Transformation of Contaminant Candidate List (CCL3) compounds
during ozonation and advanced oxidation processes in drinking water:
assessment of biological effects

Hana Mestankova^{1}, Austa M. Parker^{2*}, Nadine Bramaz¹, Silvio Canonica¹, Kristin Schirmer^{1,3,4},
Urs von Gunten^{1,3,4^}, and Karl G. Linden^{2^}*

¹ Eawag, Swiss Federal Institute of Aquatic Science and Technology, Überlandstrasse 133, P.O.
Box 611, CH-8600 Dübendorf, Switzerland

² Department of Civil, Architectural, and Environmental Engineering, University of Colorado
Boulder, Boulder, Colorado 80309, United States

³ School of Architecture, Civil and Environmental Engineering (ENAC), Ecole Polytechnique
Fédérale de Lausanne (EPFL), GR A1 465, Station 2, CH-1015 Lausanne, Switzerland

⁴ Institute of Biogeochemistry and Pollutant Dynamics, ETH Zentrum, CH-8092 Zürich,
Switzerland

* Co-primary authors contributed equally to this work.

^ Corresponding Authors: vongunten@eawag.ch and karl.linden@colorado.edu

ABSTRACT

The removal of emerging contaminants during water treatment is a current issue and various technologies are being explored. These include UV- and ozone-based advanced oxidation processes (AOPs). In this study, AOPs were explored for their degradation capabilities of 25 chemical contaminants on the US Environmental Protection Agency's Contaminant Candidate List 3 (CCL3) in drinking water. Twenty-three of these were found to be amenable to hydroxyl radical-based treatment, with second order rate constants for their reactions with hydroxyl radicals ($\cdot\text{OH}$) in the range of $3\text{--}8 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$. The development of biological activity of the contaminants, focusing on mutagenicity and estrogenicity, was followed in parallel with their degradation using the Ames and YES bioassays to detect potential changes in biological effects during oxidative treatment. The majority of treatment cases resulted in a loss of biological activity upon oxidation of the parent compounds without generation of any form of estrogenicity or mutagenicity. However, an increase in mutagenic activity was detected by oxidative transformation of the following CCL3 parent compounds: nitrobenzene ($\cdot\text{OH}$, UV photolysis), quinoline ($\cdot\text{OH}$, ozone), methamidophos ($\cdot\text{OH}$), *N*-nitrosopyrrolidine ($\cdot\text{OH}$), *N*-nitrosodi-*n*-propylamine ($\cdot\text{OH}$), aniline (UV photolysis), and *N*-nitrosodiphenylamine (UV photolysis). Only one case of formation of estrogenic activity was observed, namely, for the oxidation of quinoline by $\cdot\text{OH}$. Overall, this study provides fundamental and practical information on AOP-based treatment of specific compounds of concern and represents a framework for evaluating the performance of transformation-based treatment processes.

KEYWORDS: Ultraviolet; ozone; hydroxyl radical; mutagenicity; estrogenicity

INTRODUCTION

Advanced Oxidation Processes

The presence of micropollutants in water resources is an issue of concern in the drinking water industry (Schwarzenbach et al. 2006). To keep their concentrations in drinking waters as low as possible, treatment processes for removal of micropollutants based on either separation (e.g., membranes, activated carbon) or transformation (e.g., oxidation) may be employed (Virkutyte et al. 2010). To this end, conventional and advanced oxidation processes (AOPs) have been demonstrated to be able to degrade the majority of micropollutants (Huber et al. 2003, Rosario-Ortiz et al. 2010, Lee and von Gunten 2010). During oxidation, micropollutants are typically not mineralized and transformation products are formed, often with similar structures to the parent compounds (von Sonntag and von Gunten 2012). Therefore, an assessment of the success of such processes should not only be based on a measurement of the abatement of parent compounds but also on an assessment of the remaining effects and even the potential formation of new biological activity (von Sonntag and von Gunten 2012).

Conventional ozonation and AOPs are transformation-based treatment processes which rely on the *in situ* production of the hydroxyl radical ($\cdot\text{OH}$) that can quickly react with a wide range of micropollutants (Buxton et al. 1988). The most commonly used AOPs in water treatment are the combination of ozone with H_2O_2 and UV with H_2O_2 . Ozone- and UV-based AOPs have both been utilized to provide treatment of regulated and emerging contaminants at full-scale facilities (Virkutyte et al. 2010). In addition to the $\cdot\text{OH}$ driven oxidation processes, ozone-based AOPs include direct ozone reactions and UV-based AOPs also include an inherent direct photolysis component (Oppenlander 2007, von Sonntag and von Gunten 2012). Therefore,

in these cases the effect of direct ozone reactions or direct photolysis has to be considered as well.

Conventional ozonation and AOPs generally lead to the removal of the intended biological activity associated with micropollutants, as shown previously for estrogenic compounds, antibiotics, antiviral compounds and herbicides (Dodd et al. 2009, Lee et al. 2008, Mestankova et al. 2011, Mestankova et al. 2012, Rosenfeldt et al. 2007). However, in a few cases the oxidation of specific parent compounds leads to transformation products with similar or alternative modes of biological activity. For instance the oxidation of dimethylsulfamide, a harmless metabolite of the fungicide tolylfluanide, led to the formation of the mutagenic *N*-nitrosodimethylamine (NDMA) during ozonation of bromide- and dimethylsulfamide-containing waters (Schmidt and Brauch 2008, von Gunten et al. 2010). NDMA was also formed during ozonation of dyes containing dimethylamino functional groups (Yang et al. 2003). The identification of transformation products, with a subsequent evaluation of their biological effects for an overall risk assessment of micropollutant abatement during AOPs, is a tedious and expensive process. Therefore, the application of biological assays to samples taken during degradation is a cost efficient and useful approach to elucidate the formation of transformation products with increased biological effects or new modes of action (Escher and Fenner, 2011).

US EPA Contaminant Candidate List

The US EPA's Contaminant Candidate List (CCL) includes compounds that are being explored for possible regulation. The 1996 Amendments to the Safe Drinking Water Act require the EPA to publish a revised CCL every 5 years (USEPA 1996). This process ends in a regulatory determination for some contaminants. The most recent CCL is the CCL3, which contains 104 chemical and 12 microbial contaminants (USEPA October 8, 2009). The CCL3 list

provides insight into potential future regulatory activity, and therefore is a helpful framework to design an emerging contaminant study.

In this study, 25 compounds from the 104 chemical contaminants on the US EPA CCL3 were selected for evaluation (see below for explanation, Table 1 and supporting information (SI) Table S1). The rate of oxidative transformation of selected CCL3 compounds was tested for oxidation by $\cdot\text{OH}$, ozone or UV photolysis by measuring the kinetics of the abatement of the parent compounds. The mixtures of transformation products formed during oxidation were then tested by bioassays for mutagenic and estrogenic activity. Contaminant degradation was followed using a parallel evaluation of abatement of the parent compound and biological activity, which enables the discovery of unknown transformation products of importance.

MATERIALS AND METHODS

Rationale of Contaminant Selection

104 chemicals from the CCL3 list were grouped into 8 classes (Table 1 and SI Table S1) based on their chemical structures and/or biological modes of action. The chemical structures and available information from literature enabled an estimate of the potential for oxidative transformation of the selected compounds. The criteria for the selection of 25 out of the 104 contaminants from the CCL3 were based on: (1) being treatable by AOP (i.e., high second order rate constant for the reaction with O_3 and/or $\cdot\text{OH}$), (2) likelihood of being in the AOP influent (i.e., low octanol-water partition coefficient (K_{ow}) relative to other compounds in the class), (3) established analytical methods, and (4) regulatory relevance (i.e., based on health and occurrence data from the US EPA) (USEPA October 8, 2009). Halogenated compounds (group 1) were excluded from this study because they generally have low second order rate constants for the reaction with ozone and $\cdot\text{OH}$ (von Gunten 2003). Olefines (group 2) were selected based on their

high reactivity towards ozone and $\cdot\text{OH}$ (Onstad et al. 2005, von Sonntag and von Gunten 2012). Nitro compounds (group 3) typically have low reactivities with ozone, however, depending on other moieties of the structure they may be efficiently transformed by $\cdot\text{OH}$. Furthermore, nitro compounds are toxic and therefore interesting compounds for an assessment of biological effects during oxidative transformation (Cronin et al. 1998, Shinoda et al. 1998). Ethers, alcohols, phenols, aldehydes and other oxygen containing compounds (group 4) were chosen based on high rate constants for the reaction with $\cdot\text{OH}$, thus likely to be candidates for treatment by AOPs (Buxton et al. 1988, Stefan and Bolton 1998). The selected nitrogen-containing compounds (group 5) were expected to have high reactivities with $\cdot\text{OH}$, and in some cases (anilines) high reactivity with ozone. Organophosphorous compounds (group 6) were expected to react readily with $\cdot\text{OH}$ and possibly lead to toxicologically relevant degradation products (Nordmeyer and Dickson 1990). Amides (group 7) were also selected based on their reactivity towards $\cdot\text{OH}$ (de Laat et al. 1996). *N*-nitroso compounds (group 8) were selected based on their known biological effects (Magee and Barnes 1967, Mestnakova et al. 2014) and their potential to be photolysed (Sharpless et al. 2003, Plumlee and Reinhard 2007). Metalorganic compounds were not selected in the current study because they are expected to be mostly adsorbed to organic particles and sediments based on their high K_{ow} values (Gschwend and Wu 1985).

