ON-LINE SOLID-PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY/PARTICLE BEAM-MASS SPECTROMETRY FOR DEGRADATION STUDIES OF SOME POLAR PESTICIDES IN WATER

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Abstract

An on-line automated method for photodegradation studies of isoproturon, diuron, atrazine, fenitrothion, and metoxuron by means of liquid chromatography/mass spectrometry (LC/MS) with particle beam (PB) interface is described. Surface water samples were first spiked with 50 µg/l of each pesticide and then exposed to the radiation of the medium-pressure mercury lamp. Next, in regular intervals of 60 min, aliquots of 50-ml sample were enriched on a solid-phase extraction (SPE) cartridge and eluted on-line by a gradient of LC eluent onto analytical column for separation, followed by MS detection. Photodegradation experiment was performed twice with each pesticide, with MS operated in electron ionization (EI) and positive chemical ionization (PCI) modes and ammonia as reagent gas. Additional spectral and quantitative information was obtained from ultraviolet diode-array detector (UV DAD) placed in-line between the outlet of the analytical column and MS detector. A great number of photodegradation products (DPs) of parent pesticides were detected and in numerous instances, tentatively identified. The appearance of several degradation products is reported for the first time.

Introduction

Determination of Degradation Products (DPs) of organic contaminants is nowadays one of the major challenges in analytical chemistry of environmental pollutants. DPs are often more toxic [1,2] and/or persistent [3] in environmental matrices than their parents. On the other hand, their identification is rather difficult due to the limited knowledge about their composition. Their detection is complicated by their generally low (µg/l) concentrations, polar character, thermolability and unknown kinetics of degradation reactions. This is specially true for DPs of modern pesticides which are purposely manufactured as

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'decomposable' in order to be removed from the environment while usually their more toxic major DPs are active against detrimental species [4].

One common degradation process of these compounds in aquatic media is photochemical degradation. For this to occur in the water, the emission spectrum of the sun needs to fit the adsorption spectrum of the pollutant. However, photodegradation is possible even without this condition as an effect of the photosensitizer activity of many natural compounds, such as humic substances [3,5]. It had been suggested previously that the real-world conditions could simulated by means of the artificial UV-irradiation [6], i.e., the DPs match those appearing under natural conditions, only reaction kinetics are different. Photodegradation studies of representatives of major pesticide classes, phenylureas [3,7,8], organophosphorus [3,9-11], carbamates [12,13] and triazines [3,14,11], have been already reported. However, there are still a great number of DPs, which have neither been detected nor identified, which may be due to the types of detection system usually employed after the chromatographic separations and methods of selective trace enrichment of DPs from sample matrix.

An increasing presence of medium and highly polar pollutants in aqueous samples has already favoured the use of liquid chromatography (LC) [14,15]. In sample preparation, a direct loop injection of the sample aliquot is most often employed [10,14,16-18]. More recently, off-line and on-line solid-phase extraction (SPE) [19] are used in order to monitor analyses at levels close to those being applied in the field. Among the detection systems available for the LC methods [15], mass spectrometry (MS), an inherently selective and sensitive technique, provides molecular mass and structural information. This is especially true when a particle beam (PB) interface, which generates classical electron ionisation (EI) spectra and solvent-independent positive chemical ionisation (PCI) spectra, is used [20,21]. Such a complementary information is an obvious advantage in comparison with the most popular thermospray interface (TSP)-MS in which spectra lack structurally informative fragments [10,22,23]. A rapidly developing atmospheric pressure ionisation (API)-MS interfacing technology provides good sensitivity and information similar to PB-MS. However, the ionisation/fragmentation processes are strongly dependent on the operational parameters [20,25] rendering the interpretation of spectra more complicated. These variations are greatly reduced when using tandem MS [26].

Several systems for analysis of trace levels environmental pollutants have been developed within the decade [27]. All of them utilize an efficient solid-phase extraction (SPE) procedure coupled on-line to LC/UV DAD [28]. LC/TSP-MS [22,23], LC/PB-MS [24,29-31], GC/MS [32,24] and LC/API-MS and MS/MS [26]. From the experience obtained with LC-based techniques it can be already stated that the best set-up for identification of unknowns is LC/PB-MS. It has been demonstrated that concentration of 100-250-ml sample [24,30,31] sufficiently compensates the major backdraw of PB/MS methods – relatively poor sensitivity [33,34].

In this study DPs of environmentally relevant representatives, (in terms of amounts applied and their frequent occurrence in environmental matrices) [35,21,15], of five major classes of polar pesticides, were examined. The photochemical degradation of isoproturon, alachlor, aldricarb, trazine, diuron, fenitrothion, methiocarb and metoxuron were carried out at two concentration levels using direct loop injections and an on-line SPE/LC/UV-DAD/PB-MS [24]. The UV or UV-DAD signal was recorded in order to observe those DPs which are not adequately sensitive for the PB-MS. The aim of this study was to develop a reliable and sensitive LC/PB-MS method for identification of DPs of polar organic pollutants in surface water, which are difficult to determine by GC/MS or other LC/MS interfacing techniques. The use of the on-line SPE should allow automation of the procedure and analysis of DPs originating from parent compounds present in the sample at low-µg/l levels, which is rather close to real-world situations. The efficiency of the photolytical processes will be estimated from comparison of UV-irradiated samples and samples kept in the dark. The presented methodology and setup for the given type of analysis is reported, for the first time.

