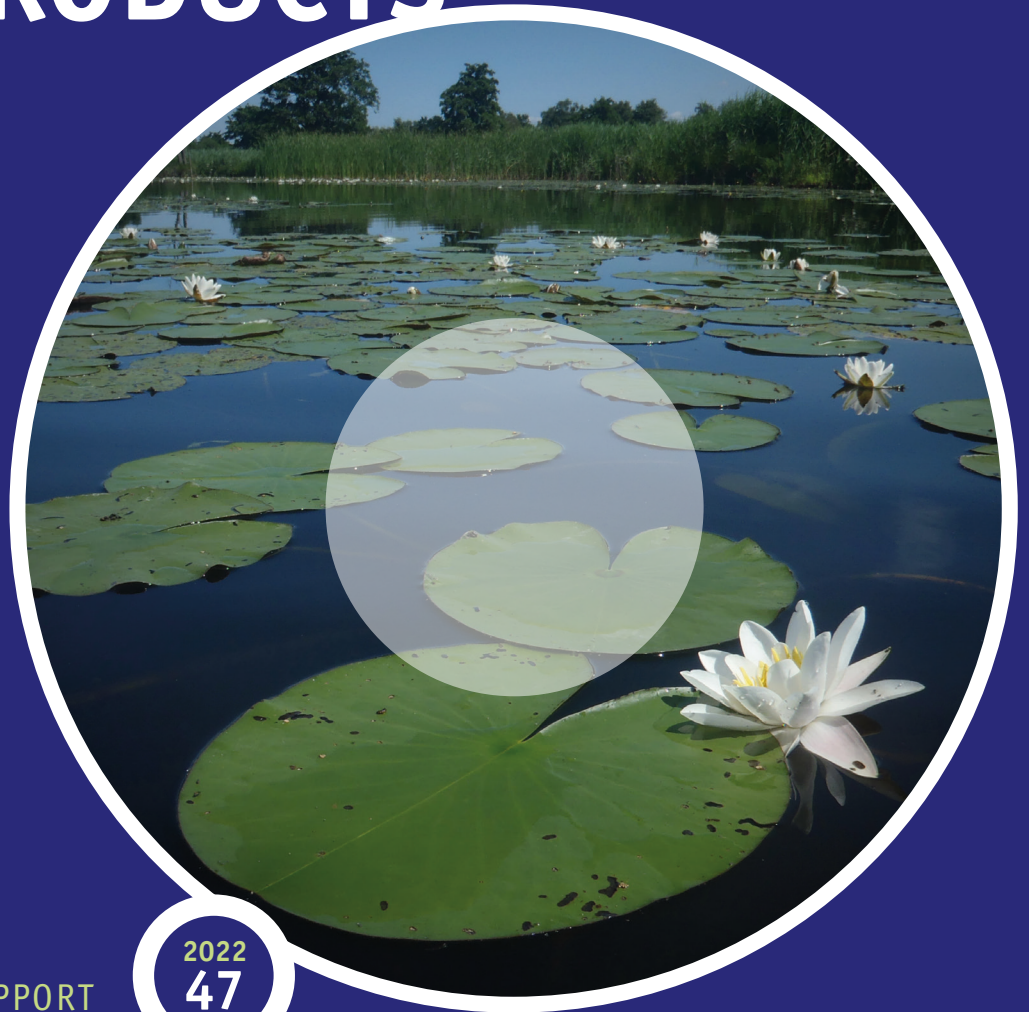




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LITERATURE STUDY ON OXIDATION PRODUCTS



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FOREWORD

FOR MOST TREATMENT PLANTS THE USE OF OZONATION WILL RESULT IN AN IMPROVED WATER QUALITY

Ozonation is one of the suitable technologies for the removal of micropollutants from wastewater. Besides the great improvement of the water quality after ozonation also oxidation products can be formed. Some of these might have a potentially negative effect on the surface water quality or drinking water sources. However, there are still many unknowns about the formation of oxidation products and their (eco)toxicity. This literature study on oxidation products elaborates on the topic of oxidation products and aims at a clear understanding of the formation, possible mitigation measures, and the (eco)toxicological effects of oxidation products formed during ozonation.

Within the Innovation Program Micropollutants from Wastewater of STOWA and the Dutch Ministry of Infrastructure and Water a multitude of technologies for the removal of micropollutants is being investigated. A fair share of these technologies are so called oxidative technologies, often comprising ozonation process. Ozonation is an efficient way to improve the water quality as ozone breakings apart the micropollutants in the water. Nevertheless, during ozonation potentially harmful oxidation products can be formed. To create more insight and understanding in the potential negative effects of ozonation the present literature study was conducted. The study is based on international scientific literature, expert experiences and practical knowledge. The study was conducted under supervision of an international expert group with backgrounds in the field of ozonation, water quality and (eco)toxicology.

The literature study provides introductions to the topics of oxidation products and ecotoxicological assessment methods and drinking water standards. Thereafter the report elaborates on the identification of the most relevant oxidation products. Bromate is identified as the most relevant one. It is a potentially carcinogenic compound which can be formed out of bromide during ozonation. Subsequently the effects of the wastewater composition on the formation of oxidation products are discussed. Primarily the concentrations of bromide and DOC are the most relevant parameters determining the formation of oxidation products. The influence of the process design of the ozonation step on the formation of oxidation products is investigated. Multiple parameters like the ozone dose, injection method and contact time are amongst the most important ones determining the oxidation product formation. Finally, preliminary conclusions are drawn on the trade-off between toxicity reduction (micropollutant abatement) and toxicity increase (oxidation product formation). For most treatment plants the employment of ozonation will result in an improved water quality and absence of negative effects. The report is ended with the most important identified knowledge gaps and suggestions for additional research. The insights of this study are used to compile the “Technical guidelines. oxidation products of ozonation; minimization of unwanted oxidation products; understanding formation and mitigation strategies” (2022-48; in Dutch).

Managing director
J. Buntsma

EXECUTIVE SUMMARY

INTRODUCTION

Ozonation is one of the few best available technologies for the removal of organic micropollutants at wastewater treatment plants (WWTPs). A point of attention regarding ozonation is the formation of oxidation products and their potential negative impact on water quality. For some oxidation products sufficient information is available on their formation during ozonation, their potential negative impact on water quality and options to mitigate their formation. However, for many others this is not the case. Within the Innovation Program Micropollutants (IPMV) of STOWA and the Dutch Ministry of Infrastructure and Water Management the formation of oxidation products and their potential (eco- and human) toxicity is a subject of discussion. These discussions often lack a comprehensive and factual underpinning. To provide a factual basis for the discussion, the present study was initiated. Based on the outcomes of this study a framework for action (*'handelingsperspectief'*) will be developed for wastewater practices in the Netherlands. The goal of the present study is to get a comprehensive overview on the formation, potential impact and possible mitigation strategies of oxidation products relevant for ozonation practices on WWTPs in the Netherlands. The study focusses on oxidation products that pose the highest threat to the aquatic environment and/or drinking water sources.

IDENTIFICATION OF OXIDATION PRODUCTS

Multiple oxidation products or groups of products have been identified, the ones that are currently known as most important are reported. **Bromate** is classified as potentially carcinogenic to humans and quality standards are included for bromate in the Dutch Drinking Water Directive. Surface water quality standard for bromate is currently being revised in the Netherlands. Pilot studies in the Netherlands revealed that bromate concentrations after ozonation of the WWTP-effluent varied from <1 up to 70 µg/l depending mostly on the applied ozone dose and wastewater composition. Concentrations of **chromate**, **chlorate** and **chlorinated compounds** are typically expected to be low and insignificant from a toxicological point of view.

NDMA, a suspected human carcinogen, is a nitroso-compounds for which a maximum value is included in the Dutch Drinking Water Directive. Formation of NDMA during ozonation depends on the concentration of its precursors in WWTP-effluent. Certain precursors are known, but there is no complete picture on precursors yet. NDMA can already be present in the secondary effluent. Results from Switzerland showed that the NDMA concentration in the secondary effluent can be higher than the NDMA formed during ozonation. **Carbonyls** (aldehydes, ketones and carboxylic acids) are formed by the reaction of ozone and dissolved organic matter (DOM). The available information on carbonyls shows that the formation of carbonyls in some cases exceeds the PNEC values. This is typically limited to two times the PNEC values.

A last category of oxidation products are the **transformation products**, these products are formed during the oxidation of organic micropollutants. Theoretically this could be hundreds of compounds, in this study a couple of examples are given. In general, the biological activity of the transformation products is lower compared to their parent compound (the micropollutant). In a few cases an increased toxicity was observed for the transformation product compared to the parent compound (e.g. acyclovir, carbamazepine and oxazepam).

Transformation products occur in low concentrations and therefore under realistic conditions the overall effect can be insignificant. However, because too little is known yet, it cannot be excluded that transformation products are formed which are more toxic than the parent compound in environmental relevant concentrations.

EFFECTS OF WASTEWATER COMPOSITION

The wastewater composition has a huge influence on the formation of oxidation products. Especially the WWTP-effluent constituents *bromide* and *DOM* (dissolved organic matter) determine to a large extent the formation of bromate and various organic oxidation products. Bromate formation is directly linked to the bromide concentration. Limited data on bromide concentrations in Dutch wastewater is available. However, the data that is available shows a high variety in bromide concentrations among the different WWTPs. The DOM levels are relatively high in the Netherlands compared to Switzerland. Therefore, a higher absolute ozone dose is required for micropollutant abatement. This might result in relatively large amounts of oxidation products. Other parameters i.e. *suspended solids*, *pH*, *alkalinity* and *temperature* are found to have a smaller effect on the formation of oxidation products in the typical ranges at which these parameters are present in WWTP-effluent. In drinking water treatment, the presence of *ammonium* can contribute to a reduced bromate formation. However, because of the different water matrix and its influence on the main pathway of bromate formation, the exact extent to which ammonium can contribute to bromate mitigation in ozonation of WWTP effluent is difficult to predict, no field results with WWTP effluent have been found. It is expected that the presence of ammonium is of minor influence but this should be further confirmed.

INFLUENCE OF PROCESS DESIGN

There is a high influence of the process design on the formation of oxidation products. Especially the *process configuration* itself is highly important. This is mainly related to the ozone dosage, in combination with the reactor design and possibly the addition of H_2O_2 . A higher *ozone dosage* results in a higher formation of bromate and several organic oxidation products (e.g. carbonyls and nitromethane). NDMA formation does not seem to be influenced much by the process design. Bromate formation can be reduced by the choice of *reactor configuration*. The *addition of H_2O_2* isn't very well studied and understood in wastewater practices. In drinking water treatment, H_2O_2 -addition is known to effectively suppress bromate formation. The *addition of other chemicals* (e.g. pH depression) doesn't look suitable in ozonation of WWTP-effluent. Due to the relatively high ozone and hydroxyl radical reaction constant rates of most micropollutants a measurable ozone exposure is not needed for the oxidation of micropollutants. Therefore, the *contact time* has little to no effect on the formation of oxidation products compared to the ozone dose.

Several *pre- or hybrid-treatment* options have been identified. Multiple of them like *coagulation* and *filtration* (e.g. sand filters) are likely to have only a minor effect on the formation of oxidation products. Hybrid-treatment processes removing DOM and/or micropollutants from the wastewater seem to be useful, e.g. *PACAS*. By the removal of DOC an equal specific ozone dose ($g O_3/g DOC$) but a lower absolute ozone dose ($g O_3/L$) suffices the aimed micropollutant removal target and thereby results in a reduced formation of oxidation products. The partial removal of (hard to oxidise) micropollutants prior to ozonation by for instance the use activated carbon allows a lower specific ozone dose resulting in less oxidation products.

Sand filtration, treatment in moving bed and fixed bed bioreactors and activated carbon filtration can be used as a *post-treatment* after ozonation. Readily biodegradable oxidation products (such as carbonyls) can be removed with biological post-treatment (e.g. biological activated carbon or sand filtration). Removal of bromate is theoretically possible but not a feasible option in practice. NDMA can be removed with most post-treatments, the removal efficiency varies per post-treatment. Transformation products formed during ozonation can be (partly) removed, especially with activated carbon. However, there is only limited literature available on the removal of transformation products. Post-treatment including activated carbon has the advantage that it can lead to an improved abatement of micropollutants.

NET-EFFECT, TRADE-OFF BETWEEN MICROPOLLUTANT ABATEMENT AND OXIDATION PRODUCT FORMATION

Several full-scale and pilot studies in the Netherlands, Switzerland and Germany have demonstrated with bioassays that the overall toxicity of organic substances in WWTP-effluent can be reduced by ozonation leading to an improved water quality. In all cases a reduced estrogenic activity was found after ozonation (with and without post-treatment). Also, for other bioassays a positive influence was found. However, in some cases, certain bioassays showed an increased toxicity (especially mutagenicity) directly after ozonation which could be completely or partly removed during aerobic biological post-treatment. There are indications that the level of ecotoxicity reduction after ozonation depends on the composition and the *origin of the wastewater* that is treated. Certain (industrial) discharges may cause an increase of toxicity after ozonation. It is not known yet which substances are causing this increased toxicity. This demonstrates the importance of field tests with pilot plant research and/or laboratory studies prior to the realisation of a full-scale ozonation.

It is difficult to assess to which extent all potential toxic organics are well represented in the bioassays after concentration of the samples, especially small hydrophilic compounds which cannot be (fully) extracted during sample pre-treatment. However, the reviewed literature includes also bioassays with native wastewater samples including all water constituents. Moreover, at WWTP Aachen-Soers the impact of ozone plus biological aerobic post-filtration on the receiving water quality is being investigated. So far, it seems that the receiving water quality improves or remains comparable after introduction of ozone with sand filtration, depending on the specific toxicological endpoints researched. If combined bioanalytical endpoints are considered, most studies observed an overall reduction in toxicity after ozonation.

Bioassays themselves and the interpretation of bioassay outcomes into an overall environmental assessment are still under development. It is currently still difficult to assess the overall ecotoxicity of the entire water sample before and after ozonation. Ongoing improvements and developments need to contribute to an improved understanding of the reduction in ecotoxicity with ozonation in the future.

DE STOWA IN BRIEF

The Foundation for Applied Water Research (in short, STOWA) is a research platform for Dutch water controllers. STOWA participants are all ground and surface water managers in rural and urban areas, managers of domestic wastewater treatment installations and dam inspectors.

The water controllers avail themselves of STOWA's facilities for the realisation of all kinds of applied technological, scientific, administrative legal and social scientific research activities that may be of communal importance. Research programmes are developed based on requirement reports generated by the institute's participants. Research suggestions proposed by third parties such as knowledge institutes and consultants, are more than welcome. After having received such suggestions STOWA then consults its participants in order to verify the need for such proposed research.

STOWA does not conduct any research itself, instead it commissions specialised bodies to do the required research. All the studies are supervised by supervisory boards composed of staff from the various participating organisations and, where necessary, experts are brought in.

The money required for research, development, information and other services is raised by the various participating parties. At the moment, this amounts to an annual budget of some 6,5 million euro.

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LITERATURE STUDY ON OXIDATION PRODUCTS

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1

INTRODUCTION

In recent years, organic micropollutants such as pharmaceuticals, consumer products, and industrial chemicals have been detected in the aquatic environment. Discharge of effluent from wastewater treatment plants (WWTPs) is identified as one of the major sources of those compounds into the environment¹. Research into several technologies to abate these compounds at the WWTPs are in progress, such as effluent fixed bed filtration, adsorption technologies and (advanced) oxidation processes. Ozonation is such an oxidation technology and has been shown to be highly effective for the abatement of organic micropollutants from wastewater². The first full-scale ozonation plants at WWTPs are currently being constructed in the Netherlands.

A point of attention regarding ozonation is the formation of oxidation (by-) products and their potential negative impact on water quality. These oxidation products are formed by the reaction of ozone with the target compounds (e.g. micropollutants) and other compounds in the water matrix. Typically, ozonation processes do not result in full degradation, i.e. mineralisation of compounds to mainly CO₂ and water. In most cases compounds are attacked by ozone and hydroxyl radicals and broken down into several oxidation products. For some oxidation products sufficient information is available on their formation during ozonation, their potential negative impact on water quality and options to mitigate their formation. However, for many others this is not the case. Regarding the vast amount of micropollutants present in wastewater, possibly thousands of oxidation products are not yet identified, so their potential negative impact on the water quality is still largely unknown.

The Innovation Program Micropollutants (IPMV) of the Dutch Foundation for Applied Water Research (STOWA) and the Dutch ministry of Infrastructure and Water Management focusses on the development of new technologies and the improvement of existing technologies for micropollutant removal from wastewater. Within this program, various technologies involve oxidation with ozone. In all those projects the formation of oxidation products and their potential (eco and human) toxicity is a reoccurring subject of discussion. These discussions often lacked a comprehensive and factual underpinning and were sometimes based on emotions. To provide a factual basis for the discussion it was decided within the IPMV that a separate study was to be conducted on this matter which is the present study.

The goal of this study is to get a comprehensive overview on the formation, potential impact and possible mitigation strategies of oxidation products relevant for oxidation practices on WWTPs in the Netherlands (based on existing knowledge as present in literature and experiences in the field). As the list of potentially formed oxidation products is endlessly long, the study focusses on oxidation products that pose the highest threat to the aquatic environment and/or drinking water sources, i.e. oxidation products that are known or suspected to have a negative impact on the chemical or (eco)toxicological water quality.

The study was supervised by a Supervisory Committee existing of representatives from Water Authorities and Drinking Water Companies and a group of International Experts in the field of wastewater, ozonation, water quality and toxicity. Supervisors assisted in the selection of relevant information for this study, interpretation of literature, quality control, and provided their expert judgement, especially for non-documented information. For the study information was gathered from international (scientific) literature sources and practical experiences, no additional laboratory or field research was performed. Effects of the wastewater matrix composition and the influence of process design on oxidation products are investigated. An evaluation is made of the trade-off between toxicity reduction by micropollutant removal and potential toxicity increase by formation of oxidation products.

1.1 SCOPE OF THE STUDY

In order to fulfil the goal of this study (a comprehensive overview on oxidation products relevant for oxidation practices on WWTPs in the Netherlands), a clear scope with boundaries of the study was set.

The study has the following scope and boundaries:

Among the various (advanced) oxidation processes only ozonation is currently identified as a competitive technology for wastewater treatment in the Netherlands. UV treatment (in combination with H_2O_2) has been tested on pilot scale but resulted in a too high energy demand and thereby increased costs and CO_2 -footprint due to the low transmission values of WWTP-effluent. Possibly the development of UV-LED will significantly lower the energy demand, however the expected time horizon for this development is 10 -15 years and thereby outside the scope of this study. Several other oxidative technologies have been tested or will be tested soon. Nevertheless, when it comes to the technology readiness level, investment and operational costs, CO_2 -footprint and removal efficiency only ozonation is currently a competitive technology. This study will therefore focus on ozonation, including the combination of ozone and hydrogen peroxide. No other (advanced) oxidation processes will be discussed.

The study has a focus on the removal of organic micropollutants present in municipal wastewater. Treatment facilities which specifically serve industrial or hospital wastewater only are not within the scope of this study. Note that in some treatment facilities for municipal wastewater the influent also consists of a portion of wastewater with an industrial and/or hospital origin. In the Netherlands the vast majority of WWTPs treating municipal wastewater are activated sludge systems. So far, the ozonation process has only been studied and realised in a post-treatment configuration where an initial treatment has been performed with activated sludge. This study therefore focusses on post-treatment of WWTP-effluents from activated sludge systems. Effluents of alternative treatment concepts like water factories are beyond the scope of this study.

In drinking water treatment ozonation has been applied for a longer period of time. As a result, there is more knowledge, experience, and literature available for this field of water treatment. This information is considered for the present study. Together with the Supervisory Committee and International Experts who guided this study, an evaluation is made to assess whether the reviewed information is relevant and useful for wastewater treatment practices or not.

1.2 STRUCTURE OF THE REPORT

In chapter 2 ecotoxicological assessment methods and drinking water standards are discussed. In chapter 3 the known relevant oxidation products after ozonation of wastewater are identified. In chapter 4 the influence of the general wastewater composition on the formation of oxidation products is described. Subsequently, the influence of process design and conditions is discussed in chapter 5. Moreover, mitigation strategies are discussed for undesired oxidation by-products. Not all oxidation products mentioned in chapter 3 will be referred to in this chapter, only the products that are currently known to be most relevant. In chapter 6 the trade-off between toxicity decrease due to micropollutant abatement and toxicity increase due to oxidation (by-)product formation is discussed. Most important knowledge gaps are discussed in chapter 7.

1.3 BRIEF INTRODUCTION INTO OZONISATION OF WWTP-EFFLUENT

The ozonisation process as referred to in this study is the treatment of WWTP-effluent by ozone. In an ozone generator oxygen is converted into ozone. The ozone is dissolved into the WWTP-effluent where it reacts with various compounds. Compounds that react with ozone are oxidised and oxidation occurs either through direct ozone oxidation or a reaction with the less selective but highly reactive hydroxyl radical. Direct ozone oxidation happens through a reaction with ozone, which is a relatively selective oxidant that attacks predominantly electron-rich organic moieties such as phenols, anilines, olefins, sulphur and amine moieties. Hydroxyl radicals are less selective and are much more reactive than ozone.

1.4 NOMENCLATURE

Oxidation products	– all oxidation products that are formed during oxidation of wastewater
Oxidation by-products	– oxidation products originating from the wastewater matrix
Transformation products	– oxidation products originating from the target compounds (i.e. organic micropollutants)

2

INTRODUCTION TO ECOTOXICOLOGICAL ASSESSMENT METHODS AND DRINKING WATER STANDARDS

The study focusses on oxidation products that pose a threat to the aquatic environment and/or drinking water sources. This chapter gives a brief overview in the assessment of both toxicological categories.

2.1 ECOTOXICOLOGICAL ASSESSMENT METHODS

The impact of oxidation products on the ecotoxicity is one of the major subjects of this study. In this chapter a brief introduction is given on the methods for ecotoxicity assessment.

Toxicity towards the aquatic environment can be assessed using biological toxicity tests with live organisms or cell cultures (bioassays). Bioassays are conducted to determine the general or specific toxicity of a sample, for instance a sample of ozonated WWTP-effluent. Thus, ideally a bioassay (or set of bioassays) covers the effects of all micropollutants, all oxidation products (oxidation by-products and transformation products) and if applicable the background toxicity that was already present in the sample prior to ozonation. Different bioassays are developed that either test for non-specific toxicity or specific modes of action (toxicological endpoints). Those bioassays are triggered by specific categories of compounds with a similar mode of action, e.g. estrogenic compounds, and typically respond to a specific group of substances. There are no bioassays that cover the full suite of modes of action of all micropollutants, let alone their oxidation products. Moreover, due to short exposure times bioassays usually only catch acute toxicity, not chronic toxicity. An estimation of chronic toxicity can be obtained by acute tests on concentrated water samples. Bioassays which evaluate the general toxicity are mainly *in vivo* tests that can detect the toxicity of the entire mixture. Examples of toxicity tests that were applied in Dutch ozone pilots are included in appendix 1.

