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Toxicity of Malathion and Diazinon Byproducts Generated Through the UV/Nano-Zn Process

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Abstract

The potential toxicity of diazinon and malathion byproducts had been poorly studied. This study aimed to determine the toxicity of malathion, diazinon, and their byproducts generated through the UV/nano-Zn process. Diazinon and malathion samples were prepared at 1, 5, and 10 mg/L concentrations. In this study, the UV/nano-ZnO process was used for the degradation of these insecticides. The contact times in reactors were 0.5, 1, and 2 h and pH was set at 6, 7, 8, and 9. The dehydrogenase enzyme assay using *Nitrobacter* and *Nitrosomonas* bacteria was used for malathion, diazinon, and their byproducts. All tests were prepared in triplicate. The probit analysis in SPSS Ver. 16.0 software was used for the calculation of EC_{50} (50% effective concentrations). According to the results, byproduct analysis and toxicity assessment were performed in the following situation in the UV/nano-ZnO process: pH 8, contact time of 2 h, initial concentration of 5 mg/L, removal efficiency of diazinon and malathion of 95.4% and 97.5%, respectively. The EC_{50} values using *Nitrobacter* and *Nitrosomonas* were 0.35 and 4.26 mg/L for diazinon and 173.3 and 279.82 mg/L for malathion, respectively. The EC_{50} values using *Nitrobacter* and *Nitrosomonas* were 2.24 and 2.82 mg/L for diazinon byproducts and 28.10 and 197.92 mg/L for malathion byproducts, respectively. This study showed that in some cases the byproducts of diazinon and malathion produced through the UV/nano-ZnO process are more toxic than diazinon and malathion (primary forms). Therefore, it can be suggested that their removal in photo-catalyst processes should be under special caution.

Keywords: Insecticide, Byproducts, Photo-Catalyst Process, UV/Nano-ZnO

1. Background

Organophosphorus Pesticides (OPPs) are widely used in agriculture in many countries to maintain and increase crop yields (1). Because of their wide use and chemical properties, these compounds can cause various degrees of contamination in different environments (2-4). Among OPPs, diazinon and malathion are the most frequently used pesticides in agriculture and are commonly detected in different environments, especially water resources (3, 5-8). These compounds can be the inhibitors of acetylcholinesterase enzyme (7). They also disrupt the function of other enzymes that are responsible for most biochemical processes (5). According to the Canadian standards, the maximum acceptable concentrations of diazinon and malathion in drinking water are 0.02 and 0.19 mg/L, respectively (8).

In the last decades, there has been great attention to the development of efficient methods for insecticide removal from aqueous environments based on advanced oxidation processes (AOPs) such as TiO_2/Ni photo-electrode (2), UV/N-doped TiO_2 nanosheets (3), UV/iodide/ZnO (9), UV/H2O2 (10), and UV/nano-ZnO (11). Among such processes, UV/nano-ZnO can be regarded as an effective treatment method for hazardous contaminants such as diazinon, malathion, and other compounds (12-16). However, the complete mineralization of such contaminants to H_2O and CO_2 normally needs different parameters such as contact time, anionic and cationic concentration, initial concentration of pollutants, etc. (17, 18). The complete treatment of these insecticides is hardly achieved because of the formation of intermediate oxidation byproducts. These byproducts may be more toxic than insecticides themselves (19, 20). For example, researchers showed that the UV/H_2O_2 treatment of drinking water increased post-chlorination disinfection byproduct (DBP) formation (19). In another study on photocatalytic degradation byproducts of diazinon, it was found that three-min UV irradiation generated several degradation byproducts using gas chromatography-mass spectrometry (GC-MS) (21).

Yet, the potential toxicity of diazinon and malathion byproducts has been poorly studied. One of the fast and reliable toxicity methods is the dehydrogenase enzyme assay using resazurin (7-hydroxy-10-oxidophenoxazin-10-ium-3-one). This bioassay is based on the function of viable and active bacteria to reduce resazurin to resorufin. Such reactions occur intracellularly (21) where initial resazurin enters the cytosol to convert to resorufin by dehydrogenase enzyme activity through accepting electrons from nicotinamide adenine dinucleotide (NADH), flavin adenine dinucleotide (FADH), nicotinamide adenine dinucleotide phosphate (NADPH), and so on (22). The reduction related to bacterial activity can cause blue resazurin to be converted to the reduced, fluorescent, and pink form. In this study, the dehydrogenase enzyme assay is performed using *Nitrosomonas* and *Nitrobacter* bacteria because they have an important role in nitrification as a key bioprocess in both natural and engineered systems (23).

