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ASSESSMENT OF THE STATE OF POLLUTION IN THE MEDITERRANEAN SEA BY ORGANOPHOSPHORUS COMPOUNDS AND PROPOSED MEASURES

In cooperation with

FAO WHO IAEA

UNEP
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1. BACKGROUND

According to the Protocol for the Protection of the Mediterranean Sea against Pollution from Land-based Sources (LBS Protocol) the Contracting Parties shall take all appropriate measures to prevent, abate, combat and control pollution of the Mediterranean Sea Area caused by discharges from rivers, coastal establishments or outfalls, or emanating from any other land-based sources within their territories.

Article 5 of this Protocol stipulates that:

- The Parties undertake to eliminate pollution of the Protocol Area from land-based sources by substances listed in Annex I to this Protocol;

- To this end they shall elaborate and implement, jointly or individually, as appropriate, the necessary programmes and measures;

- These programmes and measures shall include, in particular, common emission standards and standards for use.

Organophosphorus compounds and substances which may form such compounds in the marine environment (with the exception of those which are biologically harmless or which are rapidly converted into biologically harmless substances) are included in Annex I to this Protocol.

The Meeting of Experts for the Technical Implementation of the LBS protocol (December, 1985) proposed that the measures to be recommended to the Contracting Parties for each group of substances should be based on an "assessment document" which should be prepared by the Secretariat. According to this proposal, which was adopted by the Fifth Ordinary Meeting of the Contracting Parties to the Barcelona Convention (September, 1987), such assessments should include *inter alia* chapters on:

- sources, point of entries and amounts of pollution for industrial, municipal and other discharges to the Mediterranean Sea;

- levels of pollution;

- effects of pollution;

- present legal, administrative and technical measures at national and international level.

Prior to the preparation of this assessment document a small ad-hoc meeting on organophosphorus compounds was convened in Athens in November 1987 (FAO/UNEP/IAEA/WHO, 1987). The meeting agreed on an annotated outline of the assessment document and recommended the initiation of a pilot monitoring study to collect relevant data from the Mediterranean region. The study centred on 4 pesticide and 2 non-pesticide compounds all of which have been used as case-examples throughout the assessment document. The results of the study are found in document FIR/MEDPOL/OP/5 (FAO/UNEP/IAEA/WHO, 1989).

The present document, which was prepared by the Secretariat with the help of consultants and in close cooperation with FAO, WHO and IAEA, was reviewed by an ad-hoc meeting of experts which met in Athens on 7-8 March 1990. The document evaluates information on the contamination of the marine environment by organophosphorus compounds, the ecological and human health significance of such contamination, and makes recommendations to the Contracting Parties taking also into consideration the results of the pilot monitoring study.

2. GENERAL FACTS ON ORGANOPHOSPHORUS COMPOUNDS

2.1 Chemistry

The basic compound for the synthesis of organophosphorus compounds is phosphine (PH_3), which is alkylated or arylated to R_2PH or R_3P. The oxidation of phosphines gives phosphine oxides, $R_2P(O)H$, R_3PO, and the conversion of the C-P bond to C-O-P bond phosphites, RO_3P. The combination of these two steps results in the formation of phosphinate esters, $R_2(RO)P(O)$, phosphonite esters, $R(RO)_2P(O)$, and phosphate esters, $(RO)_3P(O)$. Side chains can be halogenated, oxygen substituted with sulphur, and occasionally with nitrogen. Within the large group of organophosphorus compounds esters, especially phosphate esters, are of the greatest importance.

According to use, organophosphorus compounds and their derivatives can be divided into the following categories:

(a) industrial use
  - flame retardants, plasticisers, solvents, antifoaming agents, industrial hydraulic fluids, lubricants, dispersants, detergents.

(b) pesticides
  - insecticides, acaricides, nematicides, anthelmintic agents, fungicides, herbicides.

In most of the phosphate esters manufactured for industrial use the organic radicals fall into the following categories:

(a) triaryl phosphates - either three identical aryl groups (cresyl, phenyl) or two phenyl and one other aryl group (e.g. monocresyl diphenyl phosphate);

(b) aryl-alkyl phosphates - either two alkyl and one phenyl (e.g. dibutyl phenyl phosphate) or one alkyl and two aryl groups (e.g.) 2-ethylhexyl diphenyl phosphate);

(c) trialkyl phosphates - three identical alkyl groups (e.g. tritributyl phosphate);

(d) trihaloalkyl phosphates - three identical halogenated alkyl groups (e.g. tris 2-chloropropyl phosphate).

As biological and environmental behaviour can be altered by structural modifications around the phosphorus centre, the search for organophosphorus compounds which can satisfy a specific requirement in stability and selectivity resulted in the synthesis of more than 50,000 organophosphorus compounds and the registration of 250 as pesticides (Hutson and Roberts, 1985). The most frequently used 100 were reviewed by WHO (1986). Though in most of the pesticides two of the organic radicals are either methyl or ethyl, the third radical is different in nearly every pesticide. Thus the third radical can be a longer alkyl group, alkyl derivative, aryl group, the combination of the two, a ring with sulphur or nitrogen. The R-O-P bond is often replaced by R-S-P and in few instances by R-N-P bond. In the first organophosphorus compound synthesized in 1944 the P=O bond was replaced by R=S bond. The majority of organophosphorus pesticides confirms to the formulae shown on Table I.

Other structures not shown in Table I are diphosphate esters and phosphoroamidates. Diphosphate esters consist of two identical organophosphorus compounds (phosphorothionate) moieties which are linked by oxygen (sulfotep) or by an organic radical sandwiched between two oxygen (temephos) or sulphur (dioxathione, ethione). In phosphoroamidates one (e.g. fenamiphos) or more (triamiphos) valence is occupied by nitrogen.

2.2 Physicochemical characteristics and environmental behaviour

Table II gives the physical-chemical properties of six organophosphorus pesticides. The molecular weights of the six compounds in Table II are in a narrow range, but the range for all organophosphorus compounds is larger. It is from 213 to 466 for pesticides and from 140 (trimethyl phosphate) to 518 (3,5,5-trimethylhexyl) for other compounds. With the exception of tributylphosphate (TBP) organophosphorus compounds have relatively low vapour pressure and volatilization loss from water is minor. The high Henry constant (the ratio of molar concentrations in the liquid and vapour phase at equilibrium) of tributylphosphate indicates its relatively high volatilization from water.

The solubility of organophosphorus compounds in water shows great variation. Thus industrial alkylphosphate esters are soluble while tricresylphosphates are practically insoluble in water. The water solubility of pesticides shows similar variation and range from 0.4 mg l^{-1} for chlorpyrifos to 4,000 mg l^{-1} for vamidithion. An exception is acephate which is highly soluble in water with a saturation concentration of 67%.

The octanol-water partition coefficient (log K_{ow} = P_{ow}) is usually below 3-4 (Bowman and Sans, 1983). As organophosphorus compounds have molecular weights below 600, the effect of lipophilicity on bioconcentration is not limited by molecular size. However compared with organochlorine compounds with similar lipophilicity, organophosphorus compounds have a lower bioconcentration potential in aquatic organisms (Saeger et al., 1979). The reason for this is their relative instability.

Table I

Chemical structures of organophosphorus pesticides. (Compiled from WHO, 1986). R denotes the twin alkyl radicals, R' indicates difference from R, X denotes the third (so called leaving) radical.

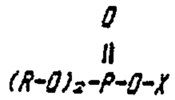
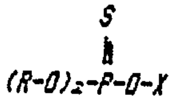
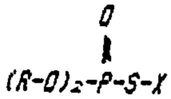
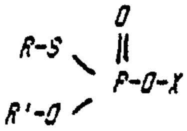
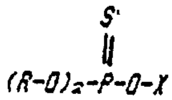
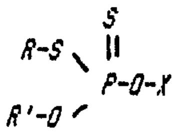
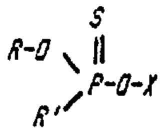
Phosphorus group	Outline of structure	Common or other name
Phosphate	 $(R\text{-}O)_2\text{-}\overset{O}{\overset{\|}{P}}\text{-}O\text{-}X$	chlorfenvinphos, crotoxyphos, dichlorvos, dicrotophos, heptenphos, mevinphos, monocrotophos, naled, phosphamidon, TEPP, tetrachlorvinphos, triazophos
Phosphorothioate S-phosphoryl	 $(R\text{-}O)_2\text{-}\overset{S}{\overset{\|}{P}}\text{-}O\text{-}X$	azothoate, bromophos, bromophos-ethyl, chloropyrophos, chloropyriphos-methyl, coumaphos, diazinon, dichlofenthion, fenchlorphos, fenitrothion, fenthion parathion, parathion-methyl, pyrazophos, pyrimiphos-ethyl, pyrimiphos-methyl, thionazin
O-alkyl	 $(R\text{-}O)_2\text{-}\overset{O}{\overset{\|}{P}}\text{-}S\text{-}X$	amiton, demeton-S-methyl, omethoate, oxydemeton-methyl, phoxim, vamidothion
S-alkyl	 $\begin{matrix} R\text{-}S \\ R'\text{-}O \end{matrix} > \overset{O}{\overset{\|}{P}}\text{-}O\text{-}X$	profenofos, trifenofos
Phosphorodithioate O-alkyl	 $(R\text{-}O)_2\text{-}\overset{S}{\overset{\|}{P}}\text{-}O\text{-}X$	amidithion, azinphos-ethyl, azinphos-methyl, dimethoate, disulfoton, formothion, malathion, mecarbam, menazon, methidathion, morphothion, plenthoate, phorate, phosalone, phosmet, prothoate, thiometon
S-alkyl	 $\begin{matrix} R\text{-}S \\ R'\text{-}O \end{matrix} > \overset{S}{\overset{\|}{P}}\text{-}O\text{-}X$	prothiofos, sulprofos
Phosphonothioate	 $\begin{matrix} R\text{-}O \\ R' \end{matrix} > \overset{S}{\overset{\|}{P}}\text{-}O\text{-}X$	EPN, trichlornat, letptophos

Table II

Physical-chemical properties of six major organophosphorus compounds.

	Ethyl-parathion	Methyl-parathion	Fenitrothion	Malathion	TBP	TCEP
Molecular weight	291.3	263.2	277.2	330.4	266.3	285.5
Boiling point (°C)	150 at 0.6mmHg	154 at 1mmHg	140 at 0.1mmHg	156 at 0.7mmHg	292 at 760mmHg	214 at 25mmHg
Melting point (°C)	6	35-38	0.3	2.85	-80	-55
Vapour pressure (mmHg/°C)	3.8-6.0X10^{-6} at 20°C	9.7-11.2X10^{-6} at 20°C	5.4X10^{-5} at 20°C	1.2X10^{-4} at 20°C	127 at 177°C	0.5 at 145°C
Henry's constant (mmHg/ppm at 25°C)	2.52X10^{-7}	1.0X10^{-7}	9.3X10^{-7}	8.62X10^{-7}	7.08X10^{-2}	4.82X10^{-7}
Water solubility (mg l^{-1})	24 at 25°C	55-60 at 25°C	30 at 20°C	145 at 25°C	280 at 25°C	7000 at 25°C
Specific gravity	1.265 at 25°C	1.358 at 20°	1.3227 at 25°C	1.23 at 25°C	0.98	1.4 - 2.2
Log Kow	3.8	2-3.4	2.3-3.3	2.9	4	1.5
Log Koc (carbon water sorption coeff.)	2.8	3.3	2.8		3.5	2.2
Persistence (half-life in water at pH7 in days at room temperature)	2-8	2-4	1-2	2-8	6	24

The bioconcentration (and bioaccumulation) of organophosphorus compounds is limited by their instability in water and biota. They are liable to hydrolysis and other mechanisms of ester cleavage. Fig. 1 illustrates the rather rapid disappearance of methyl-parathion from a pond, and Fig. 2 the disappearance of four arylphosphates from river water. Fig. 3 gives the biodegradation scheme of parathion in various biota. The importance of biological factors has been demonstrated by the difference in the persistence of parathion in biotic and abiotic water. The half life of parathion is usually few days, but in abiotic water no noticeable breakdown was observed in 40 days (Fisher and Lohner, 1987). Uptake by plants and particles is an important process which limits the availability of organophosphorus compounds for absorption by fish (Hutson and Roberts, 1985).

