SCIENTIFIC OPINION

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Safety of gaseous chlorine dioxide as a preservative slowly released in cold food storage areas

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS)

Abstract

Following a request from the European Commission, the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) provides a scientific opinion regarding the safety of use of gaseous chlorine dioxide as a preservative that is slowly released in cold food storage areas. Gaseous chlorine dioxide is generated from tubes that contain two reactants, which upon mixing slowly release gaseous chlorine dioxide. The tubes are to be used in cold storage rooms or in refrigerators for domestic use. In consideration of the reactive nature of gaseous chlorine dioxide, the Panel assessed to what extent chlorinated species are generated by the interaction of gaseous chlorine dioxide, under the proposed conditions of use, with some model food matrices. According to the applicant, the only by-products resulting from the proposed use of gaseous chlorine dioxide are chlorite and chlorate; therefore, the safety of these by-products has also been assessed. From the data provided by the applicant, under domestic refrigerator-like experimental conditions, the treatment of model food matrices with gaseous chlorine dioxide led to low amounts of by-products and did not produce chlorinated organic by-products. Chlorate was not detected in the model food matrices tested. Consequently, considering the negligible amounts in model food matrices of the parent compound and of its by-products chlorite and chlorate, together with the available toxicity database, the Panel concluded that, under the domestic refrigerator-like conditions of use proposed by the applicant, the consumption of foods treated with gaseous chlorine dioxide would not be a safety concern. The risk resulting from exposure of the consumer to chlorine dioxide by inhalation was not assessed, because this is outside the remit of the Panel. The Panel considered that a new assessment would be warranted in case of any change in the conditions of use.

Keywords: gaseous chlorine dioxide, ClO_2, preservative, food, storage, safety

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Summary

Following a request from the European Commission, the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) provides a scientific opinion regarding the safety of the use of gaseous chlorine dioxide (ClO_2) as preservative that is slowly released in cold food storage areas.

The chlorine dioxide gas is generated *in situ* from tubes that contain two reactants, which upon activation slowly release gaseous ClO_2. The tubes are to be used in cold storage rooms or in refrigerators for domestic use. The Panel noted that the mode of generation of ClO_2 proposed by the applicant is different from the ones already used and accepted so far.

The Panel is aware that the use of the material proposed by the applicant may lead to inhalation of ClO_2, but the possible risk resulting from exposure of the consumer by inhalation was not assessed because this is outside the remit of the Panel.

The evaluation of the preservative effect of gaseous ClO_2 is outside the remit of the Panel.

The applicant has submitted a dossier in support of its application for the authorisation of gaseous ClO_2 for use as a preservative in cold food storage areas. In consideration of the well-known reactive nature of gaseous ClO_2, the Panel agreed to assess, as a first step, to what extent chlorinated species are generated by the interaction of gaseous ClO_2, under the proposed conditions of use, with some model food matrices.

To this end, and upon request from the Panel, the applicant generated additional data showing that, under the measured in-use conditions in a refrigerator, only a small amount of the total releasable ClO_2 from the tube was detected. The maximum detected concentration of ClO_2 was 0.043 ppm, which corresponds to 15 µg/120 L. The mean concentration was 0.029 ppm, which corresponds to 10.2 µg/120 L. The applicant also provided experimental data showing that the release of one of the two reactants during use was very low. The data covering a period of 28 days showed that only < 0.005% of the total amount of the gaseous reactant will be released during the intended application duration.

From the data provided by the applicant, under domestic refrigerator-like experimental conditions, the treatment of model food matrices with gaseous ClO_2 led to low amounts of by-products, did not produce chlorinated organic by-products, and chlorate was not detected in the model food matrices. The Panel considered that the data indicated the absence of formation of potentially adverse chlorinated compounds in the model food matrices used by the applicant. However, the Panel noted the limitations of the model food matrices; in addition owing to the type of cold area used (a domestic refrigerator) in which a small size tube was used to generate the gaseous chlorine ClO_2, the consideration of absence of formation of potentially adverse compounds is restricted to these conditions and do not apply to larger areas where bigger tubes can be used.

According to the applicant, the only by-products resulting from the proposed use of gaseous ClO_2 are chlorite and chlorate; therefore, the safety of these by-products has been assessed. The toxicity database for chlorite and chlorate is limited, but did not indicate concern about a genotoxic or carcinogenic potential.

The ANS Panel did not perform an estimate of exposure, because no potentially adverse substances were identified in the initial step of the assessment.

The Panel concluded that, under the conditions of use proposed by the applicant, the consumption of foods treated with use of gaseous ClO_2 under the proposed domestic refrigerator-like conditions of use would not be of safety concern.