2. Objectives

This study aimed to determine the toxicity of malathion and diazinon byproducts generated during the UV/nano-Zn process.

3. Methods

3.1. Test Reagents and Chemicals

The freeze-dried *Nitrosomonas* and *Nitrobacter* bacteria and reconstitution solutions were supplied by the Iranian Research Organization for Science and Technology (IROST). Resazurin, diazinon, malathion, phosphate buffer, and borate buffer were purchased from Sigma Aldrich. The analytical grades of nano-ZnO, sulfuric acid, and sodium hydroxide were from Merck (Darmstadt, Germany). All solutions were prepared with ultra-pure water from a Milli-Q system (Millipore, Bedford, MA, USA).

3.2. Removal of Diazinon and Malathion

Diazinon and malathion samples were prepared at 1, 5, and 10 mg/L concentrations. The UV/nano-ZnO process was used for the degradation of these insecticides. The contact times in the reactor were 0.5, 1, and 2 h and pH was adjusted at 6, 7, 8, and 9 for determining conditions in which more than 90% and lower than 100% of influent insecticides were degraded. Such conditions resulted in more process byproducts and might be more similar to real conditions. All experiments were repeated three times.

Degradation processes were performed in a 500 mL annular photochemical reactor, in the axis of which a UV mercury lamp (254 - 365 nm, 80 W) was installed inside a quartz glass well (photon flux of 1.18×10^{-7} Einstein/s and average UV fluence rate of 0.58 mW/cm^2) based on Li et al., 2015. For maintaining the homogeneity of the solution, a magnetic stirrer was used at the bottom of the quartz glass. Before starting the process, the UV lamp was lighted up for 20 min to achieve a stable output. Direct UV photolysis and UV/H_2O_2 oxidation were performed in ultrapure water containing an initial 16.45 M (or 5 mg/L) diazinon, similar to those used in a previous study (14). The nano-ZnO solution was added to the reactor to obtain a final concentration of 25 mg/L. Phosphate and borate buffers were used to maintain the solution pH at 7, 8, and 9. Samples (100 μL) were withdrawn from the reactor at regular intervals for residual insecticides or their byproducts analysis. Before toxicity assessment and extraction of byproducts, nanoparticles were removed from suspension by centrifugation at 8,000 rpm for 10 min.

3.3. Extraction and Identification of Byproducts

To obtain the accurate concentration of diazinon and malathion byproducts, the compounds were extracted using solid-phase extraction after the UV/nano-ZnO process under the optimal condition. For this aim, we used a C_{18} bonded cartridge (Mega Bond Elut, Varian, Jones Chromatography Ltd., Hengoed, UK) containing 500 mg of a sorbent. The cartridge was conditioned according to the company instructions by the sequential application of 5 mL of methanol and water, followed by 10 mL of reagent. The malathion and diazinon byproducts were then extracted with 10 mL of methanol. Methanol was then removed using a stream of nitrogen gas at ambient temperature before toxicity or GC-MS analysis.

The analyses of diazinon, malathion, and their byproducts were done using Ultra-Performance Liquid Chromatography and Electrospray Ionization Mass Spectrometry (UPLC-ESI-MS/MS). This system was coupled with an ACQUITY™ UPLC BEH C8 separation column (2.1 × 100 mm, 1.7 μm particle size). A mixture of acetonitrile (Sigma-Aldrich Co., HPLC grade) and ultra-pure water (65:35 for diazinon and 30:70 for malathion) was applied as a mobile phase under the isocratic elution mode. The flow rate was manipulated at 1 cm^3/min and the UV detector was employed at the wavelengths of 202 and 210 nm for malathion and diazinon, respectively (14, 23). Other detailed operation parameters for UPLC and MS were reported in the study by Li et al. (24). Full-scan data were obtained from 50 to 500 m/z at an acquisition rate of 0.05 s per spectrum in both positive and negative electrospray ionization modes. For the determination of byproducts, Collision Induced Dissociation (CID) MS/MS experiments were conducted. Detailed operation parameters for (CID) MS/MS were reported by Li et al. (24).

3.4. Bioassay Using Dehydrogenase Enzyme and Resazurin

Nitrosomonas and *Nitrobacter* cultures (aged 48 h) were used for all tests. To ensure that an equal number of each strain was always used, a set of dose-response curves for both *Nitrosomonas* and *Nitrobacter* was prepared. According to such graphs, a final concentration of 5×10^5 CFU/mL was approved for this assay. The results of dehydrogenase toxicity were compared with the diluted colony count method to ensure its reliability. The dehydrogenase enzyme assay was used for malathion, diazinon, and their byproducts (25, 26).