The environmental behaviour of organophosphorus pesticides has been evaluated by MacKay et al. (1985) with the help of a fugacity (partial pressure of the solute) model.

2.3 Analytical methodologies

Analytical methodologies for the fractionation and clean-up of organophosphorus pesticides in real environmental samples include the use of silica-gel (Lores et al., 1987), Florisil (Neicheva et al., 1988) or gel permeation (Lawrence, 1987). As regards the analytical part, capillary gas chromatography with thermionic nitrogen-phosphorus detector is usually the preferred method, with minimum detectable amounts between 0.01-0.1 $\mu g\ l^{-1}$ in water (Wang et al., 1987, Leistra et al., 1984).

Other detectors employed in gas chromatography for the determination of organophosphorus compounds are the flame photometric detector (Prinsloo and De Beer, 1985; Onuska, 1984) or mass spectrometry in the electron impact (Wilkins et al., 1985) or chemical ionization mode (Stan and Kellner, 1982; Cairns et al., 1985). Due to the fact that some organophosphorus pesticides are thermally labile and/or polar, the use of liquid chromatographic techniques is advisable, either using ultraviolet (Osselton and Shelling, 1986) diode array (Greve and Goewie, 1985), reductive amperometric (Clark et al., 1985) and mass spectrometry detectors (Barceló et al., 1987; Farran et al., 1988; Barceló et al., 1988). Recently a review of the use of liquid chromatography in pesticide analysis has been published (Barceló, 1988a).

Mass spectrometry detection in combination with gas and liquid chromatography has been recently applied for the determination of trialkyl and triaryl phosphates, including tris-2-chloroethyl phosphate (Cairns et al., 1983; D'Agostino and Provost, 1986; Barceló, 1988).

3. METABOLISM AND TOXICITY

3.1 Metabolism

Organophosphorus compounds undergo metabolic transformation in biological systems. Some of these transformations converts an inactive compound to an active one, others have the opposite consequence. Any metabolic transformation which brings the structure of organophosphorus

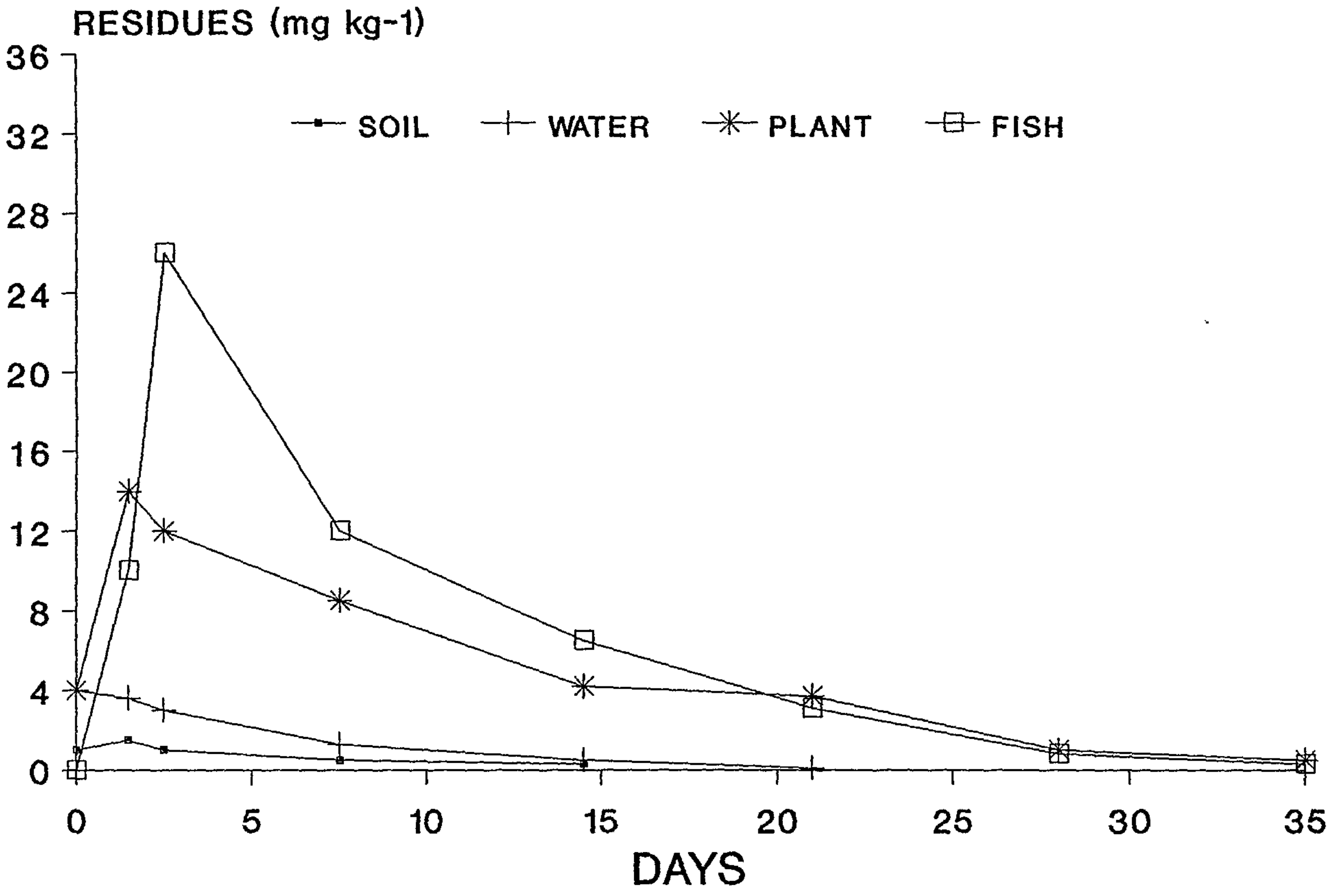


Fig. 1 Persistence of methyl parathion in a carp pond (from Sabbarhawl and Belsare, 1986)

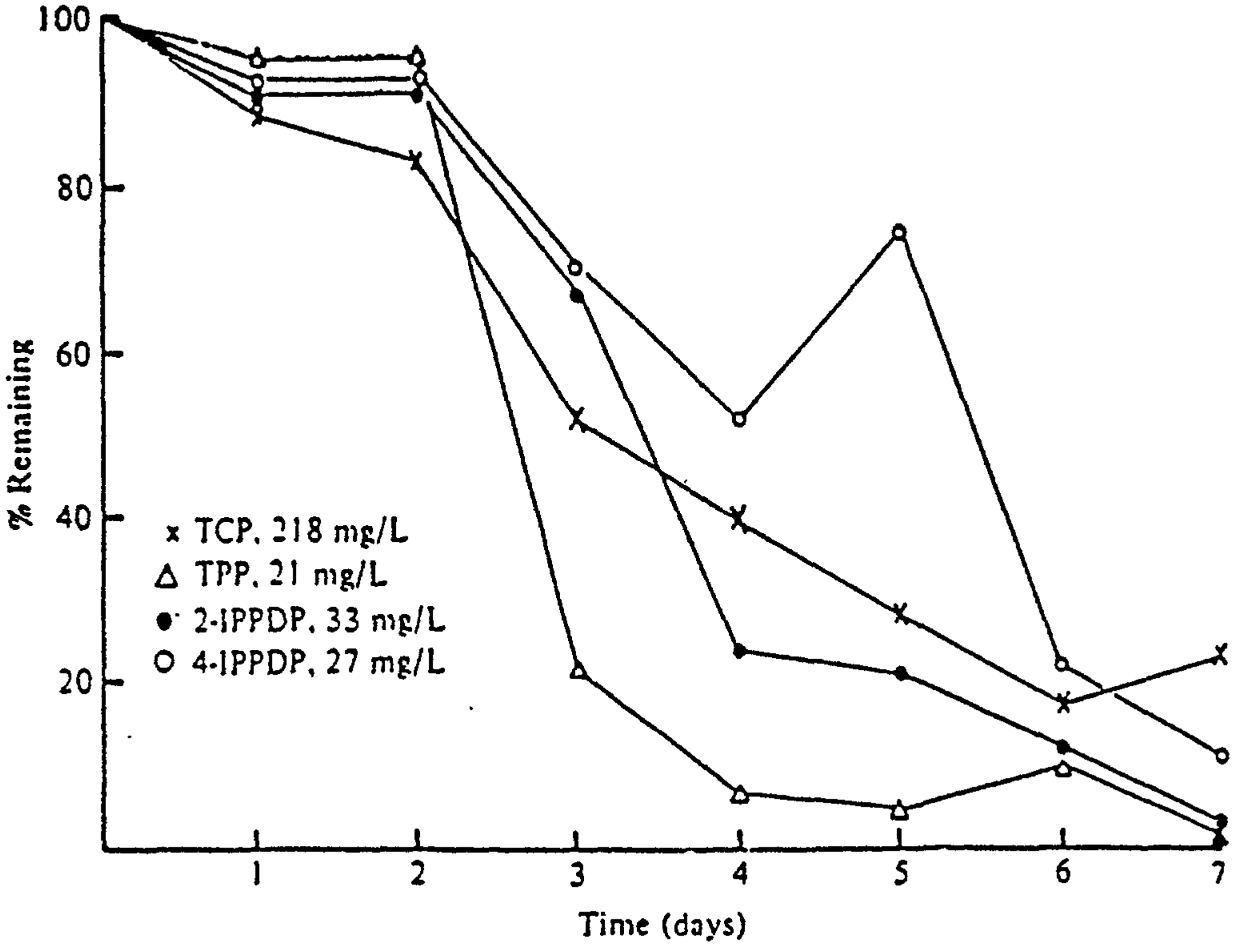


Fig. 2 Biodegradation of aryl phosphates in river water. Triphenyl phosphate (TPP), 2-isopropylphenyl diphenyl phosphate (2-IPPDP) and 4-isopropylphenyl diphenyl phosphate (4-IPPDP) were added as a mixture. TCP = tricresyl phosphate (from Latourette, 1979 in Boethling and Cooper, 1985).

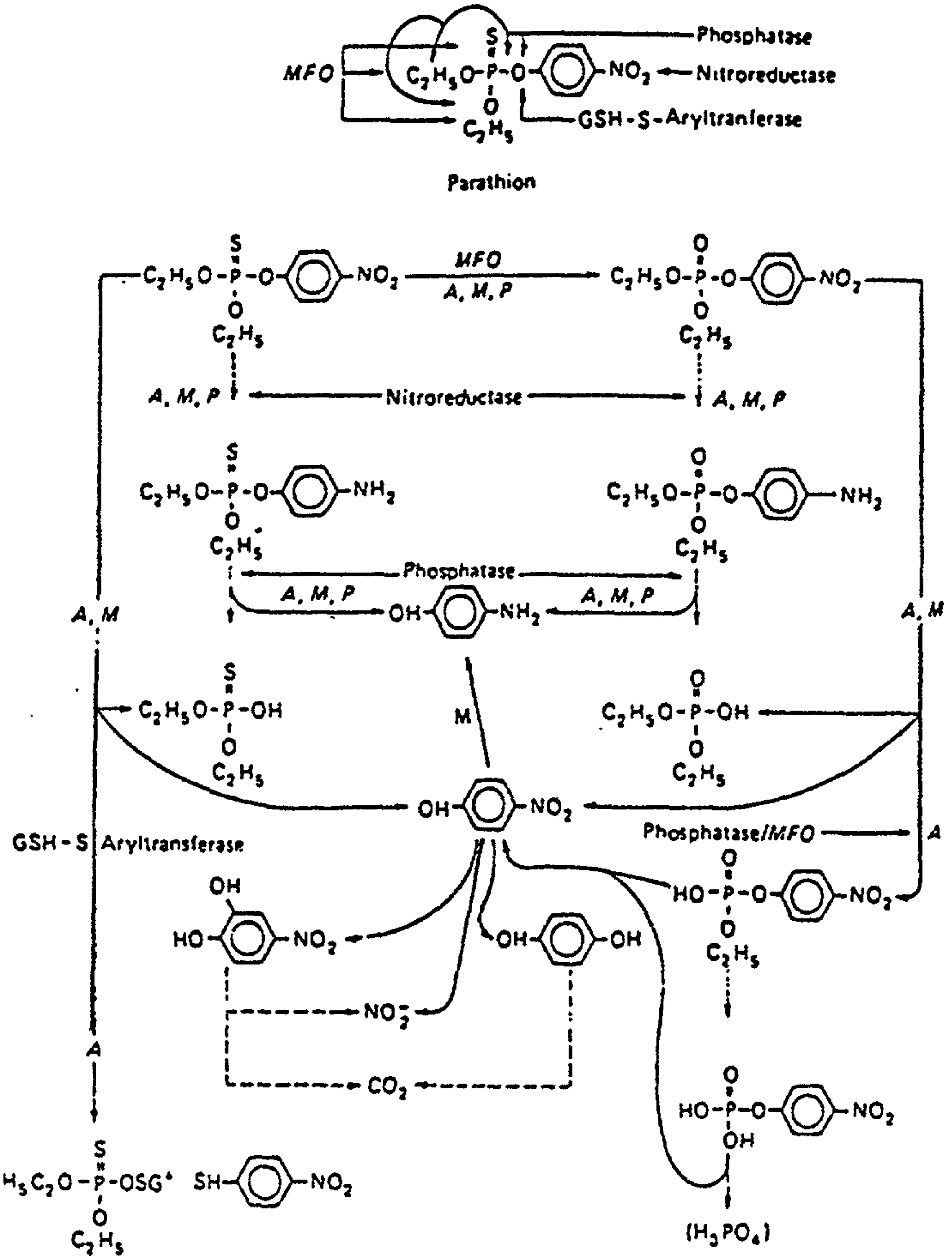


Fig. 3 A general scheme of the metabolic fate of parathion: A = animals, M = microorganisms, MFO = mixed function oxidase(s), and P = plants (from Mulla *et al.*, 1981)

