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UV/H₂O₂ advanced oxidation for abatement of organophosphorous pesticides and the effects on various toxicity screening assays

Highlights

- Advanced oxidation was applied to detoxify organophosphorous insecticides in water
- Three toxicity assays were used: estrogenicity, genotoxicity and neurotoxicity
- In general, the compounds' toxicity remained constant or decreased after treatment
- However, methamidophos degradation resulted in an increase in sample genotoxicity
- The increase is likely due to the formation of toxic transformation products

UV/H₂O₂ advanced oxidation for abatement of organophosphorous pesticides and the effects on various toxicity screening assays

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Abstract

Advanced oxidation processes (AOPs) are utilized due to their ability to treat emerging contaminants with the fast reacting and non-selective hydroxyl radical ([•]OH). Organophosphorous insecticides are common drinking water contaminants, with 12 different compounds of this class being found on the US EPA's most recent Candidate Contaminant List (CCL4). The use of the AOP UV/H₂O₂ for the treatment of organophosphorous insecticides was explored in this study, by coupling biological and analytical tools to follow the abatement of the target compounds. Four insecticides were explored for advanced oxidation treatment: acephate, dicotophos, fenamiphos, and methamidophos. All four compounds were fast reacting with [•]OH,

all reacting with second order rate constants $\geq 5.5 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$. Three major endpoints of toxicity were studied: estrogenicity, genotoxicity (mutagenicity), and neurotoxicity. None of the target compounds showed any estrogenic activity, while all compounds showed an active genotoxic (mutagenic) response (AMES II assay) and most compounds had some level of neurotoxic activity. AOP treatment did not induce any estrogenic activity, and reduced the compounds' neurotoxicity and genotoxicity in all but 1 case. Methamidophos degradation by UV/H₂O₂ resulted in an increase in genotoxicity, likely due to the formation of toxic transformation products. The increase in toxicity gradually decreased with time, possibly due to hydrolysis of the transformation products formed. This study provides insights into parent compound abatement and the changes in toxicity due to transformation products.

Keywords: Organophosphorous insecticides; estrogenicity; genotoxicity; neurotoxicity; advanced oxidation; UV/H₂O₂, transformation products

Introduction and Background

Emerging contaminants in the United States that are being explored for regulation are placed onto the US EPA's Candidate Contaminant List (CCL). On the most recent CCL list published by the EPA (CCL4), there are twelve organophosphorous insecticides (USEPA, 2016). Organophosphorous insecticides are widely used in the United States, and may find their way into the water cycle through urban stormwater or agricultural runoff, and groundwater contamination (Buchanan et al., 2009). These insecticides act by specifically binding to the enzyme acetylcholinesterase (AChE), blocking AChE and thereby inhibiting the hydrolysis of the neurotransmitter acetylcholine (ACh). ACh inhibition is not specific to insects, where the

delivery of the insecticide was meant for, but can also occur if ingested by humans. Several studies have previously reported neurological effects of organophosphorous insecticide ingestion (Wadia et al., 1974, Gromov and Rozengart, 1976, Bradwell, 1994).

New technologies are being implemented in water treatment for control of contaminants of emerging concern (CECs) such as agricultural and pest control chemicals like organophosphorous insecticides. Advanced oxidation processes (AOPs) have proven to be an effective technology for CECs abatement, due to their partial oxidation by $\cdot\text{OH}$ radical, and not by a phase transfer mechanism like sorption or air stripping (Huber et al., 2003, Huber et al., 2004, Lee et al., 2008, Keen et al., 2014, Bethi et al., 2016, Lester et al., 2016). Several types of advanced oxidation treatment including $\text{O}_3/\text{H}_2\text{O}_2$, $\text{UV}/\text{H}_2\text{O}_2$, UV/O_3 and UV/TiO_2 are available for water treatment (Braun and Oliveros, 1997). $\text{UV}/\text{H}_2\text{O}_2$ was explored in this study for organophosphorous degradation due to its practical applicability in drinking water treatment.

