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Identification of fenthion and temephos and their transformation products in water by high-performance liquid chromatography with diode array detection and atmospheric pressure chemical ionization mass spectrometric detection

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Abstract

Two organophosphorus pesticides, fenthion and temephos were oxidized with N-bromosuccinimide (NBS) in order to obtain the oxidated analogues which are the activated form of the pesticides. Analysis of the non-oxidized solution containing the pesticides, and two oxidized solutions which differed in the amount of NBS added was performed with both LC–diode array detection and NMR to confirm the presence of the degradation products. LC–atmospheric pressure chemical ionization-MS was used to unequivocally identify all the transformation products formed. With this technique it was possible to identify oxo derivatives, sulfoxides and isomers of both fenthion and temephos. Fenthion and its transformation products were characterized with both positive and negative mode of ionization whereas temephos was analyzed at an extraction voltage of 20 V to gain in sensitivity and at 40 V to enhance fragmentation. With both fenthion and temephos, the isomeric forms of these pesticides, the oxons and the sulfoxides eluted before the parental pesticide. It was observed that an increase of NBS produced fenthion sulfoxide oxon and temephos dioxon while the parental pesticides disappeared from these samples. In the case of temephos, the oxidation of this pesticide with NBS reproduce environmental conditions, since these same transformation products were also found in environmental waters that had been treated with temephos. © 1997 Elsevier Science B.V.

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1. Introduction

Organophosphorus pesticides are comprised within the ten most widely used pesticides all over the world. Among this family of pesticides, fenthion and temephos occupy a prominent position since they are applied in large quantities to combat agricultural buds and mosquito pests, respectively. These compounds, after being applied, remain in the environment for some days depending, basically, on their intrinsic properties as well as climatic conditions. During this period, these pesticides undergo chemical, physical and biological changes which result in the formation of degradation products, which may be more toxic than the parent compound [1]. In this sense, identification of oxo and sulfoxide derivatives of fenthion and temephos have been reported in waters after some days of sunlight exposure [2,3]. Another work reports the formation of temephos oxon, the sulfoxide and temephos isomer in the environment after a phytosanitary treatment [4]. The analytical protocols used for determining the parent

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compounds are in many cases not valid for determining the transformation products, since they are more polar than the parent compounds. It is obvious that there is a need to develop analytical methods capable of determining the pesticides along with their degradation products in environmental waters. The method which has been recommended by the US Environmental Protection Agency (EPA) involves gas chromatographic analysis with selective detection such as nitrogen–phosphorus or electron-capture detection [5]. These methods are not suitable to determine most of the polar degradation products such as nitrophenols without a time-consuming derivatization step. Such compounds, which are characterized as highly polar, thermolabile and non-volatile, are preferably analyzed by liquid chromatography (LC), which appears as the most appropriate way of pesticide separation. Most commonly, ultraviolet photodiode array detection (DAD) is used to identify organophosphorus pesticides and has been applied in numerous works concerning pesticide monitoring [6–8]. However, LC–DAD does not render structural information, and limits the applicability of the technique in environmental analysis, since it cannot provide confirmation or identification of unknowns. Another problem that arises for the analysis of pesticides degradation products is the lack of pure standards. In this sense, it is compulsory to use techniques which provide both quantitative as well as qualitative results. LC coupled to mass spectrometry with thermospray interface has been applied to identify pesticides and their degradation products in many matrices such as water [9], soil or sediments [10,11]. Thermospray interface has been accepted and validated by the EPA, and is at the moment the official method used by many laboratories involved in pesticide monitoring [12]. However, the thermospray process often generates few ions per compound (1 or 2) which makes identification of unknowns difficult and often it is necessary to use MS–MS [13]. As a consequence of all the above mentioned, nowadays there is a clear tendency to use atmospheric pressure ionization interfaces (API) such as ionspray (ISP) or atmospheric pressure chemical ionization (APCI) for pesticide monitoring, since that technique offers much lower limits of detection and provides more structural information compared to thermospray (TSP). Previous works related pesticide analysis include those of Molina et al. [14], who state that limits of detection of various pesticides, not TSP amenable, are at levels of pg in selected ion monitoring, indicating the suitability of the technique for pesticide residue analysis. Chiron et al. [15] and Lacorte and Barceló [16] report the coupling of ESP-MS and APCI-MS interfaces with on-line liquid–solid extraction, respectively. In both instances, detection limits of ng/l are obtained when analyzing ground water, and Lacorte and Barceló [16] report that below 20% variation is obtained from day-to-day analysis. Besides gathering acceptable detection limits, API techniques are characterized by the fact that by increasing the extraction potential more structural information is obtained, with fragment spectra similar to that obtained by collision-induced dissociation (CID). As a consequence, identification of unknowns from environmental water matrices is facilitated. However, at this point, references on the characterization of pesticide degradation products with this technique are scarce since on one hand, there is a lack of commercially available degradation products and on the other, most studied are centered in the identification and quantitation of the parental pesticides. In this sense, the present work was developed to determine two priority pesticides, fenthion and temephos, and their degradation products after samples that contained the parental pesticides were oxidized with N-bromosuccinimide (NBS) and kept in acetonitrile until analysis. The objective was to oxidize the parental pesticides with two different amounts of NBS to produce as many degradation products as possible. Afterwards, the parental solution with the intact pesticide and the oxidized samples were analyzed with LC–DAD and nuclear magnetic resonance (NMR), and LC–APCI-MS to identify unequivocally the oxidated transformation products of the above mentioned pesticides and to asses the effect of the oxidation process. This information was used to identify temephos degradation products that had been formed in the environment after spraying the area of the Ebre delta with temephos.

2. Experimental

2.1. Chemicals

HPLC-grade water and acetonitrile (Merck, Darm-

stadt, Germany) were filtered through a 0.45 μm filter before use. The pure standards fenthion, fenthion oxon and fenthion sulfoxide were obtained from Dr. Erhenstorfer (Augsbourg, Germany) and temephos and temephos sulfoxide from Promochem (Wesel, Germany). NBS was from Merck–Schuchardt (Munich, Germany).

2.2. Sample preparation

Fenthion and temephos were dissolved at a concentration of 10^{-3} *M* in acetonitrile. NBS solution was prepared in water at a concentration of 10^{-2} *M*. The NBS solution was added at each ml of the solution containing the organophosphorus pesticides in different ratios in order to obtain different oxidated compounds: (i) $R=0$ corresponds to the non-oxidized pesticide solution, (ii) $R=2$ indicates that each ml of pesticide solution contains 0.2 ml of NBS and (iii) $R=4$ indicates 0.4 ml of NBS for each ml of the pesticide solution. In all cases, this final solution was mixed in an ultrasonic bath for 1 min and analyzed by LC–DAD, NMR and LC–APCI-MS.

2.3. Chromatographic conditions

2.3.1. LC–DAD

Two HPLC pumps Jasco 880-PU (Spectroscopic Co., Japan) with a Rheodyne injector valve 7125 connected to a Waters 996 Photodiode Array Detector (Waters, Milford, MA, USA) were used. A LiChrocart cartridge column (125×4 mm I.D.) packed with LiChrospher 100 RP18 of 5 μm of C_{18} (Merck) was used. Elution was carried out with acetonitrile and water with two different gradient elution programs. The gradient used for samples which contained fenthion was: isocratic at acetonitrile–water (45:55) for 15 min, then to 100% of acetonitrile in 15 min, a condition which was kept for 5 min. The gradient used for the samples which contained temephos was: acetonitrile–water (60:40) for 15 min, then to 100% of acetonitrile in 10 min, a condition which was maintained for 5 min. In both cases, the flow-rate was kept at 1 ml/min and the system was restabilized at initial conditions in 5 min. 20 μl of sample were injected each time.