Experimental Section

Chemicals

HPLC-gradient grade methanol and HPLC-gradient grade water were obtained from J. T. Baker (Deventer, the Netherlands). Ammonium acetate (99%) came from Merck (Darmstadt, Germany). All pesticides were of 96-99% purity and were purchased from Riedel-de Haën (Seelze, Germany). Helium gas for the PB nebuliser and for degassing of solvents and ammonia reagent gas (99.99999% and 99.9996% purity, respectively) were supplied by Hoekloos (Schiedam, the Netherlands).

Samples. All experiments were conducted with river Rhine water samples taken every two weeks from the same sampling site (Lobith, the Netherlands). Prior to use, the samples were filtered through 0.45 µm acetyl cellulose filter (Schleicher & Schuell, Dassel, Germany).

Instrumentation

Solid-phase extraction. A L-6200A gradient pump (Merck-Hitachi, Darmstadt, Germany) was used to deliver methanol for conditioning of the cartridge, and the sample. 10 mm×4.0 mm I.D. cartridges packed with LiChrospher 60 RP-18 (octadecylbonded silica, 10 µm particle size) were put in the OSP-2A automated cartridge exchange unit equipped with two six-port switching valves (Merck, Darmstadt, Germany) and used for trace enrichment of investigated compounds from irradiated water samples. A Boos silica (diol-modified silica with copper phthalocyanine trisulphonic acid moiety, 20 µm particle size) was packed in the same cartridges as above; Bondesil-C18/OH material (Varian, Harbor city, USA, 40 mm particle size, 100 Å pore size) and PLRP-S material (Styrene-divinylbenzene copolymer, 15-25 µm particle size, 100 Å pore size Polymer Laboratories, Church Stretton, UK) were slurry-packed in 10mm×2.0mm I.D. and 10mm×3.0mm I.D. stainless-steel precolumns, respectively.

Liquid chromatography. An HP 1090 LC gradient system equipped with autoinjector, HP 1020 filter photometric UV detector (Hewlett-Packard, Waldbronn, Germany) and six-port switching valve (Rheodyne, Cotati, CA, USA) was used to deliver the mobile phase. A 250mm×4.6mm I.D. stainless-steel column packed with 5 µm C-18-bonded silica of 100 Å pore size (Supelcosil LC-18-DB, Supelco, Bornem, Belgium) was used for separation. An HP 1050 UV diode-array detector (Hewlett-Packard), operated at 254 nm wavelength with 4 nm bandwidth, was placed between the analytical column outlet and PB-MS in order to monitor DPs not adequately sensitive for the PB-MS.

Particle beam-mass spectrometry. A Hewlett-Packard (Palo alto, CA, USA) 5989 MS Engine, equipped with a dual EI/CI ion source, high-mass option and high-energy dynode (HED), was connected to the LC column outlet via an HP 59980B PB interface. Full-scan mass spectra were acquired within the mass range (i) 65-350 amu at 0.36 scans/sec (EI, 70 eV ionisation) and (ii) 85-350 amu at 0.32 scans/sec (PCI, 230 eV ionisation). The system performance was monitored by daily injections of 500 ng of diuron [30]. The system was controlled by the MS ChemStation G1034C (DOS Series) data system installed on the HP Vectra 486/66X computer (Hewlett-Packard).

Sample Preparation and Analytical Procedure

2-1 volumes of spiked or blank river Rhine water samples in glass beakers were kept in a cooling-water bath at the constant temperature of 20°C and irradiated under a medium-pressure mercury lamp. Because of the observed strong influence of UV-light source position on the speed of photodegradation, the lamp was kept at optimised distance 40 cm from the water surface for samples spiked at low level (50 µg/l) and 20 cm for samples spiked at high level (100-200 mg/l). At each 60 min interval, an aliquot of the sample was taken to be analysed by the LC/UV DAD/PB-MS system and the distance between the UV-lamp and water surface was corrected.

Sets of mixtures of a parent pesticide with its expected DPs (indicated in the literature and with standards available in our laboratory), were analysed prior to the actual 'irradiation' experiment in order to test an applicability of the method for a given analyte.

For the high-spiked levels an aliquot of 25 µl of the irradiated sample was directly introduced onto the column by the HP 1090 autosampler; for the low-spiked levels an on-line SPE was coupled to the system. In the latter experiments, the cartridge was first conditioned at 5 ml/min with 5 ml of methanol and, next, 5 ml of HPLC-grade. Subsequently, a 50-ml sample was enriched at a flow rate of 5 ml/min. The analytes trapped on the precolumn were then desorbed in the forward-flush mode with the LC eluent (methanol and 0.1 M ammonium acetate, pH 4) at a flow rate of 0.4 ml/min and on-line transferred to the analytical column. The gradient profile was: 50% methanol linearity changed to 90% in 20 min, this condition was held for a further 10 min and, finally, returned to the original composition in 5 min. The setup of the system is shown in Figure 1. The details of the procedure are presented in Table S1.