When water is treated with an AOP, those contaminants with sufficiently high second order rate constants for their reactions with $\cdot\text{OH}$ (typically $> 5 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$) will be oxidized and transformation products are formed. These products may or may not retain the parent compound's toxicity or could develop a new form of toxicity. The toxicity of the chemical products subsequent to a transformation based treatment process can be assessed using a technique known as effect-direct analysis (EDA) (Escher and Fenner, 2011). In an unknown water matrix with unknown transformation products, the toxic equivalents concept (TEQ) can be used to quantify toxicity. TEQ is calculated by dividing the concentration of the parent compound required to reach 50% of the maximum effect (EC_{50}) by the EC_{50} of the sample. The ratio of TEQ to its initial value (TEQ/TEQ_0) allows the evaluation of the changes in relative toxicity of the sample during degradation of the target compound (Mestankova et al., 2016). The

toxic equivalents concept expands to specific measurements of potential toxicity, such as the mutagenic equivalents concept (MEQ), measuring mutagenic potential and neurotoxic equivalents concept (NEQ), measuring potential neurotoxicity by acetylcholinesterase inhibition. The TEQ approach saves time and money on analytical techniques, and allows for a more comprehensive review of contaminant treatment by assessing mixtures of products formed. The objectives of this research were to determine the toxicity of four organophosphorous insecticides (acephate, dicrotophos, fenamiphos, and methamidophos) and, more importantly, assess the toxicity of their transformation products formed during UV/H₂O₂ AOP treatment.

Methods

Chemicals and analytical methods

All compounds were analytical grade, (Sigma-Aldrich, MO, USA), and were quantified using an Agilent HPLC ion trap mass spectrometer (MS) with an Agilent Eclipse XDB-C₈ column, following US EPA standard method 538 (detection limit 17-34 ng/L) including verification of blanks and positive controls. The standard deviations of the HPLC measurements were <3%. The HPLC mobile phases A and B were water with 1% formic acid and acetonitrile, respectively. The mobile phase eluent gradient started with 30% eluent B, followed by a 5-min linear gradient to 70% B, additional 2-min gradient to 100% B and a 2-min gradient back to 30% B, maintained for 4 min for equilibration.

Experimental Setup

UV/H₂O₂ experiments employed a quasi-collimated beam reactor, with four low-pressure (LPUV) lamps (ozone free, 15 watt, #G15T8) housed above a 4 inch aperture equipped with a manual shutter. As the UV lamps are mounted at a considerable distance away from the exposed

solution (placed in a lower chamber), the light beam reaching the sample can be considered to be quasi collimated. Each lamp emitted radiation principally at 254 nm, and incident irradiance was measured at a wavelength of 254 nm with a calibrated radiometer (International Light Inc., Model 1700/SED 240/W). In a typical quasi collimated beam apparatus, The UV fluence (i.e., dose) was calculated from the incident irradiance measurement, exposure time, depth of sample and water quality characteristics (Bolton and Linden, 2003). Fluence levels of 0 to 1000 mJ/cm² were used. Contaminants were dosed in deionized water (MilliQ resistance 18.2 MΩ·cm) at a concentration of 1 mg/L, hydrogen peroxide was added at a concentration of 5 mg/L, sample solutions were irradiated at 25.0 ± 0.5 °C, and buffered at a pH of 7.2 ± 0.1.

Yeast Estrogen Screen (YES)

The yeast estrogen screen (YES) bioassay was used to quantify the estrogenicity of the AOP treated organophosphorous insecticides (Routledge and Sumpter, 1996, Mestankova et al., 2016). The test is based on a recombinant strain of the yeast *Saccharomyces cerevisiae*, which contains a reporter gene for the human estrogen receptor hER and expression plasmids carrying the reporter gene *lac-Z* encoding the enzyme β-galactosidase. β-galactosidase releases chlorophenol red from the chromogenic substrate chlorophenol red-β-D-galactopyranoside (CPRG). A color change was measured photometrically, using a Biotek Epoch plate reader (VT, USA).

Ames mutagenicity assay

The Ames II test (*Salmonella*/microsome mutagenicity assay) uses *Salmonella typhimurium* strains with engineered mutations, which render the bacteria unable to synthesize histidine. The lack of histidine does not allow the bacteria the ability to reproduce without an external histidine source. A gene mutation, caused by an external source such as a chemical pollutant, can restore the gene function (reverse mutation) and allows the bacteria to produce histidine. TA98,

measuring frameshift mutation, and TAMix, comprised of a mixture of six strains and measuring point mutations were used in this study, following ISO 11350 (Mestankova et al., 2016). The test was considered valid if the percentage of revertant wells in the negative controls was lower than 10% (from 48 wells), and the positive controls caused $\geq 80\%$ revertants in the positive wells. Bacteria strains (TA98, TAMix) and S9 rat liver enzyme were obtained from Xenometrix (Switzerland). The S9 rat liver enzyme was added alongside of the chemical before the initial incubation, in the presence of a histidine-rich indicator in order to mimic liver function activity, inducing metabolism of compounds.

