Scientific Paper

Oxidation as a Pre-step in Determination of Organophosphorus Compounds by the AChE-TLS Bioassay†

Mojca Bavcon Kralj, Polonca Trebše, Mladen Franko

Laboratory for Environmental Research, Nova Gorica Polytechnic, Vipavska 13, P. O. Box 301, 5001-SI, Nova Gorica, Slovenia

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Abstract

The oxidation efficiency of *N*-bromosuccinimide (NBS), hypochloride and other oxidants such as m-CPBA, Oxone, CAN, iodine water, hydrogen peroxide in aqueous solution and juice samples spiked with four model thio-organophosphorus compounds (thio-OPs): azinphos-methyl, chlorpyrifos, diazinon and malathion, was studied. The complete conversion into oxons was noticed within five minutes in case of NBS, a partial conversion in case of sodium hypochlorite, and no oxo-products were observed using other oxidants. Since the NBS is efficient in acidic conditions and hypochlorite in alkaline media, the hydrolysis during the oxidation process can be significantly avoided using appropriate pHs.

All oxidation experiments were verified using GC-MS analysis. Compounds of interest were quantified by using standard solutions of thio-OPs and identification of formed oxo-OPs was based on MS spectra. The formed oxo-analogs were detected using AChE-bioassay with TLS detection, which enabled determination of parent thio-compounds at ppb levels. With a rapid (5 min) oxidizing pre-step the AChE-bioassay can serve as a fast pre-screening test (15 min) to identify samples that contain AChE inhibitors, including the thio-OPs.

Key words: organophosphorus pesticides, oxidation, AChE-TLS bioassay

Introduction

The extensive use of pesticides to enlarge the production in agriculture has lead to growing input of these pollutants into environment over the last decades. Organophosphorus (OP) pesticides are among most wide spread pollutants used for pest control with many applications, but with the growing importance as insecticides, acaricides, nematocides, and helmithicides.[1] They are a large and highly diverse family of organic chemicals that express acute lethality not only to insects but also to mammals, since they are able to inhibit acetylcholinesterase (AChE), an enzyme vital to normal nerve function.[2] Many of the commercially available organophosphorus (OP) insecticides are phosphorothionates (lipophilic), which are characterized by one thione moiety (P=S) and three -OR groups attached to a phosphorus atom, whereas their respective oxidized analogues are more polar, characterized by a double phosphorus oxygen bond (P=O), which actually makes them potent AChE inhibitors.[1]

An alternative for approaching the large amount of daily based analysis in pesticide residues covered by the employment of highly sophisticated and time-consuming chromatographic techniques are the biosensors and related bioanalytical techniques. In most bioassays for organophosphorus pesticides, the inhibition of enzymatic activity, in particular acetylcholine esterase (AChE) and butyrylcholine esterase (BChE), is most frequently exploited.[3] However, other enzymes have also been used, e.g. tyrosinase, organophosphorus hydrolase and alkaline phosphatase.[4-6] The used enzymes are usually immobilized, i.e., bound by covalent or non-specific interactions to a solid surface, where the reaction of pesticides with enzymes causes inhibition depending on the type of pesticide and its concentration.[4] Generally, the amount of detected pesticides is reported as a cumulative concentration of all present AChE inhibiting compounds, expressed in equivalents of pesticide used for calibration (usually paraoxon).

For the reasons of optimisation, reduction of time, and higher reproducibility several biosensor/bioassay set-ups are based upon flow injection analysis (FIA).[3-5] The performance of AChE-FIA assays was further improved, and LOD's lowered by introducing a highly sensitive method of optical detection relying on thermal lens spectrometry (TLS).[7-9]

OPs and their metabolites in environmental matrices are generally present in small quantities

(ppb-, rarely in ppm- concentration range). Therefore, the extraction of these compounds from solid matrices or their preconcentration from aqueous media is frequently needed and requires the use of organic solvents, depending on the solubility of extracted pesticide or group of pesticides.[10-14] Furthermore, the detection of thio-OPs, which are due to agricultural practice the dominant form of OPs in environmental matrices, requires oxidation of thio-OPs into their oxo analogues, because the thio-OPs are much weaker inhibitors or do not inhibit AChE at all.[5,15-23] For this purpose Bromine[15-18] and *N*-bromosuccinimide (NBS)[5,19-22] were introduced as selective and rapid oxidants to enhance inhibition in determination of thio-OPs by biosensors. Oxidations were performed either directly in water samples or in solvents which were then diluted in working buffer prior analysis. The first biosensor method for the detection of phosphorothionates in food was developed by H. Schulze and his research group, using NBS as an oxidizing reagent.[21-23] However the complete assay could not be performed in less than 2 h.[21]

Other methods of oxidations were also developed till now, but were mainly intended for synthesis of oxons from thio-phosphates. Oxidations were performed on P=S or P=Se moieties with alkyl or O-alkyl side chains with the use of different common oxidants: dinitrogen tetraoxide and nitric acid,[24,25] peroxide and dioxane,[26] dimethyl sulfoxide,[27-29] peroxyacids,[30-33] trifluoroacetic anhydride,[34,35] dimethyldioxiran,[36] potassium peroxymonosulfate,[37] ozone,[38] and perfluoro-*cis*-2,3-dialkyloxaziridine[39,40]. However the listed oxidants, which were mainly meant as reagents in chemical synthesis of oxons, were found most efficient in organic solvents and under drastic experimental conditions (reaction time of several hours employing reflux or extremely low/high temperatures, high concentrations of reactants).