A new assessment would be warranted in case of any change in the conditions of use.

The Panel recommended that the risk of inhalation of gaseous chlorine should be considered, particularly in the case of large cold storage areas. In this respect, the Panel draws attention to the risk of misuse e.g. large tubes for the production of ClO_2 being used in domestic refrigerators.

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1. Introduction

The present scientific opinion deals with the evaluation of the safety of gaseous chlorine dioxide (ClO_2) when used as a preservative slowly released in cold food storage areas.

1.1. Background and Terms of Reference as provided by the European Commission

1.1.1. Background as provided by the European Commission[1]

The use of food additives is regulated under the European Parliament and Council Regulation (EC) No 1333/2008 on food additives.[2] Only food additives that are included in the European Union (EU) list, in particular in Annex II to that regulation, may be placed on the market and used in foods under the conditions of use specified therein.

An application has been introduced for the authorisation of the use of gaseous ClO_2 as a preservative in food storage areas, e.g. refrigerators and cooling rooms. The gaseous ClO_2 is generated with the help of sticks that contain [redacted], which upon activation slowly release gaseous ClO_2. The sticks are to be used in cold food storage rooms or in refrigerators for domestic use.

The released gaseous ClO_2 protects foodstuffs during storage against deterioration caused by microorganisms and against the growth of pathogenic microorganisms.

1.1.2. Terms of Reference as provided by the European Commission

The European Commission asks the European Food Safety Authority (EFSA) to give an opinion, in accordance with Regulation (EC) No 1331/2008[3] establishing a common authorisation procedure for food additives, food enzymes and food flavourings, about the safety of the use of gaseous ClO_2 as a preservative that is slowly released in cold storage areas.

1.1.3. Interpretation of Terms of Reference

The EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) is aware that the use of the material proposed by the applicant may lead to inhalation of ClO_2 when opening or entering the cold food storage area, but the possible risk resulting from exposure of the consumer by inhalation was not assessed because this is outside the remit of the Panel.

The evaluation of the preservative effect of gaseous ClO_2 is outside the remit of the Panel.

1.2. Information on existing evaluations and authorisations

The applicant has provided information on existing authorised uses and evaluations of ClO_2. Most of the available information on ClO_2 (gas) has been reviewed by the World Health Organization (WHO) through its International Programme on Chemical Safety (IPCS) (WHO/IPCS, 2002).

In the EU, ClO_2 was previously evaluated by the former EFSA Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) in 2005, with respect to the toxicological risks to public health from possible reaction products (e.g. semicarbazide) deriving from its application on poultry carcasses (EFSA, 2006).

A tolerable daily intake (TDI) of 0.03 mg/kg bw (expressed as chlorite) was established by the International Programme on Chemical Safety (WHO/IPCS, 2000) and the same numerical value was set by the United States Environmental Protection Agency (EPA) as a reference dose (RfD) (EPA,

[1] This section of the opinion has been edited to temporarily remove information claimed to be confidential by the applicant, pending a decision thereon by the European Commission pursuant to Article 12(3) of Regulation (EC) 1331/2008. This does not affect the circulation of the information claimed confidential between the Commission, the Authority and the Member States.

[2] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16–33.

[3] Regulation (EC) No 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 354, 31.12.2008, p. 1–6.

2000). WHO guidelines for drinking water quality set a value of 5 ppm (= 5 mg/L) for chlorine based on a TDI of 0.150 mg/kg bw, allocating 100% of this TDI to water and assuming that a 60 kg bw individual consumes 2 L of water per day (WHO, 1996).

In 1963, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) considered 0–30 mg ClO_2/kg flour to be an acceptable treatment level for the use of ClO_2 for flour (JECFA, online).

In Germany, the use of ClO_2 as a drinking water disinfectant is based on §11 of the German 'Trinkwasserverordnung'.[4,5] According to this legislation a maximum concentration of 0.4 mg/L of ClO_2 can be used for disinfection purposes and the residual ClO_2 concentration at the end of the disinfection process should not exceed 0.2 mg/L. The legislation also foresees that the most prominent reaction product, the ion chlorite (ClO_2^-), should not exceed the maximum limit of 0.2 mg/L.

In the USA, current drinking water regulations permit a maximum residual disinfectant level of 0.8 mg ClO_2/L (= 0.8 ppm) (EPA, online).

The ANS Panel noted that, for use as an antimicrobial agent, ClO_2 is added to water used in poultry processing; as a spray or dip for red meat, red meat parts and organs and ready-to-eat meats in an amount not exceeding a residual concentration of 3 ppm.