Results and Discussion

I-General Considerations

The main aim of this study was to develop an analytical method for identification of DPs of polar pesticides in surface water. The degradation behaviour is expected to be similar for more compounds of the same class. Because of the relatively slow degradation of the analytes under natural sunlight (half-lives of known fast degrading organophosphates are about 4-12 days [20]) the process was accelerated by means of intensive UV-irradiation (See Experimental Section). Aliquots of spiked samples were analysed at regular intervals with the aim to monitor a speed of degradation of the parent compound and appearance of new DPs in chromatograms. EI and PCI spectra of each DP were used for its structural elucidation. All experiments were conducted with surface water samples at 20°C in order to simulate real-world conditions; control experiments with spiked samples kept in the dark show minor or no

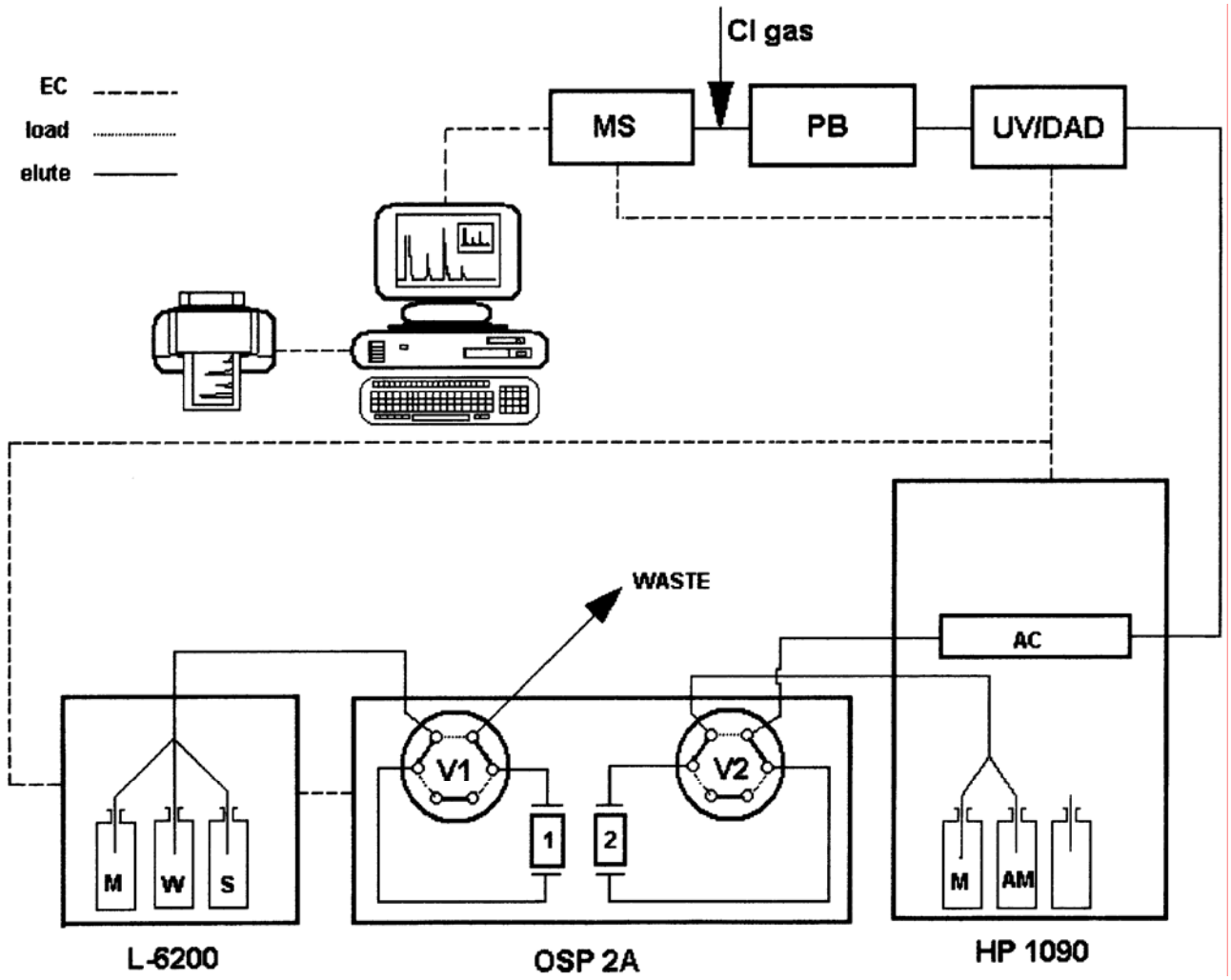


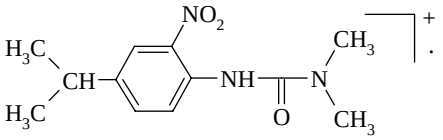
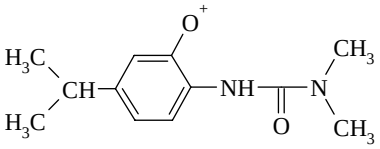
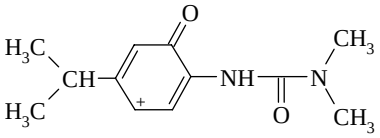
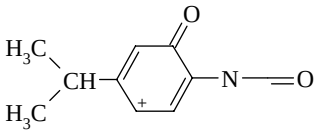
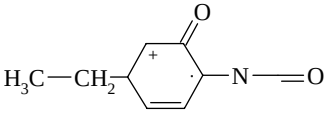
Figure 1. Schematic representation of the setup for automated photodegradation studies of pesticides in surface water. L-6200: gradient pump; OSP-2A: automated cartridge exchange and value switching unit; HP-1090: solvent delivery gradient system; UV/DAD: UV-diode array detector; PB: particle beam interface; MS-mass spectrometer; V1, V2: six-port switching valves of OSP-2A; AC: analytical column; AM: ammonium acetate; M: methanol; S: sample; W: HPLC-grade water; EC: electronical concentrations; 1,2: positions of the OSP-2A cartridge.

decrease in the amount of parent analytes after two weeks. This indicates that UV photolysis is the major degradation process of all investigated compounds, and that biodegradation or hydrolysis is less important in this regard.