It is therefore evident that modifications of existing oxidation methods are needed to avoid false negative results and provide an adequate means for a rapid routine bioanalytical assay such us required in methods for low cost screening of large number of samples.

The aim of our work was thus to search for a rapid, sufficiently effective and specific conversion of selected thio-OPs into oxo-OPs (Figure 1), which could be performed without any preconcentration or extraction step and under normal laboratory conditions in order to enable determination of thio-OPs by the FIA-AChE bioassay with TLS detection.

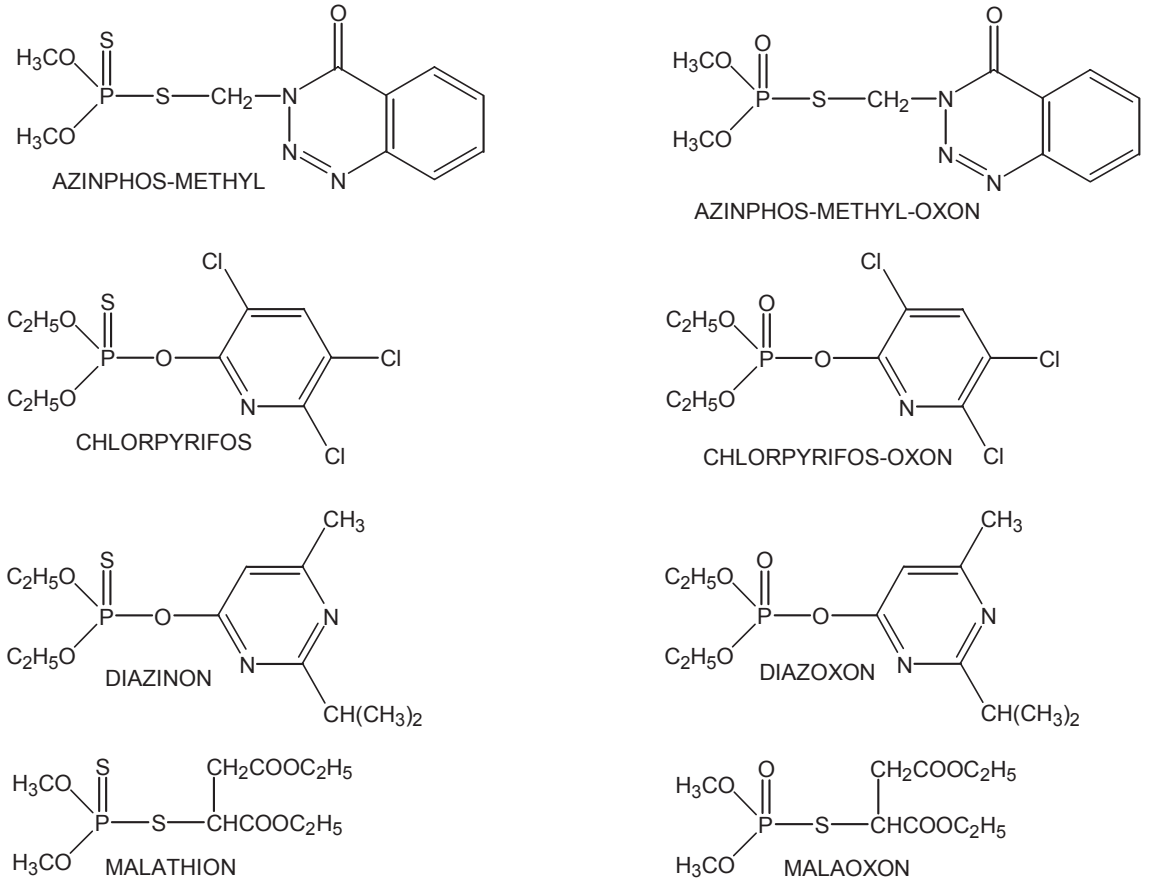


Figure 1. The chemical structures of relevant OP compounds.

Experimental

1. Materials

The pesticides were of minimum 95% purity and no further purification of the chemicals was performed. Azinphos-methyl, chlorpyrifos, diazinon, malathion and malaoxon, were provided from Pestanal.

