REGULATION (EU) NO 528/2012 CONCERNING THE MAKING AVAILABLE ON THE MARKET AND USE OF BIOCIDAL PRODUCTS

COMBINED List of endpoints of applications for approval of active substance Ozone generated from oxygen

Ozone generated from oxygen

Product types 2, 4, 5 and 11

(Disinfectants and algaecides not intended for direct application to humans or animals, food and feed area and drinking water; preservatives for liquid-cooling and processing systems)

Substance Name:	Ozone generated from oxygen
EC Name:	Ozone
EC Number:	not applicable for an in situ generated active substance
CAS Number:	not applicable for an in situ generated active substance
Index Number:	Not allocated
Applicants:	EurO3zon
	The European Ozone Trade Association Limited
Contact details of evaluating CA:	BAuA, Germany
	Ctgb, The Netherlands

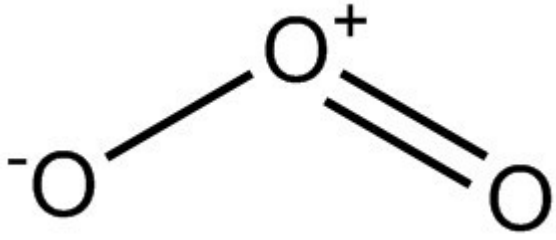
New active substance submitted under Article 8(4) in combination with Article 93 of the BPR

Date: March 2023

COMBINED List of endpoints of applications for approval of active substance Ozone generated from oxygen

1.1.1. Chapter 1: Identity, Physical and Chemical Properties, Classification and Labelling

Active substance (ISO Name)	ISO name is not applicable Common name: Ozone generated from: - Ambient air; - Ambient water; - Oxygen
Product-type	2, 4, 5 and 11

Identity	
Chemical name (IUPAC)	2-Trioxiden-2-ium-1-ide
Chemical name (CA)	Ozone
CAS No	Not applicable for an *in situ* generated active substance
EC No	Not applicable for an *in situ* generated active substance
Other substance No.	Not applicable for an *in situ* generated active substance
Minimum purity of the active substance as manufactured (g/kg or g/l)	Not applicable for in situ generated active substances. Please consult the specific generation techniques for information on the generated ozone concentrations. Ozone is an in situ generated active substance and the concentration generated can vary depending on the settings of the applied devices.
Identity of relevant impurities and additives (substances of concern) in the active substance as manufactured (g/kg)	Not applicable for in situ generated active substances. Please consult the specific generation techniques for information on the generated ozone concentrations. Ozone is an in situ generated active substance and the concentration generated can vary depending on the settings of the applied devices.
Molecular formula	O_3
Molecular mass	47.9982 g/mol
Structure	

Physical and chemical properties	
Melting point (state purity)	ca. -193°C Purity not stated (public literature)
Boiling point (state purity)	ca. -112°C Purity not stated (public literature)
Thermal stability / Temperature of decomposition	The half-life of ozone is much shorter in water than in air. Increased temperature in either medium decreases the half-life. Air: Half-Life of ozone is ca 1500 minutes (~ 25 hours) in still air at room temperature (24°C) and zero humidity (McClurkin et al, 2013). Water: Half-lives of ozone in water, at pH 7 and at 20°C, range from ca 500 -5000 s (Gardoni et al 2012). Generally accepted range for half-lives for ozone in water at 20°C is ca 20-30 minutes.
Appearance (state purity)	Colourless to pale blue with pungent odour Purity not stated (public literature)
Relative density (state purity)	1.66 (air = 1.0) (calculation from ideal gas law)
Surface tension (state temperature and concentration of the test solution)	Not applicable to gaseous ozone: based on the molecular structure of ozone, no effect on surface tension of water is expected or can be predicted. In addition, it would not be technically feasible to perform the study following the OECD test guidelines.
Vapour pressure (in Pa, state temperature)	1kPa at -158°C 10 kPa at -139.7°C 100 kPa at -111.5°C In general the vapour pressure is not required for substances with a standard boiling point of < 30°C, however they are included because they were provided by The European Ozone Trade Association Limited.
Henry's law constant (Pa m^3 mol -1)	9210.4425 Pa m^3/mol at 25°C
Solubility in water (g/l or mg/l, state temperature)	Solubility ratio: 0.31 at 20°C and pH 2.7 ((mg ozone/L H2O) / (mg ozone/L air)) In water: pH 7 at 20°C: 5 mg/L Purity not stated (public literature)
Solubility in organic solvents (in g/l or mg/l, state temperature)	The information is only required if the active substance as manufactured is delivered in an organic solvent, which is not the case for ozone. Ozone gas is generated in situ at the user's location and not stored.
Stability in organic solvents used in biocidal products including relevant breakdown products	not applicable

