Photodegradation of Insecticide Diazinon under Simulated Solar Light Irradiation Conditions and Identification of Photoproducts

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Abstract: The photodegradation of the organophosphorus insecticide diazinon with a high probability to contaminate aquatic environments under simulated solar light irradiation conditions on natural water of a river in North of Iran has been studied. During exposure to solar natural light, diazinon is broken down and produced photoproducts in environment. The degradation of the insecticide was a 32 hour process and included the formation of several intermediates that were identified using GC/ion-trap mass spectrometry with EI or CI in positive and negative ionization mode and HPLC/mass spectrometry. On the basis of the structurally identified photoproducts a possible degradation pathway is proposed for the photolysis of diazinon in natural water. Since primarily hydroxy derivatives were identified in this solution, the mechanism of degradation was probably based on hydroxyl radical attack. The initial oxidative pathways of the degradation of diazinon involved the substitution of sulfur by oxygen on the P=S bond, cleavage of the pyrimidine ester bond, and oxidation of the isopropyl group. Exact mass measurements of the derivatives allowed the elemental formula of the molecules to be determined confidently.

Keywords: photodegradation, diazinon, photoproduct, organophosphorus

1. Introduction

Diazinon [O,O-diethyl-O-(2-isopropyl-6-methylpyrimidin-4-yl) thiophosphate] (Figure 1) is an organo- phosphorus insecticide with widespread agricultural utilization. The primary environmental concerns associated with its use are bird kills, contamination of surface water, and impacts on aquatic species. Diazinon and its metabolites have been encountered during monitoring studies in various aquatic systems all over the world [1-3]. Additionally, high diazinon residues have been found in urban water-ways and effluents from sewage treatment plants [4, 5]. Toxic effects of diazinon are due to the inhibition of acetyl cholinesterase. Metabolic studies in animals have shown that the main route of metabolism is oxidation, and several metabolites with higher acetyl cholinesterase inhibition activity have been identified [6]. In the environment, diazinon appears to be mobile and persistent enough to significantly impact water resources. The abiotic environmental fate of diazinon has been well studied previously; it is stable at neutral waters, while it is rapidly hydrolyzed at acidic pH [7]. Hydrolysis products have been identified as 2-isopropyl-6-methyl-pyrimidin-4-ol and diethyl thiophosphate [8]. Diazinon is slowly photodegraded in distilled water under sunlight, while in natural waters the process is significantly faster [9, 10]. 2-Isopropyl-6-methylpyrimidin-4-ol has been identified as the main photoproduct [7]. Photolysis studies of diazinon in soil aqueous suspensions under UV light have shown that the main transformation products are its oxygen analogue, diethyl 2-isopropyl-6-methylpyrimidin-4-yl phosphate (diazoxon), its isomer O,O-diethyl S-(2-isopropyl-6-methylpyrimidin-4-yl) thiophosphate) (isodiazinon), and O,O- diethyl O-[2-(1-hydroxy-1-methylethyl)-6-methylpyrimidin-4-yl] thiophosphate (hydroxydiazinon) [8]. Because diazinon transformation products are more polar than the parent compound, they may be consequently more water soluble, more mobile, and have a greater potential to leach from soil. Therefore, these compounds would probably be found in surface and ground water at higher concentrations than the parent compound.

Since the formation of highly toxic reaction intermediates is possible, identification of the degradation products is essential [11]. The purpose of this study was to analyze the photodegradation products of diazinon using GC/ion-trap mass spectrometry and HPLC/ mass spectrometry. For identification, structural data were obtained on an ion trap mass spectrometer following electron impact ionization (EI), positive ion chemical ionization (CI) and negative ion chemical ionization (NCI). These data were complemented with exact mass measurements on both precursor and fragment ions obtained using a mass spectrometer. A degradation pathway for diazinon is proposed based on the identified intermediates and also compared with the metabolic pathways in living organisms.