In the USA a maximum of 200 ppm ClO_2 is also permitted for sanitising processing equipment used in fruit and vegetable processingas an antimicrobial agent in water used to wash fruits and vegetables that are not raw agricultural commodities in an amount that does not exceed 3 ppm.[6]

ClO_2 is authorised in the USA for use in washing whole fresh fruits and vegetables and shelled beans and peas with intact cuticles at a concentration not exceeding 5 ppm (5 mg/kg). For peeled potatoes, the maximum permitted wash concentration is 1 ppm (1 mg/kg) (WHO/FAO, 1998).

In 1967, under the authority of the Federal Insecticide, Fungicide and Rodenticide Act, the US Environmental Protection Agency (EPA) first registered the liquid form of ClO_2 for use as a disinfectant and sanitiser in a variety of sites, such as animal farms, bottling plants, and food-processing, handling and storage plants.

In 2006, the US EPA published a re-registration eligibility decision for ClO_2 as a pesticide (EPA, 2006).

In 1988, the US EPA registered ClO_2 gas as a sterilant for use on manufacturing and laboratory equipment, environmental surfaces, tools and clean rooms. Under oxidant-demand conditions, e.g. contact with naturally occurring organic and inorganic matter, ClO_2 rapidly degrades. The most prominent by-products are chlorite (ClO_2^-) and chlorate (ClO_3^-). Regarding existing information on evaluations of associated by-products of ClO_2 use, acceptable daily intake (ADI) values for chlorite and chlorate are 0.03 mg/kg bw per day and 0.01 mg/kg bw per day, respectively (JECFA, 2007, 2008).

The EFSA CONTAM Panel established a TDI for chlorate of 3 µg/kg bw per day, based on the TDI established for perchlorate (EFSA CONTAM Panel, 2015).

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of its application for the authorisation of gaseous ClO_2 for use as a preservative that is slowly released in cold food storage areas. Additional data were generated by the applicant upon request from the ANS Panel. The request for the additional data was

[4] Trinkwasserverordnung in der Fassung der Bekanntmachung vom 2. August 2013 (BGBl. I S. 2977), die durch Artikel 1 der Verordnung vom 18. November 2015 (BGBl. I S. 2076) geändert worden ist

[5] Bekanntmachung der Liste der Aufbereitungsstoffe und Desinfektionsverfahren gemäß § 11 der Trinkwasserverordnung, 17. Änderung vom 13. November 2012

[6] Code of Federal Regulations. Title 21, Part 173.300. Secondary Direct Food Additives Permitted in Food for Human Consumption: Chlorine dioxide. 4-1-12 Edition. Available at: https://www.gpo.gov/fdsys/pkg/CFR-2012-title21-vol3/pdf/CFR-2012-title21-vol3-sec173-300.pdf

discussed at a technical hearing held during a meeting of the Standing Working Group on Applications on 20 January 2015.[7]

2.2. Methodologies

The assessment was conducted in line with the principles described in the EFSA Guidance on transparency in the scientific aspects of risk assessment (EFSA Scientific Committee, 2009) and following the relevant existing Guidances from the EFSA Scientific Committee.

The approach described in the current 'Guidance for submission for food additive evaluations' (EFSA ANS Panel, 2012) could not be fully adhered to by the Panel for this evaluation of the application for the authorisation of gaseous ClO_2 for use as a preservative that is slowly released in cold food storage areas.

In consideration of the well-known reactive nature of gaseous ClO_2, the Panel agreed to assess, as a first step, to what extent chlorinated species are generated by the interaction of gaseous ClO_2, under the proposed conditions of use, with some model food matrices. According to the applicant, the only by-products possibly resulting from the proposed use of gaseous ClO_2 are chlorite and chlorate; therefore, the safety of these by-products has also been assessed.

3. Assessment

3.1. Technical data

3.1.1. Identity of the substance[8]

Because ClO_2 is produced *in situ* in a stick [redacted], the identity is described for both reactants and for the ClO_2 produced *in situ*.

ClO_2 (Chemical Abstracts Service (CAS) No. 10049-04-4), exists as a greenish-yellow to orange gas at room temperature, with a characteristic pungent chlorine-like odour (WHO/IPCS, 2002).

ClO_2 gas is strongly oxidising; it is explosive at concentrations in excess of 10% v/v at atmospheric pressure, and is easily detonated by sunlight or heat. The specific gravity is 1.642 (Budavari et al., 1996 as reported in WHO/IPCS, 2002).

