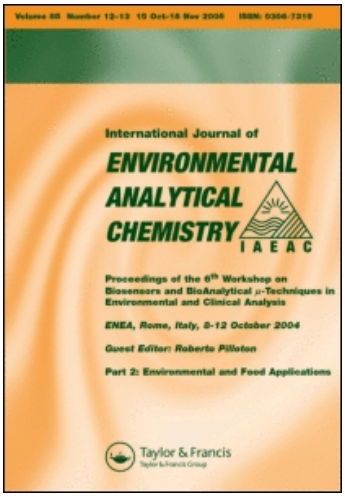

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Degradation study of selected organophosphorus insecticides in natural waters

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To cite this Article Druzina, Branko and Stegu, Matej(2007) 'Degradation study of selected organophosphorus insecticides in natural waters', International Journal of Environmental Analytical Chemistry, 87: 15, 1079 — 1093
To link to this Article: DOI: 10.1080/03067310701485341
URL: http://dx.doi.org/10.1080/03067310701485341

Intern. J. Environ. Anal. Chem.
Vol. 87, No. 15, 20 December 2007, 1079–1093

Degradation study of selected organophosphorus insecticides in natural waters

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(*Received 22 September 2006; in final form 28 May 2007*)

The degradation of 15 organophosphorus insecticides was studied in drinking, ground, and surface waters under different laboratory-controlled and environmental conditions. Surface waters originated from rivers Savinja (near the city of Celje) and Kamniska Bistrica (at the spring), which both belong to the Danube river basin. Groundwater was collected from wells (70 m deep) in Ljubljana, which are the direct source of drinking water for the capital. These matrices were selected on the basis of their different chemical composition and microbial activity. Major factors influencing the degradation were determined, such as temperature, oxygen, sunlight, pH, and type of water. The degradation of atrazine, present in many water sources in Slovenija, was followed simultaneously as a reference under the same conditions. The degradation kinetics was followed by gas chromatography with mass-selective detection, which also allowed the identification of some degradation by-products, such as oxon analogues paraoxon, dyfoxon, malaoxon, phenyl-methyl sulfoxide, fenthion sulfone, phorate sulfoxide, and phorate sulfone. The results show that the half-lives of the selected organophosphorus insecticides varied from 4 to 192 days or more, depending on the water source and experimental conditions. As a result, kinetically constants and half-lives were calculated for every tested insecticide, and major degradation products were determined.

Keywords: Organophosphorus insecticides; Environmental fate; Degradation by-products; Azinophos methyl; Chlorfenvinphos; Chlorpyrifos; Coumaphos; Diazinon; Dichlorvos; Dimethoate; Fenitrothion; Fenthion; Fonofos; Malathion; Mevinphos; Parathion; Parathion-methyl; Phorate

1. Introduction

The presence of pesticides in waters constitutes a pervasive problem, and there is a growing concern about reducing the pesticide contamination. Three sources of entry into water are possible: (1) industrial waste or effluent discharged directly into water; (2) seepage from buried toxic wastes into water supplies; and (3) contamination of surface and groundwater directly or from runoff during spraying operations.

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International Journal of Environmental and Analytical Chemistry
ISSN 0306-7319 print/ISSN 1029-0397 online © 2007 Taylor & Francis
DOI: 10.1080/03067310701485341

As the problem of organochlorine pesticides persistence in the environment became evident, insect pest-control began to rely more on the anticholinesterase organophosphorus compounds owing to their relatively rapid decomposition and low accumulation in biological food chains [1]. Currently, organophosphate compounds are the most widely used class of pesticides in industrialized countries.

Organophosphorus pesticides are derivatives of phosphoric, phosphonic, phosphorothioic, or phosphonothioic acids, comprising many chemicals with a wide range of uses [2]. They exert their acute effects in insects, fish, birds and mammals by inhibiting the acetylcholinesterase (AChE) enzyme, but may also have a direct toxic effect [2].

The fate of organophosphorus pesticides in the aquatic environment has led to numerous investigations in recent years [3–12]. In this regard, it has been reported that degradation is mainly influenced by photolysis [13], by microbial degradation [14–17] and by hydrolysis, particularly at pH > 7 [18]. The sunlight reaching the Earth has a wavelength of over 286.3 nm [19]. The majority of UV rays are absorbed in the surface water layer (down to a depth of 2 m) but they can reach deeper depths under the condition that light penetrates there. The phototransformation of a compound in surface water may result from light absorption by the pollutant itself (direct photolysis) or may be photo-induced by the dissolved natural organic matter or nitrate ions present in water, as these chromophores are known to photoproduce reactive species (indirect photolysis). In the aquatic environment, the processes of direct and indirect photolysis occur concurrently. The presence of microorganisms, algae, or humic substances accelerates photochemical reactions because these components are capable of absorbing sunlight. Some nonionic organic compounds, particularly pesticides, undergo photodegradation much faster in the presence of photosynthesizing micro organisms [20]. The biodegradation rate in the real aquatic environment depends on the characteristics of the aquatic system, presence of particulate matter, concentration of inorganic and organic nutrients, temperature, oxygen concentration, redox potential, and adaptation of the microbial population [21, 22].

The changes in the mutagenicity of fenitrothion during its biodegradation in solutions were investigated by Matsushita *et al.* [23]. Fenitrothion is completely decomposed within 12 days. The mutagenicity increased during anaerobic biodegradation, which was due to amino-fenitrothion, a metabolite formed during anaerobic biodegradation of fenitrothion.

Organophosphorus pesticides, when present in natural waters, degrade into compounds that also have activity against pests. The few studies indicate that the degradation products may exhibit higher, lesser, or similar activity to the parent pesticide. For example, as stated by Pehkonen and Zhang [24] degradation of chlorpyrifos to 3,5,6-trichloro-2-pyridinol (via hydrolysis) results in a total loss of insecticidal activity; nevertheless, the product is bioactive against several fungal pathogens or, as mentioned by Coats [25], earthworms are six times more sensitive to *p*-nitrophenol and 14 times more sensitive to 2,4-dichlorophenol than to parathion and 2,4-D, respectively.

The objectives of the present study were: (1) determination of the degradation kinetics of the 15 selected organophosphorus insecticides in different natural waters under laboratory controlled and environmental conditions, and (2) identification of degradation by-products formed during the course of the experiment.