Output of bioassays and environmental relevance

The challenge of bioassays is to put the effects into the perspective of relevance for the aquatic environment. Therefore effect-based trigger values (EBT) have been determined that can be used in order to evaluate whether the bioassay output indicates an environmental risk of the tested sample or not². In some studies, an overall assessment of environmental risks based on bioassay results has been made. Other studies only indicate the difference in effect of the original sample, after ozonation and in some studies also after a possible post-treatment step following the ozonation. This shows whether ozonation removes or increases a specific effect and whether this effect can be mitigated by a post-treatment after ozonation.

Ecotoxicity tests and influence of sample pre-treatment

In some bioassays original samples without pre-treatment are used for the assessment of ecotoxicity. However, to decrease bioassay detection limits a pre-treatment step is required in many bioassays to increase the concentrations of the target compounds in the sample^{2,3}. Moreover, concentrating the sample enables the possibility to estimate long-term (chronic) risks with short-term (acute) bioassays. Samples are concentrated using passive sampling (e.g. POCIS, Speedisk, silicon rubber) or using a large-volume extraction procedure, like for instance Solid Phase Extraction (SPE) with. In Van der Oost et al (2019), a comparison was made between these concentration techniques for polar substances such as pharmaceutical residues and crop protection agents in WWTP effluents. Because of the reproducibility and extraction recoveries for the polar substances, extraction by SPE is preferred⁴. In SPE procedures used for organic micropollutant extraction, matrix constituents like minerals and nutrients are not extracted, avoiding interference of those compounds during the toxicological assessment. Also, contamination of the bioassay by pathogens is avoided by SPE³ enlarging the conservation time of the sample².

It must be noted that inorganics, including bromate and (heavy) metals, do not adsorb to the resin, and therefore the toxicity of those compounds is not included in bioassays conducted on concentrated samples. The same applies to certain (small) organic (hydrophilic) molecules that do not adsorb well to commonly applied resins, resulting in poor recoveries during extraction⁵. Most oxidation products are more hydrophilic compared to their precursor and thus using this method, risks may be underestimated and a comparison between pre- and post-treatment will be difficult⁵. A certain loss of small hydrophilic compounds is unavoidable when concentrating a sample and it needs to be taken into account when evaluating ecotoxicity tests².

2.2 DRINKING WATER STANDARDS

The most important legislation in the Netherlands on the production and distribution of drinking water is the Drinking Water Directive (Drinkwaterbesluit). This directive provides quality standards for several categories of microbial parameters (e.g. *Escherichia coli* and Enterococci), chemical parameters (e.g. bromate and benzene) and indicator parameters (e.g. temperature and pH)⁶. These quality standards are derived from toxicological thresholds for human consumption of drinking water. As for the current study mostly chemical parameters are of interest, the chemical parameters as provided by the Drinking Water Directive are given in Appendix 2.

3

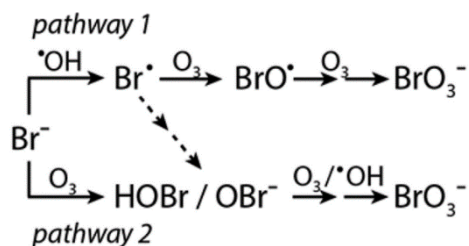
IDENTIFICATION OF MOST RELEVANT OXIDATION PRODUCTS

During ozonation numerous oxidation products are formed. In this chapter an overview is given of the identified oxidation products that are formed during ozonation of WWTP-effluent and which are currently known to be most relevant for the ecological and/or chemical water quality, and/or drinking water preparation. Oxidation products are clustered in categories of different types of compounds. Per cluster the most relevant oxidation products are described, including a brief insight in their formation mechanism and their toxicity. For a limited number of oxidation products surface- and/or drinking water quality standards are given as for many compounds no standards could be found.

3.1 BROMATE

Bromate (BrO_3^-) is a well-known ozonation by-product. Bromate is formed during the oxidation of bromide (Br^-), involving multiple chemical reactions with both ozone and hydroxyl radicals⁷. In Figure 1, a simplified scheme is presented, showing two pathways for bromate formation. Ozone decomposes in absence and presence of DOC to hydroxyl radicals, and therefore both pathways can be relevant during ozonation of WWTP effluent. This is different compared to drinking water treatment, where pathway 2 is considered more important. Depending on the water matrix, hypobromous acid (HOBr)/hypobromite (OBr^-) is an important intermediate in the formation of bromate in pathway 2, and this has been utilized in many control strategies for reducing bromate formation in drinking water treatment.^{8,9}

FIGURE 1 A SIMPLIFIED SCHEME OF THE BROMATE FORMATION FROM BROMIDE (FIGURE TAKEN FROM SOLTERMANN ET AL. 2017⁸)



In the environment, bromate is not degraded under aerobic conditions and therefore persistent¹⁰. It is also difficult to remove with conventional water treatment techniques¹¹.

Bromate is classified as potentially carcinogenic to humans and the WHO recommends a guideline value of 10 $\mu\text{g/l}$ for bromate in drinking water¹². The Drinking Water Directive 2020/2184 of the European Union also requires a maximum of 10 $\mu\text{g/l}$ bromate in drinking water. The Dutch Drinking Water Directive requires a maximum value in drinking water for

bromate of 1 µg/l and in case of disinfection the limit is 5 µg/l (as a 90-percentile value, the maximum that may occur is 10 µg/l).

Regarding surface water, in Switzerland the Ecotox Centre Eawag-EPFL has proposed an environmental quality standard for bromate of 50 µg/l. In the Netherlands there are no quality standards for bromate in WWTP-effluents **yet**, nor legislative standards for surface water. There is an ad hoc maximum permissible risk level (ad-hoc MTR) of 30 µg/l in place for bromate in surface waters. Moreover, in September 2021 the National Institute for Public Health and the Environment in the Netherlands (RIVM) published risk limits for bromate in fresh surface water. For aquatic plants and animals, a safe concentration in water of 50 µg/l is determined. For drinking water intake points, a more stringent value of 1 µg/l is proposed to meet the quality standards for drinking water¹³. Based on this information currently the Ministry of Infrastructure and Water Management is in the process of determining a bromate standard for surface water. Moreover, in September 2021 the RIVM has also recommended to regard bromate as a substance of very high concern (SVHC) because of its carcinogenicity¹⁴. Bromate itself is not an SVHC as it does not appear in the REACH regulation of the European Chemicals Agency as a SVHC. However, because of the carcinogenicity of the bromate ion other bromate compounds such as potassium bromate and nickel dibromate are listed as SVHC.

Bromate concentration after the ozonation of WWTP-effluent depends on wastewater characteristics and process conditions. Pilot studies in the Netherlands revealed that bromate concentrations after ozonation of the WWTP-effluent varied from <1 up to 70 µg/l depending mostly on the applied ozone dose and wastewater composition. In section 5.1 this is discussed in more detail.

3.2 CHROMATE

Chromate (chromium VI) is a toxic compound, more toxic than chromium (III), which can exert carcinogenic, mutagenic and teratogenic effects in biological systems¹⁵. It may be formed from chromium (III) during ozonation, possibly originating from industrial wastewaters discharges¹⁶. In the Dutch Drinking Water Directive, the maximum concentration for total chromium in drinking water is 50 µg/l. For surface water the maximum chromium concentration is 20 µg/l. There is no maximum concentration specifically for chromate.

Katsoyiannis *et al.* (2018) shows that only when the chromium (III) concentration is above 50 µg/l and the specific ozone dose is 1 g O₃/g DOC or more, more than 10 µg/l chromium (VI), a former drinking water guideline in California, could be produced when ozonating secondary wastewater effluent (DOC 6,4 mg/l)¹⁶. However, the chromium (III) concentrations measured in 70 Swiss wastewaters were generally below 1 µg/l and in only one case the chromium (III) concentration was 10 µg/l. Higher concentrations may be related to industrial discharges. In the WWTPs chromium (III) is removed mainly via the activated sludge and analysis of the sludge may indicate if there are any periodically increased chromium (III) concentrations in the wastewater¹⁰. However, because the concentration of chromium (III) discharged in the wastewater is in most cases very low, at least in Switzerland, and the chromate production is very slow, the chromate production during ozonation is generally not problematic as it leads to toxicologically insignificant concentrations.^{10,16}

3.3 CHLORATE AND CHLORINATED ORGANIC COMPOUNDS

Chlorate formation during ozonation is relevant when a pre-treatment with chlorine or chlorine dioxide is applied, typically as a disinfection step. In the Netherlands disinfection is not applied at WWTPs. Moreover, formation of chlorinated organic compounds for typical conditions during ozonation of wastewater is minimal.⁷

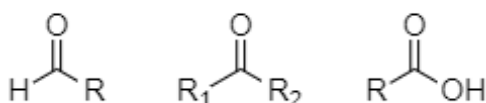
3.4 BROMINATED ORGANIC COMPOUNDS

The major intermediate in bromate formation (HOBr/OBr) can react with organic matter forming organic bromine such as bromoform, bromal, bromoacetic acid, and unknown brominated compounds¹⁷. However, low concentrations are expected because ozone also reacts with their precursors.

3.5 CARBONYLS

Carbonyls are oxidation by-products mainly formed from dissolved organic matter (DOM) which is part of the water matrix of WWTP-effluent. The water matrix of WWTP-effluent largely exists of very diverse organic molecules which are not (completely) removed and/or formed in the activated sludge process. Typically, these compounds are natural organic matter like humic acids and soluble microbial products. These organic molecules are often referred to as dissolved organic matter (DOM). They are commonly quantified as dissolved organic carbon (DOC) or total organic carbon (TOC). DOC can react with ozone, which results in the formation of oxidation by-products. Reactive compounds present are primarily aromatics and unsaturated aliphatic compounds which form formaldehydes and glyoxal respectively. Since the concentration of DOC in effluent (mg/L) is typically several orders of magnitude higher compared to organic micropollutants (ng/L - µg/L), the formation of oxidation by-products originating from DOC is typically higher than the formation of other by-products.¹⁸ It was shown that DOC with high molecular weight (1600 D) is slightly more reactive to ozone than DOC with low molecular weight (500 D)¹⁹. After ozonation, high concentrations of carbonyls were observed. These carbonyls can be aldehydes, ketones and carboxylic acids (see Figure 2: The standard structure of aldehydes, ketones, and carboxylic acids (from left to right)).^{20,21,22}. Common aldehydes formed are formaldehyde, heptanal and acetaldehyde.

FIGURE 2 THE STANDARD STRUCTURE OF ALDEHYDES, KETONES, AND CARBOXYLIC ACIDS (FROM LEFT TO RIGHT)



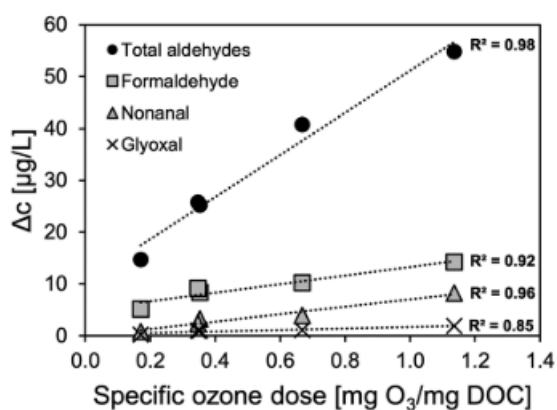
In the following sections the various carbonyls are discussed. First the aldehydes and ketones, then the carboxylic acids. Next, the α,β -unsaturated carbonyls are discussed and then the oxidation process is described. Lastly, the potential toxicity of the various carbonyls is discussed.

ALDEHYDES AND KETONES

Approximately 80% of the identified aldehydes are formaldehyde and acetaldehyde²³. Aldehydes that are formed to a lesser extent are propanal, butanal, hexanal, glyoxal, methylglyoxal, and acetone²⁴.

Composition of different carbonyl oxidation by-product formation depends a lot on the wastewater composition. Overall, when aldehydes are formed, nine compounds are commonly found (formaldehyde, acetaldehyde, butanal, heptanal, glyoxal, propanal, hexanal, methylglyoxal and acetone). At a specific ozone dose of 0.67 g O₃/g DOC, concentrations of formed aldehydes ranged from less than 1 µg/L for propanal and methylglyoxal up to 10 µg/L for formaldehyde and heptanal. Sometimes, a total carbonyl concentration of >100 µg/L is achieved²³. Moreover, the amount of oxidation by-product formation depends on the concentration of the parent compound and in most cases also on the applied ozone dose. Formation of formaldehyde, acetaldehyde, butanal, heptanal, nonanal, and glyoxal correlates well with the specific ozone dose ($R^2 > 0.8$, some shown in Figure 3). For propanal, hexanal, methylglyoxal, and acetone, there is no such correlation. Total aldehyde concentration correlates best with the ozone dose ($R = 0,98$). This reveals that the overall potential for aldehyde formation at a given specific ozone dose can be predicted even though unpredictable concentration shifts can occur between by-products.²⁴

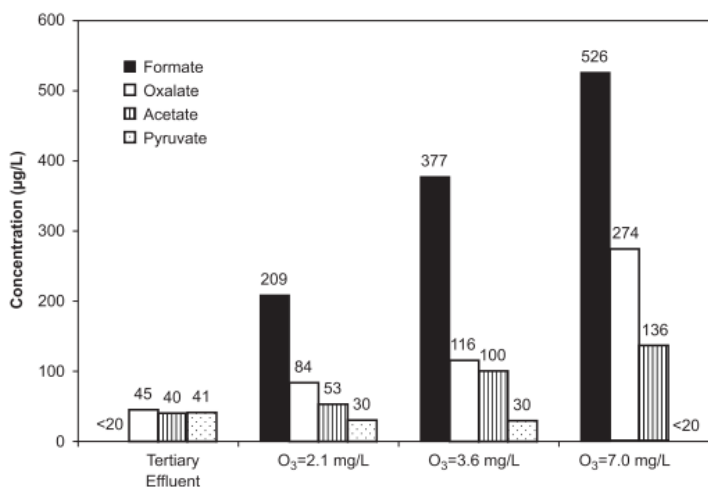
FIGURE 3 ALDEHYDE FORMATION DURING OZONE TREATMENT AT DIFFERENT SPECIFIC OZONE DOSES WITH C₀ DOC = 10 MG/L (FIGURE TAKEN FROM SAUTER ET AL. 2021²⁴)



CARBOXYLIC ACIDS

Other carbonyls formed are carboxylic acids²⁵. It was noticed that carboxylic acids were formed at much higher concentrations than aldehydes^{19,26}. Wert *et al.* (2007) analysed six different carboxylic acids after ozonation of wastewater effluent. Four of them could be detected: formate, oxalate, acetate, and pyruvate. Ketomalonate and propionate were not detected (<20 µg/L). Formation of formate, oxalate, and acetate appear to correlate with the specific ozone dose (see Figure 4). Formate is the major route, namely 55-60% of carboxylic acid formation. Wert *et al.* (2007) state that this may be the result of formaldehyde oxidation.^{26,27}

FIGURE 4 CARBOXYLIC ACID FORMATION DURING OZONATION ON PILOT-SCALE (FIGURE TAKEN FROM WERT ET AL. 2007²⁶). DOC OF THE TERTIARY EFFLUENT AFTER FLOCCULATION AND FILTRATION WAS 7.1-7.2 MG/L. THUS, SPECIFIC OZONE DOSE WAS 0.3, 0.5 AND 1,0 G O₃/G DOC RESPECTIVELY



α,β -UNSATURATED CARBONYLS

Due to recent advances in quantitative methods, other carbonyl oxidation by-products that can be measured are α,β -unsaturated carbonyls. These are formed from phenolic compounds and alkylated aromatic compounds and are generally quickly further oxidized to aldehydes and carboxylic acids. Moreover, the amounts formed of these unsaturated carbonyls which are not further oxidized, are very low. Approximately 0,3% of the total carbonyl formation are α,β -unsaturated carbonyls. Of this 0,3% around 90% is made up of propenal and 2-butenal.²³

Even though formation of α,β -unsaturated carbonyls are not common, Prassé *et al.* (2018) showed that toxic α,β -unsaturated enedials and oxoenals are formed during water treatment systems. These oxidation by-products damage proteins by reacting with lysine and cysteine moieties which can cause cellular damage.²⁸ Van Buren *et al.* (2020) investigated products of ring hydroxylation and oxidation of alkyl substituents as well as a suite of ring-cleavage products and state their concerns of toxic oxidation by-product formation when the wastewater contains benzenes or alkylbenzenes.²⁹

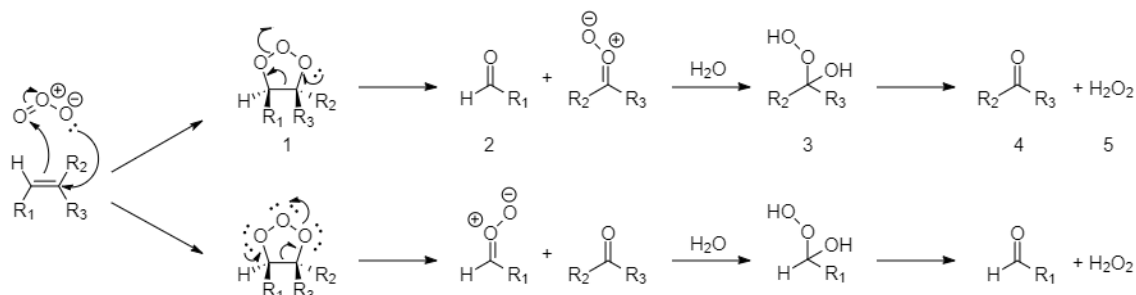
CARBONYL OXIDATION PROCESS

Transformation of the various carbonyls (aldehydes, ketones, carboxylic acids, α,β -unsaturated carbonyls) by ozonation occurs either through direct ozone oxidation or a reaction with the less selective hydroxyl radical.

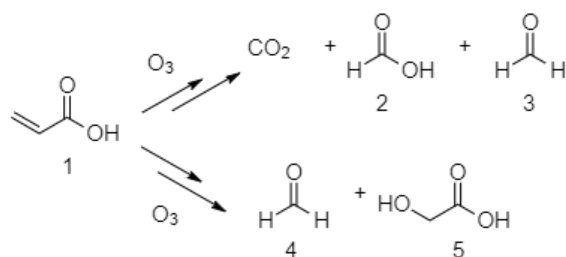
The direct ozone oxidation mechanism to form carbonyls from unsaturated hydrocarbon compounds (olefins) is well known as the Criegee mechanism³⁰. Formation rates of carbonyls strongly depend on the substitution of the olefins and may vary over 8 orders of magnitude. The reaction of olefins with ozone is depicted in Scheme 1. It is initiated by the attack of ozone on the substance, to form an ozonide (1). This is an unstable cyclic trioxide which decomposes into a carbonyl compound (2) and a hydroxy hydroperoxide (3). This hydroxy hydroperoxide then slowly decomposes into another carbonyl compound (4) and hydrogen peroxide (5). This carbonyl compound can be an aldehyde, and aldehydic acid or a carboxylic acid. When an acid is formed, water is formed instead of hydrogen peroxide. This has been observed for example for carboxy-acyclovir, an antiviral drug having a fused pyrimidine – imidazole ring with conjugated double bonds (guanine-like structure). Significant singlet

oxygen formation from the reaction of ozone with olefins was also observed for 5-chloro-uracil³¹. The oxidation mechanism has also been established for substituted ethenes. In the case of acrylic acid (see Scheme 2), attack of ozone leads to an ozonide intermediate which can lose CO₂ and form formic acid (2) and formaldehyde (3) or decompose into formaldehyde (4) and glyoxylic acid (5).³²

SCHEME 1 REACTION OF OZONE WITH OLEFINS TO FORM CARBONYLS (SCHEME ADAPTED FROM VON GUNTEN 2003³²)

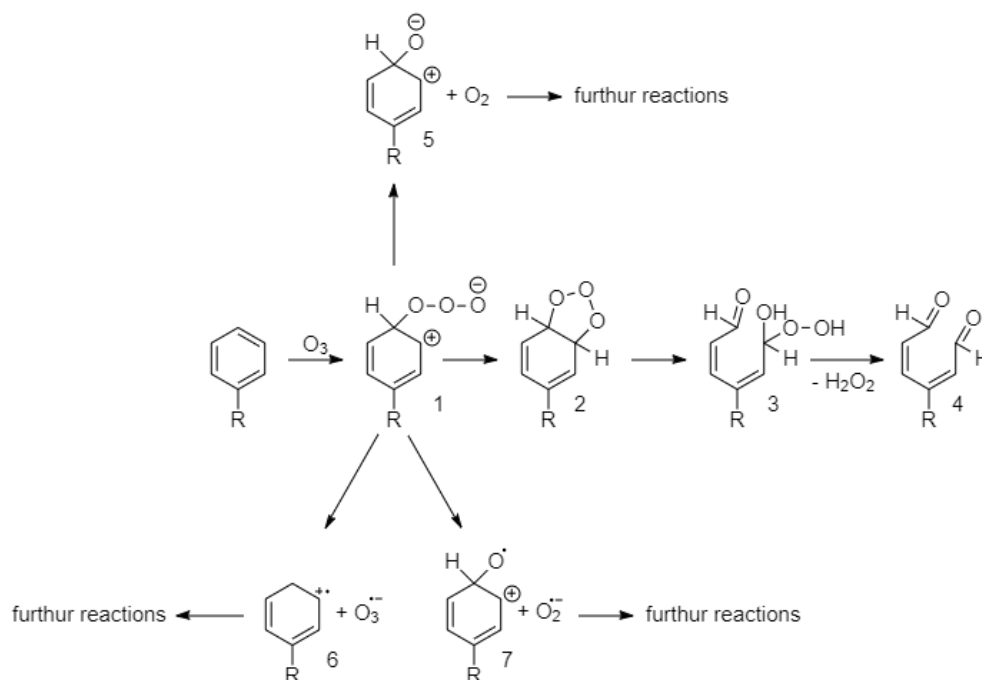
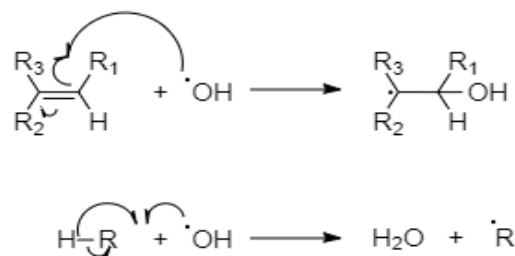


SCHEME 2 REACTION OF OZONE WITH ACRYLIC ACID TO FORM CARBONYLS (SCHEME ADAPTED FROM VON GUNTEN 2003³²)



Carbonyls are also formed when ozone reacts with activated aromatic compounds, e.g. benzene with alkyl or alkoxy (hydroxyl) substituents (see Scheme 3)³². This reaction generally occurs via the formation of an ozone adduct (1) and subsequent ozonide (2) on the benzene ring. Breakdown of the ozonide leads to cleavage of C-C bond (3) with the formation of two terminal carbonyls and H₂O₂ (4). This mechanism has been observed for benzotriazole, bezafibrate, bisphenol-A, estrone, 17 α -ethinylestradiol, metoprolol and propranolol amongst others. In competition with the ozonide formation, the ozone adduct can release singlet oxygen (5) before it is further oxidized to a carbonyl. Another competitive pathway is that the ozone adduct dissociates as a radical (6) or oxygen dissociates as a superoxide (7) before it can undergo further reaction to carbonyls.³¹

In addition, carbonyls can also be formed by the reaction with hydroxyl radicals. Laboratory tests indicate that oxidation by-product formation largely depends upon hydroxyl radical exposure. At equivalent ozone dosages, greater concentrations of aldehydes (31-47%), carboxylic acids (12-43%), and assimilable organic carbon (5-52%) were produced²⁶ when hydrogen peroxide was added to increase the hydroxyl radical. The mechanism of the oxidation of unsaturated compounds with hydroxyl radicals proceeds through the addition of the hydroxyl radical to the C-C double bond (for olefinic, aromatic, free amine, and sulphur moieties) or by abstracting a carbon-bound proton (see Scheme 4). The addition pathway is generally faster than the abstraction pathway.³³

SCHEME 3 REACTION OF OZONE WITH ACTIVATED AROMATIC COMPOUNDS (SCHEME ADAPTED FROM LEE ET AL. 2016³¹)SCHEME 4 MECHANISM OF THE OXIDATION OF UNSATURATED COMPOUNDS WITH HYDROXYL RADICALS THROUGH ADDITION (TOP) OR ABSTRACTION (BOTTOM) (SCHEME ADAPTED FROM VON GUNTEN 2003³²)

The formed carbonyls could be further oxidized to eventually CO_2 , however their oxidation rate is much lower than their formation rate and applied ozone doses are typically too low for full mineralisation into CO_2 . Therefore, accumulation of the carbonyls will occur during ozonation³².

