A review of ecotoxicity reduction in contaminated waters by heterogeneous photocatalytic ozonation.

Brent Lashuk[a], Viviane Yargeau[a*]

[a]Department of Chemical Engineering, McGill University, Quebec H3A 0C5

*Corresponding Author

Viviane Yargeau

Professor

Chemical Engineering

McGill University

3610 Rue University

Montreal, QC, Canada, H3A OC5

E-mail: viviane.yargeau@mcgill.ca

Phone: +1-514-398-2273

Fax: +1-514-398-6678

Abstract

The widespread deterioration of our water systems requires new wastewater treatment technologies to ensure environmental protection. Conventional wastewater treatments were not designed for, and are therefore ineffective, at removing contaminants of emerging concern (CECs) such as pharmaceuticals, personal care products, pesticides, and industrial chemicals. Furthermore, treatment processes capable of breaking down CECs may produce toxic transformation products more harmful than the parent chemicals. Heterogeneous photocatalytic ozonation provides a promising option with high degradation and mineralization of organic compounds. The aim of the present paper is to review ecotoxicity reduction in water treated by heterogeneous photocatalytic ozonation as a measure of process viability. The discussion investigates changes in toxicity based on a variety of toxicity tests performed to evaluate potential effects on ecosystems, the types of catalysts and radiation sources used, the nature of the target contaminants, and the type of water matrix treated. Acute toxicity testing, TiO_2 catalysts, and mercury-vapour lamps including blacklights were dominant in the reviewed studies, investigated in 86%, 84% and 79% of the papers, respectively. Pharmaceuticals were the main group of chemicals treated and the water matrices used were predominantly pure water and secondary effluent. Overall, the findings of these studies provide evidence that photocatalytic ozonation is an efficient process to remove persistent organic compounds while, most of the time, not increasing the toxicity of the effluent (as reported by 86% of the studies). Due to the wide variation in experimental set-ups, no clear correlation between reaction conditions and toxicity was determined, however, *V. fischeri* acute toxicity assays and chronic/sublethal tests appeared most sensitive to transformation products. Future studies need to a) incorporate multiple toxicity tests to produce a more reliable and inclusive ecotoxicity assessment of treated effluent and b) investigate immobilized catalysts and energy efficient radiation sources (i.e. solar and LEDs) for industrial applications.

Keywords

Advanced oxidation processes (AOPS), toxicity, bioassays, chemicals of emerging concern (CECs), wastewater

1 Introduction

Water is essential for all life on Earth, but clean supplies are being threatened with the rise in anthropogenic pollution. Contaminants of emerging concern (CECs) are chemical compounds increasingly being detected in water systems with impacts that are not fully understood. They are often low concentration pollutants, such as pharmaceuticals, personal care products (PCPs), pesticides, and industrial chemicals, which may pose a threat to human health and the environment (Petrie et al., 2015). Research has shown that wastewater treatment plants (WWTPs) are releasing these contaminants along with the treated wastewater because conventional treatment processes are ineffective for removal or degradation of several CECs (Bolong et al., 2009; Schröder et al., 2016). Once in our water systems, CECs and their transformation products (TPs) have adverse effects on aquatic organisms such as a decrease in fish fertility and cytotoxicity (Bolong et al., 2009). To reduce levels of CECs in the aqueous environment, new water treatment methods are being developed and implemented.

Advanced oxidation processes (AOPs) describe a broad family of treatment processes that incorporate chemical oxidation, namely with hydroxyl radicals, to unselectively degrade persistent organic pollutants. These processes include, but are not limited to, catalyzed and non-catalyzed ozonation, plasma, electrochemical oxidation, photocatalysis, and combined AOPs. Detailed reviews have been published recently on the use and success of AOPs for the degradation of CECs (Gomes et al., 2017a; Kanakaraju et al., 2018; Miklos et al., 2018). Chemical processes like AOPs are ideal treatment methods since they can degrade and mineralize contaminants, rather than adsorbing or concentrating contaminants for further treatment. However, these processes are often energy intensive and incomplete mineralization has the potential to produce toxic transformation products (Kanakaraju et al., 2018; Miklos et al., 2018).

One type of AOP that has recently gained significant research interest is photocatalytic ozonation. The growth in popularity can be seen in Figure 1 with the increase in publications and citations involving photocatalytic ozonation over the last two decades. Both homogeneous and heterogeneous catalysts have been used in photocatalytic ozonation. Homogeneous catalysts were shown to degrade organic pollutants (Espejo et al., 2015; Gimeno et al., 2016) and to decrease the chemical and biological oxygen demand of wastewater (Kern et al., 2013). However, the catalyst cannot be separated from the treated water, which results in secondary pollution with the addition of metal ions. This addition has even been shown to increase toxicity of the treated water when using Fe(III) due to ferrioxalate formation (Espejo et al., 2015). Unlike homogeneous catalysts, heterogeneous catalysts can be recovered from the treated water and reused. The use of immobilized catalyst also facilitates upscaling and use in industrial reactors. This technique provides an environmentally friendly approach for wastewater treatment and remediation (Mecha and Chollom, 2020). These advantages make heterogenous catalysts a more viable option and for that reason, the present review is limited to studies using heterogeneous catalysts.

The photocatalytic ozonation process combines photocatalysis (a photocatalyst and a suitable light source) and ozonation (feed of an ozone containing gas) at ambient temperatures, creating a synergy that is effective for degrading and mineralizing persistent organic pollutants through a variety of pathways as described in reviews by Mehrjouei et al. and Xiao et al. and summarized in Figure 2 (Mehrjouei et al., 2015; Xiao et al., 2015). The addition of ozone to photocatalysis addresses one of the main factors limiting efficiency of photocatalysis, which is the electron-hole recombination. The ozone present scavenges the photo-excited electrons formed at the surface of the catalyst, which increases the catalyst efficiency and contributes to the formation of additional oxidants, such as hydroxyl radicals, that reacts with the organic compounds to be removed.

The main mechanism for the degradation of organic compounds by photocatalytic ozonation is the reaction with hydroxyl radicals which can be generated through multiple mechanisms involving direct photolysis, photocatalysis, and indirectly through chain reactions (Xiao et al., 2015). The production of hydroxyl radicals from direct photolysis involves O_3 and H_2O_2 precursors. O_3 will absorb wavelengths less than 300 nm, generating reactive oxygen species that will react with water to produce hydroxyl radicals in solution (Eq. 1-2). O_3 will also react with water in the presence of UV radiation to produce H_2O_2, which can cleave directly into hydroxyl radicals under UV radiation (Eq. 3-4).

$$\mathrm{O_3} + hv \rightarrow \mathrm{O_2} + \mathrm{O^\bullet} \tag{1}$$

$$\mathrm{O^\bullet} + \mathrm{H_2O} \rightarrow 2\,{}^\bullet\mathrm{OH} \tag{2}$$

$$\mathrm{O_3} + \mathrm{H_2O} + \mathrm{hv} \rightarrow \mathrm{H_2O_2} + \mathrm{O_2} \tag{3}$$

$$\mathrm{H_2O_2} + hv \rightarrow 2\,{}^\bullet\mathrm{OH} \tag{4}$$

Through the generation of electron – hole pairs (e^- and h^+) by UV excitation of the photocatalyst (M) (Eq. 5), both e^- and h^+ can initiate hydroxyl radical formation. The h^+ on the surface of the catalyst can react with adsorbed water to readily form hydroxyl radicals (Eq. 6), a dominant mechanism in aqueous solution.

$$\mathrm{M} + hv \rightarrow \mathrm{M(e^- + h^+)} \tag{5}$$

$$\mathrm{h^+} + \mathrm{H_2O_{(ads)}} \rightarrow {}^\bullet\mathrm{OH} + \mathrm{H^+} \tag{6}$$

The photoexcited electron (e^-) initiates many reactions in photocatalytic ozonation because of the ability of oxygen species to accept electrons. Adsorbed O_3 will be reduced to the ozonide radical, which will proceed through chain reactions resulting in the production of oxygen and a hydroxyl radical (Eq. 7-9). Similarly, O_2 can be reduced to the superoxide anion radical, a highly oxidizing species which in the presence of O_3 produces the ozonide (Eq. 10-11) which as shown in Eq. 8-9 can form hydroxyl radicals in solution. The reaction with ozone and e^- to produce the ozonide radical (Eq. 7) is not only important for the formation of hydroxyl radicals. This step is also critical to the high efficiency and synergism of photocatalytic ozonation as it is responsible for the inhibition of rapid electron – hole recombination (Mena et al., 2012).

$$\mathrm{O_{3(ads)}} + \mathrm{e^-} \rightarrow \mathrm{O_3^{\bullet -}} \tag{7}$$

$$\mathrm{O_3^{\bullet -}} + \mathrm{H^+} \rightarrow \mathrm{HO_3^\bullet} \tag{8}$$

$$\mathrm{HO_3^{\bullet} \rightarrow O_2 + {}^{\bullet}OH} \tag{9}$$

$$\mathrm{O_{2(ads)} + e^{-} \rightarrow O_2^{\bullet -}} \tag{10}$$

$$\mathrm{O_2^{\bullet -} + O_3 \rightarrow O_3^{\bullet -} + O_2} \tag{11}$$

The formation of hydroxyl radicals and other reactive oxygen species in the photocatalytic ozonation leads to rapid degradation of organic compounds. With R representing organic compounds, X^* as reactive organic species, and I as intermediate organic compounds, Eq. 12-13 demonstrate the degradation of organic compounds to intermediates and eventually to complete mineralization. The high oxidative potential of hydroxyl radicals allows for unselective attacks on organic compounds which can lead to higher levels of mineralization compared to what can be achieved using conventional methods such as ozonation and chlorination.

$$\mathrm{R + X^{*} \rightarrow I + X} \tag{12}$$

$$\mathrm{I + X^{*} \rightarrow \cdots \rightarrow H_2O + CO_2 + inorganic\ ions} \tag{13}$$

Although the hydroxyl radical mechanism is dominant for the removal of most CECs, more stable pollutants such as perfluorooctanoic acid (PFOA) rely on a secondary mechanism of direct oxidation of adsorbed compounds on the catalyst surface since hydroxyl radicals alone are ineffective (Szajdzinska-Pietek and Gebicki, 2000). Degradation of PFOA by photocatalytic ozonation was found to be initiated by direct oxidation with the photo-generated hole (h^+) (Eq. 14), followed by spontaneous decarboxylation (Eq. 15). The presence of hydroxyl radicals enhanced the process (Eq. 16) and encouraged subsequent chain reactions (Eq. 17-18) (Huang et al., 2016).

$$\mathrm{C_7F_{15}COO^{-} + h^{+} \rightarrow C_7F_{15}COO^{\bullet}} \tag{14}$$

$$\mathrm{C_7F_{15}COO^{\bullet} \rightarrow C_7F_{15}^{\bullet} + CO_2} \tag{15}$$

$$\mathrm{C_7F_{15}^{\bullet} + {}^{\bullet}OH \rightarrow C_7F_{15}OH} \tag{16}$$

$$C_7F_{15}OH \rightarrow C_6F_{13}COF + H^+ + F^- \quad (17)$$

$$C_6F_{13}COF + H_2O \rightarrow C_6F_{13}COO^- + 2H^+ + F^- \quad (18)$$

The nature and toxicity of the transformation products formed during wastewater treatment is important to evaluate the potential for implementation of a process. Oftentimes, the transformation products are just as toxic, if not more so than the parent compounds (Donner et al., 2013; Magdeburg et al., 2014; Neuwoehner et al., 2010). Although transformation product formation can be monitored, toxicity should be assessed to take into consideration unknown products, the type of matrix, and the presence of different contaminants. Although a significant number of studies have been published on photocatalytic ozonation, relatively few analyzed the toxicity of photocatalytic ozonation treated waters. Recently, reviews have been published on AOPs for toxicity reduction in real wastewater (Rueda-Marquez et al., 2020), industrial wastewater (Malik et al., 2020), but neither focused specifically on photocatalytic ozonation. A recent review by Mecha and Chollom evaluating photocatalytic ozonation as a viable method for wastewater remediation also briefly covered toxicity (Mecha and Chollom, 2020) but only a few studies were selected and there was no in-depth discussion on the topic. Although these previous works mention toxicity, to our knowledge there is no detailed review on photocatalytic ozonation and toxicity, which is essential to assess the potential of this emerging approach as a wastewater treatment. The present review provides a comprehensive summary of studies monitoring ecotoxicity as an output measure of water treatment using heterogeneous photocatalytic ozonation for the removal of CECs. Ecotoxicity was specified since the release of treated wastewater has a direct impact on the environment but can hardly be linked directly to toxic effects on humans, which the term toxicity can suggest. The period of study includes all papers published from 1999 to 2020 on the topic found through Web of Science and Scopus. These toxicity findings were analyzed and discussed with a focus on the types of toxicity tests, catalysts and radiation sources, nature of the target contaminants, and the type of water matrix treated in order to determine the main trends, the potential for toxicity reduction, as well as the most promising avenues for the photocatalytic ozonation.