insecticides nearer to the structure of organophosphorus triesters, increases the ability to inhibit acetylcholinesterase and converts a proinsecticide to an active insecticide. Activation reactions are (Hutson and Roberts, 1985):

(a) oxidative desulphuration of a thiophosphoryl group (conversion of P=S in parathion to P=O in paraoxon);

(b) thioether oxidation to sulphoxide or sulphone (the conversion of C-S-C to C-S(=O) or C-S(=O)$_2$-C in fenthion or in disulfoton);

(c) S-alkyl oxidation (the conversion of P-S-R to P-S(=O)-C in metamidophos or to P-S(=O)$_2$-R in profenofos;

(d) oxidative N-demethylation (the conversion of N-R$_3$)$_2$ to NH-R$_3$ in dicrotophos or phosphamidon.

Deactivation reactions are (Hutson and Roberts, 1985):

(a) hydrolytic removal of the X radical (the leaving group), mostly dearylation;

(b) glutathione-dependent dearylation;

(c) glutathione dependent dealkylation;

(d) oxidative dealkylation.

3.2 Mode of action

The hydrolytic conversion of a triester to diester by acetylcholin-esterase is the most important step in the toxicology of organophosphorus compounds because this detoxification reaction is associated with the toxification of acetylcholin. The physiological roles of acetylcholin and acetylcholinestrase are interlinked. Acetylcholin is a transmitter of nerve impulses in cholinergic synapses and acetyl-cholinesterase prevents the build-up of toxic acetylcholin concentrations through the hydrolysis of released acetylcholin to cholin and acetate. Fig. 4 shows that the hydrolysis of organophosphorus compounds by the same reaction results in inactivation through the phosphorylation of the enzyme by the formed phosphate diester. Reactivation is either slow or absent. The hydrolysis of the diester leaves only an acidic negatively charged residue. This process, called aging, does not restore activity to the enzyme. The result of acetylcholine esterase inhibition is the accumulation of acetylcholin and the excessive stimulation of cholinergic nerves.

The mode of action is the same in different animal phyla, though even within one order (e.g. fishes) there are substantial species differences in the hydrolysis of organophosphorus compounds (Hutson and Roberts, 1985).

Besides acetylcholinesterase, organophosphorus compounds can inhibit pseudocholinesterase, carboxylesterase, and various other esterases, among them the Neuropathy Target Esterase (NTE). The reaction of organophosphorus compounds with acetylcholinesterase and NTE is not different, the difference is in the outcome.

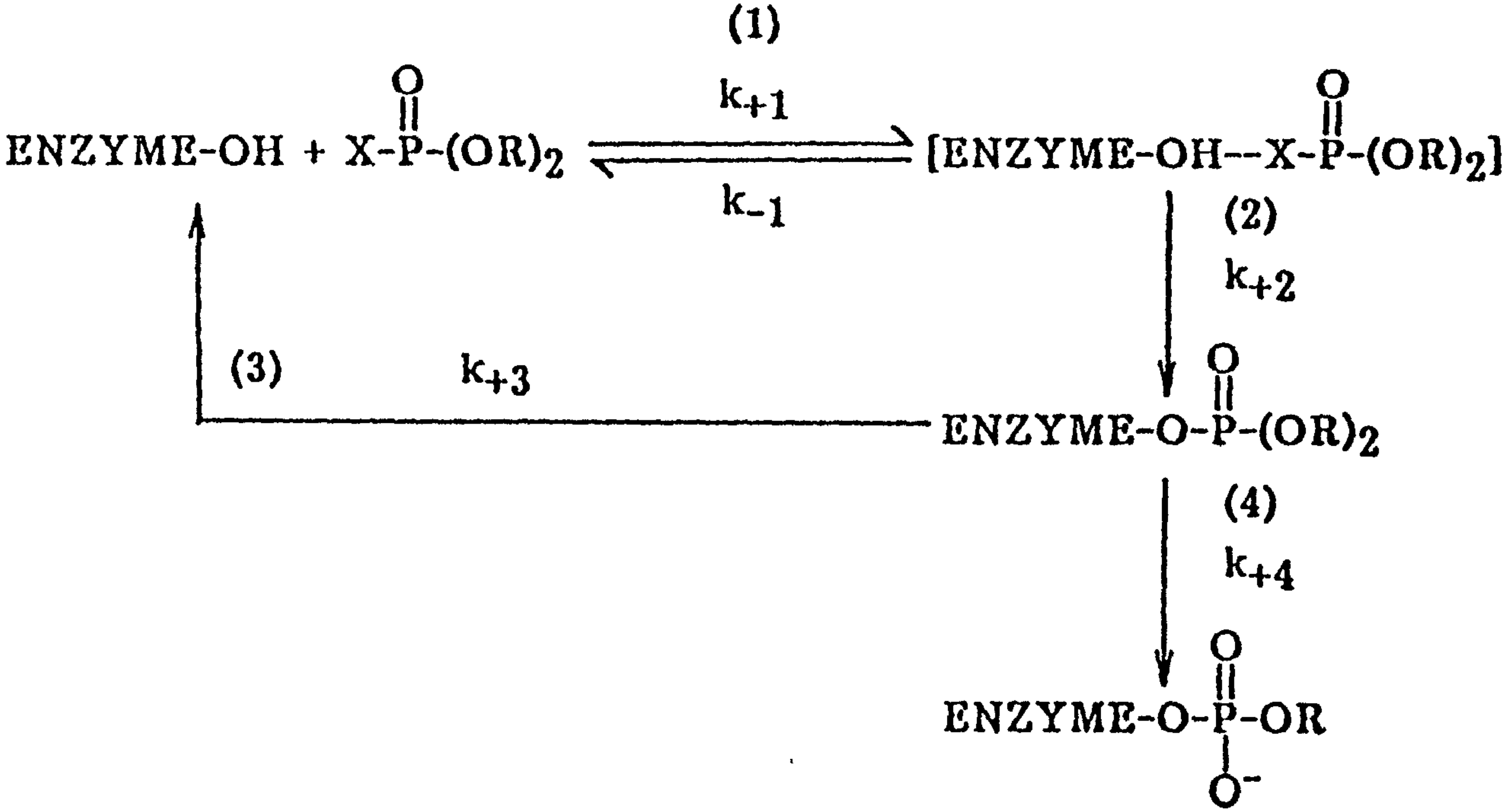


Fig. 4 Inhibition of an esterase by an organophosphorus compound (from Moretto and Johnson, 1987)

1. Formation of Michaelis complex;
2. Phosphorylation of the enzyme;
3. Reactivation reaction;
4. "Aging"

3.3 Toxic manifestations

3.3.1 In humans and other mammals

The acute toxic effects of organophosphorus compounds are the result of the excessive cholinergic stimulation in every mammalian species. In patients following the ingestion or inhalation of organophosphorus compounds the acute symptoms and signs of toxicity are either mimic the cholinergic effects of muscarine (e.g. pinpoint pupils, excessive bronchial secretion), or mimic the cholinergic effects of nicotine (e.g. fasciculation of muscles), or central nervous manifestations (e.g. anxiety, confusion). They begin within 30-60 min and are at a maximum at 2-8 hours after intake. In mild, moderate and severe intoxications the signs and symptoms in order of appearance are (Dreisbach and Robertson, 1987):

a) Mild - Anorexia, headache, dizziness, weakness, substernal discomfort, tremor of tongue and eyelids, pin-point pupil, impairment of visual acuity.

b) Moderate - Nausea, salivation, tearing, abdominal cramps, vomiting, sweating, slow pulse, spontaneous contraction of muscle fibres (fasciculation).

c) Severe - Diarrhoea, pinpoint and non-reactive pupils, excessive bronchial secretion, respiratory difficulty, pulmonary oedema, cyanosis, involuntary defecation and urination, convulsions, coma. Death is caused by respiratory failure and heart block.

The induction of acetylcholin toxicity is a common characteristic of all organophosphorus compounds, at least after metabolic activation. An other toxic effect called organophosphorus compound induced delayed polyneuropathy (OPIDP) can be induced only by few organophosphorus compounds. The reason for this is that the inhibition of neuropathy target esterase (NTE) by phosphorylation is only the first condition of delayed neuropathy. The second condition is that the aging of NTE can be produced only by some organophosphorus compounds and nerve damage (paralysis) can be produced only in sensitive species. Cat, hen, some large animals, and man, are sensitive, while rodents, baboon, monkey and marmoset are insensitive.

Organophosphorus triesters used for industrial purposes which have the ability to produce delayed neuropathy are tri-ortho-cresyl phosphate (TOCP), mono-, di- and tri-o-tolyl, o-cresyldiphenyl, o-isopropyl-phenyldiphenyl, di- and monophenyl derivatives, tributyl phosphate, tris(2-chloroethyl) phosphate (Clayton and Clayton, 1981; Muir, 1984; Moretto and Johnson, 1987; Pellin et al., 1987). The meta-and para-cresyl phosphates are not active. Pesticides which are able to cause delayed neuropathy in hens after a single dose include: amiphros, chloropyrifos, coumaphos, cyanofenphos, desbromoleptophos, EPN, dichlorvos, isofenphos, leptofos, trichlornat (WHO, 1986; Moretto and Johnson, 1987; Vilanova et al., 1987). The effect on NTE can be antagonised in a sensitive species by pretreatment with organophosphinate or sulfonyl fluorides. These become attached to NTE and inhibit its activity, but because they have no hydrolysable bond, they do not cause aging (WHO, 1986). Fig. 5 shows the reaction of NTE with organophosphorus compounds and organophosphinates.

