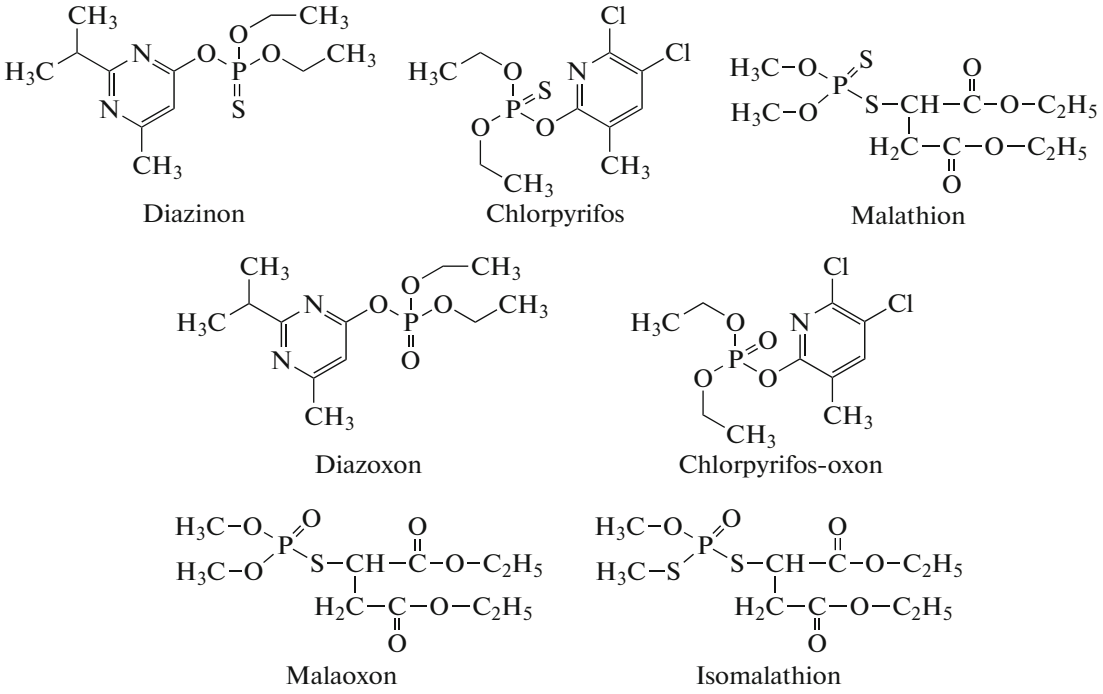
Keywords: acetylcholinesterase assays, chlorpyrifos, diazinon, malathion, *N*-bromosuccinimide, oxidation

DOI: 10.1134/S0036024423120221

INTRODUCTION

Organophosphates such as diazinon, chlorpyrifos, malathion, and parathion have been commonly used as insecticides for several decades to control a variety of insects in the agriculture and the household environment, and are among the most widespread pollutants for pest control [1, 2]. Although organophosphates are preferred in agriculture because of their relatively low persistence in the environment, they are indiscriminate pesticides, highly toxic to animals and humans as well and their use must be controlled [3–5]. The insecticide action of this large and diverse family of organic compounds is based on the inhibition of acetylcholinesterase (AChE, EC 3.1.1.7) by phosphorylation of the serine hydroxyl group in the substrate-binding domain of the enzyme, resulting in acetylcholine accumulation at cholinergic synapses in the central and peripheral nervous systems [6–8]. In addition, organophosphate interaction with other physiologically important enzymes such as ATPases and peroxidases was reported [9, 10]. Most bioassays for the detection and neurotoxic evaluation of organophosphorus pesticides involve the inhibition of AChE and butyrylcholineesterase activity [11, 12], but other enzymes such as alkaline phosphatases, organophosphorus hydrolases, and tyrosinases have also been exploited [13–15]. The decrease in enzyme activity is a function of the concentration and the inhibitory potency of all present AChE inhibitors.

Most of the commercially available insecticide preparations contain thioorganophosphates (malathion, parathion, etc.) as an active ingredient, which are characterized by one thione moiety (P=S) and three –OR groups attached to a phosphorus atom (Scheme 1). Thio forms were found as significantly weaker AChE inhibitors than their corresponding oxidized analogs, oxons (even about 1000 times) containing one double phosphorus-oxygen bond (P=O) (Scheme 1). The substitution of sulfur with oxygen makes the oxo forms more polar and consequently more potent to inhibit AChE activity [16–18]. Therefore, AChE-based bioassays are capable to detect low concentrations of P=O-containing forms (Scheme 1), whereas the limits of detection (LODs) of thio forms



Scheme 1. Structures of studied thioorganophosphates and their P=O-containing analogs.

are relatively high. For this reason, the detection of parent thioorganophosphates, being employed as insecticide preparations due to safety, requires their conversion to oxo analogs. For this purpose, oxidation pretreatment is one of the methods to improve the sensitivity of AChE bioassays for the detection of thio forms. This can be achieved by using a variety of chemical and enzymatic agents such as follows: hydrogen peroxide, bromine water, chlorine compounds [19, 20], *N*-bromosuccinimide (NBS) [21], myeloperoxidase [22, 23], chloroperoxidase [24], cytochromes [25], esterases [26], and electrochemical oxidation [27].