2.3.2. LC–APCI-MS

The eluent was delivered by a gradient system from Waters 616 pumps coupled to a Model Waters 600S controller (Waters, Milford, MA, USA). The same analytical column and gradient elution program as described in Section 2.3.1 was used. Prior to sample analysis, flow-injection (FI) APCI-MS experiments without the use of the analytical column were performed with acetonitrile–water in order to characterize the two pesticides and those transformation products which were commercially available. The flow-rate was set at 1 ml/min and the amount of each standard injected was 400 ng through a 20 μl loop.

The analyses were performed with a VG Platform from Micromass (Manchester, UK) equipped with an APCI interface. The system includes a Megalynx software for instrument control and data processing. The interface and instrument set-up is described in detail elsewhere [16]. All samples were analyzed in positive mode of ionization in scan conditions. Moreover, samples which contained fenthion, were analyzed in negative mode to gain in sensitivity. In positive mode of ionization, the cone voltage was set at 20 and 40 V, to gain in sensitivity or structural information, respectively, and in all cases the corona discharge was set at 3.5 kV. The HV lens voltage was set at 0.15 kV whereas the focus voltage varied between 10 and 86 V. The ion source was set at 200°C and the probe temperature was 500°C. In negative mode of operation, cone and corona discharge voltages were set at 40 V and 3.5 kV, respectively; HV lens voltage was set at 0.21 kV and focus voltages varied from −11 to 100. Nebulizing and drying gas were maintained at the maximum flow of 20 and 300 l/h, respectively.

2.4. NMR analysis

The oxidized samples were purified using semi-preparative chromatography with an Ultrabase UB 535 column of 250 mm-1/2 C_8 of 5 μm particle size from Shandon SFCC (France). The pure compounds collected at each fraction were analyzed with NMR spectrometry to confirm their identification. The NMR spectrometer was a Jeol 400 MHz (Japan). The following list reports the conditions used:

H NMR (C^2HCl_3). Fenthion: d: 6.9–7.1 (m, 3H, H Ar), 3.83 (d, 6H, OCH_3 $J=13.6$ Hz from P), 2.42

(s, 3H, SCH_3), 2.30 (s, 3H, $ArCH_3$). Fenthion oxon: d: 6.9–7.1 (m, 3H, H Ar), 3.84 (d, 6H, OCH_3 $J=11.4$ Hz from P), 2.41 (s, 3H, SCH_3), 2.31 (s, 3H, $ArCH_3$). Fenthion sulfoxide: d: 7.0–8.0 (m, 3H, H Ar), 3.85 (d, 6H, OCH_3 $J=13.6$ Hz from P), 2.67 (s, 3H, SCH_3), 2.35 (s, 3H, $ArCH_3$). Fenthion sulfoxide oxon: d: 7.0–8.0 (m, 3H, H Ar), 3.87 (d, 6H, OCH_3 $J=11.2$ Hz from P), 2.66 (s, 3H, SCH_3), 2.35 (s, 3H, $ArCH_3$).

H NMR ([$^2H_6$]DMSO). Temephos: d: 7.37 (d, 4H, $H_{33'55'}Ar$, $J=8.8$ Hz), 7.19 (d, 4H, $H_{22'66'}Ar$, $J=8.4$ Hz), 3.80 (d, 6H, OCH_3 $J=13.6$ Hz from P). Temephos oxon: d: 7.37 (d, 4H, $H_{33'55'}Ar$, $J=8.8$ Hz), 7.23 (d, 2H, $H_{26}Ar$, $J=8.8$ Hz), 7.19 (d, 2H, $H_{2'6'}Ar$, $J=8.4$ Hz), 3.80 (d, 3H, OCH_3 $J=13.6$ Hz from P), 3.79 (d, 3H, OCH_3 $J=11.6$ Hz from P). Temephos dioxon: d: 7.37 (d, 4H, $H_{33'55'}Ar$, $J=8.8$ Hz), 7.23 (d, 4H, $H_{22'66'}Ar$, $J=8.8$ Hz), 3.79 (d, 6H, OCH_3 $J=11.6$ Hz from P). Temephos sulfoxide: d: 7.77 (d, 4H, $H_{33'55'}Ar$, $J=8.4$ Hz), 7.38 (d, 4H, $H_{22'66'}Ar$, $J=8.0$ Hz), 3.79 (d, 6H, OCH_3 $J=11.2$ Hz from P).

2.5. Environmental water sample analysis

A water sample, collected from a rice field that had been treated with temephos and had been previously analyzed by LC–TSP (see Ref. [2]) was filtered through a 0.45 μm filter to remove the suspended particles. 1 l of this sample was solid-phase extracted with Empore C_{18} Extraction disks. Previous to sample preconcentration, the disk was conditioned with 10 ml of acetonitrile, 10 ml of methanol and 10 ml of water. Elution was carried out with 2×10 ml of acetonitrile. The sample was evaporated and finally a 100 μl extract with acetonitrile was obtained. This sample was analyzed by LC–APCI-MS with the main purpose of identifying temephos and their degradation products. In order to gather maximum sensitivity, selected ion monitoring was performed, and the ions selected covered the whole range of possible degradation products that might appear (e.g., at m/z 139, 249, 435, 457, 451, 466, 473 and 483) and included also two characteristic ions of phosphorotioates, e.g., at m/z 109 and 125. Gradient elution was performed, in this case, with acetonitrile and water with the program: from acetonitrile–water (10:90) to 100% acetonitrile in 30 min. The gradient started at high percentage of water to avoid interferences from the humic material present in the environmental waters. Cone and corona discharge voltages were set at 40 V and 3.5 kV, respectively. 20 μl of the water extract was injected onto the same analytical column as previously described in Section 2.3.

3. Results and discussion

3.1. LC–DAD

Fig. 1 shows the LC–DAD chromatogram of a standard which contained the parental pesticide fenthion and the degradation products which were formed as a result of the oxidation process. In the case of fenthion, this compound eluted at 19 min, and could be identified by retention time and spectral comparison against a standard. Early eluting peaks were identified as possible degradation products of fenthion due to spectral similarity with the original form. Fig. 2 represents the standard with temephos and their degradation products. Similarly, the degradation products of temephos present higher polarity and elute earlier than the parental pesticide. In both cases, the UV spectra of each compound is shown. The comparison of their retention time (t_R), their relative retention time (rrt) and UV spectrum with those of the standard solution (when available) permitted the identification of fenthion and temephos and some of their transformation products. The identification of the degradation products of fenthion was done through the purification of each compound and NMR analysis. The presence of oxo derivatives in the chromatographic profile is a first indication of the efficiency of the oxidative process. However, as it can be seen from Figs. 1 and 2, LC–DAD did not permit the identification of some peaks. Therefore, NMR and LC–APCI-MS were performed in order to confirm the results obtained and with the intention to identify the highest number of degradation products.