2 Discussion

2.1 Bioassays used to monitor treatment efficiency

The use of bioassays to monitor water toxicity has been a common practice for some decades (Bitton and Koopman, 1992; Tothill and Turner, 1996). The bioassays focus on different organisms in various trophic levels including microbes, algae, invertebrates, plants, and fish (Hassan et al., 2016). In addition, these assays focus on different types of toxicity (e.g. acute, chronic, phytotoxicity) allowing broader understanding of the potential toxicity of a sample. For a more comprehensive analysis of a sample's ecotoxicity, it is recommended to perform multiple assays (Farré and Barceló, 2003). Since bioassays are generally quick, simple, and low-cost methods, they offer vital insight into the feasibility of water treatment methods. Furthermore, they can detect the combined effect chemicals can have on one another in complex matrices as opposed to analytical methods that can only identify and quantify selected toxic compounds, making toxicity tests a valuable complementary method for water assessment (Escher et al., 2020).

Implementing toxicity tests when developing new methods for wastewater treatment is essential to make sure that the finished product does not present an environmental hazard. It has been shown that toxic transformation products can be formed during wastewater treatment, particularly chlorination and ozonation, but in advanced treatment methods as well (Magdeburg et al., 2014; Maya et al., 2018; Monarca et al., 2000; Petrie et al., 2015; Watson et al., 2012). The formation of these toxic compounds is due to the incomplete mineralization of contaminants or chemical reactions creating hazardous by-products during additive treatments like chlorination.

Complete mineralization of organic contaminants to their respective ions and CO_2 is the optimal result of wastewater treatment processes producing non-toxic effluent. However, this result is usually not achieved because of the complexity of the matrices and economic considerations. The synergistic combination of photocatalysis and ozonation for photocatalytic ozonation has shown promise for increased mineralization and removal of persistent organic compounds (Mecha and Chollom, 2020; Mehrjouei et al., 2015; Xiao et al., 2015). A review of the literature indicates that 36 studies conducted on heterogeneous photocatalytic ozonation included the monitoring of ecotoxicity as an output measure of treatment efficiency. These studies have been summarized in Table 1. Two studies used mammalian cells in their assays, however they were applied as a mean to assess possible toxic effects in the context of ecotoxicity, not human toxicity, and were therefore included in the present literature review. Acute toxicity assessment is reported in the majority of the studies. Phytotoxicity, chronic toxicity, cytotoxicity, estrogenicity, and oxidative stress assays were incorporated into these studies as well (Figure 3). Detailed discussion of the various toxicity tests is included in subsequent sections.

2.1.1 Acute toxicity assays

The most common practice for toxicity analysis in water is acute toxicity testing using the standardized microcrustacean *Daphnia magna* (International Organization for Standardization (ISO), 2012) or luminescent bacteria *Vibrio fischeri* (International Organization for Standardization (ISO), 2007) bioassays. This practice was used by 86% of the photocatalytic ozonation papers presented in Table 1 examining acute toxicity of the treated effluent, with the majority applying *D. magna* (References 3, 7, 10-12, 14, 15, 17, 20-22, 34, 35, Table 1) or *V. fischeri* bioassays (References 4, 5, 12, 16, 22, 24, 25, 28-33, Table 1) (Figure 4). While both bioassays are accepted methods, they may vary in effectiveness towards wastewater samples due to differences in selectivity. An example of this is the photocatalytic ozonation treatment of carbamazepine, which resulted in an increase in acute toxicity towards *V. fischeri* but showed no acute toxicity towards *D. magna* (Oropesa et al., 2018). This emphasizes the need for multiple toxicity assays to gain a more representative picture of the potential ecotoxicity. The other acute toxicity assays performed included bacteria (*S. aureus, E. coli* and *P. phosphoreum,* References 1, 2, 6), mosquito larvae (*C. pipiens,* References 8 and 9), crustaceans (*D. parvula* and *A. salina,* References 8, 9, 13, 23), and clams (*C. fluminea,* References 25, 28, 29, 31). 73% of the 11 studies which incorporated these alternative acute toxicity assays used them in tandem with other toxicity tests.

Of the 31 studies that performed acute toxicity tests, 10 showed complete or near complete detoxification and only 3 showed an increase in toxicity in the treated water relative to the untreated effluent. The remaining 18 acute toxicity studies showed either no significant difference between untreated and treated water or had significant decreases in toxicity although not complete removal.

The decreases in toxicity can be directly linked to the removal of the harmful parent(s) or intermediate(s) and the high mineralization efficiency. However, the use of different acute toxicity assays with varying sensitivities can affect the observed trend and makes direct comparison of studies difficult. Interestingly, the assay that was used in all the studies reporting an increased toxic responses was *V. fischeri* (Mahne et al., 2012; Martini et al., 2019; Oropesa et al., 2018). The responsible contaminants were textile dyes (Mahne et al., 2012), carbamazepine (Oropesa et al., 2018) and sulfamethoxazole (Martini et al., 2019). This could be due to a higher sensitivity of *V. fischeri*, although the responses of assays can also be largely affected by contaminant type (Czech et al., 2014; Parvez et al., 2006; Terasaki et al., 2009). The higher toxicity reported by Mahne et al., might also be explained by the short reaction time that was used considering that the treatment was stopped as soon as the dye solution was colourless (Mahne et al., 2012). This could have resulted in the formation of transformation products that did not have time to degrade.

2.1.2 Chronic toxicity assays

Higher removals of pollutants contribute to lower concentrations of toxic compounds and their transformation products in the environment, which may be below the threshold for acute toxicity to be observed. Chronic toxicity tests along with the analysis of sublethal effects of wastewater effluent can evaluate impacts on organisms' lifecycles such as reproduction and growth rather than focusing on short term impacts and lethality. One of the main conclusions from the comprehensive review on AOPs for water treatment by Rizzo (Rizzo, 2011) was that future work should include the chronic effects of treatments since acute toxicity tests may not be well-suited for environmentally relevant contaminant concentrations. For example, diclofenac at environmental concentrations can cause long-term and sublethal effects on fish and mussels (Gonzalez-Rey and Bebianno, 2014; Lonappan et al., 2016). Although there is more likely to be chronic ecotoxicity from the continuous release of low concentrations of contaminants from wastewater treatment plants, these assays are less prevalent in literature. Unlike quick and simple acute toxicity bioassays, chronic toxicity tests are more complex because sublethal effects are more difficult to monitor, take time to study, and commonly require specialized equipment and personnel (Oropesa et al., 2018).

For photocatalytic ozonation, only two studies have looked at the chronic toxicity of the treated water and the findings showed mixed results. These studies evaluated the microcrustaceans *Ceriodaphnia dubia* (Horn et al., 2014) and *Daphnia magna* (Oropesa et al., 2018). The study by Horn et al. evaluated photocatalytic ozonation for toxicity removal from university wastewater with immobilized TiO_2 by assessing the effects on *C. dubia* survival and reproduction. The researchers found no significant difference between untreated and treated water with both exhibiting high chronic toxicity. In the second study, Oropesa et al. 2018 used photocatalytic ozonation with TiO_2 for carbamazepine removal and observed that the undiluted treated water induced daphnid mortality four days after exposure, preventing analysis of chronic effects. This was in stark contrast to the acute toxicity tests of the same solution showing no toxicity towards *D. magna*. The samples were diluted 25% and 50% to analyse the growth, mortality, molting inhibition, reproduction age, total number of broods and viable offspring, embryo toxicity, and production of male offspring. Relative to the negative control, they found photocatalytic ozonation treated water increased the age of first reproduction, increased the number of total offspring, and showed a higher average body length for daphnids, while all other results were not significantly different. It is interesting to note the contrasting chronic toxicity results of diluted and undiluted samples, which showed that the diluted samples with concentrations closer to what would be present in the environment, were less toxic than the negative control. This also highlights the importance of performing multiple assays and taking into consideration the environmentally relevant concentrations.

2.1.3 Phytotoxicity assays

Phytotoxicity tests involve assessing toxic effects towards plants or algae and by evaluating a number of factors such as growth (e.g. root length, germination, biomass), enzyme activity, and colour. These types of tests are relatively inexpensive but require extended growth periods of days to weeks and are therefore impractical for rapid toxicity screening. For photocatalytic ozonation, phytotoxicity was the second most common type of bioassay performed with 9 studies (25%), 7 of which observed non-toxic effluent (References 18, 23, 25, 26, 28, 29, 31) and only 1 exhibiting toxic results (Santiago-Morales et al., 2012). The phytotoxicity assays involved assessing germination index (GI) in *Lepidium sativum* (water cress), root length in *Lactuca sativa* (lettuce) and *Solanum lycopersicum* (tomato), and photosynthetic pigments in *Pseudokirchneriella subcapitata* (algae) and *Spirodela polyrhiza* (duckweed). The GI values for *L. sativum*, which takes into account germination rate and radicle length as described by Fernandes et al. (Fernandes et al., 2020a), indicated that photocatalytic ozonation of paraben contaminated water produces plant-safe effluent (References 25, 26, 28-30). Similarly, the *L. sativa* and *S. lycopersicum* root length assays showed a continuous decrease in phytotoxicity throughout treatment of water contaminated with herbicides, resulting in complete detoxification (Rajah et al., 2019; Solís et al., 2016a). In contrast, both studies investigating photosynthetic pigments showed the formation of toxic transformation products that were either completely removed with further treatment (Fathinia and Khataee, 2015) or remained leaving higher toxicity effluent (Santiago-Morales et al., 2012). These two studies are the only two showing transient or persistent increased phytotoxicity suggesting that particular attention must be given to potential transformation products formed during photocatalytic ozonation of dyes to which plants might be sensitive. In all of the phytotoxicity studies, the results aligned with toxicity assays performed in tandem with the exception of Santiago-Morales et al. which showed decreases in acute toxicity towards *D. magna* and *V. fischeri* and an increase in phytotoxicity for *P. subcapitata*.

2.1.4 Cell-based assays and oxidative stress tests

Cell-based bioassays are another area of toxicity testing that focus on many different endpoints in toxicity pathways. These can look at the overall cytotoxic effects on a cell cultures, or at intermediate indicators of toxicity such as effects on cell receptors, adaptive stress responses, metabolism, and reproduction. While sublethal and cell level effects do not directly predict overall toxicity, they can be indicative of potential adverse effects. These tests are ideal to be used with *in vivo* tests and chemical analysis, and it is recommended to use a combination of cell-based tests which encompass induced drug metabolism, endocrine disruption, and adaptive stress responses (Escher et al., 2014).

Two studies incorporated cell-based assays for photocatalytic ozonation treatment which included the MTT assay (metabolic activity) and LDH assay (cytotoxicity) on mammalian cells, as well as the YES assay (estrogenic effects). Although these studies incorporate mammalian cells in the bioassays, they were used a measure of general toxicity, not toxicity to humans. Moreira et al. 2016 used both MTT and LDH assays on Caco-2, hCMEC/D3, and L929 fibroblast cell lines, as well as the YES assay (Moreira et al., 2016). The researchers observed an increase in cytotoxicity for the MTT assay with Caco-2 and hCMEC/D3 cell lines after photocatalytic ozonation treatment of wastewater containing 22 CECs, although the differences were statistically insignificant. The LDH assay showed negligible changes in toxicity between treated and untreated solutions. For the YES assay, they did not detect any estrogenic activity in the treated or untreated waters. Mecha et al. 2017 only utilized the MTT with Vero cells and were able to show significant decreases in toxicity for treated municipal wastewater (Mecha et al., 2017). This study evaluated TiO_2, Ag-TiO_2, Cu-TiO_2, and $FeTiO_2$ catalysts under UVA and solar radiation and found the largest increase in cell viability of 29% to 80% for Fe-TiO_2 catalyst under UV light.