The aim of this study was to optimize oxidation pretreatment to achieve as possible as higher sensitivity of AChE-based assays for thioorganophosphate detection. Diazinon (*O*,*O*-diethyl-*O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothionate), chlorpyrifos (*O*,*O*-diethyl-*O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate), and malathion (diethyl 2-[(dimethoxyphosphorothioyl)sulfanyl]butanedioate) were treated with the oxidizing agent, NBS in various ratios and the efficacy of the oxidation was monitored by free electric eel AChE bioassay and immobilized AChE-flow injection analysis (AChE-FIA) system. NBS was reported to be a reliable, rapid, and selective oxidizing agent for the conversion of thioorganophosphates to their oxo analogs, which does not require additional steps such as preconcentration and extraction [28].

EXPERIMENTAL

Chemicals

All chemicals were of analytical grade. Malathion (97.3%), chlorpyrifos (99.8%), and diazinon (97.3%) were purchased from Pestanal (Germany). Acetylcholinesterase from electric eel (AChE, specific activity 288 IU/mg solid, 408 IU/mg protein), acetylthiocholine iodide (ASChI), 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB), and controlled-pore glass (CPG 240, 80–120 mesh) were from Sigma Chemicals Co. (Germany). Sodium dihydrogen phosphate ($NaH_2PO_4 \cdot 2H_2O$), potassium hydrogen phosphate ($K_2HPO_4 \cdot 3H_2O$), sodium dodecyl sulfate (SDS), glutaraldehyde, 3-aminopropyl-triethoxysilan, *N*-bromosuccinimide (NBS), and ascorbic acid were provided from Merck (Germany). All the materials were used as received, without further purification.

Oxidation of Organophosphates

Organophosphate stock solutions were prepared by diluting approximately 10 mg in 10 mL ethanol and kept in the freezer until used. Working solutions were prepared daily by diluting stock solutions with deionized water to desired concentrations as needed. NBS and ascorbic acid solutions of appropriate concentrations were prepared daily.

Different organophosphate concentrations, within the concentration range from 1×10^{-9} to 5×10^{-4} mol/L, were treated with NBS in various ratios:

Table 1. IC_{50} and LOD values obtained from the sigmoidal inhibition curves for malathion and malathion oxidized with NBS in ratios: 1 : 1, 1 : 2, 1 : 10, and 1 : 20 (Fig. 1)

Malathion		Malathion : NBS			
		1 : 1	1 : 2	1 : 10	1 : 20
IC_{50}, mol/L	5.0×10^{-4}	5.6×10^{-6}	6.1×10^{-7}	3.2×10^{-8}	1.1×10^{-7}
LOD, mol/L	5.1×10^{-6}	1.5×10^{-6}	1.8×10^{-7}	1.0×10^{-8}	3.3×10^{-8}

1 : 1, 1 : 2, 1 : 10, and 1 : 20 for malathion, and 1 : 10 for diazinon and chlorpyrifos. The oxidation reaction was allowed to proceed for 10 min and was stopped by adding an excess of ascorbic acid (100 times higher concentration than NBS). Subsequently, the oxidized organophosphates were tested using AChE assays as described below.

Acetylcholinesterase Activity Assay

The activity of AChE was determined using a slightly modified Ellman's method [17, 29] in the absence (control) and presence of the tested organophosphates/their oxidized solutions. Control tubes contained corresponding ethanol concentrations. 0.02 IU commercial enzyme (in 50 mmol/L phosphate buffer pH 8.0, final volume 0.650 mL) was exposed to desired inhibitor concentrations for 20 min at 37°C. Then, the enzyme substrate, 10 μL 0.075 mol/L ASChI was added in combination with the chromogenic reagent, 20 μL DTNB (0.01 mol/L in 50 mmol/L phosphate buffer pH 7.0). The enzyme reaction was allowed to proceed for 5 min and stopped by adding 65 μL SDS (10%). The absorbance of the yellow-colored product, 5-thio-2-nitrobenzoate was measured at 412 nm (in buffer solution) using Perkin Elmer Lambda 35 UV–Vis spectrophotometer (Shelton, USA). All experiments were done in triplicate in two independent experiments.

Flow-Injection Analysis Based on AChE Bioassay (FIA-AChE Bioassay)

The activity of immobilized AChE incorporated in a flow-injection system was measured by Ellman's method [29] as previously described in details [30]. Briefly, the FIA system consisted of a HPLC pump (Dionex AMP-1), an injection valve (Waters U6K), and a bioanalytical column (40 × 4 mm) filled with 0.55 mg of beads with the immobilized AChE. The carrier buffer (flow rate 0.2 mL/min) was pumped through a flow-through cell (250 mL). The control enzyme activity of the immobilized enzyme in the bioanalytical column was measured by injecting the mixture of the substrate (ASChI) and chromogenic reagent (DTNB). Then, the sample containing the selected organophosphate was injected and the remaining enzyme activity was determined after another injecting the ASChI–DTNB mixture. The absorbance of the produced yellow compound, 5-thio-2-nitrobenzoate at 412 nm was measured spectrophotometrically (Perkin Elmer Lambda 35 UV–Vis spectrophotometer (Shelton, USA)). All measurements were performed in triplicate, in two independent experiments.