TOXICITY OF COMMONLY FORMED CARBONYLS

Toxicity for some endpoints increases due to the formation of aldehydes and organic acids. Even though carboxylic acids are formed to a larger extent, aldehydes are more important from a toxicological point of view. The main aldehydes formed (formaldehyde, acetaldehyde, glyoxal and methylglyoxal) are carcinogenic and mutagenic and have been observed to cause tumours in rats^{21,27}. However, extending consideration of toxicity of carbonyl compounds beyond a few saturated aldehydes and ketones is important because all members of this chemical family cause toxicity by forming covalent bonds with nucleophilic sites on biological targets. Formation of α,β -unsaturated carbonyls has also been noted. Furthermore, a double bond between carbons two and three in α,β -unsaturated aldehydes substantially increases the electrophilicity, and therefore the toxicity of these compounds²³.

Predicted no effect concentrations (PNECs) are being used as indications to evaluate the environmental toxicity. An inventory was made of available PNECs for commonly formed carbonyls, as listed in Table 1.

TABLE 1§ AVAILABLE PNECS FOR CARBONYL COMPOUNDS¹⁵

Compound	PNEC (mg/l)	Compound	PNEC (mg/l)
Aldehyde		Carboxylic acid	
Formaldehyde	0.44	Formate	2
Heptanal	0.006	Oxalate	0.16
Propanal	0.014	Acetate	3.058
Hexanal	0.071	α,β -unsaturated carbonyls	
Glyoxal	0.319	Propenal	0 ¹
Methylglyoxal	0.404	2-butanal	0 ²
Ketone			
Acetone	10.6		

For most carbonyls the found concentrations after ozone treatment are below PNEC values. The concentration of oxalate as displayed in Figure 4 at an ozone dose of 7 mg O₃/L exceeds the PNEC. However, for lower ozone doses the oxalate concentration is below the PNEC. For the commonly formed aldehydes acetaldehyde, butanal, and pyruvate, no PNEC values were found. For these aldehydes acute toxicity tests on the aquatic organisms fish, algae and daphnia were performed. For acetaldehyde effects in the category “harmful” were observed in fish and daphnia. However, formal Classification & Labelling of acetaldehyde for aquatic toxicity is not relevant, due to its rapid biodegradation. Butanal has been tested for acute aquatic toxicity in fish, daphnia, algae, and bacteria as well as in various protozoa and the overall pattern of results indicates that butanal is of low toxicity to aquatic organisms. Pyruvate was neither classified for acute aquatic toxicity, nor for chronic aquatic toxicity. Thus, acetaldehyde, butanal and pyruvate are not indicated as toxic to aquatic organisms.¹⁵

3.6 N-CONTAINING COMPOUNDS

Ozonation of nitrogen-containing compounds may yield specific harmful by-products, nitrosamines, of which *N*-nitrosodimethylamine (NDMA) is a well-known example. In the last years, many studies have been conducted into the ozonation of a large variety of nitrogen-containing compounds, evaluating their removal, reaction mechanisms and pathways, and elucidating transformation products.^{31,34-39} This has greatly extended the knowledge about the fate of these compounds during ozonation. Information about the toxicity of the transformation products has been found for nitrosamines and nitromethane, these will be discussed in more detail.

3.6.1 *N*-NITROSODIMETHYLAMINE (NDMA) AND OTHER *N*-NITROSAMINES

Regarding the *N*-nitrosamines, most information has been found on the *N*-nitrosamine NDMA, a suspected human carcinogen. It is regularly detected in WWTP-effluents and ozonated waters. NDMA is on the list of ‘substances of very high concern’⁴⁰. When released in surface water it is not expected to sorb on solids, but it may degrade due to biological processes or under influence of sunlight (EPA 2014). In Dutch Drinking Water Directive a maximum allowable concentration for NDMA in drinking water of 12 ng/l is included⁶.

- 1 This is the current available PNEC values for freshwater from the European Chemicals Agency. LC50-values for freshwater fish were considered to be in the range of 14 – 90 µg/l and a NOEC value of 11.4 µg/l.¹⁵
- 2 This is the current available PNEC values for freshwater from the European Chemicals Agency. LC50-values for freshwater fish were considered to be in the range of 0.65 mg/l and a NOEC value of 24.7 µg/l.¹⁵

A PNEC value for NDMA has not been found in literature. Mestankova *et al.* (2014) investigated the mutagenicity of NDMA and other N-nitrosamines for *Salmonella typhimurium* strain YG7108 and found that the mutagenicity increased in the order NDPhA << NPYR < NDPA < NDEA < NDMA (Table 2). Based on that result they conclude that (quote): “the aliphatic N-nitrosamines are more potent than the aromatic or cyclic compounds and the potency increases with decreasing aliphatic chain length”. The selected N-nitrosamines showed no mutagenicity in the strains of TA98 and TAMix, that are commonly used in the Ames test⁴¹.

TABLE 2 MUTAGENICITY OF N-NITROSAMINES IN THE YG7108 STRAIN (TABLE ADAPTED FROM MESTANKOVA ET AL. 2014⁴¹)

N-nitrosamine	EC50 (M) (95% confidence interval)	Reported lowest mutagenic concentration
NDMA	1.0×10^{-5} ($5,8 \times 10^{-6} - 1,7 \times 10^{-5}$)	5×10^{-5} M (3.700 µg/l)
NDEA	6.8×10^{-5} ($3,8 \times 10^{-5} - 1,1 \times 10^{-4}$)	Not available
NDPA	1.3×10^{-4} ($8,5 \times 10^{-5} - 2,0 \times 10^{-4}$)	Not available
NPYR	9.4×10^{-4} ($4,5 \times 10^{-4} - 2,0 \times 10^{-3}$)	7.5×10^{-4} M (75.000 µg/l)
NDPhA	Not significant	Not significant

In Switzerland, recently a test procedure was implemented to evaluate the formation of NDMA during ozonation to determine a wastewater's suitability for ozonation²⁰. In Krauss *et al.* (2009) 21 WWTPs in Switzerland were sampled for their NDMA concentration in the influent and secondary effluent. In 20 out of 21 WWTPs NDMA concentrations of <1,5 up to 33 ng/l were found in the secondary effluent (after the main biological treatment). In one WWTP the NDMA concentration in the secondary effluent varied much more, between <1,5 to 188 ng/l. Krauss *et al.* state that levels above 5 ng/l in the influent are probably caused by discharges from a non-domestic origin such as industrial discharges.⁴² In Bourgin *et al.* (2018) the effect of ozonation on the NDMA concentration is analysed. In this research relatively high concentrations of ~20 up to 190 ng/L NDMA have been observed in the secondary effluent (after the main biological treatment) of WWTP Neugut (Dübendorf, Switzerland). During ozonation at specific ozone doses of 0,54-0,97 g O₃/g DOC, the authors found that the maximum NDMA formation was 30 ng/l at a specific ozone doses of 0,54 g O₃/g DOC. Thus, an addition of about 30 ng/l on the NDMA which was already present in the secondary effluent (20-190 ng/l). These results show that only a small portion of NDMA in the effluent of the ozone reactor is formed during ozonation, most of it was already present in the wastewater (Figure 5).²⁰ Moreover, concentrations are found far above the standard for drinking water in the Netherlands of 12 ng/l. No results have been found for Dutch WWTPs. It should be further investigated what the NDMA levels are in WWTP-effluents in the Netherlands and what the effect of ozonation on NDMA formation is.

Bourgin *et al.* (2018) found that the highest NDMA formation during ozonation was not found at the highest specific ozone dose. Therefore it seems that the composition of the secondary effluent with possible varying levels of NDMA precursors and thereby NDMA formation rates has a higher impact on the NDMA formation than the applied specific ozone dose²⁰. This is confirmed by observations of other researchers such as done by Lee *et al.* (2016) and Schindler Wildhaber *et al.* (2015) who determined NDMA formation at different specific ozone doses (0,5-1,5 g O₃/g DOC) and found a similar lack of correlation. These and several other studies conclude that NDMA formation during ozonation is more affected by the changing concentrations of NDMA precursors in the WWTP-effluents than the specific ozone dose^{24,43-45}. NDMA formation is difficult to avoid during ozonation or even peroxone treatment due to the rapid reaction of the precursors with ozone^{44,46}.

For some wastewater constituents it is known that they can be precursors for NDMA formation during ozonation. However, there is no complete picture yet on precursors, kinetics and mechanisms related to NDMA formation, as of yet³¹. Some *N,N*-dimethylamino group containing compounds (those connected to sulfamide, hydrazine, hydrazides, and carbazides) are potent ozone-NDMA precursors with molar yields up to 94%^{31,46}. Pathways for NDMA formation have been proposed by Yang *et al.* (2009)⁴⁷ and further explored by other authors. Detailed studies into *N,N*-dimethylsulfamide (DMS) ozonation have revealed that the presence of bromide is a prerequisite for NDMA formation from this particular type of compound. Dimethylamine and other *N,N*-dimethylamino compounds are also precursors, at very low molar yields of below 0,05%. At sufficiently large concentrations these compounds can still contribute to significant NDMA formation. For example, some quaternary or tertiary amine-based coagulants release dimethylamine during ozonation⁴⁶. Lim *et al.* (2016) reveal that *N,N*-dimethylhydrazine compounds have inevitable high molar yields of NDMA, because of a highly efficient ozone reaction forming the compound, and a low efficiency of the hydroxyl radical reaction. To mitigate NDMA formation from *N,N*-dimethylhydrazine compounds the authors propose source control or abatement before ozonation⁴⁶. *N,N*-dimethylhydrazine moieties ((CH₃)₂N-). In several studies it has been observed that NDMA is significantly removed during biological or physical-chemical post-treatment after ozonation, although not completely^{20,24,45,48,49}. For example, Bourgin *et al.* (2018) and Hollender *et al.* (2009) observed the presence in secondary effluent, and its formation during ozonation. Post-treatment by (aerobic) sand filtration showed significant removal of the NDMA as can be seen in Figure 5 and Figure 6^{20,43}. Four other nitrosamines showed decreasing concentrations along the different treatment processes of the WWTP (Figure 6)⁴³.

FIGURE 5 NDMA CONCENTRATION MEASURED IN THE SECONDARY EFFLUENT, EFFLUENT OF OZONATION AND EFFLUENT OF SAND FILTRATION TREATMENT STEP AT WWTP NEUGUT, SWITZERLAND (FIGURE TAKEN FROM BOURGIN ET AL. 2018²⁰)

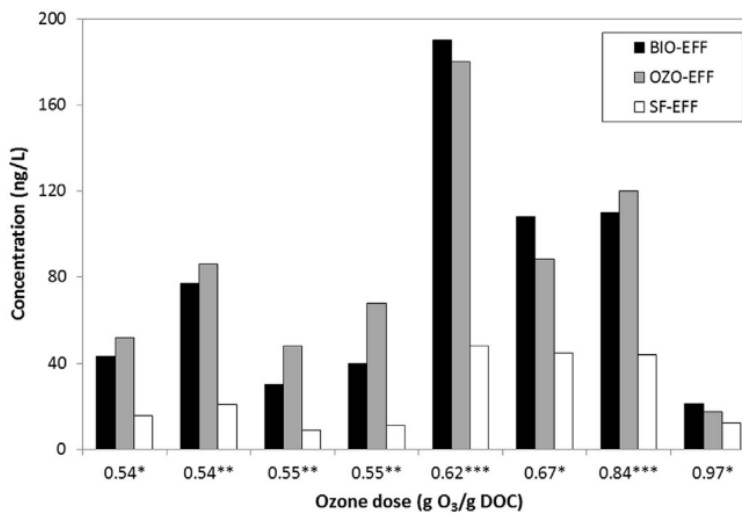
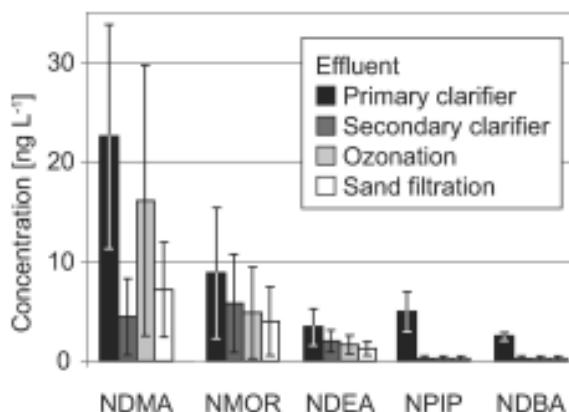


FIGURE 6 MEDIAN CONCENTRATION FOR NDMA, NMOR, NDEA, NPIP, AND NDBA ALONG THE TREATMENT PROCESS AT A WWTP (FIGURE TAKEN FROM HOLLENDER ET AL. 2009⁴³)



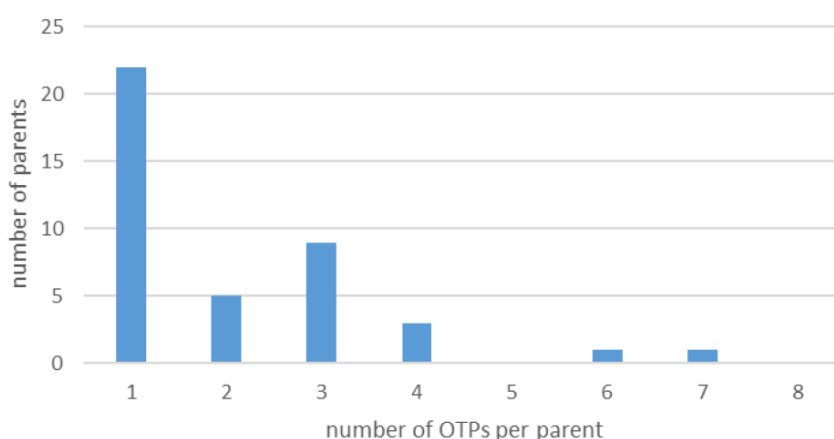
3.6.2 NITROMETHANE

During ozonation often nitromethane is formed. Shi *et al.* (2020) demonstrated that nitromethane is formed during ozonation of N-methylamine compounds, such as certain types of drugs (ephedrine and methamphetamine, and certain antidepressants), with yields of over 50%. Upon chlorination, nitromethane is converted into chloropicrin, a halonitromethane (HNM). HNMs are highly genotoxic, which makes the formation of nitromethane of interest for water treatment applications with post-chlorination or post-chloramination.⁵⁰ Shi *et al.* (2021) showed that nitromethane is typically removed in biological post-treatment⁵¹. To the best knowledge of the authors no chlorination is used in the Netherlands as post-treatment on WWTP-effluent.

3.7 TRANSFORMATION PRODUCTS

During ozonation of WWTP-effluent the aim is to oxidize micropollutants before they enter the aquatic environment. When the micropollutants are oxidized by ozone or hydroxyl radicals they are seldomly completely mineralized into CO₂. Thus, residual fractions of the original micropollutant are formed, the so-called transformation products. Identification of transformation products formed out of the wide array of micropollutants present in wastewater is already a complex task, let alone the toxicological assessment of these compounds. Nevertheless, for various micropollutants studies have been conducted to research the formation pathway(s) of transformation products and identify the final products. Therefore, in literature already quite some identified transformation products can be found. Gulde *et al.* (2021) tested the ozonation of 70 micropollutants in lab-experiments. With a broad screening method using LC-HR-MS they registered 1749 potential transformation product signals after the ozonation tests. In the same study the formation of transformation products in four full-scale ozonation plants at Swiss WWTPs was investigated. After ozonation at ozone doses of 0.23 to 0.55 g O₃/g DOC a total of 84 transformation products could be identified and linked to 40 micropollutants, and chemical structures could be proposed. For some micropollutants multiple transformation products were detected, 6 for carbamazepine and 7 for sitagliptin (see Figure 7)⁵².

FIGURE 7 AMOUNT OF TRANSFORMATION PRODUCTS (OTP) ASSIGNED TO 40 PARENT MICROPOLLUTANTS (FIGURE TAKEN FROM GULDE ET AL. 2021⁵²)



The question that will be addressed in this chapter is if the toxicity of the transformation products is higher than their parent compounds and if the transformation products are present at relevant concentrations. A limited amount of literature is available on the toxicity and concentrations of the transformation products. This section summarizes the information that has been found on the toxicity of specific micropollutants before and after ozonation. Note that a summation of all possibly formed transformation products is not provided. Therefore, the transformation products for which information is presented in this chapter should be regarded as examples.

3.7.1 ESTROGENS AND THEIR TRANSFORMATION PRODUCTS

Steroid estrogens can be well removed during ozonation. 17α -Ethinylestradiol (EE2), the hormone used in the contraceptive pill, and which has a significant share of the estrogenic activity in WWTPS effluent, can be oxidized during ozonation, both directly with ozone or with hydroxyl radicals⁵³. In Huber *et al.* (2004) the effect of ozonation on the estrogenic activity in an aqueous water sample containing EE2 has been studied in bench-scale tests. Elimination of EE2 led to a proportional reduction of estrogenicity, the latter measured with a yeast estrogen screen (an *in vitro* yeast assay). The results show that the estrogenic activity after ozonation was with 99.5% reduced. The residual EE2 in the solution could explain most the residual estrogenicity.⁵⁴ Also, in Lee *et al.* (2008) a reduction in estrogenic activity during ozonation of EE2 has been reported. They found that slight modification of the chemical structures of EE2 already resulted in a substantial reduction of the estrogenic activity of >87%.⁵³

3.7.2 ANTIBIOTICS TRANSFORMATION PRODUCTS

In Dodd *et al.* (2009) the changes in antibacterial potencies during ozonation of antibiotics is tested. The research included 13 antibiotics representing several structural classes:

- macrolides: roxithromycin, azithromycin, tylosin
- fluoroquinolones: ciprofloxacin, enrofloxacin
- beta-lactams: penicillin G, cephalexin,
- Sulfonamides: sulfamethoxazole
- Others: trimethoprim, lincomycin, tetracycline vancomycin and amikacin

Experiments showed that the antibacterial activity is reduced by ozonation for all tested substances. For 11 out of the 13 antibiotics it has been observed that the removal of the parent compound led to an equivalent removal of the antibiotic activity. Only for the two

beta-lactams penicillin G and cephalixin this was not the case. Though the antibacterial activity for these two antibiotics reduced substantially during ozonation, the bioassays indicate that oxidation products may have been formed with some biological activity. The biologically active oxidation product(s) from penicillin G formed with the direct reaction with ozone appeared to be recalcitrant to further oxidation by ozonation. Other biological activated oxidation products formed could be further deactivated by ozonation. This research shows that generally ozonation is effective in the reduction of antibiotic activity reduction.⁵⁵

Lange *et al.* (2006) investigated clarithromycin as a model compound for macrolide antibiotics. Toxicity tests were performed using *P. putida* DOT-T1E growing in the presence of the original and ozone-treated antibiotic. During ozonation, one mol of clarithromycin is degraded per mol ozone, which allowed the authors to calculate the amount of antibiotic left in the treated solutions. The resulting growth inhibition showed a correlation with the remaining concentration. Also, in this article it is concluded that with ozonation the biological activity is eliminated.⁵⁶

Continuous ozonation of the antibiotic ofloxacin (OFX) was studied by Carbajo *et al.* (2015) in both a synthetic water matrix and sewage treatment plant effluent. Aquatic toxicity assays (with *P. putida* and *P. subcapitata*) were performed before and after ozonation. Results show that with decreasing ofloxacin concentration the toxicity is reduced, implying that the influence of the oxidation products on the aquatic toxicity is negligible.⁵⁷

3.7.3 ACYCLOVIR TRANSFORMATION PRODUCT: COFA

During biological treatment the antiviral drug acyclovir (ACV) is transformed into C-ACV, which is transformed by ozonation into COFA. COFA is biologically stable and highly polar, which means it cannot be removed by sand filtration or activated carbon filtration. An initial toxicity screening of C-ACV and COFA using *V. fischeri* by Prasse *et al.* (2012) showed no bacterial toxicity of C-ACV but they did find inhibition with COFA⁵⁸. Schluter-Vorberg *et al.* (2015) used ACV, C-ACV and COFA in different toxicity tests. No toxicity was observed for acyclovir up to 100 mg/L, C-ACV was found to reduce the reproduction level of *Daphnia magna* by 40% at 102 mg/L, and COFA inhibited the growth of green algae (EC10 of 14.1 mg/L) a factor 7 more than ACV. The predicted genotoxicity of C-ACV and COFA was not increased compared to that of ACV⁵⁹. It was concluded that ozonation can potentially increase toxicity through COFA formation, but that this is highly unlikely because in practice the concentrations are much lower than those mentioned above. When comparing the EC10 with the measured environmental concentrations of COFA (0,001 µg/l), the authors stipulate that de EC10 does not indicate an unacceptable environmental risk. Rather, COFA could be seen as an example demonstrating that some transformation products can be more toxic than their parent compound⁵⁹.