One of the most common methods to evaluate potential toxicity at a cellular level is by evaluating adaptive stress responses through oxidative stress assays. These can be performed with *in vitro* assays or by analyzing stress induced biomarkers in organisms. These tests are of importance in the development of AOPs as reactive oxygen species are generated during treatment, which have the potential to cause oxidative stress. Only one study investigated the oxidative stress effects of photocatalytic ozonation, which was performed by analysing superoxide dismutase (SOD), catalase (CAT), and lipid peroxidation (LPO) levels in *D. magna* (Oropesa et al., 2016). At a 6.25% dilution (comparable to environmental concentrations), the researchers showed that there were increased levels of CAT in CEC spiked secondary wastewater treated by TiO_2/O_3/Solar Light (SL), indicating an elevated presence of H_2O_2. In respect to SOD and LPO activity, no statistically significant changes were observed using the TiO_2 catalyst. In contrast, Fe_3O_4/O_3/SL treatment of the same solution had no significant differences for any of the three oxidative stress biomarkers indicating a potentially safer treatment method. It should be noted that this study also evaluated acute toxicity in *D. magna* and found the effluent was non-toxic for either catalyst after photocatalytic ozonation treatment.

2.1.5 Toxicity tests for the optimization of wastewater treatment

Toxicity tests demonstrate that the final product of photocatalytic ozonation treatment is rarely more toxic than the original solution, however, toxicity tests can also be used for monitoring treatments and optimizing the processes. This can be achieved by employing multiple endpoints to assess the evolution of toxicity over time rather than just initial and final values. When analysis of multiple endpoints was performed, the formation of toxic transformation products was observed in the early stages of photocatalytic ozonation treatment (References 8, 9, 16, 21, 23, 24). However, as treatment was continued past the time required to degrade the parent compounds, these studies showed the acute toxicity would decrease to or below the toxicity levels of the untreated water. This was especially prevalent in studies pertaining to pesticides, for which intermediates have been previously shown to be toxic, but a prolonged treatment was shown to effectively remove both parent compounds and toxic intermediates (References 5, 8, 9, 23, 24). These studies are good examples of the use of toxicity testing as an output measure to optimize the treatment conditions.

Oxidized intermediates, especially carboxylic acids, formed from incomplete mineralization of organic compounds can have toxic effects. Final increases in oxalic acid and formic acid were noted with photocatalytic ozonation (Martini et al., 2019; Oropesa et al., 2016) while other studies have showed that these acids were not formed or were completely removed (References 3, 6, 24, 32). Oxalic acid was also the contaminant of interest in Tichonovas et al. 2017 and was completely removed by photocatalytic ozonation (Tichonovas et al., 2017). These differences between studies could be explained by the type of catalyst and reaction conditions but demonstrates the potential for photocatalytic ozonation to remove the short organic acids that are difficult to remove by other methods of treatment such as ozonation (Rajah et al., 2019; Solís et al., 2016c). This further suggests that photocatalytic ozonation has the potential to avoid or control the formation of toxic transformation products.

Along with monitoring toxicity over time, evaluating residual toxicity with respect to the environment is good practice. The residual toxicity can still present environmental hazards even if toxicity levels are much lower than the untreated water. The recent studies of Gmurek et al., Gomes et al., and Fernandes et al. investigated photocatalytic ozonation for the removal of parabens, discussed residual toxicity in respect to acute toxicity (Fernandes et al., 2020a; Gmurek et al., 2019; Gomes et al., 2019). In accordance with Miralles-Cuevas et al., *V. fischeri* exhibiting luminescence inhibition (LI) levels above 30% are considered toxic (Miralles-Cuevas et al., 2017). Gmurek et al. showed a decrease of the highly toxic paraben solution to as low as 36% LI for the TiO_2 catalyst, and a range from 43% to 61% LI for doped catalysts. Gomes et al. demonstrated that in municipal wastewater with an original LI of 51%, the initial spiking with parabens resulted in 100% LI and photocatalytic ozonation treatment could decrease the LI to 48%. Fernandes et al. found that with the 10% N doped TiO_2 catalyst a decrease from 99% to 63% LI was shown for *V. fischeri*. Each of these studies of treated effluents exhibit LI values above 30% meaning they are still toxic. Furthermore, Fernandes et al. also showed that with the same catalyst the decrease in acute toxicity towards the clam *C. fluminea* was 84% to 45% - nearly a 50% decrease in toxicity, though the effluent remained toxic. All of these results demonstrate drastic reductions in acute toxicity; however, the residual toxicity of treated solutions is still important for real-world application.

2.1.6 Summary of toxicity assays for photocatalytic ozonation

In the studies reviewed, 86% of the toxicity results showed no increase in toxicity after photocatalytic ozonation treatment, which correlates to the observed high removal percentages of contaminants. Increases in toxicity were reported as the result of toxic transformation products produced during water treatment. The breakdown of types of toxicity testing used can be found in Table 2, where the number of studies which observed increased toxicity is also noted since this parameter is important for implementation of new water treatment technologies. Acute toxicity tests were performed most often (31 of 36 studies), and only 10% resulted in an increase in toxicity. The *V. fischeri* acute toxicity assay was the only test to show increased toxicity in treated effluent which suggests that this may be a more sensitive technique for acute toxicity testing in water treatment. Chronic toxicity tests were only implemented in two studies, yielding inconclusive results; one study showed no significant differences (Horn et al., 2014) and the other showed highly toxic effects when undiluted but at environmentally relevant concentrations, growth was actually enhanced by the treated effluent (Oropesa et al., 2018). Phytotoxicity analysis was the second most common type of testing performed after acute toxicity. However, only one study which assessed phytotoxicity showed an increase toxicity towards *P. subcapitata* contradicting non-toxic results for *V. fischeri* and *D. magna* acute toxicity tests with the same solution (Santiago-Morales et al., 2012). In cell-based assays, which encompassed cytotoxicity, estrogenicity, and oxidative stress, the only negative response found was for oxidative stress with an increased production of CAT due to elevated levels of H_2O_2 (Oropesa et al., 2016).

While acute toxicity assays provide relatively quick insight into the effectiveness of a water treatment process, a single assay produces a limited view at overall toxicity. As can be seen with the toxicity testing done for photocatalytic ozonation (Table 2), chronic toxicity and oxidative stress assays have the highest rate of increased toxicity, thus appear to be more sensitive to transformation products in the treated effluent. These tests are underrepresented due to the need for specialized lab equipment and greater time and cost inputs but can give crucial insight into the effects of contaminants at environmentally relevant concentrations. Of the 36 studies, 15 groups conducted multiple toxicity assays of which 20% showed disagreement in results within their study. This supports the notion that multiple bioassays should be employed for toxicity analysis to gain a better understanding of the overall toxicity and emphasis should be put on incorporating more types of assays besides acute tests. In addition, toxicity assays should be used over the course of the treatment to optimize treatment time and operating parameters to maximize the efficiency of the process and safety of the effluent. Many of the tests showed significant decreases in toxicity over the treatment but in several cases, the effluent remained at toxic levels. This review further demonstrates that wastewater guidelines focusing on the presence of specific chemical compounds should, in future, incorporate thresholds for toxicity levels and involve bioanalytical techniques for assessing the hazardous effects of treated mixtures, as suggested in recent publications (Escher et al., 2020; Neale et al., 2020).

2.2 Catalysts applied in photocatalytic ozonation treatment

The selection of a catalyst for photocatalytic experiments is based on light absorption and interaction with contaminants. In photocatalytic ozonation, the most common material used is pure TiO_2 or in combination with other transition metals (Mehrjouei et al., 2015). This is based TiO_2's high photocatalytic activity, low price, and availability. Issues arise in photocatalysis using TiO_2 due to the rapid electron hole recombination, however, this is less of an issue for photocatalytic ozonation because of the electron scavenging ability of O_3 (Huang et al., 2016; Mena et al., 2012). It is also important to consider the band gap of the catalyst; catalysts with larger band gaps (e.g. TiO_2) will require lower wavelengths of light. To increase energy efficiency and economic feasibility it is beneficial to use a catalyst that can absorb higher wavelengths of light such as UVA and visible wavelengths. Doping large band gap catalysts with metals having lower band gaps can reduce the energy required to excite the electrons into the conduction band, decrease electron-hole regeneration and increase efficiency (Yang et al., 2018). A drawback to decreasing the band gap is the decrease the oxidative potential of a catalyst, which may reduce degradation efficiency. With respect to the interaction of the catalyst with the contaminant, surface area and point of zero-charge of the catalyst are important. Higher surface areas mean more active sites and less mass-transfer limitations, which is why some researchers have incorporated activated carbon and carbon nanotubes into their catalysts (Xiao et al., 2015). The point of zero charge influences the adsorption potential of a catalyst in combination with the pH of solution and pKa of the target compounds. This is important since one of the main mechanisms in photocatalytic ozonation is the direct reduction/oxidation of the contaminant on the catalysts surface (Figure 2). For example, the point of zero of Degussa P25 TiO_2 is 6.9, (Kosmulski, 2006), meaning at a pH 5, the catalyst has an overall positive charge, which would allow negatively charged analytes (i.e. carboxylic acids) to readily adsorb and react.

2.2.1 TiO_2-based catalysts

TiO_2-based catalysts were present in 84% the studies for photocatalytic ozonation with toxicity testing. Commercially available or synthesized TiO_2 powders, doped TiO_2 powders, and immobilized TiO_2 catalysts were used (Figure 5). The results of the toxicity tests with TiO_2 catalysts showed only 10% – 16% of the studies increased toxicity (Table 3, References 11, 12, 16, 22, 33). For powdered TiO_2 catalysts, commercially available P25 TiO_2 was used nearly 75% of the time (References 1-4, 6-14, 32) compared to synthesized TiO_2 powders (References 5, 29, 31-33). This is not surprising since P25 is a well-studied and very active photocatalyst but has drawbacks due to safety with handling of the nano-particles (Baranowska-Wójcik et al., 2020) and high band-gap energy. Silver and nitrogen doped TiO_2 were the most common doped catalysts, although gold, platinum, palladium, copper, iron, and cerium were also used. Ce-TiO_2 was the only doped catalyst to show an increase in phytotoxicity after treatment of wastewater spiked with galaxolide and tonalide (Santiago-Morales et al., 2012). In two studies comparing Ag-TiO_2, Au-TiO_2, Pt-TiO_2, Pd-TiO_2, and TiO_2, all the catalysts resulted in decreased toxicity with Ag-TiO_2 showing the most potential with the highest degradation efficiency (Gomes et al., 2017b) and low associated cost (Gmurek et al., 2019).

Powdered catalysts are useful for determining if a treatment is suitable to degrade compounds and remove toxicity, however, powders are very difficult to remove and recycle and are therefore not practical for industrial application (Mehrjouei et al., 2015). Considering this, immobilized catalysts may provide a solution, but they have a lower surface area resulting in mass transfer limitations and decreased efficiency. In terms of toxicity studies using immobilized catalysts for photocatalytic ozonation treatment, the results were promising with only one out of the seven studies reporting an increase in toxicity. The increase in toxicity observed in that study was attributed to the very short reaction time only allowing for decolourization of the solution and not mineralization or further degradation of the transformation products (Mahne et al., 2012). The immobilized catalysts all incorporated TiO_2 as the photocatalyst and the supports included acrylic ramps (Horn et al., 2014; Machado et al., 2007), Ahlstrom photocatalytic paper (Mahne et al., 2012), ceramic plates (Fathinia and Khataee, 2015), glass Raschig rings (Moreira et al., 2016), and glass rods (Tichonovas et al., 2018, 2017), with no specific trends in preferred support material.