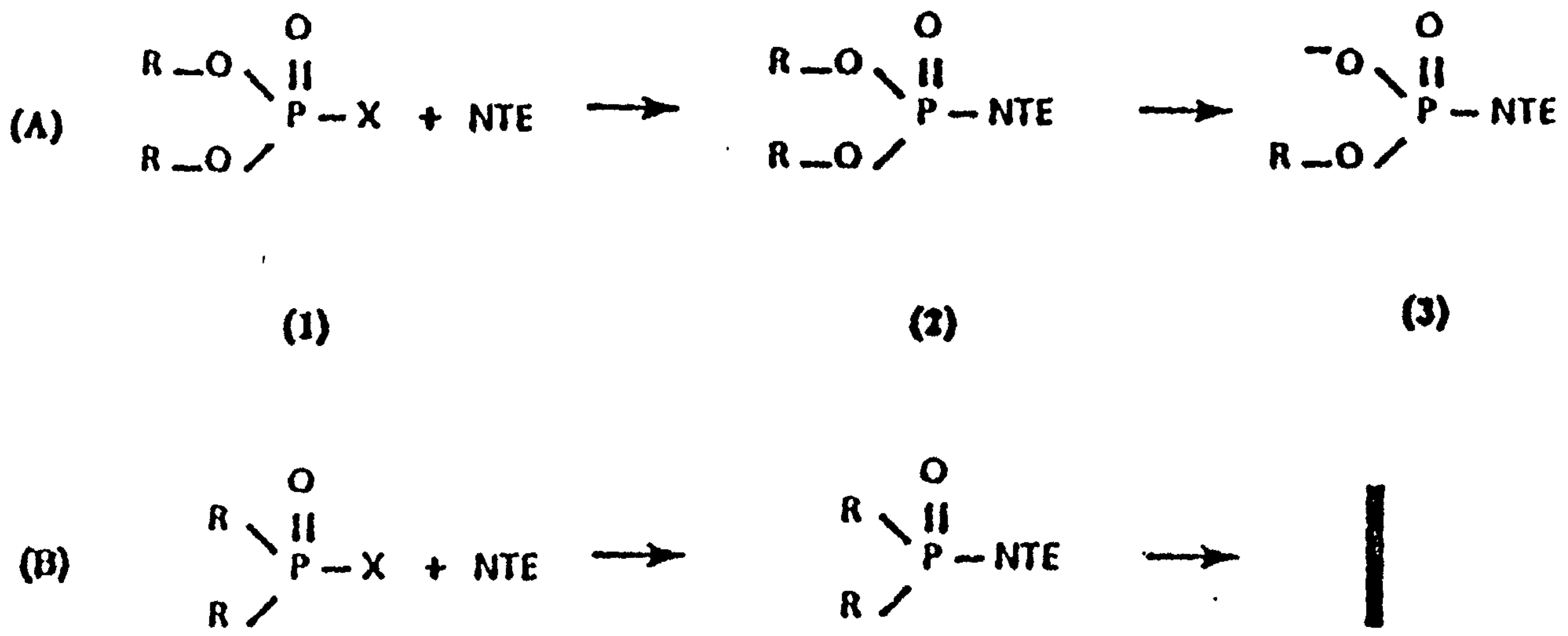


Fig. 5 Reaction of NTE with an organophosphorus compound (A) or an organophosphinate (B) (from Moretto and Johnson, 1987)

1. enzymatic activity, no known physiological role;
2. inhibited enzyme, no toxic effect;
3. in A. aged inhibited enzyme, initiation of the mechanism of OPIDP;
3. in B. no aging possible; initiation of the mechanism of OPIDP impossible, blockade of the effect of neuropathic organophosphorus compounds

The signs and symptoms of delayed neuropathy is tingling sensation, weakness, and ataxia. This progresses to paralysis of the lower limb. The upper limbs are affected only in severe cases.

There is no correlation between the potencies of organophosphorus compounds to inhibit acetylcholinesterase and induce delayed neuropathy.

Experimental evidences indicate that organophosphorus esters are either not mutagenic in vitro or only weakly mutagenic. Acceptable tests do not indicate carcinogenicity. One report claimed that trichlorphon is teratogenic in pigs, but few teratogenic effects have been reported for other compounds (WHO, 1986).

3.3.2 In ecosystems

Organophosphorus insecticides frequently caused death from acute poisoning of the wild fauna. Thus the use of phosphamidon to control leafroller caterpillars on conifers in Montana, USA, caused a 87% decrease in the bird population (Ramade, 1987). In an other incidence 5,000 to 25,000 birds died following a fenthion application at 95.0 ml/hectare (Seabloom et al., 1973). Contrary to the terrestrial environment there is hardly any information on the effects of organophosphorus compounds on the aquatic ecosystem where toxic mechanisms may not be restricted to an anticholinesterase effect.

No information is available on disturbances of marine ecosystems by organophosphorus compounds. In freshwater ecosystems temporary effects have been reported occasionally. At ambient environmental levels, short-term intoxication is not likely to occur and no hazard is to be expected from ethyl-parathion or methyl-parathion applications though in agricultural areas, crustaceans may be affected temporarily. For fenitrothion, results of extensive monitoring programs carried out in Canada in zones sprayed with this pesticide, revealed a temporary disturbance of populations of aquatic invertebrates, but no effects were noted on aquatic vertebrates. Although the doses used in Japan may be up to ten times higher than those in Canada, no evidence of impingement on aquatic ecosystems has been reported in scientific literature. No information has been found on ambient levels of fenitrothion and the eventual consequences in aquatic biotopes in the EEC countries. With regard to malathion it has been shown that chronic exposure to concentrations of 15 $\mu g\ l^{-1}$ can result in 80% mortality of some species of marine crustaceans; it should be emphasized that such concentrations are quite close to ambient levels resulting from practical application rates in mosquito control programmes.

Monitoring studies of river water, drinking water, sediments and biota, in the United States, Canada, Western Europe and Japan have revealed the presence of TBP at concentrations less than one $\mu g\ l^{-1}$; taking into account the very low persistence of this compound, the hazard at such low ambient levels can be considered to be negligible.

3.4 Effect levels

3.4.1 In animal experiments

The acute toxicities of organophosphorus pesticides show wide variation. The most toxic disulfoton, mevinphos, and parathion, have an oral LD50 in rats of below 5 mg kg^{-1} and the LD50s of the least toxic bromophos, malathion and primiphos-methyl are over 2,000 mg kg^{-1} (WHO, 1986). Most of industrial organophosphorus compounds have oral LD50 values over 500 mg kg^{-1} in the rat.

Compared with a pure compound the technical grade may be more or less toxic. Impurities in technical malathion increases toxicity (WHO, 1986).

WHO (1986) tabulates data on 17 organophosphorus pesticides with potential to cause delayed neuropathy in hens. The single dose which elicited delayed neuropathy was in the range of 25 to 150 mg kg^{-1} for mipafox (N,N-diisopropylphosphorodiamidic fluoride), EPN, desbromoleptophos, chlorpyrifos, cyanofenphos, isofenphos, dichlorvos, and cuamophos.

3.4.2 In humans

As the common consequence of different organophosphorus pesticides is the inhibition of acetylcholinesterase, exposure and the severity of adverse effects are usually related to the decrease in red blood cell or plasma acetylcholinesterase activity. A decrease by 30-50% or more indicates exposure and necessitates removal. Signs of poisoning does not occur until blood levels are below 50%, while severe poisoning is usually associated with depression below 20%. Because normal variation in enzyme activity is large, determination must start before exposure and thereafter monitoring should be repeated at weekly intervals.

The possibility to monitor exposure to organophosphorus compounds with the potential to cause delayed neuropathy has been explored, but its feasibility needs further research.

Differences in the toxicity of various organophosphorus compounds are reflected by differences in the levels of both threshold limit values for inhalation exposure and acceptable daily intakes. The American Conference of Governmental Industrial Chemists set the following threshold atmospheric concentrations for occupational inhalation exposure: 0.05 mg m^{-3} for TEPP; 0.1 mg m^{-3} for fonofos, mevinphos, parathion, phosdrin and tri-o-cresyl phosphate; 0.2 mg m^{-3} for dioxathion, parathion-methyl; 0.5 mg m^{-3} for EPN; 3 mg m^{-3} for naled and triphenyl phosphate; 5 mg m^{-3} for crumofate and tributyl phosphate; 10 mg m^{-3} for malathion and temephos (Clayton and Clayton, 1981).

The acceptable daily intakes of 47 organophosphorus pesticides are listed by WHO (1986). They range from 0.00002 mg kg^{-1} for phorate through 0.005 mg kg^{-1} for parathion to 0.1 mg kg^{-1} for crufomate.

3.4.3 In ecosystems

In view of the widespread use of organophosphorus compounds it is not surprising that extensive studies have been performed over the years on the dose(or concentration)- effects of these compounds to various groups of terrestrial and aquatic organisms, as well as to mammal species (as surrogates for man), to determine the hazard of these chemicals to the environment.

With regard to the impact of organophosphorus pesticides on the aquatic environment, extensive and very detailed literature studies have been made during recent years on request of the EEC, and extensive reviews have been published on parathion, methyl-parathion and fenitrothion by Persoone et al. (1985) and on malathion by Premazzi (1983).

Table III summarizes the information collected by Persoone et al. (op.cit.) relevant to this particular report, on the acute toxicity of parathion, methyl-parathion and fenitrothion to estuarine and marine biota. This Table shows that crustaceans are the most sensitive marine organisms. The two species which gave exceptionally low LC_{50} values were Homarus americanus (0.1 µg l^{-1}) and Penaeus sp. (0.02 µg l^{-1}).

The major ecotoxicological as well as toxicological data for the 4 pesticides and the non-pesticide organophosphorus compounds have been summarized in Table IV.

From these tables, it can be seen that organophosphorus pesticides are very toxic to aquatic biota. Insect larvae and crustaceans are extremely sensitive to intoxication by these products, although there are wide differences in sensitivity between different species of the same group. Acute effects on freshwater insects and crustaceans, as well as on marine crustaceans, are reported in scientific literature at concentrations below 1 µg l^{-1}. Other categories of biota are in general less sensitive, though acute effects often occur at concentrations below 1 mg l^{-1}. The 96-hour LC50 of chlorpyrifos to atherinid fishes is reported to range from 0.4 to 6.7 µg l^{-1} (Borthwick et al., 1985).

Chronic effects have been found at and even below the 0.1 µg l^{-1} level for the most sensitive biota (insects and crustaceans); fish species can be affected by 50 µg l^{-1} of some organophosphorus pesticides when exposed for weeks to the compounds.

Experimental field studies have revealed that aerial application of organophosphorus pesticides (at practical rates) can lead to substantial mortalities of non-target insects and crustaceans.

In contrast to the wealth of ecotoxicological information available for organophosphorus pesticides, there are but few data on the effects of non-pesticide organophosphorus compounds to biota. Muir (1984) and Boethling and Cooper (1985) have attempted to collect and interpret some of the data reported in literature on alkyl and arylphosphate toxicity. Although some of the non-pesticide organophosphorus compounds are also very toxic to aquatic biota, the range is highly variable and species - as well as chemical - dependent.

Table III

Effect concentrations (in µg l^{-1}) of three important organophosphorus pesticides to estuarine and marine biota (from Persoone *et al.*, 1985).

Pesticide	Group of organisms	Effect	Concentration
PARATHION	ALGAE	reduction of photosynthesis	1000
	INVERTEBRATES		
	Molluscs	mortality (LC50)	330-10000
	Crustaceans	mortality (LC50)	0.2-4300
	VERTEBRATES		
	Fish	mortality (LC50)	15-65
METHYL-PARATHION	INVERTEBRATES		
	Molluscs	mortality (LC50)	1200-25000
	Crustaceans	mortality (LC50)	0.33-28
	VERTEBRATES		
	Fish	mortality (LC50)	60-76000
FENITROTHION	ALGAE	growth (EC50)	3000-3900
	INVERTEBRATES		
	Coelenterates	mortality (LC50)	30000
	Molluscs	mortality (LC50)	2500-6700
	Crustaceans	mortality (LC50)	<0.1-410
	VERTEBRATES		
	Fish	mortality (LC50)	670-5000

Acute toxicity levels indeed range from 150-200 µg l^{-1} for some compounds on different aquatic biota (insects, crustaceans, fish, and quite surprisingly also lake phytoplankton), to over 6700 mg l^{-1} for other compounds on particular species of fish.

Chronic effects (mortality and growth impairment) resulting form long-term exposure (several weeks) to aryl phosphates has been observed at concentrations as low as a few µg l^{-1} in freshwater crustaceans as well as in fish.

Table IV

Major toxicological data on 4 pesticide and 1 non-pesticide organophosphorus compound of concern to the Mediterranean.