3.7.4 CARBAMAZEPINE TRANSFORMATION PRODUCTS

Ozonation of carbamazepine and four of its suspected transformation products were evaluated for removal, formation during ozonation and toxicity to zebra fish by Pohl *et al.* (2019). They concluded that the ozonation of carbamazepine at a concentration of 25, 50 and 100 mg/l increased toxicity in an embryo toxicity test. Identification of the transformation products revealed that carbamazepine-10,11-epoxide (the main therapeutically active carbamazepine metabolite) was detected in the highest concentrations after ozonation, and 10,11-dihydrocarbamazepine was the second most abundant transformation product, followed by 10,11-dihydro-10-hydroxycarbamazepine. Oxcarbazepine formation was not

detected. The authors suspect that carbamazepine-10,11-epoxide was one major driver of the increased toxicity after ozonation.⁶⁰ It must be noted that this compound is also formed during metabolism of carbamazepine in the liver of humans and therefore it can be present already in the wastewater prior to ozonation. In the Netherlands the average concentration of carbamazepine-10,11-epoxide in WWTP-effluent is 1 µg/l⁶¹. Apart from this, the carbamazepine concentrations applied in the tests by Pohl *et al.* (2019) are a much higher compared to concentrations in WWTP-effluent (on average in the Netherlands this is 0.5 µg/l)^{60,61}. Thus, the research shows that the transformation product of carbamazepine can be more toxic than its parent compound. However, the research does not include a risk assessment and it does not show whether this toxic effect is also observed during ozonation of carbamazepine at typical concentrations in WWTP-effluent.

3.7.5 DICLOFENAC TRANSFORMATION PRODUCT: 2,5-DIHYDROXYPHENYLACETIC ACID

Diclofenac (DCF) is one of the main representatives of the non-steroidal anti-inflammatory drugs and is found in many waters worldwide. Matejczyk *et al.* (2020) evaluated the toxicity and biological effects of DCF, its microbial degradation metabolites 4'-hydroxy-DCF (4'-OHDCF) and 5-hydroxy-DCF (5-OHDCF) and 2,5-dihydroxyphenylacetic acid, an oxidation product formed during ozonation of DCF. Several toxicity tests were conducted. One toxicity test was done with the *Chironomus aprilinus* larvae, resulting in a LC50 after 48h of DCF of 105 mg/l, and 20 mg/l for the oxidation product 2,5-dihydroxyphenylacetic acid. In comparison, for the two microbial degradation metabolites 4 and 5-OHDCF the LC50 was 79 and 63 mg/l resp. The oxidation product after ozonation thus had a lower LC50 value for *C. aprilinus* larvae compared to the parent compound. Opposite results were found during viability tests with *E. coli*. The strongest inhibition was found for DCF and the biological metabolites 4- and 5-OHDCF (mean value ~60 and ~65% respectively, compared to ~75% for 2,5-dihydroxyphenylacetic acid) at a (environmentally very high) concentration of 10 mg/l. This indicates the varying sensitivity of different species towards these substances.⁶²

In a study on zebrafish embryotoxicity, Pohl *et al.* (2019) found a reduced toxicity after ozonation of diclofenac. After almost complete removal (97%) of diclofenac by ozonation, no toxicity was observed anymore. The initial (high) diclofenac concentrations were 3,4, 7,5 and 15 mg/l. Thus, the transformation products of diclofenac were less toxic than diclofenac itself. It must be noted that the concentrations of diclofenac, and thus also the concentrations of the transformation products, were much higher than expected in WWTP-effluent (µg/l range).⁶⁰

3.7.6 DIURON (HERBICIDE) TRANSFORMATION PRODUCTS

Diuron is an herbicide inhibiting the photosynthesis which can be removed by oxidation. In oxidation experiments Mestankova *et al.* (2011) studied the toxicity of diuron before and after ozonation. Because of the sensitivity of plants and or green algae towards diuron, a combined algae test was applied as a bioassay with the inhibition of photosynthesis and growth rate as endpoints. Results showed that the toxicity of the oxidation products, after both oxidation with hydroxyl radicals and ozone, was insignificant compared to the toxicity of the parent compound. The test indicates that the oxidation compounds have a lower toxicity towards algae compared to the parent compound and/or that the concentration in which they are formed is not sufficient to cause any increase in toxicity.⁶³

3.7.7 HYDROCHLOROTHIAZIDE TRANSFORMATION PRODUCT: CHLOROTHIAZIDE

Chlorothiazide is the main TP of direct ozonation of hydrochlorothiazide. Bourgin *et al.* (2017) studied the formation of chlorothiazide during ozonation of surface water, and found that after an initial increase in concentration, the compound was removed during prolonged ozonation. The specific ozone dose varied from 0,5-3 g O₃/g DOC.⁶⁴ Bioluminescence tests with ozone-treated hydrochlorothiazide samples by Borowska *et al.* (2016) showed no toxic effects and the authors conclude that the parent compounds and the corresponding transformation products are not harmful towards the tested bacteria⁶⁵.

3.7.8 OXAZEPAM TRANSFORMATION PRODUCTS

After ozonation of high concentrations (10 mg/l) oxazepam, Pohl *et al.* (2019) found effects on zebrafish larvae locomotion, but no toxic responses in recorded lethal or sublethal endpoints applying a embryo toxicity test. The recorded effect indicates that ozonation of oxazepam could potentially have an impact on the swimming behaviour of the larvae, but it must be noted that the high concentrations applied in this test (10 mg/l) are not environmental relevant. At the lowest concentration tested (0,1 mg/l) this effect was not observed⁶⁰.

3.7.9 OVERALL RELEVANCE OF TRANSFORMATION PRODUCTS

The various transformation products described in section 3.7 are only examples of all transformation products that can be present in ozonated WWTP-effluent because of the vast amount of micropollutants possibly present in WWTP-effluent. However, it does show that biological activity of parent compounds in certain environmentally relevant groups (antibiotics and estrogens) are eliminated with ozonation. Elimination of the biological activity as a result of the elimination of the micropollutant during ozonation has been observed for example with the estrogen 17 α - ethinylestradiol, many antibiotics and the herbicide diuron. For diclofenac a reduced toxicity was also observed. In a few cases an increased toxicity was observed for the transformation product in relation to the parent compound (e.g. acyclovir, carbamazepine and oxazepam). However, for these three compounds the research in the found literature was not performed at environmental realistic concentrations which raises the question of their environmental relevance. Also in Lee & Von Gunten (2016) it is mentioned that although in certain cases toxicity may have been observed for an individual transformation product, under realistic conditions the overall effect can be insignificant due to the low concentrations of the transformation product³¹. Nevertheless, the limited knowledge on the effects of transformation products at environmental relevant concentrations hinders conclusive conclusions to be drawn on their toxicity in regard to their parent products, and their toxicity in general.

3.8 SUMMARY

Bromate is classified as potentially carcinogenic to humans and quality standard of 1 ug/l are included for bromate in the Drinking Water Directive. Surface water quality standard for bromate is currently being revised in the Netherlands. Pilot studies in the Netherlands revealed that bromate concentrations after ozonation of the WWTP-effluent varied from <1 up to 70 μ g/l depending mostly on the applied ozone dose and wastewater composition. Common (biological) post-treatment technologies do not remove bromate.

Chromate, chlorate and chlorinated compounds have been identified as potential oxidation products but in municipal wastewater treatment the concentrations are typically expected to be low and insignificant from a toxicological point of view.

From the nitroso-compounds most information has been found for **NDMA**. For NDMA, a suspected human carcinogen, a maximum value of 12 ng/l is included in the Dutch Drinking Water Directive. Formation of NDMA during ozonation depends on the concentration of its precursors in WWTP-effluent. Certain precursors are known, but there is no complete picture yet on precursors. NDMA can also be present in the secondary effluent. Results from Switzerland showed that the NDMA concentration in secondary effluent can be higher than the NDMA formed during ozonation. NDMA can be removed to a certain extent by biological or physical-chemical post-treatment after ozonation.

Organic oxidation products which are formed out of dissolved organic matter (DOM) and organic transformation products formed out of micropollutants have been addressed in this chapter. In comparison with DOM, it must be noted that the concentration of micropollutants in the secondary effluent is typically a 1.000 to 10.000-fold lower. As DOM also reacts with ozone and hydroxyl radicals it consumes the largest amount of the dosed ozone. As a result, probably higher concentrations of DOM related oxidation products are formed compared to concentrations of transformation products from the micropollutants.

Oxidation products mainly formed from DOM are **carbonyls**. Three groups of carbonyls can be distinguished: aldehydes, ketones and carboxylic acids. Altogether, when comparing the PNEC values of the carbonyls with the detected concentrations described in literature it becomes clear that after ozonation in some cases the values of several individual carbonyls may exceed PNEC values (this is before post-treatment). The exceedance is typically limited to two times the PNEC value. Carbonyls are easily biodegradable, for instance in a biological post-treatment.

The **transformation products** included in this chapter are only examples of all transformation products that can be present in ozonated WWTP-effluent because of the vast amount of micropollutants possibly present in WWTP-effluent. However, it does show that biological activity of parent compounds in certain environmentally relevant groups (antibiotics and estrogens) are eliminated with ozonation. In a few cases an increased toxicity was observed for the transformation product in relation to the parent compound (e.g. acyclovir, carbamazepine and oxazepam). However too little is known about the effects at environmental realistic concentrations of these identified products. The limited knowledge on the effects of transformation products at environmental relevant concentrations hinders conclusive conclusions to be drawn on their toxicity in regard to their parent products, and their toxicity in general.

In chapter 6 the overall net toxicity of organic oxidation products altogether before and after ozonation of WWTP effluent is discussed.

4

EFFECTS OF WASTEWATER COMPOSITION ON OXIDATION PRODUCT FORMATION

This chapter elaborates on the influence of the general wastewater composition on the oxidation products that can be formed during ozonation in WWTP-effluent. The parameters discussed are: bromide, pH, ammonium, nitrite, alkalinity, temperature, dissolved organic matter and suspended solids. In chapter 3 also specific precursors of organic oxidation products are mentioned, these will not be discussed in more detail in this chapter.

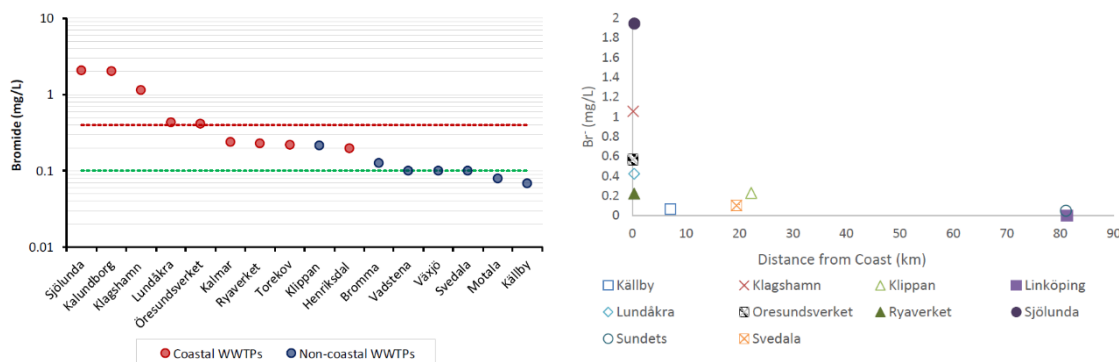
4.1 BROMIDE

The bromide concentration in the WWTP-influent is an important parameter for the extent to which bromate formation takes place. The occurrence of bromide in the wastewater can have a natural source (e.g. bromide is present in oceans at approximately 65 mg/l and in surface waters in the range of 15-200 µg/l)¹¹, but it can also have a major anthropogenic source. In Switzerland it was found that high bromide concentrations (>100 µg/l) in wastewater could be related to industrial discharge⁶⁶. Bromine is used in several industries. Industrial products containing bromine are for example bromine containing flame retardants and biocides. Use of bromine in industrial processes often leads to the release of bromide into surface waters, directly or indirectly via the WWTP. Research in Switzerland revealed that the chemical and waste industry (e.g. municipal waste incinerators) contribute primarily to the bromide load in river water⁶⁶. A high share of the bromide concentration may thus originate from point sources. Reduction of the bromide sources discharging on the sewer system may therefore be an option to reduce bromate formation⁸.

In most of the Swiss WWTPs bromide concentrations are low (<50 µg/l) and are considered to have only a minor impact on bromate formation: it leads to bromate concentrations of <10 µg/l with a specific ozone dose of 0,4-0,6 g O₃/g DOC⁶⁶.

In Sweden a relation was found between the bromide concentration in wastewater and the distance of a WWTP to the coast (Figure 8). Bromide levels at coastal WWTPs were much higher than those of non-coastal WWTPs. The relation is explained by the impact of deposition of seaborne aerosols and seawater intrusion into the sewer systems⁶⁷.

FIGURE 8 LEFT: BROMIDE CONCENTRATIONS IN WASTEWATER OF COASTAL SWEDISH WWTPS AND NON-COASTAL WWTPS (FIGURE TAKEN FROM FALÅS ET AL. IN PREPARATION⁶⁷). RIGHT: RELATION BETWEEN BROMIDE CONCENTRATIONS IN WASTEWATER OF SWEDISH WWTPS AND DISTANCE FROM THE COAST (FIGURE TAKEN FROM DELL 2020⁶⁸)



There is no extensive overview of bromide concentrations in wastewater in the Netherlands as bromide is not frequently analyzed at most WWTPs. A first indication could be derived from the bromide concentration in drinking water in the Netherlands. Bromide concentrations of about 135 µg/l, ranging from 110 to 160 µg/l, were found to be present in drinking water in the western part of the Netherlands. In the Watson database⁶¹ bromide concentrations can be found for a few of the Dutch WWTPs, mainly located in the mid-eastern part of the Netherlands. The average concentration is around 190 µg/l, minimal and maximal concentrations are <10 and 430 µg/l (according to the Watson database, in pilot plant research much higher values were observed)⁶¹. For a couple of ozonation pilot and demo studies bromide concentrations are determined on a more frequent basis. At WWTP de Groote Lucht bromide concentrations ranged from 200-700 µg/l, at WWTP Aarle-Rixtel from 200-1,000 µg/l^{69,70}. At WWTP Wervershoof an average concentration of 300 µg/l was found, at WWTP Houten the average concentration was 58 µg/l (varying from 24-94 µg/l) and at WWTP Winterswijk the average concentration was 110 µg/l (varying from 60-210 µg/l)⁷⁰. Based on these measurements the conclusion can be drawn that bromide concentrations are highly variable among the WWTPs in the Netherlands and that locally very high concentrations of bromide can be found. For most WWTPs in the Netherlands the bromide concentration is (much) higher compared to Switzerland.

4.2 pH, AMMONIUM, NITRITE, ALKALINITY AND TEMPERATURE

With respect to influence on the formation of oxidation products of the parameters pH, ammonium and alkalinity, only their influence on the formation of bromate has been found in literature. No literature has been found on the influence of these parameters on the formation of the other oxidation products.

The pH influences the ozone decomposition rate, the hydroxyl radical exposure, and also the equilibrium between HOBr and OBr⁻ ($pK_a = 8.8$). In drinking water treatment, influence of pH has been well studied. Lowering the pH, known as “pH depression”, is an existing strategy to mitigate bromate formation during disinfection in drinking water treatment. A decrease in pH from 8 to 6 has shown to reduce bromate formation by 60%. Lowering the pH (e.g. to 6.0) results in an increased ozone stability (ideal for disinfection), a decreased hydroxyl radical exposure and also it shifts the equilibrium of HOBr/OBr⁻ to HOBr, the latter reducing the formation of bromate by ozone via pathway 2 (Figure 1)^{8,9,26}. A decrease in hydroxyl radical exposure would also lead to mitigation of bromate formation, however, this will reduce the efficiency of micropollutant abatement⁸.

No field results with WWTP-effluent have been found to confirm the influence of pH in ozonation of WWTP-effluent. Furthermore, the influence may differ per WWTP as the influence of pH depends on the composition of the water matrix. When aiming at disinfection, one of the purposes in drinking water treatment, lowering the pH may be a feasible solution. However, when lowering the pH, the hydroxyl radical exposure is lowered and for the abatement of micropollutants in wastewater treatment, both ozone and the hydroxyl radicals are necessary. Therefore, this option for mitigating bromate formation is not considered to be an appropriate strategy.⁸

Ammonium can react with HOBr/BrO⁻ to form bromamine (NH₂Br), reducing the bromate formation via pathway 2⁷. Therefore, ammonium addition is a control option in drinking water treatment for minimizing bromate formation. In ozonation experiments related to drinking water treatment the bromate formation was reduced by approximately 50% with 200 µg/l NH₄⁺ from 8 µg/l to 4 µg/l bromate⁹. However, as mentioned in section 3.1, in treatment of WWTP effluent both pathways in bromate formation (Figure 1) play a role. The exact extent to which ammonia can contribute to the bromate mitigation and if this is significant, is therefore also difficult to predict in this water matrix. Some model calculations take this reaction into account and predict that bromate formation increases at low ammonium concentrations⁷². However, no field results with WWTP effluent have been found. If there is a measurable effect this is expected to be relatively small, because the pathway 1 (Figure 1) seems to be quite important during ozonation of WWTP-effluent. This should be further confirmed.

Bromamine is toxic but can be transformed with ozone to nitrate and Br⁻⁷³. Moreover, it will be removed in biological post-treatment. The drawback of the presence of ammonium during ozonation of WWTP-effluent, is that it may lead to uncontrolled nitrification. Nitrite control is very important in ozonation processes as nitrite reacts rapidly with ozone to nitrate and is thereby regarded as an ozone scavenger. Furthermore, the reaction of ozone and nitrite may also lead to (toxic) by-products. A small part (2%) of the nitrite can react with ozone to peroxyxynitrite or to NO₂-radicals. The peroxyxynitrite can lead to NO₂-radicals which can lead to nitration. NO-radicals can react with amine-compounds to nitrosamines. High concentrations of nitrite can therefore lead to unwanted oxidation products⁷⁴.

Alkalinity can influence the bromate formation kinetics and pathway^{7,75}. Carbonate is a hydroxyl radical scavenger and at higher alkalinity more carbonate is present. No additional information was found in literature how the alkalinity influences the ozonation process at WWTPs. It is expected that the carbonate scavenging is typically quite small as high DOM levels as present in WWTP effluent are more important for the bromate formation. Nevertheless, carbonate scavenging could be of importance because the formed carbonate radicals can react with some bromine intermediates. The exact influence of carbonate on bromate formation in WWTP-effluents is not well known as no literature on this topic have been found.

Temperature influences the reaction rates and the ozone decomposition. For example, the ozone decay rate is higher at increasing temperature. Contradicting dependence of temperature on bromate formation has been found in drinking water research^{76,77}.

4.3 DISSOLVED ORGANIC MATTER

Composition of wastewater has a significant influence on the ozonation process. Elevated concentrations of major ozone scavengers like alkalinity, dissolved organic matter (DOM), and NO_2^- lead to higher ozone consumption and thus more oxidation products. Among those scavengers DOM commonly receives the most attention as it is present at highest concentrations and therefore can scavenge much of the dosed ozone. DOM is typically expressed as dissolved organic carbon (DOC), the sum of all grams dissolved carbon atoms in a water sample. In the Netherlands the DOC concentration is on average 11 mg/l (with a range of 8-20 mg/l). In Switzerland and (parts of) Germany this concentration is generally significantly lower. There DOC concentrations of on average 6 mg/l were found with a range of 3-9 mg/l⁷⁸. The amount of DOM that reacts with ozone depends on the DOC concentration and the ozone dosage. For example, Liu *et al.* (2020) studied the ozonation of municipal- and industrial wastewater with DOC concentrations of 8,7 and 9,6, and 11,4 and 12,3 mg/l respectively. Higher levels of DOC resulted in higher hydroxyl radical scavenging rates and subsequent elevated oxidation by-product production⁷⁹⁻⁸¹. This was supported by studying the ozone stability which varied significantly between the different water matrices. At equivalent ozone doses, complete ozone depletion was achieved in 0,2-5 min in secondary effluent, 3-20 min in surface water, and 10-30 min in ground water⁸². Moreover, type of DOC influences the ozonation process, whether DOC consists of aliphatic compounds, aromatic compounds, etcetera. Because of its influence on the ozone chemistry and hydroxyl radical formation, DOC also influences the bromate formation⁷.

Composition of DOC depends on the wastewater origin (domestic, agricultural, industrial, etc.), specific location, time of the day and year and operating conditions (temperature, pH, flow, etc.). Characterization of DOC is rather complex and is not standardized. Characterization into fractions based on molecular weight, hydrophobicity, optical characteristics, or a combination thereof has been studied^{83,84}. This categorization can help understand which fractions of DOC influence ozone demand and thus ozone dose.

Agbaba *et al.* (2015) showed that the DOM removal depends upon the applied ozone dose, as well as the DOC concentration (5.2 - 9.9 mg/l) and fraction distribution (see Figure 9). Ozonation alters the DOM distribution of the various DOM fractions. The relative contribution of high-molecular weight natural organic matter decreased whereas the share of smaller molecules increased. Moreover, experiments were conducted with three samples. Two of those had a similar DOM characterisation (A and B), the third one (C) had a significantly lower hydrophobic fraction (FAF). The authors observed that the hydrophobic DOM fraction in matrices A and B were oxidized by ozone to hydrophilic DOM, whereas the hydrophilic fraction of water matrix C did not change. Increase in hydrophilicity of DOM was followed by the increase of total aldehyde content. Noteworthy is that in samples A and B predominantly aldehydes such as formaldehyde, acetaldehyde, and glyoxal were formed. In sample C predominantly acetaldehyde and methylglyoxal were formed (see Figure 10). These results indicate that differences in the type of DOM, in particular regarding the hydrophilicity, affect the formation of polar oxidation products such as aldehydes.⁸⁵ The breakdown of high-molecular weight DOM fractions into smaller molecules during oxidation is generally well known. The exact end-products and effects on the distributions of the various DOM fractions is much less known and is likely to differ per water matrix.