Partition coefficient (log POW) (state temperature)	$logP_{Ow}$ = - 0.87 (estimation)
Dissociation constant	Not applicable to ozone
UV/VIS absorption (max.) (if absorption > 290 nm state ε at wavelength)	254 nm ε = 3 000 $L \cdot mol^{-1} cm^{-1}$ 575 nm ε = 1.31 $L \cdot mol^{-1} cm^{-1}$ 603 nm ε = 1.36 $L \cdot mol^{-1} cm^{-1}$ 749 nm ε = 0.117 $L \cdot mol^{-1} cm^{-1}$

Physical Hazards	
Explosives	not applicable
Flammable gases	non-flammable gas
Flammable aerosols	not applicable
Oxidising gases	oxidising gas 1
Gases under pressure	not applicable
Flammable liquids	not applicable
Flammable solids	not applicable
Self-reactive substances and mixtures	not applicable
Pyrophoric liquids	not applicable
Pyrophoric solids	not applicable
Self-heating substances and mixtures	not applicable
Substances and mixtures which in contact with water emit flammable gases	not applicable
Oxidising liquids	not applicable
Oxidising solids	not applicable
Organic peroxides	not applicable
Corrosive to metals	not applicable
Desensitised explosives	not applicable
Auto-ignition temperature(liquids and gases)	not applicable
Relative self-ignition temperature for solids	not applicable
Dust explosion hazard	not applicable

Classification and labelling[1]	
with regard to physical hazards	Ox. Gas 1, H270
with regard to human health hazards	Acute Tox. 1; H330, ATE 10 ppmV (gases) Muta. 2, H341 Carc. 2; H351 STOT SE1; H370 (respiratory system, nervous system, cardiovascular system) (C ≥ 0.002 % STOT SE 1; H370, 0.0005 % ≤ C < 0.002 % STOT SE 2; H371) STOT RE1; H372 (respiratory system, nervous system) (C ≥ 0.05 % STOT RE 1; H372, 0.01 % ≤ C < 0.05 % STOT RE 2; H373)
with regard to environmental hazards	Aquatic acute 1; H400 (M=100) Aquatic chronic 1; H410 (M=1)

[1] RAC opinion was adopted in March 2023 and will be published on the ECHA website: https://echa.europa.eu/registry-of-clh-intentions-until-outcome/-/dislist/details/0b0236e180dfd06a

1.1.2. Chapter 2: Methods of Analysis

Analytical methods for the active substance	
Technical active substance (principle of method)	photometric determination with DPD or Iodometric titration methods are available
Impurities in technical active substance (principle of method)	The impurities could be analysed with the following methods: • Infrared spectrometry measuring the absorption at a specific wavelength and calculation of the concentration using Lambert-Beer law • flame ionization detection measuring the created current when the substances are burned. • electrochemical hydrogen sensors measuring change in conductivity • HPLC with different detectors (conductivity, UV, amperometric detection)

Analytical methods for residues	
Soil (principle of method and LOQ)	Relevant residues of ozone in soil are not expected because of rapid degradation upon contact with organic substances.
Air (principle of method and LOQ)	Relevant residue: ozone EN 14625 UV photometric measuring principle; LOQ: 10 - 500 µg/m³ OSHA method, ion chromatography with UV detection at 200 nm, LOQ: 100 µg/m³

Water (principle of method and LOQ)	**Relevant residue: ozone** DIN 38408-3 photometric determination with DPD; LOQ: 0.02 mg/L (drinking water) DIN 38408-3 indigo colorimetric method; LOQ: 0.01 mg/L (surface and drinking water) ASTM D7677-11, amperometric sensor, LOQ: 1 µg/L (drinking water). **Relevant residue: bromates** ISO 11206:2011; ion chromatography and post column reaction; LOQ: 7.7 µg/L (swimming water); 2.6 µg/L (drinking water) **Relevant residue: trihalomethanes** DIN 38407-43:2014; Headspace GC-MS; LOQs (drinking water): bromoform 0.44 µg/L, dibromochloromethane 0.28 µg/L, bromodichloromethane 0.15 µg/L, chloroform 0.14 µg/L LOQs (surface water and waste water - also acceptable for swimming water): bromoform 0.54 – 1.2 µg/L dibromochloromethane 0.18 – 1.7 µg/L, bromodichloromethane 0.2 – 0.81 µg/L, chloroform 0.2 – 0.89 µg/L **Relevant residue: chlorate and chlorite** DIN EN ISO 10304-4:1999: HPLC with different detectors (conductivity, UV, amperometric detection); LOQs (drinking water): chlorate 90 µg/L, chlorite 100 µg/L LOQs (swimming pool water): chlorate 159 µg/L, chlorite 100 µg/L
Body fluids and tissues (principle of method and LOQ)	Relevant residues of ozone in body fluids and tissues are not expected because ozone is not taken up systemically in the body.
Food/feed of plant origin (principle of method and LOQ for methods for monitoring purposes)	Contact with food and feeding stuff is unlikely from the intended use. Residue analytical methods for ozone in food and feeding stuff are not required.
Food/feed of animal origin (principle of method and LOQ for methods for monitoring purposes)	Contact with food and feeding stuff is unlikely from the intended use. Residue analytical methods for ozone in food and feeding stuff are not required.