Two methods of sample preparation were utilized in the study: the direct loop injections of a small sample volume (25 µl), and the on-line SPE of 50-ml sample. Because of known lower sensitivity of PB interface [21], amounts of DPs introduced for analysis should be in the 10-500 ng range which implies that spiking levels of parent pesticides should be approximately ten times higher, i.e., ca. 100 mg/l (25 µl injection) or 50 µ/l (50 ml injection). However, even using these amounts may not be sufficient to 'see' DPs with low conversion efficiency from the parent compound or those with an early breakthrough on the SPE cartridge [28]. An original amount of the parent analyte is a relevant parameter studies of this type and experiments with 'high' (mg/l) concentrations obviously do not reflect real field situations. Moreover, at these levels the solubility of pesticides in water is limited and addition of organic modifier, which should not affect photolysis processes (e.g. methanol) [3], is required. Low-µg/l concentrations of parent compounds are closer to those actually being applied in field and therefore the discussion is primarily focused on data obtained by the SPE method.

Schemes of proposed degradation pathways are simplified; all DPs are drawn as if originated from the

Table 1. Main fragment ions obtained from EI spectra of DP3 and DP4 of isoproturon

Fragment	m/z
	251
	221
	221
	176
	161

parent compound. This, of course, may not be true and some of the DPs can be intermediates for others.

II-Trace Enrichment

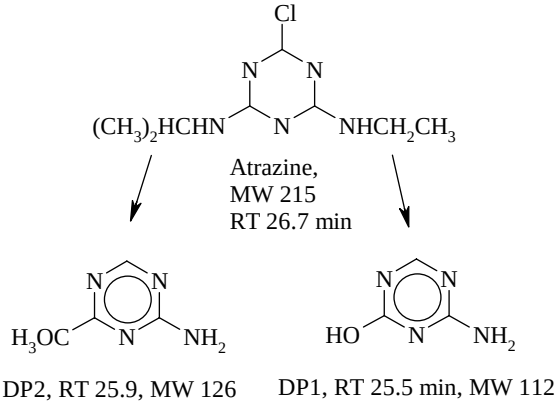
A major criterion for the successful enrichment of an analyte on the SPE cartridge/precolumn is its sufficiently large breakthrough volume on a sorbent used [36]. Several sorbents were tested to find the suitable material for enrichment of both the less polar parent pesticides and their, expectedly, more polar DPs in one run. A mixture of aldicarb with its sulfone and sulfoxide oxidation products were selected as the 'most extreme' example. Aldicarb is the only pesticide in the test set with an aliphatic structure, i.e., with the lowest probability to be trapped in the reversed phase system [37]. From the four sorbent materials (see Experimental Section) the C-18/OH and PLRP-S gave the worst performance. The trapping efficiency of the Boos silica material was the highest of all but a problem with the elution of the analytes from the cartridge occurred (broad peaks). The LiChrospher 60 RP18 material gave satisfactory retention for all three compounds and was therefore used further in the study. A typical sample volume for enrichment of environmental water samples in on-line systems is 100-250 ml [19,28,30]. However, with regards to low breakthrough volumes of the polar DPs the volume in this study was adjusted to 50 ml as a compromise.

III-Structural Elucidation

Both EI and ammonia PCI mass spectra were employed to identify the DPs of each pesticide. As previously described [38], the ammonia PCI mass spectra are complementary to EI spectra because of the possibility to determine molecular mass of an analyte by generating the characteristic ions $[M+H]^+$ and $[M+NH_4]^+$. After molecular mass determination, the EI spectrum was searched for structurally informative ions. In this study, we attempted to assign the structure of at least five ions from each EI spectrum (if present) which should be, according to EPA directives [39], enough for its tentative identification. In several instances (see below) PCI fragments could be employed for an additional confirmation of the structure. One of the aims of this study was to focus on the identification of stable pesticide DPs in surface water. Chromatograms obtained after a sufficient duration of irradiation (20-24 h is an equivalent of several weeks photolysis under natural conditions [19]) were evaluated. In cases of faster degrading compounds the total disappearance of parent compounds or all DPs from chromatograms indicated the end of experiment.

Atrazine. After 24 h of irradiation, the parent compound could not be detected and two stable DPs were present in chromatograms (Scheme 1). Their concentration gradually increased since the beginning of the experiment. Similar to the other compounds, the experiment was stopped here because a further prolongation of the UV-irradiation may lead to the total degradation of analytes to, e.g., nitrate or acetate whose detection is in the domain of ion chromatography.