FIGURE 9 DISTRIBUTION OF DOM FRACTIONS OF THREE WATER MATRICES A (A), B (B), AND C (C). HAF – HUMIC ACID FRACTION, FAF – FULVIC ACID FRACTION, HPIA – HYDROPHILIC ACID FRACTION, HPI-NA – NONACIDIC HYDROPHILIC FRACTION (FIGURE TAKEN FROM AGBABA ET AL. 2015⁸⁵)

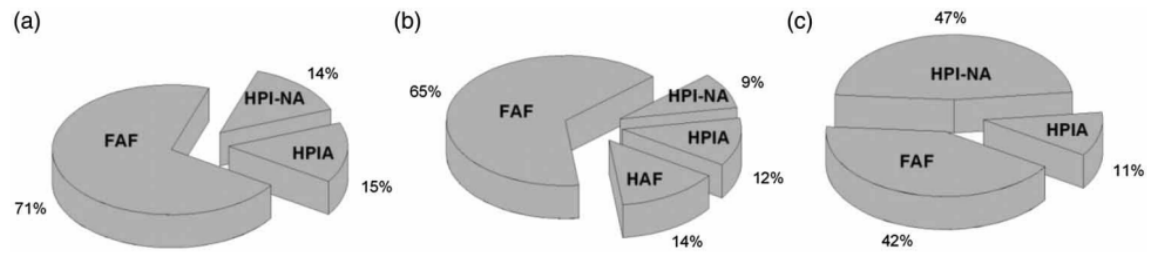
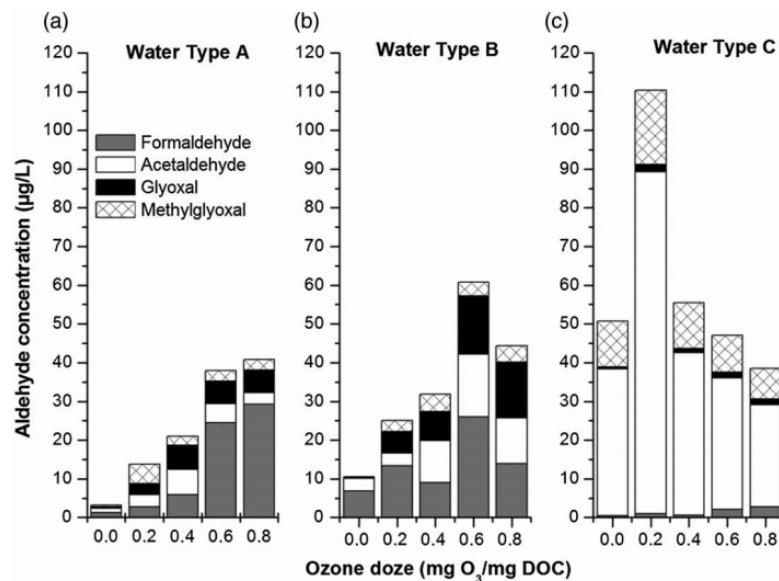


FIGURE 10 INDIVIDUAL ALDEHYDE CONCENTRATIONS IN RAW AND OZONATED WATERS FOR THE DIFFERENT WATER MATRICES (FIGURE TAKEN FROM AGBABA ET AL. 2015⁸⁵)



Igos *et al.* (2021) demonstrated that at increasing specific ozone doses DOM removal increased and subsequently also oxidation -product formation increased. However, this result was obtained at suspended solid (SS) concentrations below 2 mg/l. At SS concentrations of 2-7 mg/l the DOM removal was negligible⁸⁶.

In summary, both the concentration and the composition of DOM do not only influence rates of ozone consumption but also impact the formation of oxidation by-products, such as aldehydes. Moreover, DOM is a crucial water quality parameter affecting the ozonation process and the oxidation product formation^{87,88}.

4.4 SUSPENDED SOLIDS

Various studies have found that suspended solids (SS) have a minor effect on the ozonation process. Pak *et al.* (2016) report that ozone can be scavenged by SS⁸⁹. The ozonation of SS can result into dissolved compounds, DOC. Initially high molecular-weight compounds are formed, when sufficient ozone is present also low molecular-weight compounds are formed^{68,89}.

Zucker *et al.* (2015) conducted ozonation tests after filtration of WWTP-effluent. The outcome of this study is that filtration, and thereby a lower concentration SS, improved micro-

llutant removal. Moreover, they found a relation between particle size-distribution of SS and micropollutant removal. An increasing level of filtration, i.e. filtering over smaller pore sizes, resulted in an improved micropollutant removal (Figure 11). The authors state that the sorption of micropollutants to the SS was considered negligible as previously shown by other studies. In Table 3 the particle size-distribution of the tested effluent is given. Mechanism behind the observed relation is not fully understood. However, they state that due to the large surface area of small particles, fine SS may inhibit micropollutant oxidation due to particle interaction.⁹¹

FIGURE 11 REMOVAL OF THREE FAST-REACTING MICROPOLLUTANTS IN DIFFERENT PORE-SIZE FILTERED EFFLUENTS. SPECIFIC OZONE DOSE IS 0,17 G O₃/G DOC. NFE: NONFILTERED EFFLUENT. DOC = 10,3 MG/L AND PH = 7.8 AND REMAINED UNCHANGED DURING FILTRATION AND OZONATION. ERROR BARS INDICATE STANDARD DEVIATIONS BETWEEN TRIPLICATE MEASUREMENTS (FIGURE FROM ZUCKER ET AL. 2015 ⁹¹)

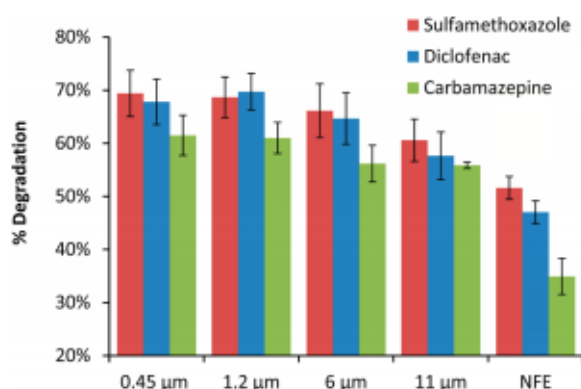


TABLE 3 AVERAGE PARTICLE SIZE-DISTRIBUTION IN WWTP-EFFLUENT FROM A WWTP IN ISRAEL, PARTICLES SMALLER THAN 2 µM COULD NOT BE QUANTIFIED⁹¹

Size (µm)	2 – 3	3 – 5	5 – 10	10 – 15	15 – 30	30 – 50	50+	Total (no./mL)
Percentage (%)	51	17	22	6,2	2,9	0,55	0,090	37,838

Huber *et al.* (2005) demonstrated that reaction of ozone with SS was not a relevant process. This was demonstrated by the addition of 15 mg/l of SS with an estimated average particle size of >50 µm and found only marginally influenced micropollutant oxidation, a decrease of less than 5%. However, they state that due to the large surface area of small particles, fine SS may inhibit micropollutant oxidation due to particle interaction⁹².

In conclusion, the presence of SS in WWTP-effluent, after a decent and well-functioning settling step, has a minor impact on the ozonation of micropollutants. Although the exact mechanism is not fully understood, when present in high concentrations SS is likely to function as an ozone scavenger and thereby negatively influences the oxidation process. Putting things in perspective, for typical WWTP-effluents SS has a minor impact on the ozonation process, DOC is regarded as the water quality parameter that has a much stronger influence^{92,93}.

4.5 SUMMARY

The wastewater composition has a huge influence on the formation of oxidation products. Especially the WWTP-effluent constituents **bromide** and **DOM** determine to a large extent the formation of bromate and various organic oxidation products, such as NDMA. Bromate formation is directly linked to the bromide concentration. Limited data on bromide concen-

trations in Dutch wastewater is available. However, the data that is available shows a high variety in bromide concentrations among the different WWTPs. The DOM levels are relatively high in the Netherlands compared to Switzerland. Therefore, a higher absolute ozone dose is required for micropollutant abatement. This might result in relatively large amounts of oxidation products. Other parameters like **suspended solids, pH, alkalinity and temperature** are found to have a smaller effect on the formation of oxidation products in the typical ranges at which these parameters are present in WWTP-effluent. In drinking water treatment, the presence of **ammonium** can contribute to a reduced bromate formation. However, because of the different water matrix and its influence on the main pathway of bromate formation, the exact extent to which ammonium can contribute to bromate mitigation in ozonation of WWTP effluent is difficult to predict. Some model calculations suggest a significant effect, however no field results with WWTP effluent have yet been found. It is expected that the presence of ammonium is of minor influence on bromate formation but this should be further confirmed.

5

INFLUENCE OF PROCESS DESIGN ON OP FORMATION AND MITIGATION

In this chapter the influence of the process design of the ozonation process itself and pre-, hybrid- and post-treatment processes on the formation and mitigation of oxidation products are discussed. The discussion on mitigation strategies is limited to processes and configurations that are found reasonably applicable by the Supervisory Committee and International Experts.

5.1 PROCESS CONFIGURATION

With respect to the formation of oxidation products the configuration of the process is an important factor, especially in relation to the bromate formation. For most of the other oxidation products mentioned in chapter 3 the relation with process configuration is less present or yet unknown. By several means the process configuration and process conditions can be influenced to mitigate oxidation product formation. In this section the following process parameters are discussed:

- Ozone dosage
- Contact time
- Reactor configuration
- Addition of chemicals

5.1.1 OZONE DOSAGE

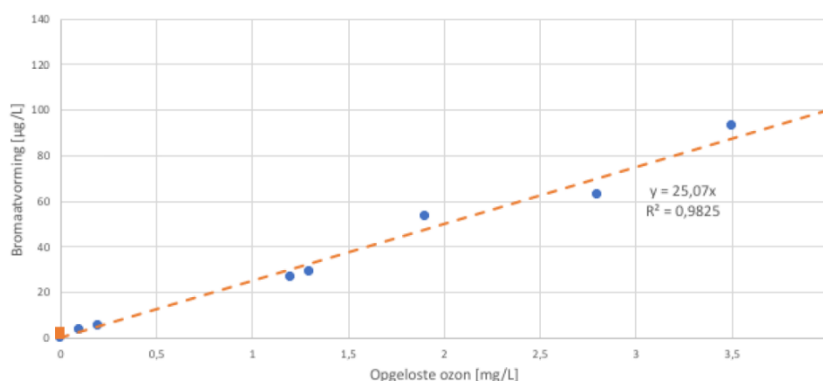
In this section the effect of ozone dosage on bromate formation is discussed, and shortly also the effect on organic by-products.

BROMATE FORMATION

Two important parameters in the bromate formation are the ozone dosage and the bromide concentration. Generally, the higher the ozone dose, the higher the bromate concentration. And the higher the bromide concentration, the higher the bromate concentration. But note that also other factors play a role, such as reactor configuration which is discussed in section 5.1.3. Key in the formation of bromate is the residual ozone concentration. Residual ozone concentration depends among others on the specific ozone dose. Residual ozone concentration is the fraction of ozone which does not rapidly (within seconds) react with the matrix, i.e. it is the fraction that is larger than the spontaneous ozone demand. This residual concentration is available for the oxidation of other compounds, for instance bromide. Maintaining a low residual ozone concentration in the water is therefore necessary to mitigate the formation of oxidation by-products such as bromate. This is illustrated in Figure 12 showing the results of Delfos *et al.* (2020)⁹⁴.

FIGURE 12

BROMATE FORMATION IN RELATION THE DISSOLVED OZONE CONCENTRATION IN BENCH-SCALE EXPERIMENTS WITH WWTP-EFFLUENT WERVERSHOOF (FIGURE TAKEN FROM DELFOS ET AL. 2020)⁹⁴



This section describes the combined influence of the ozone dosage and the bromide concentration on the bromate formation. It must be noted that the influence of ozone dose and bromide concentration on bromate formation cannot be assessed fully independently from the local conditions, especially regarding the reactor design and wastewater characteristics. Nevertheless, the assessment of the ozone dose and bromide concentration only, can provide a good insight into bromate formation, as shown in multiple examples in this section. In Figure 13 the relation between the specific ozone dose and the bromate yield in Swiss WWTP-effluents is provided. The bromate yield (mg bromate/mg bromide) increases clearly with increasing ozone dosage. A specific ozone dose of 0,4 – 0,6 g O₃/g DOC, which is often the dosing range for micropollutant removal in Switzerland, resulted in less than 3% bromate yield (on molar basis)⁸. The first ozone plant in Switzerland for the treatment of WWTP-effluent was built in Neugut. Experiences from this full-scale ozone plant are provided by Bourgin *et al.* (2017). Reported bromide concentrations in the wastewater are on average 60 µg/l (35-85 µg/l). At a specific ozone dose of <0,45 g O₃/g DOC the bromate yield was low (<1%). Recommended specific ozone dose for sufficient removal of micropollutants in Neugut was 0,55 g O₃/g DOC, including a margin to compensate potential nitrite peaks. Today, WWTP Neugut reaches sufficient abatement of micropollutants with 0,37 – 0,42 g O₃/g DOC. A specific ozone dose of 0,55 g O₃/g DOC resulted in a bromate yield of 3%, meaning that at a bromide concentration of 60 µg/l about 1,8 µg/l of bromate is formed. At a maximum ozone dose of 0,92 g O₃/g DOC and a bromide concentration of 60 µg/l, the bromate formation reached up to 5,7 µg/l²⁰. It must be noted that these findings are based on a WWTP in Switzerland. In two Dutch ozone pilot studies the relation between the bromide concentration, ozone dose and bromate formation was also explored (see Figure 14). In both WWTPs the bromide concentration was >200 µg/l and up to 1000 µg/l. This is very high compared to the majority of the Swiss WWTPs, where the bromide concentration was <50 µg/l.

Results from the pilot at WWTP Aarle-Rixtel where a side-stream ozone injection system was applied show that with >300 µg/l bromide and a specific ozone dose of 0,7 g O₃/g DOC the bromate formation was approximately 5-10 µg/l. At a higher specific ozone dose of 1,2 g O₃/g DOC, the bromate formation increased to 10-35 µg/l. At 0,3 and 0,5 g O₃/g DOC the bromate formation was <1 µg/l⁶⁹. Figure 14 shows the impact of an increased specific ozone dose on the removal efficiency of micropollutants. At an ozone dose of 0,3 g O₃/g DOC the abatement efficiency of 16 micropollutants was approx. 50% (compared to WWTP-effluent concentrations), at 0,7 g O₃/g DOC this increased to 60-70% and the abatement is maximum 90% with 1,2 g O₃/g DOC.

Results from de pilot at WWTP de Groote Lucht show that bromide concentrations of 200-600 µg/l, and a specific ozone dose of 0,6-0,9 g O₃/g DOC resulted in approximately 10 µg/l bromate. But a slightly higher specific ozone dose of 0,94 resulted in 20 µg/l bromate. Higher ozone dosages up to 1.2 g O₃/g DOC resulted in 20-70 µg/l bromate (note that the different ozone doses were applied during the course of the pilot and that the wastewater composition may varied over time, possibly (partly) explaining the range in bromate formation at similar ozone doses).⁷⁰ In both Dutch pilots the bromide concentration was relatively high (≥ 200 µg/l) compared to the Swiss situation. There are no results available yet about ozonation and bromate formation in Dutch WWTPs with low bromide influent concentrations. However, based on Swiss and German guidelines and experiences, it is expected that with a low bromide concentration of <100 µg/l and a specific ozone dose of 0,5-0,7 g O₃/g DOC (usually sufficient to achieve a 80% removal of indicator micropollutants in Switzerland) the bromate concentration will remain below 10 µg/l^{10,73}.

FIGURE 13 THE RELATION BETWEEN THE SPECIFIC OZONE DOSE AND THE BROMATE YIELD. RESULTS FROM SEVERAL WWTPS IN SWITZERLAND COMBINED (LEFT, FIGURE FROM SOLTERMANN ET AL. 2016⁸ AND THE RESULTS OF THE OZONE INSTALLATION AT WWTP NEUGUT IN SWITZERLAND (RIGHT, FIGURE TAKEN FROM BOURGIN ET AL. 2017²⁰)

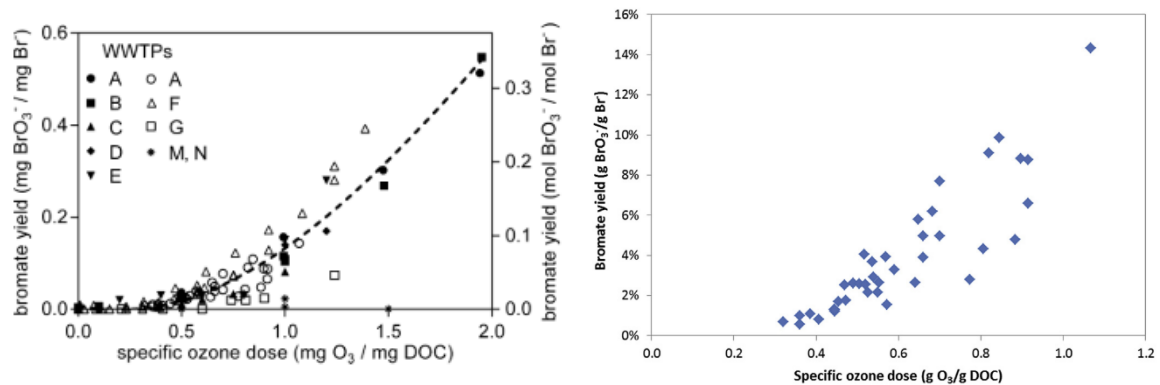


FIGURE 14 RESULTS FROM THE PILOT IN AARLE-RIXTEL. THE BROMATE FORMATION AT DIFFERENT BROMIDE CONCENTRATION AND A FIXED OZONE DOSE OF 1,2 G OZONE/G DOC (LEFT) AND THE BROMATE FORMATION AT DIFFERENT SPECIFIC OZONE DOSES AND A MINIMUM BUT VARYING BROMIDE CONCENTRATION OF 0,3 MG/L (FIGURE TAKEN FROM KRAS ET AL. 2020⁶⁹)

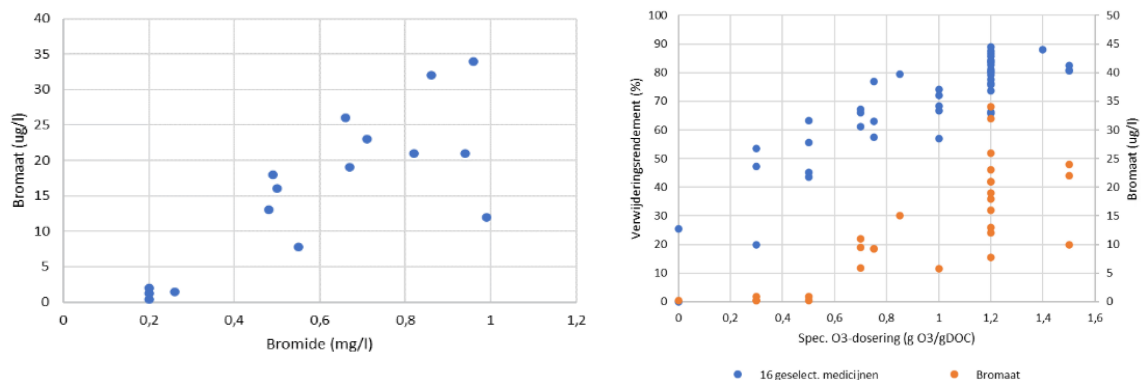
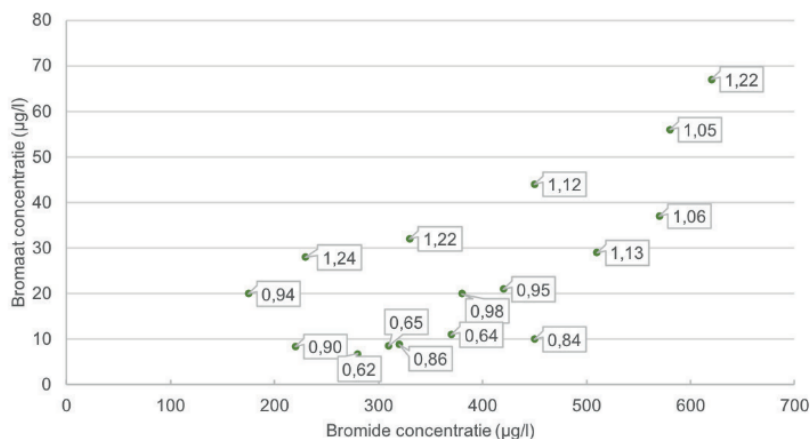


FIGURE 15

BROMATE FORMATION IN WWTP DE GROOTE LUCHT. PRESENTED ARE THE BROMIDE AND BROMATE CONCENTRATION AFTER OZONATION AND THE DATA LABELS THE SPECIFIC OZONE DOSE (G O₃/G DOC) IS GIVEN (FIGURE TAKEN FROM BEEN ET AL. 2018⁷⁰)



At WWTP Winterswijk a pilot has been performed with ozone followed by UV treatment. Bromide concentration in the influent of the pilot varied from 60-210 µg/l and was on average 110 µg/l. The DOC-concentration varied from approximately 10 to 20 mg/l. Specific ozone doses of 0,4 – 1 g O₃/g DOC were tested for micropollutant removal. During these pilot tests the bromate concentration remained below the detection limit of 10 µg/l in the ozone effluent. To investigate the bromate formation further, bromide was spiked up to 1.000 µg/l. At a specific ozone dose of 0,95 g O₃/g DOC the bromate concentration remained below 10 µg/l. At (very) high specific ozone doses of 1,96 and 3,13 g O₃/g DOC and spiked bromide concentrations of around 700 µg/l the bromate concentration was respectively 41 and 68 µg/l.⁷¹

Currently also at WWTP Wervershoof the applicability of ozone is being researched. This pilot is still running, and results are not available yet. However, bench scale tests with WWTP Wervershoof effluent are available. These tests show that with a bromide concentration of about 300 µg/l and a specific ozone dose of 0,36 and 0,80 g O₃/g DOC the bromate formation is below 10 µg/l (0,23 µg/l and 5,4 µg/l respectively). At 1,52 g O₃/g DOC the bromate is about 30 µg/l (Figure 18).⁹⁴

Organic by-products

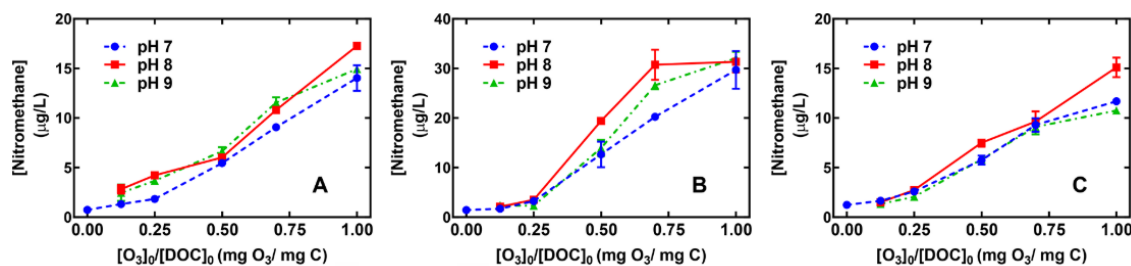
Higher specific ozone dose leads to an increase in formation of oxidation by-products (see Figure 15). Wert *et al.* (2007) showed that at an increased ozone dose of 3,6 mg/l to 7,0 mg/l, total aldehyde and carboxylic acid concentrations increased from 114 µg/l to 211 µg/l and 623 µg/l to 937 µg/l, respectively²⁶. Moreover, Park *et al.* (2016) showed that with higher specific ozone dose, acetaldehyde and formaldehyde formation increased, and the relative portion of formaldehyde and acetaldehyde changed with changes in specific ozone doses⁹⁵.

Nitro-compounds

Several studies have reported the formation of NDMA at different specific ozone doses. There seems no correlation between the NDMA formation and the applied ozone dose. Several studies have concluded that the decisive factor for NDMA formation during ozonation are the concentrations of its precursors in the WWTP-effluents^{20,24,43-45}.

For nitromethane, Shi *et al.* (2020) reported increasing concentrations with increasing ozone doses, as shown in Figure 16 for three different treatment plant effluents⁵⁰.