2. Experimental

2.1. Chemicals and Reagents

Diazinon Technical (95.0%) and granules 10 % were a gift from Abyek Chemical Complex. Analytical grade standard of diazinon of high purity was obtained from Fluka and was used without further purification and all the chemicals and solvents were obtained from E-Merck (Darmstadt, Germany).
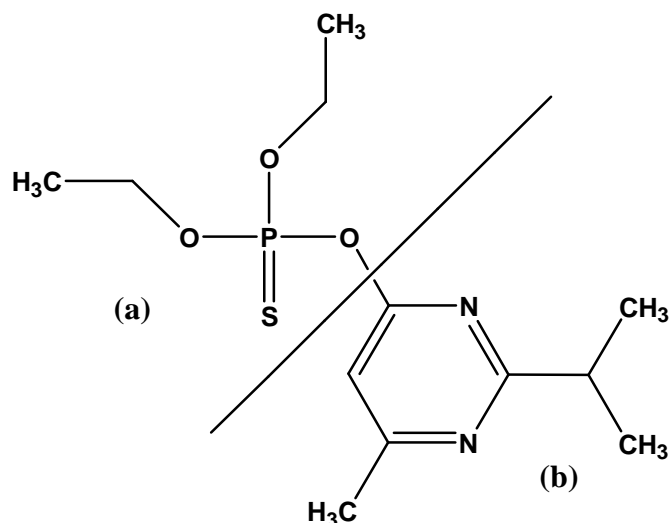
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Figure 1. Molecular structure of the diazinon. Thiophosphate moiety (a) and pyrimidine group (b) are shown

2.2. Water sampling

Natural waters used in the experiments were collected from the Mazandaran province in North Iran and was natural water of Babolrood River. The natural water sample collected from the top meter of water body in 2.5-L pre-cleaned amber glass bottle and stored at 4°C prior to use. Water sample was used without previous treatment and filtration. Physicochemical characteristics of sample of Babolrood River natural water are given in table 1.

TABLE 1. Physicochemical properties of the selected natural waters

Water type	pH	C (μS/cm)	TDS[1] (mg/L)	TOC[2] (mg/L)	Salinity (°/oo)	NO$_3^-$ (mg/L)
Babolrood River	7.81	382	208	2.93	0.4	3.28

[1]TDS: Total Dissolved Solids, [2]TOC: Total Organic Carbon

2.3. Photolysis in natural waters under simulated solar irradiation

The photochemical stability of diazinon was tested using a Sunset CPS+ photo simulator from Heraeus (Hanau, Germany), equipped with a xenon arc lamp (1500 W) and special glass filters restricting the transmission of wavelength below 290 nm. The light source was on the top of the reactor and average irradiation intensity of 750 W/m^2 was maintained throughout the experiments measured by an internal radiometer. Chamber and black panel temperatures were regulated by pressurized air-cooling circuit and monitored using thermocouples supplied by the manufacturer. The temperature of samples did not exceed 25°C using tap water cooling circuit for the UV-reactor. The distance between the irradiated solutions and the lamps was 17 cm. Irradiation experiments were carried out by exposing 200 ml of aqueous solution of diazinon (20 mg/L) containing 0.2 % sodium azide (for biological degradation

resistance) under artificial solar irradiation. During irradiation, the samples were magnetically stirred. All experiments were carried out at room temperature and pH 6. A dark control experiment was also conducted in this experiment. The treated water sample was processed for diazinon photodegradation on 32 hour after treatment that almost is equal with sunlight natural irradiation at 8 sunny days.

2.4. Extraction procedure

Treated water samples (200 ml) were taken in a separator funnel and 10 g sodium chloride was added. The diazinon residues and probably photoproducts were extracted by liquid-liquid partitioning thrice with 15% dichloromethane in acetone. The extract is passed through anhydrous sodium sulphate to remove residual water. The organic layers were combined and concentrated to near dryness on a rotary vacuum flash evaporator, followed by nitrogen gas manifold evaporator. The process was repeated thrice after adding 5 ml acetone in order to eliminate the traces of dichloromethane. The final volume (2 ml) was made in acetone for analysis. The final sample was stored in the dark place while awaiting analysis.

2.5. Analytical Procedures

2.5.1. HPLC analysis

Analyses were carried out using a Waters (Milford, MA) Model TM 600 gradient pump and a 717 autosampler. Data were processed using Waters Millenium software (version 2.0). The analytical column was Waters C$_{18}$, 4.6 × 250 mm, the mobile phase was isocratic acetonitrile/water, 70:30 (v/v), at a flow rate of 1 mL/min, and the eluate was monitored at 254 nm. The irradiated solutions were filtered through 0.45 μm Millipore Millex-LCR filters before HPLC analysis. The injection volume was 20 μL.