3.2. LC–NMR

One of the main problems of LC–DAD is its limitation in identifying the nature of a degradation product for which there is no standard available. For

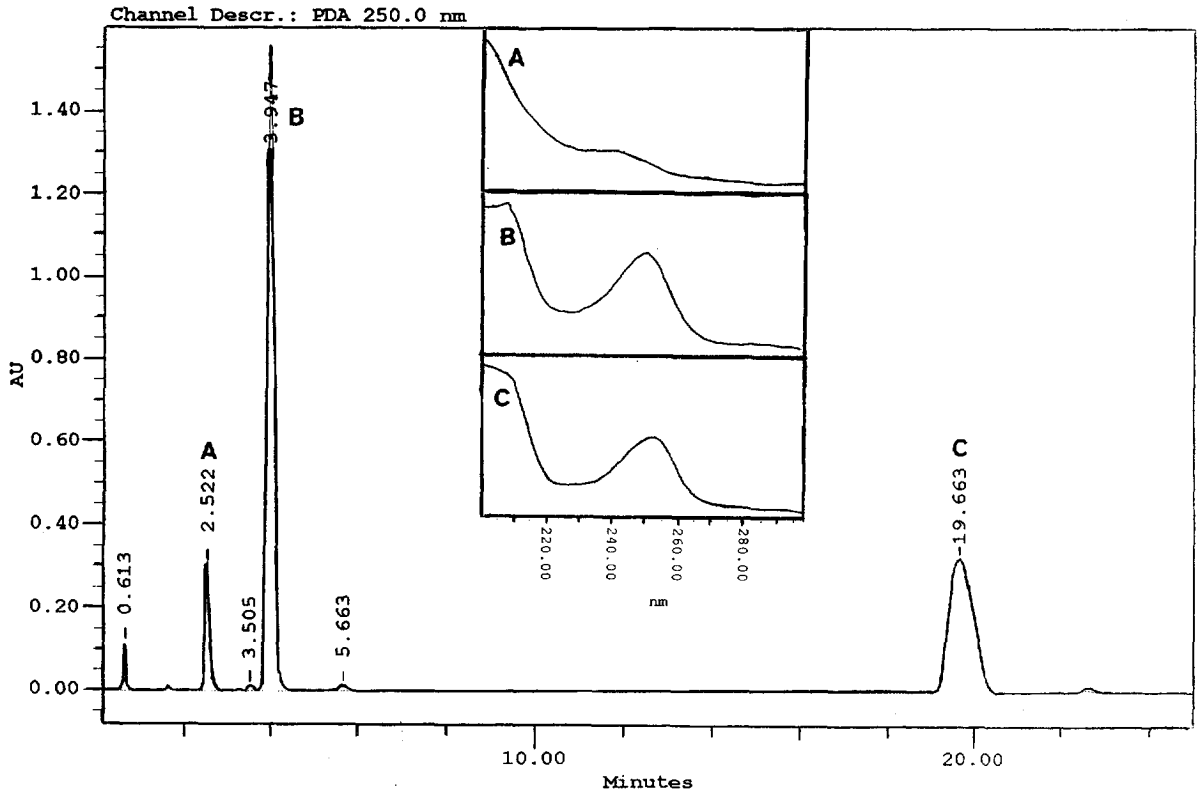

Fig. 1. LC–DAD chromatogram obtained at 250 nm which corresponds to oxidized fenthion. Peak identification: A = fenthion sulfoxide, B = fenthion oxon and C = fenthion. Analytical conditions described in Section 2.3. Amount injected = 400 ng through a 20 μl loop. The analytical conditions were: isocratic at 45% of acetonitrile and 55% of water for 15 min, then to 100% of acetonitrile in 15 min, condition which was kept for 5 min. In both cases, the flow-rate was kept at 1 ml/min and the system was restabilized at initial conditions in 5 min.

such reason, the oxidized samples were purified (95% purity) and each compound was analyzed with NMR to identify the degradation products formed. In the case of fenthion, the peaks that eluted at 2.522 and 3.947 were identified as fenthion sulfoxide and fenthion oxon. Oxidation from phosphorotioate (P=S) to phosphate (P=O) was identified by a decrease of phosphorus–proton coupling constant of CH_3O (13.6 Hz for P=S to 11.4 Hz for P=O). Proton chemical shift of CH_3S was increased by oxidation of sulfide (2.42 ppm for sulfide to 2.66 ppm for sulfoxide). Similarly, temephos oxon and dioxon were identified unequivocally by NMR analysis. The phosphorus–proton coupling constant decreased from 13.6 to 11.6 Hz. The effect of sulfide oxidation was the increase of the aromatic proton chemical shifts (+0.4 ppm for $H_{33'55'}$ and +0.15 ppm for $H_{22'66'}$).

3.3. LC–APCI-MS

LC–APCI-MS is characterized by the fact that it renders enough structural information as to identify pesticides transformation products in water samples and it provides limits of detection below 0.1 μg/l. The analytical parameters, such as method reproducibility, repeatability and detection limits have been described in a recent paper [16] in which also few organophosphorus pesticides are characterized. In this study where transformation products are involved, flow injection analysis (FIA) were carried out prior to sample analysis to characterize all the standards available (fenthion, fenthion oxon and sulfoxide and temephos and temephos sulfoxide). The results obtained with FIA are identical to those obtained with the oxidized samples, and therefore they are not reported. APCI-MS gave, in all cases