RESULTS AND DISCUSSION

Effect of Malathion, Diazinon, and Chlorpyrifos Oxidation on Acetylcholinesterase Assay Sensitivity

Firstly, different malathion concentrations (within the concentration range 1×10^{-9}–5×10^{-4} mol/L) were oxidized with NBS in the following ratios: 1 : 1, 1 : 2, 1 : 10, and 1 : 20 in order to optimize the organophosphate oxidation, which results in a maximal sensitivity and low LOD of AChE assay. The results, expressed as the dependence of remaining enzyme activity (% of control) on malathion concentration, obtained after 20 min exposure of electric eel AChE towards malathion and its oxidized solutions are presented in Fig. 1.

It can be observed that the investigated malathion/malathion oxidized solutions inhibit the enzyme activity in a concentration-dependent manner but with different inhibitory potencies. The experimental plots fitted a sigmoidal function for all cases. The inhibition parameters, IC_{50} (inhibitor concentration with the capability to inhibit 50% of the enzyme activity after a given exposure time) and LOD (inhibitor concentration resulting in 10% enzyme inhibition) values were determined from the corresponding sigmoidal inhibition curves and shown in Table 1.

Obviously, the inhibition curves for malathion oxidized with NBS in all investigated ratios are shifted to lower concentrations compared to unoxidized malathion. This suggests that oxidation with NBS results in an increased AChE sensitivity, thus the assay capability to detect lower thioorganophosphate concentrations. Furthermore, it can be seen that the inhibition curve for the 1 : 10 ratio (Fig. 1, open asterisk) is shifted to the lowest malathion concentrations, suggesting that malathion oxidation is the most efficient in the case of 10 times higher NBS concentration. The values of the inhibition parameters (Table 1) show that IC_{50} for oxidized malathion (3.2×10^{-8} mol/L,

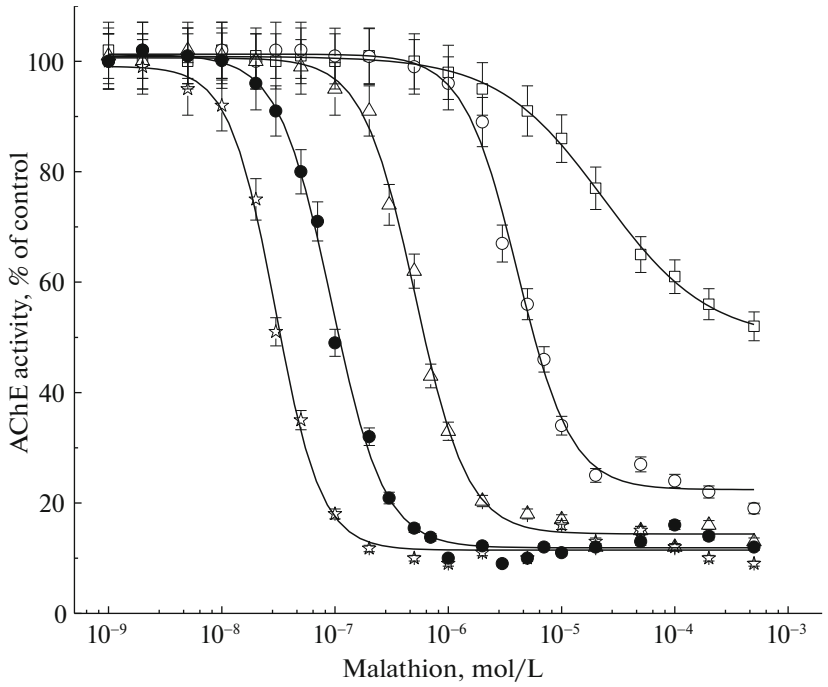


Fig. 1. Dose-dependent electric eel AChE inhibition after 20 min exposure to malathion (open square) and malathion previously oxidized with NBS in ratios: 1 : 1 (open circle), 1 : 2 (open triangle), 1 : 10 (open asterisk), and 1 : 20 (solid circle).

malathion : NBS = 1 : 10) is several thousand times lower than for untreated malathion (IC_{50} = 5.0 × 10^{-4} mol/L). Thus, it is possible to detect 1.0 × 10^{-8} mol/L malathion after its oxidation using AChE bioassay. This LOD concentration is about 500 times lower than LOD obtained for pure malathion (5.1 × 10^{-6} mol/L).

As the oxidation of malathion with NBS in the ratio 1 : 10 was found to be the most favorable for the thioorganophosphate detection by AChE assay, the influence of the oxidation of thioorganophosphates, diazinon and chlorpyrifos on AChE sensitivity was investigated as well. Diazinon and chlorpyrifos (within the concentration range 1 × 10^{-9}–2 × 10^{-4} mol/L) were treated with the oxidizing agent under the same conditions (10 times higher NBS concentration) and tested by electric eel AChE assay (20 min preincubation). Dose-dependent inhibition curves fitted by sigmoidal function were obtained for both untreated thioorganophosphates and after their oxidation (Fig. 2).