Acetylcholinesterase inhibition assay

The AChE inhibition assay measures the kinetic inhibition of the enzyme acetylcholinesterase. The level of neurotoxicity of the mixtures is proportional to the time-based inhibition of the production of acetylcholinesterase. Experiments were performed in a 96 well plate, according to a well referenced standard method by Ellman et al. (1961). Lyophilized Electric Eel enzyme (AChE 0.26U/mg) was used, and the enzyme activity was assessed to ensure validity. Solutions of paraoxon and parathion were used as positive controls for the experiments, with concentrations ranging from 1.82×10^{-6} to 4×10^{-4} M used in a 96 well plate. Positive controls were made in ethanol, and pipetted into a 96 well plate, and the ethanol was allowed to evaporate. Samples were then redissolved in 50 μ L of 0.05 M phosphate buffer at pH 7. Five μ L of *N*-bromosuccinimide (NBS), (Sigma-Aldrich, 99%) was used to provide bromination of the contaminants in question, as an alternative type of oxidation (other than hydroxyl radicals) that could lead to a toxic byproduct (Lee et al., 2002). After bromination, the samples were thoroughly mixed on a shaker table for 2 minutes, and 5 μ L of ascorbic acid (Sigma-Aldrich, 99%) was added to all wells, and the plates were shaken again for 1 minute. Samples that were

not exposed to bromination were treated identically, but without the addition of NBS. A 40 μ L aliquot of acetylcholinesterase enzyme solution was added into every well and placed on the shaker table for thorough mixing for 10 minutes. After this time, 40 μ L of acetylthiocholine solution (Sigma-Aldrich, 98%) and dithionitrobenzoic acid solution (DTNB) (Sigma-Aldrich, 99%) were added to each well, plates were mixed for 1 minute on a shaker table and immediately read on a plate reader (Biotek Epoch, VT, USA) for absorbance at 420 nm for 10 minutes each at 30 second intervals.

Toxic equivalents concept (TEQ)

If the parent compound dominates the overall toxicity, the disappearance of the parent compound is directly proportional to the decrease in the relative TEQ (TEQ/TEQ_0) of the mixture and no further action involving the identification of the transformation products would be warranted. In contrast, a deviation from the parent compound degradation proportionality indicates the formation of toxic transformation products, and the EDA would then be expanded to identify the toxic compounds (Biran et al., 2010).

Results and Discussion

Kinetic Data

The second order rate constants for the reactions of all the examined insecticides with \cdot OH radical are $\geq 5.5 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$ (Mestankova et al., 2016), indicating that they are all expected to be significantly degraded in a water matrix treated with an AOP. In contrast, the second order rate constants for the reactions of this class of compounds with ozone are very low ($\leq 0.65 \text{ M}^{-1}\text{s}^{-1}$, Mestankova et al. 2016). In addition, only marginal degradation of acephate, dicrotophos and

methamidophos were observed through direct photolysis. Some direct photolysis could be seen for fenamiphos; however, it did not result in any toxicity increase

Estrogenic Activity

Figure 1 shows the estrogenic activity observed while using the YES assay during the $\cdot\text{OH}$ radical-based abatement of the four selected insecticides (using UV/ H_2O_2). All insecticides showed a negative response (i.e., no estrogenic activity) in comparison to the positive control, for estrogenic activity.