In each of microplate test wells, 100 μL of *Nitrosomonas* or *Nitrobacteria* suspension was introduced, followed by 100 μL of dilution water (for test controls), 2% dimethyl sulfoxide (for solvent controls), or various concentrations of malathion, diazinon, or their byproducts (test wells). Also, a control test was run with an equal concentration of malathion and diazinon that remained after the treatment of these insecticides with the UV/nano-ZnO process in the optimum conditions. All microplates were incubated for 24 h at 30°C. Resazurin measurement was done at the beginning and end of experiments (30 min) after shaking with a vortex mixer for one minute to resuspend bacterial cells. For this aim, a Fluoroskan Ascent (Labsystems International) was applied to detect the remained resazurin fluorescence in 96-well microplates at the wavelength of 610 nm (26-28).

3.5. Statistical Methods

The microplates were prepared in triplicate for the solutions of each byproduct concentration. The probit analysis in SPSS Ver. 16.0 software was used for EC50, NOEC, and 100% mortality calculation.

4. Results and Discussion

The degradation of diazinon and malathion under different conditions is described in Table 1. As shown in this table, irradiation under different pHs gave the highest degradation at pH 9 for both insecticides. However, in this study, pH 8 was chosen for the assessment of optimal conditions because the difference between the results of pH 8 and 9 was not statistically significant (P value > 0.05) and pH 8 is more suitable for most bacteria. Therefore, the effects of the initial concentration of insecticides and contact time were assessed at pH 8. The results in Table 2 show that the removal efficiency of diazinon and malathion increased with increasing contact time. However, in contrast, Table 3 shows that removal percentages decreased by increasing the initial concentration of insecticides.

Table 1. Removal Efficiency of Diazinon and Malathion Under UV/Nano-ZnO Process in Different Solution pHs[a]

Parameters	Solution pH			
	6	7	8	9
Removal of diazinon, %	76.1	82.0	87.6	90.3
Removal of malathion, %	84.9	92.2	95.5	96.9

[a] Contact time: one hour; initial concentration of insecticide: 5 mg/L

Table 2. Removal Efficiency of Diazinon and Malathion Under UV/Nano-ZnO Process at Different Contact Times[a]

Parameters	Contact Time, h		
	0.5	1	2
Removal of diazinon, %	68.6	87.6	95.4
Removal of malathion, %	73.2	95.5	97.5

[a] pH: 8; initial concentration of insecticide: 5 mg/L

Table 3. Removal Efficiency of Diazinon and Malathion Under UV/Nano-ZnO Process at Different Initial Concentrations of Insecticides[a]

Parameter	Initial Concentration of Insecticides, mg/L		
	1	5	10
Removal of diazinon, %	97.2	95.4	87.1
Removal of malathion, %	98.5	97.5	83.3

[a] pH: 8; contact time: 2 h

According to the results of Tables 1-3, the UV/nano-ZnO process could efficiently and rapidly remove diazinon and malathion. However, researchers showed that other AOPs can also be efficient and rapid. In this regard, most recent studies presented heterogeneous catalytic ozonation using nano-MgO for toluene removal (29) and UV/nano-CuO for textile wastewater treatment (30). In another study, Kamani et al. reported photocatalyst decolorization of C. I. Sulphur Red 14 from solutions by UV/nano-ZnO (31). However, in most of such studies, the assessment of byproduct toxicity was not performed. Thus, in this study, a general condition was chosen for the toxicity assessment of byproducts.

According to Table 3, the difference between the results of 1 and 5 mg/L concentrations was not statistically significant (P value > 0.05). Hence, the initial concentration of 5 mg/L was chosen for toxicity assessment because the possibility of byproduct generation was more at this concentration. According to the results of Tables 1-3, the byproduct analysis and toxicity assessment were performed in the following conditions of the UV/nano-ZnO process: pH 8, contact time of 2 h, initial concentration of 5 mg/L, and removal efficiencies of 95.4% and 97.5% for diazinon and

malathion, respectively.