Procedure for the Assessment of the Selected CCL3 Compounds

Figure 1 outlines the experimental procedure for this study, with the following goals: (1) Assessment of the feasibility of treatment for the selected CCL3 compounds by oxidation processes, (2) determination of the removal of potential biological effects of the parent compounds and (3) investigation of the potential formation of toxic degradation products with similar or different modes of action than the parent compounds.

The evaluation of second-order rate constants for the reaction of the selected micropollutants with ozone and $\cdot\text{OH}$ (and the efficiency of photolysis) provided a framework for selecting compounds for further study. Compounds with second order rate constants for the reaction with $\cdot\text{OH}$ below $5 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ were not selected because a very low removal efficiency for such compounds can be expected in AOPs. Therefore, AOPs would not be a treatment process of choice, except for *N*-nitrosamines, which also undergo efficient transformation via direct photolysis (Sharpless et al. 2003). The selected compounds underwent biological effects testing following oxidative transformation, including both mutagenicity (Ames II) and estrogenicity (YES) tests. Overall, 45 individual treatment scenarios (via $\cdot\text{OH}$, ozone, or direct UV photolysis) were evaluated across the compounds with both bioassays. The details of the treatments applied to each chemical are presented in SI, Table S2.

Figure 1 here

Oxidation Experiments with Hydroxyl Radical and Ozone

Each compound was evaluated individually in unique experiments. All chemicals used as the parent compounds were standards of analytical grade of $\geq 99\%$ purity (suppliers and purity indicated in SI, Table S1). All aqueous solutions were prepared using deionized water obtained from a Milli-Q water purification system (Millipore). Chemicals used for preparation of media or reaction mixtures were $\geq 95\%$ purity. Oxidation experiments for compounds from groups 3, 5, and 7 were carried out as previously described (Mestankova et al. 2011). Hydroxyl radicals for the degradation of the parent compounds in aqueous solutions ($\text{pH} = 7$, 5 mM phosphate buffer) were generated in systems based on the photolysis of hydrogen peroxide (1 mM, 34 mg/L or higher) by a medium pressure mercury vapor UV lamp filtered to emit between 308-410 nm to minimize UVC direct photolysis. The initial concentration of the parent compound was typically

100 μM , where solubility permitted. Oxidation experiments for compounds from groups 1, 2, 4, and 6 were carried out based on photolysis of hydrogen peroxide by a low pressure UV mercury vapor lamp (near-monochromatic at 254 nm: LPUV/ H_2O_2) in a quasi-collimated beam system. Four LPUV lamps (ozone free, 15 W, General Electric #G15T8) were housed above a 4-inch aperture equipped with a manual shutter. Incident UV irradiance was measured by a calibrated radiometer (International Light Inc., Model 1700/SED 240/W). UV fluence (i.e. UV dose) was calculated by multiplying the average irradiance by the exposure time in seconds including factors based on Bolton and Linden (2003).

Ozonation experiments were carried out with solutions containing 100 μM or lower concentrations of the parent compound at pH 7 (5 mM phosphate) and in the presence of 10 mM *t*-butanol to scavenge $\cdot\text{OH}$ such that ozone was the only oxidant. The solutions were stirred in borosilicate glass vials and spiked with various volumes of an ozone stock solution via a gas tight glass syringe to achieve the desired ozone dose and extent of degradation (Mestankova et al. 2011). An ozone stock solution (~ 1.3 mM) was produced by sparging O_3 -containing oxygen through ice-cooled Milli-Q water (Bader and Hoigné 1981).

Direct Photolysis Experiments

Direct photodegradation experiments were performed using near-monochromatic light at 254 nm (Mestankova et al. 2011). More details are given in SI, Text S1.

Analytical Methods

Samples from degradation experiments were analyzed by HPLC with UV detection, HPLC-MS, or GC-MS (SI, Table S1 for specific analytical methods). Standard analytical methods were used, and parameters including compound purity and column sizing are given in

the SI, Table S1. Quantification of the parent compound and both bioassays were performed within 12 hours of parent compound degradation. The standard deviations of the HPLC measurements were < 3%, with quantification limits < 1 µM.

Determination of Rate Constants

Second order rate constants for the reaction of $\cdot\text{OH}$ with the selected contaminants (k_{OH}) were measured in systems based on the photolysis of hydrogen peroxide by competition kinetics with *p*-chlorobenzoic acid (pCBA) as a competitor ($k_{\text{OH}} = 5.0 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$) (Elovitz et al. 2000, Huber et al. 2003, von Sonntag and von Gunten 2012). Second-order rate constants for the reaction of fast-reacting compounds with ozone (k_{O_3}) were determined by competition kinetics with cinnamic acid ($k_{\text{O}_3} = 3.8 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$ for pH 7 – 8 (Leitzke et al. 2001)). Competition kinetics experiments were performed in duplicate by plotting $\ln([C]/[C]_0)$ ($[C]$: concentration of target compound) versus $\ln([\text{competitor}]/[\text{competitor}]_0)$, which allows a calculation of the unknown rate constant from the slope of the straight line of such a graph (Elovitz et al. 2000, Elovitz and von Gunten 1999, Huber et al. 2003, von Sonntag and von Gunten 2012). In the case of compounds reacting slowly with ozone, k_{O_3} values were determined in excess of either ozone or the target compound at pH 2 under pseudo-first order conditions (Hoigné and Bader 1983a, von Sonntag and von Gunten 2012). The excess of ozone or target compound, respectively, was ≥ 20 . The ozone concentration during the experiments was measured by the indigo method (Bader and Hoigné 1981).

Bioassays

The Ames (*Salmonella*/microsome mutagenicity assay) test is a widely accepted bacterial reverse mutation assay. It is used worldwide as a screening assay, and has been recognized for its

180 ease of use, rapidness, broad applicability, high sensitivity and reproducibility. The correlation
181 between mutagenicity and carcinogenicity has been widely evaluated because carcinogenicity is
182 of utmost human health concern and can result from mutagenicity. Original data in the 1970's
183 showed a high correlation between carcinogenicity and mutagenicity detected by the Ames test
184 (90% from 174 chemicals tested, including almost all of the known human carcinogens of that
185 time (McCann et al. 1975)). A later study showed lower specificity for rodent carcinogens
186 (58%), and for the non-carcinogens the specificity was 73% (Ashby et al. 1989). Several
187 carcinogens are non-mutagenic; therefore, the Ames assay may underestimate carcinogenicity
188 due to the inability to measure the specific mode of carcinogenicity (Ashby et al. 1989). A
189 disadvantage of the Ames test is that prokaryotic cells differ from eukaryotic cells with respect to
190 uptake and metabolism. To test the metabolites of the chemicals, a crude preparation of enzymes
191 is obtained from the homogenized liver of rats (previously pre-treated with a substance for
192 enzyme induction). This preparation is known as the S9 fraction and with added enzyme
193 cofactors is called S9-mix. Addition of the S9-mix allows for the metabolization of AOP
194 transformation products to mimic further transformation in a living organism. The presence and
195 absence of the S9-mix indicates the metabolized and non-metabolized forms of the compound in
196 question, respectively. The Ames test was performed as described in ISO 11350 (ISO 2012).
197 Two mutations contained within bacteria were used in the Ames II assay: TAMix, which is
198 comprised of a mixture of six Salmonella strains (measuring base pair substitution), and TA98
199 (measuring frameshift mutations). Positive controls were used as the reference compounds (see
200 equation 1). 4-nitro-o-phenylenediamine (4-NODP) was used as a positive control for frameshift
201 mutations (TA98), and 2-nitrofurantoin (2-NF) for base pair substitutions (TAMix). 2-

aminoanthracene (2-AA) was used as the positive control for both frameshift and base pair mutations in the presence of the S9-mix. Additional details are given in SI, Text S2.

The recombinant yeast estrogen screen (YES) test was used to detect and quantify the estrogenicity in the treated samples (Routledge and Sumpter 1996). Under well controlled and proper experimental conditions the yeast-based YES assay is a sensitive and reproducible test across laboratories for the screening of compounds interacting with the estrogen receptor (Dhooge et al. 2006). It was reported that false negative rates were 22% and false positive 7% on the estrogenic agonisms (for 105 chemicals tested (Kolle et al. 2010)). The suitability of this assay for pre-screening of water bodies was evaluated (Brix et al. 2010) and no significant difference was found between two laboratories. A low coefficient of variation (<9%) was reported also for positively identified compounds for 3 laboratories in a previous study (Dhooge et al. 2006). The YES test was performed as described by Rutishauser et al. (Rutishauser et al. 2004) (for details see SI, Text S2).