FIGURE 16 NITROMETHANE FORMATION FROM OZONATION. A, B AND C SHOW THE RESULTS OF DIFFERENT TREATMENT PLANT EFFLUENTS. ERROR BARS REPRESENT THE RANGE OF EXPERIMENTAL DUPLICATES (FIGURE TAKEN FROM SHI ET AL. 2020⁵⁰)



5.1.2 CONTACT TIME

For disinfection purposes of (drinking) water an important parameter is the combination of the ozone concentration and contact time, the so-called Ct-value or ozone exposure. Deactivation of microorganisms improves at higher Ct-values. For oxidation of micropollutants and the subsequent formation of oxidation products the Ct-value seems of lesser importance. Due to the relatively high ozone and hydroxyl radical reaction constant rates of most micropollutants a measurable ozone exposure is not needed for the oxidation of micropollutants as the rate of abatement of micropollutants by ozone is given by $-\ln(c_t/c_0) = k_{O_3} \cdot O_3\text{-exposure} + k_{OH} \cdot OH\text{-exposure}$. It was found that the contact time had little to no effect compared to the ozone dose^{96,97}.

Xu *et al.* (2002) found that there was no significant difference between different contact times, revealing the fast kinetics of the reaction between ozone and unsaturated and aromatic compounds to oxidation products. According to the authors, the critical factor for water quality improvement is the ozone dose transferred into the water⁹⁶. For WWTP-effluent this is typically close to 100%.

Bromate formation increases with increasing ozone exposure. Lowering the exposure by shortening the contact time thus reduces the formation of bromate¹⁷. However, this also impacts the micropollutant removal, especially for slow reacting micropollutants this is the case. Thus, there is a trade-off in the applied ozone exposure between the mitigation of bromate formation and the abatement of micropollutants. This trade-off depends on the local conditions (e.g. water matrix) and abatement goals.

5.1.3 REACTOR CONFIGURATION

Key in the mitigation of bromate formation is to avoid the presence of residual ozone, i.e. a low ozone exposure. In theory this can be achieved by an unlimited amount of very small ozone inputs into the process. In practice this can be done by the use of membranes, multiple static mixers or multiple injection points for dosing ozone¹⁸. For instance, one could think of a series of contact tanks dividing the total ozone dose over different tanks instead of one contact tank with one ozone injection point. Various studies that are described in this section have shown that a dosing strategy based on small portions of ozone to the process is effective in the minimisation of bromate formation. In these studies, the reactor configuration is often tested in combination with the addition of H₂O₂. Therefore, it is difficult to assess the effect of the reactor configuration itself. The effect of H₂O₂ addition on the formation of oxidation products is discussed in section 5.1.4.

Merle *et al.* (2017) describes the MEMBRO₃X process in which ozone is transferred to the water through the pores of PTFE hollow fibre membranes. By dosing the ozone in many small portions in presence of H₂O₂, the bromate formation is minimized. For the treatment

of groundwater (180 µg/l bromide) with the MEMBRO₃X process the bromate production was <0,5 µg/L, compared to 8 µg/L bromate in the reference test with a conventional ozone/ H₂O₂ process. The MEMBRO₃X process was also tested with river- and lake water which have a higher DOM content compared to groundwater. Also, for these two matrices a lower bromate formation was found compared to the conventional ozone/H₂O₂ process (up to 50% less bromate).⁹⁸ No studies for the MEMBRO₃X process have been found for the treatment of WWTP-effluent, nor cost-effectiveness assessments.

Speth *et al.* (2002) describes the HiPOx[®] process for ozonation related to groundwater treatment. In this system ozone is dosed in small portions at various points in the reactor, and H₂O₂ is added in excess, aiming to reduce bromate formation. However, a comparison with the conventional ozone / H₂O₂ process is not made⁹⁹.

In a Dutch drinking water research, the dosing of ozone in a number of small portions (maximum 6 injection ports) in combination with H₂O₂ was investigated. A static mixer was applied after each injection port. At an ozone dose of 1 g O₃/m³ (with a DOC of approx. 4-5 mg/l¹⁰⁰ this would correspond to 0,2-0,3 g O₃/g DOC) the bromate remained below 1 µg/l, also without H₂O₂ (the bromide concentration was 320 µg/l). At a specific ozone dose of 2 g O₃/m³ the addition of H₂O₂ resulted in a lower bromate concentration. Without H₂O₂ the bromate concentration was 9 µg/l, with 5 mg/l H₂O₂ the bromate was reduced to 3 µg/l and with 10 mg/l H₂O₂ the bromate concentration was 0,5 µg/l¹⁰¹.

At a full-scale Dutch drinking water treatment plant of Waternet (0,25 – 0,32 g O₃/g DOC, 6 mg/L DOC and 90 µg/l bromide) the ozone injection system was changed from diffusors into a single injection point, a so-called side-stream injection. In the side-stream a high dose of ozone is injected, thereafter this side stream is brought into a contact tank and mixed with the main water stream of the treatment plant. After changing the injection system from diffusors to the side-stream the bromate concentration increased significantly. Even though the hydraulic retention time of the side stream is limited it was estimated that roughly half of the bromate is formed in the side-stream itself due to the high ozone concentration in the side-stream (Waternet, personal communication, June 2021).

Currently, within the 'Innovation program on Micropollutants' STOWA is investigating the applicability of ozone in combination with ultrasound processes. This technology combines ultrasound and an innovative ozone dosing and mixing system. As the ozone dosing can most likely be lowered with this technology and the residual ozone is expected to be low, the bromate concentration is likely to be lower compared to conventional ozonation. This will be further investigated in a pilot study¹⁰².

5.1.4 ADDITION OF CHEMICALS

Chemicals can be added prior or during the ozonation process to influence the oxidation, particularly the bromate formation. The most common additive during ozonation is H₂O₂ (as already discussed in the previous section). Also, ammonium and acid or base to alter the pH are known additives, mainly if disinfection is the main target. Effect of the addition of these chemicals on the formation of oxidation products in relation to the treatment of WWTP-effluent for micropollutant removal is discussed in this section.

Hydrogen peroxide (H₂O₂)

Bromate

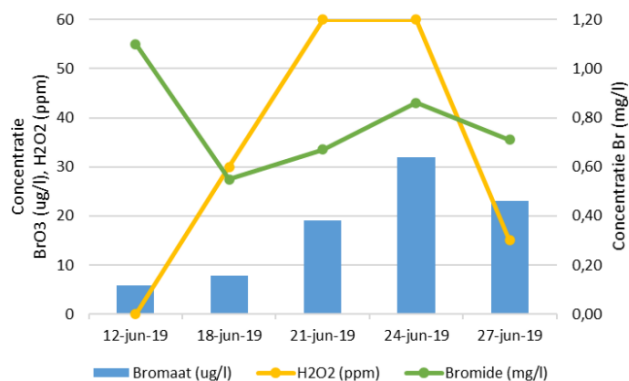
Bromate formation can be mitigated by the application of hydrogen peroxide during ozonation. The combination of ozone and H₂O₂, also referred to as the peroxone process, originates from the drinking water treatment domain. As shown in the simplified scheme in section 3.1, the bromate formation can occur via two pathways. Pathway 2 is considered to be the main pathway during drinking water treatment. In this pathway the intermediate HOBr is formed. H₂O₂ can reduce HOBr, thereby partially mitigating the formation of bromate^{8,103}. However, the situation in wastewater treatment may be different. Presence of higher concentrations of DOM accelerates the ozone transformation to hydroxyl radicals, possibly making pathway 1 more important compared to drinking water treatment⁸. Therefore, the addition of H₂O₂ may be less effective in wastewater treatment compared to drinking water treatment by the HOBr pathway. Nevertheless, since ozone is also a decisive reactant in pathway 1, the addition of H₂O₂ limits the lifetime of the ozone, which in turn reduces the extent of Br oxidation⁸. Therefore, potentially a reduction of bromate formation can be obtained when applying ozone/ H₂O₂ in wastewater treatment. In bench-scale laboratory research, Soltermann *et al.* (2017) investigated the addition of 5 and 10 mg/L H₂O₂ (0,4 and 0,8 mg H₂O₂/mg O₃). The results show that with the specific wastewater used (7,8 mg/L DOC) and an ozone dose of 1,5 g O₃/g DOC, a reduction in bromate formation of 40-55% and 65% could be obtained with H₂O₂ doses of 5 and 10 mg/L, respectively. It is concluded that with H₂O₂ addition bromate formation can be mitigated during ozonation of WWTP effluent but at high concentrations of > 5 mg/l.⁸

In the same study it is mentioned that the effectiveness of H₂O₂ on bromate mitigation depends strongly on the wastewater composition. This was also shown in Lee *et al.* (2016). In this study, secondary effluents of 10 different WWTPs were sampled (from Switzerland, U.S. and Australia). Ozone was dosed at 1 and 1,5 g O₃/g DOC and H₂O₂ was added in a H₂O₂:O₃ ratio of 0,5 and 1 on molar basis (equal to 0,4 and 0,7 mg H₂O₂/mg O₃, respectively). H₂O₂ addition in mg/L was thus varying, depending on the ozone dosage and the DOC of the effluent. The DOC of the effluents differed from 4,7 to 26,4 mg/L. In some of these effluents the addition of H₂O₂ reduced the bromate formation. But there was a huge difference between the effluents. The range in bromate reduction was -50% to + 48% at a specific ozone dose of 1 g O₃/g DOC.⁴⁴ Concentrations of H₂O₂ in this research were relatively low for bromate mitigation during ozonation of WWTP effluent. Likely higher concentrations of H₂O₂ concentration are needed for bromate mitigation.

In the Dutch ozone pilot at Aarle-Rixtel also H₂O₂ was applied in order to reduce bromate formation. In contrast to the expectations the addition of H₂O₂ did not result in a reduced bromate formation in this research⁶⁹. H₂O₂ dosages were 30 and 60 mg/l in the first test days (~3 to 5 mg H₂O₂/mg O₃) and in the last test day 15 mg/l (~1 mg H₂O₂/mg O₃).

FIGURE 17

BROMATE FORMATION DURING OZONATION WITH 1 – 1,2 G O₃/G DOC WITH H₂O₂ ADDITION. PILOT-SCALE RESEARCH (FIGURE TAKEN FROM KRAS ET AL. 2020⁶⁹). THE DOC WAS 8-12 MG/L. THE H₂O₂ DOSING WAS 30 AND 60 MG/L IN THE FIRST DAYS (~3 TO 5 MG H₂O₂/MG O₃) AND IN THE LAST DAY 15 MG/L (~1 MG H₂O₂/MG O₃)

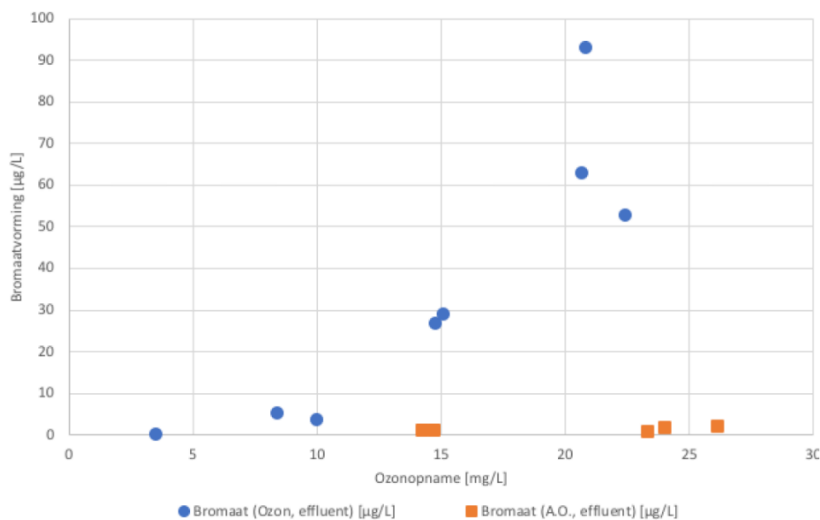


Also, in the bench-scale tests with WWTP Wervershoof effluent the mitigation of bromate with H₂O₂ was investigated (Figure 18). At (very) high ozone doses (1,61 up to 2,91 g O₃/g DOC) H₂O₂ was dosed at a ratio of 4 mg H₂O₂/mg O₃. The results show that the bromate concentration lowered substantially to maximum 2,1 µg/l at 2,9 g O₃/g DOC (25 mg O₃/l) applying 83 mg/l H₂O₂⁹⁴. Dosing of H₂O₂ has not (yet) been investigated at pilot-scale at Wervershoof. In contrast to the pilot results of Aarle-Rixtel the bench-scale results with Wervershoof effluent do show a significant effect in bromate mitigation using H₂O₂, although the ratio H₂O₂/O₃ between both researches is quite similar. The cause of this difference is unknown. Possibly it is related to differences in the research set-up (e.g. reactor configuration) and/or in the specific wastewater characteristics. For example, at Aarle-Rixtel the bromide concentration was higher than at Wervershoof (approx. 600-800 µg/l vs. 300 µg/l respectively). To our knowledge there is currently no full-scale experience with dosing H₂O₂ for bromate mitigation in WWTP-effluent.

The reaction of H₂O₂ with ozone could also result in lower ozone exposure to micropollutants. This may lead to a reduced effectiveness of the micropollutant removal, specifically for compounds reacting directly with ozone. In practice this effect seems not very pronounced as the abatement of micropollutants stays more or less similar in the presence or absence of H₂O₂. In the pilot research at Aarle-Rixtel an equal removal of micropollutants was observed with or without the addition of H₂O₂. Residual H₂O₂ after ozonation may be removed by (biological) post-treatment systems⁸.

FIGURE 18

BENCH-SCALE RESULTS ON THE RELATION BETWEEN THE OZONE DOSE AND THE BROMATE FORMATION FOR WWTPS WERVERSHOOF EFFLUENT (BLUE DOTS) AND THE EFFECT OF H₂O₂ ADDITION (ORANGE DOTS). THE BROMIDE CONCENTRATION WAS ABOUT 300 µG/L. THE DOC WAS 8,03-8,70 MG/L, THUS THE OZONE DOSE OF 13,0 AND 22,7 MG/L (ORANGE DOTS) CORRESPONDED WITH 1,6 AND 2,8 G O₃/G DOC. THE H₂O₂ DOSING WAS 4 MG H₂O₂/MG O₃ (FIGURE TAKEN FROM DELFOS ET AL⁹⁴)



Organic oxidation products

In the presence of H₂O₂ the lifetime of ozone becomes shorter, therefore the formation of ozone-induced oxidation products may be altered. This may result in a different overall distribution of oxidation products compared to ozonation without H₂O₂ addition. Remucal *et al.* (2020) have shown that the reaction of ozone with DOM results in different oxidation products than the reaction of hydroxyl radicals with DOM¹⁰⁴.

Ammonium (NH₄⁺)

Typically, ammonium in the WWTP-effluent is a parameter that is controlled by a process controller aiming at low levels. Nevertheless, concentrations of ammonium in the effluent differ per WWTP. The influence of ammonium on the formation of bromate is discussed in section 4.2. It is concluded that the exact extent to which ammonia can contribute to the bromate mitigation in WWTP-effluent and if this is significant is difficult to predict. No field results with WWTP-effluent have been found but the effect is expected to be relatively small. This should be further confirmed.

pH depression

Also, pH depression is already explained in section 4.2. In short, pH influences the bromate formation, but for the abatement of micropollutants in wastewater treatment, this option for mitigating bromate formation is not considered to be an appropriate strategy⁸.

5.2 PRE- OR HYBRID-TREATMENT

There are several treatment steps identified which can be applied prior to ozonation to improve the oxidation process. These processes typically enable the elimination of specific matrix constituents, primarily DOC removal or abatement of ammonium or nitrite, or the partial elimination of micropollutants. No removal technologies at pilot or full-scale have been found for the removal of bromide from secondary effluent. In literature regarding drinking water treatment some suggestions are made for technologies for the abatement of bromide, e.g. membrane filtration, ion exchange and precipitation¹⁰⁵. Although these

suggestions are not extensively assessed or tested, they seem not feasible as they are not cost-effective for wastewater treatment.

5.2.1 COAGULATION AND FILTRATION

Enhanced coagulation (EC) using ferric chloride (FeCl_3) or polyaluminum chloride (PACl) have been evaluated as a pre-treatment process to improve ozonation efficiency by lowering DOC levels. Wert *et al.* (2011) researched EC using FeCl_3 and showed it reduced DOC concentrations between 10% and 47% at FeCl_3 -dosages of respectively 10 to 30 mg Fe/l. The removal was highly dependent on the type of wastewater. For three different WWTP-effluents respectively 26, 28 and 47% DOC removal was observed at the highest applied dose of FeCl_3 . Polysaccharides and humic substances were particularly well removed by EC which implies less ozone scavenging by these substances after EC. In general, compounds with a higher apparent molecular weight were removed by EC. Noteworthy is that EC lowers the pH of wastewater, at highest dose of FeCl_3 the pH dropped from 7,0-7,2 till 6,3-6,7.¹⁰⁶ Effect of pH change on the ozonation process is discussed in 5.1.1.

There has also been a study into simultaneous electrocoagulation and ozonation (E-HOC) in one integrated unit to remove DOC. Jin *et al.* (2020) found that at a pH of 5 and a specific ozone dosage of 1,5 g O_3 /g DOC, 58,6% DOC removal efficiency was achieved. Ibuprofen was the only micropollutant tested in this set-up. Removal efficiency of ibuprofen increased compared to the application of ozone without E-HOC pre-treatment and could be attributed to the enhanced hydroxyl radical formation due to the E-HOC process. However, when the pH was increased to 7 and 9, the synergistic effects of ozone and coagulants decreased significantly.¹⁰⁷

5.2.2 FILTRATION

As stated in chapter 4, Zucker *et al.* (2015) and Huber *et al.* (2005) found that there is a minor impact of suspended solids on the efficiency of micropollutant ozonation process for micropollutant removal. This impact, though minor, increases by lowering the SS concentration. In particular the smallest particles may have the most impact on the ozonation process, thus applying a filtration step for the removal of fine particles might improve the efficiency of the ozonation process.^{91,92}

In the pilot at WWTP De Groote Lucht, two configurations were applied: sand filtration – ozone – sand filtration, and only ozone - sand filtration. Between both configurations no major differences were found in micropollutant removal^{69,70}. To our best knowledge there are no cases where a filtration step is applied prior to ozonation on full-scale in wastewater treatment.

5.2.3 BIOLOGICAL TREATMENT

De Wilt *et al.* (2018) demonstrated that a 38% removal of total organic carbon (TOC) could be obtain in a biological treatment step treating WWTP-effluent with a TOC concentration of 17 mg/l. In the subsequent ozonation step the absolute ozone dose could thereby be lowered proportionally. In addition, multiple micropollutants were biodegraded during biological treatment which positively contributed to the goal of micropollutant removal. The biological treatment followed by ozonation can therefore be regarded as a hybrid-configuration. In the same biological process ammonium and nitrite were completely oxidized to nitrate. The complete ammonium removal might trigger the formation of bromate during ozonation, whereas the abatement of nitrite results in less ozone scavenging.¹⁰⁸

5.2.4 PACAS

On lab-scale it was tested that the addition of powdered activated carbon (PAC) to wastewater reduced the DOC concentration. At a PAC-dose of 10 mg/l added to secondary effluent the DOC concentration was reduced by 10%. Moreover, a broad array of micropollutants was already removed from the wastewater by adsorption to the PAC. In a hybrid-configuration a subsequent ozonation process can thereby be operated at a lower ozone dosage to meet a certain target for micropollutant removal.¹⁰⁹

5.3 POST-TREATMENT

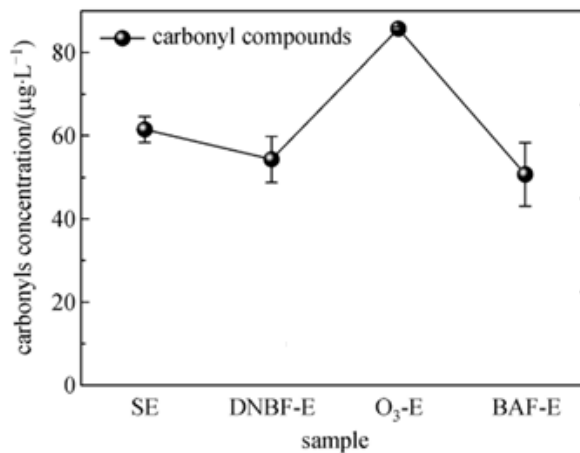
After ozonation, oxidation products are present in the water matrix. Certain types of oxidation products can be removed with a post-treatment step. Several biological post-treatment steps investigated are fixed bed or moving bed biological filtration, sand filtration, and biological activated carbon (BACF). Another well-investigated post-treatment step is the granular activated carbon (GAC). Apart from oxidation products removal, the aim of a post-treatment step after ozonation can include the additional removal of micropollutants. First the different post-treatments are introduced, followed by a comparison in section 5.3.4. Toxicity removal during post-treatment will be discussed in chapter 6.

5.3.1 MOVING BED AND FIXED BED BIOREACTORS

Moving bed and fixed bed bioreactors are characterized by the application of a carrier material used for attached growth of micro-organisms. In a moving bed biofilm reactor, carrier material is mixed in the tank while in a fixed bed bioreactor the carrier material is stationary. Itzel *et al.* (2020) evaluated a moving bed bioreactor as a post-treatment for ozonation at full-scale at WWTP Warburg, Germany. Non-targeted screening was used to determine the removal of unknown oxidation products. From this analysis, out of the 241 oxidation products that were found, 230 products were eliminated with the biological post-treatment, resulting in a 95% overall removal percentage.¹¹⁰ Schollee *et al.* (2018) found that around 55% of non-target features formed during ozonation from hierarchical cluster analysis were removed during post-treatment with moving bed or fixed bed bioreactors, similar to sand filtration¹¹¹. This is supported by Zhang *et al.* (2014), who investigated the combination of ozonation and a biological aerated filter¹¹².

Li *et al.* (2015) researched the influence of a bioreactor on the carbonyl concentration. A pilot-scale aerated bioreactor was used with volcano rock as a filter media. As seen in Figure 19 the carbonyl concentrations increased after ozonation and decreased again after biological post-treatment to levels of the secondary effluent¹¹³.

FIGURE 19 CONCENTRATIONS OF CARBONYLS IN WWTP-EFFLUENT. SECONDARY EFFLUENT (SE), DENITRIFICATION BIOFILTER EFFLUENT (DNBF-E), OZONE EFFLUENT (O₃-E) AND BIOLOGIC AERATED FILTER (BAF-E) (FIGURE ADAPTED FROM LI ET AL. 2015¹¹³)



5.3.2 SAND FILTRATION

Sand filtration is one of the oldest wastewater treatment technologies known. A typical sand filter is a lined watertight box (concrete or plastic-lined) and filled with sand.¹¹⁴ There are two predominant removal mechanisms taking place in sand filters: filtration and biodegradation. During filtration particles like metal precipitates that have reacted with phosphorus species or other suspended solids are filtered out. Biodegradation in sand filters is typically linked to nitrification and/or denitrification for nitrogen removal. The (partial) biodegradation of organic molecules can also take place in (aerobic) sand filters.

In Switzerland and Germany sand filtration is the most common applied post treatment after ozonation. In most cases the sand filter was already present upon realisation of the ozonation process for enhanced phosphorus removal, nitrification and SS removal. Denitrification, and thus dosage of a carbon-source, isn't common in Switzerland. This means that the studied sand filters are mostly aerobic, whereas for denitrification anoxic circumstances are required.