3.1.2. Specifications[9]

The physical and chemical properties of ClO_2 and the reactants that are mixed to generate it were retrieved by the applicant from published literature and other evaluations (Table 1).

[7] http://www.efsa.europa.eu/sites/default/files/applicationswg.pdf

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Table 1: Physical and chemical properties of chlorine dioxide (ClO_2), [redacted] [according to US EPA (2006), WHO/IPCS (2002) and Budavari et al. (1989)]

	ClO_2 gas	[redacted]	[redacted]
CAS registry number	10049-04-4		
Molecular formula	ClO_2		
Molecular weight (g/mol)	67.46		
Melting point (°C)	− 59		
Boiling point (°C)	11		
Water solubility (g/L)	3.0 at 25°C and 34 mmHg		
Conversion factor in air	1 ppm = 2.8 mg/m^3 at 20°C		
Vapour pressure (kPa at 20°C)	101		
Relative vapour density[(a)]	2.3		

3.1.3. Manufacturing process[10]

[redacted]

The Panel noted that the mode of generation of ClO_2 proposed by the applicant is different from the ones used and accepted so far, e.g. by the United States Environment Protection Agency (EPA).

In general, ClO_2 can be produced in several ways: (a) mixing a solution of chlorine with a solution of $NaClO_2$; (b) acidification of chlorates with hydrochloric or sulphuric acid; (c) reduction of chlorates in

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acid medium; (d) reacting acids with chlorites; and (e) electrolysis, using sodium chloride, $NaClO_2$ and water (Dychdala, 2001).

Since ClO_2 is unstable as a gas and becomes explosive at concentrations of > 10% v/v in air, depending on the temperature and pressure (National Safety Council Data Sheet 525 – ClO_2, 1967 as reported in EPA, 1999), the generation process has to be carried out on-site at the point of use.

3.1.4. Methods of analysis in food[11]

Different methods were used by the applicant to measure ClO_2 release from the tubes and for the determination of ClO_2, ClO_2^- and [redacted] in a cold food storage area under realistic conditions of use.

Ultraviolet (UV) spectroscopy was used for the determination of ClO_2, with a resolution of 1.0 nm in the scanning area of 190–700 nm. The signals at the absorption maximum of ClO_2 (360 nm) and of ClO_2^- (260 nm) were registered.

Infrared (IR) spectroscopy was used for detection of the possible gaseous reactant released and ClO_2 in activated and non-activated tubes. A Fourier transform single-beam device equipped with a Teflon cuvette (length, 23.8 cm) with sodium chloride discs for gas-phase measurements was used. The spectra were measured in a range of 4100–500 cm^{-1}. Nitrogen was used as the reference gas. Ion chromatography was also used to detect chloride ions. A Dionex Model DX500 ion chormatograph equipped with a CD20 conductivity detector GP40 gradient pump and a Dionex Ionpac AS4A anion exchange column (250 × 4 mm) with a guard column was used. The aqueous mobile phase contained 0.5 mM $NaHCO_3$ and 0.75 mM Na_2CO_3. The suppressor-regenerant used was 20 mM H_2SO_4.

For the study of in-use conditions of the tube, an electrochemical sensor for the detection of the ClO_2 released was used, consisting of electrochemical cells with an electrolyte, and different electrodes. The electrochemical reaction generates an electrical signal, which is proportional to the ClO_2 concentration. According to the data provided, the pocket size detector had a detection range between 0 and 2 ppm, with a tolerance of 0.03 ppm.

Upon request from the Panel the applicant has submitted data on the interaction between gaseous ClO_2 and different model food matrices (Documentation provided to EFSA n.2).

The different surrogate matrices (tryptophan and octenoic acid) and their oxidation products after the treatment with Cl_2 or ClO_2 were analysed with the high-performance liquid chromatography (HPLC)–UV/mass spectrometry (UV/MS) technique. A Hewlett Packard 1100 HPLC with a Bruker-DAD detector (200–400 nm) and a Bruker Esquire ESI-MS ion-trap system were used. An HPLC column (Agilent Eclipse, 5µm, 50 × 4.6 mm) was used, and acetonitrile and formic acid in water were used as eluents at a flow rate of 0.6 mL/min.

3.1.5. Stability of the substance, and reaction and fate in food

Most of the published data concern the occurrence of disinfection by-products in water, emerging after ClO_2 use. The chemistry of ClO_2 in water and disinfection by-products, has been described in detail by Rice and Cotruvo (1978).