For the oxidation studies different oxidants were used: *N*-bromosuccinimide – NBS (Fluka), cerium ammonium nitrate - CAN and *m*-chloroperoxybenzoic acid – *m*CPBA (Merck), iodine from RDH, potassium iodide from Lachema, potassium hydrogen monopersulfate - Oxone from Merck, 30% hydrogen peroxide from Carlo Erba, sodium hypochloride with 6% active chlorine from Šampionka Renče (Renče, Slovenia). Reduction and removal of oxidants were achieved using ascorbic acid from Kemika Zagreb (Zagreb, Croatia).

Solvents used for extraction methods were obtained from different suppliers: ethyl acetate from Carlo Erba, hexane from RDH. Solvents were dried over anhydrous sodium sulphate purchased from RDH.

For the bioassay measurements acetylcholinesterase (AChE) from electric eel (950 IU mgL^{-1}), acetylthiocholine iodide (ASChI), 5,5'-dithio-bis (2-nitrobenzoic acid) (DTNB), pyridine-2-aldoxime methiodide (2-PAM), controlled-pore glass (CPG 240, 80-120 mesh) were purchased from Sigma Chemicals. Glutardialdehyde (25%), di-potassium hydrogenophosphate, and 3-amino propyltriethoxysilane were obtained from Merck.

2. Oxidation procedure

Pesticide stock solutions were prepared by dilluting approximately 10 mg of pesticide in 10 mL of ethanol. Standard pesticide stock solutions were held in refridgerator until used. Working solutions were prepared daily by dilluting 1 mL of standard stock solution in ethanol to 100 mL of water or apple juice. Working solutions of 10 ppm were then further diluted to desired concentrations as neeeded.

Oxidants and solutions of reducing agents were prepared daily. The volumes of the analytes, oxidants and reducing agents solutions were always in 1 : 1 : 1 volume ratio. In the oxidation experiments, the pesticide concentration ranged from 300 ppb to 10 ppm, the concentration of oxidants was varied to obtain different pesticide to oxidant molar ratios (from 1:1, 1:2, 1:5, 1:10, 1:50, 1:100, 1:500, to 1:1000), whereas the concentration of reducing agent was always set 10 – times higher than oxidant concentration. The oxidation reaction was allowed to proceed for 5 min only, and was interrupted by the addition of a reducing agent.

3. Extraction of pesticides

For the GC analysis of samples two different extraction techniques were used - liquid-liquid extraction (LLE) and solid phase extraction (SPE). LLE extraction was used for oxidation studies in apple juice to avoid exceeding break-through volumes. SPE cartridges (100 mg Strata C18) are specified to retain a mass of solute (analyte plus retained contaminants) that is equivalent to approximately 5% of sorbent mass (in our case 5 mg). Thus SPE was not used in juice analysis, due to low reproducibility. Previously developed LLE method[41] was used instead.

SPE method was used for extraction of pesticides from water samples. General procedure was as follows: cartridge conditioning step: 3 mL of ethyl acetate, 3 mL of methanol, 5 mL of deionised water; sample load: 10 mL of water samples; analyte elution: 3 mL of ethyl acetate; preconcentration step on rotary evaporator. Samples were then rediluted either in ethyl acetate (GC-MS) or in hexane (GC-ECD; GC-MS).

As mentioned, LLE was generally used for extraction of apple juice samples. 10 mL of oxidant solution was added to a 10 mL sample of spiked apple juice, and after 5 min 10 mL of reducent solution was added. After that, an aliquot of 10 mL was taken and extracted with ethyl acetate (60 mL) with the addition of 50 mL Na_2SO_4 water solution (10%). The organic phase was separated and dried over anhydrous Na_2SO_4, evaporated to dryness, the residue was redissolved in 1 mL hexane and analysed by GC/ECD and GC/MS.