Table 1. Physicochemical properties and acute toxicity of the selected organophosphorus insecticides [26–29].

Insecticide	M ($g\,mol^{-1}$)	Solubility (water, $mg\,L^{-1}$)	Vapour pressure (MPa)	Octanol/water partition coefficient ($\log K_{ow}$)	Soil sorbtion coefficient ($\log K_{oc}$)	Acute toxicity LD_{50} ($mg\,kg^{-1}$ rat)
Azinphos-methyl	317	28–30	<1	2.8–3	3	4.4–16
Chlorfenvinphos	360	145	0.5	3.1–3.9	2.5	10–39
Chlorpyrifos	351	1.4–2	2.4–2.5	4.7–5.3	3.6	95–270
Coumaphos	363	0–1.5	0.013	4.1	–	13–41
Diazinon	304	40–60	0.097	3.1	3	300–400
Dichlorvos	221	10,000	290	1.5	1.5	25–80
Dimethoate	229	>10,000	1.1	0.5–0.8	1.3	180–330
Fenitrothion	277	14–30	18	3.3–3.4	2.6	250–800
Fenthion	278	2–4	4	3.8–4.1	3.2	180–298
Fonofos	246	13–17	28–30	3.9	2.9	3.2–18.5
Malathion	330	125–145	5.3	2.8–2.9	3.3	1000
Mevinphos	224	>10,000	17	1.3	1.6	3–12
Parathion	291	12.4–24	5	3.8	–	2–60
Parathion-methyl	263	55–70	1.3	3.5–3.8	3.7	6–50
Phorate	260	50	110	3.9	3	1.1–3.7

2. Experimental

2.1 *Chemicals*

All compounds included in this study were certified reference materials for residue analysis, purchased from Dr. Ehrenstorfer GmbH. The physicochemical properties of these insecticides are shown in table 1, and their structures are shown in figure 1. Organic solvents used in sample preparation and analysis were all pesticide-grade (Riedel de Haen). Other chemicals were of reagent-grade from various suppliers. All glassware for sampling and analysis were rinsed with pesticide-grade solvents (ethanol and hexane) before use. Solid-phase extraction was performed on Supelclean ENVI-Chrom P (SDVB) solid-phase extraction tubes (6 mL, 500 mg). This is a highly cross linked, neutral, specially cleaned styrenedivinylbenzene copolymer resin used to retain hydrophobic compounds with some hydrophilic functionality under reverse-phase conditions; particle size: 80–160 μm; spherical shape; pore size: 110–175 Å; surface area: $900\,m^2\,g^{-1}$.

2.2 *Water sampling*

Surface waters originated from rivers Savinja and Kamniska Bistrica, which both belong to the Danube river basin. Samples were taken in the middle reaches of the Savinja River near the city of Celje and at the spring of Kamniska Bistrica River. All samples were collected at the river banks in the top 20-cm layer. Groundwater was collected from the Jarski Prod A3 Well, 70 m deep near Ljubljana, which belongs to the Ljubljansko Polje groundwater body. This is one of the major wells used as a direct source of drinking water for the capital. No further purification of the groundwater is necessary before distribution to end users. All samples taken were refrigerated at 4°C in the dark prior to use. Their physicochemical characteristics are given in table 2.

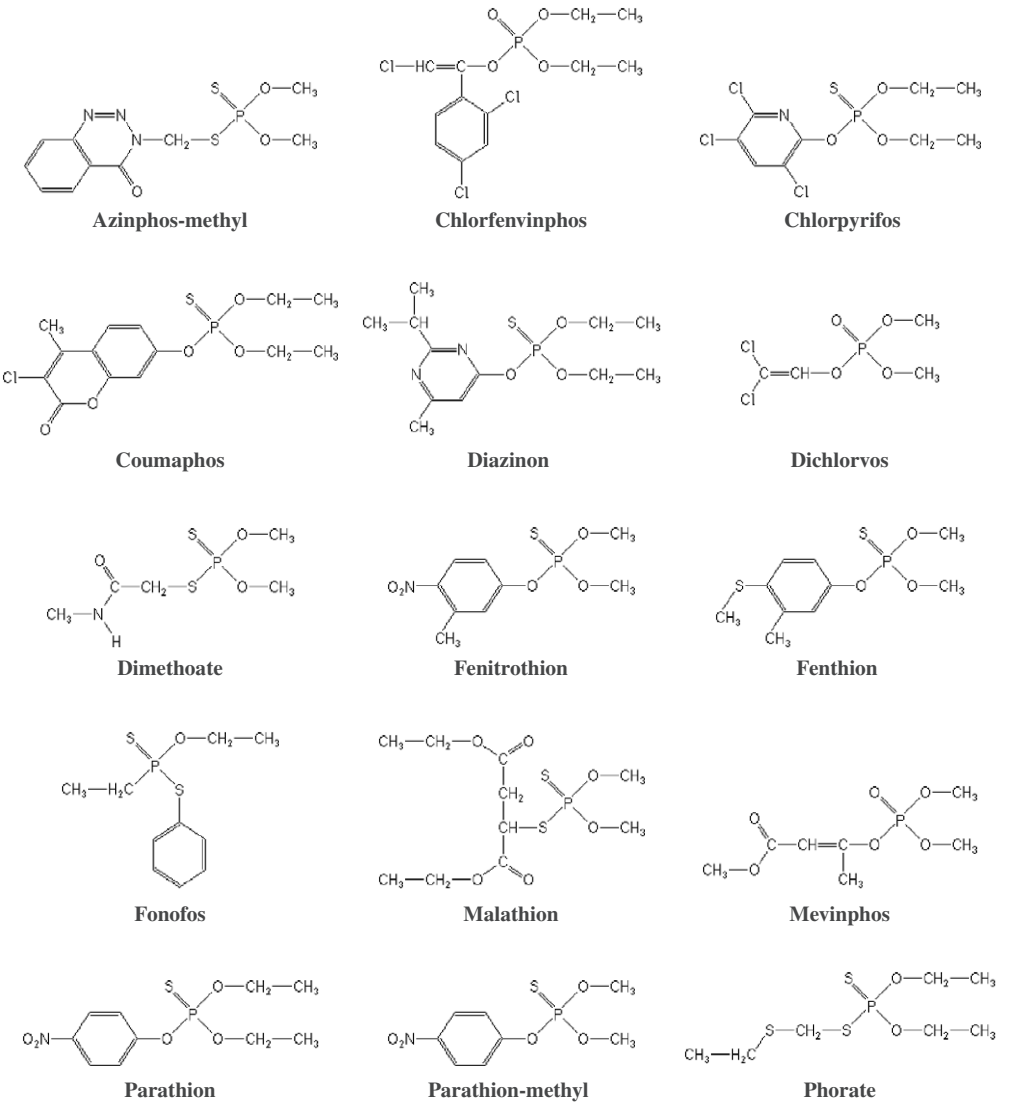

Figure 1. Structures of the organophosphorus compounds used in this experiment.