	PARATHION (1)	PARATHION-METHYL (1)	FENITROTHION (1)	MALATHION (2)	TBP (3)
Acute aquatic toxicity ($\mu g\ l^{-1}$)	0.2-10000	0.3-76000	0.1-30000	0.3-30000	(8.8)
Chronic aquatic toxicity ($\mu g\ l^{-1}$)	0.1	0.1	0.1	0.1	
Acute oral LD50 in rats ($mg\ kg^{-1}$) (4)	3.6-13	14-24	250-500	2800	3000
Chronic toxicity for mammals ($mg\ kg^{-1}$)	1-3	2-28	0.2-9	120	
Bioaccumulation	moderate	low to moderate	low to moderate	low	moderate
Bioconcentration factor (BCF)	up to 750	7 (insuff.data)	up to 940 (mosses) 180-250 (fish)	no data	
Mutagenicity	- (to be confirmed)	± (organism dependent)	-	+	-
Carcinogenicity	"equivocal" evidence	-	-	-	-

(1) Persoone *et al.*, 1985
(2) Premazzi (1983)
(3) Muir (1984)
(4) Clayton and Clayton (1981)

Table V

No effect levels (µg l^{-1}) of three major organophosphorus pesticides for estuarine and marine biota (from Persoone et al., 1985).

Pesticide	Group of organisms	Reported in literature	Extrapolated from acute and chronic toxicity data
PARATHION	ALGAE	-	100
	INVERTEBRATES		
	Molluscs	25-30	600
	Crustaceans	<0.1*	<0.1*
	VERTEBRATES		
	Fish	<10*	4
METHYL-PARATHION	INVERTEBRATES		
	Molluscs	-	>1000
	Crustaceans	0.10-0.32	<0.2
	VERTEBRATES		
	Fish	56-10000 *	-
FENITROTHION	ALGAE	<100	300
	INVERTEBRATES		
	Coelenterates		3000
	Molluscs		25
	Crustaceans		<0.1
	VERTEBRATES		
	Fish	10	100-500

* lowest concentration at which a sublethal or chronic effect was noted

The acute aquatic toxicity figure for TBP (8.8 mg l^{-1}) in Table IV (which is the only effect data which could be found for the aquatic environment) originates from an experiment with fish and should be interpreted with caution. It is indeed possible (and very likely) that as for other pesticide and non-pesticide organophosphorus compounds, several groups of aquatic biota are much more sensitive to TBP than fish.

In the reports on organophosphorus pesticides of Persoone et al. (1985), NOEL's (no observed effect level) are given for parathion, methyl-parathion and fenitrothion, which are either experimental or have been extrapolated from acute or chronic toxicity data (Table V).

From this table it appears that for the most sensitive biota in the marine environment (crustaceans) no-effect levels for organophosphorus pesticides are below 0.1 μg l^{-1}.

A few experimental no-effect levels have also been reported in scientific literature for non-pesticide organophosphorus compounds. For the waterflea Daphnia NOEL's range from 17 μg l^{-1} to 460 μg l^{-1} for one particular arylphosphate ester (Boethling and Cooper, 1985); the 30-fold difference found between the lowest and the highest NOEL is due to differences in the commercial formulation of the same organophosphorus ester. Exposure of rainbow trout for more than one month to levels of arylphosphates of as low as 0.2 μg l^{-1} has been shown to cause many adverse effects: behavioral, morphological as well as physiological.No information is available on no-effect levels for TBP to aquatic biota, and only one figure has been found for the chronic effect on one fish species; it would be meaningless to try to derive a "safe concentration" for the aquatic environment from this sole figure.

On the basis of all toxicological information available on non-pesticide organophosphorus compounds, it seems however, that the NOEL's for the most toxic non-pesticide organophosphorus compounds to the most sensitive biota in the marine environment, are of the same order of magnitude as those of organophosphorus pesticides, i.e. below 0.1 μg l^{-1}.

4. PRODUCTION AND USE

The available figures on production and use of both organophosphorus pesticides and non-pesticides is scarce and fragmentary at the national as well as at the international level. In addition, different figures are reported by different sources and this indicates the difficulties in obtaining accurate and reliable data. Table VI lists organophosphorus compounds presently manufactured and used. Very few data exist for countries that border the Mediterranean. It is known, e.g., that 21,000 tons of organochlorine and organophosphorus pesticides were used in Italy in 1983, of which approximately 19,000 tons were organophosphorus insecticides (ISTAT, 1984). Cyprus, Greece, Malta and Turkey reported consumption data on organophosphorus compounds to the Coordinating Unit for MAP. According to these data Cyprus imported 313 and 389 tons of organophosphorus pesticides in 1987 and 1988 respectively but these figures do not refer to active ingredient. Similarly Malta imported in 1986 45 tons of organophosphorus pesticides. Turkey imported in 1985 130 tons of pesticide and non-pesticide organophosphorus compounds while this figure in 1986 was reduced to 67 tons. Greece reported the following consumption figures (in tons) for 1988: azinphos-ethyl 80, azinphos-methyl 15, fenitrothion 5, fenthion 130, malathion 50, parathion 30, parathion-methyl 100 and dichlorvos 10. The consumption of parathion, malathion and other organophosphorus pesticides in Mediterranean countries as reported to FAO is given in Table VII.

Despite the weak data base available, a slight increase in the total consumption of organophosphorus compounds over the years may be expected. For some insecticides, such as parathion-methyl, a substantial decrease in consumption can be noted in the EEC countries: from 3,500 tons in 1974 to 1,000 tons in 1984.

Table VI

Organophosphorus compounds currently manufactured and used.

A. Pesticides

- acephate
- amidithion
- azinphos-ethyl
- azinphos-methyl
- bromophos
- bromophos-ethyl
- carbophenothion
- chlorpyrifos
- chlorfenvinphos
- chlorthion
- coumpahos
- demeton
- demeton-S-methyl
- demeton-S-methylsulfon
- dialifor
- diazinon
- dichlorvos
- dicrotopos
- dimethoate
- dioxathion
- disulfoton
- ethion
- fensulfothion
- fenthion
- fenitrothion
- fonofos
- formothion
- leptophos
- malathion
- methamodophos
- methidation
- mevinphos
- monocrotopos
- naled
- omethoate
- oxydemeton-methyl
- parathion-ethyl
- parathion-methyl
- phenkapton
- phorate
- phosalone
- phosmet
- phosphamidon
- phoxim
- pirimiphos-ethyl
- pirimiphos-methyl
- prothoate
- pyrazophos
- ronnel
- sulfotepp
- temephos
- tetrachlorvinphos
- thiometon
- triamiphos
- triazophos
- trichlorfon
- vamidothion
- zinophos

B. Non-pesticides

- dibutyl phenyl phosphate
- cresyl diphenyl phosphate
- cumylphenyl diphenyl phosphate
- 2-ethylhexyl diphenyl phosphate
- isodecyl diphenyl phosphate
- isopropylphenyldiphenyl phosphate
- tributyl phosphate
- tricresyl phosphate
- triphenyl phosphate
- tris (2-chloroethyl) phosphate
- tris (2-chloropropyl) phosphate
- tris (1,3-dichloropropyl) phosphate
- tris (2-ethylhexyl) phosphate

Table VII

Consumption of organophosphorus pesticides (in kg) as reported to FAO by some Mediterranean countries.

Country	Parathion						Malathion						Other organophosphorus					
	1974-76	1981	1982	1983	1984	1985	1974-76	1981	1982	1983	1984	1985	1974-76	1981	1982	1983	1984	1985
Algeria																		
Cyprus	222	1782	842	1400			89	255	212	200			132	534	591	500		
Egypt	397						3573	2080					54267	7200				
France																		
Greece	2873						2837						6493					
Israel													8557	11280	7550	5860		
Italy	23147	24231	18591	24315	26000		8997	6068	5524	5752	5000		87204	149558	144956	158910		
Lebanon																		
Libya																3100		500
Malta									350			200		250				
Monaco																		
Morocco																		
Spain																		
Syria																		
Tunisia																		
Turkey	6408	1750	1837				1939	550	577				20764	11000	11550			
Yugoslavia																		

Source: FAO Production yearbooks

The total production of non-pesticide organophosphorus compounds in the United States increased from 47,000 tons in 1977 to 77,000 tons in 1986. As indicated above, there are compounds such as TBP which are now consumed to a larger extent in Europe than in the United States of America.

Based on world production and use of organophosphorus pesticides, ethyl-parathion, methyl-parathion, fenitrothion and malathion certainly are among the most important organophosphorus pesticides. Some more information follows on these 4 major compounds.

Parathion

Total production in the EEC countries was estimated at 15,000 tons in 1974, 3,500 tons in 1979 and 6,000 tons in 1984; consumption values decreased from 3,500 to 1,000 tons. In the United States the production of this pesticide had decreased from 7,530 tons in 1965, to 6,940 tons in 1970, to 4,535 tons in 1972 while the consumption in 1982 was estimated to be 3,125 tons. In 1989 the total production of parathion and parathion-methyl in the United States was 40,000 tons and in the EEC countries 12,000 tons out of which 1,000 tons were utilised.

Parathion-Methyl

The EEC production and use of this organophosphorus pesticide in 1974 was approximately 15,000 and 3,500 tons respectively. In 1984 while the production decreased to 8,000 tons, the consumption dropped to 1,000 tons. In 1982 in the United States the production was 23,000 tons and the consumption 7,700 tons. Since total world production of parathion-methyl in 1973 was as high as 60,000 tons, one may thus expect a decrease in production and use of this pesticide in the EEC countries in the years to come. (ECDIN)

Fenitrothion

This organophosphorus insecticide is quite well-known in Canada since it has been applied by aerial spraying on forests in amounts near 1,000 tons/year since 1967 to combat spruce budworm pests. Data available for the EEC countries indicate a total use of 800 tons for 1979 and 300 tons for 1989.

Malathion

Total production of this pesticide was 30,000 tons in 1980-81. In the Mediterranean countries 2,100 tons (active ingredient) of malathion were sprayed during the same period (40% of which was in Spain), versus 9,700 tons in Asia. In the EEC countries the production of malathion has increased from 5,750 tons in 1974 to 10,000 tons in 1984 with an annual use close to 2,000 tons. In the United States the annual use of this pesticide over the period 1972-1982 has varied only slightly: from 7,350 tons (1972) to 6,350 tons (1982). The total consumption of malathion during the period 1980-81 is shown in Table VIII (Premazzi, 1983). In 1988 the total production was 25,000 tons and in the EEC countries 14,000 tons out of which 1,000 tons were utilised.

Table VIII

Total consumption of malathion in various continents and countries (period 1980-1981) (Premazzi, 1983).

Area	tons/yr active constituent	Area	tons/yr active constituent
Western Europe	1.515	Asia	9.680
Spain	220	Pakistan	4.000
Italy	250	Sri Lanka	2.150
France	220	India	1.500
Greece	150	South Korea	400
Portugal	15	Iran	350
Finland	10	Turkey	200
Germany	10	Iraq	200
Belgium	5	Japan	170
Netherlands	5	Taiwan	150
North America		Malaysia	150
USA	7.000	Thailand	100
Canada	350	Nepal	100
Central and South		Philippines	80
America	4.037	Lebanon	50
Brazil	1.650	Syria	50
Argentina	800	Saudi Arabia	20
Cuba	450	Indonesia	10
Columbia	400	Africa	905
Mexico	300	Morocco	300
Guatemala	170	Kenya	150
El-Salvador	150	Egypt	140
Venezuela	50	South Africa	130
Uruguay	20	Algeria	50
Peru	18	Libya	50
Nicaragua	15	Tunisia	40
Ecuador	9	Zambia	20
Chile	5	Ethiopia	10
Australia	250	Tanzania	10
New Guinea	100	Nigeria	5
		New Zealand	10
	TOTAL	23.850	

For non-pesticidal organophosphorus compounds the total production in the United States in 1986 has been estimated to be 77,000 tons. For two of the most commonly produced and used compounds: tributylphosphate (TBP) and tris(2-chlorethyl)phosphate (TCEP) only a few data could be found for the former. In the EEC countries 10,000 tons of TBP were produced and used in 1979 as well as in 1983, versus only 2,500 tons in 1972. The consumption in the United States during 1983 was 1,360 tons. (Muir, 1984; ECDIN/EEC).