4. Analysis of pesticides

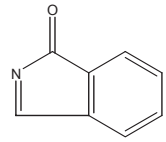
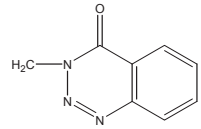
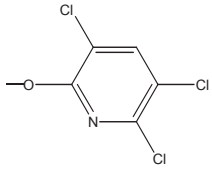
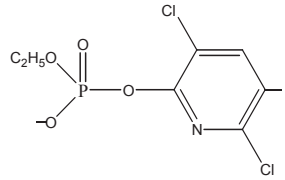
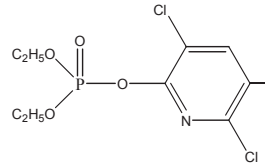
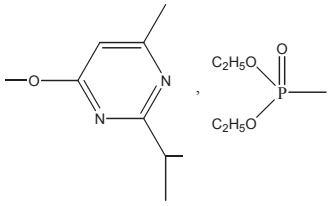
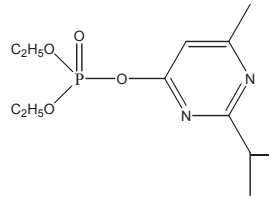
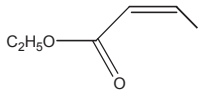
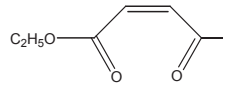
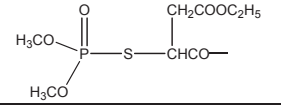
Ethyl acetate and hexane extracts were analysed by gas chromatography coupled with electron capture detector GC-μECD (HP 6890) and gas chromatography – mass spectrometry (Varian, Saturn 2100T). A non-polar SPB-1 column (100% polydimethylsiloxane; 30 m × 0.53 mm; film tickness 3 μm) was used for separation in GC-μECD system, whereas a CP-Sil 8 CB low bleed/MS column (5% phenyl - 95% methylpolysiloxane, 30 m × 0.25 mm; film tickness 0.25 μm) was used in the GC-MS system. Temperature programe was the same in both systems: injector was held at 220 °C, oven temperature started at 80 °C, and was increasing with a gradient of 10 °C/min till 290 °C and mantained constant for 5 min. The injection volume was in all cases 1 μL, the exception was only in case of azinphos-methyl. Due to low sensitivity to this pesticide 2 μL of extracts were injected in GC-MS. In all cases two replicates were performed. The target and qualifier ions of OPs analyzed by GC-MS are summarized in Table 1.

5. Calibration curves

Different ranges of calibration curves were prepared, according to the type of matrix and concentration of pesticide.

For the oxidation studies in water samples, a SPE extraction was used. The respective extraction

Table 1. List of target and qualifier ions for formed oxo-OPs

Organophosphate	Target ion	Qualifier ions	
Azinphos-methyl-oxon	***77*** 	**132** 	**160** 
Chlorpyrifos-oxon	***197*** 	**270** 	**298** 
Diazoxon	***137*** 	**273** 	
Malaoxon	***99*** 	**127** 	**268** 

recoveries were: (92 ± 10)% for azinphos methyl, (88 ± 4)% for chlorpyrifos, (89 ± 2)% for diazinon, (93 ± 5)% for malathion, and (95 ± 10)% for malaoxon. The r-square values of individual regression lines ranged from r^2 = 0.9867 for malaoxon to r^2 = 0.999 for malathion. When the oxidations were performed in apple juice, which is a far more complex matrix than water, the SPE gave low recoveries and irreproducible results. For this purpose a liquid-liquid extraction was performed yielding the respective extraction recoveries: (45 ± 2)% for chlorpyrifos, (66.5 ± 0.3)% for diazinon, (97 ± 3)% for malathion, and (93 ± 9)% for malaoxon. The r-square values of individual regression lines ranged from r^2 = 0.9934 for malaoxon to r^2 = 1.000 for diazinon. Despite the low recoveries in case of some pesticides, e.i. chlorpyrifos and diazinon, the described method enabled the determinations with the RSD in the range of 5-15%.

6. Toxicity measurements using AChE bioassay

6.1 Solutions

0.05 M phosphate buffer containing 45mM NaCl and 12μM $MgCl_2$ (pH 8.0) was prepared in deionised water. The pH was adjusted to 8.0 with 37% HCl. The substrate acetylthiocholine iodide (ASChI) solution was prepared by dissolving 5 mg of ASChI in 3 mL of phosphate buffer and mixing with 1 mL solution of 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB), which was prepared by dissolving 4 mg of DTNB in 10 mL of phosphate buffer. The solution was prepared prior analysis and kept on ice to avoid non-enzymatic hydrolysis. Pyridine-2-aldoxime (2-PAM) was used as a regenerator of inhibited enzyme and was prepared in 4mM concentration in phosphate buffer.

6.2 Enzyme immobilisation

A simple cross-linking with glutaraldehyde and binding to the activated controlled porosity glass according to immobilisation procedure described in literature[7-9] was chosen. The procedure included a pre-cleaning step, where the glass beads (CPG-240) were boiled in nitric acid; an aminoalkylating step, where the dried glass beads were activated with 3-aminopropyl triethoxysilane; the cross-linking with glutaraldehyde under reduced air pressure; and the final step of AChE immobilisation. The immobilised enzyme was then packed into a column (60 × 2,1 mm i.d.) as needed.

6.3 Determination of enzyme activity

The enzyme activity was assayed according to Ellman.[42] Acetylthiocholine iodide (ASChI) was applied as the enzyme substrate with DTNB as a chromogenic reagent. The product of enzymatic reaction is 5-thio-2-nitrobenzoate with absorption maximum at 410 nm (in buffer solution) and was detected optically.