Only a few publications have concentrated on reaction products of ClO_2 in food. The Joint FAO/WHO Expert Meeting 'Benefits and risks of the use of chlorine-containing disinfectants in food production and food processing' has published a summary report of the available publications (FAO/WHO, 2009). Since most of the studies used aqueous ClO_2 for the determination of by-products, which, in addition, were not quantified in most cases, the results can only be roughly applied to this application.

After spray treatment of carcasses with water containing ClO_2, the only by-products are chlorite and chlorate (EFSA, 2006). Approximately 5 % of the starting ClO_2 concentration remains as ClO_2 after treatment. Chlorite and chlorate were detected at concentrations of 66% and 28%, respectively. ClO_2 may interact with organic matter in solution, or protein and fat compounds of poultry carcasses. Under that condition, no semicarbazide formation was reported and the extent of incorporation of

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chlorine into lipids was very low, the major reaction of chlorine being oxidation rather than chlorination (EFSA, 2006).

The reactions of ClO_2 with carbohydrates generally result in oxidation products (Fukuyama et al. 1986). A residual 1% was assumed by the applicant to be a mixture of different undetermined by-products with unknown toxicity profiles.

Upon request from the Panel, the applicant has submitted data on the interaction between gaseous ClO_2 and different model food surrogate matrices (Documentation provided to EFSA n. 3). The surrogate matrices analysed were:

a) water (unbuffered) to simulate condensed water within the refrigerator;

b) water (buffered system; pH 6), as most food has a high content of water and a pH between 5 and 7;

c) L-tryptophan as a naturally occurring amino acid with an aromatic ring, a double bond and two amino functions in the molecule;

d) 3-octenoic acid, an unsaturated fatty acid mimicking food rich in fat.

Purging of these surrogate matrices with ClO_2 (1.3 µM in 10 min) was conducted under domestic refrigerator-like conditions. Gaseous chlorine (1.3 µM in 10 min, corresponding to 2.6 µM Cl) was used as a positive control in order to demonstrate the analytical capacity to detect chlorinated by-products in the tested surrogate matrices.

The simulation involving tryptophan showed that 98 % of the ClO_2 was absorbed by the matrix, but none was dissolved without reaction. After exposure to ClO_2, 64 % of the starting amount of tryptophan could be detected. Oxidation products of tryptophan could be identified (mostly di-oxidized forms) but no chlorinated tryptophan derivatives were detected. Further analysis of the matrix solution for inorganic chlorinated species showed that the majority (68 M% of the 100 M% nominal present chlorine) of ClO_2 was converted to chloride (37 M%) and chlorite (31 M%). Chlorate was not detected.

The simulation involving 3-octenoic acid showed that most of the ClO_2 gas absorbed by the matrix (97%) was dissolved in the aqueous solution without reaction with 3-octenoic acid (approx. 70%). Oxidation products of 3-octenoic acid were detected in low amounts (maximum 4%), mostly as mono-oxidized forms. Chlorite was the major inorganic chlorine-containing species, with 40 M%, whereas chloride accounted only for 4.1 M%. Chlorate was not detected.

The respective positive controls, which involved exposure of the food surrogate matrices to chlorine gas, showed the formation of chlorinated hydrocarbons, for both L-tryptophan and 3-octenoic acid, The ratios between chlorinated by-products and the total by-products were 2.5 M% for L-tryptophan and 82 M% for 3-octenoic acid. In both simulations, chloride was the main inorganic species formed as a result of purging the surrogate matrices with chlorine gas.

On request by the ANS Panel, the applicant also conducted a literature search on the possible residual presence of ClO_2 in foods, and no new relevant data contradicting the current experimental data showing an almost total absence of such residual presence were identified (Documentation provided to EFSA n.3).

3.1.6. Technological function

The applicant proposed the use of ClO_2 as a preservative; the maximal possible amount of ClO_2 in the tube is 0.0242 g, and the maximum possible daily release (involving a 30-day period) is 0.80 mg.

According to the applicant, the released gaseous ClO_2 protects foodstuffs during storage against deterioration caused by microorganisms, and against the growth of pathogenic microorganisms. The microbicidal efficacy was determined by treating a refrigerator containing agar plates with *Pseudomonas aeruginosa* and *Salmonella* species. The Panel noted that there was no information on whether gaseous ClO_2, under the conditions of use proposed by the applicant, had any antimicrobial effect in foods.

3.2. Exposure data

3.2.1. Proposed uses and use levels[12]

As proposed by the applicant, gaseous ClO_2 could be generated in tubes of different size and volume, depending on the volume of the cold area that is intended to be treated (Table 2).