2.5.2. GC/MS analysis

A Hewlett-Packard system consisting of a 5890 GC system equipped with electron capture detector (ECD) and capillary column HP-1 (30 m × 0.32 mm i.d., 0.25 µm film thickness of 5% diphenyl / 95% dimethyl polysiloxane), and a 5971 mass spectrometer. Chromatographic data were monitored by Xcalibur® 1.4 software. The helium carrier gas velocity was 30 cm/s, the injector temperature was 210 °C, and the transfer line temperature was set at 275°C. The oven program was 1 min at 60°C, 20°C/min to 80°C, 5°C/min to 196°C, and 20°C/min to 270°C (10   min). The mass spectrometer detector was tuned by maximum sensitivity autotune. Mass spectra database searches were carried out using the Wiley (New York, NY) Registry of Mass Spectral Data, 6th edition, and the NIST Mass Spectral Search Program, version 1.6d.

3. Result and Discussion

The intermediate products formed in this photodegradation of diazinon in the aqueous solution after 32 hours were investigated by HPLC/GC/MS analysis. Six products were identified by the molecular ion and mass fragment ions, and also through comparison with NIST library data. The structures of the six photoproducts are represented in Scheme 1, together with the parent compound. The similarities of these compounds to the NIST library data were more than 85%. By interpreting the mass spectra, the product no. 1 (2-isopropyl-6-methyl-pyrimidin-4-ol, IMP) was formed from diazinon due to the cleavage of P-O (pyrimidine ring) bond. The product no. 2 (diazoxon) was found to be the product which was substituted the S atom to O atom. Further hydroxyl radical attack could yield to hydroxyl derivative of diazoxon (product no. 3). The product nos. 4 and 6 were formed due to hydroxylation of isopropyl group on diazinon. The product no.5 was formed by further oxidizing hydroxydiazinon (product no. 4) on the isopropanol group. In addition to these six compounds, other degradation products still possibly would exist in the photodegradation system but were not detected because of their low concentration, extraction efficiency and limited sensitivity in GC/MS. Based on the intermediate products listed in Table 2, as well as the literature data [12, 13], the possible degradation pathway for diazinon is proposed in Scheme 1. As can be seen, two possible degradation pathways are proposed (Figure 1): one pathway involved the cleavage of the P-O (pyrimidine group) bond yielding IMP. In the photodegradation process, loss of the pyrimidine group probably occurred either through oxidative desulfuration by OH radical attack [14] on the thiono group to give diazoxon followed by hydrolysis, or through an oxidation mechanism acting directly on diazinon. These oxidative mechanisms have already been observed for most thiophosphates in metabolic studies [15]. In the second pathway, the thiophosphoric moiety of diazinon was

preserved. Hydroxylation of the primary or secondary carbon atoms of the isopropyl group gave hydroxydiazinon or 2-hydroxydiazinon, respectively, which after further oxidation led to diazinon aldehyde and diazinon methyl ketone. A similar transformation pathway was observed following for the oxygen analogue diazoxon formed by oxidation of the P=S bond of diazinon to a P=O bond which led to the formation of the hydroxylated derivatives. IMP is the hydrolysis product of diazinon detected in natural waters while photochemical transformation of the parent molecule yields the production of diazoxon and hydroxydiazinon, respectively [8]. Hydroxylated metabolites of diazinon including hydroxydiazoxon have also been observed in metabolism studies [16].

TABLE 2. Identified products by GC/MS in the photocatalytic degradation of dizainon

Product No.	RT (min)	M.W.	Characteristic ions (m/z)	Compound
1	26.1	152	137,152,124	2-isopropyl-6-methyl-pyrimidin-4-ol
2	29.9	288	137,151,273	Diazoxon
Diazinon	30.5	304	137,152,179	Diazinon
3	32.0	304	111,153,289	Hydroxydizoxon
4	32.7	320	153,169,320	Hydroxydiazinon
5	33.7	304	153,180,199	Diazinon methyl ketone
6	35.2	320	151,122,195	2-hydroxydiazoxon