Toxic Equivalent Concept (TEQ)

The mutagenicity/estrogenicity in the samples was analyzed based on the toxic equivalent (TEQ) concept (Escher et al. 2008), which was used in previous studies (Dodd et al. 2009, Lee et al. 2008, Mestankova et al. 2011). This approach is suitable for quantification of the biological activity of mixtures and allows an evaluation of the development of these effects due to degradation of target compounds. The biological activity of samples taken during degradation, containing the known parent compounds and unknown degradation products, can be directly compared with the extent of the parent compound degradation.

TEQ (or more specifically MEQ and EEQ for mutagenicity and estrogenicity, respectively) is calculated by dividing the equivalent concentration required to reach 50% of the

maximum effect (EC_{50}) of the reference compound (17 β -estradiol for EEQ and for MEQ positive controls of the test) by the EC_{50} of each sample (Eq. 1).

$$MEQ \text{ or } EEQ = \frac{EC50 \text{ (reference compound)}}{EC50 \text{ (sample)}} \quad (1)$$

A positive control for the bioassay is used as the reference compound. If the parent compound presents mutagenicity or estrogenicity, the MEQ or EEQ, respectively of each sample can be normalized to its initial value (MEQ_0 or EEQ_0) as a measure of the development of the specific activity during degradation of the parent compound (Eq. 2).

$$\frac{MEQ}{MEQ_0} \text{ or } \frac{EEQ}{EEQ_0} = \frac{EC50_0 \text{ (sample)}}{EC50 \text{ (sample)}} \quad (2)$$

If this ratio is plotted against the extent of degradation (C/C_0), in an ideal case the decrease of the parent compounds results in the corresponding stoichiometric decrease of the specific activity of the mixture of the transformation products. In this case, the degradation products do not show a significant biological response and these data are on a straight line with a slope of -1. If transformation products are causing a significant biological effect, a positive deviation from the “ideal case” is expected (points above the line with a slope of -1).

RESULTS AND DISCUSSION

Kinetic Data

Table 1 shows the selected compounds from the CCL3 with their measured and previously published second order rate constants for the reactions with ozone and $\cdot OH$.

Table 1. Rate constants for the reactions of the selected compounds from the CCL3 with ozone and $\cdot OH$. References to values from literature are given in brackets (superscripts), chemicals with rate constants in bold were studied for biological effects.

	$k_{O_3} (M^{-1}s^{-1})$	$k_{OH} (M^{-1}s^{-1})$
1. Halogenated compounds		
1,2,3 - Trichloropropane	0.87 ± 0.03	$< 5 \times 10^8$
2. Olefines		
Dimethipin	$(1.2 \pm 0.1) \times 10^5$	$(6.7 \pm 0.4) \times 10^9$
3. Nitro compounds		
Nitrobenzene	0.09 (Hoigné and Bader 1983a)	$3.9\text{-}4.2 \times 10^9$ (Buxton et al. 1988, García Einschlag et al. 2003)
Nitroglycerin	0.04 ± 0.01	$< 5 \times 10^8$
4. Ethers, alcohols, and phenols		
1,4-Dioxane	0.3 (Eibenberger 1980a, Thomas 1965)	$2.5\text{-}3.1 \times 10^9$ (Hoigné and Bader 1983b)
Oxirane, methyl	0.09 ± 0.02	$(5.7 \pm 0.4) \times 10^9$
Methyl-tert-butyl ether (MTBE)	0.14 (Acero et al. 2001, Eibenberger 1980b)	1.0×10^9 (Acero et al. 2001)
Cumene hydroperoxide	$(2.0 \pm 0.67) \times 10^5$	$(2.8 \pm 0.2) \times 10^9$
5. Nitrogen containing compounds		
4,4'-Methylenedianiline	$> 9 \times 10^7$ (Hoigné and Bader 1983a)	$> 5 \times 10^9$ (estimated)
Aniline	0.9×10^8 (Hoigné and Bader 1983b)	$0.9\text{-}1.7 \times 10^{10}$ (Christensen 1972, DeLaat et al. 1996, Qin et al. 1985, Solar et al. 1986)
Quinoline	51 (Wang et al. 2004)	7×10^9 (Wang et al. 2004)
6. Organophosphorous compounds		
Acephate	0.23 ± 0.01	$(7.1 \pm 0.2) \times 10^9$
Dicrotophos	0.03 ± 0.01	$(5.5 \pm 0.2) \times 10^9$
Methamidophos	0.02 ± 0.01	$(8.2 \pm 0.4) \times 10^9$
Fenamiphos	0.65 ± 0.01	$(6.2 \pm 0.2) \times 10^9$
7. Amides		
Metolachlor	3 (DeLaat et al. 1996)	6.1×10^9 (DeLaat et al. 1996)
Metolachlor ethanesulfonic acid (ESA)	5.0 ± 0.4	7.1×10^9 (DeLaat et al. 1996)
Metolachlor oxanilic acid (OA)	7.0 ± 0.1	5.6×10^9 (DeLaat et al. 1996)
N-methyl-2-pyrrolidone	2 (Muruganandham et al. 2007)	4.9×10^9 (Gligorovski et al. 2009)

Molinate	500 (Chen et al. 2008)	$1.3-6.9 \times 10^9$ (Reifferscheid and Heil , Shemer et al. 2006)
----------	------------------------	---

8. *N*-nitroso compounds

<i>N</i> -nitrosodimethylamine (NDMA)	0.05 (Lee et al. 2007)	4.3×10^8 (Mezyk et al. 2004)
<i>N</i> -nitrosodiethylamine (NDEA)	0.08 (Mestankova et al. 2014)	7×10^8 (Mezyk et al. 2008)
<i>N</i> -nitrosodi- <i>n</i> -propylamine (NDPA)	0.04 (Mestankova et al. 2014)	2.3×10^9 (Landsman et al. 2007)
<i>N</i> -nitrosopyrrolidine (NPYR)	0.33 (Mestankova et al. 2014)	1.75×10^9 (Landsman et al. 2007)
<i>N</i> -nitrosodiphenylamine (NDPhA)	8.5 (Mestankova et al. 2014)	6.5×10^9 (Mestankova et al. 2014)

Second order rate constants for reactions with $\cdot\text{OH}$ and ozone that were not available in the literature were experimentally determined. Of the 25 compounds in this study, two (1,2,3-trichloropropane and nitroglycerin) had second order rate constants for the reaction with $\cdot\text{OH} < 5 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$, a benchmark for which an effective abatement of a target compound is not feasible by AOPs, unless the compound can undergo efficient direct photolysis (e.g., NDMA). Most organic contaminants react with $\cdot\text{OH}$ with second order rate constants $\geq 10^9 \text{ M}^{-1}\text{s}^{-1}$. A benchmark for rate constants of the reaction of a target compound with ozone was set to $> 10 \text{ M}^{-1}\text{s}^{-1}$, below which the reaction with ozone does not contribute significantly to the abatement of the parent compound. Direct UV photolysis was not relevant for most compounds evaluated for treatment with some exceptions, including nitrobenzene, dimethipin, cumene hydroperoxide, aniline, fenamiphos, and *N*-nitrosamines (Table 2), which were tested for formation of mutagenic and estrogenic compounds by direct photolysis.

Mutagenic and Estrogenic Activity of Parent Compounds

Based on the kinetic study, the 23 parent compounds that were determined to be treatable by AOPs (Table 1, $^{\bullet}\text{OH}$ rate constant $\geq 5 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$) were tested for parent compound mutagenicity and estrogenicity. In addition, 1,2,3-trichloropropane was evaluated based on its current importance to US EPA.

Most parent compounds tested yielded no responses in the Ames II or the YES assay, i.e. neither mutagenicity nor estrogenicity. Among the few compounds causing mutagenicity in the Ames II test were cumene hydroperoxide, (group 4, in the TAMix strain, without the addition of the S9-mix) and group 6 chemicals (Parker et al. in prep.). Only one parent compound was found to be estrogenic in the YES assay: 4,4'-methylenedianiline (MDA) (group 5) with an EC_{50} value of $1.1 \times 10^{-4} \text{ M}$. In agreement with our findings, it was previously reported that MDA has a weak positive response with an EC_{50} of $1.9 \times 10^{-4} \text{ M}$ (using the recombinant yeast assay with 5 days of incubation) (Hamblen et al. 2003). MDA, which is structurally related to bisphenol A, shows low estrogen binding affinity (Blair et al. 2000). The responses of all parent compounds evaluated under the Ames and YES assays are provided in Table S3 (SI).

Development of Mutagenic Activity during AOPs

The development of the mutagenic activity following degradation by $^{\bullet}\text{OH}$, ozone, and direct photolysis of each compound is given in Table 2. In most cases no increase occurred but for some compounds an increase in biological activity from transformation products was observed (e.g., the case of quinoline, aniline, and several *N*-nitrosamine compounds). In the following sections, mutagenicity development during degradation of individual classes of compounds is discussed in more detail.