Scheme 1

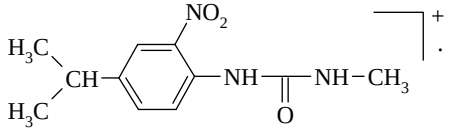
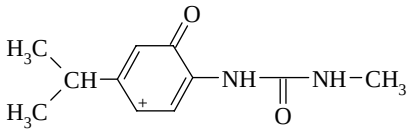
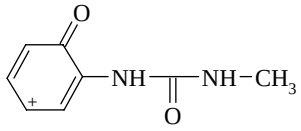
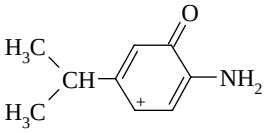


DP2, RT 25.9, MW 126 DP1, RT 25.5 min, MW 112

Both DP1 and DP2 probably originate from intermediate transformation products of desalkylated atrazine, formed and decomposed immediately at the beginning of the experiment. This is supported by an analysis of standard compounds of deethylatrazine and deisopropylatrazine [16,11] which were not detected in any of our samples. A difference between the desalkylated and desaminated DPs is in the substitution of chlorine by hydroxy (DP1) or methoxy (DP2) group. Hydroxylation, as the main photodegradation pathway, and methoxylation of atrazine has already been reported [14,18], however, an additional loss of the amino group is reported for the first time. According to the literature [18], a small amount of methanol is required for formation of the methoxy analogue. In our experiments, atrazine solutions, prepared in plain surface water (spiked at 50 µg/l level) and with 5% methanol addition (spiked at 100 mg/l), provided identical spectra of DPs in both EI and PCI modes. In the former case the source of methyl is presumably a large amount of carbon-containing compounds (humic and fulvic acids) in surface water or free groups from alkyl-cleavages of atrazine. Regarding the interpretation of EI spectra, all characteristic ions are related to the opening of the aromatic ring, a common behaviour for triazine pesticides [40]. The only exception is ion m/z 111 in the spectra of DP2 which can be assigned to a $[H_2NC_3HN_3O]^{+\circ}$. This suggests that DP2 is a possible precursor of DP1 (m/z 112) formed after the replacement of methyl group with hydrogen. DP1 gives the base peak $[HNCHNCO]^{+\circ}$ (m/z 70) and DP2 gives the base peak $[H_2C(OCH_3)NC]^{+}$ (m/z 71).

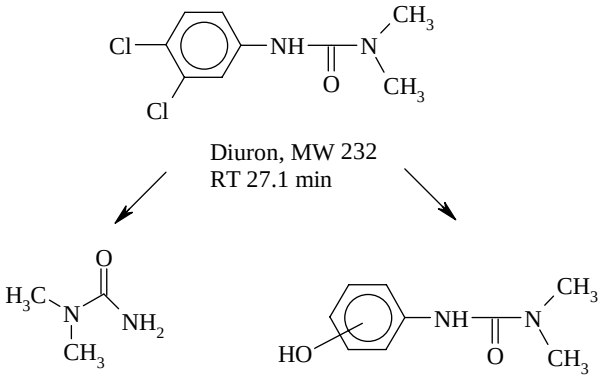
Diuron. Seven DPs and a small amount of parent compound were observed in the irradiated samples after 27.5 h (Table 2). EI spectra of diuron are dominated by the $[(CH_3)_2NCO]^{+\circ}$ ion (m/z 72). This abundant ion was also present in the spectra of DP3, DP4, DP5 and DP7 which indicates that the group stayed unchanged (Scheme 2). EI and PCI spectra of DP3 and DP4 suggest that compounds are isomers with a molecular mass of 180, the mass spectra reflect the replacement of the aromatic chlorines with hydroxy groups in meta and para positions. DP5 was tentatively identified as monuron, the compound appeared already in the first chromatogram and seems to be an immediate DP which degrades after 240 min of irradiation [3]. DP2 is presumably the aliphatic reminder of diuron molecule after rather unexpected break of the aryl-N bond. The molecular mass of DP6 is suggested from the PCI spectrum dominated by ion m/z 200; no signal was observed in the EI mode. The structure of the DP is given on the base of assumptions that (i) the missing spectrum indicates some change in the structure of aliphatic part of diuron molecule, otherwise the abundant ion m/z 72 should be present in the EI trace; (ii) PCI spectrum shows that the molecule still possess one chlorine and; (iii) the ion m/z 200 in PCI spectrum is a protonated molecule, i.e. the molecular mass is 199. Molecular mass of DP7 is 15 amu higher than that of the parent pesticide. The EI spectrum still shows characteristic ion m/z 72 which suggest that substitution should happen at the aryl part of the molecule; the ion m/z 248 in the PCI spectrum is assigned to the protonated molecule (mol. wt. 247) and ions m/z 214 and 180 are results of subsequent losses of two chlorines (34 and 68 amu from the molecule). All this suggests that diuron molecule did not change dramatically and a structure of DP7 is proposed as N'-hydroxydiuron. Similar to the degradation pathways of the other phenylureas such as isoproturon and metoxuron (See below), diuron is presumably substituted by a nitro group in the ortho-position (DP of mol. wt. 277) and after the expulsion of the nitroso group (30 amu, mol. wt. 247) the reactive positively charged oxygen forms a hydroxy group with the anilino hydrogen.