Table 2: Proposed tube size and volume for the intended volume to be treated

Size	Volume of the tube (mL)	Intended volume/area for treatment
Small	8	140–200 L
Medium	50	5 m^3
Large	80	10 m^3

ClO_2 is generated *in situ* by mixing two reactants in defined volumes and concentrations. The maximal possible content of ClO_2 in the tube does not exceed 0.3% of the total fluid in the tube.

Following a request from the Panel, the applicant provided data showing that, under the measured in-use conditions in a refrigerator (120 L of volume), only a small amount of the total releasable ClO_2 from the tube was detected in the refrigerator. The maximum detected concentration of ClO_2 was 0.043 ppm, which corresponds to 15 µg/120 L. The mean concentration is 0.029 ppm, which corresponds to 10.2 µg/120 L. The mean release at 7°C over the 30-day period of the study was 327 µg/day corresponding to 56% of the total possible release (Documentation provided to EFSA n. 3).

[redacted]

3.3. Exposure estimate

An estimate of exposure was not performed by the ANS Panel, because no potentially adverse substances were identified in the initial step of the assessment.

The Panel is aware of the use of chlorine dioxide as a disinfectant for foods (e.g.poultry), and this use could be an additional source of exposure via food to this substance and its reaction products for the consumers.

3.4. Biological and toxicological data

The applicant carried out a literature search on the biological and toxicological data relevant to gaseous ClO_2. The keywords 'Chlorine dioxide' + 'mode of action' were searched for on the literature databases PubMed and Scopus. Since the post-submission mainly focused on current findings concerning residues or by products of ClO_2 in treated foods and the mode of action of ClO_2, the search results including efficacy data and alternative fields of application were excluded. The ANS Panel considered this literature search to be adequate.

Most of the available biological and toxicological data for ClO_2 (gas) are available in the review by the WHO/IPCS (WHO/IPCS, 2002). In addition to ClO_2, the applicant provided some data for the by-products chlorites and chlorates. The risk for human health resulting from the presence of chlorate in food was also recently assessed by the CONTAM Panel of EFSA (EFSA CONTAM Panel, 2015).

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3.4.1. Absorption, distribution, metabolism and excretion

Absorption

The rapid appearance of ^{36}Cl in plasma following oral administration of ClO_2 ($^{36}ClO_2$; 1.5 M/kg bw) or chlorite ($^{36}ClO_2^-$; 0.15 mg/kg bw) has been shown in laboratory animals, with peak levels 1–2 h after dosing. By the use of 72-h urinary excretion rates for ^{36}Cl, absorption rates of 30–35% of intragastrically administered ClO_2 or chlorite were estimated. The absorption rate constant and half-time were 3.77/h and 0.18 h, respectively (Abdel-Rahman et al., 1982).

Distribution

In rats, orally absorbed ^{36}Cl (from $^{36}ClO_2$ or $^{36}ClO_2^-$ exposure sources) is slowly cleared from the blood and is widely distributed throughout the body. In rats, 72 h following dosing, the highest concentrations were found in the blood, stomach and small intestine. Relatively high concentrations were also seen in the lung, kidney, liver, testes, spleen, thymus and bone narrow (Abdel-Rahman et al., 1982).

Metabolism

ClO_2 rapidly dissociates, predominantly into chlorite (which itself is highly reactive) and Cl^-, which is ultimately the major metabolite of both ClO_2 and chlorite in biological systems (Abdel-Rahman et al., 1982).

In rats, 72 h following single oral (gavage) administration of radiolabelled ClO_2, chlorite accounted for approximately 21% of the radioactivity in plasma samples. Chlorate was not found in plasma. Cl^- accounted for approximately 80% of the radioactivity in a plasma sample (Abdel-Rahman et al., 1979a).

Excretion

Urine is the primary route of excretion of orally administered radioactivity from radiolabelled ClO_2 or chlorite. Urine is the primary route of ^{36}Cl elimination, predominantly in the form of Cl^-. In rats, 72 h following single oral (gavage) administration of $^{36}ClO_2$, 31% and 4.5% of the radiolabel had been excreted in the urine and faeces, respectively, mainly in the form of Cl^-. The ratio of $^{36}Cl^-$ to $^{36}ClO_2^-$ was 4 to 1, and no parent compound was detected. Chlorate was found in only minor amounts in the urine (Abdel-Rahman et al., 1979a, 1979b).

3.4.2. Acute toxicity

Chlorite

No data were provided.

Chlorate

No data were provided.

3.4.3. Short-term and subchronic toxicity

Chlorite

No data were provided.

Chlorate

No data were provided.