282 **Table 2. Evaluation of the mutagenic activity by the TA98 and TAMix Ames bioassays in absence (-S9) and presence (+S9) of**
 283 **the S9-mix after treatment of selected CCL3 compounds with $\cdot\text{OH}$, ozone or UV light (direct photolysis, $\lambda = 254 \text{ nm}$).**
 284

	oxidation by $\cdot\text{OH}$ radical				oxidation by ozone				direct photolysis			
strains:	TA98		TAMix		TA98		TAMix		TA98		TAMix	
metabolic activation:	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
1. Halogenated compounds												
1,2,3-trichloropropane	-☒→	-☒→	+☒→	-☒→	-☒→	-☒→	+☒→	-☒→	-☒→	-☒→	+☒→	-☒→
2. Olefines												
Dimethipin	-→-	-→-	-→-	-→-	-→-	-→-	-→-	-→-	-→-	-→-	-→-	-→-
3. Nitro compounds												
Nitrobenzene	- +	-→-	-→-	-→-	-→-	-→-	-→-	-→-	- +	-→-	-→-	-→-
4. Ethers, Alcohols, and Phenols												
1,4-Dioxane	-→-	-→-	-→-	-→-	-☒→	-☒→	-☒→	-☒→	-☒→	-☒→	-☒→	-☒→
Oxirane, methyl	-→-	-→-	-→-	-→-	-☒→	-☒→	-☒→	-☒→	-☒→	-☒→	-☒→	-☒→
Methyl-tert-butyl ether	-→-	-→-	-→-	-→-	-☒→	-☒→	-☒→	-☒→	-☒→	-☒→	-☒→	-☒→
Cumene hydroperoxide	-→-	-→-	+ -	-→-	-→-	-→-	+ -	-→-	-→-	-→-	+ -	-→-
5. Nitrogen-containing compounds												
Aniline	-→-	-→-	-→-	-→-	-→-	-→-	-→-	-→-	-→-	- +	-→-	-→-
4,4'-Methylenedianiline	-→-	-→-	-→-	-→-	-→-	-→-	-→-	-→-	n.a.	n.a.	n.a.	n.a.
Quinoline	- +	- +	-→-	-→-	- +	- +	- +	- +	n.a.	n.a.	n.a.	n.a.
6. Organophosphorous compounds												
Acephate	+ - [?]	-→-	-→-	-→-	-☒→	-☒→	-☒→	-☒→	-☒→	-☒→	-☒→	-☒→
Dicrotophos	-→-	-→-	+ - [?]	-→-	-☒→	-☒→	-☒→	-☒→	-☒→	-☒→	-☒→	-☒→
Methamidophos	-→-	+ +	-→-	-→-	-☒→	-☒→	-☒→	-☒→	-☒→	-☒→	-☒→	-☒→
Fenamiphos	-→-	-→-	+ -	-→-	-☒→	-☒→	-☒→	-☒→	-→-	-→-	+ -	-→-
7. Amides												
Metolachlor	-→-	-→-	-→-	-→-	-☒→	-☒→	-☒→	-☒→	-→-	-→-	-→-	-→-

Metolachlor ethanesulfonic acid	- → -	- → -	- → -	- → -	-☒→	-☒→	- ☒→	-☒→	- → -	- → -	- → -	- → -
Metolachlor oxanilic acid	- → -	- → -	- → -	- → -	-☒→	-☒→	- ☒→	-☒→	- → -	- → -	- → -	- → -
2-methyl-2-pyrrolidone	- → -	- → -	- → -	- → -	-☒→	-☒→	- ☒→	-☒→	-☒→	-☒→	- ☒→	-☒→
Molinate	- → -	- → -	- → -	- → -	- → -	- → -	- → -	- → -	- → -	- → -	- → -	- → -

8. *N*-nitroso compounds (Mestankova et al. 2014)

<i>N</i> -nitrosodimethylamine	- → -	- → -	- → -	- → -	-☒→	-☒→	- ☒→	-☒→	- → -	- → -	- → -	- → -
<i>N</i> -nitrosodiethylamine	- → -	- → -	- → -	- → -	-☒→	-☒→	- ☒→	-☒→	- → -	- → -	- → -	- → -
<i>N</i> -nitrosodi- <i>n</i> -propylamine	- → -	- → -	- ↗ +	- ↗ +	-☒→	-☒→	- ☒→	-☒→	- → -	- → -	- → -	- → -
<i>N</i> -nitrosopyrrolidine	- → -	- → -	- ↗ +	- ↗ +	-☒→	-☒→	- ☒→	-☒→	- → -	- → -	- → -	- → -
<i>N</i> -nitrosodiphenylamine	- → -	- → -	- → -	- → -	- → -	- → -	- → -	- → -	- ↗ +	- ↗ +	- → -	- → -

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

-☒→ Insufficient degradation was achieved for testing.

+☒→ Parent compound activity was positive, but insufficient degradation was achieved for testing.

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

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

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

- ↗ + Parent compound activity was negative, and after treatment the activity increased.

Group 2 - Olefines

The only olefin tested from the CCL3 list was dimethipin. Dimethipin showed no parent compound response for either of the Ames assay strains tested (TAMix, TA98). Dimethipin is amenable to oxidation by $\cdot\text{OH}$ and by ozone, and also direct photolysis. When subjected to either of these processes, dimethipin showed no formation of mutagenic activity.

Group 3 - Nitro compounds

Direct photodegradation of nitrobenzene (NB) (Tables 1 and S1 (SI)) leads to formation of significant mutagenic activity in the strain TA98 in the absence of the S9-mix, as shown in Figure 2. The mutagenicity was quantified as MEQ-2-NF (for explanation see Materials and Methods). Further photolytic degradation of NB leads to the removal of the detected mutagenicity. Also during oxidation of NB by $\cdot\text{OH}$ (via the UV/H₂O₂ process) a weak mutagenicity formation was observed. However, the observed formation of the mutagenic activity during degradation of aromatic nitro compounds should be interpreted cautiously due to documented false positives tests of these compounds in the Ames test (*McCann and Ames 1976; Maragos et al. 1993; Kirkland et al. 2007*).

Direct photolysis and oxidation of NB by $\cdot\text{OH}$ leads to a formation of 2-nitrophenol, 3-nitrophenol, 4-nitrophenol, phenol, nitrohydroquinone, nitrocatechols and catechol (Beltran et al. 1998, Chen et al. 2005, Chen et al. 2006, Matthews and Sangster 1967). NB itself is non-mutagenic in the strains TA98 and TA100 in the absence and presence of S9, only the substituted NBs were observed to be mutagenic (Assmann et al. 1997). 4-Nitrophenol was reported to be mutagenic in the TA98 in the absence of S9 (Yang et al. 2003) in contrast to earlier studies (Shimizu and Yano 1986). Mutagenicity was also reported for phenol, an additional possible transformation product of NB (Kamber et al. 2009, Kirkland et al. 2011).

Figure 2 here

Group 4 - Ethers, alcohols, and phenols

In group 4 (Tables 1 and S1 (SI)) only cumene hydroperoxide exerted mutagenicity. Its degradation by photolysis, oxidation by ozone and $\cdot\text{OH}$ led to the removal of its mutagenic activity. The degradation of cumene hydroperoxide by all three treatment methods together with the mutagenic equivalents (MEQ) is shown in Figure 3.

Figure 3 here

The MEQ results show that the mutagenic activity of the parent compound decreases at or below the line for stoichiometric loss of mutagenicity (slope = -1). This means that mutagenicity is lost proportionally to the degradation of the parent compound. During degradation of other compounds from this group, no significant mutagenicity was revealed.

Group 5 - Nitrogen-containing compounds

None of the parent compounds of group 5 (Tables 1 and S1 (SI)) were mutagenic in the Ames tests (TA98 and TAMix). However, degradation of aniline and quinoline led to the formation of mutagenic compounds, which were detectable by the Ames test (TA98 and TAMix) (Table 2). The direct photolytic transformation products of aniline induced mutagenicity in the strain TA98 in presence of S9 (Figure 4).

Figure 4 here

Based on a previous study, the photolysis of aniline leads to the formation of hydrazobenzene, 4-aminodiphenylamine, 2-aminodiphenylamine, benzidine and azobenzene (Zechner et al. 1976). Benzidine has been recognized as a human carcinogen by the US EPA and

the IARC and it was positively tested for mutagenicity in the strain TA98 in the presence of the S9-mix (Chung et al. 2000, Sinsheimer et al. 1992). Azobenzene is also considered to be a probable human carcinogen (B2) and was tested positively in TA100 in the presence of the S9-mix. Hydrazobenzene showed the same results in TA100 in presence of the S9-mix. (Zeiger 1987). However, in the present study no significant mutagenicity was observed in the TAMix strains with and without S9, which are able to detect the same type of mutations as TA100.