Table 2. Main fragment ions obtained from EI spectrum of DP5 of isoproturon

Fragments	m/z
	237
	207
	165
	150

Fenitrothion. Two DPs were observed after 23 h of irradiation. However, fenitrothion degrades slowly and a high peak for the parent compound was still present in chromatograms (Table S2). Results indicate that the molecule of fenitrothion decomposes at the P-O bond (Scheme 3) with consequent formation of substituted phenol and dimethoxyphosphorothionate [18]. The latter

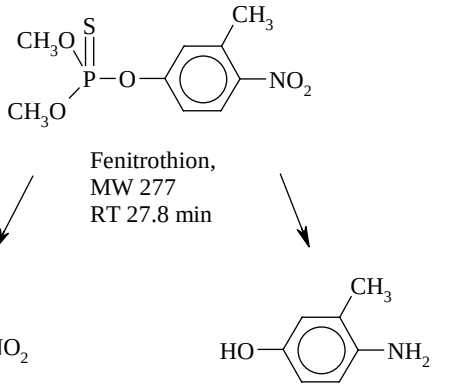
Scheme 2



DP1, RT 7.4 min, MW 88

Two isomers, differing in the position of OH on the ring
DP3, RT 16.2 min, MW 180
DP4, RT 17.6 min, MW 180

Scheme 3



DP1, MW 123, RT 24.4 min

DP2, MW 123, RT 30.8 min

Table S1. OSP-2A time table for automated analysis of pesticide photogradation products: 50 ml sample enriched on cartridge packed with LiChrospher 60 RP-18 material

Time	Percent***			Flow	Comment (event No.)*
(min)	A	B	C	(ml/min)	
0.0	100	0	0	0	close clamp (31), VI-load position (10)
0.1	100	0	0	5	V2-load position (20)
1.1					V1-elute position (11)
2.1	100	0	0	5	
2.2	0	100	0	5	V1-load position (10), remove MeOH from
3.2					V1-elute position (11), remove MeOH from
3.7	0	100	0	5	V1-load position (10)
3.8	0	0	100	5	fill tubings (5 ml sample)
4.8					V1-elute position (11), enrichment of sample
14.8	0	0	100	5	
14.9	0	0	100	0	stop sample flow, V1-start position (10)
15.0					open clamp (30)
15.1					move cartridge one position forward (42), close
15.2					V2-elute position, start HP 1090** and MS
15.3	100	0	0	5	clean tubings (3.5 ml MeOH)
16.0	100	0	0	5	
16.1	100	0	0	0	stop MeOH flow

*VI, V2-six-port switching values of the OSP-2A
**See experimental section
***A, methanol (MeOH); B, HPLC-grade water; C, sample

Table S2. Major ions in EI and PCI spectra of DPs of the studied pesticides

Compound	RT	EI		PCI			
	(min)	BP	Other ions (m/z)	$[M+H]^+$	$[M+NH_4]^+$	Other ions (m/z)	MW
Atrazine	26.7	200	68, 122, 138, 158, 173, 215	216		112, 154, 182, 188	215
DP1	25.5	70	85, 112	1113	130	88, 111	112
DP2	25.9	71	69, 83, 84, 86, 111, 126	127	144	102, 88, 111, 156	126
Fenitrothion	27.8	125	109, 122, 138, 247, 260, 277	278	295	248, 108, 124, 128, 143, 233, 265	277
DP1	24.4	123	77, 106, 122	124	141		123
DP2	30.8	212		124	141	108	123
Diuron	27.0	72	124, 13, 160, 187, 232	233	250	199, 165, 216	232
DP1	7.4			89			88
DP2	12.3			89	106		88
DP3	16.2	72	107, 135, 180	181		110, 89, 91, 106, 165	180
DP4	17.6	135	72	181		110, 165, 180	180
DP5	24.8	72	99, 13, 198	199	216	165	198
DP6	26.4	72	202, 247	248		89, 106, 180, 214	247
DP7	27.1			200		166	199
Isoproturon	26.7	72	91, 146, 161, 191, 206	207	224	193, 191	206
DP1	24.5	72	135, 177, 221, 237, 251	268		89, 149, 238	267
DP2	26.2	72	203, 213, 250	297	314	89, 106, 151, 166, 222, 237, 267	296
DP3	27.4	135	150, 161, 165, 176, 180, 207, 237	238	255	92, 151, 193, 208, 222	237
DP4	27.9	135	150, 161, 165, 176, 180, 207, 237	238	255	92, 151, 193, 208, 222	237
DP5	29.4	72	161, 176, 205, 221, 251	252	269	89, 151, 207, 222, 236	251
Metoxuron	23.4	72	106, 140, 168, 183, 228	229	246	181, 195, 212, 215	228
DP1	16.3	72	79, 107, 135, 180	181	198	106, 110, 127	180
DP2	17.8	165	72, 94, 122, 139, 150, 210	211	228	106, 110, 140, 165, 181, 195	210
DP3	27.4	72	183, 198, 227, 243, 273	274	291	89, 106, 173, 210, 216, 229, 244	273
DP4	27.8	127	68, 84, 109, 170	211	228	199, 216	210