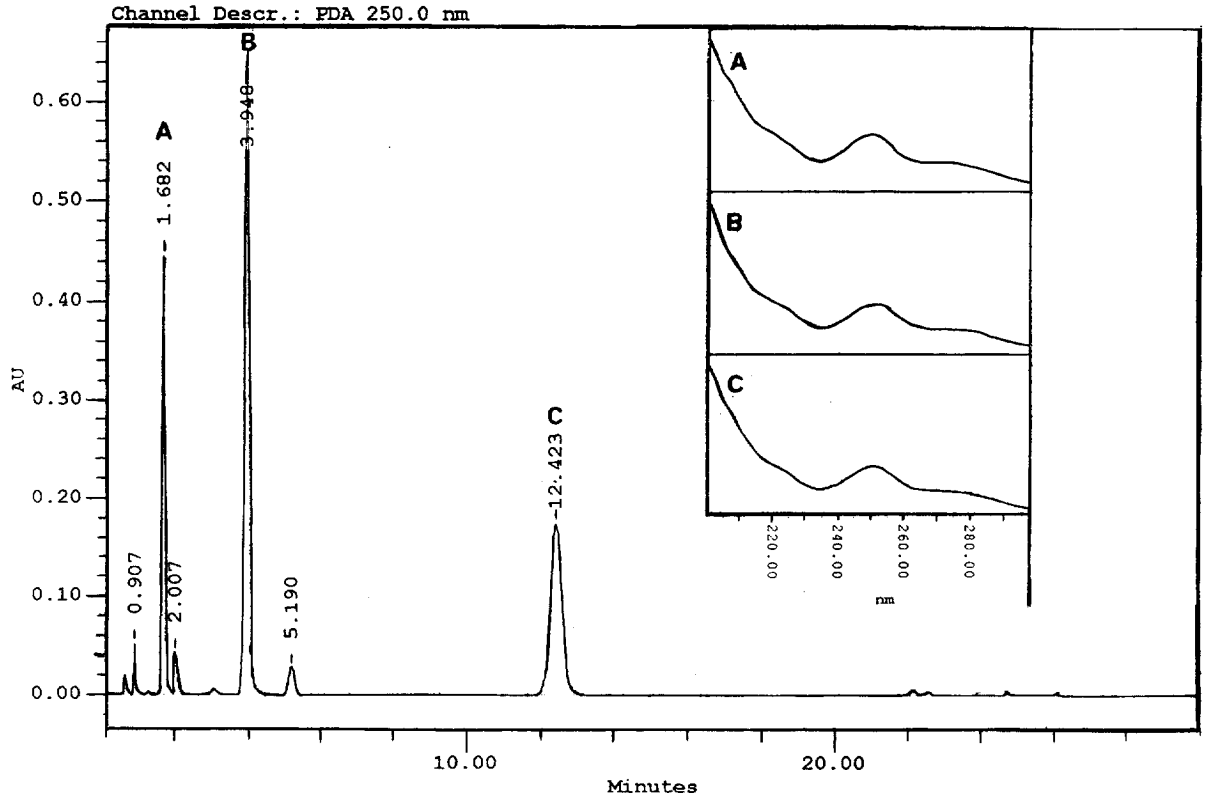

Fig. 2. LC–DAD chromatogram obtained at 250 nm which corresponds to oxidized temephos. Peak identification: A = temephos dioxon, B = temephos oxon and C = temephos. Analytical conditions described in Section 2.3. Amount injected = 400 ng through a 20 μl loop. The analytical conditions were: at 60% of acetonitrile and 40% of water for 15 min, then to 100% of acetonitrile in 10 min, condition which was maintained for 5 min.

more than three different fragment ions which permit the unequivocal identification and confirmation of a pesticide. If the results herewith obtained are compared with those of Lacorte and Barceló [2] who utilized a TSP interface, it is clear that more structural information is obtained in using the APCI interface. Moreover, at an extraction voltage of 40 V, it is possible to obtain molecular peak and fragment ions at low m/z values, which allow compound identification. In the case of fenthion, both positive (PI) and negative (NI) modes of ionization were applied in order to detect as many transformation products as possible and as a mean of confirmation of the results obtained with PI.

The oxidative reaction produced oxo-analogues, sulfoxides and isomeric forms of fenthion and temephos. All these compounds could be identified by LC–APCI-MS. Figs. 3 and 4 illustrate the schema of the possible transformation routes followed by fenthion and temephos after oxidation. All the transformation products were unequivocally identified following the rules depicted below:

3.3.1. Samples that contained fenthion

For fenthion, NI offers more sensitivity due to the fact that the aromatic moiety is stabilized by a delocalized electron. The typical ions of all the degradation products identified, which include oxo and sulfoxide derivatives, are reported in Table 1. Moreover, the isomer of fenthion was also identified and it eluted before their original compound. This can be explained by the more polar structure P=O of the thiophosphate group as compared to the P=S bond, which results in a lesser affinity towards the

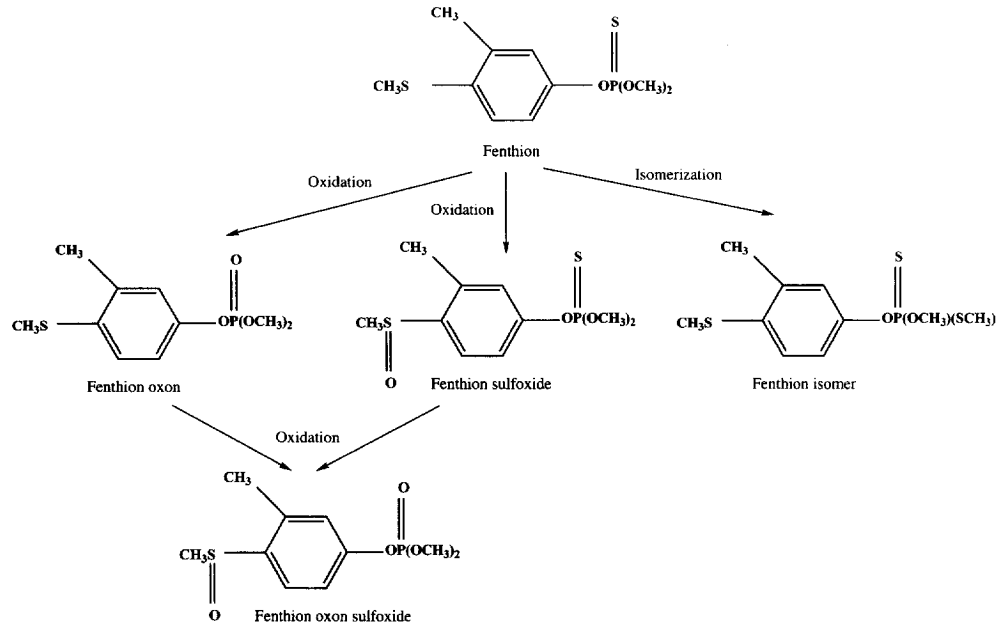

Fig. 3. Structural formula of fenthion and their degradation products and schema of the oxidative pathways after oxidation of fenthion with NBS.

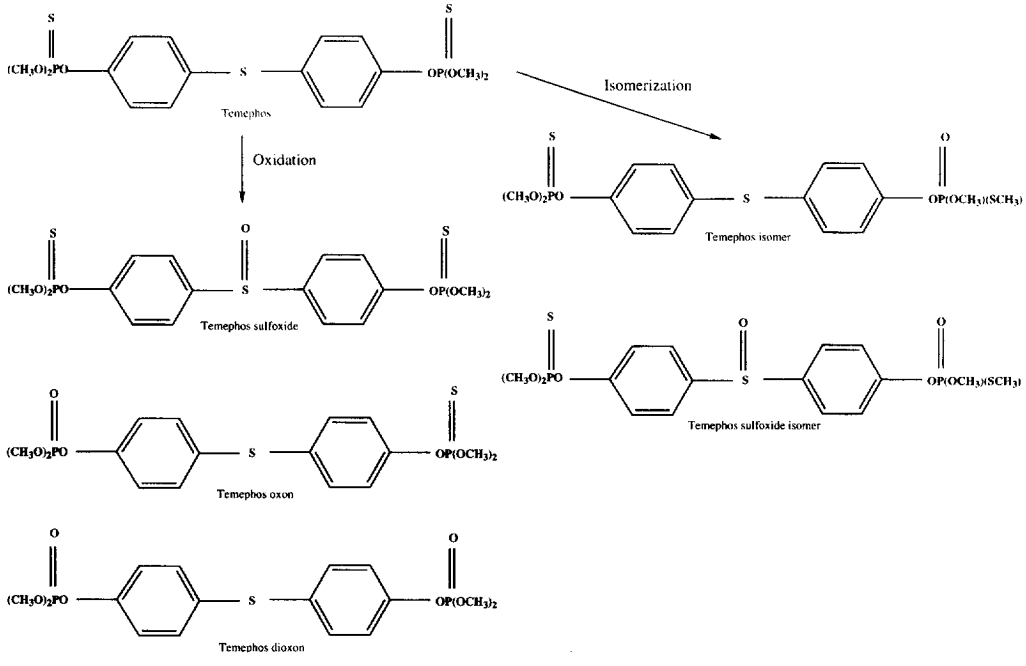

Fig. 4. Structural formula of temephos and their degradation products and schema of the oxidative pathways after oxidation of temephos with NBS.