2.2.2 Other metal catalysts

Other transition metal catalysts used were WO_3 (Figueredo et al., 2019), ZnO (Biglari et al., 2017; Yeber et al., 1999), and Fe_3O_4 (Espejo et al., 2014; Gimeno et al., 2016; Oropesa et al., 2016), none of which increased the effluent toxicity. The study by Oropesa et al. showed a direct comparison between TiO_2 and Fe_3O_4, finding that Fe_3O_4 had not induced oxidative stress under solar light photocatalytic ozonation whereas TiO_2 did (Oropesa et al., 2016). This highlights the main benefit from using these alternative metals to TiO_2, which is the lower band gaps of the semiconductors and therefore the lower excitation wavelength required, which may reduce operating costs. The degradation efficiency of the ZnO, Fe_3O_4, and WO_3 catalysts were compared to P25 TiO_2 in four of the studies. Similar degradation results were shown for ZnO (Yeber et al., 1999), lower TOC removal was observed with WO_3 (Figueredo et al., 2019), and Fe_3O_4 showed contrasting results with both lower overall degradation (Gimeno et al., 2016) and higher TOC removal (Oropesa et al., 2016). Although these results show metal-based catalysts besides TiO_2 can be effective for degradation and toxicity reduction, there are currently very few of these studies and more research is needed to comment on the risk of formation of toxic transformation products.

2.2.3 Carbon-based catalysts

Photocatalytic ozonation with carbon-based catalysts such as activated carbon (AC), carbon nanotubes (CNT) and doped CNT is interesting due to a combination of high surface area, strong adsorption properties, high radical formation and O_3 decomposition abilities of CNT, with the photocatalytic behavior of metal oxide catalysts. Three studies in the review have investigated such catalysts, References 32, 33,

36. Bare CNT as well as TiO_2/CNT and Fe/CNT have been analyzed for effluent toxicity with photocatalytic ozonation (Martini et al., 2019; Orge et al., 2017). Although both studies resulted in high degradation levels, Martini et al. showed an increase in toxicity for all catalysts used (TiO_2, CNT, TiO_2/CNT, Fe/CNT) when removing sulfamethoxazole while Orge et al. showed that photocatalytic ozonation treatment of metolachlor with TiO_2/CNT could decrease toxicity. Conflicting results were also reported in terms of removal of transformation products: Martini et al. found an increase in carboxylic acids in the final solution including oxalic and oxamic acid (Martini et al., 2019) and conversely, Orge et al. reported a removal of the oxalic and oxamic acid intermediates formed in the treatment (Orge et al., 2017). The use of AC was demonstrated using olive stone activated carbon for the degradation of clopyralid (Rajah et al., 2019). This work showed that AC photocatalytic ozonation effectively degrades and completely eliminates the phytotoxicity in the treated solutions at a higher rate than photolytic ozonation and catalytic ozonation, with higher mineralization.

2.2.4 Summary of the potential of the various catalysts to impact residual toxicity

The increases in toxicity observed in the photocatalytic ozonation studies with respect to the catalysts used can be seen in Table 3. Increased residual toxicity was more frequently reported with the use of TiO_2 catalyst, but this is likely proportional to the representation of the number of studies which used TiO_2. Alternative catalysts including WO_3, Fe_3O_4, ZnO, and AC were demonstrated as effective catalysts in photocatalytic ozonation systems and did not elevate toxicity levels. The use of CNTs demonstrated an increase in toxicity in one study but not in another study. The small number of studies for each type of catalyst and the difference in contaminants and matrices used make it difficult to draw conclusions on possible links between the types of catalyst used and the impact on toxicity. However, considering the importance of immobilized catalysts for industrial use of photocatalytic ozonation, it is key to note that the studies showed immobilized catalysts gave comparable results to powdered catalysts in terms of toxicity reduction while also achieving high degradation of parent compounds. In addition, the use of doped catalysts and alternative metals with lower band gaps is beneficial for energy efficiency and compatibility with solar radiation. Doping catalysts is thus an important consideration for catalyst design since this can create heterojunctions between different crystalline phases and different semi-conductors, which can increase degradation efficiency, and therefore toxicity reduction. Finally, the incorporation of high surface area and high adsorption catalysts, particularly ones that incorporate carbon sources, demonstrate a unique opportunity to enhance surface reactions and increase removal of toxicity and contaminants. This could prove beneficial with photocatalytic ozonation and the removal of very stable compounds such as PFOA which rely on direct surface reactions to initiate their degradation (Huang et al., 2016).

2.3 Radiation types applied

The choice of radiation source in photocatalytic ozonation is largely dependent on the wavelength required to excite the valence electrons on the photocatalyst. As an example, TiO_2 has a large band gap of 3.2 eV, requiring wavelengths lower than 390 nm for electron excitation. This is one of the main drawbacks of TiO_2 as a photocatalyst because the requirement for higher energy radiation sources which limits the use of solar radiation since only a very small portion of sunlight contains adequate energy. UV sources encompass a large portion of the overall cost of photocatalytic ozonation, so development of treatment systems based on more efficient light sources (i.e. LEDs) or an increased use of solar light in photocatalytic ozonation systems is needed (Xiao et al., 2015).

The dominant radiation sources used in photocatalytic ozonation toxicity studies were mercury lamps (Figure 6), which ranged from UV-C to visible wavelengths, mainly corresponding to the use of TiO_2 powdered and immobilized catalysts. Although there is a high energy cost associated with the use of these lamps, they are relatively inexpensive. They can also emit wavelengths less that 300 nm to directly interact with aqueous O_3 and H_2O_2 to produce hydroxyl radicals and enhance contaminant degradation. Filtered mercury lamps (“blacklights”), with a maximum wavelength of 365-370 nm, were used in over one-third of the studies to test photocatalytic activity at near visible wavelengths. The slightly higher wavelength was used mainly with doped TiO_2 catalysts due to the reduction in catalyst band gap energy.

Concentrated solar radiation or synthetic solar radiation (xenon arc lamps) were also common. With the increase in wavelength, most of these studies focused on non-TiO_2 catalysts such as Fe_3O_4 (Gimeno et al., 2016; Oropesa et al., 2016), AC (Rajah et al., 2019), and WO_3 (Figueredo et al., 2019). At these wavelengths, none of the non-TiO_2 catalysts had an increase in toxicity, but TiO_2 under concentrated solar and the same contaminants as Fe_3O_4, showed increase in oxidative stress (Oropesa et al., 2016). The Ce-TiO_2 under UVA-vis Xe-arc lamp radiation also showed an increase in phytotoxicity (Santiago-Morales et al., 2012). The use of solar radiation would be the most cost-effective approach for photocatalytic ozonation, however further studies with solar photocatalysts catalysts and efficiencies are needed.

Only one study incorporated LEDs (382 nm) into the design of their photocatalytic ozonation reactor using immobilized TiO_2 (Moreira et al., 2016). LEDs are an energy efficient alternative to fluorescent UV lamps with higher output and lower energy costs. Using high intensity LEDs (2 x 10W), the researchers were able to demonstrate near complete removal of 22 compounds from wastewater and a complete removal in drinking water, while observing a decrease or no statistically significant change in toxicity. Moreira et al. incorporates both an immobilized catalyst and an energy efficient light source for photocatalytic ozonation; a practical method for upscaling reactors and more research should follow this example.

2.3.1 Summary of the potential of the various radiation types in combination with specific catalysts to lower residual toxicity

The radiation source in combination with the selected catalyst plays an important role in the detoxification of contaminated water. Table 4 summarizes this data for photocatalytic ozonation. All radiation sources showed at least one occurrence of increased toxicity except LEDs. As shown in Section 2.2, types of TiO_2

catalyst were responsible for nearly all of the results with increased toxicity. In combination with radiation source, a higher percentage of studies showed an increase in toxicity when combining TiO_2 based catalysts with UVA-vis radiation. This could be due to the limitations of TiO_2-based catalysts needing photo-excitation by lower wavelengths because of its higher band gap energy. The addition of hydroxyl radicals and other reactive oxygen species formed in solution under UVC with ozone and hydrogen peroxide (shown above in Eq. 1-4) could also help degradation and lead to less toxic effluent. However, a recent study tested the effects of different UV radiation sources (A, B, and C) on the degradation of two pharmaceuticals using photocatalytic ozonation and an immobilized TiO_2 catalyst and found the TiO_2 catalyst with UVA radiation was favoured (Fathinia et al., 2020). The highest degradation resulted when performing photocatalytic ozonation under UVA light, pointing to the synergistic heterogeneous reactions on the catalyst surface having the greatest contribution to the production of reactive oxygen species and, in turn, degradation. This also supports using lower energy and less expensive radiation sources for increased economic feasibility, such as LEDs with their high efficiency and long lifetimes.

A final consideration when assessing the effects of radiation source on toxicity reduction is the intensity or photon flux of the light source used. More powerful light sources increase the intensity and photon flux and allow for greater excitation of the photocatalyst. However, the efficiency of the catalyst must be considered so that the system is not employing excess energy. For example, the black lights incorporated into the studies in Table 1 (References 4, 8, 9, 12, 23-26, 28-31, 35) had a relatively low intensity and photon flux but were still capable of high degradation rates and decreasing toxicity. This is also a limitation when using solar radiation. Mecha et al. reported the radiation intensity from the parabolic solar collector to be 3.76 e-3 mW/cm^2, which is orders of magnitude lower than artificial radiation sources (Mecha et al., 2017). Although this can be addressed with increasing reaction times, it may hinder the production of reactive oxygen species and lead to lower levels of toxicity reduction and degradation. The synthesis of catalysts with increased catalytic performance based on optimal physical and chemical properties such as adsorption affinity, band gap, or charge transfer capabilities is required for use in systems with lower intensity radiation sources to address potential limitations from photocatalyst excitation.

2.4 Nature of the contaminants and types of matrices assessed

2.4.1 Types of compounds investigated

The contaminants studied for photocatalytic ozonation toxicity fall into four general categories: pharmaceuticals, industrial compounds, pesticides, and personal care products (PCPs). The most studied group of compounds is pharmaceuticals (Table 5), which is consistent with the high occurrence of these chemicals in the aquatic environment (López-pacheco et al., 2019). Pharmaceuticals were the focus of 3 of the 5 studies which had an increase in toxicity, while the other two studies were based on industrial chemicals and PCPs.

The pharmaceutical toxicity studies pointed towards carbamazepine and sulfamethoxazole as the compounds responsible for elevated toxicity levels. Treated secondary effluent of a mixture of carbamazepine, diclofenac, hydrochlorothiazide, ketorolac, metoprolol, and sulfamethoxazole had no acute toxicity but induced higher catalase levels, indicative of oxidative stress (Oropesa et al., 2016). Included in these compounds that induced oxidative stress are carbamazepine, which also produced chronic and acutely toxic effluent (Oropesa et al., 2018), and sulfamethoxazole, which induced an increase in acute toxicity after treatment (Martini et al., 2019). Interestingly, other photocatalytic ozonation treatment involving sulfamethoxazole and carbamazepine showed complete detoxification (Beltrán et al., 2008; Espejo et al., 2014; Gimeno et al., 2016). The studies of Espejo et al., Oropesa et al. 2016, and Gimeno et al. had both sulfamethoxazole and carbamazepine and were all performed with the same matrix, contaminants, and similar catalysts and radiation sources. The main difference was that Oropesa et al. investigated oxidative stress in addition to the *D. magna* acute toxicity assay, where the elevated catalase levels were found for the TiO_2 catalyst. Beltran et al. and Martini et al. both looked at sulfamethoxazole alone in ultra-pure water. Beltran et al., with a TiO_2 catalyst and a high-pressure mercury lamp (238 – 579 nm), demonstrated complete detoxification towards *D. magna*. Martini et al. found an increase in toxicity towards *V. fischeri* for all of their catalysts (TiO_2, CNT, TiO_2/CNT, Fe/CNT) using a medium pressure mercury lamp (>350 nm), suggesting that the difference in toxicity test or the radiation level may explain the contrasting results. However, the study by Oropesa et al. 2018 investigated carbamazepine alone in ultrapure water showed no acute toxicity for *D. magna* but had increase in both acute toxicity (*V. fischeri*) and chronic toxicity (*D. magna*) using a TiO_2 catalyst. This supports the finding of their previous study (Oropesa et al., 2016) where there was no acute toxicity for *D. magna* although there were increased levels of catalase found in the organisms. Overall, these contrasting findings for carbamazepine and sulfamethoxazole may stem from the toxicity test performed where none of the studies showed acute toxicity towards *D. magna*, but each study which looked at *V. fischeri* for acute toxicity or sublethal effects on *D. magna* showed increases. These results coincide with other AOPs showing that the transformation products for sulfamethoxazole (Yang et al., 2017) and carbamazepine (Donner et al., 2013; Jelic et al., 2013) can be more toxic than the parent compound and suggest that under optimal conditions photocatalytic ozonation can eliminate the transformation products and toxicity.