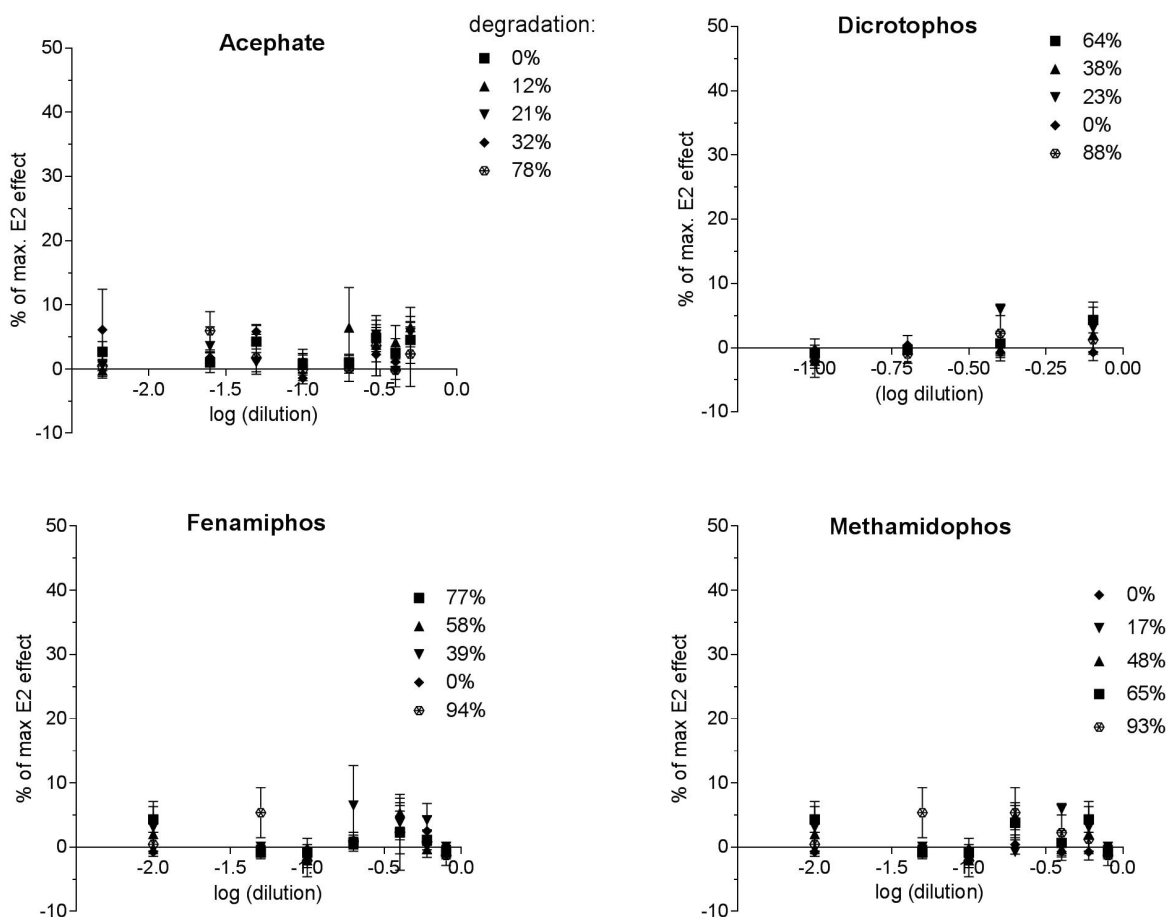


Figure 1. Estrogenic activity (YES assay) presented as % of maximum effect of the reference compound estradiol (E2), measured in triplicate during abatement of insecticides by hydroxyl radical in the AOP UV/ H_2O_2 .

While no estrogenic activity was noted with the YES assay for the parent compound and none developed from the transformation products, estrogenic activity has been observed previously for several organophosphorous insecticides, using alternative estrogenic assays (Raun Andersen et al., 2002).

Mutagenic Activity

Table 2 presents a summary of the data describing the mutagenic activity of all four insecticides. All four compounds showed at least one positive response to the various strains used in the AMES assay, with and without metabolic activation. The AMES test was utilized in a more qualitative comparative sense. A compound was considered mutagenic if the sample exhibited greater than 2 times the number of revertant wells as compared to the negative controls, where the number of revertant wells in the negative control indicates the extent of spontaneous mutation.

Table 2. Mutagenicity results of acephate, dicotophos, methamidophos, and fenamiphos using the AMES assay with and without S9 rat liver enzyme, following 90% abatement by $\cdot\text{OH}$ (adpted from Mestankova et al. 2016).

strains:	TA98 (Frameshift)		TAMix (Base-pair substitution)	
metabolic activation:	-S9	+S9	-S9	+S9
Acephate	+↘-?	-→-	-→-	-→-
Dicotophos	-→-	-→-	+↘-?	-→-
Methamidophos	-→-	+↗+	-→-	-→-
Fenamiphos	-→-	-→-	+↘-	-→-

+/- Positive/negative response from the AMES assay

-→- Parent compound activity was negative, and after treatment, sample was negative.

+↘- Parent compound activity was positive, and after treatment, activity decreased proportional to parent compound.

+↘-? Parent compound displayed weak activity, and activity decreased with treatment, but was too weak to conclusively determine.

+↗+ Parent compound activity was positive, and after treatment, activity increased.

Acephate showed a low positive initial response in the TA98 strain, without the addition of metabolic activation by the S9 rat liver enzyme, and this response decreased with increasing AOP treatment (increasing UV dose and $\cdot\text{OH}$ exposure). Dicotophos and fenamiphos caused positive responses in the TAMix combination strains without the addition of S9 in the parent compound form, and the response decreased with increasing extent of AOP treatment. Methamidophos showed a parent compound response with metabolic activation in the TA98 strain, and showed an increase in this response with increasing $\cdot\text{OH}$ exposure during AOP treatment.

Because of the observed unusual mutagenicity trend of methamidophos during AOP treatment, its mutagenic kinetics was further evaluated. The compound's mutagenicity (TA 98,

indicating a frameshift mutation in the presence of S9) first decreased with $\cdot\text{OH}$ radical induced degradation, then increased with increasing extent of advanced oxidation treatment (Figure 2).