5.3.3 ACTIVATED CARBON FILTRATION

Two different techniques can be distinguished related to activated carbon filtration: granular activated carbon filtration (GACF) and biological activated carbon filtration (BACF). GACF relies on adsorption for the removal of micropollutants and other organics, while BACF relies on biodegradation. Of course, biological activity is also observed in GACF and therefore it is difficult to define when an activated carbon filter is a BAC or GAC filter. GAC filters are regenerated more often, thus the applied bedvolumes (BVs) give an indication whether the filter is more a GAC or BAC system. Typically, above the 10.000 – 30.000 BVs one can state that a GACF turns into a BACF, this is however highly dependent on the applied load (combination of flow and matrix). In the examples given in this section the number of BVs is mentioned if provided.

BACF can adsorb and biodegrade oxidation products to reduce the effluent toxicity¹¹⁵. The combination of ozonation with a BACF can lead to significant and additional micropollutant removal and lowering of toxicity¹¹⁶. Marron *et al.* (2020) showed that carbonyls are reduced over 90% after BACF²³. Shi *et al.* (2021) has observed that BACF can also remove nitromethane. Removal percentages at two pilot plants were 77% and 95% respectively⁵¹. Activated carbon can also enhance ozone decomposition to hydroxyl radicals.

Noteworthy is that Vatankhah *et al.* (2019) and Valdés *et al.* (2002) investigated the impact of ozone on the surface properties of BAC and found that continuous exposure of ozone ultimately caused structural damage to the BAC surface and led to a decrease in BET (Brunauer-Emmett-Teller) surface area^{117,118}.

GAC filtration can reduce toxicity and remaining micropollutants¹¹⁹. The use of ozone followed by GAC filtration is currently being investigated at WWTP Horstermeer in the Netherlands at pilot-scale. There are no results of this pilot available yet.

5.3.4 COMPARISON BETWEEN THE DIFFERENT POST-TREATMENT OPTIONS

According to Bourgin *et al.* (2018) and Schollée *et al.* (2018) who both investigated biological post-treatments (sand filtration, moving bed, and/or fixed bed bioreactor, and granular activated carbon), sand filtration performed the best in terms of removal of DOC, AOC (assimilable organic carbon) and SS. Between sand filtration and moving bed and fixed bed bioreactors, sand filtration showed the best relative degradation of NDMA by 66%, while moving bed- and fixed bed filtration showed slightly lower relative eliminations (48% and 41%, respectively). The removal rate of NDMA with GAC depends on the treated bedvolumes. A relatively fresh filter of GAC (12.500-16.500 BVs) showed an abatement of 83% for NDMA, while a preloaded GAC filter (35.000-40.000 BVs) exhibited a similar efficiency as sand filtration (with 56% removal of NDMA). Bourgin *et al.* (2018) and Schollée *et al.* (2018) both state that GAC filters outperform biological filters, due to an additional micropollutant adsorption.^{20,111}

Gulde *et al.* (2021) identified 84 transformation products formed during ozonation, originating from 40 micropollutants. Post sand filtration led to an abatement of 6% of these oxidation products. Abatement by granular activated decreased with increasing bed volumes. At 16.000 BVs, 53% of the identified oxidation products were removed, at 35.000 BVs this decreased to 40%.⁵²

Sauter *et al.* (2021) investigated three different deep-bed filters parallel to each other. A BAC filter, dual-media filter with sand and BAC and dual media filter with sand and anthracite (the latter two included a coagulation step to remove phosphorus). All three filters were run up to 50.000 BVs. It was found that all three filters achieved an efficient removal of biodegradable oxidation products, with only a limited impact of changes in the tested empty bed contact time (EBCT) or filtration rate. The use of BAC instead of non-adsorptive filter material resulted in a higher removal of DOC, dissolved oxygen, and micropollutants. Furthermore, they demonstrated that the integration of enhanced phosphorus removal into post-treatment is feasible with relatively low efforts by inline coagulant dosing (FeCl_3) in the filter influent.²⁴

For the removal of micropollutants and oxidation products Ternes *et al.* (2017) have compared 4 post-treatment processes after ozonation of WWTP-effluent: GAC_{nae} (nonaerated granular-activated carbon), GAC_{ae} (aerated granular-activated carbon), BF_{nae} (nonaerated biofilter) and BF_{ae} (aerated biofilter). A total of 30 substances was divided into four categories; (A) micropollutants not or hardly biodegradable in the activated sludge system, (B) micropollutants regulated or proposed for regulation under the Water Framework Directive, (C) metabolites of micropollutants formed during biological wastewater treatment, and (D) oxidation products formed during ozonation. The average performance of the post-treatment processes and ozonation per category is provided in Table 4. Note that for category D a negative removal efficiency is given, this represents the formation of oxidation products. Some of those oxidation products were already found in the WWTP-effluent at very low concentrations. Therefore, negative removal efficiencies are not -100%.¹¹⁹

TABLE 4 THE AVERAGE REMOVAL (CAT. A, B AND C) OR FORMATION (CAT. D) OF FOUR SELECTED CATEGORIES OF MICROPOLLUTANTS/METABOLITES/OXIDATION PRODUCTS. SPECIFIC OZONE DOSE OF $0,98 \pm 0,24 \text{ G O}_3/\text{G DOC}$, EBCT 28-34 MIN AND TREATED BVS OF 24.000-27.000^{119,120}

	Ozone (%)	Ozone + GAC _{nae} (%)	Ozone + GAC _{ae} (%)	Ozone + BF _{nae} (%)	Ozone + BF _{ae} (%)
Category A	82	91	89	85	80
Category B	86	86	86	86	86
Category C	94	97	97	92	92
Category D	-37	-27	-24	-31	-31

All post-treatment processes showed a removal of oxidation products (category D). The removal efficiency of the aerated GAC was the highest. Note that only three oxidation products were assessed. The reduction of toxicity in post-treatment processes is discussed in Chapter 7.

Bromate can only be transformed to bromide under specific anaerobic conditions⁷. Therefore, in a typical biological post-filtration step, such as sand filtration, bromate is not removed^{45,70,121}. The anaerobic degradation requires relatively long retention times: in an anaerobic fixed-film bioreactor on the reduction of bromate in groundwater, 90% bromate reduction was achieved in 40-80h¹¹. Because of the required anaerobic condition, which is not easily met after ozonation, and the long hydraulic retention time, bromate reduction as post-treatment has not been implemented yet at full-scale and isn't regarded as a feasible option^{7,18}.

Bromate can be removed with GACF, however only with fresh AC^{64,122}. With fresh AC a chemical reduction of bromate takes place¹²². As bromate is a very hydrophilic small ion without a carbon backbone, adsorbed bromate will easily be displaced by organic compounds, for which GAC has a higher affinity. Since the removal only takes place with fresh AC, GACF is not considered a feasible option for bromate reduction.

5.4 SUMMARY

There is a high influence of the process design on the formation of oxidation products. Especially the **process configuration** itself is highly important. This is mainly related to the ozone dosage, in combination with the reactor design and possibly the addition of H₂O₂. A higher **ozone dosage** results in a higher formation of bromate and several organic oxidation products (e.g. carbonyls and nitromethane). NDMA formation isn't influenced much by the process design. A **reactor configuration** with multiple ozone injection points reduces the bromate formation compared to a single point injection. The **addition of H₂O₂** isn't very well studied and understood in wastewater practices. In drinking water treatment, H₂O₂ is known to effectively suppress bromate formation. For wastewater treatment the required ratio H₂O₂:O₃ for bromate mitigation seems to be about 4:1 (weight basis). The available literature, however, is limited and not always congruent. The effect and the required ratio should therefore be confirmed in further research. The **addition of other chemicals** (e.g. pH depression) doesn't look beneficial in ozonation of WWTP-effluent. Due to the relatively high ozone and hydroxyl radical reaction constant rates of most micropollutants a measurable ozone exposure is not needed for the oxidation of micropollutants. Therefore, the **contact time** has little to no effect on the formation of oxidation products compared to the ozone dose.

Several **pre-treatment** options have been identified. Multiple of them like **coagulation** and **filtration** (e.g. sand filters, not membranes) are likely to have only a minor effect on the formation of oxidation products. Pre-treatment processes removing DOC and/or micropollutants from the wastewater seem to be the most useful, e.g. **PACAS**. By the removal of DOC an equal specific ozone dose ($\text{g O}_3/\text{g DOC}$) but a lower absolute ozone dose ($\text{g O}_3/\text{L}$) suffices the aimed micropollutant removal target and thereby results in a reduced formation of oxidation products. The partial removal of (hard to oxidise) micropollutants prior to ozonation allows a lower specific ozone dose resulting in less oxidation products.

Sand filtration, biological treatment in moving bed and fixed bed bioreactors, BACF and GACF can be used as a **post-treatment** after ozonation. Readily biodegradable oxidation products (such as carbonyls) can be removed with biological post-treatment (e.g. BACF). Removal of bromate is theoretically possible but not a feasible option in practice. NDMA can be removed with most post-treatments, the removal efficiency varies per post-treatment. Transformation products formed during ozonation can be (partly) removed, especially with AC. However, there is only limited literature available on the removal of transformation products. Post-treatment including AC (GACF and BACF) has the advantage that it can lead to an improved abatement of micropollutants.

6

NET-EFFECT OF OXIDATION, TRADE-OFF BETWEEN TOXICITY REDUCTION (MICROPOLLUTANT ABATEMENT) AND TOXICITY INCREASE (OXIDATION PRODUCT FORMATION)

6.1 INTRODUCTION

Previous chapters show the available knowledge on the formation of oxidation products, the associated toxicity, the potential impact of oxidation products like bromate on the drinking water production and the possible mitigation strategies. Ozonation can eliminate toxicity by oxidizing the micropollutants, but also oxidation products can be formed that are potentially toxic and can jeopardize the ecological quality of surface waters and the drinking water production. The most relevant inorganic oxidation by-product is bromate which is an undesired compound for drinking water production. The main identified organic oxidation by-products are carbonyls like formaldehyde and acetaldehyde, and NDMA. Those compounds mainly pose a direct toxic effect for the aquatic environment. Moreover, NDMA is also known to be relevant for drinking water production. In addition, out of the micropollutants themselves transformation products are formed, which (in the examples mentioned in 3.7) are commonly less toxic than their parent compounds.

The question arises what is the net effect of ozonation of WWTP-effluent? Is the overall toxicity reduced after ozonation? Does the type of toxicity alter due to ozonation? What is the net effect on the aquatic environment and drinking water production? In this chapter these questions will be addressed.

For the determination of the net-effect of oxidation both the results of ecotoxicity tests and chemical analyses are of importance. With bioassays typically the overall toxicity of (extractable) organic compounds is evaluated. It is difficult in bioassays to include the effects of small polar substances and very volatile compounds, which are not extracted from aqueous samples during sample pre-treatment. Some results have been published on bioassays performed with the native water sample (without pre-treatment) and of course the identified compounds (certain carbonyls as described section 3.5) may be included using chemical analysis. Inorganic substances, like bromate, are typically also not included in bioassays on concentrated extracts, but these are identified in chapter 3 and can be measured and evaluated separately.

The results of the chemical analysis and ecotoxicity tests on individual compounds are already discussed in the preceding chapters. The outcomes of ecotoxicity assessments on the total water matrix obtained in pilot or full-scale ozone installation will be presented in this chapter, after which an overall summary is provided.

As a disclaimer to this chapter, it must be noted that answering the above-mentioned questions is complicated as there are still a lot of knowledge gaps in this field of research. The amount of available literature on experiences with effects-based assessments focusing on a broad array of toxicological endpoints is limited, especially regarding long-term effects.

6.2 FIELD RESULTS ECOTOXICITY TESTS

Bioassay results from pilot and full-scale ozonation installations in the Netherlands, Switzerland and Germany are summarized in this section. When available also the effect of ozonation on environmental risks in the surface water is provided. In Postma (2019), ecological effects on advanced treatment are discussed as well. In that study it is concluded that the bioassay results from Germany and Switzerland can be extrapolated to the Dutch situation¹²³.

6.2.1 THE NETHERLANDS

The use of bioassays to evaluate toxicity of treated wastewater focused on removal of organic micropollutants is still under development. The first application of bioassays to evaluate toxicity of treated wastewater samples in the Netherlands was the PACAS pilot in Papendrecht in 2018. The same bioassay panel was thereafter used for the ozonation pilots at Aarle-Rixtel and De Groote Lucht⁶⁹. Appendix 1 shows the SIMONI (smart integrated monitoring) selection of bioassays for surface water assessment that have also been performed in these pilots on WWTP effluents. In total 14 types of bioassays were included. In 2020, within the Innovation Program Micropollutants of STOWA, a protocol was released for standardization of sample pre-treatment and with recommended bioassays to be applied in studies on micropollutant removal in WWTP effluent within the Netherlands¹²⁴. The procedure applied in the earlier mentioned pilots, was thus further improved (SPE instead of XAD sample pre-treatment). Further improvement in quality and costs is still ongoing.

- Ozonation pilot at Aarle-Rixtel⁶⁹:
 - At four sampling days samples have been taken for the determination of toxicity reduction using bioassays. The bioassays demonstrated that the ecotoxicity was reduced with 50-75% after the combination of ozonation plus biological post-treatment. This is based on the reduction in SIMONI Risk Indication (SRI, formerly known as SIMONI score), a value comprising the outcomes of the various bioassay altogether.
 - The SRI, based on the results of 14 bioassays, was mainly determined by the results of the following four bioassays: the PXR CALUX (related to detoxification), the Nrf2 CALUX (related to oxidative stress), and to a lesser extent also by ER CALUX (estrogenic activity) and PAH CALUX (related to the presence of poly aromatic hydrocarbons). The toxicity measured with these four bioassays was reduced after ozonation and biological post-treatment during all four sampling days. An increased activity of Nrf2-CALUX (oxidative stress) was measured on one sampling day.
 - Two samples were taken directly after ozonation (prior to post-treatment). Based on these two results, the impression is that the reduction in ecotoxicity was mainly attributed to ozonation. The reduction in ecotoxicity during the biological post-treatment was negligible. Only for the PAH CALUX assay most of the toxicity reduction took place in the biological post-treatment.
- Ozonation pilot at De Groote Lucht⁷⁰:
 - In the ozone pilot at WWTP De Groote Lucht, the ecotoxicity expressed as SRI was reduced after the combination of ozonation plus sand filtration by approximately 60%.

- Based on two samples, the sand filter did not seem to contribute to the reduction of the ecotoxicity.
- Most bioassays showed a reduced effect after ozonation. These include bioassays measuring the effect of toxic components such as pesticides, polycyclic aromatic hydrocarbons (PAHs), PCBs, and also estrogenic and antibiotic activity. One bioassay showed an increased response in all experiments, namely the Nrf2 CALUX, related to oxidative stress.

6.2.2 SWITZERLAND

In Switzerland bioassay evaluation at several ozonation studies have been conducted.

- At the full-scale ozone plant at WWTP Neugut, a broad range of bioassays was performed, both *in vitro* and *in vivo*, to assess the effect of the full-scale ozone installation, a full-scale aerobic sand filtration and other pilot post-treatment options.¹²⁵
 - The results show that the toxicity level directly after ozonation was very low. Only in one case a mutagenic effect was measured after ozonation. This toxicity was reduced by the sand filter and completely eliminated by the three other post-treatments (fixed bed bioreactor, moving bed bioreactor and GAC filtration).
 - Overall, the bioassays have shown that the ozonation reduces the ecotoxicity of the water and improves the quality of the effluent significantly.
 - Since the ozonation did not result in clearly measurable toxic effects, it was difficult to make a comparison from an ecotoxicological point of view between four different types of post-treatments (sand filtration, GAC filtration, fixed bed and moving bed).
 - It was concluded that WWTP Neugut, which treats wastewater originating mainly from households and partially from food industry, is a suitable location to apply ozone followed by sand filtration for abatement of micropollutants.
- At a pilot ozone installation followed by aerobic sand filtration at WWTP Wüeri in Regensdorf ecotoxicity tests have been performed both *in vitro* and *in vivo* and also with and without concentrating the water sample. The results are as follows¹²⁶:
 - Four bioassays showed a reduction in toxicity after ozonation and aerobic sand filtration: acetylcholinesterase-inhibition test, estrogenic activity and algae test (both growth rate and photosynthesis inhibition tests). The water sample was concentrated in these bioassays.
 - In five bioassays performed with native water sample (without sample pre-treatment) a reduced effect (2 bioassays) or no effect (3 bioassays) on toxicity was observed after ozonation plus sand filtration. These five bioassays include reproduction test with *C. dubia*, growth inhibition of *P. subcapitata* and *L. minor*, fish egg test with *D. rerio* and test with *G. fossarum* on food selection, feeding behaviour and population dynamics.
 - On-site flow through tests showed >70% elimination of toxicity in a Fish-Early-Life-Stage test after ozone and sand filtration. Tests with three other aquatic organisms did not show elimination of toxicity but neither an increase in toxicity. These three other tests include: reproduction tests with snails (*P. antipodarum*), life cycle tests with mosquitoes (*C. riparus*) and reproduction tests with glossworms (*L. variegatus*). It is mentioned though that directly after ozonation (thus before sand filtration) an inhibiting effect was observed in the Fish-Early-Life-Stage test and the glossworms test, which was eliminated again after aerobic sand filtration.

- Recently, findings of a pilot study at WWTP Glarnerland, a municipal WWTP receiving some industrial discharges, were published. Here, WWTP-effluent was treated with ozone prior to GAC filtration. Bioassays with algae and water fleas were conducted, but the most striking results were obtained with the Ames-test that determines mutagenic activity. This test is not included in the Dutch SIMONI bioassays-panel, where genotoxicity is measured using p53 CALUX.⁷⁴
 - Results from Glarnerland show a high to very high mutagenic activity after ozonation, (using strain TA98 + S9 and TA98 – S9). Mutagenic activity was sometimes also observed in other ozonation studies, but at WWTP Glarnerland this effect was higher compared to those other studies.
 - After GAC-filtration this mutagenic activity was reduced to levels equal or lower than those in the original sample before ozonation. This was regardless of the number of bed volumes of the GAC-filter (up to 40.000 BV).
 - Mainly because of the high inexplicable mutagenic activity it was concluded that ozonation is not suitable for WWTP Glarnerland, so it was decided not to continue with ozonation on that specific location.
- Laboratory ozonation tests by Schindler Wildhaber *et al.* (2015) have been performed on different WWTP-effluents from Switzerland. This laboratory research is included in this section because it provides more insight in the influence of wastewater composition on the toxicity before and after ozonation. The research included the following bioassays:⁴⁵
 - Four bioassays have been conducted with SPE pre-treatment: the Ames-test (for determining mutagenic activity), yeast estrogen and androgen screen (for endocrine androgenic, anti-androgenic, estrogenic and anti-estrogenic activity) and the combined algae test.
 - Two bioassays have been conducted with the native sample, without SPE: the toxicity test with cladoceran *C. dubia* and the embryo toxicity test with the zebrafish *Danio rerio*.

Using the results of all bioassays and the results about matrix effects on ozone stability micropollutant elimination and the formation of bromate and NDMA, an overall evaluation of the toxicity was made⁴⁵:

- For 3 out of 7 WWTP-effluents the bioassays showed that ozonation resulted in a significantly improved water quality. Also, the water matrix effects on the ozone stability were comparable to reference data. These were all WWTP-effluents which predominantly receive wastewater originating from households. Concluded was that ozonation for these three effluents is a feasible treatment for micropollutant removal and the reduction of ecotoxicity.
- For 1 of the WWTP-effluents receiving industrial wastewater, a decrease in toxicity and mutagenicity was observed after ozonation. The matrix effects on the ozone stability were only partly comparable to reference data. No further remarks were made.
- For 1 of the WWTP-effluents receiving industrial wastewater, mutagenicity wasn't always reduced and the *C.dubia* test indicated an increase in toxicity due to ozonation. The matrix effects on the ozone stability were only partly comparable to reference data. It was recommended to conduct more tests to confirm this.
- For the 2 WWTP-effluents with a high industrial contribution, including landfill leachate and discharges from various industries for one WWTP, and discharges from a chemical industry and a waste incinerator for the other WWTP, the results indicated a decrease in algal toxicity but an increase in at least one bioassay (mutagenicity, toxicity towards *C.dubia* or fish embryo toxicity) after ozonation. After a biological

post-treatment, however, this toxicity disappeared. Based on these bioassay results, the low elimination efficiency of the micropollutants, and differences in the matrix effects on ozone stability compared to reference data, at this stage ozonation was not recommended for these WWTPs.

The research of Schindler Wildhaber *et al.* (2015) demonstrates the importance of the origin of wastewater treated in the WWTP for the ozonation process. WWTPs receiving little to no industrial wastewater seem to be more suitable for the removal of micropollutants and reduction of ecotoxicity by ozonation. Ozonation of wastewater with a high industrial contribution of certain industries such as chemical industry and waste incinerators, may lead to increased toxicity. At those WWTPs ozonation is possibly less favourable. Chemical analysis and ecotoxicological tests could provide clarity on this topic.

6.2.3 GERMANY

In Aachen-Soers a full-scale ozone installation is operational since 2018. The ozonation is followed by post-nitrification and an aerobic sand filter. Ecotoxicity tests have been performed before and after the introduction of the ozonation process. The final results are not yet published but from preliminary results communicated via presentations the following becomes clear^{127,128}:

- Estrogenic activity is 100% reduced after ozonation.
- The androgenic activity was already low in the secondary effluent and no detectable increase during ozonation was observed.
- Ames-test with strains YG1041 und YG1042 indicated an increase of mutagenic activity after ozonation. With strain T98 no mutagenic activity after ozonation was observed. The YG strains seem to be more sensitive than the T98 and T100 for the mutagenic action of nitro-aromatic compounds¹²⁹.
- No negative effect is observed for algae, lemna (duckweed) and for fish embryos in a fish embryo toxicity test. The multicellular primary producers were slightly affected by wastewater pollution at the WWTP. An improvement was observed after ozonation.
- In situ tests with snails (reproduction rate) and rainbow trout (general fitness, endocrine and genotoxic potential), upstream and downstream of the WWTP in the receiving surface water (river Wurm) in 2017-2019 showed very comparable results before and after the installation of the ozonation process at WWTP Aachen-Soers. It must be noted that in situ tests are difficult to interpret because other (unknown) extrinsic environmental factors may also play a role.
- Since the introduction of the ozonation process, the receiving water quality seems to improve in terms of macrophytes biodiversity and SPEAR_{pesticide} (a biological indicator linking pesticide contamination to the composition of invertebrate communities)¹³⁰. More measurements are needed to confirm the link between the application of ozonation and the improved water quality of the receiving surface water in more detail.^{128,131}

Outcomes of pilot studies in Germany are:

- Ozonation pilot (location not provided)¹¹⁹:
 - Experiences with an ozonation pilot show that the endocrine activity of the treated wastewater decreased clearly, but the mutagenic activity increased strongly, and therefore the effect-based assessment index was negative.
 - Filtration with GAC reduced the mutagenic activity, this was not the case for a biological filter with expanded clay, favouring the configuration ozone + GAC instead of ozone + biofiltration¹¹⁹. The pilot run for almost two years, so probably there was also a biological activity on the GAC (thus it was probably also a BAC).