IC_{50} and LOD values for diazinon, chlorpyrifos, and after their oxidation with NBS, obtained from the inhibition curves (Fig. 2), are given in Table 2.

It is obvious (Fig. 2) that AChE is significantly less sensitive to diazinon ($IC_{50} > 2.0 \times 10^{-4}$, Table 2) than chlorpyrifos ($IC_{50} = 3.6 \times 10^{-6}$, Table 2) and malathion ($IC_{50} = 5.0 \times 10^{-4}$, Table 1). However, values in Table 2 show that the oxidation treatment was the most efficient for diazinon, resulting in LOD decrease about 1000 times related to untreated diazinon (from 1.3 × 10^{-5} to 1.3 × 10^{-8} mol/L), whereas chlorpyrifos oxidation reduced LOD value about 30 times (from 3.1 × 10^{-7} to 1.0 × 10^{-8} mol/L). Thus, oxidation with NBS, as a pre-step in the determination of thioorganophosphates by AChE assay, enables the detection of about 10 nmol/L thioorganophosphates by AChE assay under the described conditions.

Table 2. IC_{50} and LOD values obtained from AChE inhibition curves for diazinon, chlorpyrifos, and after their oxidation with NBS (1 : 10) (Fig. 2)

Thio organophosphate	IC_{50}, mol/L	LOD, mol/L	Oxidized thio organophosphate	IC_{50}, mol/L	LOD, mol/L
Diazinon	$>2.0 \times 10^{-4}$	1.3×10^{-5}	Diazinon + NBS	4.6×10^{-8}	1.3×10^{-8}
Chlorpyrifos	3.6×10^{-6}	3.1×10^{-7}	Chlorpyrifos + NBS	3.2×10^{-8}	1.0×10^{-8}

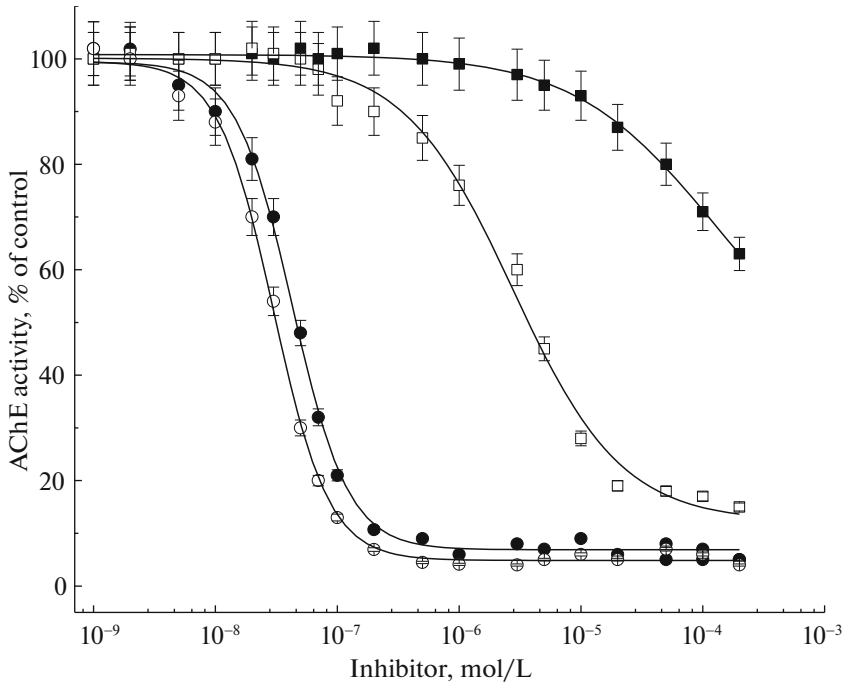


Fig. 2. Concentration-dependent influence of diazinon (solid square), chlorpyrifos (open square) and previously oxidized thioorganophosphates with NBS (1 : 10), diazinon (solid sircle), and chlorpyrifos (open circle) on AChE activity after 20 min preincubation.

The effect of thioorganophoshate oxidation on the sensitivity and LOD of immobilized AChE incorporated in the bioanalytical FIA-system was studied for malathion, diazinon, and chlorpyrifos. The response of FIA-AChE assay towards the investigated thioorganophosphates and their oxidized solutions previously treated with NBS (ratio 1 : 10) was followed within the concentration range 1×10^{-8}–5×10^{-4} mol/L. The obtained inhibition curves describing the dependence of the immobilized AChE activity on the organophosphate concentrations were concentration-dependent and followed a sigmoidal shape (Fig. 3).

The inhibitory parameters as the indicators of FIA-AChE assay sensitivity, IC_{50} and LOD values obtained from the corresponding inhibition sigmoidal curves for the investigated thioorganophosphates before and after their oxidation are presented in Table 3.