Table 1
Characterization by LC–APCI-MS of fenthion and the degradation products formed as a result of the oxidation process

Compound identified	t_R (min)	PI	NI
F. sulfoxide oxon M_r=278	0.99	109(45) $[(CH_3O)_2PO]^+$ 125(14) $[(CH_3O)_2PO_2]^+$ 154(23) $[M-(CH_3O)_2PO_2+H]^+$ 264(93) $[M-CH_3+H]^+$ 279(100) $[M+H]^+$	n.d.
Fenthion sulfoxide M_r=294	2.76	109(21) $[(CH_3O)_2PO]^+$ 125(10) $[(CH_3O)_2PS]^+$ 154(20) $[M-(CH_3O)_2PS.CH_3]^+$ 278(39) $[M-O]^+$ 280(100) $[M-CH_3+H]^+$ 295(95) $[M+H]^+$	154(100) $[M-(CH_3O)_2PS.CH_3]^-$ 169(60) $[M-(CH_3O)_2PS]^-$ 279(9) $[M-CH_3]^-$
Fenthion oxon M_r=262	4.55	109(13) $[(CH_3O)_2PO]^+$ 137(58) $[M-(CH_3O)_2PO_2]^+$ 216(37) $[M-(CH_3O)CH_3]^+$ 231(97) $[M-CH_3O]^+$ 263(100) $[M+H]^+$	125 (16) $[(CH_3O)_2PO_2]^-$ 138(100) $[OC_6H_3-SCH_3]^-$ 153(77) $[M-(CH_3O)_2PS]^-$ 247(43) $[M-CH_3]^-$
Fenthion isomer M_r=278	6.70	125(51) $[(CH_3O)(CH_3S)PO]^+$ 137(21) $[M-(CH_3O)(CH_3S)PO_2]^+$ 153(81) $[M-(CH_3O)(CH_3S)PO]^+$ 200(12) $[M-(CH_3O)(CH_3S)]^+$ 216(45) $[M-(CH_3S)CH_3]^+$ 231(100) $[M-CH_3S]^+$ 279(50) $[M+H]^+$	n.d.
Fenthion M_r=278	20.82	109(100) $[(CH_3O)_2PO]^+$ 125(54) $[(CH_3O)_2PS]^+$ 137(22) $[M-(CH_3O)_2PSO]^+$ 153(26) $[M-(CH_3O)_2PS]^+$ 231(27) $[M-CH_3S]^+$ 278(42) $[M]^+$ 279(17) $[M+H]^+$	138(82) $[OC_6H_3-SCH_3]^-$ 141(48) $[(CH_3O)_2POS]^-$ 153(84) $[M-(CH_3O)_2PS]^-$ 263(100) $[M-CH_3]^-$

Information on molecular mass (M_r), retention time in minutes (t_R) and ions formed (m/z and % abundance) is given. Results are reported for extraction volatge of 40 V and corona discharge of 3.5 kV. Positive (PI) and negative (NI) modes of ionization were used.
n.d.=Not detected.

stationary phase. In this sense, fenthion sulfoxide oxon was the first eluting compound due to the double structure P=O and S=O.

Samples were injected at an extraction voltage of 40 V. The high voltage increased the fragmentation of the analytes and fragment ions at low m/z values were released as a result of the high CID. In this way, identification of the different compounds was enhanced. The means used to identify all the transformation products were:

3.3.1.1. Fenthion

The characteristic ions of fenthion under PI were at m/z 109, which appeared as base peak; m/z 125, which corresponds to a typical fragment ion of dimethyl-phosphorothiolates and m/z 278 which corresponds to the molecular peak. The other fragment ions are listed in Table 2. In NI mode, the loss of the methyl group from the aromatic moiety renders m/z as base peak. The presence of fragment ions at m/z 263 and 153 is in accordance with

Table 2
Fenthion and the transformation products identified by LC–APCI-MS

t_R (min)	rrt	Relative area (%)		Compounds identified
		$R=2$	$R=4$	
0.99	0.048	1.4	100	F. sulfoxide oxon, m/z 264
2.76	0.132	100	43	F. sulfoxide, m/z 280
4.55	0.225	91	0.37	F. oxon, m/z 263
6.7	0.321	3.6	n.d.	F. isomer, m/z 231
20.82	1.00	1.3	n.d.	Fenthion, m/z 109

t_R = retention time; rrt = relative retention time; n.d. = not detected; R = molar ratio NBS/fenthion.

previous works which used APCI interface [16]. The characterization of fenthion by both modes of ionization at different ions (at least three) is a way to identify and confirm the presence of a pesticide in a sample.

3.3.1.2. Fenthion isomer

This transformation product eluted at 6.7 min, and has the same molecular mass as its parental pesticide. Since the process of isomerization changes the sulfur of the dimethyl-phosphorotioate group with the oxygen of the methoxy group, it is expected that this compound would render the same fragmentation pattern as the parental compound. In this case, the fragment ions at m/z 125, 137, 153, 231 and 278 correspond to equivalent loses. As an example, the ion at m/z 125 corresponds, in the case of fenthion to $[(CH_3O)_2PS]^+$ while in the case of its isomer it is related to $[(CH_3O)(CH_3S)PO]^+$. However, the two compounds could be distinguished by the fact that the isomer presented the ion at m/z 200 which correspond to the loss of $[(CH_3O)(CH_3S)]$. Fenthion isomer was also identified as such due to the absence of fragment ion at m/z 109.

3.3.1.3. Fenthion oxon

Fenthion oxon presented the protonated molecule at m/z 263 as base peak. The presence of the fragment at m/z 109 $(CH_3O)_2PO$ and the absence of $(CH_3O)_2PS$ indicates that the compound corresponds to a oxo-derivative. The other specific ions at m/z 137, 216 and 231 correspond to looses of fragments of the molecule, equivalent to those observed for fenthion (Table 1). In the NI mode, the structure at m/z 125 corresponds to $(CH_3O)_2PO_2]^-$ which corresponds a characteristic fragment ion of the dimethylphosphates. The other ions formed are equivalent to those obtained for fenthion.

3.3.1.4. Fenthion sulfoxide

Fenthion sulfoxide formed an ion at m/z 280 as base peak, which corresponds to a loss of the methyl group from the aromatic moiety and the addition of a proton. Identification of the sulfoxide was done also at m/z 295 $[M+H]^+$ and at m/z 278 which corresponds to the cleavage of the sulfoxide bond and consequent loss of the oxygen group from the molecule. Moreover, it presents the ions at m/z 109 and 125 like fenthion. The other ions formed, at lower abundances, are listed in Table 1. Moreover, the fenthion sulfoxide was also identified by retention time comparison against a standard.

3.3.1.5. Fenthion sulfoxide oxon

The presence of another peak which followed the same fragmentation pattern as fenthion sulfoxide and had as base peak the ion at m/z 279 was a clear evidence that the compound that eluted immediately before was the sulfoxide oxon. It also presented the ion at m/z 264 which corresponds to a loss of a methyl group and the fragment ion at m/z 154, similar to the sulfoxide. Diagnostic ions at m/z 109 and 125 are useful for further confirmation of the results.