2.3 *Experimental conditions*

Degradation tests were divided into two separate parts: indoor laboratory tests with total control over experimental conditions and outdoor tests with environmental conditions. Test aqueous solutions of the selected insecticides were prepared from primary standard solutions in acetone ($1\,g\,L^{-1}$). Selected matrix (surface or groundwater with no previous treatment of filtration or sterilization) was spiked with primary standard solutions to a final concentration of $1\,mg\,L^{-1}$ in 5-L reaction reservoirs (stoppered glass bottles and glass beaker). The mixtures were homogenized by magnetic stirring for the equilibration period of 8 h. Indoor and outdoor experiments were

Table 2. Characteristics of selected natural waters.

Parameter	Savinja river	Kamniska Bistrica river	Groundwater
pH	7.9	7.8	7.4
Conductivity ($\mu S\,cm^{-1}$)	380	182	517
Turbidity (NTU)	1.8	0.36	<0.1
TOC ($mg\,L^{-1}$)	9.21	4.11	<0.1
Hardness (°N)	12.9	4.9	15.4
HCO_3^- ($mg\,L^{-1}$)	244	104	256
NH_4^+ ($mg\,L^{-1}$)	0.22	<0.04	<0.04
NO_3^- ($mg\,L^{-1}$)	11.5	<1.0	15.2
NO_2- ($\mu g\,L^{-1}$)	91	<3	<3
Mn ($\mu g\,L^{-1}$)	3	<2	<2
Fe ($\mu g\,L^{-1}$)	50	<50	<50
PO_4^{3-} ($mg\,L^{-1}$)	<0.03	0.12	<0.03
Coliform bacteria total ($MLN\,100\,mL^{-1}$) faecal	24,000	2	<2
	9000	<2	<2

carried out simultaneously for 4 months during the period of December 2005 to the end of March 2006.

In the indoor part, two different matrixes were used (groundwater and Savinja River), and six different conditions were applied: four test portions of groundwater with pH values of 6 (test no. 2), 7 (test nos 1 and 3) and 8.5 (test no. 4), respectively, and two test portions of surface water with no pH adjustment were prepared. Simple acid/base additions were used to adjust the starting pH of groundwater test solutions. No additional buffer solutions were added to natural waters. Three reaction reservoirs of groundwater with different pH values and one reaction reservoir with surface water (test no. 6) were stoppered, covered with aluminium foil and placed in the laboratory at approximately 25°C. The fourth groundwater test portion with pH 7 (test no. 1) and the second surface water test portion (test no. 5) were placed in the 'dark room' and were incubated at 4 ± 2°C. With these conditions, hydrolysis (pH, temperature) and microbial degradation influences on the degradation kinetics of selected pesticides were determined.

In the outdoor part of the experiment, the influence of light (solar radiation), air (oxygen), and volatility on the degradation process was determined. For this purpose, water from Kamniska Bistrica River was used. One test portion in stoppered glass bottle (test no. 7) and one test portion in open glass beaker (test no. 9) were placed on the sun-exposed place in front of the Institute. A dark control experiment (test no. 8) was also conducted by exposing stoppered glass bottle, filled with the same pesticide solutions and covered with aluminium foil in the same environmental conditions. Meteorological data for Ljubljana for the time period from December 2005 to March 2006 are shown in table 3.

2.4 *Extraction and analysis*

On regular time intervals, determined by investigators, 50-mL aliquots of pesticide solutions were taken from each reaction reservoir and analysed on a gas chromatograph with a mass-selective detector. Prior to sampling, each reaction reservoir was

Table 3. Meteorological data for Ljubljana.

Month	T_1 (°C)	T_2 (°C)	T_3 (°C)	T_4 (°C)	T_5 (°C)	t_s (h)	N_{cd}
December 2005	0.2	2.9	−2.3	7.3	−10.9	60	3
January 2006	−1.6	0.9	−4.3	7.5	−15.7	74	2
February 2006	0.5	4.3	−2.7	12.5	−10.8	83	2
March 2006	4.5	8.6	0.6	16.2	−8.3	94	2

T_1: average temperature; T_2: average maximum temperature; T_3: average minimum temperature; T_4: absolute maximum temperature; T_5: absolute minimum temperature; t_s: number of hours of solar radiation; N_{cd}: number of clear days.

termostated to room temperature and homogenized. Samples were first diluted to a volume of 500 mL with ultra-pure water (to ensure better flow through SPE column, especially with turbid samples), homogenized, and purified by means of solid-phase extraction. Prior to extraction, 5 mL of methanol and 0.5 mL of atrazine D5 internal standard solution (1 $\mu g\,mL^{-1}$ in acetone) were added to each sample, and solid-phase extraction tubes were placed on a vacuum manifold and conditioned with methanol and ultra-pure water. After equilibrating for 1–2 h, samples were loaded on wet solid-phase extraction columns, dried with nitrogen, and extracted with 10 mL of ethyl acetate. Extracts were concentrated to a volume of 0.5 mL and quantitatively transferred to gas-chromatography vials. Part of the extract was further diluted by a factor of 10 with ethyl acetate for quantization purpose in GC/MSD sim mode. The remaining part of the extract was injected directly for qualitative GC/MSD analysis in full scan mode. In the final sampling stages (after 2–3 months), 1-L aliquots of undiluted test solutions were used to increase the concentration of the degradation products for GC/MSD detection in full scan mode. With each series of samples, the recovery of the whole process was also determined by standard addition of pesticide solution to ultra-pure water on the concentration level of 1 $\mu g\,L^{-1}$ of the sample. Control samples and blank samples (ultra-pure water only) were treated in the same manner as the other samples.