5. ORGANOPHOSPHORUS COMPOUNDS IN THE MEDITERRANEAN AREA: INPUTS, LEVELS, AND HAZARDS

On the basis of the quantities currently produced and/or used in four Mediterranean countries (France, Israel, Italy and Spain) and taking into account the toxicity, persistence and bioaccumulation six organophosphorus compounds have been tentatively selected from the compounds listed in Table VI; these six substances will be treated hereafter more in detail as "case-examples" for the Mediterranean. Four of the six compounds are pesticides (ethyl-parathion, methyl-parathion, fenitrothion and malathion), the other two are non-pesticide organophosphorus compounds [tributyl phosphate (TBP) and tris(2-chloroethyl) phosphate (TCEP)].

5.1 Sources, inputs and levels in the Mediterranean

The types of marine areas at risk from contamination by organophosphorus discharges are, in order of decreasing importance:

- river mouths and estuaries
- lagoons
- shallow waters
- marshes

By far the largest input of organophosphorus compounds in the marine environment comes through transportation of the compounds to the sea via surface waters. Industrial effluents containing organophosphorus residues may, however, also be discharged directly into shallow waters through pipelines from on-shore plants.

A third source of pollution may arise from spraying of crops with organophosphorus pesticides on fields nearby the sea and last but not least aerial transportation of both non-pesticide and pesticide organophosphorus compounds from various point or non-point sources may eventually also add to the pollution of coastal waters, lagoons and marshes.

Information on point source inputs of the organophosphorus compounds into the Mediterranean is scarce. Most of the information available is related to surface waters in Italy. In the Tiber river maximum concentrations of malathion of 0.5 - 0.6 $\mu g\ l^{-1}$ have been detected during the years 1970-73 (Leone and Puccetti, 1971, 1978). Values have also been published for different pesticides in surface waters of the Ferrara Province during the period 1975-84 (Baldi et al., 1986). For malathion, the concentrations were usually below 0.4 $\mu g\ l^{-1}$, although, in 1977 and 1982, 2.6 and 4.2 $\mu g\ l^{-1}$ respectively were noted. Parathion-methyl concentrations were usually lower than 1 $\mu g\ l^{-1}$; a maximum level of 11.1 $\mu g\ l^{-1}$ was found in 1981. Parathion-methyl values were essentially below 0.1 $\mu g\ l^{-1}$, except in 1976 where a 4.4 $\mu g\ l^{-1}$ concentration was recorded. No fenitrophion has been detected in any water sample in this Italian Province. In France, 2 to 4 $\mu g\ l^{-1}$ parathion-ethyl and malathion have been reported in the river Saône in 1968 (Mestres et al., 1969), as compared to values below 8 $\mu g\ l^{-1}$ in the drainage waters of the Camargue in 1978 (Podjelski and Dervieux 1978). In the latter drainage waters, the concentration of the pesticide decreased from 5 $\mu g\ l^{-1}$ to 0.2 $\mu g\ l^{-1}$ three days after application.

In Spain, values have been reported for fenitrothion in lake Albufera at Valencia (Carrasco et al., 1987). For the period 1983-85, concentrations varied from 0.1 to 2.0 µg l^{-1}; these values decreased each time to non detectable levels (below 0.05 µg l^{-1}) within two weeks. Fenitrothion levels in Gambusia affinis up to 306 ng g^{-1} have been reported (Barceló et al. 1990) and in soils from the Ebro delta up to 1 ng g^{-1} (Durand, 1989). For the non-pesticide TBP, data were published in 1987 for the Besos river mouth in Spain, (Gomez-Belinchon et al., 1988); the organophosphorus levels ranged from 0.02 to 1 µg l^{-1}, with some peaks of 14 µg l^{-1}. In the Llobregat estuary TBP and TCEP were found at concentrations of up to 0.3 and 0.4 µg l^{-1} respectively (Barceló, 1990). In Italy, TBP has been monitored in surface and drinking waters in Northern Italy in 1987. TBP levels in the river Po varied from 0.02-0.03 µg l^{-1} in Ferrara, to 0.04-0.13 µg l^{-1} in Turin (Galassi et al., 1986).

In Greece, organophosphorus pesticides (diazinon, azinphos, methyl and parathion-methyl) have been detected in the Ioannina lake and in the Kalamas river during the period 1984-1985 (Albanis et al., 1986). Fig. 6 shows that the concentration of these compounds fluctuate seasonally, with the highest concentrations in the summer when rainfall is lower and pesticides are intensively used.

The comparison of the sources and inputs of organophosphorus compounds into the Mediterranean with other regions in the world is limited by the availability of data. Most of the information available at present reports contamination levels in surface waters (mainly lakes and river waters) in western Europe, Canada and the United States. Concentrations in seawater or sediments are scarce or lacking. A pilot survey organised in the framework of the Long-term Programme for Pollution Monitoring and Research in the Mediterranean (FAO/UNEP/IAEA/WHO, 1989) found lower values in two Mediterranean estuaries (Ebro and Llobregat in Spain) and in the Northern Adriatic coast (Lido delle Nazioni and Marina di Ravenna in Italy) than those reported for surface waters. The study was not supported by data quality assurance. The summary of the findings is as follows:

(a) Fenitrothion and malathion were not detected in any of the sampling sites. Parathion-methyl was present only in the Llobregat estuary, where concentrations ranged from <1.0 to 30 ng l^{-1}. Parathion was detected at the two Adriatic sites where concentrations ranged from <1.0 to 3 ng l^{-1}. Phorate was not detected while diazinon was detected at every site with the exception of Ebro estuary. Concentrations were low in the Adriatic (<1.0 to 2 ng l^{-1}) but in the Llobregat estuary they ranged from <1.0 to 30 ng l^{-1}.

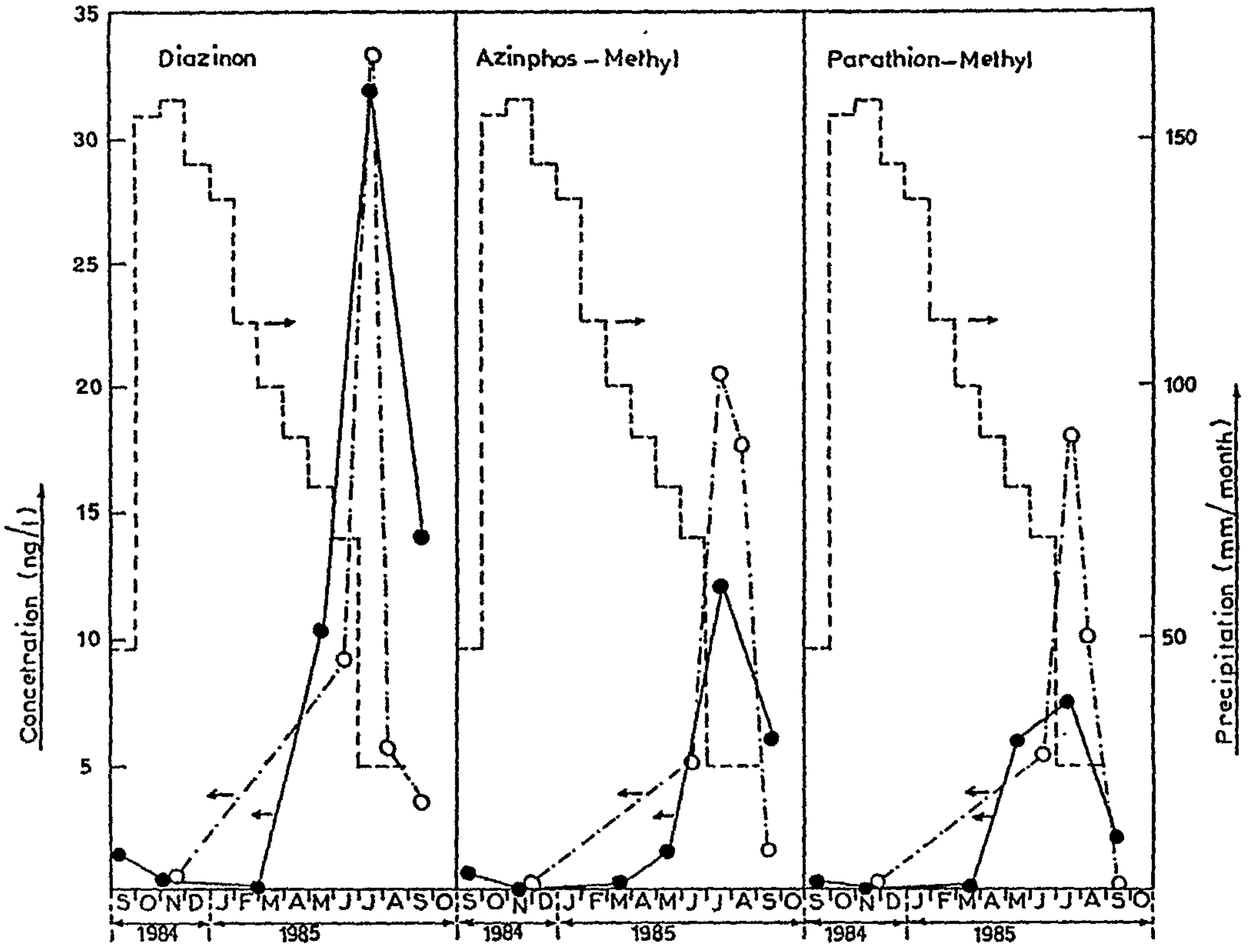


Fig. 6 Mean values of the pesticides Diazinon, Azinphos-methyl and Parathion-methyl in the Ioannina lake (.-.) and in the Kalamas river (o-.-.-o) in Greece over one year period. The dotted line (---) represents the average height of rainfall (mm/month) in the area for a fifteen year period (from Albanis *et al.*, 1986)

(b) Industrial organophosphorus compounds usually gave higher concentrations than pesticides. The concentration of tributyl phosphate was 1-300, <1.0 to 20, 8 to 34 and 3 to 1000 ng l^{-1} at the Llobregat estuary, Ebro estuary, Lido Nazioni and Marina di Ravenna respectively. The corresponding concentrations were for tris-2-chloroethyl phosphate: <1.0 to 400, <1.0, <1.0 to 100 and <1.0 to 300; for tris-isobutyl phosphate: <1.0 to 900, <1.0, 40 to 200, 13-64. Trischloro-isopropyl phosphate was estimated only at the two Adriatic sites, where concentrations ranged from non detectable to 31 in Lido Nazioni and from non detectable to 9 ng l^{-1} in Marina di Ravenna.

(c) The pilot study included two other areas which were the Haifa Bay and the Nile delta. At these sites no organophosphorus pollution could be detected but the detection limits (500 and 700 ng l^{-1}) were high and therefore the negative results only exclude the possibility of toxic contamination.

(d) The survey included estimations in biota. In the Adriatic sites all the organophosphorus compounds were below the detection limit in the biota (mussels). In the Ebro estuary organophosphorus pesticides were detected in _Mullus barbatus_ the maximum values of which were: diazinon, 12 ng g^{-1}; fenitrothion, 16 ng g^{-1}; paraoxon, 16 ng g^{-1}; parathion-methyl 2.2 ng g^{-1}. Industrial organophosphorus compounds could not be detected in biota. In the canals of the Ebro delta the presence of azinphos-ethyl, coumaphos, dioxathion, fenchlorphos (Ronnel), fenitrothion, fenthion, malathion, and tetrachlorvinphos was identified in _Gambusia affinis_. Maximum concentrations were 306 ng g^{-1} fresh weight for fenitrothion, and 9 to 35 ng g^{-1} for the others.

Ambient **parathion** levels in river and lake water in Europe and the United States range from non-detectable to 0.07 µg l^{-1}, most samples showing no contamination. In the Netherlands, Leistra _et al._ (1984) report that leaching of organophosphorus compounds (such as e.g. parathion) from glasshouse soils can contribute substantially to the pollution of water courses (0.1 to 2.0 µg l^{-1}).

For **parathion-methyl** concentrations are between 0.02 to 0.04 µg l^{-1} in most cases, in different lakes and surface waters in the United States. Following a four year survey of 144 estuaries in 21 states of the United States, residues in aquatic biota only showed 2 cases of contaminated fish (out of 1524 samples).

For **fenitrothion**, concentrations in Canadian waters range from 2.3 to 16.3 mg l^{-1} and mainly result from run-off from contaminated soils following spraying. Within a few days the levels had usually decreased to values below 1 mg l^{-1}. The median fenitrothion concentrations detected in aquatic biotopes in Quebec after spraying ranged from 1 to 8 µg l^{-1} (Morin _et al._, 1986). One study has reported levels of up to 0.7 mg l^{-1} (Moody _et al._, 1978).