Fig. 5 shows the ion chromatogram of the different transformation products of fenthion. Table 2 indicates the different compounds that were identified in the different samples after fenthion had been oxidized with NBS and the normalized abundance of

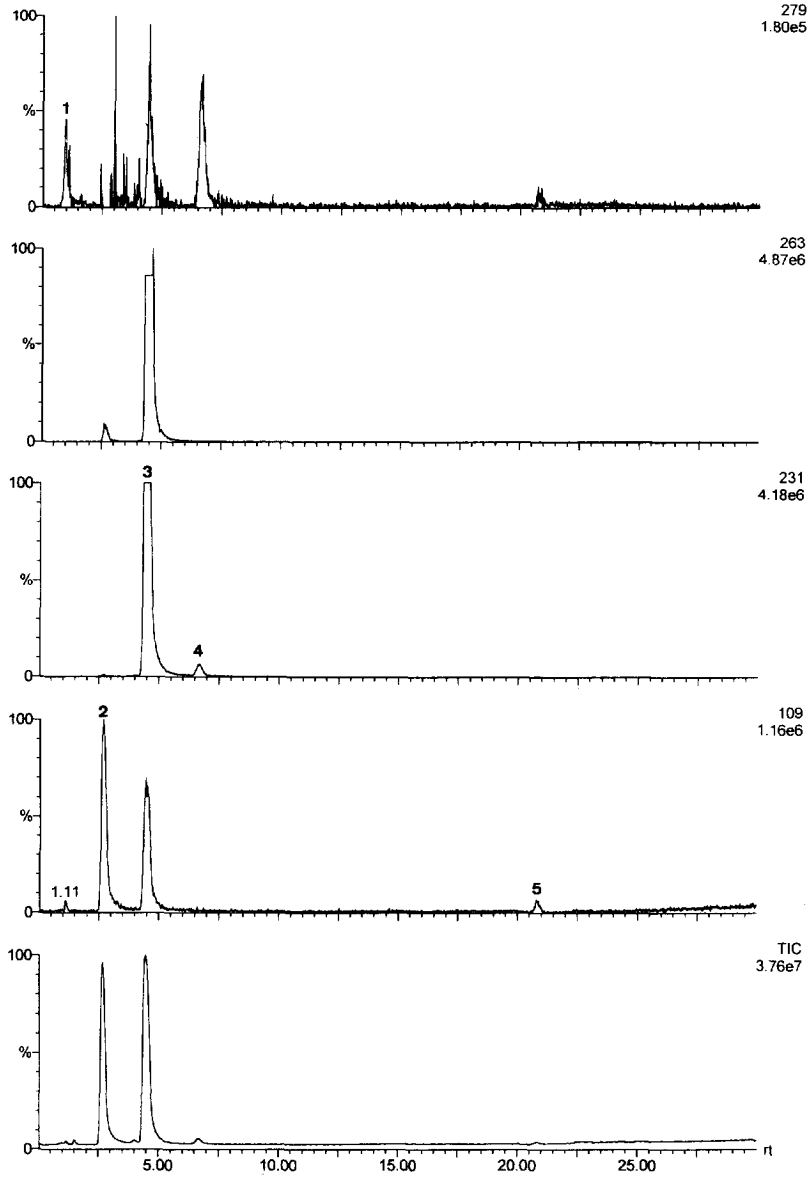

Fig. 5. TIC and selected ion chromatogram obtained by LC–APCI-MS with positive ionization mode and 40 V of extraction voltage of a sample that contained fenthion and was oxidized with NBS. Peak identification: 1 = fenthion sulfoxide isomer, 2 = fenthion sulfoxide, 3 = fenthion oxon, 4 = fenthion isomer and 5 = fenthion. Amount injected = 400 ng.

each compound, calculated from the most abundant ion of each compound, in the samples $R=2$ and $R=4$. It can be seen from this table that as a result of the oxidative process, fenthion disappears from the non-oxidized sample ($R=0$) and produce fenthion oxon and fenthion sulfoxide. However, an increase of the ratio of NBS/pesticides produces of a stronger oxidation effect, with the formation fenthion sulfoxide oxon, which is the most abundant compound at $R=4$.

3.3.2. Sample that contained temephos

The difficulty to detect temephos along with their transformation products is related to the fact that temephos has a symmetrical structure with double dimethoxy-thiophosphate group which gives a vast and complex combination of locations which can undergo the processes of oxidation and isomerization and as a consequence, they generate a large number of transformation products. LC–APCI-MS permitted the identification of temephos and six transformation products. Table 3 reports the transformation products of temephos detected in the different samples. Similarly as in the case of fenthion, the oxidative process results in the formation of temephos oxon as the most abundant compound in $R=2$, and temephos is hardly detected (see Table 3). Since temephos has a double thiophosphate group that can be oxidized, the second most abundant peak corresponds to temephos dioxon, which appears as the most intense peak when increasing the amount of NBS ($R=4$). These values were obtained at 20 V of extraction voltage to enhance sensitivity. Table 4 lists the main ions and abundances of each compound at 20 and 40 V of extraction voltage. Fig. 6 illustrates the ion chromatograms of the different compounds. Compound identification was as follows:

3.3.2.1. Temephos

Temephos was identified both by retention time comparison against a standard and by ion formation comparison with the results obtained by FIA. Temephos eluted at 17.03 min and at an extraction voltage of 20 V the protonated molecule was the base peak while at 40 V, it presented the fragment ion at m/z 125 as base peak.

3.3.2.2. Temephos isomer

This compound presents the same molecular mass as its parental form but elutes at 6.5 min. At an extraction voltage of 20 V a unique fragment ion was formed at m/z 467, which was not sufficient to identify this compound. At 40 V, the base peak corresponded to m/z 249 which corresponds to half of the molecule (see Table 4). The other most abundant ions corresponded to the characteristic ion at m/z 125 $[(CH_3O)_2PS]^+$, m/z 419 and 467 (protonated molecule).

3.3.2.3. Temephos oxon

This compound eluted before their parental pesticide, similarly to fenthion. The protonated molecular ion was formed at an extraction voltage of 20 V while at 40 V several fragment ions that permitted their identification. Evidence that it corresponds to temephos oxon is that it has the ion at m/z 125 which can correspond to both $[(CH_3O)_2PS]^+$ and $[CH_3O)_2PO_2]^+$ structures. It presents m/z 233 as base peak which corresponds to the fragment 125 plus the aromatic moiety and the sulfur atom. This fragment alone does not indicate whether this compound is the temephos oxon or the temephos oxon isomer. The distinction is done through retention time information. Besides, the ion at m/z 249 which belongs to $[(CH_3O)_2PSO-C_6H_4-S]^+$, corresponds

Table 3
Temephos and the transformation products identified by LC–APCI-MS

t_R (min)	rrt	Relative area (%)		Compounds identified
		$R=2$	$R=4$	
1.65	0.098	0.7	0.03	T. sulfoxide oxon, m/z 467
1.76	0.104	67.6	100	T. dioxon, m/z 435
2.19	0.130	6.7	4.6	T. oxon isomer, m/z 451
3.67	0.218	0.4	n.d.	T. sulfoxide, m/z 483
4.96	0.295	100	16	T. oxon, m/z 451
6.68	0.397	3.8	0.2	T. isomer, m/z 467
16.8	1.000	0.3	n.d.	Temephos, m/z 467

t_R = retention time; rrt = relative retention time; n.d. = not detected; R = molar ratio NBS/temephos.