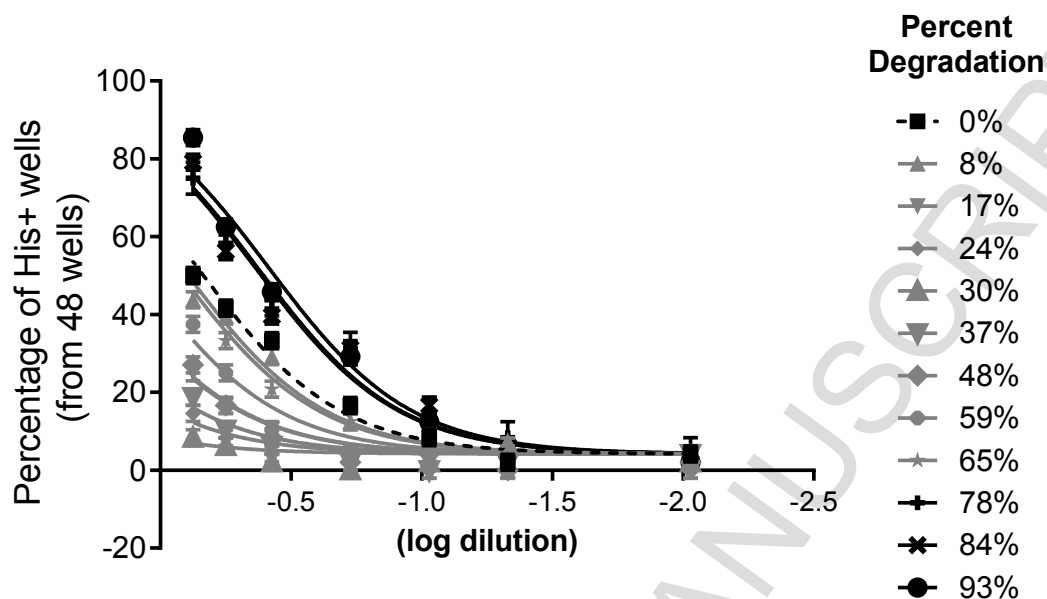


Figure 2. Mutagenicity measured in triplicate by the AMES bioassay (TA98 + S9) during abatement of Methamidophos. The dashed line indicates the parent compound response (0% abatement), solid gray lines (8 – 65% abatement) and solid dark lines (78, 84, and 93% abatement) indicate decreased and increased toxicity signals, respectively, due to transformation product formation.

In order to quantify the responses of the parent compound and oxidation products of methamidophos' abatement by $\cdot\text{OH}$, the MEQ approach was employed (Figure 3). The red line in Figure 3 indicates a stoichiometric toxicity signal degradation response if the mutagenicity is lost by the abatement of the parent compound. This indicates that no toxic oxidation products are formed from the AOP treatment. For $C/C_0 > 0.7$ the data points fall below the line for stoichiometric MEQ/MEQ₀ abatement (slope of -1). This could be caused by transformation products formed during the parent compound abatement, which reduce the number of the revertant wells. A similar effect was observed for the photolysis of *N*-nitrosamines in a previous study (Mestankova et al. 2014). For $C/C_0 < 0.4$, the AOP treatment of methamidophos led to a

mutagenicity increase above the line of the parent compound, indicating an increase in toxicity from transformation products, having an MEQ/MEQ₀ ratio of >1.