For industrial chemicals, the furniture industry dyes Reactive Black 5 and Reactive Blue 19 gave acutely toxic solutions after photocatalytic ozonation treatment (Mahne et al., 2012). Again, this was likely from transformation products that were not degraded since the reactions were ceased after the solution became colourless. Pesticides treated by photocatalytic ozonation did not increase toxicity, however, the synthetic musks galaxolide and tonalide of the personal care product group caused increased phytotoxicity after treatment (Santiago-Morales et al., 2012). The authors attributed the increase in toxicity to incomplete mineralization and heightened concentrations of toxic transformation products. For galoxide and tonalide, this increase in toxicity was observed towards *P. subcapitata* for multiple different oxidative treatments (O_3, O_3/H_2O_2, Xe/O_3, Xe/Ce-TiO_2 and Ce/O_3/Ce-TiO_2) while there was a decrease with UV/O_3 and O_3/Ce-TiO_2 even though degradation efficiencies were similar (Santiago-Morales et al., 2012). There was no incorporation of UV/O_3/Ce-TiO_2 in this study although separately these systems, UV/O_3 and O_3/Ce-TiO_2, were most effective at removing toxicity.

2.4.2 Matrix effect

The matrix is an important consideration in the viability of a water treatment process as the chemical makeup can affect efficiency as well as the nature of the transformation products. Preliminary stages of testing a treatment process usually involve using pure water. Once shown to be effective, synthetic and real wastewater effluent are used. Real wastewater is challenging to work with as it is a complex mixture of organic and inorganic substances, and the chemical makeup constantly changes, which adds variability to the results and decreases reproducibility of experiments over time. The composition varies greatly depending on the percentage of influent from municipal sources and industrial sources, location, and season, making it even more challenging to find a universal treatment process. The changes in parameters can not only affect the degradation of the target contaminants but it can lead to the formation of toxic intermediates. The matrix may also enhance wastewater treatment processes; for example, the Ag-TiO_2 photocatalytic ozonation was a more efficient treatment method in real wastewater than ultrapure water for the removal of parabens (Gomes et al., 2019). To address effects from matrix variability, novel treatment approaches should be tested in a variety of different matrices to determine the effectiveness and robustness of the method.

The analysis of effluent toxicity after photocatalytic ozonation treatment was performed on a variety of different matrices shown in Figure 7. Although nearly half of the studies were performed in pure water, there was a significant number of studies that incorporated real wastewater (40%). These included secondary effluent, industrial wastewater (textile, pulp and paper, and furniture industries), and hospital wastewater. Furthermore, synthetic wastewater, surface water, and reverse osmosis concentrate were also used as photocatalytic ozonation matrices. The toxicity results did not show a large correlation between the matrix and increased toxicity. However, of the 14% of studies showing an increase in toxicity, pure water (5.5%) and secondary effluent (5.5%) had the largest percentages, consistent with the large proportion of studies that looked at each respective solution. Overall, it was shown that photocatalytic ozonation with respect to toxicity is effective in a variety of matrices.

2.4.3 Summary of the potential for contaminant type and matrix to impact toxicity

As shown in Table 6, multiple types of matrices were used with photocatalytic ozonation and increase toxicity was reported in difference matrices, including pure water, secondary effluent, and industrial wastewater. The application of photocatalytic ozonation in different matrices resulting in high degradation and decreases in toxicity (in 86% of the studies) demonstrate that this technique is robust and suitable for upscale. The responsible types of compounds associated with the increases in toxicity were mainly pharmaceuticals, but also involved PCPs and industrial compounds. For both matrices and compounds, the type which showed the most increases in toxicity corresponded to the matrix/compounds tested most frequently. The findings did not suggest that any particular matrix or group of compounds were more likely to cause an increase in toxicity. However, with respect to individual chemicals in the pharmaceutical group, incomplete mineralization of sulfamethoxazole and carbamazepine gave increases in toxicity most often when paired with the *V. fischeri* bioassay which has also been observed with other AOPs (Donner et al., 2013; Yang et al., 2017).

3 Conclusion

The necessity to keep our water systems free of contaminants for human and environmental health is a more important issue now than ever before. New processes to remove CECs from wastewater and other contaminated water need to be incorporated into conventional treatment facilities to improve the quality of the treated water released into the environment. The present review of studies based on heterogenous photocatalytic ozonation demonstrates that it is an efficient process to remove persistent organic compounds while not causing the formation of toxic transformation products (13%) or even decreasing residual toxicity (73%), with only some studies reporting increasing toxicity (14%). Many different toxicity tests have been used to evaluate toxicity that cover a range of toxicity levels, such as acute and phytotoxicity, over various trophic levels. Acute toxicity assays were performed most often followed by phytotoxicity. Of the 56 total toxicity tests performed, only 6 showed increases in toxicity including 3 for acute toxicity, and one for each chronic toxicity, phytotoxicity, and oxidative stress. *V. fischeri* assays appeared to be the most affected by the treated effluent and were responsible for all results of elevated acute toxicity, even when performed in tandem with other toxicity tests that yielded contrasting results. It is observed, however, that chronic toxicity and oxidative stress tests may be more sensitive to environmentally relevant concentrations of contaminants and transformation products although they are incorporated into far fewer studies than acute toxicity and phytotoxicity assays.

The catalysts and light sources implemented were also reviewed showing great variance in reactor conditions. Catalysts containing TiO_2 were the most common, in 84% of the studies, and due to their prevalence were involved in most of the increased toxicity effluents. The radiation sources used were mainly mercury-vapour lamps including blacklights (79%), but solar radiation, xenon lamps, and LEDs were also used. The light sources involved with the highest percentage of increased toxicity were solar light and xenon lamps, indicating the importance of developing and using compatible band-gap catalysts with higher wavelength radiation sources. The differences in wavelength and intensity of these lamps can have great effect on photocatalytic ozonation efficiency and the formation of reactive species. Direct comparison between methods to assess effect of matrices and contaminants is difficult due to the variety of different reaction conditions, compounds, and toxicity tests involved. However, overall photocatalytic ozonation was shown to decrease effluent toxicity in most studies while achieving high levels of removal of the target contaminants. Photocatalytic ozonation shows great potential to be incorporated into current wastewater treatment facilities to improve the quality of treated effluent.

While all the reviewed studies included at least one toxicity assay, it is recommended that future toxicity studies incorporate multiple toxicity tests to increase the reliability of test results. This should include sublethal or chronic toxicity tests as these may be more sensitive toxic effects prevalent at environmentally relevant concentrations. These toxicity tests should also be used to assess residual toxicity and to monitor and optimize treatments. Multiple endpoints can be incorporated to model degradation and toxicity trends to gain perspective on the effluent properties over time and how these can be optimized for detoxification in large scale reactors. Furthermore, emphasis on conducting toxicity tests along with chemical analysis is key since high removals of parent compounds does not always lead to non-toxic effluent.

This review showed that immobilized catalysts and other photocatalysts besides TiO_2 are effective for degradation and detoxification. Future studies can further develop and optimize catalysts to increase the efficiency of photocatalytic ozonation for industrial application. This includes, but is not limited to, modifying band gaps, using cost effective catalysts, and immobilizing catalysts. Less expensive radiation sources such as solar radiation and LED lights and eliminating complex catalyst recovery procedures can decrease operational costs and facilitate the implementation of photocatalytic ozonation at a large-scale.

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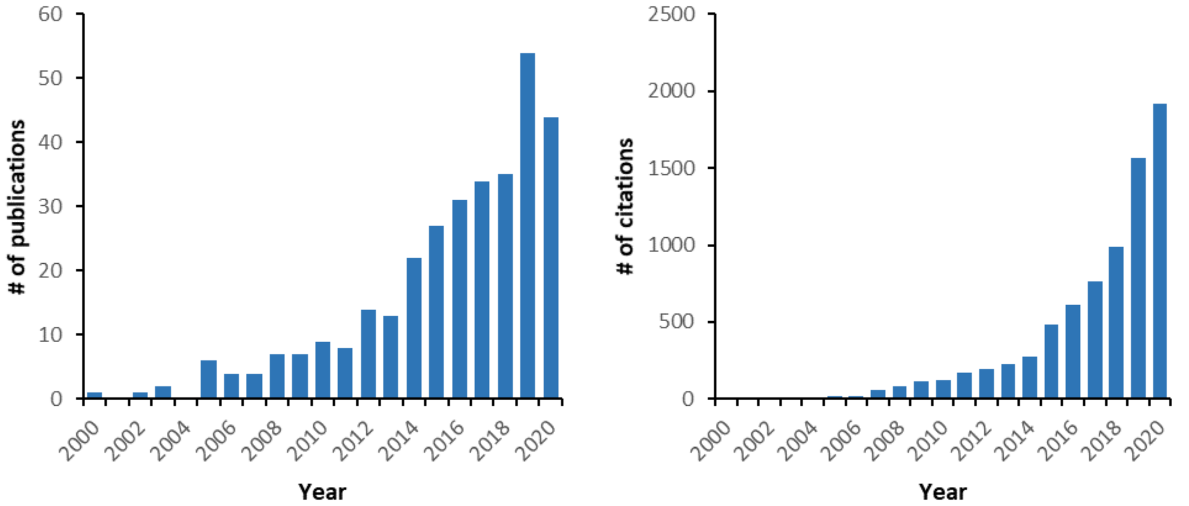

Figure 1. Publication data from Web of Science under search “photocatalytic ozonation”. Total number of publications per year (left) and total number of citations per year (right) from 2000 to 2020, inclusive.

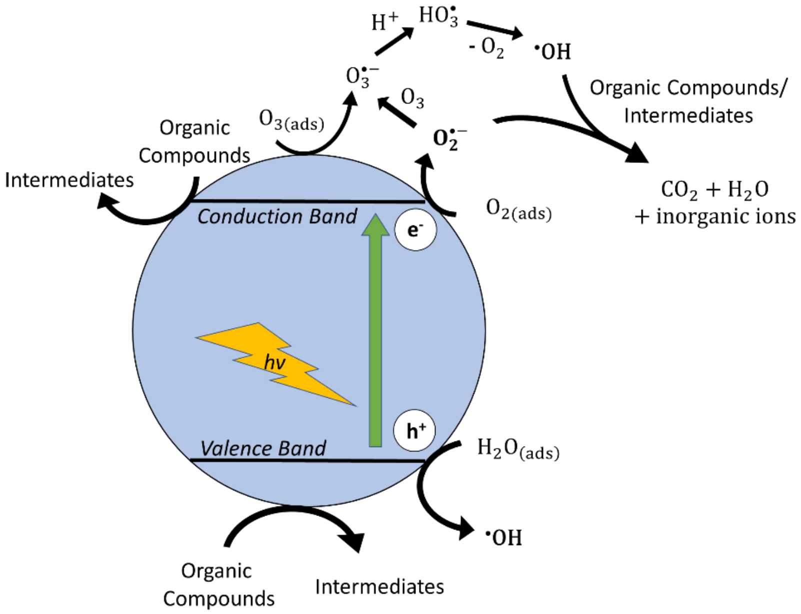

Figure 2. Photocatalytic ozonation surface degradation mechanisms for organic pollutants. (Only catalyst surface reactions are presented, for clarity the image does not include aqueous photolysis and ozone photolysis reactions)

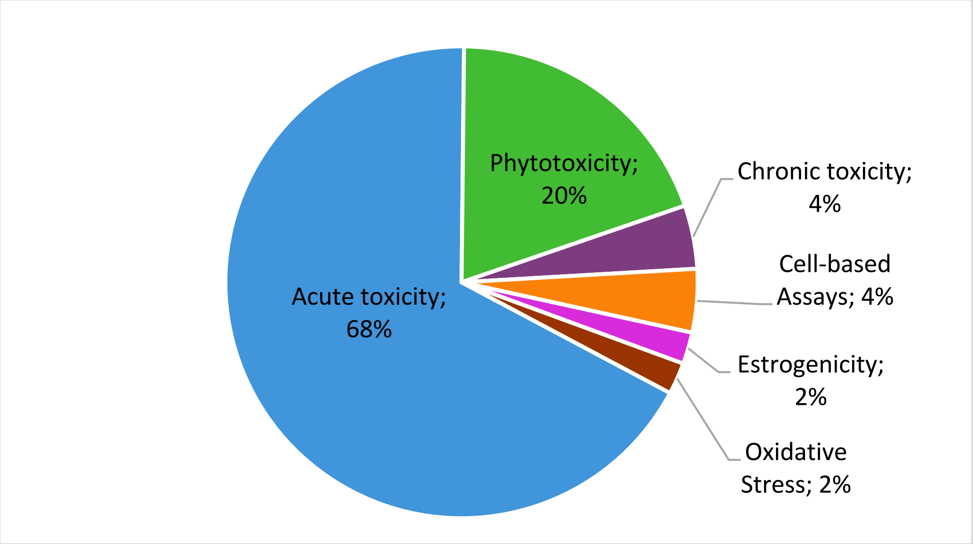

Figure 3. Distribution of types of toxicity tests in photocatalytic ozonation studies where the percentage is in respect to the total number of tests performed (46 toxicity tests, 36 studies).

