Chemosphere 182 (2017) 477–482

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

UV/H_2O_2 advanced oxidation for abatement of organophosphorous pesticides and the effects on various toxicity screening assays

Austa M. Parker [a], Yaal Lester [c], Emily K. Spangler [a], Urs von Gunten [b, d], Karl G. Linden [a, *]

[a] Department of Civil, Environmental, and Architectural Engineering, University of Colorado Boulder, Boulder CO 80309, USA
[b] Eawag, Swiss Federal Institute of Aquatic Science and Technology, Überlandstrasse 133, P.O. Box 611, CH-8600 Dübendorf, Switzerland
[c] The Water Research Center, Tel Aviv University, 69978 Tel Aviv, Israel
[d] School of Architecture, Civil and Environmental Engineering (ENAC), Ecole Polytechnique Federale de Lausanna (EPFL), CH-1015 Lausanne, Switzerland

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.

ARTICLE INFO

Article history:
Received 15 December 2016
Received in revised form
25 April 2017
Accepted 30 April 2017
Available online 2 May 2017

Handling Editor: Shane Snyder

Keywords:
Organophosphorous insecticides
Estrogenicity
Genotoxicity
Neurotoxicity
Advanced oxidation
UV/H_2O_2
Transformation products

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/H_2O_2 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, dicrotophos, fenamiphos, and methamidophos. All four compounds were fast reacting with •OH, all reacting with second order rate constants $\geq 5.5 \times 10^9$ $M^{-1}s^{-1}$. 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 one case. Methamidophos degradation by UV/H_2O_2 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.

1. 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

* Corresponding author.
E-mail address: karl.linden@colorado.edu (K.G. Linden).

http://dx.doi.org/10.1016/j.chemosphere.2017.04.150
0045-6535/

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 •OH radical, and not by a phase transfer mechanism like sorption or air stripping (Huber et al., 2003, 2004; Lee et al., 2008; Keen et al., 2016; Bethi et al., 2016; Lester et al., 2016). Several types of advanced oxidation treatment including O_3/H_2O_2, UV/H_2O_2, UV/O_3 and UV/TiO_2 are available for water treatment (Braun and Oliveros, 1997). UV/H_2O_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 •OH (typically $> 5 \times 10^8$ $M^{-1}s^{-1}$) will be oxidized and transformation products are formed. These products may or may not retain the parent compound's effect 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-directed 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 (Escher et al., 2008). 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 an evaluation of the changes in relative toxicity of the sample during degradation of the target compound (Mestankova et al., 2016). The TEQ 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/H_2O_2 AOP treatment.

2. Methods

2.1. 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-C_8 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.

2.2. Experimental setup

UV/H_2O_2 experiments employed a quasi-collimated beam reactor, with four low-pressure (LP) UV lamps (ozone free, 15 W, #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). 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 0–1000 mJ/cm^2 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.

2.3. 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).

2.4. 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 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 ≥ 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.

2.5. 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