1.1.3. Chapter 3: Impact on Human Health

Absorption, distribution, metabolism and excretion in mammals	
Rate and extent of oral absorption:	No data submitted. 100% default if needed for exposure assessment
Rate and extent of dermal absorption*:	No data submitted. 100% default if needed for exposure assessment
Rate and extent of inhalation absorption	100% default if needed for exposure assessment

Distribution:	Based on the physicochemical properties of ozone, it is expected that the majority of the substance reacts with the tissue at the site of contact. Reaction products might be expected to distribute more widely.
Potential for accumulation:	Not assessed
Rate and extent of excretion:	Based on the physicochemical properties of ozone, it is expected that the majority of the substance reacts rapidly and completely with the tissue at the site of contact.
Toxicologically significant metabolite(s)	No data submitted. Based on the physicochemical properties of ozone, it is expected that the majority of the substance reacts rapidly and completely with the tissue at the site of contact.

* The dermal absorption value is applicable for the active substance and might not be usable in product authorization

Acute toxicity	
Rat LD50 oral	No studies submitted, waiving acceptable
Rat LD50 dermal	No studies submitted, waiving acceptable
Rat LC50 inhalation	**EurO3zon:** 1-10 ppm (range, derivation of actual value not possible **EUoTa**: <12 ppm Acute Tox. 1; H330, ATE 10 ppmV (gases) STOT SE1; H370 (respiratory system, nervous system, cardiovascular system) (C ≥ 0.002 % STOT SE 1; H370, 0.0005 % ≤ C < 0.002 % STOT SE 2; H371)
Skin corrosion/irritation	no classification
Eye irritation	no classification
Respiratory tract irritation	Irritant to the respiratory tract, NOAEC = 0.06 ppm (LOAEC 0.08 ppm)
Skin sensitisation (test method used and result)	No data submitted No evidence from the scientific open literature that ozone is a dermal sensitiser (systematic literature search was not conducted)
Respiratory sensitisation (test method used and result)	Not a causal factor for the development of allergic asthma, but causes Airway Hyperresponsiveness NOAEC for Inhalation elicitation: ≤ 50 ppb

Repeated dose toxicity	
Short term	
Species / target / critical effect	Increased number of bradyarrhythmic episodes; morphological changes, cell death and altered neurogenesis in different brain regions; oxidative stress

Relevant oral NOAEL / LOAEL	No studies submitted, waiving acceptable
Relevant dermal NOAEL / LOAEL	No studies submitted, waiving acceptable
Relevant inhalation NOAEL / LOAEL	LOEC systemic: 0.1-0.12 ppm STOT RE1; H372 (respiratory system, nervous system)(C ≥ 0.05 % STOT RE 1; H372, 0.01 % ≤ C < 0.05 % STOT RE 2; H373)

Genotoxicity	Positive evidence for somatic cell mutagenicity and genotoxicity obtained from *in vivo* studies - which are further supported by positive *in vitro* tests. Further indication for the genotoxic and mutagenic potency is given in epidemiological studies**.** Muta. 2, H341
Carcinogenicity	
Species/type of tumour	Lung tumor formation in many different and independent studies in mice. All of these studies used different times and protocols. Lung effects were above the historical control data and followed a positive trend (NTP study). Both sexes of A/J mice and $B6C3F_1$ mice developed lung tumours. The 2-year NTP study is in compliance with Food and Drug Administration Good Laboratory Practice Regulations. The carcinogenic effects in the lungs - the first site of contact after inhalative exposure – are mechanistically plausible taken into account the genotoxic effects of ozone or its oxidation products in the lung. Therefore, lung carcinogenicity could be attributed to genotoxic effects (initiation events) and further oxidative stress (initiation and tumour promoting events). Carc. 2, H351
Relevant NOAEL/LOAEL	Not determined for neoplastic effects.