Obviously, the inhibition curves for all untreated thioorganophosphates are significantly shifted to lower concentrations after their treatment with the oxidizing agent (Fig. 3). Furthermore, IC_{50} and LOD values (Table 3) indicate that the NBS-induced oxidation affected the most the decrease of chlorpyrifos detection (about 200 times). The IC_{50} of untreated chlorpyrifos (1.7×10^{-4} mol/L) achieved 1.2×10^{-6} mol/L after 10 min oxidation with NBS, whereas the corresponding LOD value was decreased from 4.1×10^{-5} to 1.8×10^{-7} mol/L. In the case of diazinon and malathion, the sensitivity of FIA-AChE assay was increased about several dozen times. Actually, LOD values decreased from 1.8×10^{-5} and 3.4×10^{-5} mol/L to 1.3×10^{-6} and 7.2×10^{-7} mol/L for diazinon and malathion, respectively (Table 3). Accordingly, the FIA-AChE assay can detect the thioorganophosphates at low micromolar concentrations after their

Table 3. IC_{50} and LOD values obtained from FIA-AChE inhibition curves for diazinon, chlorpyrifos, malathion, and after their oxidation with NBS (1 : 10) (Fig. 3)

Thio organophosphate	IC_{50}, mol/L	LOD, mol/L	Oxidized thio organophosphate	IC_{50}, mol/L	LOD, mol/L
Diazinon	$>2 \times 10^{-4}$	1.8×10^{-5}	Diazinon + NBS	5.1×10^{-6}	1.3×10^{-6}
Chlorpyrifos	1.7×10^{-4}	4.1×10^{-5}	Chlorpyrifos + NBS	1.2×10^{-6}	1.8×10^{-7}
Malathion	1.8×10^{-4}	3.4×10^{-5}	Malathion + NBS	3.1×10^{-6}	7.2×10^{-7}

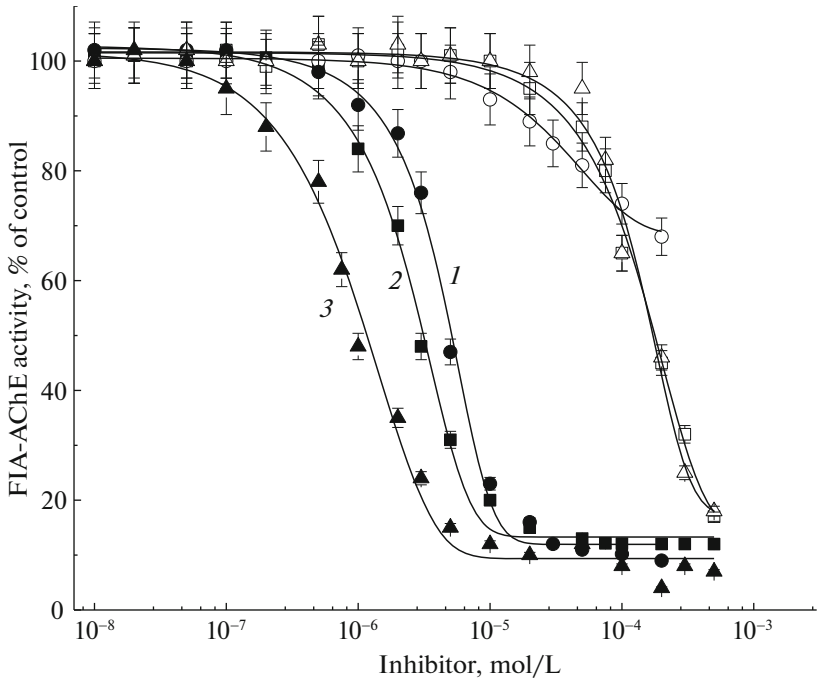
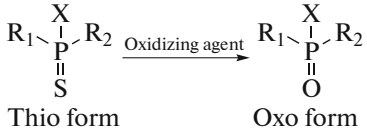


Fig. 3. Concentration-dependent inhibition of immobilized AChE activity in FIA system induced by diazinon (open circle), malathion (open square), chlorpyrifos (open triangle), oxidized diazinon (*1*), oxidized malathion (*2*), and oxidized chlorpyrifos (*3*). Oxidation of the thioorganophosphates was carried out with NBS (ratio 1 : 10) for 10 min.

oxidation with NBS under optimal conditions prior to injecting them into the FIA system.

Thioorganophosphate insecticides, which are used as commercial preparations due to greater safety for human health during their application compared to oxo forms, are over 100 times weaker AChE inhibitors related to their oxo analogs [16, 17]. Thus, the detection limit of organo-thiophosphates, i.e., the lowest concentration that can be detected by AChE bioassay is significantly higher than the detection limit of the corresponding oxons [30]. One of the ways to decrease the detection limit of thioorganophosphates is to convert them into related oxo forms by the action of an oxidizing agent (Scheme 2), which results in a greater decrease in enzyme activity at the same concentrations.