During oxidation of quinoline (Q) with ozone, formation of mutagenicity was detected in both strains (in absence and presence of the S9-mix) during oxidation by ozone, but only in the TA98 strain during oxidation by $\cdot\text{OH}$ (Table 2). In a prior study, quinoline itself exhibited a positive response for base-pair substitution in TA100 in the presence of the S9-mix (Plumlee and Reinhard 2007). However, in the present study, the mutagenic potency of the parent compound Q was not detected in any strain used in this study, perhaps because the experimental conditions of the Ames test was not sensitive enough to detect the mutagenicity of quinoline.

Several possible transformation products of Q were shown to be mutagenic *in vitro*. Mutagenic activity of 5-hydroxyquinoline and 8-hydroxyquinoline (5-OH-Q and 8-OH-Q) (more details on the formation of hydroxyquinolines are given in the section on estrogenicity below) was detected in the TAMix (in presence of the S9-mix). It was previously demonstrated that 5-OH-Q and 8-OH-Q showed mutagenicity in TA98 and TA100 in presence of the S9-mix (Sideropoulos and Specht 1984, Smith et al. 1997, Willems et al. 1992). Also some other derivatives of quinoline were mutagenic in the TA100 in presence of the S9-mix (Willems et al. 1992).

Group 6 - Organophosphorous compounds

The results from the study of the mutagenic activity of organophosphorous compounds are described in detail elsewhere (Parker et al. in prep.). Briefly, a degradation of the

organophosphorous insecticide methamidophos by $\cdot\text{OH}$ led to the formation of mutagenic activity in the TA98 strain in presence of the S9-mix. Fenamiphos and dicrotophos showed positive activity in the TAMix strain in absence of the S9-mix, and $\cdot\text{OH}$ degradation (UV/H₂O₂) was effective at removing this mutagenic signal. Acephate showed a weak response in the TA98 strain in absence of the S9-mix, and oxidation by $\cdot\text{OH}$ (UV/H₂O₂) was effective for the removal of this activity.

Group 7 - Amides

No mutagenicity formation was observed during degradation of chemicals from group 7.

Group 8 – N-nitroso compounds

The results of this class of compounds have been published in another study (Mestankova et al. 2014); here only a brief summary will be provided. The UV photolysis of the non-mutagenic NDPhA leads to the formation of mutagens detectable in the TA98 strain (with and without the S9-mix). Oxidation of both *N*-nitrosodi-*n*-propylamine and *N*-nitrosopyrrolidine by $\cdot\text{OH}$ revealed the formation of direct mutagens detectable in the TAMix strains (Zeiger 1987).

Development of Estrogenic Activity

An overview of all the results from estrogenicity testing is given in Table S4 (SI). 23 parent compounds were tested in the YES assay and only 4,4'-methylendianiline (MDA) showed estrogenic activity in the YES test. The development of the estrogenic activity of MDA as EEQ/EEQ₀ was measured during its degradation with ozone and $\cdot\text{OH}$ (Figure 5).

Figure 5 here

Both processes (ozone and $\cdot\text{OH}$) lead to a decrease of the overall estrogenic activity, following a slight initial increase due to $\cdot\text{OH}$. The EEQ decreased stoichiometrically with the

decrease of the concentration of MDA during oxidation as indicated by the values being close to the line with a slope of -1 .

From the 23 target compounds tested, quinoline (Q) is the only one resulting in a formation of estrogenicity detected by YES test during its oxidation by $\cdot\text{OH}$. The formation of estrogenic activity was confirmed under two different UV/H₂O₂ experimental set-ups: (1) a medium-pressure mercury vapor lamp (including a band-pass filter in the wavelength range of 308-410 nm) in a solution containing 5 mM (170 mg/L) H₂O₂ and (2) a low pressure mercury vapor lamp (emitting primarily at 254 nm) with 1 mM (34 mg/L) H₂O₂ (Figure 6).

Figure 6 here

The formation of the estrogenic activity was also confirmed for a lower Q concentration of 1 μM (~ 0.13 mg/L) (Figure S6), which is closer to environmentally relevant conditions. The oxidation of 100 μM (~ 13 mg/L) Q leads to a maximum estrogenic activity of approximately 2×10^{-10} M EEQ. Given that the potency of Q relative to E2 is 3×10^{-4} , a concentration of 0.24 mg/L would therefore be needed to lead to an estrogenicity comparable to 1 ng/L 17 β -estradiol. The levels of estrogenicity measured were well below the effect concentrations relevant to humans (Brand et al., 2013). The oxidation of Q by ozone did not result in any estrogenic activity, nor did the direct photolysis of Q with a low-pressure lamp.

The degradation of Q by $\cdot\text{OH}$ was previously studied in detail (Nicolaescu et al. 2005a, b, Zhang et al. 2003). Hydroxyl radical attack occurs preferentially on the electron-rich benzene ring by an OH addition reaction, leading to a hydroxylation of Q in positions 5-7. Further oxidation of these products can lead to the formation of quinolone-5,8-dione. The pyridine ring is less susceptible to an $\cdot\text{OH}$ attack, but it still occurs, as the products of its hydroxylation are also detected in systems where Q is transformed by $\cdot\text{OH}$. While one of the main degradation products,

8-hydroxyquinoline, was reported to not exhibit estrogenic activity by the recombinant yeast assay with 3 days exposure (Miller et al. 2001), together with other hydroxylated quinolines (6- and 7-OH-Q) it exhibited a clear positive response for estrogenic activity by the YES 5-day assay (Schultz and Sinks 2002). All hydroxylated quinolines (with a hydroxyl group in positions 2-8) were evaluated by the YES test in the present study. The EC₅₀ values for the estrogenic hydroxylated quinolines determined in this study (3 days exposure YES test) are presented in Table 3 together with literature data of the YES test with 5-days exposure. Hydroxylated quinolines with a hydroxyl group on the pyridine ring did not exhibit an estrogenic response.

Table 3. EC₅₀ values of estrogenic hydroxylated quinolines in the YES test compared with previously published data, in which the compounds were tested with the recombinant yeast assay with longer incubation times (Schultz and Sinks 2002) (5 days, instead of 3).

	This study (3-d EC₅₀, M) (95% confidence interval)	5-d EC ₅₀ (M) (Schultz and Sinks 2002)
6-OH-Q	8.0×10^{-4} ($6.5-9.7 \times 10^{-4}$)	3.4×10^{-4}
7-OH-Q	1.4×10^{-3} ($1.1-1.6 \times 10^{-3}$)	6.9×10^{-4}
8-OH-Q	6.6×10^{-8} ($3.1 \times 10^{-8}-1.46 \times 10^{-7}$)	3.4×10^{-7}

8-OH-Q is the most potent of the estrogenic compounds among the hydroxyquinolines, however, no *in vivo* data on its estrogenicity are available. A relatively high toxicity, i.e. impairment of viability, of 8-OH-Q towards yeast cells probably causes the ambiguous results obtained for its estrogenicity testing (note also the large 95 % confidence interval of EC₅₀).

The absence of estrogenicity in samples taken during oxidation by ozone can be explained by the formation of different transformation products. The reactions of Q with ozone (relatively slow) take place at the benzene ring leading to the cleavage of 5,6 or 7,8 bonds

(Andreozzi et al. 1992) and the formation of quinolinic acid, which can be further oxidized to a *N*-oxide derivative. A minor ozone attack was observed on the pyridine ring through formation of an *N*-oxide.

CONCLUSIONS

Advanced oxidation processes are able to degrade the selected compounds from the CCL3. Based on a thorough kinetic assessment of the 25 CCL3 compounds studied, 23 were found to be amenable to hydroxyl radical-based treatment, with second-order $\cdot\text{OH}$ rate constants in the range of $3\text{--}8 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$. All compounds tested in the groups containing olefines, ethers, alcohols and phenols, amides, and *N*-nitroso compounds were degradable by $\cdot\text{OH}$.

Ozonation, photolysis and AOPs are relevant not only for the abatement of the parent compound, but also for the removal of biological activity as illustrated by the mutagenic activity removal of cumene hydroperoxide or the estrogenic activity removal of MDA. 6 of the 23 evaluated compounds exhibited transformation products causing mutagenic or estrogenic activity detectable by *in vitro* assays. Mutagenic activity (via $\cdot\text{OH}$, ozone, or direct photolysis) occurred in 9 treatment cases with the most frequent detected in the TA98 strain (frameshift mutation). The formed mutagens were mostly direct mutagens, meaning they do not need metabolic activation by the S9 enzymes to become mutagenic. Estrogenic activity (via $\cdot\text{OH}$, ozone, or direct photolysis) was generated only from the oxidation of quinoline by $\cdot\text{OH}$. However, when examined at environmentally relevant concentrations, quinoline oxidation produced only low estrogenic activity, probably not relevant in an overall risk assessment.