Organophosphorus compounds may reach the sea when mangrove swamplands are sprayed for mosquito control. In Florida fenthion was sprayed aerially as a thermal fog at a rate of 28.0 mg m^{-2} from approximately 30 meters height and deposition on the recipient surface water (a ditch which communicated with the estuarine through a river) and concentration were estimated after three different test runs. Depositions were 1.86, 0.125, and 0.03 mg m^{-2} and peak concentrations 1.6, 0.16 and 0.16 ng l^{-1} respectively. Concentrations declined with zigzags (due to tidal changes) to non-detectable levels in 24 hours (Wang et al., 1987).

Malathion concentrations in surface water of 0.3 µg l^{-1} at Essex in the United Kingdom and 0.01 - 0.07 µg l^{-1} in the river Rhine in the Federal Republic of Germany have been reported.

Contamination of sediments has been found to occur in the vicinity of pesticide manufacturing plants. In Denmark, marine sediments close to a company producing organophosphorus pesticides contain levels of malathion, parathion and methyl parathion ranging from 1 ng g^{-1} up to 3.8 ng g^{-1} (Kjolholt, 1985).

For the non-pesticide organophosphorus compounds, and particularly for TBP, concentrations in the river Rhine vary from 0.03 to 0.3 µg l^{-1}, with occasional values of 1 µg l^{-1}. TBP has frequently been detected in different lakes and rivers in the U.K., Norway, Switzerland, the Federal Republic of Germany, the Netherlands, the U.S.A. and Canada. The maximum levels were 10 µg l^{-1} in the river Wall in the Netherlands and 2 µg l^{-1} in the Delaware river in the U.S.A.

Concentrations of phosphate esters in sediments and fish tissue can, however, be 100 to 1000 fold higher than those in water, (ECDIN/EEC; Muir, 1984; N.R.C., 1975).

Nevertheless surveys carried out in the United States and Japan suggest that with the exception of a few compounds (such as TPB), phosphate esters are not widespread contaminants of aquatic environments.

5.2 Hazards to marine biota and humans

5.2.1 Marine biota

Despite the fact that neither a monitoring programme has been carried out in the Mediterranean, nor reported concentration values are supported by quality assurance data, it is most probable that the levels of organophosphorus compounds in this sea are too low to present hazard to the aquatic biota. The concentration of organophosphorus pesticides in rivers which flow into the Mediterranean sea ranges from non-detectable to 5.0 µg l^{-1}, with occasional peaks of 11 µg l^{-1}. For industrial pesticides, concentrations in rivers range from 0.02 to 1.3 µg l^{-1}, with an outlier maximum of 14 µg l^{-1} for TBP. Average concentrations for both agricultural and industrial organophosphorus compounds are below 0.1 µg l^{-1}. Assuming a dilution of 1/1,000 in coastal waters, concentrations most likely do not exceed 0.1 ng l^{-1}. This concentration is three orders lower than concentrations toxic to the most sensitive aquatic species (see Tables V, VI and VII). However the FAO/UNEP/IAEA/WHO (1989) pilot study indicated that in two estuaries and two Adriatic sites 5 miles from the coast the concentration of organophosphorus pesticides exceeded 0.1 ng l^{-1}, though remained below the lowest toxic concentration for the most sensitive species. The difference in the concentration of industrial organophosphorus compounds between surface waters and estuarine or sea water was less striking, and the maximums approached the concentration which may cause harm to sensitive species after prolonged exposure.

5.2.2 Human health hazard

The route of exposure to organophosphorus compounds in sea or seaproducts is either dermal (contact with contaminated water) or oral (ingestion of contaminated seafood or water). Intoxication can occur when intake exceeds the no-effect level. Considering the environmental instability of organophosphorus compounds, low bioconcentration potential, and low reported concentrations in surface waters, it is reasonable to conclude that organophosphorus compounds in the marine environment are not human health hazards. This view is supported by the comparison of non-toxic levels of exposure with concentrations found by the FAO/UNEP/IAEA/WHO (1989) pilot survey.

Parathion has a recommended occupational exposure limit of 0.1 mg m^{-3}. As the respiration volume of a 70 kg man is 12 m^3 during 8 h work, the average permissible daily intake during a 5 day working week is 0.86 mg per day. The acceptable daily intake (ADI) of parathion given by FAO/WHO (WHO, 1986) is 0.005 mg kg^{-1} (0.35 mg/70 kg day^{-1}). In either case the pollution of seafood must exceed 1.6 µg g^{-1} fresh weight to deliver intake above the acceptable level when one fishmeal (200 g) is consumed daily. As the ADI of malathion is 0.02 mg kg^{-1} and the NOEL reported by the National Research Council of Canada for fenitrothion is 0.33 mg kg^{-1}, the toxicologically hazardous concentration in seafood for these two pesticides is over 7.0 and 115 µg g^{-1} fresh weight respectively. In the study sites of the FAO/UNEP/IAEA/WHO (1989) survey the concentration of industrial organophosphorus compounds did not reach the detection limit while the maximum concentration of pesticides remained about one thousand times below the concentration which could supply the allowable daily intake for consumers of one fishmeal per day.

6. EXISTING NATIONAL AND INTERNATIONAL REGULATIONS FOR THE PREVENTION OF MARINE POLLUTION BY ORGANOPHOSPHORUS COMPOUNDS

There is only one regulation in the Mediterranean area which limits aquatic pollution by organophosphorus compounds. In Italy L319/1976 sets the acceptable concentration for total organophosphorus pesticides to 0.1 mg l^{-1} in effluents. Regulations outside the area are also uncommon and mainly refer to surface waters, foodstuff, and crops.

The maximum allowable concentration of parathion is 3 µg l^{-1} in surface waters in the USSR and Sweden. In Mexico it is 100 µg l^{-1} in estuaries and 1 µg l^{-1} in coastal waters. In the U.S.A., the Environmental Protection Agency has set the water quality criteria for the protection of freshwater and marine aquatic biota at 0.04 µg l^{-1} for parathion and 0.1 µg l^{-1} for malathion. In the USSR the maximum allowable surface water concentration is 50 µg l^{-1} for malathion and 250 µg l^{-1} for fenitrothion.

In the U.S.A. the emission limits are regulated for hazardous substances by the Water Pollution Control Act. Thus the discharge of parathion-methyl from any point source into USA waters requires a permit. Inspection, monitoring, and reporting requirements are specified after issue of permit. Even if it is not stated in the permit, discharge of the substance must be reported when it exceeds the highest of the following levels: (a) 100 $\mu g\ l^{-1}$, (b) 5 times the maximum concentration established by the director of EPA.

It is obligatory that owners/operators of vessels or offshore facilities should notify the USA government of any discharge of parathion, parathion-methyl and malathion in or on navigable waters adjoining shorelines or the contiguous zone. Discharges of more than 45.4 kg (100 pounds) parathion-methyl, 4.5 kg (10 pounds) malathion and 0.45 kg (1 pound) parathion in any 24 hour period are considered as violation of the Federal Water Pollution Control Act.

Other international regulations deal with allowable concentrations in the working atmosphere (see 2.4.2) and in foodstuffs. In Netherland and the Federal Republic of Germany maximum residue levels in vegetable and fruit are 0.05 $mg\ kg^{-1}$ for fenitrothion and parathion, 0.1 $mg\ kg^{-1}$ for parathion-methyl and 0.5 $mg\ kg^{-1}$ for malathion.

In 1982 EEC prepared a list of 129 potentially dangerous substances based on their toxicity, persistence and bioaccumulation. This is in fact List I of the Annex to Directive 76/464/EEC. In February 1990 the Commission proposed to the Council that priority should be given to some of these substances for the purpose of fixing limit values and quality objectives. Among them are the following organophosphorus compounds: azinphos-methyl, azinphos-ethyl, fenitrothion, fenthion, malathion, parathion, parathion-methyl and dichlorvos.

7. RATIONALE FOR ESTABLISHING MARINE POLLUTION CONTROL MEASURES IN THE MEDITERRANEAN REGION

The scientific rationale for control depends on whether (a) the levels actually encountered in the marine environment are close to those which are likely to prove harmful to the ecosystem or cause adverse effects in man through any exposure route; (b) harmful levels may eventually be reached if inputs are not controlled.

As at present only limited data are available on the degree of marine pollution by organophosphorus compounds, a definite answer to the above points cannot be given and further studies are required. However the limited data produced in the framework of the MED POL pilot monitoring study indicate that concentrations of organophosphorus pesticides in water even though they largely lie below 10 $ng\ l^{-1}$, in one case they reached values of up to 30 $ng\ l^{-1}$ (Llobregat estuary, Spain). Similarly, concentrations of non-pesticide organophosphorus compounds eventhough generally low, they occasionally reached concentrations of 1000 $ng\ l^{-1}$.

As it is well documented that 100 ng l^{-1} of certain organophosphorus compounds can cause damage in crustaceans, which is the most sensitive group of marine organisms to these compounds, it is considered necessary that the situation must be investigated further through appropriate research and monitoring work, so that it can be definitively decided whether and what specific marine pollution control measures should be implemented for the protection of marine life.

As organophosphorus compounds released into the marine environment are not persistent in water or biota they would not present a hazard to human health through the marine food chain at the concentrations reported in the pilot study (maximum concentration in biota, 306 ng g^{-1} F.W. for fenitrothion). It is not therefore considered necessary that any measures specifically aimed at protecting human health should be indicated at this stage.

8. RECOMMENDATIONS FOR ADOPTION BY THE CONTRACTING PARTIES

On the basis of the assessment document (UNEP(OCA)/MED WG.12/4) prepared by the Secretariat in close collaboration with FAO, WHO and IAEA the following recommendations concerning organophosphorus compounds are submitted to the Scientific and Technical Committee with a view to their transmission by the Secretariat to the next meeting of the Contracting Parties:

(i) The Contracting Parties adopt the following statement:

Organophosphorus compounds is the general name for a large group of organic compounds containing phosphorus. The search for new compounds has led to the synthesis of more than 50,000 organophosphorus compounds and the registration of 250 of them as pesticides. However, the commonly used compounds do not exceed 100 while a number of these compounds are used by industry and are termed as non-pesticides.

The available information on the production and use of both organophosphorus pesticides and non-pesticides is scarce, fragmentary and in many cases contradicting. Based on world production and use of organophosphorus pesticides, parathion, parathion-methyl, malathion and fenitrothion are certainly among the most important ones.

Eventhough in many cases they are looked at and treated as a group, organophosphorus compounds have varying physical and chemical properties eg. their solubility in water can vary as much as 10,000 times. In general however they are considered unstable in water but some of them can have a very high toxicity. The "no-observed effect level" for crustaceans which appear to be the most sensitive marine biota to organophosphorus compounds is well below 100 ng l^{-1}.

Organophosphorus compounds reach the marine environment through rivers, agricultural run-off and industrial point sources. Information on their levels in the Mediterranean is very scarce. A MED POL study indicated that both pesticide and non-pesticide organophosphorus compounds can be detected in Mediterranean river estuaries and coastal areas subjected to pollution from these compounds. Some compounds were also detected in fish. However in general these concentrations are lower than those found in freshwater systems.

Only very few countries have so far taken measures for the control of water pollution by organophosphorus compounds and in most of these cases it is in the form of water quality criteria.

It is generally accepted that on the basis of available information there is no human health hazard from the consumption of seafood and therefore specific measures are not warranted at the present stage.

As far as the aquatic ecosystems are concenred it is evident that fresh water ecosystems are exposed to a bigger danger than the marine ones. However the present insufficient data available do not allow for specific recommendations to be made and further studies are necessary.

(ii) The Contracting Parties agree:

(a) that it is not considered pertinent at the present stage and on the basis of available evidence to apply any specific measures for the protection of human health from hazards through the consumption of sea food containing organophosphorus compounds;

(b) to request the Secretariat to organise relevant studies on organophosphorus compounds which will provide data useful for the formulation of marine pollution control measures to be proposed to the Contracting Parties by 1993;

(c) to provide the Secretariat to the Barcelona Convention with the fullest information possible on:

(i) quantities of organophosphorus compounds produced and consumed in the country

(ii) present national legislative and administrative measures employed for the control of pollution by organophosphorus compounds

(iii) available data on effects and levels of organophosphorus compounds in Mediterranean coastal and estuarine waters.