Table 4
Characterization by LC–APCI-MS of temephos and the degradation products formed as a result of the oxidation process

Compound identified	t_R (min)	20 V	40 V
T. sulfoxide oxon M_r=466	1.65	467(100) $[M+H]^+$	n.d.
Temephos dioxon M_r=434	1.76	435(100) $[M+H]^+$ 457(8) $[M+Na]^+$	125(42) $[(CH_3O)_2PO_2]^+$ 203(36) $[S-C_6H_4-PO(OH)-CH_3O]^+$ 233(100) $[(CH_3O)_2PO_2-C_6H_4-S]^+$ 435(93) $[M+H]^+$ 457(10) $[M+Na]^+$
T. oxon isomer M_r=450	2.07	451(100) $[M+H]^+$ 473(23) $[M+Na]^+$	141(29) $[(CH_3O)(CH_3S)PO_2]^+$ 249(29) $[(CH_3O)(CH_3S)PO_2-C_6H_4-S]^+$ 451(100) $[M+H]^+$ 473(43) $[M+Na]^+$
Temephos sulfoxide M_r=482	3.56	279(23) $[(CH_3O)POH-(C_6H_4)_2SO]^+$ 341(64) $[M-(CH_3O)_2PSO]^+$ 483(100) $[M+H]^+$	99(63) $[PH(OH)_2SH]^+$ or $[P(OH)_4]^+$ 113(55) $[POS(OH)_2]^+$ 139(100) $[C_6H_4-PO_2]^+$ 483(9) $[M+H]^+$
Temephos oxon M_r=450	4.88	451(100) $[M+H]^+$	125(36) $[(CH_3O)_2PO_2]^+$ or $[(CH_3O)_2PS]^+$ 233(100) $[(CH_3O)_2PO_2-C_6H_4-S]^+$ 249(37) $[(CH_3O)_2PSO-C_6H_4-S]^+$ 265(13) $[(CH_3O)(CH_3S)PO_2-C_6H_4-S-O]^+$ 403(20) $[M-CH_3S]^+$ 451(50) $[M+H]^+$
Temephos isomer M_r=466	6.55	467(100) $[M+H]^+$	125(54) $[(CH_3O)_2PS]^+$ 155(15) $[PSO-C_6H_4]^-$ 249(100) $[M-(CH_3O)_2PSO-C_6H_4]^+$ 405(15) $[M-(CH_3O)_2+H]^+$ 419(49) $[M-(CH_3O)(OH)+H]^+$ 467(45) $[M+H]^+$
Temephos M_r=466	17.03	125(26) $[(CH_3O)_2PS]^+$ 141(20) $[(CH_3O)_2POS]^+$ 467(100) $[M+H]^+$	109(77) $[(CH_3O)_2PO]^+$ 125(100) $[(CH_3O)_2PS]^+$ 249(81) $[(CH_3O)_2PSO-C_6H_4-S]^+$ 451(26) $[M-CH_3]^+$ 467(47) $[M+H]^+$

Information on molecular mass (M_r), retention time in minutes (t_R) and ions formed (m/z and % abundance) is given. Results are reported for extraction volatge of 20 and 40 V and corona discharge of 3.5 kV. Positive mode of ionization (PI) was used.

to the other end of the molecule with the intact structure (as temephos).

3.3.2.4. Temephos sulfoxide

This compound was identified by retention time information in comparison with a pure standard, and at 20 V, it presented the protonated molecule at m/z 483 as base peak. At an extraction voltage of 40 V an ion at m/z 139 as base peak was produced, corresponding to $[C_6H_4PO_2]$, which corresponds to a transposition of the S with the O of the methoxy group.

3.3.2.5. Temephos oxon isomer

At an extraction voltage of 20 V this compound presented the ion at m/z 451 as base peak, corresponding to the protonated molecule and an adduct

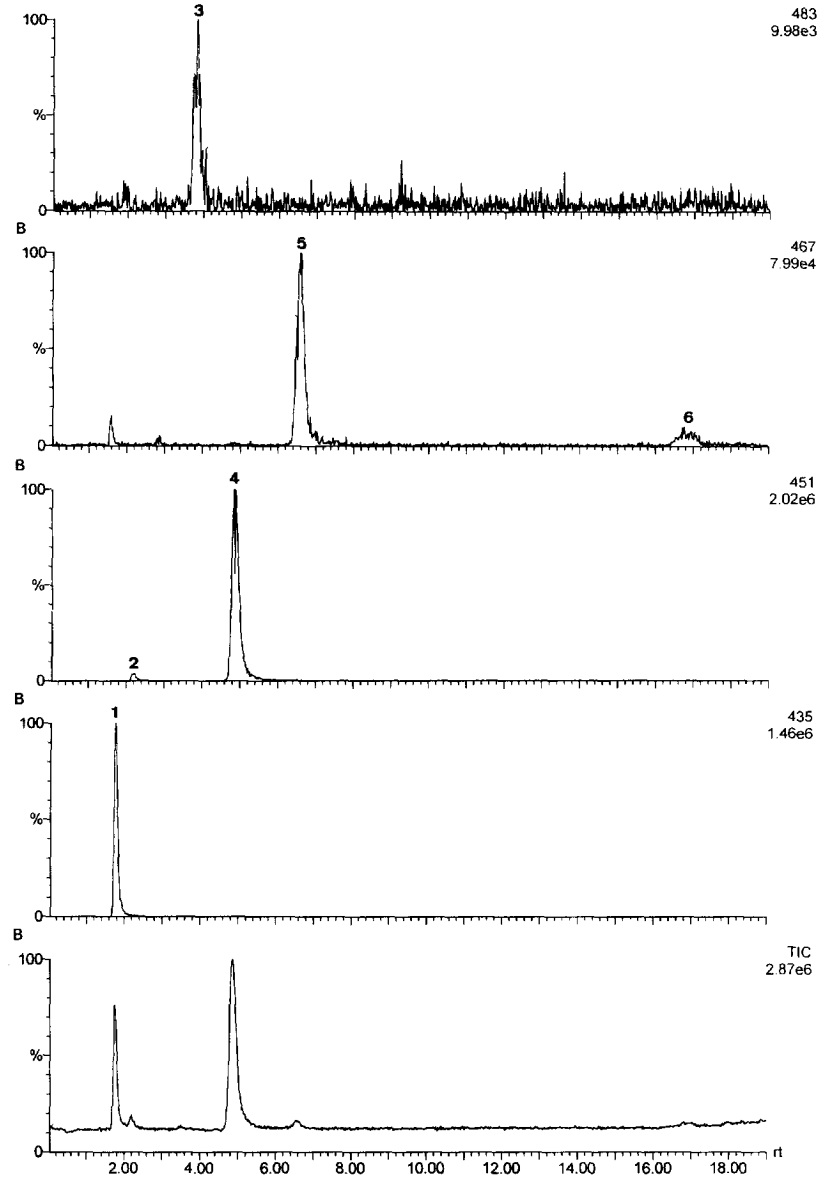

Fig. 6. TIC and selected ion chromatogram obtained by LC–APCI-MS with positive ionization mode and 40 V of extraction voltage of a sample that contained temephos and was oxidized with NBS. Peak identification: 1 = temephos dioxon, 2 = temephos oxon isomer, 3 = temephos sulfoxide, 4 = temephos oxon, 5 = temephos isomer and 6 = temephos. Amount injected = 400 ng.

with sodium at m/z 473. Moreover, at 40 V, more fragment ions were formed. Similarly as in the case of temephos oxon, the fragment ion at m/z 249 was formed. The ion at m/z 141 corresponds to $[(CH_3O)(CH_3S)PO_2]^+$ structure, and belongs to the isomerization of one end of the molecule.