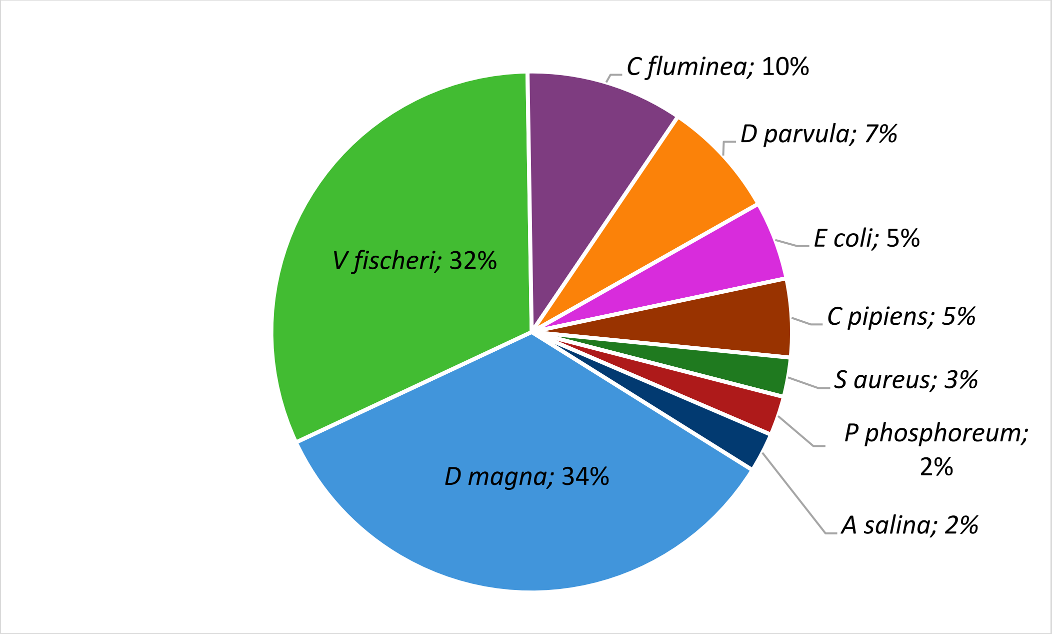

Figure 4. Distribution of types of acute toxicity tests used (41 toxicity tests, 31 studies).

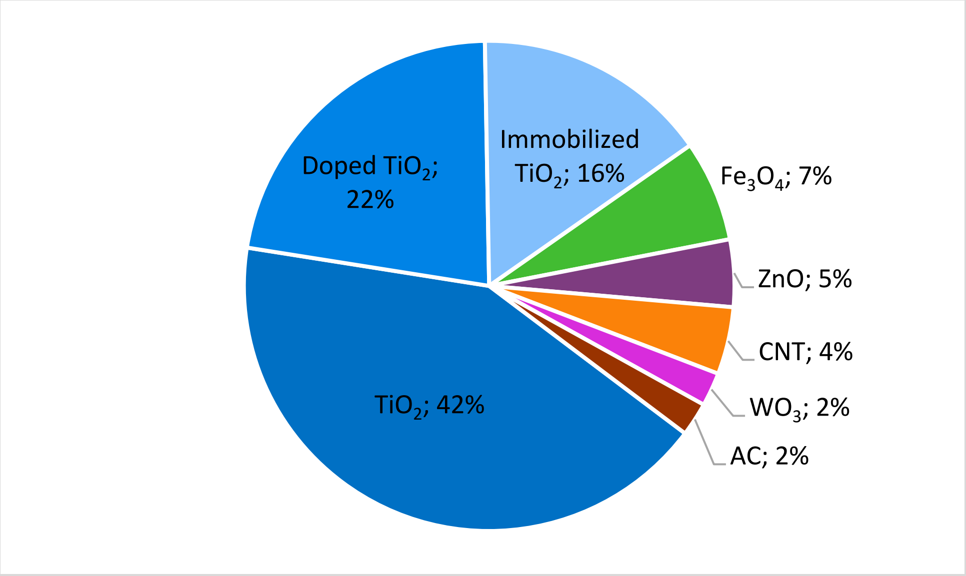

Figure 5. Catalyst distribution among photocatalytic ozonation toxicity studies (36 studies).

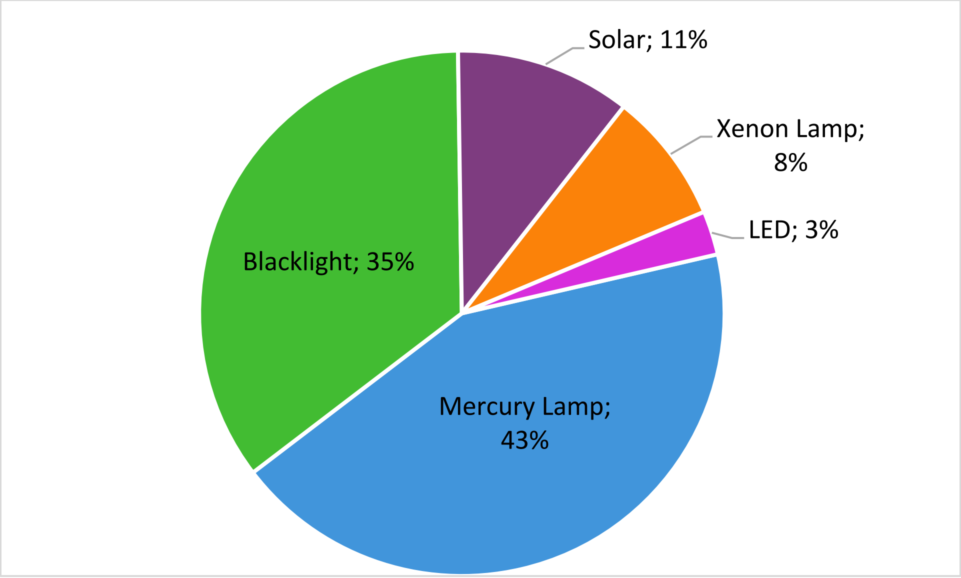

Figure 6. Distribution of radiation sources used in photocatalytic ozonation toxicity studies (37 sources, 36 studies).

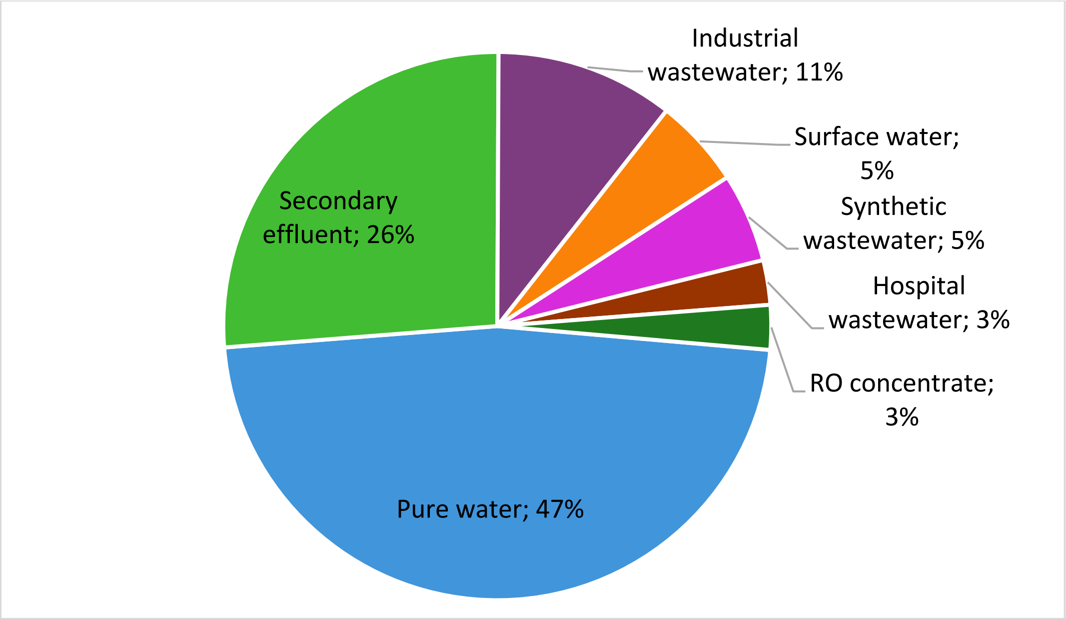

Figure 7. Matrices tested in photocatalytic ozonation toxicity studies (38 matrices, 36 studies).

Table 1: Overview of heterogeneous photocatalytic ozonation studies published before the end of 2020, which include toxicity testing.

#	Catalyst and Reference	Contaminant and Matrix	Radiation source	Ozone Exposure [1]	Removal of contaminant [2]	Bioassay	Changes in toxicity reported
TiO_2							
1	TiO_2 (P25) (Yeber et al., 1999)	Contaminant: Organochlorine compounds Matrix: Pulp and paper mill effluent after ClO2 bleaching	>254 nm, high pressure Hg, 125 W, 12 mW/cm^2	- ; - ; 2000 mg O_3/h; - ; -	TOC: High	*Photobacterium phosphoreum* (acute toxicity)	Approximately 40% decrease in toxicity
2	TiO_2 (P25) (Gomes De Moraes et al., 2000)	Contaminant: Dyes, surfactants, additives Matrix: Textile industry effluent	High pressure Hg, 125 W, 3.11 mW/cm^2	- ; 200 mL; - ; 15 L/h; 14 mg O_3/L	Colour: High TOC: Med	*Escherichia coli* (acute toxicity)	Decrease in effluent toxicity by half
3	TiO_2 (P25) (Beltrán et al., 2008)	Contaminant: Sulfamethoxazole Matrix: Ultrapure water	238-579 nm, high pressure Hg, 700 W, 3.08 e-5 E/L/s	- ; 900 mL; - ; 30 L/h; 10 mg O_3/L	Parents: High TOC: High	*Daphnia magna* (acute toxicity)	Complete detoxification after treatment
4	TiO_2 (P25) (Zhou et al., 2011)	Contaminant: Organic compounds Matrix: RO concentrate from wastewater reclamation plant	365 nm, black light, 9 W, 7.7 +/- 0.1 mW/cm^2	- ; 350 mL; 17.6 mg O_3/h; - ; -	Raw RO concentrate COD: Low DOC: Med Coagulated RO Concentrate COD: Med DOC: Low	*Vibrio fischeri* (acute toxicity)	Approximately 40% decrease in toxicity for raw RO concentrate and 50% decrease for coagulation pre-treatment
5	TiO_2 (Beduk et al., 2012)	Contaminant: Malathion, parathion Matrix: Deionized water	254 nm, medium pressure Hg	2 mg O_3/L; 1000 mL; - ; - ; -	Parents: High	*Vibrio fischeri* (acute toxicity) *Daphnia magna* (acute toxicity)	No toxicity towards *V. fischeri* and near complete detoxification for *D. magna*
6	TiO_2 (P25) (Moreira et al., 2015)	Contaminant: Amoxicillin, diclofenac Matrix: Water	>300 nm, medium pressure Hg	- ; 250 mL; - ; 150 Nm^3/min ; 50 g O_3/Nm^3	Parents: High TOC: High	*Escherichia coli* (acute toxicity) *Staphylococcus aureus* (acute toxicity)	Reduction in toxicity for amoxicillin treated water and neither untreated nor treated water had effects with diclofenac
7	TiO_2 (P25) (Quiñones et al., 2015)	Contaminant: Acetaminophen, antipyrine, BPA, caffeine, metaprolol, testosterone Matrix: Secondary effluent	Concentrated solar radiation	- ; 5000 mL; - ; 40 L/h; 13 mg O_3/L	Parents: High TOC: Very low	*Daphnia magna* (acute toxicity)	Decrease in inhibition from 27% to 14%.