9. REFERENCES

Albanis, T.R., P.J. Pomonis and A.Th. Sdoukos (1986), Organophosphorus and carbomate pesticide residues in the aquatic system of Ioannina basin and Kalamas river (Greece), Chemosphere, 15:1023-1034.

Baldi, M., A. Bovolenta, L. Penazzi, A. Fantini e D. Finessi (1986), Residui di organoclorurati e di organofosforati in acque superficiali della provincia di Ferrara. Dieci anni di indagni: 1975-1984, Boll.Chim.Igien., 37:449-480.

Barceló, D. (1988), Application of thermospray liquid chromatography-mass spectrometry for determination of organophosphorus pesticides and trialkyl and triaryl phosphates, Biomed.Environ. Mass.Spectrom., 17:363-369.

Barceló, D. (1988a), A review of liquid chromatography in environmental pesticide analysis, Chromatographia, 25:928-936.

Barceló, D., F.A. Maris, R.B. Geerdink, R.W. Frei, G.J. De Jong and U.A.Th. Brinkman (1987), Comparison between positive, negative and chloride-enhanced negative chemical ionization of organophosphorus pesticides in on-line liquid chromatography-mass spectrometry, J.Chromatogr., 394:65-76.

Barceló, D., C.Porte, J. Cid and J. Albaigés (1990), Determination of organophosphorus compounds in coastal environments by gas chromatography nitrogen phosphorus and main spectrometric detection. Intern.J.Environ.Anal.Chem. 38:199-209.

Boethling, R.S. and J.C. Cooper (1985), Environmental fate and effects of triaryl and tri-alkyl/aryl phosphate esters, Residue Reviews, 94:49-99.

Borthwick, P.W., J.M.Jr Patrick and D.P. Middaugh (1985), Comparative acute sensitivities of early life stages of Atherinid fishes to chlorpyrifos and thiobencarb. Arch.Environ.Contam.Toxicol., 14:465-473.

Bowman, B.T. and W.W. Sans, (1983), Determination of octanol-water partition coefficients (Kow) of 61 organophosphorus and carbamate insecticides and their relationship to respective water solubility (S) values, J.Environ.Sci.Health, B18(6):667-683.

Cairns, Th., E.G. Siegmund, R.A. Jacobson, T. Bang, G. Petzinger, W. Morris and D. Heikes (1983), Application of mass spectrometry in the regulatory analysis of pesticides and industrial chemicals in food and feed commodities, Biomed.Mass.Spectrom., 10:301-315.

Cairns, Th., E.G. Siegmund and J.E. Froberg (1985), Identification of Diazinon and its metabolites in spinach by chemical ionization mass spectrometry, Bull.Environ.Contam.Toxicol., 35:291-295.

Carrasco, J.M. M. Planta, V. Gomez-Casals and V. Moragues (1987), Pesticide residues in lake Albufera, Valencia, Spain, J.Assoc.Off.Anal.Chem., 70:752-753.

Clark, G.J. A.R. Goodin and J.W. Smiley (1985), Comparison of ultraviolet and reductive amperometric detection for determination of ethyl and methyl parathion in green vegetables and surface watger using high-performance liquid chromatography, Anal.Chem., 52:2223.

Clayton, G.D. and F.E. Clayton eds, (1981), Patty's Industrial Hygiene and Toxicology. J. Wiley & Sons, New York. vol. 2. pp.2359-2383, 4801-4843.

D'Agostino, P.A. and L.R. Provost (1986), Capillary column ammonia chemical ionization mass spectrometry of organophosphorus chemical warfare agents and simulants, Biomed.Mass.Spectrom., 13:231-236.

Dreisbach, R.H. and W.O. Robertson (1987), Handbook of Poisoning. 12th edition. Appleton & Lange, Norwalk, Connecticut. pp.110-118.

Durand, G., R. Furteza and D. Barceló (1989), Determination of chlorotriazines and organophosphorus pesticides in soil samples by two different clean-up methods. Chromatographia, 28:597-604.

E.C.D.I.N. Service (CEC, Joint Research Centre, Ispra), Italy.

Farran, A., J. De Pablo and D. Barceló (1988), Identification of organophosphorus insecticides and their hydrolysis products by liquid chromatography in combination with UV and thermospray-mass spectrometry detection, J.Chromatogr., 455:163-172.

FAO/UNEP/IAEA/WHO (1987), Report of the FAO/UNEP/IAEA/WHO Ad-hoc Meeting on Organophosphorus compounds (Athens, 18-20 November 1987). Document FAO/OP/4 14p. (mimeo).

FAO/UNEP/IAEA/WHO (1989), Report of the FAO/UNEP/IAEA/WHO Review Meeting on the Organophosphorus Pilot Survey (Barcelona, 24-26 January 1989), Document FIR/MEDPOL/OP/5, 29 p.(mimeo).

Fisher, S.W. and T.W. Lohner (1987), Changes in the aqueos behavior of parathion under varying conditions of pH. Arch.Environ.Contam. Toxicol., 16:79-84.

Galazzi, S., I. Camoni, V. Leoni, A. Liberatori and L. Vigano (1986), Gruppo 5: pesticidi clorurati e fosforati, Convegno per la revisione dei limiti della lagge 319 per gli scariehi en acque superficiali, Roma.

Gomez-Belinchon, J.I., J.O. Grimalt and J. Albaigés (1988), Analysis and persistence of tributyl phosphates in riverine and marine coastal waters. Chemosphere, 17:2189-2197.

Greve, P.A. and C.E. Goewie (1985), Developments in the determination of organophosphorus pesticides, Intern.J.Environ.Anal.Chem., 20:29-39.

Hutson, D.H. and T.R. Roberts (1985), Insecticides. J. Wiley & Sons, Chichester. vol. 5 pp.11-21, 64-74, 256-261.

Kjolholt, J. (1985), Occurence of organophosphorus compounds in polluted marine sediments near a pesticide manufacturing plant, Chemosphere, 14:1763-1770.

Latourette, H.K. (1979) in Boethling and Cooper (1985).

Lawrence, J.F.. (1987), Analytical methodology for organophosphorus pesticides used in Canada, Intern.J.Environ.Anal.Chem. 29:289-303.

Leistra, M., L.G.M.Th. Tuinstra, A.M.M. Van Der Burg and S.J.M. Crum (1984), Contribution of leaching of diazinon, parathion, tetrachlorvinphos and trizophos from glasshouse soils to their concentration in water sources, Chemosphere, 13:403-413.

Leoni, V. and G. Puccetti (1971), Cromatografia su strato sottile di pesticidi organo-fosforici in rapporto a studi di contaminazione ambientale (acque superficiali italiane). Il Farmaco, 7:382.

Leoni, V. and G. Puccetti (1978), Stato di inquinamento da pesticidi del fiume Tevere e del suo bacino imbrifero. Quaderno, no.27, I.R.S.A.

Lores, E.M., J.C. Moore and P. Moody (1987), Improved silica gel clean up method for organophosphorus pesticides, Chemosphere, 16:1065-1069.

Mackay, D., S. Paterson, B. Cheung and W. Brock Neely (1985), Evaluating the environmental behaviour of chemicals with a level III fugacity model, Chemosphere, 14:335-374.

Mestres, R., G. Leonardi, Ch. Chevalier and J. Tourte (1969), Pesticides residues XIX. Determining pesticide residues in natural waters. 1. General analytical methods. Ann.Fals. Exp.Chim., 62(685):75-85.

Moody, R.P., R. Greengalh, L. Lockhart and P. Weinberger (1978), The fate of fenitrothion in an aquatic ecosystem, Bull.Environ. Contam.Toxicol., 19:8-14.

Moretto, A. and M.K. Johnson (1987), Toxicology of organophosphorus compounds and carbamates In Toxicology of pesticides: experimental, clinical and regulatory aspects, Edit. by L.G. Costa et al., Springer Berlin, Verlag, pp.33-48.

Morin, R., G. Gaboury and G. Mamarbachi (1986), Fenitrothion and Aminocrab residues in water and balsam for foliage following spruce budworm spraying programs in Quebec, 1979 to 1982, Bull.Environ.Contam.Toxicol., 36:622-628.

Muir, D.C. (1984), Phosphate esters in the handbook of environmental chemistry, ed. by O. Hutzinger, Springer-Verlag Berlin, vol. 3, part C, pp.41-66.

Mulla, M.S., L.S. Mian and J.A. Kawechi (1981), Distribution, transport and fate of the insecticides malhation and parathion in the environment. Residue Rev., 81:1-159.

Neicheva, R., E. Kovacheva and G. Marudov (1988), Determination of organophosphorus pesticides in apples and water by gas-liquid chromatography with electron capture detection, J.Chromatogr., 437:249-253.

N.R.C. (1975), Associate Committee on Scientific Criteria for Environmental Quality, Fenitrothion: The effects of its use on environmental quality and its chemistry, N.R.C.C., Publication No. 14104.

Onuska, F.I. (1984), Pesticide residue analysis by open tubular column gas chromatography: Trials, tribulations and trends, J.High Resolut.Chromatogr.Chromatogr.Comm., 7:660-670.

Osselton, MN.D. and R.D. Shelling (1986), Chromatographic identification of pesticides, J.Chromatogr., 368:265.

Pellin, M., J.L. Vicedo and E. Vilanova (1987), Sensitivity to tri-o-cresylphosphate neurotoxicity on n-hexane exposed hens as a model of simultaneous hexacarbon solvent and organophosphorus occupational intoxication, Arch.Toxicol., 59:311.

Persoone, G., P. Vanhaecke and E. Gobbers (1985), Evaluation of the impact of parathion, methyl-parathion, fenitrothion and fenthion on the aquatic environment. Report to the EEC, XI/785/83, 579 p.

Podjelski, J. and A.M. Dervieux (1978), Persistence of four pesticides in rice field water in the Camargue; characterization of their effects on the ecosystem, Trav.Soc.Pharm.Montpellier, 38(2):153-163.

Premazzi, G. (1983), Evaluation of the impact of malathion on the aquatic environment, EEC, Joint Research Centre, Ispra Establishment, Italy, 67 p.

Prinsloo, S.M. and P.R. De Beer (1985), Gas chromatographic retention data for pesticides on nine packed columns: I. Organophosphorus pesticides using flame photometric detection, J.Assoc.Off. Anal.Chem., 68:1100.

Ramade, F. (1987), Ecotoxicology. J.Wiley & Sons, Chichester. 136 p.

Saeger, V.W., O. Hicks, R.G. Kaley, P.R. Michale, J.P. Mieure and E.S. Tucker (1979), Environmental fate of selected phosphate esters, Environ.Sci.Technol., 13:840-844.

Sabbarhawl, A.K. and D.K. Belsare (1986), Persistence of methyl parathion in a carp rearing pond, Bull.Environ.Contam.Toxicol., 37:705-709.

Seabloom, R.W., G.L. Pearson, L.W. Oring and J.R. Reilly (1973), An incident of fenthion mosquito control and subsequent avian mortality. J.Wildl.Dis., 9:18-20.

Stan, H.J. and G. Kellner (1982), Negative ionization mass spectrometry of organophosphorus pesticides, Biomed.Mass. Spectrom. 9:483-492.

Vilanova, E., M.K. Johnson and J.L. Vicedo (1987), Interaction of some unsubstituted phosphoramidate analogs of methamidophos (O,S-Dimethyl Phosphrodithioamidate) with acetylcholinesterase and neuropathy target esterase of hen brain, Pestic.Biochem.Physiol., 28:224-238.

Wang, T.C., R.A. Lenahan and J.W.Jr Tucker (1987), Deposition and persistence of aerially-applied fenthion in a Florida estuary, Bull.Environ.Contam.Toxicol., 38:226-231.

W.H.O. (1986), Organophosphorus insecticides: a general introduction, Environmental Health Criteria, WHO, Geneva.

Wilkins, J.P.G., A.R.C. Hill and D.F. Lee (1985), Organophosphorus sulphides, sulphoxides and sulphones. Parts 2. Characterization by gas chromatography-mass spectrometry, Analyst, 110:1045-1051.