MW, monoisotopic molecular mass; RT, retention time; BP, m/z of base peak; MC, mother compound; *italic*, spectra obtained using loop injection of sample spiked with 100-200 µg/ml of a pesticide; **underlined bold**, molecular peak in EI spectra and base peak in PCI spectra.

compound and its transformation (oxon) analogues [41,16,10] are rather polar and were not observed in any the samples. As already stated for aldicarb, more information and sensitivity on low-molecular mass DPs was provided by the PCI mode of operation in this case (Table S2). Molecular mass of DPI (123) was tentatively recognized from the PCI spectrum, ions m/z 106 and 77 in the EI spectrum are exhibited by reduction of nitro to nitroso group (m/z 106) and by the cleavage of nitro group (m/z 77), respectively. DP2 of the same molecular mass as DP1 was not detected in the EI mode, a compound with a single ion m/z 212 in spectrum, detected at its retention time, is probably coming from the matrix. The structure of the DP2 is proposed from the PCI spectrum as 4-methoxyphenol. The DP is probably formed from 3-methyl-4-nitrophenol, observed by other authors [18,41], after expulsion of nitroso group from the nitrosubstituent (see also isoproturon below). A structurally informative ion m/z 108, which is not present in the PCI spectrum of DP1, is due to the loss of methyl group. The reaction between the aromatic methyl and nitro/oxo substituents was not unexpected; an analogy can be found in the formation of ion $[M\text{-}OH]^+$ (m/z 260 from 277 (mol. wt.)) of fenitrothion presumably due to their ortho orientation.

Isoproturon. Five degradation products were observed after 22 h of irradiation. At this time, the parent compound was not detected in the sample. Surprisingly, spectra of all DPs exhibit ions which suggests a substitution of a nitro group on the aromatic ring

(Scheme 4, Table S2). Since such a substitution is quite unusual, the proposed elucidation of the EI spectra obtained for DP3 and DP4 (Table 1) and DP5 (Table 2) is reported in detail. The PCI spectra confirmed the structures, especially important are ions related to the expulsion of the nitroso group (30 amu) from the nitro substituent with the transition from m/z 252 to 222 (DP5) and 238 to 208 (DP3, DP4) which is characteristic for nitro substituted aromates. Similarly, the two subsequent losses of 30 amu in the PCI spectrum of DP1 (transitions from m/z 297 to 267 and from 267 to 237) suggest the presence of two nitro substituents. Demethylation of the aliphatic part of the molecule in DP3 and DP4 is also indicated changes in EI spectra which are no longer dominated by a characteristic ion $[(CH_3)_2NCO]^+$ (m/z 72) except for 4-isopropylaniline (m/z 135). DP1 is formed probably due to hydroxylation of the isopropyl group [42] in molecule of DP5. When proposing structures of isoproturon DPs, one should bear in mind that the exact positions of nitrogroups on the aromatic ring or hydroxygroup on isopropyl can not be derived from the mass spectra. An ultimate confirmation would be an analysis of synthesized standard compounds. However, this was out of the scope of this study.

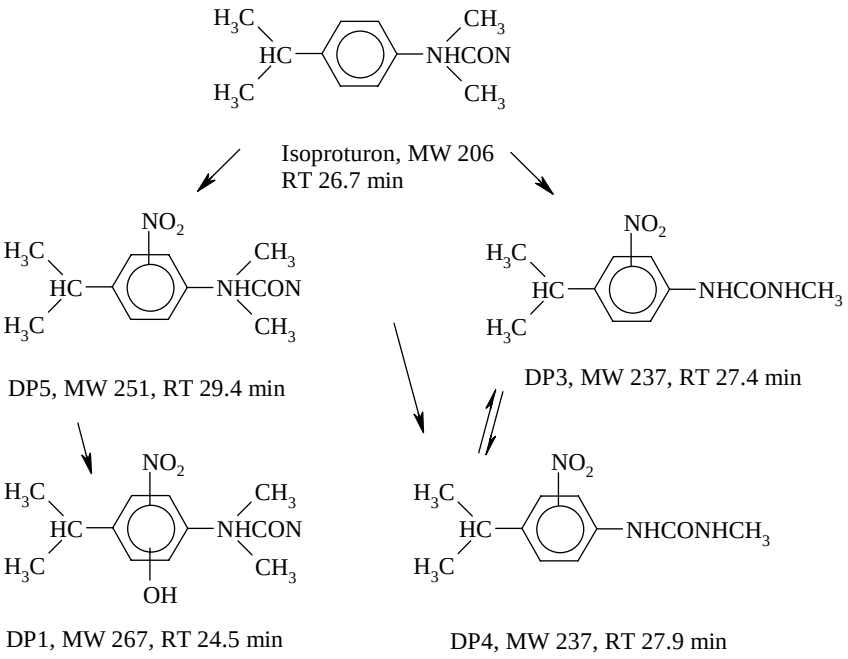
Metoxuron. Two DPs (DP1 and DP2, Scheme 5) were observed in samples spiked with 100 mg/l of metoxuron after 21 h of irradiation. Degradation of metoxuron spiked at 50 µg/l level followed a rather different pathway and DP3 and DP4 appeared in chromatograms. Their concentration gradually increased throughout the whole experiment whereas DP1 and DP2 were not detected. The expected transformation products, monomethyl and desmethyl metoxuron, were not detected in any of the samples as confirmed by analysis of their standards. PCI spectrum of DP1 shows without any doubt that the molecular mass of the compound to be 180 (Table S2). Neither EI nor PCI spectra exhibit a chlorine isotopic pattern and their elucidation is consistent with the proposed structure of demethoxylated metoxuron with chlorine replaced by a hydroxy group. Similarly, the molecular mass of DP2 is 210 and metoxuron is hydroxylated on the position of chlorine. The base peak of DP2 (M/z 165) is related to the neutral loss of $[(CH_3)_2NH]$ (45 amu). Elucidation of the DP3 structure is analogous to that isoproturon and diuron DPs (See above) which suggest that the substitution of the aromatic ring with a nitro (or amino) group is characteristic for phenylureas, however, only in samples spiked at low (50 µg/l) levels. The PCI spectrum unambiguously gives the molecular mass of 273, the molecular ion is present also in the EI spectrum. An expulsion of nitroso group from nitrosubstituent (loss of 30 amu) is indicated by the transitions from m/z 273 to 243 in the EI spectrum and from m/z 274 to 244 in the PCI spectrum. PCI and EI spectra of DP4 show that DP3 goes through further decomposition losing $[(CH_3)_2NH]$ with chlorine being replaced by the hydroxy group. Ions m/z 216 and 199 in the PCI spectrum suggest conversion of NCO to nitroso group with a loss of 12 amu. This reaction is probably taking place competitively to the loss of 30 amu (see above).