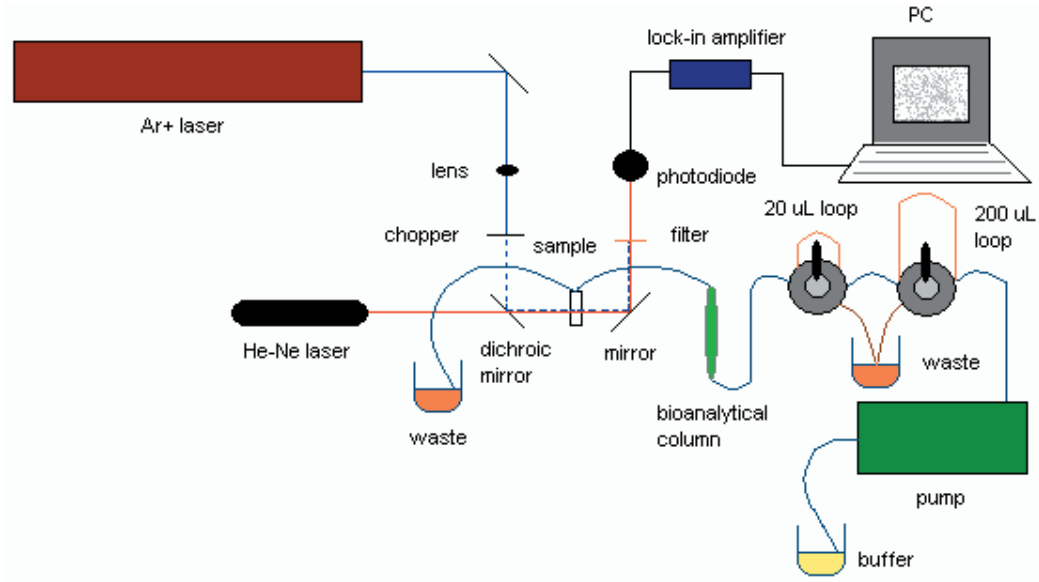


Figure 2. A schematic diagram of the bioanalytical FIA set up with TLS as a detection unit.

6.4 FIA set-up

The flow-injection analysis manifold (Figure 2) used in this work consisted of a HPLC pump, two injection valves, a reactor with immobilised enzyme and the detection unit. The carrier phosphate buffer (pH 8,0) was pumped through the system at a flow rate of 0.5 mL min^{-1}. To determine the initial enzyme activity (a_0) in the bioanalytical column the substrate was injected through the first injection valve equipped with a 20 μL injection loop (for up to 5-times). This was followed by injection of the sample containing the pesticide through the second injection valve with a 200 μL injection loop. The determination of final enzyme activity (a_f) was carried out by another injection of the substrate through the first injection valve (for up to 5-times) and calculation of remaining enzyme activity (A_r) according to the formula: $A_r = a_f / a_0$, where a_0 and a_f are averages of four signal intensities before and after the injection of sample, respectively (when five injections were made, the first signal was neglected).

Each bioanalytical column was used for several determinations of the pesticide. When the activity of the enzyme dropped to 90% of the initial value, than a reactivation using 2-PAM was performed. When the reactivation with 2-PAM could not reach the initial activity, the enzyme was replaced with the fresh one. 10 mg of glass beads with immobilised enzyme was weighted before being filled in the bioanalytical column, making the analysis more reproducible, since the initial activity was equal all the time. The biosensor was daily calibrated with 500 ppb malaoxon (Figure 3), which caused about 50% inhibition and was afterwards reactivated with 2-PAM. With this a reliable intercheck of the system was assured.

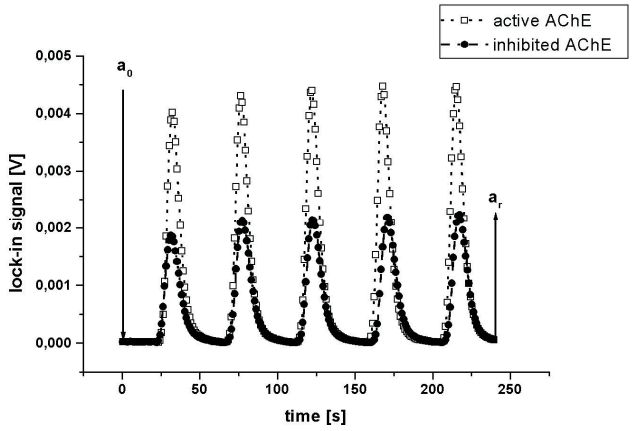


Figure 3. The evolution of TLS signals before and after inhibition with 500 ppb malaoxon