3.4.4. Genotoxicity

Chlorite

$NaClO_2$ has given positive results in some, but not all, *in vitro* genotoxicity assays and in one of the two available *in vivo* mouse micronucleus assays involving intra-peritoneal administration. Negative results were obtained in several *in vivo* assays involving oral administration of $NaClO_2$ to mice (JECFA, 2008).

Chlorate

Some positive results have been obtained in bacterial mutation assays *in vitro* with chlorate, but no positive results have been obtained in *in vivo* genotoxicity assays.

3.4.5. Chronic toxicity and carcinogenicity

Chlorite

$NaClO_2$ was not carcinogenic in a number of long-term studies, however, the Panel noted that these were not conducted according to current standards (JECFA, 2008).

Chlorate

Groups of 50 male and 50 female F344/N rats were exposed to sodium chlorate in the drinking water for 2 years at doses equivalent to approximately 5, 35 and 75 mg/kg bw per day for males and 5, 45 and 95 mg/kg bw per day for females. There were positive trends in the incidence of thyroid gland follicular cell carcinoma in male rats and thyroid gland follicular cell adenoma and carcinoma (combined) in male and female rats. The incidence of thyroid gland follicular cell hypertrophy was significantly increased in all exposed male groups and in the mid-dose and high-dose groups of females. Thyroid gland focal follicle mineralisation occurred in most females in the mid-dose and high-dose groups. The incidence rates of haematopoietic cell proliferation in the spleens of high-dose males and bone marrow hyperplasia in the mid-dose and high-dose male groups were significantly greater than in controls. On the basis of the negative *in vivo* genotoxicity data and the nature of the histopathological observations, JECFA concluded that a non-genotoxic mode of action was likely for the induction of thyroid tumours by sodium chlorate. This mode of action is likely to be mediated via decreased levels of serum thyroid hormones, leading to increased release of thyroid-stimulating hormone (TSH) and the consequent stimulation of thyroid cell proliferation and thyroid gland growth, which can lead to thyroid tumours in rodents (JECFA, 2008).

Because a no observed adverse effect level (NOAEL) was not identified in this study, JECFA (2008) applied a benchmark dose (BMD) approach to derive a point of departure on the dose–response curve. Rats are considered to be highly sensitive to the effects of agents that disrupt thyroid hormone homeostasis. JECFA considered that humans are likely to be less sensitive than rats to these effects, and that a safety factor for interspecies variation was not required. The rat thyroid gland follicular cell hypertrophy data were modelled by JECFA in order to derive the BMD for a 10% increase in follicular cell hypertrophy (BMD_{10}) and the corresponding 95% lower confidence limit ($BMDL_{10}$). The $BMDL_{10}$ values for chlorate ranged from 1.1 to 4.4 mg/kg bw per day, with the lowest value representing the best fit.

3.4.6. Reproductive and developmental toxicity

Chlorite

In a two-generation reproductive study (Gill et al., 2000), Sprague-Dawley rats (30 per sex per dose) received drinking water containing $NaClO_2$ at 0, 35, 70 or 300 mg/L for 10 weeks, and were then paired for mating. Males were exposed through mating, and then killed. Exposure of the females continued through mating, pregnancy and lactation and until necropsy following weaning of their litters. Dosing continued through two generations, with chlorite doses for the F_0 animals of 0, 3.0, 5.6 or 20.0 mg/kg bw per day for males and 0, 3.8, 7.5 or 28.6 mg/kg bw per day for females. For the F_1

animals, chlorite doses were 0, 2.9, 5.9 or 22.7 mg/kg bw per day for males and 0, 3.8, 7.9 or 28.6 mg/kg bw per day for females.

There were reductions in water consumption, food consumption and body weight gain in both sexes in all generations at various times throughout the experiment, primarily in the 70-mg/L and 300-mg/L groups; these were attributed to a lack of palatability of the water. At 300 mg/L, reduced pup survival and reduced bw at birth and throughout lactation in the F_1 and F_2 generations, lower thymus and spleen weights in both generations, a lowered incidence of pups showing a normal righting reflex, delays in sexual development in males and females in the F_1 and F_2 generations and lower red blood cell parameters in the F_1 generation were noted.

Chlorate

No data were provided.

3.5. Discussion[13]

In general, ClO_2 reacts as an electron acceptor, and hydrogen atoms present in activated organic C–H or N–H structures are thereby not replaced by chlorine (Hoigné and Bader, 1994). Thus, ClO_2 acts primarily as an oxidant rather than as a chlorinating agent. Furthermore, ClO_2 is likely to be less reactive and produces fewer by-products than chlorine in the reaction during food processing. Because of the higher selectivity, it engages in only a few side reactions. In that respect, the Panel noted that after spray-treatment of carcasses with water containing ClO_2, the only identified by-products were chlorite and chlorate (EFSA, 2006).