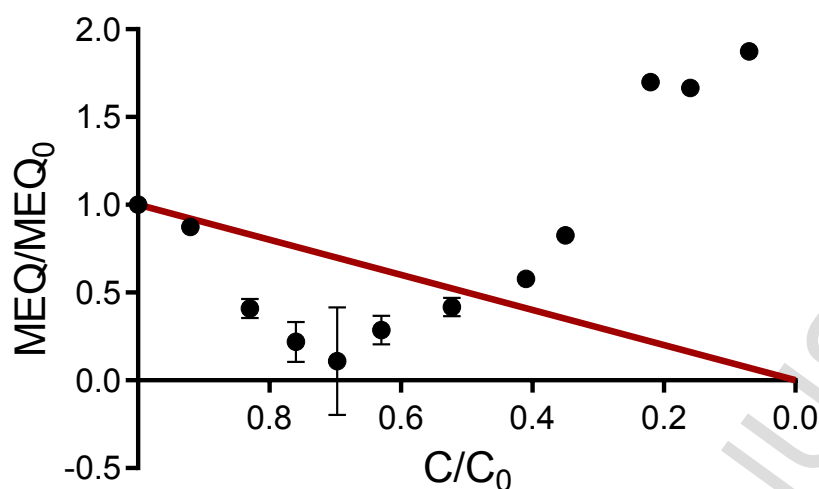
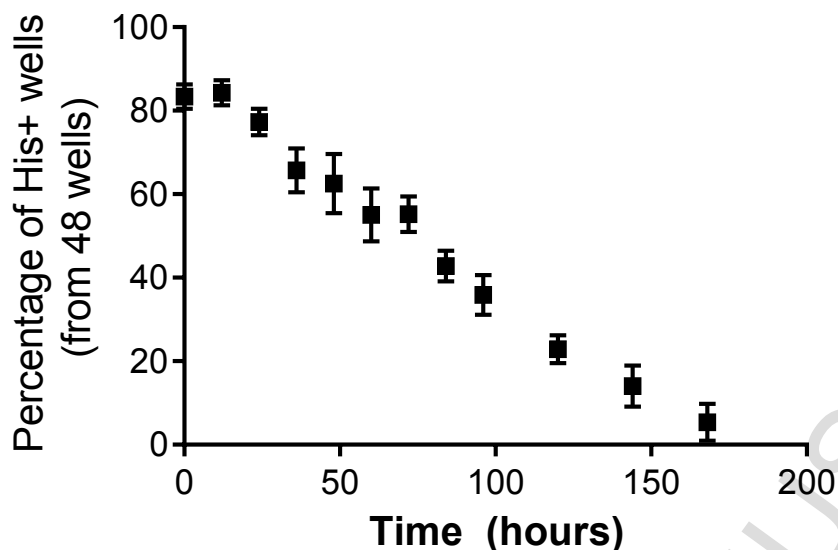


Figure 3. Mutagenic equivalents (MEQ) of methamidophos in TA98 strain in the presence of S9 measured in triplicate as a function of the abatement by $\cdot\text{OH}$. The solid line indicates an ideal response with relative toxicity correlating to the relative abatement of the parent compound concentration. The X-axis indicates the relative abatement of the parent compound by showing the fraction remaining. Note the reverse axis values.

Identification of the transformation products responsible for this increase in toxic response in the AMES assay was not successful, using various solid and liquid-phase extractions combined with gas and liquid chromatography analytical identification methods. Overall, our analytical procedure did not provide sufficiently solid results and hence, we cannot draw any conclusions regarding the identity of the toxic product. During 170 hours following AOP treatment, the observed toxicity response decreased gradually to its original level, suggesting a further transformation, potentially a hydrolysis of the mutagenic primary transformation products (Figure 4). It should be emphasized that methamidophos did not undergo direct photolysis (data not shown); hence the transformation product is undoubtedly the result of $\cdot\text{OH}$ reaction.

237



238

239 **Figure 4.** Evolution of the relative mutagenicity measured in duplicate by the AMES bioassay
 240 (strain TA98 + S9 enzyme) of methamidophos treated with hydroxyl radicals to 90% degradation
 241 in a laboratory water matrix. A decrease in toxicity over time due to further transformation
 242 product degradation can be observed.

243

244 *Neurotoxic Activity*

245 The neurotoxic activity of most of the organophosphorous compounds tested could be detected
 246 by the AChE assay. Figure 5 shows the neurotoxic equivalents for dicrotophos, fenamiphos and
 247 methamidophos, as measured by AChE inhibition, before and after treatment with the AOP
 248 UV/H₂O₂ with or without bromination of the oxidation products using NBS (Lee et al., 2002).

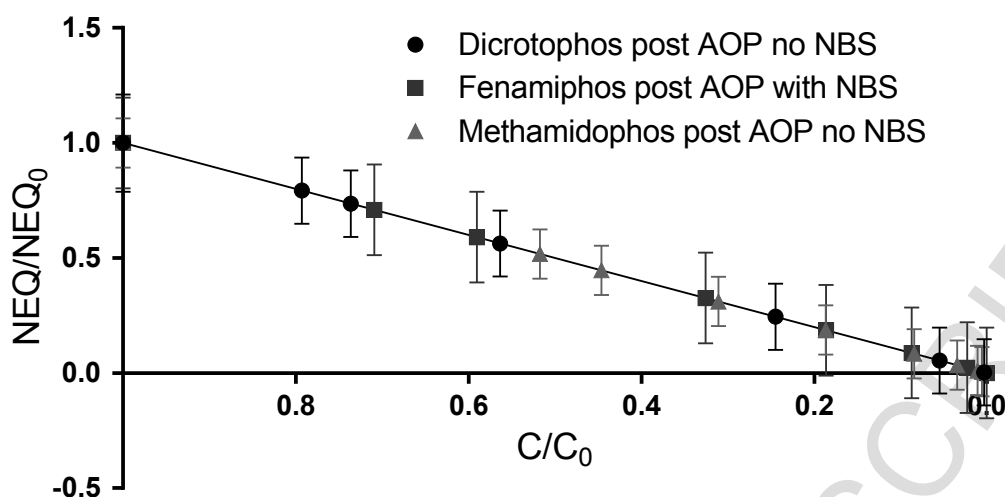


Figure 5. Relative acetylcholinesterase inhibition measured in triplicate, expressed as neurotoxic equivalents (NEQ), of dicrotophos, fenamiphos, and methamidophos with or without NBS in the AChE assay, with the solid line indicating ideal stoichiometric abatement of the effects (slope = -1).