Results and discussion

1. Testing different oxidants

To investigate the efficiency of oxidation in matrices such as water and fruit juice several oxidants (NBS, sodium hypochlorite, peroxide, CAN, mCPBA, iodine and Oxone) and pesticides (azinphos-methyl, chlorpyrifos, diazinon and malathion) in different molar ratios (from 1:1, 1:2, 1:5, 1:10, 1:50, 1:100, 1:500, to 1:1000 pesticide : oxidant molar ratio) were tested in order to determine the most appropriate ratio. Since the kinetics of oxidation is favourable in acidic conditions

Table 2. The oxidation efficiency of selected oxidants (molar ratios are given in brackets beside each oxidant) on thio-OPs (column in italics) conversion to oxo-OPs (column in bold)

Oxidant / OPs	*Remaining diazinon*	Formed diazooxon	*Remaining malathion*	Formed malaoxon	*Remaining chlorpyrifos*	Formed chlorpyrifos-oxon
NBS (1:50)	*4%*	**det. ↓**	*0%*	**det. ↑**	*15%*	**det. ↑**
NBS (1:100)	*0%*	**n.d.**	*0%*	**det. ↓**	*0%*	**det. ↓**
NBS (1:500)	*0%*	**n.d.**	*0%*	**det. ↓**	*0%*	**det. ↓**
NBS (1:1000)	*0%*	**n.d.**	*0%*	**det. ↓**	*0%*	**det. ↓**
CAN (1:50)			*68%*	**n.d.**		
H_2O_2 (1:50)			*82%*	**n.d.**		
H_2O_2 (1:500)	*100%*	**n.d.**			*100%*	**n.d.**
I_2 (1:50)			*90%*	**n.d.**		
***m*-CPBA (1:50)**			*35%*	**n.d.**		
Oxone (1:50)			*77%*	**n.d.**		
Oxone (1:500)	*66%*	**n.d.**			*72%*	**n.d.**
hypochlorite (1:50)	*21%*	**det. ↑**			*28%*	**det. ↓**

det. ↑ – maximal signal area of detected oxon
det. ↓ – signal area of detected oxon in decrease
n.d. – oxon not detected

(reaction within 5 min), the pH of oxidant solution was corrected to pH 2.0 with acids, peroxide with perchloric acid, CAN with nitric acid, Oxone is acidic by its self, whereas in the case of NBS (pH 5.3), sodium hypochlorite and iodine pH was not corrected.

The comparison of data obtained from GC-MS chromatograms and spectra showed that an efficient conversion into oxons in water samples was achieved using NBS and hypochlorite in the molar ratio of 1:10. No conversion into oxons was noticed using oxidants from 1:1 to 1:10 in juice samples, due to naturally present reducing compounds. Thus a higher molar ratio (1:50) was used, yielding satisfactory results in case of NBS and hypochlorite. By increasing the concentration of oxidants which were not efficient at lower concentrations, a disappearance of formed oxons compared to amounts expected from stoichiometry was observed (Table 2).

This can be explained by the consecutive oxidation of the oxons, resulting in the formation of more polar compounds, which were not efficiently extracted in ethyl acetate and therefore not detected by GC-MS. At oxidations using lower pesticide:oxidant molar ratio (1:50 and lower), this phenomenon was not observed.

Chromatograms of samples from oxidation of malathion to malaoxon using various oxidants are collected in Figure 4, and show that the only efficient oxidant in case of malathion was NBS.

Similar results were obtained with other OPs (data not shown). The only exception was noticed in case of diazinon, which expressed promoted hydrolysis under experimental conditions governed by the low pH of the juice (pH 3.2). For this purpose, the use of sodium hypochlorite, which was corrected to basic pH 12.0; seemed to be an alternative to avoid induced hydrolysis (Figure 5). In fact, diazinon is a particularity, since it is susceptible to acidic hydrolysis, whereas the majority of OPs are hydrolyzed in basic pH, like most esters.[1]

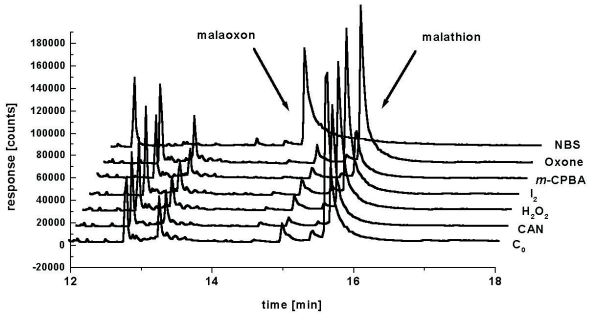


Figure 4. GC-MS chromatograms of malathion oxidation to malaoxon by various oxidants