Various oxidizing agents to treat thioorganophosphates such as hydrogen peroxide, bromine water, NBS, and enzymes were reported [19, 21–26], as well as indirect electrochemical oxidation [27]. As NBS proved to be an effective oxidizing agent [28], it was



Scheme 2. Conversion of thioorganophasphate to corresponding oxon.

used to treat malathion in various concentration ratios of 1 : 1, 1 : 2, 1 : 10, and 1 : 20 in order to find optimal conditions resulting in complete oxidation of the thio form. The dependence of the remaining AChE activity on malathion concentrations before and after oxidation with the investigated NBS concentrations (Fig. 1, Table 1) showed that oxidation was the most effective for the treatment of malathion with 10 times higher NBS concentration. In the case of 1 : 10 malathion-NBS ratio, the inhibition curve moves to the greatest extent towards lower concentrations, which induces a decrease in the detection limit of malathion, i.e. an increase in the sensitivity of AChE. However, if the concentration of NBS is further increased in relation to the concentration of malathion (ratio 1 : 20) (Fig. 1), there is a shift of the inhibition curve to the right towards higher concentrations, which indicates a decrease in the sensitivity of AChE and an increase in the LOD of malathion (Table 1). This could result from the disappearance of the formed oxon compared to amounts expected from stoichiometry due to the effect of the increased concentration of NBS. This assumption was experimentally proven by the GC-MS method, which showed a reduced peak of malaoxon with an increase in malathion : NBS ratio [28]. Moreover, if IC_{50} values for malaoxon (Table 4) and oxidized malathion (1 : 10) (Table 1) are compared, a similarity can be observed ($IC_{50} \approx 10^{-8}$ mol/L) suggesting the conversion of malathion into malaoxon after the addition of the oxidizing agent. Similar inhib-

Table 4. IC_{50} values for electric eel AChE (20 min preincubation) and FIA-AChE bioassays induced by malaoxon, diazoxon, chlorpyrifos-oxon, and isomalathion

AChE assay		FIA–AChE assay	
organophosphate	IC_{50}, mol/L	organophosphate	IC_{50}, mol/L
Malaoxon	2.1×10^{-8}	Malaoxon	3.4×10^{-6}*
Diazoxon	5.1×10^{-8}**	Diazoxon	3.1×10^{-6}
Chlorpyrifos-oxon	3.0×10^{-8}**	Chlorpyrifos-oxon	1.1×10^{-6}
Isomalathion	3.3×10^{-7}	Isomalathion	2.7×10^{-6}*

* [30], ** [17].

itory potencies of malaoxon (Table 4) and malathion after 1 : 10 NBS treatment (Table 3) can be noticed for FIA-AChE based assay as well ($IC_{50} \approx 3 \times 10^{-6}$ mol/L). Dose-dependent inhibition curves for isomalathion and oxo forms of malathion, diazinon, and chlorpyrifos—malaoxon, diazoxon, and chlorpyrifos-oxon obtained after 20 min exposure to electric eel AChE and for AChE-FIA bioassay are shown in Figs. 4 and 5, respectively. IC_{50} values determined from these sigmoidal inhibition curves for electric eel AChE and FIA-AChE bioassays are presented in Table 4.

Similar values of the inhibitory parameters for diazoxon and chlorpyrifos-oxon (Table 4) and their parent thio forms after the NBS-induced oxidation in the most efficient ratio (Tables 2 and 3) indicate a potential stoichiometric conversion of the thio to oxo forms. Nevertheless, it should take into account the fact that iso forms of thioorganophosphates such as isomalathion are strong AChE inhibitors as well, similar to the corresponding oxons (Figs. 4 and 5, Table 4), and its potential formation could also result in the decrease of the enzyme activity. Furthermore, the mixtures of malathion, diazinon, or chlorpyrifos and their related compounds were reported to possess additive inhibitory effects to AChE [16, 17], and thus induce an additional enzyme inhibition. Consequently, AChE responses would not result only from oxidized thioorganophosphate action in an analyzed solution and the assay could not be regarded as appropriate for its detection.

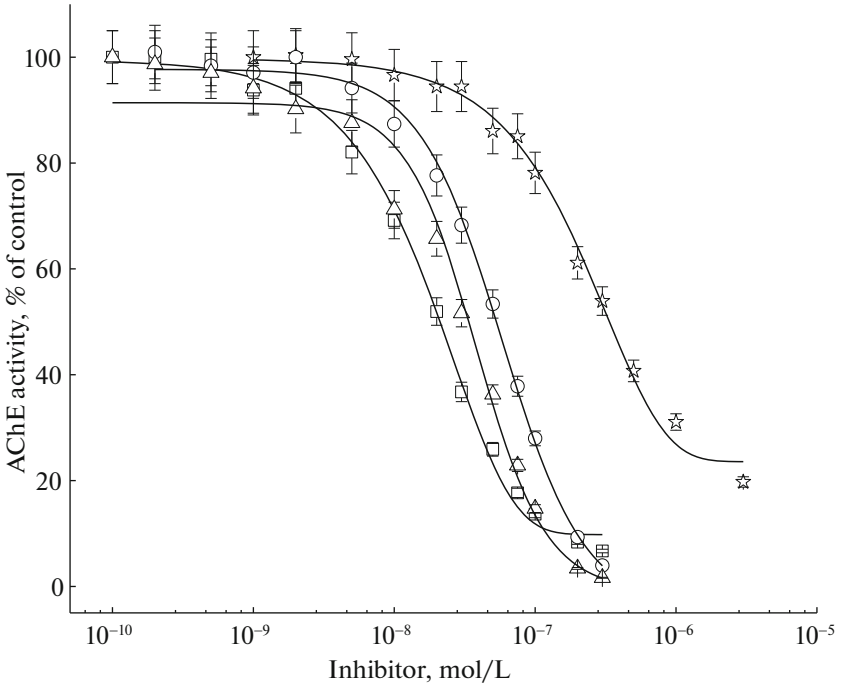


Fig. 4. Concentration-dependent inhibition of electric eel AChE activity after 20 min exposure to diazoxon (open circle) [17], malaoxon (open square), isomalathion (open asterisk), and chlorpyrifos-oxon (open triangle) [17].