Dicrotophos and methamidophos both showed a positive response in the assay without the additional bromination, and the neurotoxicity decreased proportionally to that of the parent compound during treatment with the AOP UV/H₂O₂. Fenamiphos has previously been shown to exhibit a positive response in the AChE assay (Worek et al., 2004), which is consistent with these results. The response decreased with increasing oxidative treatment with the AOP UV/H₂O₂, proportionally to the abatement of the parent compound. Acephate was not responsive in our AChE assay either before or after AOP treatment, with and without the addition of NBS.

All organophosphorous insecticides that exhibited a positive parent compound neurotoxicity response showed that the UV/H₂O₂ was effective for decreasing the neurotoxicity potential measured by the AChE activity. No new toxicity, compared to the parent compound, was detected in the AChE assay following treatment with the AOP UV/H₂O₂. Overall, treatments with the AOP UV/H₂O₂ are effective for destroying the neurotoxic activity (measured by AChE) of the selected organophosphorous insecticides.

Conclusions

It is well accepted that AOPs, while often effective in the degradation of water contaminants, may produce a large number of transformation products. These products are difficult to identify (due to their large amount and low concentration) and may occasionally be more toxic than the parent compound. In this paper we showed that UV-based AOP effectively degraded four organophosphorous insecticides. In addition, we used effect-directed analysis and various toxicity tests to evaluate the toxicity of the transformation products. Results showed that in most cases toxicity of the treated sample decreased with degradation of the parent compound, implying that the generated transformation products were not toxic. A different trend was observed in the case of methamidophos; where, genotoxicity (using the AMES II assay) of the sample decreased in the first stage of the treatment, and then increased with increasing extent of degradation. This trend may indicate the formation of toxic transformation products during treatment, although none could be identified. Further investigation showed that the sample response slowly decreased with time, likely due to the hydrolysis of the toxic transformation products. The results of this study suggest that while AOPs are efficient in removing many organic water contaminants, they may generate toxic oxidation products which warrant subsequent treatment (such as biofiltration). The results are particularly interesting in view of the recently revised California DPH Groundwater Replenishment Reuse regulations (CDPH, 2011), which recommends AOP as a final process in full-advanced-treatment facilities, treating wastewater for indirect potable reuse.

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References

- Bethi, B., Sonawane, S. H., Bhanvase, B. A. & Gumfekar, S. P. 2016. Nanomaterials-based advanced oxidation processes for wastewater treatment: A review. *Chemical Engineering and Processing* 109, 178–189.
- Biran, A., Yagur-Kroll, S., Pedahzur, R., Buchinger, S., Reifferscheid, G., Ben-Yoav, H., Shacham-Diamand, Y. & Belkin, S. 2010. Bacterial genotoxicity bioreporters. *Microbial Biotechnology*, 3, 412-427.
- Bolton, J. R. & Linden, K. G. 2003. Standardization of Methods for Fluence (UV Dose) Determination in Bench-Scale UV Experiments. *Journal of Environmental Engineering*, 129, 209-215.
- Bradwell, R. H. 1994. Psychiatric Sequelae of Organophosphorous Poisoning - a Case-Study and Review of the Literature. *Behavioural Neurology*, 7, 117-122.
- Braun, A. M. & Oliveros, E. 1997. How to evaluate photochemical methods for water treatment. *Water Science and Technology*, 17-23.
- Buchanan, I., Liang, H. C., Khan, W., Liu, Z. K., Singh, R., Ikehata, K. & Chelme-Ayala, P. 2009. Pesticides and Herbicides. *Water Environment Research*, 81, 1731-1816.
- Ellman, G. L., Courtney, K. D., Andres Jr, V. & Featherstone, R. M. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, 7, 88-90, IN1, 91-95.
- Elovitz, M.S., U.v. Gunten, and H.P. Kaiser, 2000. Hydroxyl radical/ozone ratios during ozonation processes. II. The effect of temperature, pH, alkalinity, and DOM properties. *Ozone Science and Engineering*, 22: p. 123-150.
- Elovitz, M.S., et al., 2008. Hydroxyl radical rate constants: comparing UV/H(2)O(2) and pulse radiolysis for environmental pollutants. *Journal of Water Supply Research and Technology-Aqua*, 57(6): p. 391-401.
- Escher, B. I., Bramaz, N., Mueller, J.F., Quayle, P., Rutishauser, S., Vermeirssen, E., 2008. Toxic equivalent concentrations (TEQs) for baseline toxicity and specific modes of