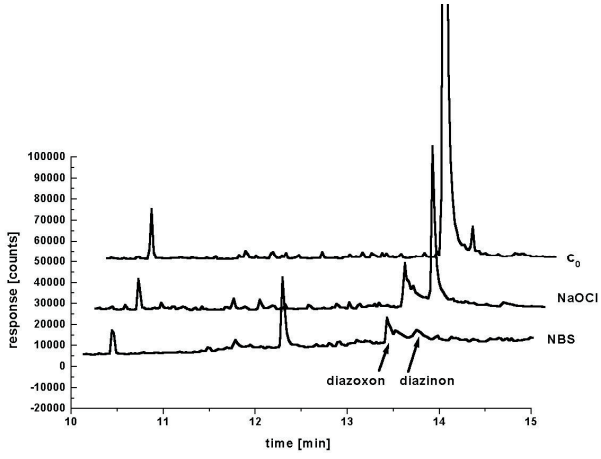


Figure 5. GC-MS chromatograms of diazinon oxidation to diazoxon by NBS and sodium hypochlorite

3. The effect of pH and reducing agents on oxidation efficiency in apple juice

Since the AChE bioassay should be performed in neutral-basic pH range (7.4 – 8,0); a correction of pH is needed prior analysis of acidic juices (pH 3.2). In this experiment the oxidation efficiency after the use of three different reducing agents was determined before and following the adjustment of juice's pH. The experiment was run in natural apple juice (pH 3.2) and pH corrected apple juice (pH 7.4), using a final concentration of malathion of 9,0 ppm. The concentration of NBS was 50-times higher, whereas the concentrations of ascorbic acid (pH 3.2), sodium ascorbate (pH 7.2), and glucose (pH 6.6) as reducing agents were 500-times higher compared to the malathion concentration. Glucose was chosen as a substitute for ascorbic acid or ascorbate, since it is normally present in juices and should thus not interfere with the bioassay. Sodium hydroxide was used to correct the pH of apple juice. As can be seen in Figure 6 the effect of pH of reducing agents is negligible, whereas the effect of the pH of matrix, thus apple juice, is far more important. Since the oxidations are favourable in acidic conditions, the corrections of pH prior bioanalytical measurements should be done after the oxidation.

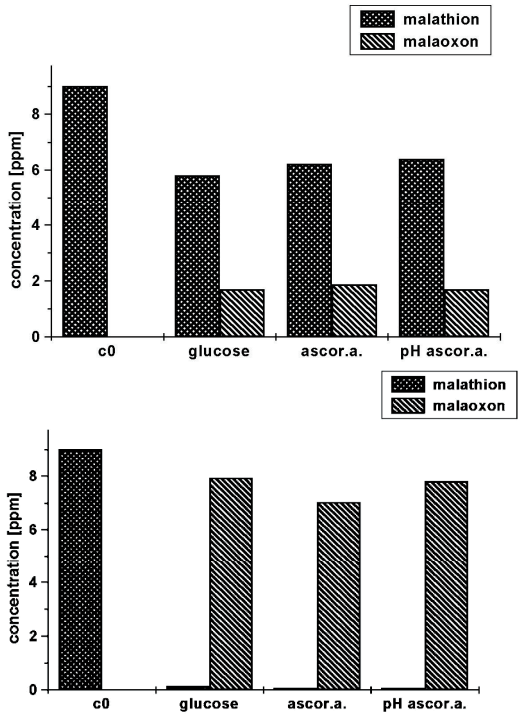


Figure 6. The conversion of malathion to malaoxon in pH corrected apple juice (pH 7.2) – left and in natural apple juice (pH 3.2) – right

4. The effect of oxidation procedure on the AChE-bioassay

Prior to inhibition measurements with AChE bioassay, NBS as an oxidant and ascorbic acid as a reducing agent, were tested as potential inhibitors of AChE. Their presence resulted in no decrease of enzyme activity (Figure 7).

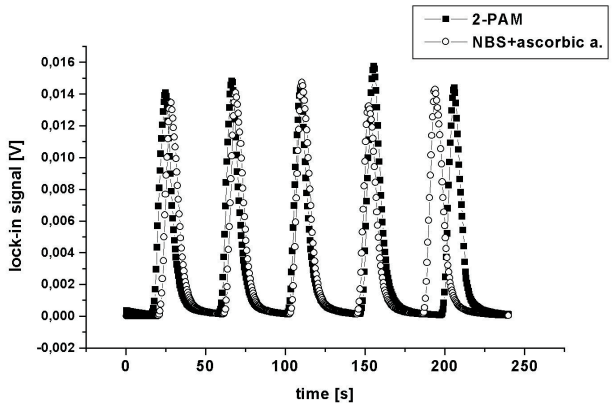


Figure 7. The effect of NBS and ascorbic acid on the activity of AChE

To evaluate the suitability of the described AChE - bioassay for detection of phosphorothionates (thio-OPs), samples spiked with the selected thio-OPs (azinphos-methyl - 15.4 ppm, chlorpyrifos - 20.8 ppm, malathion – 9 ppm) were oxidized with NBS. The oxidized samples were split in two. One part was chromatographically separated on the GC-MS system to check for the quantitative conversion of the thio-OPs into the oxo-OPs, which was satisfactory achieved in 5 min. The second part of the sample was checked for the inhibitory power of the formed oxo-OPs on the AChE enzyme. No decrease in activity of the enzyme due to native thio-OPs substances was observed, whereas a complete inhibition was achieved when the oxidized samples were injected into the bioanalytical column. To obtain a reasonable inhibition curve supplementary dilutions of samples were needed, since oxons are far more powerful inhibitors.