- Ozonation pilot fed with effluent from WWTP Neuss¹³²:
 - For two on site *in vivo* tests an increase in toxicity after ozonation was shown, which was reduced by sand-filtration. This toxicity concerned a decrease in biomass and reproduction of the annelid *L. variegatus* and increased genotoxicity detected with the comet assay using haemolymph of the zebra mussel.
 - Sand filtration reduced the observed genotoxicity in on site *in vivo* tests to levels comparable to the secondary effluent. Applying GAK as a post-treatment resulted in an even higher reduction of the toxicity.
 - Reduction of the estrogenic activity after ozonation was demonstrated with a reproduction test with the mudsnail *P. antipodarum*.¹³²
- Ozonation pilot at WWTP Eriskirch

Removal of endocrine (estrogenic, anti-estrogenic and androgenic) activity and genotoxic effects were investigated *in vitro*, and reproductive effects *in vivo*. Results show that³:

- Ozonation decreased the estrogenic activity (in concentrated samples) with 83% and in combination with sand filtration with 86%.
- Results of the anti-estrogenic activity before and after ozonation are less clear, and also the contribution of the sand filter. In the original samples (without concentrating) the anti-estrogenic activity decreased after ozonation and increased again after sand filtration. In the concentrated samples it was the opposite. Giebner et al (2018) provided three possible explanations for the increase in anti-estrogenic activity: 1) the anti-estrogenicity increased due to the removal of estrogenic activity 2) the formation of anti-estrogenic transformation products 3) false-positive responses in the bioassay.
- Androgenicity was low in the secondary effluent and remained low after ozonation.
- Mutagenicity (using an Ames-test) showed that minor mutagenic effects were detected in the secondary effluent, but these increased by 160% after ozonation. This mutagenicity was reduced again after either sand filtration (84% reduction) or GAK filtration (45% reduction). The mutagenicity induced by ozonation was measured using strain YG7108, which is highly sensitive to alkylating agents and carcinogens like nitrosamines. The result of the bioassay may thus be explained by the formation of these kinds of oxidation products.
- Lastly, a reproduction test was performed with mudsnail *P. antipodarum*, in native water samples taken before and after ozonation and after ozone + sand filtration or GAK. A decreased reproduction of 11% was measured after ozonation, and 15% after ozonation + GAK. This decrease was, however, not statistically significant. After ozonation + sand filtration the reproduction decreased significantly with 46%. The reproduction test was also performed with concentrated samples, but no negative effect on the reproduction of mudsnail *P. antipodarum* was observed in those samples. The explanation provided for this difference between the native water samples and the concentrated samples is that the compounds causing the reproduction effect are non-extractable (e.g., because of ionic, polar or volatile characteristics).

6.3 SUMMARY

- Several full-scale and pilot studies in the Netherlands, Switzerland and Germany have demonstrated with bioassays that the overall toxicity of organic substances in WWTP-effluent can be reduced significantly by ozonation leading to an improved water quality. Also in Postma (2019) it is concluded that advanced wastewater treatment (e.g. ozone in combination with post-treatment) is expected to have a positive influence on the water quality and aquatic biocenosis¹²³.

- In all cases a reduced estrogenic activity was found after ozonation (with and without post-treatment). Also for other bioassays a positive influence was found. However, in some cases, certain bioassays showed an increased toxicity (especially mutagenicity) directly after ozonation, which could be completely or partly removed during aerobic biological post-treatment.
- There are indications that the level of ecotoxicity reduction after ozonation depends on the composition and the origin of the wastewater that is treated. Certain (industrial) discharges may cause an increase of toxicity after ozonation. It is not known yet which substances are causing this increased toxicity.
- It is difficult to assess to which extent all potential toxic organics are well represented in the bioassays after concentration of the samples, especially small hydrophilic compounds which cannot be (fully) extracted during sample pre-treatment. Identified polar organics can be analysed and evaluated separately (such as certain carbonyls, see chapter 3), but many unknown oxidation products may also be formed. However, the above-mentioned literature includes also bioassays with native wastewater samples including all water constituents. Moreover, at WWTP Aachen-Soers the impact of ozone plus aerobic sand filtration on the receiving water quality is being investigated. So far, it seems that the receiving water quality improves or remains comparable after introduction of ozone with sand filtration, depending on the specific toxicological endpoints researched.
- Inorganics are also typically not included in concentrated samples due to their loss during pre-treatment. Bromate is the main relevant inorganic compound (chapter 3). Its formation during ozonation will be highly site-specific and its impact should be evaluated separately for each case.
- Bioassays themselves, pre-treatment methods and the interpretation of bioassay outcomes into an overall environmental assessment are still under development. It is currently still difficult to assess the ecotoxicity of the entire water sample before and after ozonation. Ongoing improvements and developments need to contribute to an improved understanding of the reduction in ecotoxicity with ozonation in the future. Specific effect-based trigger values (EBT) need to be developed for all bioassays and extraction methods, in order to assess the potential ecological risks in the receiving surface waters.

7

MOST IMPORTANT KNOWLEDGE GAPS

In this literature study the available literature was reviewed. Besides this, experiences with studies on a field scale were discussed, including oxidation products formation, assessment and mitigation. All information obtained is provided in the previous chapters. However, also several knowledge gaps have been identified which could not be assessed by the current available literature or practical field experience. The most important knowledge gaps for a good understanding and evaluation of oxidation product formation, assessment and mitigation during ozonation of WWTP-effluent are discussed in this chapter.

7.1 ASSESSMENT OF ECOTOXICITY RELEVANT FOR THE AQUATIC ENVIRONMENT

As shown in chapter 3 a wide variety of oxidation products may be formed. The concentrations in which they are formed generally do not exceed the available PNEC values, except for a few oxidation products. However, for most oxidation products no PNEC values are available. In addition, there are many yet unknown oxidation by-products. The assessment of ecotoxicity using bioassays as described in chapter 6 indicates that in almost all cases the ecotoxicity is reduced after ozonation. Only in a few cases, certain bioassays showed an increased toxicity (especially mutagenicity) directly after ozonation, which could be completely or partly removed during aerobic biological post-treatment. Furthermore, the limits of the current evaluation of ecotoxicity were discussed, e.g., sample pre-treatment, type of bioassay, etc., and the difficulty to interpret the obtained results to assess the impact on the aquatic environment. In general, long-term effects of complex mixtures of compounds on the aquatic environment are still very difficult to evaluate.

Based on the evaluation of available literature, several knowledge gaps are identified which should be further investigated for a proper consideration of pro's and con's of ozonation with respect to (eco)toxicity:

The most important identified knowledge gaps are:

- A limited prioritization on the relevance of the various bioassays to evaluate ecotoxicity of ozonated samples.
- The extent to which acute bioassays cover chronic mixture effects.
- There is no complete insight to which extent the bioassay results are influenced by the pre-treatment (extraction method) of the sample, e.g., to which extent oxidation products are less or equally recovered compared to their precursors in the extract used for bioassays. For chemical analyses, however, low recoveries can be corrected.
- Bioassays are often performed at higher concentrations compared to the concentrations measured in WWTP-effluents. It may be assumed that short-term effect at higher concentrations are estimates of long-term effects at low concentrations¹³³.
- Selection of the most relevant bioassays for WWTP quality assessment; only a few bioassays have an ISO NORM and can be performed by commercial labs.
- Derivation of effect-based trigger values (EBT) to make bioassays feasible for environmental and human risk assessment.

7.2 EFFECT OF MATRIX COMPOSITION AS A WHOLE

In chapter 4 the influence of the various matrix constituents on the formation of oxidation products is discussed. For quite some individual matrix constituents the impact on the ozonation process and thereby on the formation of oxidation products is known, e.g., the presence of bromide and possible formation of bromate.

WWTP-effluent is a relatively complex matrix with enormous varieties of different compounds and huge differences between effluents, e.g., due to geographical location, share of industrial wastewater, etc. The impact of individual matrix constituents is largely known for quite some compounds. However, the cohesion between the various oxidation pathways of the individual constituents and the interaction between matrix constituents during ozonation is not fully understood. Especially due to the wide variation in composition of WWTP-effluents, the net outcome of an ozonation step in terms of oxidation products depends on the local composition of the water matrix and thereby results of one location might not be valid for another. For example, the presence of ammonium is reported to have both positive and negative effects on the ozonation process. A low concentration of ammonium might be beneficial to avoid elevated bromate formation. However, ammonium can also result in uncontrolled nitrification and thereby scavenge ozone, which eventually results in the formation of nitrosamines (see section 4.2). Thereby the question arises what the net effect of the presence of ammonium is, and how this net effect is influenced by the presence of other matrix constituents.

The example of ammonium illustrates the existing knowledge gap on the net effect of the interaction of the various matrix constituents on the ozonation process and the overall impact that this has on the formation of oxidation products and their associated ecotoxicity. On the other hand, the pilot and full-scale studies so far already give quite a clear overview of the effect of ozonation on the ecotoxicity. In most examples ozonation reduces the ecotoxicity, however in specific cases the ecotoxicity is increased (e.g. high share of industrial wastewater). Thus, with demo-, pilot-, or laboratory tests this knowledge gap can be overcome for individual locations. Therefore, albeit the differences in water matrices between WWTPs the above-mentioned knowledge gap does not hinder to study the feasibility of the application of ozone treatment for an individual WWTP. Possibly the outcomes of ozone tests at multiple locations will fill this knowledge gap in a more general manner. However, most likely at least at tens of WWTPs tests must be performed in order to draw more general conclusions on this matter.

7.3 IMPACT OF REACTOR CONFIGURATION AND H₂O₂ ADDITION

Chapter 4 discusses the influence of process design on the formation of especially bromate, and some other oxidation products. Regarding bromate formation, several options to mitigate the formation are described, see section 5.1 on ozone dosage, contact time, reactor configuration and addition of chemicals.

7.3.1 REACTOR CONFIGURATION

Especially for bromate control the effect of the reactor configuration is well known. Bringing the ozone via multiple injection points into the process mitigates the bromate formation compared to single point injection. However, much less is known about the impact of reactor configuration on the other oxidation products. With respect to the reactor configuration the most important knowledge gaps therefore are:

- The extent to which the formation of oxidation products is related to the reactor configuration of the ozone installation.
- What the effect on the formation of oxidation products of the reactor configuration is compared to the effects of other parameters such as the ozone dose and the water matrix.

7.3.2 H₂O₂ ADDITION

The addition of H₂O₂ to the ozonation is a well-known strategy to prevent bromate formation in drinking water production. For ozonation of WWTP-effluent only a very limited amount of bench-scale and pilot studies have investigated the effect of H₂O₂ addition. To our best knowledge there are no experiences yet on full-scale ozonation combined with H₂O₂ addition. This knowledge gap is an important one to be investigated for possible control strategies of bromate formation. Questions related to this knowledge gap are:

- How effective is the addition of H₂O₂ to control the formation of bromate and other oxidation products in ozonation of WWTP-effluent?
- What concentrations of H₂O₂ should be dosed and on which factors does the dose depend, e.g. DOC or ozone dosage?

7.4 POST-TREATMENT

Research on various post-treatment options show that with sand filtration, moving bed and fixed bed bioreactors or (biological) granular activated carbon, biodegradable oxidation products such as NDMA and carbonyls, and transformation products can be removed. The extent of abatement depends on the type of the post-treatment, e.g., biological process, adsorptive process, and the configuration of the post-treatment such as retention time, addition of chemicals, environmental conditions. There is insufficient insight if and if so what kind of post-treatment at a minimum should be implemented for the reduction of oxidation products, i.e., which combination of type and configuration of post-treatment technologies. So far in literature no unambiguous factual answer could be found to the question whether ozonation should be followed by a post-treatment or not.

The most important identified knowledge gaps on post-treatment are:

- Which oxidation products (carbonyls, NDMA, transformation products) should be removed in a post-treatment step and to which extent or below what concentrations, probably depending on local situations?
- What is the most optimal kind of the post-treatment, i.e., type and configuration of the post-treatment for a specific removal need?
- What are the design principles of a post-treatment technology for oxidation product removal?

7.5 PFAS

During the writing of this study polyfluoroalkyl substances (PFAS) or PFAS related products were not identified as oxidation products of ozonation of WWTP-effluent. Little literature is available on the formation of PFAS related oxidation products. Moreover, the available information shows that oxidation, including ozonation, is not an effective method for PFAS removal^{134,135}. This could suggest that formation of specific oxidation products from PFAS is negligible, however this is not known. In contrast, ozone is used in one of the analytical methods to analyse total-PFAS, the TOP-assay, to convert specific PFASs into the detectable group of compounds perfluoroalkyl acids (PFAA). Whether this conversion also occurs during ozonation of WWTP-effluent and thereby influences the formation of oxidation products is unknown.

7.6 SUMMARY

The most relevant identified knowledge gaps relate to:

- The assessment of the ecotoxicity of (ozonated) WWTP-effluent: prioritization of the various bioassays, the extent to which acute bioassays cover long-term chronic mixture effects, influence of sample pre-treatment on the bioassay result and derivation of effect-based trigger values to make bioassays feasible for environmental and human risk assessment.
- Understanding the effect of the water matrix on the formation of oxidation products including bromate.
- The effect of the reactor configuration on oxidation product formation, and in particular for bromate mitigation the effectiveness of H_2O_2 and required dose.
- The most optimal post-treatment step if removal of certain oxidation products is desired.
- The effect of ozonation on PFAS conversions.
- The most optimal post-treatment step if removal of certain oxidation products is desired.
- The effect of ozonation on PFAS conversions.

8

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APPENDIX 1

OVERVIEW OF BIOASSAYS

Overview of the Dutch package of bioassays (source: STOWA, 2016. Posthuma L., De Zwart D., Osté L., Van der Oost R., Postma J. *Ecologische Sleutelfactor Toxiciteit. Deel 1: Methode voor het in beeld brengen van de toxiciteit, STOWA rapport 2016-15A*)¹³⁶.

Currently the microtox, daphniatox, PAH-Calux, ER-Calux, PXR-Calux, GR-Calux and P53-Calux (-S9) are required for demonstration research in the Netherlands (source: *Handreiking voor uitvoeren van biologische effectmonitoring bij vergaande zuivering van RWZI-effluenten versie 0.7, 03-04-2020*)¹²⁴.

EINDPUNT	BIOASSAY	RESPONS OP STOFFEN
Algemene Toxiciteit (<i>in vivo</i>)		
Bacteriën	Microtox	Alle stoffen
Fytoplankton	Algaltokit	Alle stoffen
Zooplankton	Daphniatoxkit	Alle stoffen
	In situ Daphnia	Alle stoffen
Celkweek	Cytotox CALUX	Alle stoffen
Specifieke Toxiciteit (<i>in vitro</i>)		
Estrogene activiteit	ER CALUX	Natuurlijke en synthetische estrogenen, pseudo-estrogenen, bisfenol A, alkylfenolen, medicijnen, pesticiden
Anti-androgene activiteit	Anti-AR CALUX	Pesticiden, insecticiden, herbiciden, gebromeerde vlamvertragers, (pseudo-) androgenen, anabole steroïden, antibiotica, groeipromotors, estrogenen, polychloorbiphenylen (PCB's)
Glucocorticoïde activiteit	GR CALUX	Verschillende medicijnen, corticosteroïden
Omzetting giftige stoffen	PXR CALUX	Pesticiden, polycyclische aromatische koolwaterstoffen (PAK's), alkylfenolen, triazine pesticiden, medicijnen, PCB's
Omzetting giftige stoffen	DR CALUX	Polychloor dibenzo dioxinen (PCDD's) en furanen (PCDF's), PCB's, PAK's, gebromeerde stoffen
Omzetting giftige stoffen	PAH CALUX	Polycyclische aromatische koolwaterstoffen (PAK's)
Vetmetabolisme	PPARg CALUX	Organotin verbindingen, perfluorverbindingen (bijv. PFOS, PFOA), esters, vetzuurderivaten
Antibiotica activiteit	RIKILT WaterSCAN	Vijf klassen antibiotica (tetracyclines, quinolonen, macroliden/ -lactam, sulfonamiden en aminoglycosiden), biociden (bijv. triclosan)
Oxidatieve stress	Nrf2 CALUX	Algemene chemische stress, reactieve stoffen, fungiciden, insecticiden, fenolen, medicijnen, estrogenen
Genetische toxiciteit	P53 CALUX	Gechloreerde stoffen, aromatische aminen, PAK's

Overview of the bioassays recommended in Switzerland. The first three are recommended as a minimum), source: VSA. (2017) Abklärungen Verfahrenseignung Ozonung – Empfehlung¹⁰.

Tabelle 1. Übersicht über die Biotests, die verwendeten Testorganismen, sowie die nachweisbaren Effekte (nach Kienle und Langer, 2016).

Biotest	Testorganismus	Nachweisbare Effekte (Wirkung)
Ames-Test	Bakterien (Salmonellen)	Vererbare Veränderung des Erbguts (Mutagenität)
Chronischer Fortpflanzungstest mit Wasserflöhen	Wasserfloh	Hemmung der Fortpflanzung, verringertes Überleben
Kombinierter Algentest	Grünalgen	Hemmung der Photosynthese (u.a. Wirkung bestimmter Herbizide) und Hemmung des Wachstums
Fischartest	Zebrabärbling	Erhöhung der Sterblichkeit
UmuC-Test	Bakterien (Salmonellen)	Schädigung des Erbguts (Gentoxizität)
Lumineszenz-Hemmtest	Leuchtbakterien	Hemmung der Biolumineszenz

APPENDIX 2

CHEMICAL PARAMETERS IN THE DUTCH DRINKING WATER DIRECTIVE

Overview of the chemical parameters as taken up in the Drinking Water Directive of the Netherlands.

The table is taken from 'Bijlage A. behorend bij hoofdstuk 3 van het Drinkwaterbesluit, tabel II. Chemische Parameters'.⁶

Parameter	Maximum waarde	Eenheid	Opmerkingen
Acrylamide	0,10	µg/l	Noot 1
Antimoon	5,0	µg/l	
Arseen	10	µg/l	
Benzeen	1,0	µg/l	
Benzo(a)pyreen	0,010	µg/l	
Boor	0,5	mg/l	
Bromaat	1,0	µg/l	Bij desinfectie geldt een maximale waarde van 5,0 µg/l (als 90 percentielwaarde, met een maximum van 10 µg/l)
Cadmium	5,0	µg/l	
Chroom	50	µg/l	
Cyaniden (totaal)	50	µg/l	Noot 3
1,2-Dichloorethaan	3,0	µg/l	
Epichloorhydrine	0,10	µg/l	Noot 1
Fluoride	1,0	mg/l	
Koper	2,0	mg/l	Noot 2
Kwik	1,0	µg/l	
Lood	10	µg/l	Noot 2
Nikkel	20	µg/l	Noot 2
Nitraat	50	mg/l	Noot 4
Nitriet	0,1	mg/l	Noot 4
N- nitrosodimethylamine (NDMA)	12	ng/l	
Polycyclische aromatische koolwaterstoffen (PAK's) (som)	0,10	µg/l	Som van gespecificeerde verbindingen met concentratie hoger dan de detectiegrens. Noot 5
Polychloorbifenylen (PCB's) (individueel)	0,10	µg/l	Per stof.
PCB's (som)	0,50	µg/l	Som van gespecificeerde verbindingen met concentratie > 0,05 µg/l. Noot 6
Pesticiden (individueel)	0,10	µg/l	Per stof. Noot 7. Voor aldrin, dieldrin, heptachloor en heptachloor-epoxide geldt een maximum waarde van 0,030 µg/L.
Pesticiden (som)	0,50	µg/l	Som van afzonderlijke pesticiden met concentratie hoger dan de detectiegrens.
Seleen	10	µg/l	
Tetra- en trichlooretheen (som)	10	µg/l	
Trihalomethanen (som)	25	µg/l	Noot 8
Vinylchloride	0,10	µg/l	Noot 1

Noten:

- ¹⁾ Deze parameterwaarde heeft betrekking op de residuele monomeerconcentratie in het water, berekend aan de hand van specificaties inzake de maximum migratie van de overeenkomstige polymeer in contact met water, of betreft een feitelijk gemeten waarde.
- ²⁾ Deze waarde geldt voor een monster van voor menselijke consumptie bestemd water dat via een passende steekproefmethode aan de kraan verkregen is, en dat representatief mag worden geacht voor de gemiddelde waarde die de gebruiker wekelijks binnen krijgt. Deze methode is beschreven in de «VROM-Inspectierichtlijn Harmonisatie Meetprogramma Drinkwaterkwaliteit».
- ³⁾ Met behulp van de methode moet het totaal aan cyanide in elke vorm worden bepaald.
- ⁴⁾ Ten aanzien van de concentraties nitraat en nitriet dient tevens te worden voldaan aan de voorwaarde dat $[\text{nitraat}]/50 + [\text{nitriet}]/3 < 1$, waarbij de rechte haken de concentratie in mg/l uitdrukken, voor nitraat in NO_3^- , en voor nitriet in NO_2^- .
- ⁵⁾ De gespecificeerde verbindingen zijn: pyreen, benzo(a)antraceen, benzo(ghi)peryleen, fenantreen, indeno(1,2,3-cd)pyreen, anthraceen, benzo(b)fluorantheen, benzo(k)fluorantheen, chryseen en fluorantheen
- ⁶⁾ De gespecificeerde verbindingen zijn: PCB nr. 28, 52, 101, 118, 138, 153 en 180.
- ⁷⁾ Onder pesticiden wordt verstaan: organische insecticiden, organische herbiciden, organische fungiciden, organische nematociden, organische acariciden, organische algiciden, organische rodenticiden, organische slimiciden en soortgelijke producten (onder meer groeiregulatoren). De norm van 0,1 µg/l geldt ook voor humaan toxicologisch relevante metaboliëten, afbraak- en reactieproducten van pesticiden. Voor metaboliëten van pesticiden en afbraak- of reactieproducten, die niet humaan toxicologisch relevant zijn, geldt een norm van 1,0 µg per liter.
- ⁸⁾ De maximumwaarde geldt bij het gebruik van chloor(verbindingen) voor desinfectie; in de overige situaties geldt de maximumwaarde genoemd in Tabel IIIc bij gehalogeneerde alifatische koolwaterstoffen. De gespecificeerde verbindingen zijn: chloroform, bromoform, dibroomchloormethaan en broomdichloormethaan. De concentratie broomdichloormethaan mag niet hoger zijn dan 15 µg/l. De somwaarde van 25 µg/l geldt als 90 percentiel, met een maximum van 50 µg/l. Voor drinkwatervoorzieningen op mijnbouwinstallaties als bedoeld in [artikel 1, onderdeel o, van de Mijnbouwwet](#), geldt als somwaarde 100 µg/l, waarbij het gehalte broomdichloormethaan maximaal 60 µg/l mag zijn.