The system showed a reasonable capability towards identification purpose as some of the data previously published were confirmed and many new DPs were identified that had never been observed by others. The majority of the DPs eluted at shorter retention times than the parent pesticide which indicates their higher polarity. In general, hydroxylation, dealkylation, demethoxylation, dechlorination, oxidation and cleavages of the functional groups at the 'weak' bonds were primary degradation reactions. Interesting degradation pathways, including substitution of the aromatic ring with nitro group, were observed for phenylureas isoproturon, metoxuron and diuron. It can be stated that photolysis is the major decomposition process for the investigated pesticides [43]; comparative analyses of spiked river Rhine water samples, stored for two weeks in the dark, showed almost unchanged amounts of all parent analyses.

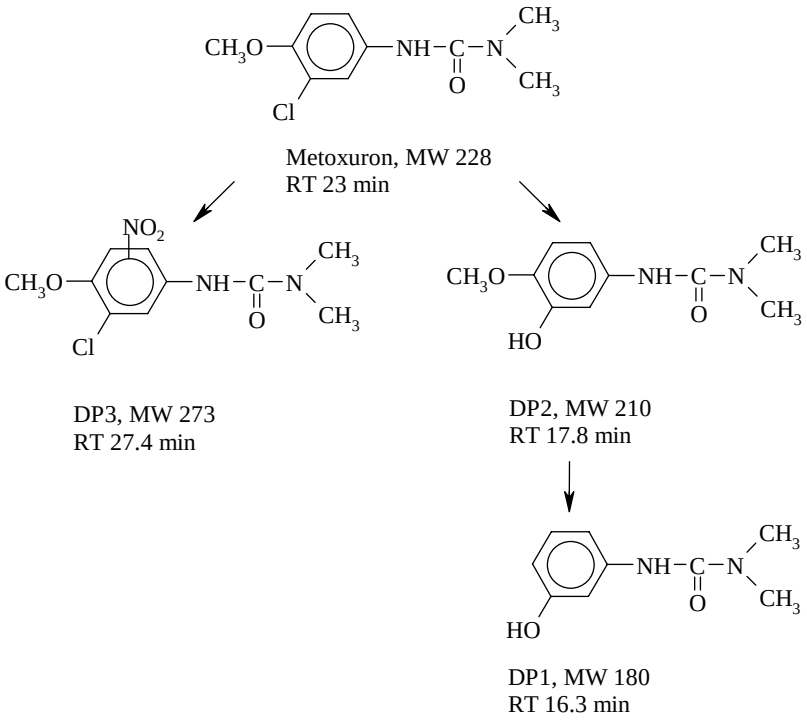
All pesticides, but atrazine, exhibited few different DPs at the two concentration levels (see above). There is a clear advantage in performing the degradation experiments at concentrations closer to those actually applied in real-world. Another disadvantage of experiments with samples spiked at high concentration level is the low solubility of many pesticides requiring the addition of an organic modifier to the sample. Despite the fact that methanol or acetonitrile are considered to be photochemically inert [44], their presence in the sample is certainly far from the 'fields' situation. On the other hand, several of the DPs were detectable only by the loop injection methods due to their early breakthrough, i.e., specific sorbents or higher spiking levels of parent pesticides (100-500 µg/l) should be used in the SPE method.

On-line SPE.LC.PB-MS can obviously help to detect, and also identify, a substantial number of DPs, many of which cannot be determined by means of the more popular GC/MS or LC/TSP-MS. An interesting simplification of the system is offered by means of a single short analytical column (SSC) used for both trace enrichment and separation [45]. Promising results from this study (not shown) are encouraging for further studies. However, the PB-MS comes to a limitation when analyzing highly polar and ionic analytes and therefore the current research is devoted to the utilization of atmospheric pressure ionization (API) interfaces preferably connected to a tandem MS [26].

Scheme 4



Scheme 5



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