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[1] Ozone dose; volume of water; ozone flow rate; gas flow rate; ozone inlet concentration

[2] High: > 75%, Med: 50 – 75%, Low: 25 – 50%, Very low: < 25%

#	Catalyst and Reference	Contaminant and Matrix	Radiation source	Ozone Exposure [1]	Removal of contaminant [2]	Bioassay	Changes in toxicity reported
8	TiO_2 (P25) (Solís et al., 2015)	Contaminant: 4-chloro-2-methylpheoxyacetic acid (MCPA), 4-chloro-2-methyl phenol (CMP) Matrix: Ultrapure water	365 nm (350-400nm), 4 x 15 W black light, 1.14 e-6 E/L/s	- ; 1000mL; - ; 30 L/h; 5 mg O_3/L	Parents: High TOC: Med	*Daphnia parvula* (acute toxicity) *Culex pipiens* (acute toxicity)	Initial increase in toxicity followed by overall decrease for both assays.
9	TiO_2 (P25) (Solís et al., 2016b)	Contaminant: Clopyralid, triclopyr, picloram Matrix: Ultrapure water	365 nm (350-400nm), 4 x 15 W black light, 1.14 e-6 E/L/s	- ; 1000mL; - ; 30 L/h; 5 mg O_3/L	Parents: High TOC: High	*Daphnia parvula* (acute toxicity) *Culex pipiens* (acute toxicity)	High toxicity of intermediates to *D. parvula*, less so for *C. pipiens*, overall effluent showed decrease in toxicity after 180 mins
10	TiO_2 P25 (Gimeno et al., 2016)	Contaminant: Acetaminophen, antipyrine, caffeine, carbamazepine, diclofenac, hydrochlorothiazide, ketorolac, metoprolol, sulfamethoxazole Matrix: Secondary effluent	Concentrated solar radiation	- ; 1800 mL; - ; 35 L/h; 13 mg O_3/L	Parents: High COD: Med TOC: Low	*Daphnia magna* (acute toxicity)	Complete detoxification
11	TiO_2 P25 (Oropesa et al., 2016)	Contaminant: Acetaminophen, antipyrine, caffeine, carbamazepine, diclofenac, hydrochlorothiazide, ketorolac, metoprolol, sulfamethoxazole Matrix: Secondary effluent	Concentrated solar radiation	- ; 5000 mL; - ; 35 L/h; 13 mg O_3/L	Parents: High TOC: Low	*Daphnia magna* (acute toxicity) *Daphnia magna*: superoxide dismutase (SOD), catalase (CAT), and lipid peroxidation (LPO) levels measured for sublethal effects (oxidative stress)	No acute toxicity towards *D. magna.* At 6.25% dilution (comparable to environmental concentrations), significantly higher CAT values, no statistically significant differences for SOD or LPO levels
12	TiO_2 (P25) (Oropesa et al., 2018)	Contaminant: Carbamazepine Matrix: Ultrapure water	370 nm (350-400 nm range), 15 W black light, 1.4 e-6 E/L/s	- ; 4000 mL; - ; 35 L/h; 13 mg O_3/L	Parents: High	*Vibrio fischeri* (acute toxicity) *Daphnia magna* (acute and chronic toxicity)	Undiluted effluent had an increase in acute toxicity towards *V. fischeri* (diluted samples were not toxic), no acute toxicity towards D. magna. Undiluted treated water too toxic for chronic reproductive D. magna test. Diluted effluent showed decreased toxicity - increased the number of juveniles and body length relative to the untreated water.
13	TiO_2 (P25) (Doná et al., 2019)	Contaminant: Methylparaben Matrix: Ultrapure water	UVC, high pressure Hg, 125 W, 6.6 e-7 E/L/s	30 mg O_3/L; 1000 mL; - ; - ; -	Parents: High TOC: High	*Artemia salina* (acute toxicity)	Negligible toxicity for pretreated and treated water

#	Catalyst and Reference	Contaminant and Matrix	Radiation source	Ozone Exposure [1]	Removal of contaminant [2]	Bioassay	Changes in toxicity reported
14	TiO_2 P25 (Figueredo et al., 2019)	Contaminant: Primidone Matrix: Ultrapure water	UV-Vis: 300-800 nm, Xe lamp, 1500 W, 55 mW/cm^2, 8.2 e-5 E/L/s Vis: 390-800 nm, Xe lamp, 1500 W, 55 mW/cm^2, 7.75 e-5 E/L/s	- ; 500 mL; 200 mg O_3/h; 20 L/h; 10 mg O_3/L	Parents: High TOC: High	*Daphnia magna* (acute toxicity)	No toxicity for pretreated and treated water
				Immobilized TiO_2			
15	Immobilized TiO_2 on acrylic ramp (Machado et al., 2007)	Matrix: Hospital secondary wastewater	254/365 nm peaks, low pressure Hg, 30 W	- ; 6000 mL; 5.8 mg O_3/h; - ; -	COD: Low	*Daphnia magna* (acute toxicity)	Complete detoxification
16	Immobilized TiO_2 on Ahlstrom photocatalytic paper (Mahne et al., 2012)	Contaminant: Reactive Blue 19 (RBL19), Reactive Red 22 (RRD22), Reactive Black 5 (RBK5) Matrix: Synthetic textile wastewater	355 nm, low pressure Hg	- ; 280 mL; 130 mg O_3/h; - ; -	Colour: High COD: Low	*Vibrio fischeri* (acute toxicity)	Decrease in toxicity only for RRD22, not RBL10 or RBK5. Initial increase in toxicity from intermediates but decrease as treatment progressed.
17	Immobilized TiO_2 on acrylic ramp (Horn et al., 2014)	Matrix: University wastewater	UVC, Hg lamp, 30 W	- ; 300 ml; 160 mg O_3/h; - ; -	N/A	*Daphnia magna* (acute toxicity) *Ceriodaphnia dubia* (chronic toxicity)	No significant differences in toxicity between untreated and treated effluent.
18	Immobilized TiO_2 on ceramic plates (Fathinia and Khataee, 2015)	Contaminant: Phenazopyridine Matrix: Water	354 nm, 100 W	- ; 2000 mL; 5L O_3/h; - ; -	Parents: High	*Spirodela polyrhiza* (phytotoxicity)	Decrease in toxicity with treatment. Initial increase in toxicity due to intermediates.
19	Immobilized TiO_2 on Raschig rings (Moreira et al., 2016)	Contaminant: Diclofenac, ibuprofen, naproxen, azithromycin, clarithromycin, erythromycin, sulfamethoxazole, trimethoprim, diphenhydramine, clopidogrel, propranolol, hydrochlorothiazide, atorvastatin, bezafibrate, carbamazepine, citalopram, venlafaxine, fluoxetine, norfluoxetine, EE2, E2, isoproturon Matrix: Surface water and wastewater	382 nm, high intensity LEDs, 2 x 10 W	- ; - ; - ; 15 Ncm^3/min; 50 g O_3/Nm^3	Parents: High TOC: Very Low	YES assay (estrogenic activity) MTT assay (cytotoxicity) LDH assay (cytotoxicity)	Wastewater: Decrease or no statistically significant change in toxicity for all cell lines with MTT and LDH assays. YES showed no effects with treated or untreated water. Surface water: No toxicity for any assays

#	Catalyst and Reference	Contaminant and Matrix	Radiation source	Ozone Exposure [1]	Removal of contaminant [2]	Bioassay	Changes in toxicity reported
20	Immobilized TiO_2 on glass rods (Tichonovas et al., 2017)	Contaminant: 2-naphthol, phenol, oxalic acid, phthalate, methylene blue, D-glucose Matrix: Synthetic wastewater	254 nm, Hg lamp, 40 W	- ; 1600 mL; - ; 660 L/h; 1.3 mg O_3/L	TOC: High	*Daphnia magna* (acute toxicity)	Complete detoxification
21	Immobilized TiO_2 on glass rods (Tichonovas et al., 2018)	Matrix: Furniture industry wastewater	254 nm, Hg lamp, 40 W	- ; 1600 mL; - ; 660 L/h; 1.3 mg O_3/L	TOC: High	*Daphnia magna* (acute toxicity)	Increase in toxicity initially followed by complete detoxification
				Doped TiO_2			
22	Ce-TiO_2 (Santiago-Morales et al., 2012)	Contaminant: Galaxolide, tonalide Matrix: Pre-ozonated secondary effluent	290-400 nm, visible light Xe-arc lamp, 1.05 e-6 E/L/s	- ; 1300 mL; 0.19 Nm^3 O_3/h; - ; -	Parents: High	*Daphnia magna* (acute toxicity) *Vibrio fischeri* (acute toxicity) *Pseudokirchneriella subcapitata* (phytotoxicity)	Decrease in acute toxicity towards *D. magna* and not significantly different for *V. fischeri* Increase in toxicity towards *P. subcapitada*
23	N-TiO_2 (Solís et al., 2016a)	Contaminant: Clopyralid, triclopyr, picloram Matrix: Ultrapure water	365 nm (350-400nm), 4 x 15 W black light, 1.14 e-6 E/L/s	- ; 1000 mL; - ; 30 L/h; 10 mg O_3/L	Parents: High TOC: High	*Daphnia parvula* (acute toxicity) *Lactuca sativa* (phytotoxicity) *Solanum lycopersicum* (phytotoxicity)	High initial toxicity of intermediates for acute toxicity assay but decreased to non-toxic after treatment. Complete detoxification for phytotoxicity assays
24	N-TiO2$_2$ (Solís et al., 2016c)	Contaminant: Diuron Matrix: Ultrapure water	365 nm (350-400nm), 4 x 15 W black light, 1.14 e-6 E/L/s	- ; 1000 mL; - ; 30 L/h; 10 mg O_3/L	Parents: High TOC: High	*Vibrio fischeri* (acute toxicity)	Initial increase in toxicity followed by overall decrease
25	N-TiO_2 (0-15% N) (Fernandes et al., 2020a)	Contaminant: Methylparaben, ethylparaben, proplylparaben, benzylparaben, butylparaben Matrix: Ultrapure water	365 nm, 3 x 6 W black light, 0.89 mW/cm^2, 5.75 e-7 E/L/s	16.5 – 43.5 mg O_3/L TOD; 2000 mL; - ; 12 L/h; -	Parents: High TOC: Low	*Vibrio fischeri* (acute toxicity) *Corbicula fluminea* (acute toxicity) *Lepidium sativum* (phytotoxicity)	Decrease in acute toxicity for both assays although still toxic, no phytotoxicity
26	10% N-TiO_2 (Fernandes et al., 2020b)	Contaminant: Methylparaben, ethylparaben, proplylparaben, benzylparaben, butylparaben Matrix: Secondary effluent and river water	365 nm, 3 x 6 W black light, 0.89 mW/cm^2, 5.75 e-7 E/L/s	45 – 70 mg O_3/L TOD; 2000 mL; - ; 12 L/h; -	Parents: High TOC: Very low	*Lepidium sativum* (phytotoxicity)	Decrease in phytotoxicity, even enhanced germination.
27	TiO_2 Ag-TiO_2 Cu-TiO_2 Fe-TiO_2 (Mecha et al., 2017)	Contaminant: Phenol Matrix: Municipal wastewater	UV: 366 nm, medium pressure Hg, 150W, 70 mW/cm^2 Solar: Parabolic solar collector, 3.76 e-3 mW/cm^2	- ; 700 mL; 1250 mg O_3/L/h; - ; -	N/A	MTT assay (cytotoxicity)	Decrease in toxicity for all catalysts with solar and UV radiation