According to the applicant, if gaseous ClO_2 is pure, under the proposed conditions of use, it does not chlorinate organic material and therefore does not form trihalomethanes and other chlorinated disinfection by-products. Typically, ClO_2 will react with compounds that have activated carbon bonds, such as phenols, or with other active compounds, such as sulphides, cyanides, and reduced iron and manganese compounds (Fukayama et al., 1986; SCVPH, 2003).

In response to a request by EFSA, the formation of chlorinated substances and oxidation by-products in food surrogate matrices in the presence of ClO_2 gas was studied. The Panel considered that the model matrices used had some limitations. 3-octenoic acid is a C8 fatty acid, whereas most fatty acids in food are C18 or higher, it is therefore not a common food constituent. Instead of tryptophan, other, more realistic, surrogate matrices such as whey protein solutions could have been used. In the data provided by the applicant for the assay with tryptophan, only oxygen-containing tryptophan derivatives were detected. However, it is mentioned that not all ClO_2-derived tryptophan derivatives could be captured by the applied analytical methods and that some of the chlorine is not accounted for in this analysis.

While recognising some limitations of these studies, the Panel considered that their results indicated the absence of formation of adverse chlorinated compounds in the model food matrices used by the applicant.

As regards detection of the ClO_2 released during use, the Panel considered that the experimental approach proposed by the applicant was adequate, as were the methods used for ClO_2, chlorite and [redacted] determination. The maximum rate of gas release was five times higher at 25°C than at 6°C, and was reached in less time (1 vs 5.2 days). The mean release of ClO_2 at 7°C was 327 µg/day. According to the data provided, the sensor used had a detection range between 0 and 2 ppm, with a tolerance of 0.03 ppm, which is adequate for the concentrations to be measured during the assay, as the door of the refrigerator should be kept closed.

[redacted]

The toxicity database for chlorite and chlorate is limited, but did not indicate any concern about a genotoxic or carcinogenic potential (JECFA, 2008).

[13] This section of the opinion has been edited to temporarily remove information claimed to be confidential by the applicant, pending a decision thereon by the European Commission pursuant to Article 12(3) of Regulation (EC) 1331/2008. This does not affect the circulation of the information claimed confidential between the Commission, the Authority and the Member States.

Overall, the Panel considered that, under the domestic refrigerator-like experimental conditions, the treatment of model food matrices with gaseous ClO_2 as proposed by the applicant:

(a) led to low amounts of by-products in these model food matrices;

(b) did not produce chlorinated organic by-products in these model food matrices.

(c) chlorate was not detected. (7°C in the dark).

4. Conclusions

The Panel concluded that, under the conditions of use proposed by the applicant, the consumption of foods treated with gaseous ClO_2 under the proposed domestic refrigerator-like conditions of use would not be of safety concern.

A new assessment would be warranted in case of any change in the conditions of use.

5. Recommendation

The Panel recommended that the risk of inhalation of gaseous ClO_2 should be considered, particularly in the case of large cold storage areas. In this respect, the Panel draws attention to the risk of misuse e.g. large tubes for the production of ClO_2 being used in domestic refrigerators.

Documentation provided to EFSA

1. Evaluation document for Knick'n'clean® sticks. Technical dossier. September 2013 (revised October 2013). Submitted by Fraunhofer ITEM on behalf of Knick´n´clean®.

2. Oral clarification provided during a technical hearing held during the 3rd meeting of the Standing Working Group on Applications.

3. Additional information submitted by Fraunhofer ITEM on behalf of Knick'n'clean® in response to a request from EFSA. July 2015.

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Abbreviations

ADI	acceptable daily intake
AFC	EFSA Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food
ANS	EFSA Panel on Food Additives and Nutrient Sources Added to Food
BMD/BMD_{10}	benchmark dose/benchmark dose for a 10% increase in an effect
CONTAM	EFSA Panel on Contaminants in the Food Chain
EPA	United States Environmental Protection Agency
FAO	Food and Agriculture Organization of the United Nations
HPLC	High-performance liquid chromatography
IPCS	International Programme on Chemical Safety
IR	infrared
JECFA	Joint FAO/WHO Expert Committee on Food Additives
MS	mass spectrometry
NOAEL	no observed adverse effect level
RfD	reference dose
SCVPH	EU Scientific Committee on Veterinary Measures relating to Public Health
TDI	tolerable daily intake
TSH	thyroid-stimulating hormone
UV	ultraviolet
WHO	World Health Organization