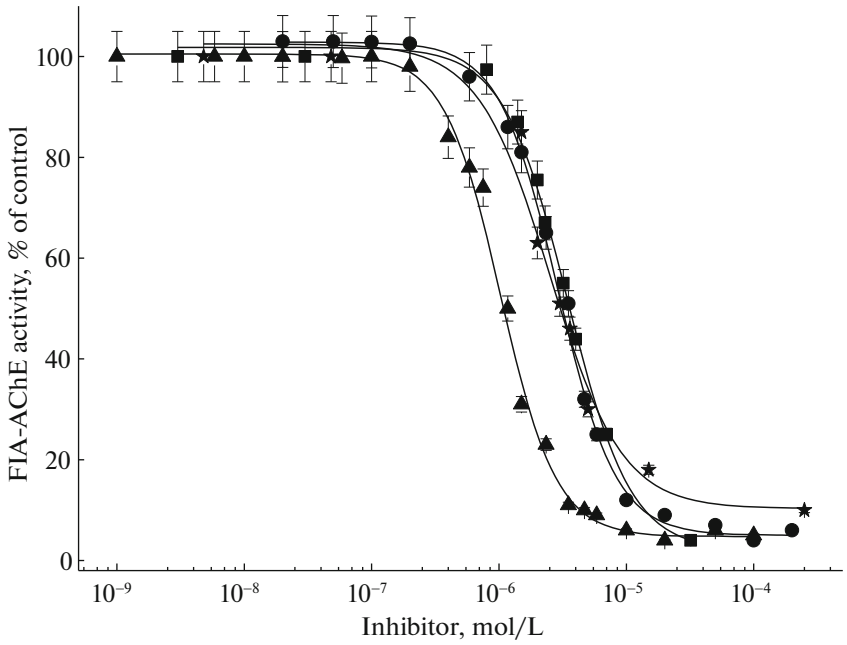


Fig. 5. Concentration-dependent inhibition of immobilized AChE activity in FIA system induced by diazoxon (solid circle), malaoxon (solid square) [30], isomalathion (solid asterisk) [30], and chlorpyrifos-oxon (solid triangle).

CONCLUSIONS

The oxidation pretreatment of the thioorganophoshate, malathion with the oxidizing agent, NBS in the ratio 1 : 10 was found to be optimal and resulted in the highest increase in the sensitivity of AChE-based assay. This oxidation pre-step was shown to be the most efficient for diazinon, which decreased LOD of free electric eel AChE assay about a thousand times and enabled the determination of the investigated thioorganophoshates—diazinon, malathion, and chlorpyrifos in 10 nmol/L concentration range. The results for FIA-AChE assay indicated an increase in the sensitivity of the immobilized enzyme up to two hundred times after the thioorganophosphate oxidation with NBS (ratio 1 : 10), which can detect the thioorganophosphates at low micromolar concentrations.

FUNDING

This study was supported by the Ministry of Education, Science, and Technological Development of the Republic of Serbia (contract no. 451-03-9/2022-14/200017).

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

1. M. B. Čolović, T. Lazarević-Pašti, and V. M. Vasić, in *Chlorpyrifos: Toxicological Properties, Uses and Effects on Human Health and the Environment* (Nova Science, New York, 2015), p. 87.
2. D. Dara and A. P. Drabovich, J. Environ. Sci. **125**, 735 (2023). https://doi.org/10.1016/j.jes.2022.03.033
3. T. D. Lazarević-Pašti and M. B. Čolović, in *Organophosphorus Pesticides: Structural Characteristics, Mechanisms of Toxicity, and Effects of Exposure on Health* (Nova Science, New York, 2016), p. 1.
4. T. Thosapornvichai, C. Huangteerakul, A. N. Jensen, and L. T. Jensen, Environ. Toxicol. Pharm. **96**, 104000 (2022). https://doi.org/10.1016/j.etap.2022.104000
5. X. Wu, J. Li, Z. Zhou, Z. Lin, S. Pang, P. Bhatt, S. Mishra, and S. Chen, Front. Microbiol. **121**, 717286 (2021). https://doi.org/10.3389/fmicb.2021.717286
6. M. B. Čolović, D. Z. Krstić, T. D. Lazarevic-Pašti, A. M. Bondžić, and V. M. Vasić, Curr. Neuropharmacol. **11**, 315 (2013). https://doi.org/10.2174/1570159X11311030006
7. A.-L. Oropesa, S. Sánchez, and F. Soler, Ibis **159**, 510 (2017). https://doi.org/10.1111/ibi.12476
8. J. J. Chaves, F. Soler, M. Perez-Lopez, and A. L. Oropesa, Rev. Toxicol. **34**, 124 (2017).
9. M. B. Čolović, V. M. Vasić, N. S. Avramović, M. M. Gajić, D. M. Djurić, and D. Z. Krstić, Toxicol. Lett. **233**, 29 (2015). https://doi.org/10.1016/j.toxlet.2015.01.003
10. Y. Zhao, C. Fang, C. Jin, Z. Bao, G. Yang, and Y. Jin, Pestic. Biochem. Phys. **182**, 105028 (2022). https://doi.org/10.1016/j.pestbp.2021.105028
11. M. B. Čolović, D. Z. Krstić, V. M. Vasić, A. M. Bondžić, G. S. Uščumlić, and S. D. Petrović, Hem. Ind. **67**, 217