Reproductive toxicity **Developmental toxicity**	
Species/ Developmental target / critical effect	There was no clear association between pre- and postnatal exposure to ozone and developmental effects in mice and rats. Developmental effects in foetus and offspring occurred at higher concentrations and interpreted as non-specific consequence of general toxicity and growth retardation.
Relevant maternal NOAEL	NOAEC: 0.4 ppm
Relevant developmental NOAEL	NOAEC: 0.4 ppm

Fertility

Species/critical effect	No impairment of sexual function and fertility in male rats and mice and female rats.
Relevant parental NOAEL	Not determined.
Relevant offspring NOAEL	Not determined.
Relevant fertility NOAEL	NOAEC: 0.5 ppm (male fertility) and NOAEC: 0.6 ppm (female reproductive success)

Neurotoxicity	
Species/ target/critical effect	No data for neurotoxicity submitted. No effects observed in available studies.

Developmental Neurotoxicity	
Species/ target/critical effect	No effects observed in available studies.

Immunotoxicity	
Species/ target/critical effect	No effects observed in available studies.

Developmental Immunotoxicity	
Species/ target/critical effect	No effects observed in available studies.

Other toxicological studies

NOAEC (short-term exposure): 60 ppb
In a controlled human volunteer study by Adams a NOAEC of 40 ppb was derived based on changes in lung function (FVC, FEV1.0) and symptoms score at LOAEC of 80 ppb. Two 6.6 h exposure studies by Schelegle, E. S.and Kim, C. S derive a LOAEC of 70 ppb and 60 ppb, respectively. As supporting studies on airway inflammation reported upregulation of lung cytokines and immune cells from the level 80 ppb, a NOAEC 60 ppb from changes in lung function can be used for Risk Assessment for short-term exposure.

MEL (medium-term, long-term exposure): 25 ppb
For ozone there is no indication for the existence of NOAECs/NOAELs from the relevant epidemiological studies submitted for the critical effect mortality. In the absence of suitable information, the existence of a threshold for this effect cannot be assumed and a minimal effect level (MEL) of 25 ppb is proposed in analogy to the DMEL approach under REACH. The following effect levels may be associated with this MEL:

- According to the epidemiological mortality studies by Jerret, M., every 10-ppb increase in exposure to ozone was associated with an increase in the risk of death from respiratory causes of about 2.9 % in single-pollutant models and 4 % in two-pollutant models. This corresponds to approx. 10 % extra risk at 25 ppb ozone.
- When a threshold rather than a linear model was applied to the data by Jerret, M., a most likely threshold of 56 ppb for death from respiratory causes (p=6 %) was identified. However, the existence of such threshold is unclear.
- Airway Hyperresponsiveness was described in rodents by Depuydt, G.F., 1999 with a LOAEC of 50 ppb

Medical data	
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Summary

	Value	**Study**	**Safety factor**
AELlong-term	Not derived		

AELmedium-term	Not derived		
AELshort-term	Not derived		
ADI[2]	Not derived		
ARfD	Not derived		
NOAEC professional	60 ppb (120 µg/m^3)	Human volunteer, acute inhalation	1
MEL	25 ppb (50 µg/m^3)	Epidemiology, mortality	-

MRLs	
Relevant commodities	Drinking water ozone (0.05 mg/L), trihalomethanes (0.1 mg/L), bromate (0.01 mg/L) natural mineral water, spring water ozone (0.05 mg/L), bromoform (0.001 mg/L), bromate (0.003 mg/L)

Reference value for groundwater	
According to BPR Annex VI, point 68	

Dermal absorption	
Study (in vitro/vivo), species tested	No study available
Formulation (formulation type and including concentration(s) tested, vehicle)	No study available
Dermal absorption values used in risk assessment	Not derived (Default: 100%)

1.1.4. Chapter 4: Fate and Behaviour in the Environment

Route and rate of degradation in water	
Hydrolysis of active substance and relevant metabolites/ degradants (DT50) (state pH and temperature)	A true hydrolysis of ozone will not occur since "the cleavage of chemical bonds by the addition of water" is not feasible. Nonetheless, ozone will degrade in the presence of water, according to the level of dissolved organic matter. DT_{50} (mean) = 16 min; pH 8, 15°C DT_{50} in the sewer: 4 minutes
pH 5	-
pH 9	-
Other pH: [indicate the value]	-

[2] If residues in food or feed.