Calibration for each pesticide was acceptable, if linearity correlation coefficient was greater than 0.98. The LOD of the SIM method for all selected pesticides was 0.02 $\mu g\,L^{-1}$. We determined the LOD from the signal-to-noise ratio, which should be more than 3. The LOQ of the SIM method for all pesticides was 0.05 $\mu g\,L^{-1}$. This represents the lowest concentration point on calibration curve. The measurement uncertainty of the method was different for each analyse and ranged from 20 to 30% at the concentration level of 0.1 $\mu g\,L^{-1}$. Recoveries of selected organophosphorus insecticides ranged from 80 to 110%, respectively.

2.5 *Chromatographic conditions*

The analysis of the organophosphorus insecticides was performed using a Hewlett Packard 6890N gas chromatograph with a 5973N mass-selective detector, equipped with a 7683 series auto sampler and injector. A 30-m HP5-MS capillary column with 0.25-mm internal diameter and 5% phenyl-methyl-polysiloxane as a stationary phase (J&W Scientific, Folsom, CA) was used under the following conditions (sim and full scan mode): temperature programme 50°C (hold 1 min), 20°C min^{-1} to 130°C (0 min), 4.5°C min^{-1} to 220°C (0 min), 15°C min^{-1} to 300°C (hold 10 min); flow conditions: carrier gas helium; constant flow: the initial flow rate was 1.0 mL min^{-1} for 25 min, then

raised 0.3 to 1.5 mL min^{-1}. A split/splitless injector was used at 250°C, 1 L (sim mode) or 2 µL (full scan mode) of extracts was injected via pulsed splitless injection: pulse time: 1 min, pulse pressure: 3 bar, purge flow: 49.7 mL min^{-1} purge time: 1.5 min, total flow: 53.6 mL min^{-1}. The mass-selective detector conditions were as follows: electron impact ionization, MSD transfer line heater: 280°C; MS Quad: 150°C; MS source: 230°C.

Depending on the organophosphorus compound, two or three characteristic ions (m/z) were used in conformation and quantification steps in sim mode: atrazine D5 m/z: 205, 220; atrazine m/z: 200, 215; dichlorvos m/z: 109, 185; mevinphos m/z: 127, 192, 109; phorate m/z: 260, 231; dimethoate m/z: 125, 229; fonofos m/z: 109, 246, 137; diazinon m/z: 304, 179; parathion-methyl m/z: 109, 263; fenitrothion m/z: 277, 260; malathion m/z: 173, 125; fenthion m/z: 278, 169; chlorpyrifos m/z: 196, 314; parathion-ethyl m/z: 291, 261; chlorfenvinphos m/z: 267, 323, 269; azinphos-methyl m/z: 160, 132; coumaphos m/z: 362, 226, 210.

3. Results and discussion

3.1 *Degradation kinetics in natural waters*

The degradation rates of the studied insecticides in different natural waters followed a first-order degradation curve shown in figures 2 and 3. $C_t = C_0 \exp(-kt)$ where C_t is the concentration of an insecticide at time t, C_0 is the insecticide initial concentration, and k is the rate constant. The half-life ($t_{1/2}$) corresponds to a period of time at which the pesticide concentration is equal to half of the initial concentration given by the equation $t_{1/2} = \ln 2/k$. The most persistent pesticides in our experiment, regarding all experimental conditions, were atrazine (our reference herbicide) and fonofos with half-lives over 200 days, except when exposed to sunlight in closed/opened reaction reservoir ($t_{1/2} \sim 82/18$ days for Atrazine and $t_{1/2} \sim 62/15$ days for Fonofos). Half-lives and reaction constants were calculated for all studied insecticides (tables 4 and 5).

An increase in the pH value of the matrix generally leads to a greater degradation rate [30–33]. Over the pH range used in our experiment (6–8.5) faster degradation at higher pH values was measured only for five insecticides, especially for chlorpyrifos, coumaphos, malathion, and, to a lesser extent, for dimethoate and fenthion. In a slightly acidic test solution (pH ~ 6), the most obvious decrease in degradation rate was observed for chlorpyrifos, fenthion, and malathion. On the other hand, diazinon expressed a better resilience at higher pH values. For all other compounds, these small pH variations do not seem to influence the degradation rate to any great degree. During the course of the experiment, the pH of the test solutions was also followed. For groundwater, a slight increase in pH value was noticed for a slightly acidic test solution from 6 to 6.4 and a decrease in case of a slightly basic solution from a pH of 8.5 to 7.0 after 4 months. The same decrease in pH was also measured in both the Savinja River test solutions (from a pH of 7.9 to 6.5 after 4 months). No significant pH changes were measured in the case of the Kamniska Bistrica River (outdoor experiments). From our indoor experiments, temperature and matrix (chemical and microbiological composition) influences on degradation kinetics also were determined. Degradation is significantly faster at higher temperatures in both matrixes for all tested insecticides,