Based on the results from this study, the possibility of generating biological effects as indicated in the Ames II and YES assays during advanced oxidation processes, while rare, cannot be completely ruled out. If ozone- or UV-based AOPs are implemented for drinking water

treatment from water resources with significant anthropogenic contamination, the use of bioassays to assess potential risks associated with AOPs, similar to the current study should be considered.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the Water Research Foundation for project funding under project #4241, as well as the National Science Foundation GRFP for funding Austa Parker. Furthermore, we would like to thank the Swiss Federal Office for the Environment for funding Hana Mestankova. The authors would also like to thank Mike Thurman, Imma Ferrer, Emily Spangler and Elisabeth Salhi for their laboratory assistance and research support during this study.

REFERENCES

- Acero, J.L., Haderlein, S.B., Schmidt, T.C., Suter, M.J.F. and von Gunten, U. (2001) MTBE Oxidation by Conventional Ozonation and the Combination Ozone/Hydrogen Peroxide: Efficiency of the Processes and Bromate Formation. *Environmental Science & Technology* 35(21), 4252-4259.
- Andreozzi, R., Insola, A., Caprio, V. and D'Amore, M.G. (1992) Quinoline ozonation in aqueous solution. *Water Research* 26(5), 639-643.
- Ashby, J., Tennant, R.W., Zeiger, E. and Stasiewicz, S. (1989) Classification according to chemical structure, mutagenicity to Salmonella and level of carcinogenicity of a further 42 chemicals tested for carcinogenicity by the U.S. National Toxicology Program. *Mutation Research/Genetic Toxicology* 223(2), 73-103.
- Assmann, N., Emmrich, M., Kampf, G. and Kaiser, M. (1997) Genotoxic activity of important nitrobenzenes and nitroanilines in the Ames test and their structure-activity relationship. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* 395(2-3), 139-144.
- Bader, H. and Hoigné, J. (1981) Determination of ozone in water by the indigo method. *Water Research* 15(4), 449-456.
- Beltran, F.J., Encinar, J.M. and Alonso, M.A. (1998) Nitroaromatic Hydrocarbon Ozonation in Water 2. Combined Ozonation with Hydrogen Peroxide or UV Radiation. *Industrial & Engineering Chemistry Research* 37, 32-40.
- Blair, R.M., Fang, H., Branham, W.S., Hass, B.S., Dial, S.L., Moland, C.L., Tong, W., Shi, L., Perkins, R. and Sheehan, D.M. (2000) The Estrogen Receptor Relative Binding Affinities of 188 Natural and Xenochemicals: Structural Diversity of Ligands. *Toxicological Sciences* 54(1), 138-153.
- Bolton, J.R. and Linden, K.G. (2003) Standardization of methods for fluence (UV dose) determination in bench-scale UV experiments. *Journal of Environmental Engineering* 129(3), 209-215.
- Brand, W., de Jongh, C.M., van der Linden, S.C., Puijker, L.M., van Leeuwen, C.J., van Wezel, A.P., Schriks, M., Heringa, M.B. (2013): Trigger values for investigation of hormonal activity in drinking water and its sources using CALUX bioassays. *Environment International* 55, 109–118
- Brix, R., Noguerol, T.-N., Piña, B., Balaam, J., Nilsen, A.J., Tollefsen, K.-E., Levy, W., Schramm, K.-W. and Barceló, D. (2010) Evaluation of the suitability of recombinant yeast-based estrogenicity assays as a pre-screening tool in environmental samples. *Environment International* 36(4), 361-367.
- Buxton, G.V., Greenstock, C.L., Helman, W.P. and Ross, A.B. (1988) Critical Review of Rate Constants for Reactions of Hydrated Electrons, Hydrogen Atoms and Hydroxyl Radicals (OH/O⁻) in Aqueous Solution. *Journal of Physical and Chemical Reference Data* 17, 513-886.

500 Chen, B., Yang, C. and Goh, N.K. (2005) Direct photolysis of nitroaromatic compounds in
501 aqueous solutions. *Journal of Environmental Sciences* 17(4), 598-604.

502 Chen, B., Yang, C. and Goh, N.K. (2006) Photolysis pathway of nitroaromatic compounds in
503 aqueous solutions in the UV/H₂O₂ process. *Journal of Environmental Sciences* 18(6), 1061-
504 1064.

505 Chen, W.R., Wu, C., Elovitz, M.S., Linden, K.G. and Suffet, I.H. (2008) Reactions of
506 thiocarbamate, triazine and urea herbicides, RDX and benzenes on EPA Contaminant Candidate
507 List with ozone and with hydroxyl radicals. *Water Research* 42(1-2), 137-144.

508 Christensen, H. (1972) Pulse radiolysis of aqueous solutions of aniline and substituted anilines.
509 *International Journal for Radiation Physics and Chemistry* 4(3), 311-333.

510 Chung, K.T., Chen, S.C., Wong, T.Y., Li, Y.S., Wei, C.I. and Chou, M.W. (2000) Mutagenicity
511 studies of benzidine and its analogs: structure-activity relationships. *Toxicol Sci* 56(2), 351-356.

512 Cronin, M.T.D., Gregory, B.W. and Schultz, T.W. (1998) Quantitative structure-activity
513 analyses of nitrobenzene toxicity to *Tetrahymena pyriformis*. *Chemical Research in Toxicology*
514 11(8), 902-908.

515 DeLaat, J., MaoualaMakata, P. and Dore, M. (1996) Rate constants for reactions of ozone and
516 hydroxyl radicals with several phenyl-ureas and acetamides. *Environmental Technology* 17(7),
517 707-716.

518 Dhooge, W., Arijs, K., D'Haese, I., Stuyvaert, S., Versonnen, B., Janssen, C., Verstraete, W. and
519 Comhaire, F. (2006) Experimental parameters affecting sensitivity and specificity of a yeast
520 assay for estrogenic compounds: results of an interlaboratory validation exercise. *Analytical and*
521 *Bioanalytical Chemistry* 386(5), 1419-1428.

522 Dodd, M.C., Kohler, H.E. and von Gunten, U. (2009) Oxidation of Antibacterial Compounds by
523 Ozone and Hydroxyl Radical: Elimination of Biological Activity during Aqueous Ozonation
524 Processes. *Environmental Science & Technology* 43(7), 2498-2504.

525 Eibenberger, J. (1980) "Pulse radiolytic investigations concerning the formation and the
526 oxidation of organic radicals in aqueous solutions" Ph.D., Thesis, Vienna Univ., Vienna, Austria,
527 95p

528 Elovitz, M.S., Gunten, U.v. and Kaiser, H.P. (2000) Hydroxyl radical/ozone ratios during
529 ozonation processes. II. The effect of temperature, pH, alkalinity, and DOM properties. *Ozone*
530 *Science and Engineering* 22, 123-150.

531 Elovitz, M.S. and von Gunten, U. (1999) Hydroxyl radical/ozone ratios during ozonation
532 processes. I. The R_{ct} concept. *Ozone Science & Engineering* 21(3), 239-260.

533 Escher, B.I., Bramaz, N., Mueller, J.F., Quayle, P., Rutishauser, S., Vermeirssen, E., (2008)
534 Toxic equivalent concentrations (TEQs) for baseline toxicity and specific modes of action as a
535 tool to improve evaluation of ecotoxicity tests on environmental samples. *Journal of*
536 *Environmental Monitoring* 10,, 612-621.

537 Escher, B.I. and Fenner, K. (2011) Recent Advances in Environmental Risk Assessment of
 538 Transformation Products. *Environmental Science & Technology* 45(9), 3835-3847.
 539

540 García Einschlag, F.S., Carlos, L. and Capparelli, A.L. (2003) Competition kinetics using the
 541 UV/H₂O₂ process: a structure reactivity correlation for the rate constants of hydroxyl radicals
 542 toward nitroaromatic compounds. *Chemosphere* 53(1), 1-7.

543 Gligorovski, S., Rouse, D., George, C.H. and Herrmann, H. (2009) Rate Constants for the OH
 544 Reactions with Oxygenated Organic Compounds in Aqueous Solution. *International Journal of*
 545 *Chemical Kinetics* 41, 309-326.

546 Gschwend, P.M. and Wu, S. (1985) On the constancy of sediment-water partition coefficients of
 547 hydrophobic organic pollutants. *Environmental Science & Technology* 19(1), 90-96.

548 Hamblen, E.L., Cronin, M.T.D. and Schultz, T.W. (2003) Estrogenicity and acute toxicity of
 549 selected anilines using a recombinant yeast assay. *Chemosphere* 52(7), 1173-1181.

550 Hoigné, J. and Bader, H. (1983a) Rate constants of reactions of ozone with organic and inorganic
 551 compounds in water--I: Non-dissociating organic compounds. *Water Research* 17(2), 173-183.

552 Hoigné, J. and Bader, H. (1983b) Rate constants of reactions of ozone with organic and
 553 inorganic compounds in water--II: Dissociating organic compounds. *Water Research* 17(2), 185-
 554 194.