Table 4 lists the diazinon and malathion byproducts detected in the UV/nano-ZnO effluent. The main goal of this section of the study was to identify a broad spectrum of non-target byproducts to demonstrate if the toxicity of effluents is due to these byproducts. The number of detected byproducts was higher for diazinon (14 different byproducts) than for malathion (9 different byproducts). However, the table only shows byproducts with the distinguishing accuracy of more than 50%. The effluent had more byproducts but according to the applied method, their distinguishing accuracy was lower than 50% and thus, they are not presented in this table. Therefore, this can be one of the different aspects of this study and previous ones (21, 24). For example, Li et al. studied the disinfection byproducts of diazinon solutions via UV and UV/H_2O_2 processes and detected trichloroacetic acid, chloroform, dichloroacetic acid, dichloroacetonitrile, monochloroacetic acid, and 1,1,1-trichloroacetone (24). In their study, the disinfection byproducts increased significantly with an increase in solution pH, UV dose, and H_2O_2 concentration. Therefore, other reasons for the difference between the results of studies can be solution pH, UV dosage, and application of H_2O_2 instead of nano-ZnO (24).

In this study, EC_{50}, NOEC, and 100% mortality of diazinon and malathion were obtained after a 30 min exposure of *Nitrobacter* and *Nitrosomonas* bacteria to these toxic substances (Table 5). According to Table 5, the EC_{50} values of diazinon were 0.35 and 4.26 mg/L for *Nitrobacter* and *Nitrosomonas*, respectively. The corresponding values for malathion were 173.3 and 279.82 mg/L, respectively. Therefore, diazinon was more toxic than malathion to both the tested bacteria.

According to Table 5, the NOEC of diazinon was close to zero for *Nitrobacter*. This shows that the minimum value of this insecticide can have adverse effects on *Nitrobacter* bacteria. In the case of *Nitrosomonas* bacteria, at concentrations of less than 0.04 mg/L of diazinon and 13.52 mg/L of malathion, it can be expected to see no adverse effect for 30 min. The third section of Table 5 shows the concentrations that induced 100% inhibition in dehydrogenase enzyme activity of bacteria. According to these results, for 100% destruction of *Nitrosomonas* and *Nitrobacter* bacteria, diazinon at 133.3 and 328.2 mg/L and malathion at 137,735.2 and 5,788.8 mg/L are needed, respectively.

Table 6 shows the toxicity of byproducts of diazinon and malathion produced through the UV/nano-ZnO process. According to these results, diazinon byproducts were more toxic than malathion byproducts. Diazinon byproduct EC50 values were 2.24 and 2.82 mg/L for *Nitrobacter* and *Nitrosomonas*, respectively. These values for malathion were 28.10 and 197.92 mg/L, respectively. This difference in toxicity could be due to the difference in produced byproducts (Table 4).

Table 4. Diazinon and Malathion Byproducts Generated Through UV/Nano-ZnO Process[a]

Insecticides/Byproduct	Accuracy Percent	Time, min
Diazinon byproducts		
1. diethyl phosphate (DEP)	93	1.36
2. diethyl thiophosphate (DETP)	87	1.61
3. Methylene Chloride	56	3.56
4. Cyclotetrasiloxane, octamethyl	80	7.04
5. 1-Tridecene	68	9.10
6. 2-isopropyl-6-methyl-4-pyrimidinol (IMP)	91	10.19
7. diazinon methyl ketone	83	11.56
8. 1-Octadecene	70	12.88
9. O-analog diazinon (diazoxon)	90	13.65
10. Diazinon	97	14.75
11. 1-hydroxy isopropyl diazoxon	65	15.01
12. hydroxydiazinon	58	15.65
13. 1-hydroxy isopropyl diazinon	69	16.34
14. 2-Hydroxydiazoxon	92	17.98
Malathion byproducts		
1. Phthalic anhydride	56	6.15
2. n-Decanoic acid	87	6.58
3. Cyclotetradecane	97	10.6
4. 9-Hexadecenoic acid	95	13.14
5. Pentadecane	96	13.74
6. Cyclododecane	95	14.87
7. Methyl pentadecyl ether	55	16.36
8. Nonadecane	97	17.46
9. Cyclododecane	89	19.63

[a]Obtained using head space and solid-phase extraction followed by UPLC-ESI-MS/MS

The toxicity results of insecticides and their byproducts showed that *Nitrobacter* was more sensitive than *Nitrosomonas*. Thus, it can be said that *Nitrobacter* is more suitable than *Nitrosomonas* to be used as an indicator for toxicity assessment of insecticides and their byproducts. This difference in sensitivity can be related to the difference in strains so that *Nitrosomonas*, unlike *Nitrobacter*, can generate membranes. These membranes use electrons produced during ammonia oxidation (23).