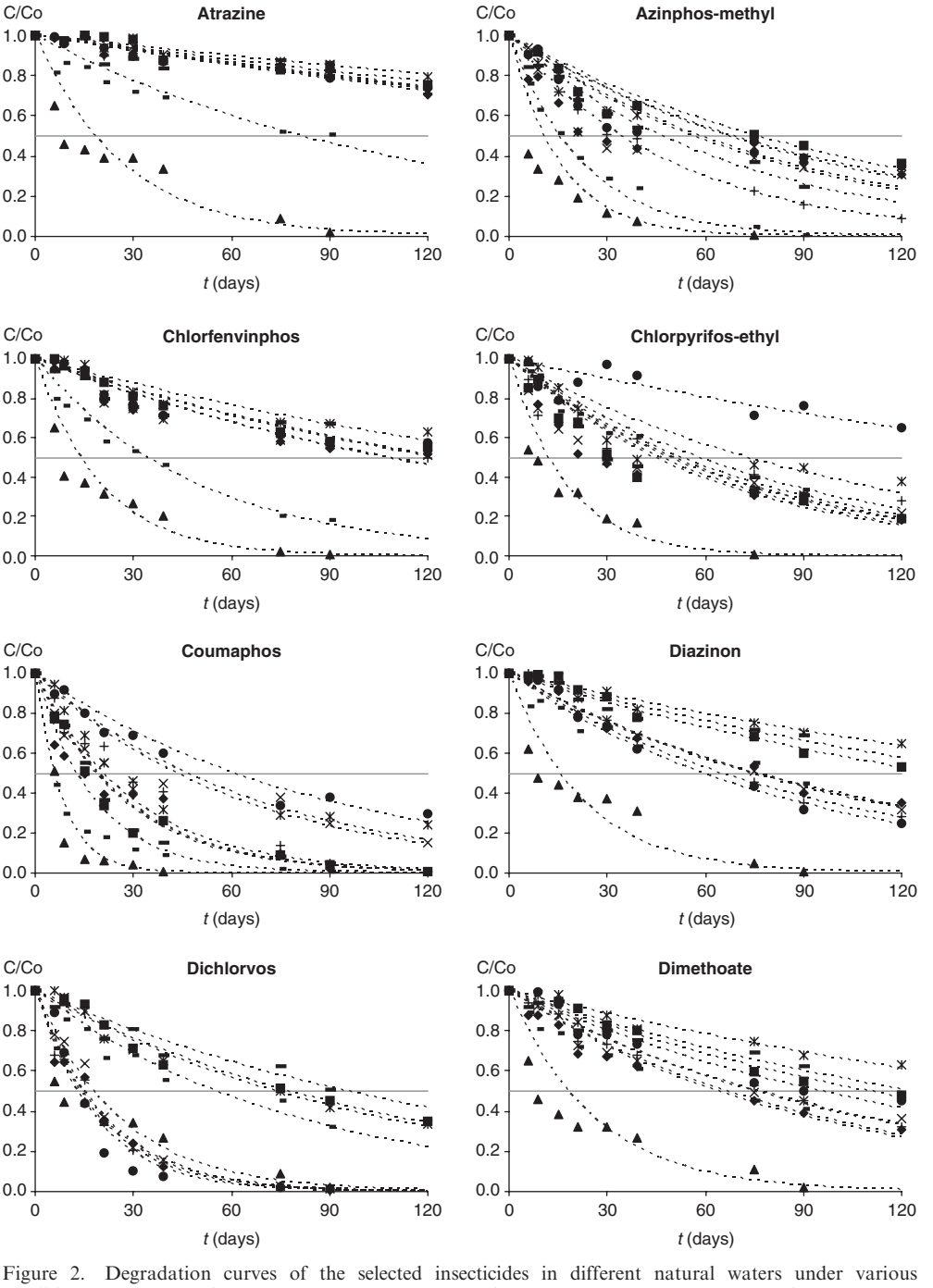

Figure 2. Degradation curves of the selected insecticides in different natural waters under various conditions: ■ . . . Groundwater (pH ~ 7; 4°C); ● . . . Groundwater (pH ~ 6; 25°C); × . . . Groundwater (pH ~ 7; 25°C); ◆ . . . Groundwater (pH ~ 8.5; 25°C); * . . . Savinja river (pH ~ 8; 4°C); + . . . Savinja river (pH ~ 8; 25°C); - . . . Kamniska Bistrica river (pH ~ 8; outside; closed); — . . . Kamniska Bistrica river (pH ~ 8; outside; closed; dark); ▲ . . . Kamniska Bistrica river (pH ~ 8; outside; open).