555 Huber, M.M., Canonica, S., Park, G. and von Gunten, U. (2003) Oxidation of Pharmaceuticals
 556 during Ozonation and Advanced Oxidation Processes. *Environmental Science & Technology*
 557 37(5), 1016-1024.

558 ISO (2012) Water Quality -- Determination of the genotoxicity of water and waste water --
 559 Salmonella/microsome test (Ames test).

560 Kamber, M., Flückiger-Isler, S., Engelhardt, G., Jaech, R. and Zeiger, E. (2009) Comparison of
 561 the Ames II and traditional Ames test responses with respect to mutagenicity, strain specificities,
 562 need for metabolism and correlation with rodent carcinogenicity. *Mutagenesis* 24(4), 359-366.

563 Kirkland, D.J., Aardema, M., Banduhn, N., Carmichael, P., Fautz, R., Meunier, J.R. and Pfuhler,
 564 S. (2007) In vitro approaches to develop weight of evidence (WoE) and mode of action (MoA)
 565 discussions with positive in vitro genotoxicity results. *Mutagenesis* 22(3), 161-175.

566 Kirkland, D., Reeve, L., Gatehouse, D. and Vanparys, P. (2011) A core in vitro genotoxicity
 567 battery comprising the Ames test plus the in vitro micronucleus test is sufficient to detect rodent
 568 carcinogens and in vivo genotoxins. *Mutation Research/Genetic Toxicology and Environmental*
 569 *Mutagenesis* 721(1), 27-73.

570 Kolle, S.N., Kamp, H.G., Huener, H.A., Knickel, J., Verlohner, A., Woitkowiak, C., Landsiedel,
 571 R. and van Ravenzwaay, B. (2010) In house validation of recombinant yeast estrogen and
 572 androgen receptor agonist and antagonist screening assays. *Toxicology in Vitro* 24(7), 2030-
 573 2040.

574 Landsman, N.A., Swancutt, K.L., Bradford, C.N., Cox, C.R., Kiddle, J.J. and Mezyk, S.P. (2007)
 575 Free Radical Chemistry of Advanced Oxidation Process Removal of Nitrosamines in Water.
 576 Environmental Science & Technology 41(16), 5818-5823.

577 Lee, C., Yoon, J. and von Gunten, U. (2007) Oxidative degradation of N-nitrosodimethylamine
 578 by conventional ozonation and the advanced oxidation process ozone/hydrogen peroxide. Water
 579 Research 41(3), 581-590.

580 Lee, Y., Escher, B.I. and von Gunten, U. (2008) Efficient Removal of Estrogenic Activity during
 581 Oxidative Treatment of Waters Containing Steroid Estrogens. Environmental Science &
 582 Technology 42(17), 6333-6339.

583 Lee, Y. and von Gunten, U. (2010) Oxidative transformation of micropollutants during
 584 municipal wastewater treatment: Comparison of kinetic aspects of selective (chlorine, chlorine
 585 dioxide, ferrateVI, and ozone) and non-selective oxidants (hydroxyl radical). Water Research
 586 44(2), 555-566.

587
 588 Leitzke, A., Reisz, E., Flyunt, R. and von Sonntag, C. (2001) The reactions of ozone with
 589 cinnamic acids: formation and decay of 2-hydroperoxy-2-hydroxyacetic acid. Journal of the
 590 Chemical Society, Perkin Transactions 2 (5), 793-797.

591 Magee, P.N. and Barnes, J.M. (1967) Carcinogenic Nitroso Compounds. Advances in Cancer
 592 Research 10, 163-246.

593 Matthews, R.W. and Sangster, D.F. (1967) Production of isomeric nitrophenols in radiolysis of
 594 aqueous nitrobenzene solution. The Journal of Physical Chemistry 71(12), 4056-4062.

595 Maragos, C.M., Andrews, A.W., Keefer, L.K. and Elespuru, R.K. (1993) Mutagenicity of
 596 glyceryl trinitrate (nitroglycerin) in Salmonella typhimurium. Mutation Research/Genetic
 597 Toxicology 298(3), 187-195.

598
 599 McCann, J., Choi, E., Yamasaki, E. and Ames, B.N. (1975) Detection of carcinogens as
 600 mutagens in the Salmonella/microsome test: assay of 300 chemicals. Proc. Natl. Acad. Sci. USA
 601 72(12), 5135-5139.

602 McCann, J. and Ames, B.N. (1976) Detection of carcinogens as mutagens in Salmonella-
 603 microsome test: 2. Assay of 300 chemicals - discussion. Proc. Nat. Acad. Sci. USA 73(3), 950-
 604 954.

605 Mestankova, H., Escher, B., Schirmer, K., von Gunten, U. and Canonica, S. (2011) Evolution of
 606 algal toxicity during (photo)oxidative degradation of diuron. Aquatic Toxicology 101(2), 466-
 607 473.

608 Mestankova, H., Schirmer, K., Canonica, S. and von Gunten, U. (2014) Development of
 609 mutagenicity during degradation of N-nitrosamines by advanced oxidation processes. Water
 610 Research 66(0), 399-410.

611 Mestankova, H., Schirmer, K., Escher, B.I., von Gunten, U. and Canonica, S. (2012) Removal of
 612 the antiviral agent oseltamivir and its biological activity by oxidative processes. *Environmental*
 613 *Pollution* 161, 30-35.

614 Mezyk, S., P., Landsman, N., A., Swancutt, K., L., Bradford, C., N., Cox, C., R., Kiddle, J., J.
 615 and Clore, T., J. (2008) Disinfection by-products in drinking water. Karanfil, T., Krasner, S.W.,
 616 Westerhoff, P. and Xie, Y. (eds), pp. 319-333, American Chemical Society.

617 Mezyk, S.P., Cooper, W.J., Madden, K.P. and Bartels, D.M. (2004) Free radical destruction of
 618 N-nitrosodimethylamine in water. *Environmental Science and Technology* 38(11), 3161-3167.

619 Miller, D., Wheals, B.B., Beresford, N. and Sumpter, J.P. (2001) Estrogenic Activity of Phenolic
 620 Additives Determined By an In Vitro Yeast Bioassay. *Environ Health Perspectives* 109(2), 132-
 621 138.

622 Muruganandham, M., Chen, S.H. and Wu, J.J. (2007) Mineralization of N-methyl-2-pyrrolidone
 623 by advanced oxidation processes. *Separation and Purification Technology* 55(3), 360-367.

624 Nicolaescu, A.R., Wiest, O. and Kamat, P.V. (2005a) Mechanistic Pathways of the Hydroxyl
 625 Radical Reactions of Quinoline. 1. Identification, Distribution, and Yields of Hydroxylated
 626 Products. *The Journal of Physical Chemistry A* 109(12), 2822-2828.

627 Nicolaescu, A.R., Wiest, O. and Kamat, P.V. (2005b) Mechanistic Pathways of the Hydroxyl
 628 Radical Reactions of Quinoline. 2. Computational Analysis of Hydroxyl Radical Attack at C
 629 Atoms. *The Journal of Physical Chemistry A* 109(12), 2829-2835.

630 Nordmeyer, D. and Dickson, D.W. (1990) Biological activity and acetylcholinesterase inhibition
 631 by nonfumigant nematocides and their degradation products on *Meloidogyne incognita*. *Journal*
 632 *of Nematology* 13(2), 229-232.

633 Onstad, G.D., Strauch, S., Meriluoto, J., Codd, G.A. and von Gunten, U. (2005) Selective
 634 oxidation of key functional groups in cyanotoxins during drinking water ozonation.
 635 *Environmental Science & Technology* 41(12), 4397-4404.

636 Oppenlander, T. (2007) Photochemical Purification of Water and Air: Advanced Oxidation
 637 Processes (AOPs) - Principles, Reaction Mechanisms, Reactor Concepts, Wiley.

638 Parker, A.M., Spangler, E., von Gunten, U. and Linden, K.G. Effect-Directed Analysis for the
 639 Treatment of Organophosphorous Insecticides in Drinking Water using Advanced Oxidation. In
 640 preparation.

641 Plumlee, M.H. and Reinhard, M. (2007) Photochemical attenuation of N-nitrosodimethylamine
 642 (NDMA) and other nitrosamines in surface water. *Environmental Science & Technology* 41(17),
 643 6170-6176.

644 Qin, L., Tripathi, G.N.R. and Schuler, R.H. (1985) Radiation chemical studies on the oxidation
 645 of aniline in aqueous solution. *Z. Naturforsch. A. Phys., Phys. Chem., Kosmophys* 40A, 1026-
 646 1039.

647 Reifferscheid, G. and Heil, J. (1996) Validation of the SOS/umu test using test results of 486
648 chemicals and comparison with the Ames test and carcinogenicity data. *Mutation*
649 *Research/Genetic Toxicology* 369(3-4), 129-145.

650 Rosario-Ortiz, F.L., Wert, E.C. and Snyder, S.A. (2010) Evaluation of UV/H₂O₂ treatment for
651 the oxidation of pharmaceuticals in wastewater. *Water Research* 44(5), 1440-1448.