#	Catalyst and Reference	Contaminant and Matrix	Radiation source	Ozone Exposure [1]	Removal of contaminant [2]	Bioassay	Changes in toxicity reported
28	0.1%Ag-TiO_2 0.5%Ag-TiO_2 0.1%Pt-TiO_2 0.5%Pt-TiO_2 (Gomes et al., 2017c)	Contaminant: Methylparaben, ethylparaben, proplylparaben, benzylparaben, butylparaben Matrix: Ultrapure water	365 nm, 3 x 6 W black light, 0.89 mW/cm^2, 5.75 e-7 E/L/s	46 – 63 mg O_3/L TOD; 2000 mL; - ; 12 L/h; -	Parents: High	*Vibrio fischeri* (acute toxicity) *Corbicula fluminea* (acute toxicity) *Lepidium sativum* (phytotoxicity)	Significant decrease in toxicity for all catalysts. 5% Ag-TiO2 had greatest reduction in toxicity with complete detoxification for *C. fluminea* and *L. sativum* and lowest inhibition for *V. fischeri*
29	TiO_2 Ag-TiO_2 Au-TiO_2 Pd-TiO_2 Pt-TiO_2 (Gomes et al., 2017b)	Contaminant: Methylparaben, ethylparaben, proplylparaben, benzylparaben, butylparaben Matrix: Ultrapure water	365 nm, 3 x 6 W black light, 0.89 mW/cm2, 5.75 e-7 E/L/s	46 – 84 mg O_3/L TOD; 2000 mL; - ; 12 L/h; -	Parents: High TOC: Low	*Vibrio fischeri* (acute toxicity) *Corbicula fluminea* (acute toxicity) *Lepidium sativum* (phytotoxicity)	Significant decrease in toxicity for all catalysts, complete detoxification for *C. fluminea* and *L. sativum* and slight inhibition for *V. fischeri*
30	Ag-TiO_2 (Gomes et al., 2019)	Contaminant: Methylparaben, ethylparaben, proplylparaben, benzylparaben, butylparaben Matrix: Secondary effluent	365 nm, 3 x 6 W black light, 0.89 mW/cm^2, 5.75 e-7 E/L/s	28 – 60 mg O_3/L TOD; 2000 mL; - ; 12 L/h; -	Parents: High	*Vibrio fischeri* (acute toxicity)	Significant decrease in toxicity but still very toxic towards *V. fischeri* after treatment.
31	TiO_2 Ag-TiO_2 Au-TiO_2 Pd-TiO_2 Pt-TiO_2 (Gmurek et al., 2019)	Contaminant: Methylparaben, ethylparaben, proplylparaben, benzylparaben, butylparaben Matrix: Ultrapure water	365 nm, 3 x 6 W black light, 0.89 mW/cm^2, 5.75 e-7 E/L/s	< 65 mg O_3/L TOD; 2000 mL; - ; 12 L/h; -	Parents: High COD: Med TOC: Low	*Vibrio fischeri* (acute toxicity) *Corbicula fluminea* (acute toxicity) *Lepidium sativum* (phytotoxicity)	Decrease in acute toxicity for all catalyst combinations but still toxic towards *V. fischeri*. Complete detoxification towards *C. fluminea* and *L. sativum*
			Other Catalysts				
32	TiO_2 (P25 and synthesized) TiO_2/CNT (Orge et al., 2017)	Contaminant: Metolachlor Matrix: Ultrapure water	*>350 nm, medium pressure Hg, 150W*	- ; 250 mL; - ; 9 L/h; 50 mg O_3/L	Parents: High TOC: High	*Vibrio fischeri* (acute toxicity)	Decrease in toxicity for all catalysts. TiO2 (synthesized) and TiO2/CNT had lowest final toxicity
33	TiO_2 CNT TiO_2/CNT Fe/CNT (Martini et al., 2019)	Contaminant: Sulfamethoxazole Matrix: Ultrapure water	>350 nm, medium pressure Hg, 50 mW/cm^2, UV dose 4.6 e5 mJ/cm^2, photon flux 1.61 e-7 $E/cm^2/s$	- ; 250 mL; - ; 9 L/h; 50 mg O_3/L	Parents: High TOC: High	*Vibrio fischeri* (acute toxicity)	Increased toxicity for all catalysts
1	ZnO (Yeber et al., 1999)	Contaminant: Matrix: Pulp mill effluent after ClO2 bleaching	>254 nm, high pressure Hg, 125 W, 12 mW/cm^2	- ; - ; 2000 mg O_3/h; - ; -	TOC: High	*Photobacterium phosphoreum* (acute toxicity)	Approximately 30% decrease in toxicity
34	ZnO (Biglari et al., 2017)	Contaminant: Phenols Matrix: Paper and pulp industry effluent	254 nm, 125W, 1.025 mW/cm^2	- ; 1000 mL; 552 mg O_3/h; - ; -	Parents: High	*Daphnia magna* (acute toxicity)	Toxicity below regulatory limits

#	Catalyst and Reference	Contaminant and Matrix	Radiation source	Ozone Exposure [1]	Removal of contaminant [2]	Bioassay	Changes in toxicity reported
35	Fe_3O_4 (Espejo et al., 2014)	Contaminant: Acetaminophen, antipyrine, caffeine, carbamazepine, diclofenac, hydrochlorothiazide, ketorolac, metoprolol, sulfamethoxazole Matrix: Secondary effluent	365 nm, 2x 15 W blacklight lamps, 1.18 e-6 E/L/s	- ; 1000 mL; - ; 35 L/h; 13 mg O_3/L	Parents: High TOC: Low	*Daphnia magna* (acute toxicity)	No toxicity after 24h and 48h toxicity tests
10	Fe_3O_4 (Gimeno et al., 2016)	Contaminant: Acetaminophen, antipyrine, caffeine, carbamazepine, diclofenac, hydrochlorothiazide, ketorolac, metoprolol, sulfamethoxazole Matrix: Secondary effluent	Concentrated solar radiation.	- ; 5000 mL; - ; 35 L/h; 13 mg O_3/L	Parents: Med COD: Low TOC: Very Low	*Daphnia magna* (acute toxicity)	Complete detoxification
11	Fe_3O_4 (Oropesa et al., 2016)	Contaminant: Acetaminophen, antipyrine, caffeine, carbamazepine, diclofenac, hydrochlorothiazide, ketorolac, metoprolol, sulfamethoxazole Matrix: Secondary effluent	Concentrated solar radiation.	- ; 4000 mL; - ; 35 L/h; 13 mg O_3/L	Parents: High TOC: Med	*Daphnia magna* (acute toxicity) *Daphnia magna*: superoxide dismutase (SOD), catalase (CAT), and lipid peroxidation (LPO) levels measured for sublethal effects (oxidative stress)	No acute toxicity towards D. magna. At 6.25% dilution (comparable to environmental concentrations), no statistically significant differences for CAT, SOD, or LPO levels.
36	Olive stone activated carbon (Rajah et al., 2019)	Contaminant: Clopyralid Matrix: Ultrapure water	300-800 nm, Xe-arc lamp, 50 mW/cm^2	- ; 500 mL; - ; 30 L/h; 15 mg O_3/L	Parents: High TOC: High	*Lactuca sativa* (phytotoxicity)	Complete detoxification
14	WO_3 (Figueredo et al., 2019)	Contaminant: Primadone Matrix: Ultrapure water	UV-Vis: 300-800 nm, Xe lamp, 1500 W, 55 mW/cm^2, 8.2 e-5 E/L/s Vis: 390-800 nm, Xe lamp, 1500 W, 55 mW/cm^2, 7.75 e-5 E/L/s	- ; 500 mL; 200 mg O_3/h; 20 L/h; 10 mg O_3/L	Parents: High TOC: Med (pure H_2O) TOC: Low (Secondary Effluent)	*Daphnia magna* (acute toxicity)	No toxicity for pretreated and treated water

Table 2. Summary of the types of bioassays used and corresponding increases in toxicity.

Assay	**Number of studies**	**Increase in toxicity**
Acute	31	3
Chronic	2	1
Phytotoxicity	9	1
Cytotoxicity	2	0
Estrogenicity	1	0
Oxidative Stress	1	1

Table 3. Summary of the types of catalysts used and corresponding increases in toxicity

Catalyst	**Number of studies**	**Increase in toxicity**
TiO_2	19	3
Doped – TiO_2	10	1
Immobilized TiO_2	7	1
WO_3	1	0
ZnO	2	0
Fe_3O_4	3	0
CNTs	2	1
AC	1	0

Table 4. Summary of radiation types, associated catalysts, and residual toxicity. (i) represents immobilized catalysts.

Radiation type	**Number of studies**	**Catalyst(s)**				**Increase in toxicity**
		TiO_2	*TiO_2 (i)*	*Doped TiO_2*	*Other*	
Hg (UVC- vis)	16	6	6	1 (Ag/Cu/Fe)	4 (X-CNT, ZnO)	2 (TiO_2 (i) (Mahne et al., 2012), TiO_2/CNT (Martini et al., 2019))
Blacklight (365-370 nm)	13	4	0	8 (N/Ag/Au/Pt/Pd)	1 (Fe_3O_4)	1 (TiO_2 (Oropesa et al., 2018))
Solar (UVA-Vis)	4	3	0	1 (Ag/Cu/Fe)	2 (Fe_3O_4)	1 (TiO_2 (Oropesa et al., 2016))
Xe (UVA-Vis)	3	1	0	1 (Ce)	2 (AC, WO_3)	1 (Ce-TiO_2 (Santiago-Morales et al., 2012))
LEDs (382 nm)	1	0	1	0	0	0

Table 5. Overview of the compounds and groups of compounds in photocatalytic ozonation toxicity studies. In red are the main compounds associated with studies that show an increase in toxicity after treatment.

Pharmaceuticals	Industrial	Pesticides	Personal Care Products
Acetaminophen Amoxicillin Antipyrine Atorvastatin Azithromycin Bezafibrate Carbamazepine Citalopram Clarithromycin Clopidogrel Diclofenac Diphenhydramine E2 (Estradiol) EE2 (Ethinyl estradiol) Erythromycin Fluoxetine Hydrochlorothiazide Ibuprofen Ketorolac Metaprolol Naproxen Norfluoxetine Phenazopyridine Primidone Propranolol Sulfamethoxazole Testosterone Trimethoprim Venlafaxine	Bisphenol-A Caffeine D-Glucose Methylene Blue Oxalic acid Phenol Phthalate Reactive Black 5 Reactive Blue 19 Reactive Red 22 2-naphthol	Clopyralid Diuron Isoproturon Malathion Metolachlor Parathion Picloram Triclopyr 4-chloro-2-methylpheoxyacetic acid (MCPA) 4-chloro-2-methyl phenol (CMP)	Benzylparaben Butylparaben Ethylparaben Galaxolide Methylparaben Proplylparaben Tonalide
29 (51%)	11 (19%)	10 (18%)	7 (12%)

Table 6. Summary of matrices used and corresponding increases in toxicity with the responsible compound type.

Matrix	**Number of studies**	**Increase in toxicity (Compound type)**
Pure water	18	2 (Pharmaceuticals)
Secondary effluent	10	2 (Pharmaceuticals, PCPs)
Industrial wastewater	4	1 (Industrial)
Surface water	2	0
Synthetic wastewater	2	0
Hospital wastewater	1	0
RO concentrate	1	0