3.3.2.6. *Temephos dioxon*

Moreover, the oxidation process lead to the formation of temephos dioxon, with two phosphate groups. This compound, with a molecular mass of 434, presented m/z at 435, which corresponds to the protonated molecule, but also exhibited an adduct

with sodium at m/z 457. The base peak at 40 V corresponded to the structure $[(CH_3O)_2PO_2-C_6H_4-S]^+$ at m/z 233, ion which was also found in the case of temephos oxon, and it means that the two former methoxy-phosphorotioate groups of temephos have been oxidized.

3.3.2.7. Temephos sulfoxide oxon

This compound could only be identified at an extraction voltage of 20 V. The base peak at m/z 467 and its early elution time (1.65 min) was evidence that it belonged to a highly polar degradation product which had undergone a double oxidation with the production of a sulfoxide oxon.

3.4. Environmental application

Fig. 7 shows the total ion current (TIC) and the selected ion chromatograms of a rice field water sample. Retention times do not correspond with the conditions used in the oxidized samples, since a gradient which started with a high percentage of water was used in order to minimize early eluting interferences from the water. Each peak was quali-

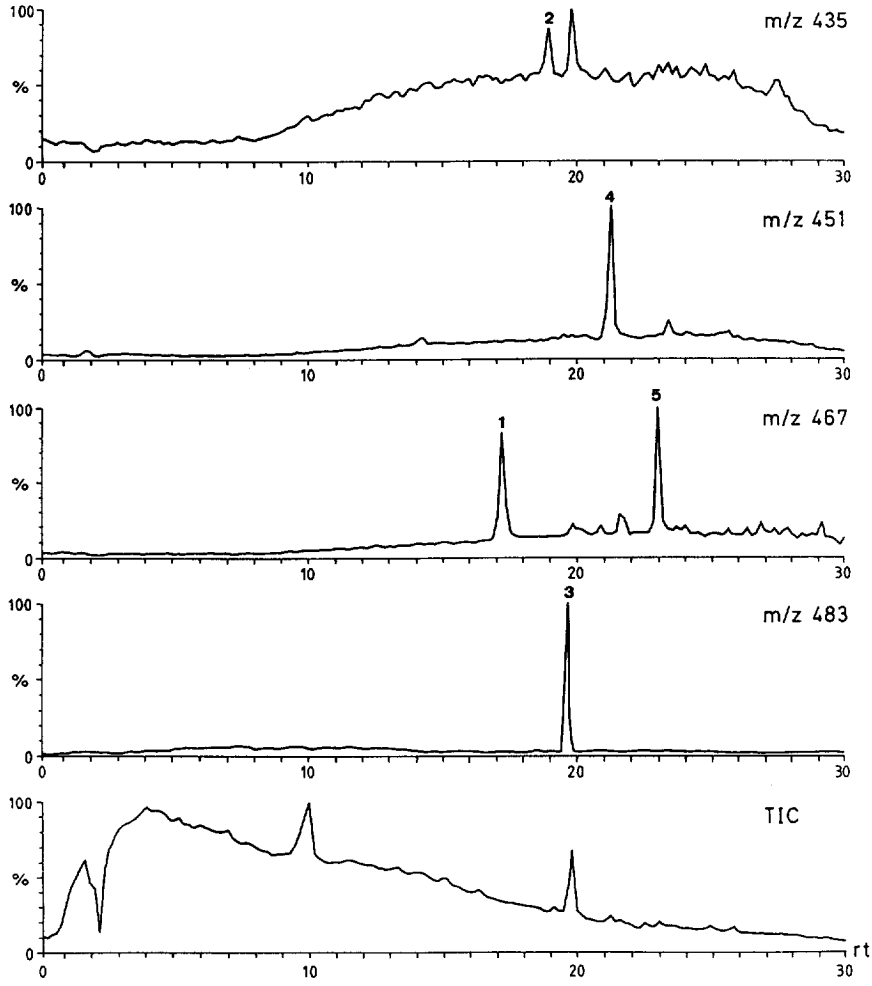

Fig. 7. TIC and selected ion chromatograms which correspond to estuarine water samples that had been treated with temephos. Peak identification: 1=temephos sulfoxide oxon, 2=temephos dioxon, 3=temephos sulfoxide, 4=temephos oxon and 5=temephos.

tatively identified as a degradation product of temephos by spectral comparison against the oxidized samples. The first eluting compound was temephos sulfoxide oxon, similarly to what was observed with the oxidized samples that contained temephos. The other transformation products eluted with the same order as the oxidized standards, with temephos dioxon, the sulfoxide, the oxon and finally temephos. The characterization of such degradation products in environmental waters was enhanced by the information we previously obtained from the NBS oxidized samples. However, quantification of these compounds was impossible, due to the lack of standards. Temephos sulfoxide oxon was identified by the fact that presented the ion at m/z 467 as base peak and the ions at m/z 109 and 125, which are characteristic fragment ions of the phosphate pesticides (such as temephos oxon). The identification of this compound was forced by retention time information, since the temephos oxon sulfoxide was the only degradation product with molecular mass of 466 that presented lower retention time that temephos oxon and sulfoxide.

The second eluting degradation product was temephos dioxon, which could be easily characterized because of the presence of the ions at m/z 435 $[M+H]^+$ as base peak and 457 $[M+Na]^+$. Next at 19.83 min eluted temephos sulfoxide which presented the ion at m/z 483 as base peak and had the ion at m/z 125 corresponding to $[(CH_3O)_2PS]$. Temephos oxon and temephos, eluting at 21.2 and 22.9 min were characterized because of the presence of the ions at m/z 451 and 467 as base peaks, respectively, corresponding to the protonated molecules.

4. Conclusions

The oxidation of fenthion and temephos with NBS was performed at two different ratios of oxidant/pesticides to asses the effect of the oxidative process. In all cases, oxo-analogues were formed, and were identified with LC–DAD, NMR and LC–APCI-MS. Even though LC–DAD rendered absorbance spectral information, only by using NMR was it possible to identify the transformation products of the two pesticides. However, LC–APCI-MS provided more structural information and four transformation products of fenthion were detected while in the case of temephos, six transformation products were unequivocally identified. The characterization of all the transformation products was done under PI and NI chemical ionization, in the case of fenthion, and under different extraction voltages when analyzing temephos. More structural information was gathered at an extraction voltage of 40 V, while at 20 V more sensitivity was obtained and therefore, this condition was used to measure the relative areas of temephos and its transformation products in the two samples that were oxidized. In all cases, the oxidative process was found to be highly efficient, and the oxo analogues of both fenthion and temephos were formed as most abundant compound. However, an increase in the ratio of NBS/pesticide lead to the formation of fenthion sulfoxide oxon and temephos dioxon, respectively.

As regards to the environmental water samples, temephos and some transformation products were identified by LC–APCI-MS from a water sample that had been treated with temephos. Thus, LC–APCI-MS at different extraction voltages can be used to detect and identify transformation products that can be formed in the environment as a result of biological and chemical degradation.

Further work will include the use of a biosensor based in the inhibition of acethylcholinesterase for determining the concentration of fenthion and temephos, along with their degradation products, in water. Such approach will indicate the toxicity of these degradation products against the eel acethylcholinesterase, and will permit to asses the inhibitory activity of the biosensor in the different degradation products identified.

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