652 Rosenfeldt, E.J., Chen, P.J., Kullman, S. and Linden, K.G. (2007) Destruction of estrogenic
653 activity in water using UV advanced oxidation. *Science of The Total Environment* 377(1), 105-
654 113.

655 Routledge, E.J. and Sumpter, J.P. (1996) Estrogenic activity of surfactants and some of their
656 degradation products assessed using a recombinant yeast screen. *Environmental Toxicology and*
657 *Chemistry* 15(3), 241-248.

658 Rutishauser, B.V., Pesonen, M., Escher, B.I., Ackermann, G.E., Aerni, H.-R., Suter, M.J.F. and
659 Eggen, R.I.L. (2004) Comparative analysis of estrogenic activity in sewage treatment plant
660 effluents involving three in vitro assays and chemical analysis of steroids. *Environmental*
661 *Toxicology and Chemistry* 23(4), 857-864.

662 Schmidt, C.K. and Brauch, H.-J. (2008) N,N-Dimethylsulfamide as Precursor for N-
663 Nitrosodimethylamine (NDMA) Formation upon Ozonation and its Fate During Drinking Water
664 Treatment. *Environmental Science & Technology* 42(17), 6340-6346.

665 Schultz, T.W. and Sinks, G.D. (2002) Xenoestrogenic gene expression: Structural features of
666 active polycyclic aromatic hydrocarbons. *Environmental Toxicology and Chemistry* 21(4), 783-
667 786.

668 Schwarzenbach, R.P., Escher, B.I., Fenner, K., Hofstetter, T.B., Johnson, C.A., von Gunten, U.
669 and Wehrli, B. (2006) The challenge of micropollutants in aquatic systems. *Science* 313(5790),
670 1072-1077.
671

672 Sharpless, C., Seibold, D. and Linden, K. (2003) Nitrate photosensitized degradation of atrazine
673 during UV water treatment. *Aquatic Sciences* 65(4), 359-366.

674 Shemer, H., Sharpless, C.M., Elovitz, M.S. and Linden, K.G. (2006) Relative Rate Constants of
675 Contaminant Candidate List Pesticides with Hydroxyl Radicals. *Environmental Science &*
676 *Technology* 40(14), 4460-4466.

677 Shimizu, M. and Yano, E. (1986) Mutagenicity of Mono-Nitrobenzene Derivatives in the Ames
678 Test and Rec Assay. *Mutation Research* 170(1-2), 11-22.

679 Shinoda, K., Mitsumori, K., Yasuhara, K., Uneyama, C., Onodera, H., Takegawa, K., Takahashi,
680 M. and Umemura, T. (1998) Involvement of apoptosis in the rat germ cell degeneration induced
681 by nitrobenzene. *Archives of Toxicology* 72(5), 296-302.

682 Sideropoulos, A. and Specht, S. (1984) Evaluation of microbial testing methods for the
683 mutagenicity of quinoline and its derivatives. *Current Microbiology* 11(2), 59-65.

684 Sinsheimer, J.E., Hooberman, B.H., Das, S.K., Brezzell, M.D. and You, Z. (1992) The in vivo
685 and in vitro genotoxicity of aromatic amines in relationship to the genotoxicity of benzidine.
686 Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 268(2), 255-264.

687 Smith, C.J., Hansch, C. and Morton, M.J. (1997) QSAR treatment of multiple toxicities: the
688 mutagenicity and cytotoxicity of quinolines. Mutation Research/Fundamental and Molecular
689 Mechanisms of Mutagenesis 379(2), 167-175.

690 Solar, S., Solar, W. and Getoff, N. (1986) Resolved multisite OH-attack on aqueous aniline
691 studied by pulse radiolysis. International Journal of Radiation Applications and Instrumentation.
692 Part C. Radiation Physics and Chemistry 28(2), 229-234.

693 Stefan, M.I. and Bolton, J.R. (1998) Mechanism of the degradation of 1,4-dioxane in dilute
694 aqueous solution using the UV hydrogen peroxide process. Environmental Science &
695 Technology 32(11), 1588-1595.

696 Thomas, J.K. (1965) Rates of reaction of the hydroxyl radical. Transactions of the Faraday
697 Society 61, 702-707.

698 USEPA (1996) 1996 Amendments to the Safe Drinking Water Act., pp. 104-182, 74 FR 53590.

699 USEPA (October 8, 2009) Drinking Water Contaminant Candidate List 3 - Final, 74 FR 51862.

700 Virkutyte, J., Varma, R.S. and Jegatheesan, V. (2010) Treatment of Micropollutants in Water
701 and Wastewater, IWA Publishing.

702 von Gunten, U. (2003) Ozonation of drinking water: Part I. Oxidation kinetics and product
703 formation. Water Research 37(7), 1443-1467.

704 von Gunten, U., Salhi, E., Schmidt, C.K. and Arnold, W.A. (2010) Kinetics and Mechanisms of
705 N-Nitrosodimethylamine Formation upon Ozonation of N,N-Dimethylsulfamide-Containing
706 Waters: Bromide Catalysis. Environmental Science & Technology 44(15), 5762-5768.

707 von Sonntag, C. and von Gunten, U. (2012) Chemistry of ozone in water and wastewater
708 treatment. From basic principles to applications, IWA Publishing, London.

709 Wang, X., Huang, X., Zuo, C. and Hu, H. (2004) Kinetics of quinoline degradation by O₃/UV in
710 aqueous phase. Chemosphere 55(5), 733-741.

711 Willems, M.I., Dubois, G., Boyd, D.R., Davies, R.J.H., Hamilton, L., McCullough, J.J. and van
712 Bladeren, P.J. (1992) Comparison of the mutagenicity of quinoline and all
713 monohydroxyquinolines with a series of arene oxide, trans-dihydrodiol, diol epoxide, N-oxide
714 and arene hydrate derivatives of quinoline in the Ames/Salmonella microsome test. Mutation
715 Research/Genetic Toxicology 278(4), 227-236.

716 Yang, L., Xu, J. and Liu, Z. (2003) Study on the mutagenicity of phenolic compounds by the
717 Ames test. Journal of Northeast Normal University (Natural Sciences Edition) 35(1), 82-85.

718 Zechner, J., Prangova, L., Grabner, G.I. and Getoff, N. (1976) Photolysis of aniline in aqueous
719 solution: spectroscopic and kinetic studies and product analysis. . Journal of Physical Chemistry
720 102(1-4), 137-150.

721 Zeiger, E. (1987) Carcinogenicity of Mutagens: Predictive Capability of the Salmonella
722 Mutagenesis Assay for Rodent Carcinogenicity. Cancer Research 47(5), 1287-1296.

723 Zhang, W.-B., An, T.-C., Xiao, X.-M., Fu, J.-M., Sheng, G.-Y. and Cui, M.-C. (2003)
724 Photochemical Degradation Performance of Quinoline Aqueous Solution in the Presence of
725 Hydrogen Peroxide. Journal of Environmental Science and Health, Part A 38(11), 2599-2611.

726

727

Figure 1. Experimental procedure for testing of the selected CCL3 compounds and their transformation products (TPs): (i) Assessment of the susceptibility of the target compounds to oxidation by $\cdot\text{OH}$, (ii) effect removal during oxidative transformation of parent compound, (iii) test for toxic transformation products with alternative modes of action. The main criteria for the feasibility of an oxidative process was based on the second order rate constant for the reaction with $\cdot\text{OH}$. Typically, compounds with low $\cdot\text{OH}$ rate constants do not react with ozone. Photolysis was also a selection criteria but except for a few compounds (see below), it is typically overruled by $\cdot\text{OH}$ radical reactions in AOPs.

Figure 2. Mutagenic equivalents MEQ-2-NF (2-NF: 2-nitrofurán) during degradation of nitrobenzene by UV photolysis in the TA98 strain without the S9-mix. For explanation of MEQ-2-NF see Materials and Methods section.

Figure 3. Evolution of relative mutagenicity during cumene hydroperoxide abatement by ozone, direct photolysis and $\cdot$ OH in the TAMix strains without the addition of the S9-mix.

Figure 4. Evolution of mutagenicity (expressed as mutagenic equivalents MEQ-AA (AA: 2-aminoanthracene) during transformation of aniline by direct photolysis in the TA98 strain in presence of the S9-mix. For explanation of MEQ-AA see Materials and Methods section.

Figure 5. Evolution of EEQ/EEQ_0 during degradation of 4,4'-methylendianiline (MDA, 100 μ M, pH 7) by ozone and $\cdot$ OH. EEQ measured by the YES assay.

Figure 6. Estrogenic activity (in EEQ) during transformation of quinoline (100 μ M) by $\cdot$ OH: (a) medium-pressure lamp (filtered to emit between 308-410 nm) in a photoreactor with 5 mM H_2O_2 ; (b) low-pressure lamp with 1 mM H_2O_2 .