- action as a tool to improve evaluation of ecotoxicity tests on environmental samples. *J. Environ. Monit.*, 10,, 612-621.
- Escher, B. I. & Fenner, K. 2011. Recent Advances in Environmental Risk Assessment of Transformation Products. *Environmental Science & Technology*, 45, 3835-3847.
- Gromov, A. E. & Rozengart, V. I. 1976. Changes in Microcirculation during Poisoning by Organophosphorous Cholinesterase-Inhibitors. *Bulletin of Experimental Biology and Medicine*, 81, 33-35.
- Hoigné, J. and H. Bader, 1983. Rate constants of reactions of ozone with organic and inorganic compounds in water--I: Non-dissociating organic compounds. *Water Research*, 17(2): p. 173-183.
- Huber, M. M., Canonica, S., Park, G.-Y. & von Gunten, U. 2003. Oxidation of Pharmaceuticals during Ozonation and Advanced Oxidation Processes. *Environmental Science & Technology*, 37, 1016-1024.
- Huber, M. M., Ternes, T. A. & von Gunten, U. 2004. Removal of Estrogenic Activity and Formation of Oxidation Products during Ozonation of 17 α -Estradiol. *Environmental Science & Technology*, 38, 5177-5186.
- Keen, O.S., Love, N.G., Aga, D.S. & Linden, K.G. 2016. Biodegradability of iopromide products after UV/H₂O₂ advanced oxidation. *Chemosphere*, 144, 989-994.
- Lee, H. S., Kim, Y. A., Cho, Y. A. & Lee, Y. T. 2002. Oxidation of organophosphorus pesticides for the sensitive detection by a cholinesterase-based biosensor. *Chemosphere*, 46, 571-576.
- Lee, Y., Escher, B. I. & von Gunten, U. 2008. Efficient Removal of Estrogenic Activity during Oxidative Treatment of Waters Containing Steroid Estrogens. *Environmental Science & Technology*, 42, 6333-6339.
- Lester, Y., Love, N., Singh, R., Aga, D. & Linden, K.G. 2016. Advanced oxidation and biofiltration: Integrative method for treating trace contaminants and oxidation products in wastewater. *Water Environment Research*, 88, 1985-1993.
- Mestankova, H., Schirmer, K., Canonica, S. and von Gunten, U. (2014) Development of mutagenicity during degradation of N-nitrosamines by advanced oxidation processes. *Water Research* 66, 399-410.

- Mestankova, H., Parker, A. M., Bramaz, N., Canonica, S., Schirmer, K., von Gunten, U. & Linden, K. G. 2016. Transformation of Contaminant Candidate List (CCL3) compounds during ozonation and advanced oxidation processes in drinking water: Assessment of biological effects. *Water Research*, 93, 110-120.
- Raun Andersen, H., Vinggaard, A. M., Høj Rasmussen, T., Gjermansen, I. M. & Cecilie Bonefeld-Jørgensen, E. 2002. Effects of Currently Used Pesticides in Assays for Estrogenicity, Androgenicity, and Aromatase Activity in Vitro. *Toxicology and Applied Pharmacology*, 179, 1-12.
- Routledge, E. J. & Sumpter, J. P. 1996. Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen. *Environmental Toxicology and Chemistry*, 15, 241-248.
- Shemer, H., et al., 2006. Relative Rate Constants of Contaminant Candidate List Pesticides with Hydroxyl Radicals. *Environmental Science & Technology*, 2006. 40(14): p. 4460-4466.
- USEPA, November 17, 2016. Drinking Water Contaminant Candidate List 4. <https://www.epa.gov/ccl/chemical-contaminants-ccl-4>
- Wadia, R. S., Sadagopa.C, Amin, R. B. & Sardesai, H. V. 1974. Neurological Manifestations of Organophosphorous Insecticide Poisoning. *Journal of Neurology Neurosurgery and Psychiatry*, 37, 841-847.
- Worek, F., Thiermann, H., Szinicz, L. & Eyer, P. 2004. Kinetic analysis of interactions between human acetylcholinesterase, structurally different organophosphorus compounds and oximes. *Biochemical Pharmacology*, 68, 2237-2248.