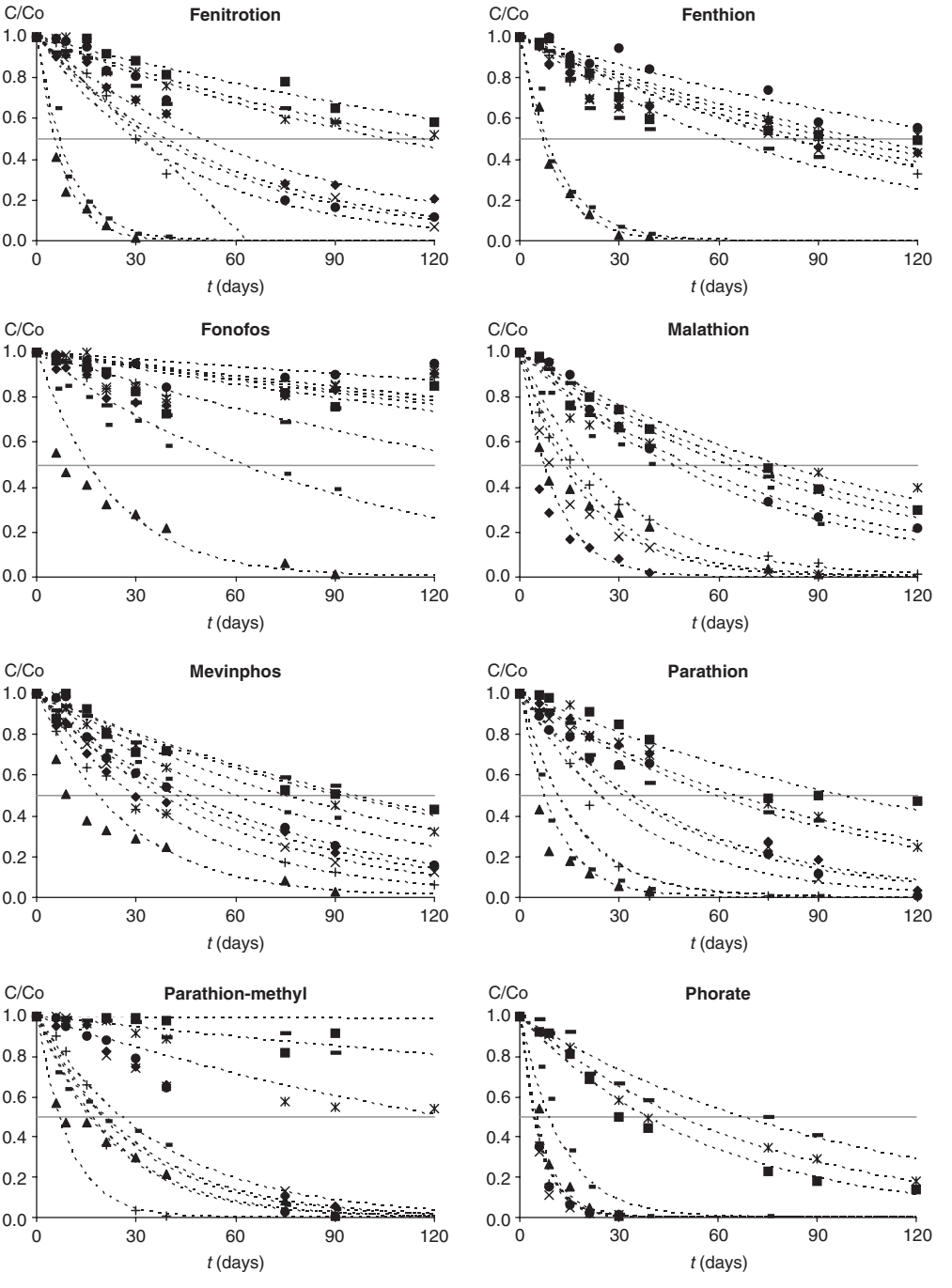

Figure 3. Degradation curves of the selected insecticides in different natural waters under various conditions: ■ . . . Groundwater (pH ~ 7; 4°C); ● . . . Groundwater (pH ~ 6; 25°C); × . . . Groundwater (pH ~ 7; 25°C); ◆ . . . Groundwater (pH ~ 8,5; 25°C); * . . . Savinja river (pH ~ 8; 4°C); + . . . Savinja river (pH ~ 8; 25°C); - . . . Kamniska Bistrica river (pH ~ 8; outside; closed); — . . . Kamniska Bistrica river (pH ~ 8; outside; closed; dark); ▲ . . . Kamniska Bistrica river (pH ~ 8; outside; open).

Table 4. Half-lives $t_{1/2}$ (days) and first-order reaction constants k (per day) of studied insecticides in groundwater.

	Groundwater (pH ~ 7; 4°C)		Groundwater (pH ~ 6; 25°C)		Groundwater (pH ~ 7; 25°C)		Groundwater (pH ~ 8.5; 25°C)	
Compound	$t_{1/2}$	k (10^{-2})	$t_{1/2}$	k (10^{-2})	$t_{1/2}$	k (10^{-2})	$t_{1/2}$	k (10^{-2})
Atrazine	Stable	–	Stable	–	Stable	–	Stable	–
Azinophos-methyl	75.3	0.92	66.6	1.04	56.3	1.23	58.2	1.19
Chlorfenvinphos	123.8	0.56	121.6	0.57	108.3	0.64	106.6	0.65
Chlorpyrifos	46.2	1.50	192.5	0.36	49.9	1.39	44.4	1.56
Coumaphos	18.7	3.70	60.8	1.14	43.0	1.61	18.4	3.77
Diazinon	130.8	0.53	59.2	1.17	73.7	0.94	75.3	0.92
Dichlorvos	77.0	0.90	12.7	5.44	14.8	4.67	13.6	5.08
Dimethoate	110.0	0.63	94.9	0.73	76.2	0.91	66.0	1.05
Fenitrothion	157.5	0.44	38.5	1.80	36.1	1.92	49.1	1.41
Fenthion	94.9	0.73	141.4	0.49	81.5	0.85	86.6	0.80
Fonofos	Stable	–	Stable	–	Stable	–	Stable	–
Malathion	68.6	1.01	51.3	1.35	13.1	5.27	7.1	9.79
Mevinphos	91.2	0.76	45.6	1.52	37.3	1.86	41.7	1.66
Parathion	97.6	0.71	23.9	2.90	33.5	2.07	31.4	2.21
Parathion-methyl	stable	–	45.5	1.10	47.2	1.06	47.6	1.05
Phorate	38.3	1.81	4.2	16.48	4.0	17.34	4.2	16.52

Table 5. Half-lives $t_{1/2}$ (days) and first-order reaction constants k ($\times 10^{-2}$) (per day) of the studied insecticides in surface waters.

	River Savinja (pH ~ 8; 4°C)		River Savinja (pH ~ 8; 25°C)		River Kamniska Bistrica (pH ~ 8; outside; closed)		River Kamniska Bistrica (pH ~ 8; outside; dark)		River Kamniska Bistrica (pH ~ 8; outside; open)	
Compound	$t_{1/2}$	k	$t_{1/2}$	k	$t_{1/2}$	k	$t_{1/2}$	k	$t_{1/2}$	k
Atrazine	Stable	–	Stable	–	81.5	0.85	Stable	–	18.2	3.81
Azinophos-methyl	68.6	1.01	34.3	2.02	15.3	4.53	46.5	1.49	10.6	6.54
Chlorfenvinphos	154.0	0.45	105.0	0.66	34.1	2.03	123.8	0.56	13.6	5.08
Chlorpyrifos	72.9	0.95	57.8	1.20	52.1	1.33	48.8	1.42	11.8	5.85
Coumaphos	46.2	1.50	20.7	3.35	12.5	5.56	19.1	3.62	5.6	12.37
Diazinon	182.4	0.38	64.8	1.07	75.3	0.92	150.7	0.46	15.5	4.47
Dichlorvos	72.9	0.95	14.3	4.85	55.4	1.25	94.9	0.73	17.7	3.91
Dimethoate	169.0	0.41	74.5	0.93	63.6	1.09	123.8	0.56	18.0	3.84
Fenitrothion	117.5	0.59	31.7	1.58	6.5	10.61	105.0	0.66	5.1	13.47
Fenthion	103.4	0.67	80.6	0.86	7.8	8.92	61.3	1.13	6.5	10.60
Fonofos	Stable	–	Stable	–	61.9	1.12	Stable	–	15.2	4.55
Malathion	77.9	0.89	19.8	3.50	45.3	1.53	62.4	1.11	14.7	4.73
Mevinphos	74.5	0.93	29.2	2.37	59.7	1.16	94.9	0.73	18.3	3.79
Parathion	64.8	1.07	11.4	6.07	11.3	6.13	58.7	1.18	7.2	9.57
Parathion-methyl	123.8	0.56	18.1	2.76	19.7	3.52	Stable	–	15.8	4.38
Phorate	48.1	1.44	4.3	16.10	8.5	8.15	67.3	1.03	4.7	14.88

except for atrazine and fonofos, which seem to be stable at 25°C in all indoor experiments and chlorpyrifos, coumaphos, and fenthion, where the pH is the dominant factor influencing the hydrolysis.