4. Conclusions

Information on degradation products is necessary to understand the environmental fate of pesticides and to establish important degradation pathway, which will allow us to get a better knowledge of the transformation of target compounds in the environment. Moreover, the study of contaminant photochemical behavior is a key issue in terms of the formation of toxic transformation products. The photodegradation of diazinon under simulated solar light irradiation conditions on natural water of a river in North of Iran has been observed to proceed essentially through a hydroxylation mechanism occurring rapidly by attack of the photochemically generated OH radicals. The main transformation intermediates identified were oxidation products of the isopropyl group of diazinon and its oxygen analogue diazoxon. Loss of the thiophosphoric moiety yielded the pyrimidinol IMP. The results reported here show that the combination of HPLC/GC/MS with EI, positive and negative ions and exact mass measurements represent a powerful analytical approach for the confirmation of the molecular structure of photodegradation intermediates. These photoproducts are extremely stable and actually more toxic than the parent compound. Toxicity and persistence of photoproducts indicate that these compounds should be considered along with the metabolites in residue and toxicology evaluations.
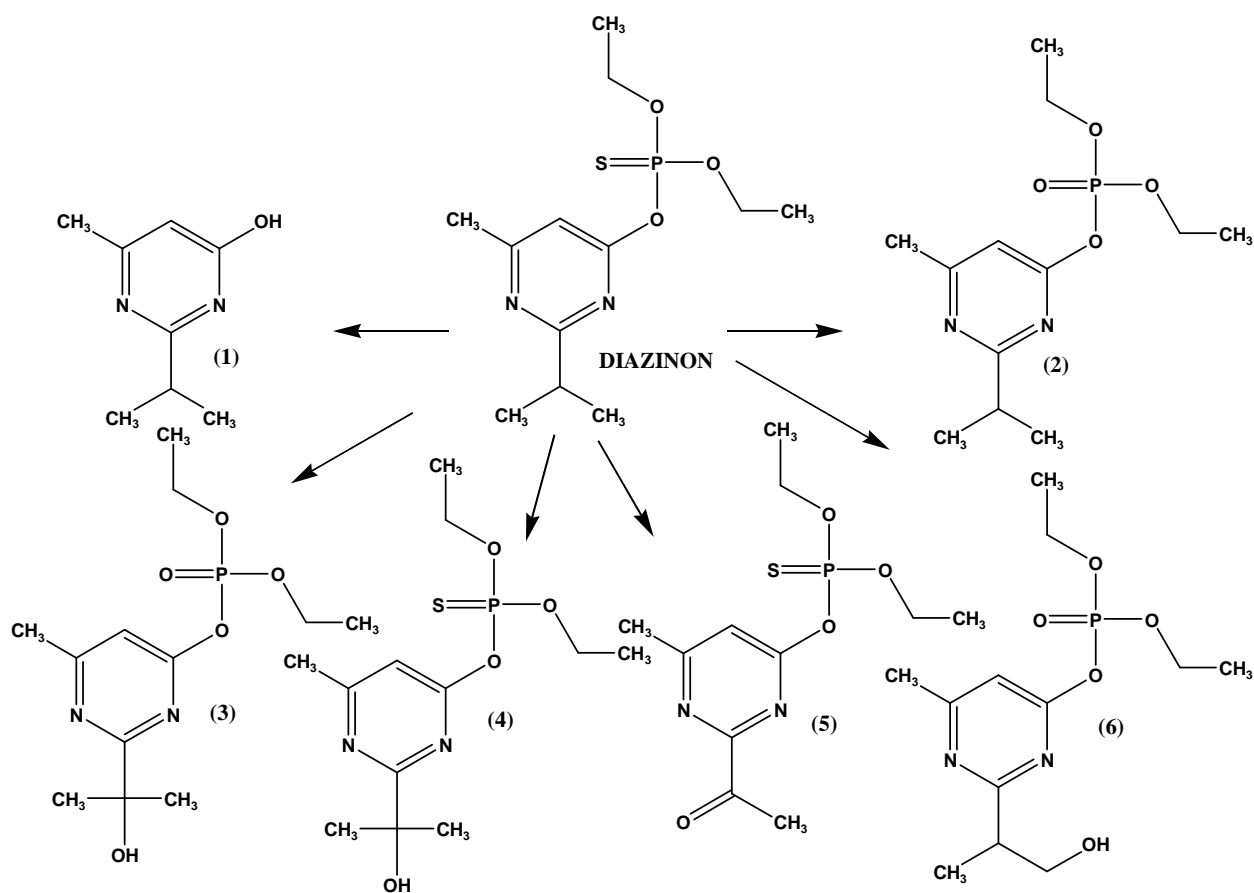
Scheme 1. Scheme of the proposed photodegration pathway for diazinon: 2-isopropyl-6-methyl-pyrimidin-4-ol, (IMP) (1), dizoxon (2), hydroxydizoxon (3), hydroxydiazinon (4) diazinon methyl ketone (5), 2-hydroxydiazoxon (6)

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