(2013).
https://doi.org/10.2298/HEMIND120323060C

12. Y. Zhao and R. Zheng, Int. J. Electrochem. Sci. **16**, 211221 (2021).
https://doi.org/10.20964/2021.12.30
13. J. Li, H. Chang, N. Zhang, Y. He, D. Zhang, B. Liu, and Y. Fang, Talanta **2531**, 124092 (2023).
https://doi.org/10.1016/j.talanta.2022.124092
14. Y.-H. Chu,Y. Li, Y.-T. Wang, B. Li, and Y.-H. Zhang, Food Chem. **254**, 80 (2018).
https://doi.org/10.1016/j.foodchem.2018.01.187
15. A. Sahin, K. Dooley, D. M. Cropek, A. C. West, and S. Banta, Sens. Actuators, B **158**, 353 (2011).
https://doi.org/10.1016/j.snb.2011.06.034
16. D. Krstić, M. Čolović, K. Krinulović, D. Djurić, and V. Vasić, Gen. Physiol. Biophys. **26**, 247 (2007).
https://pubmed.ncbi.nlm.nih.gov/18281741/
17. M. B. Čolović, D. Z. Krstić, G. S. Uščumlić, and V. M. Vasić, Pestic. Biochem. Phys. **100**, 16 (2011).
https://doi.org/10.1016/j.pestbp.2011.01.010
18. T. Matsushita, Y. Fujita, K. Omori, Y. Huang, Y. Matsui, and N. Shirasaki, Chemosphere **261**, 127743 (2020).
https://doi.org/10.1016/j.chemosphere.2020.127743
19. X. Yang, J. Dai, L. Yang, M. Ma, S.-J. Zhao, X.-G. Chen, and H. Xiao, J. Sci. Food Agric. **98**, 2624 (2018).
https://doi.org/10.1002/jsfa.8755
20. W. Li, R. Wu, J. Duan, C. P. Saint, and J. van Leeuwen, Water Res. **105**, 1 (2016).
https://doi.org/10.1016/j.watres.2016.08.052
21. A. A. Gouda, A. S. Amin, R. E. Sheikh, and M. A. Akl, Chem. Ind. Chem. Eng. Q. **16**, 11 (2010).
https://doi.org/10.2298/CICEQ090417003G
22. T. Lazarević-Pašti, T. Momić, A. Onija, Lj. Vujisić, and V. Vasić, Microchim. Acta **170**, 289 (2010).
https://doi.org/10.1007/s00604-010-0324-2
23. T. Lazarević-Pašti, M. Čolović, J. Savić, T. Momić, and V. Vasić, Pestic. Biochem. Phys. **100**, 140 (2011).
https://doi.org/10.1016/j.pestbp.2011.03.001
24. A. Boškin, C. D. Tran, and M. Franko, Environ. Chem. Lett. **7**, 267 (2009).
https://doi.org/10.1007/s10311-008-0161-2
25. I. Lyagin and E. Efremenko, Int. J. Mol. Sci. **22**, 1761 (2021).
https://doi.org/10.3390%2Fijms22041761
26. G. P. Cetrangolo, J. Rusko, C. Gori, P. Carullo, G. Manco, M. Chino, and F. Febbraio, Sensors (Basel) **20**, 1365 (2020).
https://doi.org/10.3390%2Fs20051365
27. T. D. Lazarević-Pašti, A. M. Bondzić, I. A. Pašti, and V. M. Vasić, Pestic. Biochem. Phys. **104**, 236 (2012).
https://doi.org/10.1016/j.pestbp.2012.09.004
28. M. Bavcon Kralj, P. Trebše, and M. Franko, Acta Chim. Slov. **53**, 43 (2006). http://acta-arhiv.chem-soc.si/53/53-1-43.pdf
29. G. L. Ellman, K. D. Courtney, V. Andreas, and R. M. Featherstone, Biochem. Pharm. **7**, 88 (1961).
https://doi.org/10.1016/0006-2952(61)90145-9
30. D. Krstić, M. Čolović, M. Bavcon-Kralj, M. Franko, K. Krinulović, P. Trebše, and V. Vasić, J. Enzym. Inhib. Med. Ch. **23**, 562 (2008).
https://doi.org/10.1080/14756360701632031