A microbiological analysis (coliform bacteria and enterococci) of selected matrixes was carried out at the beginning and end of the degradation test. Only in Savinja River was significant microbiological activity found, and this decreased eightfold in the test solution at 25°C and even more at 4°C after 3 months of test duration. If the

degradation kinetics of test solutions with and without microbiological activity are compared (Savinja and groundwater at 25°C), a significant degradation rate increase is observed, especially for azinphos-methyl, parathion, and parathion-methyl and also for diazinon, fenitrothion, and mevinphos. Since both matrixes have similar pH values, which is the main factor influencing hydrolysis, and both have equal experimental conditions (25°C, closed reaction reservoirs—no solar radiation, no air), anaerobic microbiological degradation can be the cause of faster degradation. In general, regarding indoor experiments, the best persistence to hydrolysis and biodegradation was found for atrazine and fonofos, as well as for chlorfenvinphos, fenthion, and dimethoate.

From the results of outdoor experiments, the influences of sunlight (photo degradation), air (oxygen), and evaporation (volatility) on degradation kinetics can be calculated. Molecular species that can be found in natural waters and can absorb light, besides the insecticides in question, are dissolved organic matter (DOM) and inorganic compounds, which play an important role in the photochemistry of insecticides [34]. Both the optical filter effect of organic matter and the sensitization effect of humic and other substances of natural waters can attribute to the complexity of photodegradation rates of all the investigated insecticides. Organic matter can act as one of the important sunlight-absorbing components of the aquatic environment [35]. Particulate matter such as sediments and microorganisms suspended in a water column may scatter incident light, greatly reducing the penetration of light beneath the surface. The sensitization effect of humic substances depends on the binding affinity of insecticides to the radical source of the humic material. The resulting excited states of the DOM and reactive transients that were produced from DOM could participate in energy transfer, electron transfer, and free-radical reaction.

In addition, inorganic molecular species such as nitrate, nitrite, and carbonate radicals can enhance the photodegradation rate of organophosphorus insecticides in natural waters [36]. Such influences were not the subject of our study, and no conclusions were made regarding the chemical composition of natural waters.

Because the tests were carried out in winter, with average temperatures below 5°C, the hydrolysis factor can be excluded, as can be clearly seen from the blank test results. Concentration changes in test solution in a closed reaction reservoir (test no. 7) were mainly due to photolysis, since the presence of air (oxygen) and evaporation factor were negligible. As can be seen from table 6, atrazine and all selected organophosphorus compounds, except chlorpyrifos, were subjected to extensive photodegradation. Even higher degradation rate constants were observed for a test solution in an opened reservoir for all compounds except fenthion and fenitrothion, which seem to degrade equally in a closed or opened reaction reservoir. Volatilization and the presence of oxygen can be the cause of higher degradation rates. Photodegradation seems to be the dominant degradation pathway in the case of atrazine, azinphos-methyl, chlorfenvinphos, fenitrothion, fenthion, fonofos, parathion ethyl, and parathion methyl. Methyl parathion was found to be more stable than ethyl parathion under all experimental conditions. If similar organophosphorus compounds are compared, such as parathion, fenitrothion, and fenthion, the addition of the methyl group (fenitrothion, fenthion) or the replacement of NO_2 by a sulfur-methyl group (fenthion) seems to modify the behaviour of the molecule under photolysis: fenitrothion and fenthion are more sensitive to photodegradation. This may be due to the fact that an organophosphorus compound with an alkyl group attached to the aromatic ring tends to have higher

Table 6. Main degradation by-products of organophosphorus insecticides in our experiment.

Groundwater (indoor; pH ~ 8.5; 25°C)		River Kamniska Bistrica (outdoor; pH ~ 8; closed)	
Name	Spectral data (*m*/*z*)	Name	Spectral data (*m*/*z*)
Main degradation by-products formed via hydrolysis and photolysis (% area)			
O,*O*,*S*-Trimethyl phosphorodithioate (0.1%)	125, 109, 93	Dimethyl paraoxon (0.4%)	109, 96, 230, 247, 200
O,*O*-Diethyl-*S*-methyl phosphorodithioate (0.2%)	200, 156, 144, 123, 11, 97	*O*,*O*-Diethyl-*S*-methyl phosphorodithioate (0.1%)	200, 156, 144, 123, 11, 97
O,*O*-Diethyl phosphorodithioate (0.2%)	186, 121, 97, 142, 109	*O*,*O*-Diethyl phosphorodithioate (0.2%)	186, 121, 97, 142, 109
2-Chloro-1-(2,4-dichlorophenyl) ethanol (0.5%)	175, 147, 111	2,4-Dichlorobenzyl alcohol (0.05%)	141, 176, 159, 111
2-Isopropyl-4-methyl-6-hidroxy pirimidine (0.5%)	137, 152, 124, 109	Dyfoxon (0.5%)	230, 93, 121, 110
3,5,6-Trichloro-2-hidroxy piridine (0.4%)	197, 169, 134, 107 172, 187,145	Desethylatrazine (0.2%)	
3-Methyl-4-nitrophenol (0.2%)	153, 136, 108, 77	Phorate sulfone (1.2%)	199, 153, 125, 97
Aminoparathion (0.3%)	205, 233, 125, 109, 97	Aminoparathion (0.3%)	205, 233, 125, 109, 97
O,*O*-Diethyl-*S*-ethyl phosphorodithioate (0.3%)	214, 186, 137, 121, 109	Malaoxon (0.1%)	268, 195, 127, 109, 99
4-Nitrophenol (0.2%)	139, 123, 109, 93	Phorate sulfoxide (0.6%)	199, 153, 125, 97
3-Methyl-4-methythio phenol (0.2%)	154, 139, 95	Fenthion sulfone	
(0.4%)	125, 109, 93		
1-(2,4-Dichlorophenyl) ethanone (0.5%)	175, 147, 111	Phenyl-methyl sulfoxide (0.2%)	140, 125, 97
1,2,3-Benzotriazin-4-ol (0.3%)	147, 104, 92, 76	1,2,3-Benzotriazin-4-ol	
(0.4%)	147, 104, 92, 76		

quantum yields (because such a group may supply a photosensitive position for a photoreaction to occur) than another with an electron withdrawing group such as NO_2 [37]. Degradation of fenitrothion and parathion (both methyl and ethyl parathion), whose main structural difference is the presence of a methyl group in orto position of the aromatic ring, indicates that this is a photosensitive position for photoreactions to occur. It has been reported that the oxidation of the methyl group attached to the aromatic ring is the predominant photoreaction for fenitrothion [38].