Previous studies showed that trace amounts of insecticide residues (at μg/L or even ng/L levels) in the food

Table 5. Results of EC_{50}, NOEC, and 100% Mortality of Diazinon and Malathion Using Resazurin Bioassay by *Nitrobacter* and *Nitrosomonas* Bacteria

Parameters/Compounds	Bacteria	Value, mg/L	Lower Bond	Upper Bond
EC_{50}, mg/L				
Diazinon	*Nitrobacter*	0.351	0.061	0.862
	Nitrosomonas	4.269	1.465	7.841
Malathion	*Nitrobacter*	173.378	83.701	432.947
	Nitrosomonas	279.828	116.515	611.385
NOEC, mg/L				
Diazinon	*Nitrobacter*	0.001	0.000	0.013
	Nitrosomonas	0.056	0.000	0.315
Malathion	*Nitrobacter*	0.218	0.004	1.34
	Nitrosomonas	13.527	0.107	48.841
100% mortality, mg/L				
Diazinon	*Nitrobacter*	133.388	15.542	1019103
	Nitrosomonas	328.294	92.189	10350.39
Malathion	*Nitrobacter*	137735.28	1.69 E + 04	1.38 E + 07
	Nitrosomonas	5788.807	1695.916	571434.5

Table 6. Results of EC_{50}, NOEC, and 100% Mortality of Diazinon and Malathion Byproducts Using Resazurin Bioassay by *Nitrobacter* and *Nitrosomonas* Bacteria

Parameters/Compounds	Bacteria	Value	Lower Bond	Upper Bond
EC_{50}, mg/L				
Diazinon byproducts	*Nitrobacter*	1.246	0.486	3.187
	Nitrosomonas	2.821	2.081	3.773
Malathion byproducts	*Nitrobacter*	28.107	17.61	46.471
	Nitrosomonas	197.927	171.238	229.043
NOEC, mg/L				
Diazinon byproducts	*Nitrobacter*	0.006	0.000	0.037
	Nitrosomonas	0.02	0.007	0.042
Malathion byproducts	*Nitrobacter*	1.926	0.409	4.217
	Nitrosomonas	35.323	25.474	45.533
100% mortality, mg/L				
Diazinon byproducts	*Nitrobacter*	243.968	41.908	17946.46
	Nitrosomonas	400.156	203.709	982.337
Malathion byproducts	*Nitrobacter*	410.224	177.405	2191.125
	Nitrosomonas	1109.06	855.319	1550.565

chain could cause potentially different destructive effects on cells, such as mutagenicity, cytotoxicity, and genetic malformations, as well as endocrine-disrupting effects for humans or animals (3, 6-8). Among AOPs, UV/nano-ZnO is regarded as an effective removal method for such insecticides from drinking water (12-16). However, according to the results of this study, the complete degradation of insecticides to H_2O and CO_2 normally takes place under special conditions (18). This study showed that in normal conditions, the complete mineralization of diazinon and malathion is hardly achieved, leading to the production of intermediate byproducts. In this regard, previous studies showed that these insecticide byproducts may be more toxic with chlorine compound than the pesticide themselves (19, 20). In this study, the concentration of each byproduct was not measured. But similar to this

study, 2-isopropyl-6-methyl-4-pyrimidinol was reported as a byproduct of diazinon. However, in a previous study, it was reported as a major degradation byproduct in the UV and UV/H_2O_2 processes, which is less toxic than its parent pesticide (6, 14). Other byproducts, such as diazoxon and hydroxyl diazinon, were also detected in previous studies, with diazoxon being thought to be more toxic than diazinon (6).

In this study, we used *Nitrobacter* and *Nitrosomonas* in bioassay tests and such strains cannot tolerate acidic pH. Therefore, for the investigation of the effect of pH, it is suggested that such toxicity assessments be conducted using acidophilus bacteria in acidic pHs in future studies.

4.1. Conclusions

This study aimed to determine the toxicity of malathion and diazinon and their byproducts produced through the UV/nano-Zn process. The results showed that diazinon was more toxic than malathion to both tested bacteria. This study showed that in some cases, the toxicity of diazinon and malathion byproducts produced through UV/nano-ZnO was more than the toxicity of diazinon and malathion themselves (primary forms). Therefore, their removal in photo-catalytic processes should be under special conditions.

Footnotes

Authors' Contribution: Study concept and design: Ramin Khaghani. Analysis and interpretation of data: Mohammad Reza Zare. Drafting of the manuscript: Mohammad Reza Zare and Ramin Khaghani. Critical revision of the manuscript for important intellectual content: Mohammad Reza Zare. Statistical analysis: Mohammad Reza Zare.

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