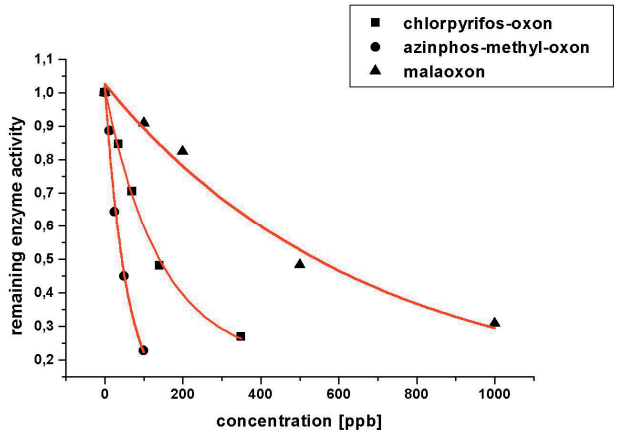


Figure 8. Inhibition curves for detection of malathion, chlorpyrifos and azinphos-methyl after oxidation to their respective oxons by the FIA AChE-TLS bioassay

After the oxidation inhibitions of AChE activity from 10 to 80% were observed for chlorpyrifos in 35 – 350 ppb concentration range, for malathion in 100 – 1000 ppb concentration range, and for azinphos-methyl in the 12.5 – 100 ppb concentration range (Figure 8). The lower limits of given concentration ranges are also representing the LOD values for determination of corresponding pesticides, which are in all cases well below the maximal residue limits for OPs in foodstuffs.[44,45]

Conclusions

The oxidation with different commonly used oxidants (NBS, sodium hypochlorite, m-CPBA, Oxone, CAN, iodine water, hydrogen peroxide) and reducing agents (glucose, ascorbic acid) has shown that a reliable, rapid and selective oxidation of phosphorothionates (model compounds: malathion, diazinon, chlorpyrifos, azinphos-methyl) to their respective oxons (malaoxon, diazoxon, chlorpyrifos-oxon, azinphos-methyl-oxon) can be achieved with NBS and sodium hypochlorite within five minutes. The oxidizing and reducing agents were directly added to water or juice. Time of analysis was therefore reduced in contrast to previously reported oxidation procedures, since no preconcentration, extraction or other analytical step was needed. The AChE bioassay based on the investigated oxidation procedure enables determination of thio-OPs in the ppb concentration range in less than 15 minutes.

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Povzetek

Proučevali smo učinkovitost kemijske oksidacije žveplovih organofosfatnih pesticidov (azinfos-metil, klorpirifos, diazinon in malation) v okso-organofosfatne analoge (azinfos-metil-okson, klorpirifos-okson, diazinon-okson in malaokson) v vodnih raztopinah in vzorcih sadnega soka. V ta namen smo uporabili *N*-bromsukcinimid (NBS), natrijev hipoklorit, *m*-kloroperoksibenzojsko kislino, kalijev peroksimonosulfat, cerijev amonijev nitrat, jodovico ter vodikov peroksid. Učinkovito oksidacijo v času krajšem od petih minut smo dosegli z uporabo NBS-a. Delno smo lahko oksidirali še diazinon v diazokson z uporabo natrijevega hipoklorita. Vsi ostali oksidanti pa so se v danih pogojih izkazali kot neprimerni. Z izbiro NBS-a v kislih pogojih in natrijevega hipoklorita v alkalnih se lahko učinkovito izognemo stranskih reakcijam hidrolize.

Z uporabo plinske kromatografije na masno detekcijo smo spremljali potek oksidacijskih eksperimentov in spremljali izginevanje žveplovih ter nastajanje kisikovih analogov na podlagi masnih spektrov. Nastale okso-produkte smo detektirali z uporabo imobilizirane acetilholinesterazne v pretočnem sistemu za injekcijsko analizo in spektrometrije na osnovi termičnih leč (AChE-TLS). To nam je omogočilo določevanje tio-OP v ppb koncentracijskem območju. S pomočjo hitre (5 min) oksidacije, lahko analizo posameznega vzorca s pomočjo AChE-TLS bioanalitske tehnike opravimo v 15 minutah. S tem je omogočen lažji in temeljitejši nadzor in odkrivanje vzorcev onesnaženih s holinesteraznimi inhibitorji, vključno z žveplovimi analogi.