Photolytic / photo-oxidative degradation of active substance and resulting relevant metabolites/ degradants	Photolytic degradation of ozone in water will progress in the same manner as in the troposphere. Ozone is decomposed into O_2 and oxygen atoms O(1D) (excited state) and O(3P) (ground state). In the gas phase and below 300 nm, the main processes (quantum yield, $\Phi \approx 0.9$) lead to the formation of O(1D) and singlet oxygen, $O_2(1\Delta g)$, as well as oxygen in its ground state, $O_2(3\Sigma g-)$. Quantum yield direct ozone photolysis is $\Phi = 0.5$,
Readily biodegradable (yes/no)	Study technically not feasible as ozone contains no carbon atoms.
Inherent biodegradable (yes/no)	Study technically not feasible as ozone contains no carbon atoms.
Biodegradation in freshwater	Study technically not feasible as ozone contains no carbon atoms.
Biodegradation in seawater	Study technically not feasible as ozone contains no carbon atoms.
Non-extractable residues	N/A
Distribution in water / sediment systems (active substance)	N/A
Distribution in water / sediment systems (metabolites/ degradants)	N/A

Route and rate of degradation in soil	
Mineralization (aerobic)	N/A
Laboratory studies (range or median, with number of measurements, with regression coefficient)	N/A
DT50lab (20°C, aerobic):	-
DT90lab (20°C, aerobic):	-
DT50lab (10°C, aerobic):	-
DT50lab (20°C, anaerobic):	-
Degradation in the saturated zone:	N/A
Field studies (state location, range or median with number of measurements)	N/A
DT50f:	-
DT90f:	-
Anaerobic degradation	N/A
Soil photolysis	N/A
Non-extractable residues	N/A
Relevant metabolites - name and/or code, % of applied a.i. (range and maximum)	N/A
Soil accumulation and plateau concentration	N/A

Adsorption/desorption	
Ka , Kd	log K_{OC} = 0.57 (QSAR estimate based on log K_{OW}) for a.s..
Kaoc , Kdoc	
pH dependence (yes / no) (if yes type of dependence)	

Fate and behaviour in air	
Direct photolysis in air	Ozone: Half-life in air: Approximately 12 hours. Several processes contribute to the decomposition of ozone in air. The actual half-life depends on such parameters as air movement, temperature and humidity.
Quantum yield of direct photolysis	0.5
Photo-oxidative degradation in air	Latitude: - Season: - DT_{50}: -
Volatilization	

Reference value for groundwater	
According to BPR Annex VI, point 68	Not applicable

Monitoring data, if available	
Soil (indicate location and type of study)	Data not available
Surface water (indicate location and type of study)	Data not available
Groundwater (indicate location and type of study)	Data not available
Air (indicate location and type of study)	Data not available

1.1.5. Chapter 5: Effects on Non-target Species

Toxicity data for aquatic species (most sensitive species of each group)			
Species	**Time-scale**	**Endpoint**	**Toxicity**
Fish			
Oncorhynchus mykiss	96h	LC_{50}	9.3 µg/L
Oncorhynchus mykiss	3 months	NOEC	2.3 µg/L
Invertebrates			
Daphnia magna	48 h 24 h	NOEC EC_{100}	11 µg/L 21 µg/L
Litopenaeus vannamei	21 d	NOEC	60 µg/L OPO (Ozone-produced oxidants measured as chlorine equivalent)
Algae			
Nannochloropsis oculate	3 d	NOEC	50 µg/L TRO (total residual oxidants)
Microorganisms			
No experimental data submitted			

Effects on earthworms or other soil non-target organisms	
Acute toxicity to ……..	No experimental data submitted

Reproductive toxicity to ……	No experimental data submitted

Effects on soil micro-organisms	
Nitrogen mineralization	No experimental data submitted
Carbon mineralization	No experimental data submitted

Effects on terrestrial vertebrates	
Acute toxicity to mammals	No experimental data submitted
Acute toxicity to birds	No experimental data submitted
Dietary toxicity to birds	No experimental data submitted
Reproductive toxicity to birds	No experimental data submitted

Effects on honeybees	
Acute oral toxicity	No experimental data submitted
Acute contact toxicity	No experimental data submitted

Effects on other beneficial arthropods	
Acute oral toxicity	No experimental data submitted
Acute contact toxicity	No experimental data submitted
Acute toxicity to ……..	No experimental data submitted

Bioconcentration	
Bioconcentration factor (BCF)	No experimental data submitted
Depuration time (DT50)	No experimental data submitted
Depuration time (DT90)	No experimental data submitted
Level of metabolites (%) in organisms accounting for > 10 % of residues	No experimental data submitted

1.1.6. Chapter 6: Other End Points

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