3.2 *Degradation by-products*

The use of GC-MS allowed determination of some transformation by-products of the tested compounds. GC-MS scan analysis was performed for all tested solutions after 3 months of experiment duration. The most abundant of these were identified using the Nist and Wiley library (table 6). The difference between indoor and outdoor experiments that we described earlier is confirmed by these results. Degradation by-products resulting from indoor experiments are mainly due to hydrolysis, and compounds found in outdoor test solutions (opened/closed reaction reservoir exposed to sunlight) are mainly due to photolysis (oxidation) (table 7). The oxon analogues (such as paraoxon, dyfoxon, malaoxon) of organophosphorus insecticides were found to be the primary products in outdoor experiments through the photodegradation process, which is in agreement with other studies [39, 40]. The main difference between these and their parent compounds is the substitution of sulfur by oxygen in the P = S bond. These oxo metabolites represent activated forms of the organophosphorus pesticides, with a considerably stronger inhibition of acetylcholinesterase activity than that exhibited by the parent compounds [9, 41]. In the case of fonofos, fenthion, and phorate, the oxidative process led to the production of phenyl-methyl sulfoxide, fenthion sulfone, phorate sulfoxide, and phorate sulfone.

In all test solutions, especially from indoor experiments, the formation of the respective phenols of some tested insecticides was observed as a consequence of splitting

Table 7. Main degradation by-products of individual organophosphorus insecticides.

Organophosphorus compound	Main degradation by-products	
	Outdoor (pH ~ 8; sunlight; no air)	Indoor (pH ~ 8.5; 25°C; dark; no air)
Atrazine	Desthylatrazine	–
Azinphos-methyl	–	1,2,3-Benzotriazin-4-ol
Chlorfenvinphos	–	2,4-Dichlorobenzyl alcohol, 1-(2,4-dichlorophenyl) ethanone, 2-chloro-1-(2,4 dichlorophenyl) ethanol
Chlorpyrifos	–	3,5,6-Trichloro-2-hidroxy piridine
Diazinon	–	2-Isopropyl-4-methyl-6-hidroxy pirimidine
Fenitrothion	–	3-Methyl-4-nitrophenol
Fenthion	Fenthion sulfone	3-Methyl-4-methylthio phenol
Fonofos	Dyfoxon, phenyl-methyl sulfoxide	–
Malathion	Malaoxon	–
Parathion	Paraoxon, aminoparathion	Aminoparathion, 4-nitrophenol
Parathion-methyl	–	4-Nitrophenol
Phorate	Phorate sulfoxide, phorate sulfone	–

of the P–O, C–N, or C–O bond (table 7). Another side reaction observed in natural waters (in some indoor and outdoor test solutions) was the formation of aminoparathion, a by-product in the degradation process of ethyl parathion. Finally, different alkyl phosphorodithioate esters were identified as degradation by-products in all tested solutions, and the most abundant were: *O*,*O*,*S*-trimethyl phosphorodithioate, *O*,*O*-diethyl-*S*-methyl phosphorodithioate, *O*,*O*-diethyl phosphorodithioate and *O*,*O*-diethyl-*S*-ethyl phosphorodithioate.

4. Conclusions

On the basis of our experiments, we can conclude that the behaviour of most widely used and toxic organophosphorus insecticides such as azinophos methyl, chlorfenvinphos, chlorpyrifos, coumaphos, diazinon, dichlorvos, dimethoate, fenitrothion, fenthion, fonofos, malathion, mevinphos, parathion, parathion methyl, and phorate, in groundwater and two distinct surface waters under various conditions, were investigated. The predominant degradation routes and degradation kinetics, depending on certain laboratory-controlled and environmental conditions, were determined. We found that degradation kinetics of organophosphorus insecticides follow a first-order reaction curve under all tested conditions in both natural matrixes. The half-lives varied from 4 to 192 days or more, depending on water source and experimental conditions. Beside our reference herbicide atrazine, fonofos exhibited the best persistence, whereas phorate degraded faster in natural waters. Faster degradation at higher pH values was measured for only five insecticides, chlorpyrifos, coumaphos, malathion, and, to lesser extent, dimethoate and fenthion; on the other hand, diazinon expressed a better resilience at higher pH values. For all other compounds, these small pH variations (6–8.5) do not seem to influence the degradation rate to any great degree. Higher temperatures increased the degradation rates of all tested insecticides except for chlorpyrifos, coumaphos, and fenthion, where the pH is the dominant factor influencing the hydrolysis. Atrazine and all selected organophosphorus compounds, except chlorpyrifos, are subjected to extensive photodegradation. Photodegradation seems to be the dominant degradation pathway in the case of atrazine, azinphos-methyl, chlorfenvinphos, fenitrothion, fenthion, fonofos, parathion ethyl, and parathion methyl, in spite of the fact that outdoor experiments were carried out in winter time with average temperatures ranging from −2 to 5°C. Volatilization (especially for chlorpyrifos) and the presence of oxygen can be the cause of even higher degradation rate constants observed for the test solution in an opened reaction reservoir.

The GC-MS analysis showed different degradation by-products depending on the test conditions. We found that the degradation of organophosphorus pesticides in natural waters involves different routes, such as oxidation (parathion to paraoxon, malathion to malaoxon, fonofos to dyfoxon, etc.), hydrolysis (diazinone to 2-isopropyl-4-methyl-6-hidroxy pirimidine, chlorfenvinphos to 2-chloro-1-(2,4-dichlorophenyl) ethanol, fenitrothion to 3-methyl-4-nitrophenol, fenthion to 3-methyl-4-methylthio phenol, parathion to 4-nitrophenol, etc.), reduction (parathion to aminoparathion), etc.

Test solutions, with hydrolysis as the primary degradation pathway, exhibited mainly the formation of respective phenols and alkyl phosphorodithioate esters. With photodegradation as the primary route, the formation of oxon analogues (such as

paraoxon, dyfoxon, malaoxon, phenyl-methyl sulfoxide, fenthion sulfone, phorate sulfoxide, and phorate sulfone) was observed.

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