Application for authorisation of food additive in accordance with Regulation (EC) No 1331/2008

Gaseous chlorine dioxide from Knick´n´clean® sticks in the food storage area

Post submission of data regarding residues in/on food after chlorine dioxide (gas) treatment and mode of action (MoA) of chlorine dioxide (gas)

31.07.2015

Introduction

Aim of this statement is to summarize the open discussion points from the scientific hearing on 20.01.2015 and the follow up discussions.

The following issues will be addressed:

1) Summary of the analytical measurements performed using Knick'n'clean® sticks and chlorine dioxide gas focussing on release rates
   ("KnicknClean_Release rates_2015_final")
   - Release rates of chlorine dioxide gas from the stick at different temperatures
   - Release of HCl from the stick
   - Measurement of ClO_2 gas from Knick'n'clean® sticks in a refrigerator as field measurement
2) Summary of the analytical measurements of residues and by-products in model matrices using ClO_2 gas and Cl_2 gas
   ("KnicknClean_Residues in model matrices_2015_final")
   - Description of the used model matrices
   - Explanation for the choice of test matrices and use scenarios
   - Summary of the results of the comparison of ClO_2 gas and Cl_2 gas treatment
3) Literature search on the mode of action (MoA) of chlorine dioxide (gas)
4) Literature search on (newly) published literature on residues of chlorine dioxide gas in/on food.
5) Overall summary on mode of action (MoA) of chlorine dioxide and residues in/on food

1) Summary of the analytical measurements performed using Knick'n'clean® sticks and chlorine dioxide gas focussing on release rates

for detailed results please refer to "KnicknClean_Release rates_2015_final"

A single Knick'n'clean® stick contains on average 2.69±0.16 mM hydrochloric acid and 0.459±0.028 mM sodium chlorite. Mixing of those two solutions (= activation of Knick'n'clean® sticks) results in the formation of ClO_2 which is subsequently released from the stick after diffusion through the outer synthetic tube. The ClO_2 release from a Knick'n'clean® stick is time delayed due to diffusion processes.

At 25.5°C the maximal ClO_2 release was reached after one day with a maximum of 1.54 µg/min. During the total study period of 18 days, 46 M% of the theoretically possible ClO_2 was released.

Lowering the temperature to 7°C (simulating conditions in a refrigerator) leads to reduced release rates of ClO_2 from the Knick'n'clean® stick. The maximal release of 0.324 µg/min was reached after 5.2 days. During the total study period (174 days), 66 M% of the theoretically possible ClO_2 was released. This is in agreement with the stated yield from literature of 68%. Regarding an operation period of 30 days the mean release rate is 327 µg/day (56% of total possible release).

Under the measured in-use conditions in a refrigerator only a small amount of the total releasable ClO_2 from the stick was detected. The maximal detected concentration of ClO_2 was 0.043 ppm which corresponds to 15 µg/120 L. The mean concentration (30 d) is 0.029 ppm which corresponds to 10.2 µg/120 L.

Additionally to the experiments dealing with the release of gaseous ClO_2 from the stick it could be shown that the release of gaseous hydrochloric acid (HCl) from the Knick'n'clean® stick can be neglected.

2) Summary of the analytical measurements of residues and by-products in model matrices using ClO_2 gas and Cl_2 gas

for detailed results refer to "KnicknClean_Residues in model matrices_2015_final")

In the context of ClO_2 gas release from Knick'n'clean® sticks the formation of chlorinated substances and oxidation by-products in food surrogate matrices was studied. The surrogate matrices analysed in this experiment were

1. water (unbuffered) to simulate condensed water within the refrigerator
2. water (buffered system; pH 6) as most food has a high content of water and a pH between 5 and 7;
3. L-Tryptophan as natural occurring amino acid with an aromatic ring, a double bond and two amino functions in the molecule and
4. 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 fridge-like conditions. Gaseous chlorine Cl_2 (1.3 µM in 10 min corresponding to 2.6 µM Cl) was used as positive control in order to demonstrate the analytical capacity to detect chlorinated by-products in the tested surrogate matrices.

As a result in none of the surrogate matrices chlorinated by-products could be detected after their exposure to chlorine dioxide gas.

The simulation involving tryptophan showed that 98% of the chlorine dioxide gas is absorbed by the matrix, but none is 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). The analysis of the reaction solution for inorganic chlorinated species showed that the majority of ClO_2 was converted to chloride (37 M%[1]) and chlorite (31 M%[1]). Chlorate was not found.

The simulation involving 3-octenoic acid showed that most of the chlorine dioxide gas absorbed by the matrix (96%) is dissolved without reaction (74%). From the initially present 3-octenoic acid an amount of 96% could be found after treatment. Oxidation products of 3-octenoic acid were detected in low amounts (max. 4%) mostly as mono-oxidized form. Chlorite was the major inorganic chlorine containing species with 40 M%[1] whereas chloride accounted only for 4.1 M%[1]. Chlorate was not found.

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1 M% are based on the amount of chlorine in the absorbed chlorine dioxide.

The respective positive controls which involved the exposure of the food surrogate matrices with chlorine gas (Cl_2) showed in both cases, L-tryptophan and 3-octenoic acid, the formation of chlorinated hydrocarbons. The ratio between chlorinated by-products and the total by-products was for L-tryptophan 2.5 M% and for 3-octenoic acid 82 M%. In both simulations chloride was the main inorganic species formed as a result of purging the surrogate matrices with chlorine gas.

Summary of the results of the Cl_2 / ClO_2 gas treatment in selected matrices

Matrix	**Tryptophan**		**3-Octenoic acid**	
Treatment	**Cl_2**	**ClO_2**	**Cl_2**	**ClO_2**
Residual gas after treatment	0%	2%	0 %	3 %
Gas dissolved in matrix	n.a.	0%	n.a.	74 %
Gas converted in matrix	100 %	~80 %	100 %	22 %
Chloride Cl^-	180 M%	37 M%	117 M%	4.1 M%
Chlorite ClO_2^-	n.d.	31 M%	n.d.	12 M%
Chlorate ClO_3^-	n.d.	n.d.	n.d.	n.d.
Hypochlorous acid HOCl	n.d.	n.d.	n.d.	n.d.
Ratio chlorinated vs oxidized species	2.5 M%	< 0.4M%	82 M%	< 4M%

< is equal detection limit

3) Literature search on the mode of action (MoA) of chlorine dioxide gas

Search Strategy

The keywords "Chlorine dioxide" + "mode of action" were searched on the literature databases "Pubmed" and "Scopus".

Since this post submission mainly focusses on current findings concerning food residues of chlorine dioxide treatment and the mode of action of chlorine dioxide, the search results including efficacy data and alternative fields of application were excluded.

Search term	Results (Pubmed/Scopus)	Number excluded* (Pubmed/Scopus)	Number included (Pubmed/Scopus)
Chlorine dioxide + mode of action	5 / 40	1 / 37	4 / 3

* Already cited in the original dossier, ClO_2 gas treatment only discussed in another field of use (e.g. hospital disinfection efficiency etc.), ClO_2 discussed only in the focus of microbial efficacy; report on combination treatments, doubled search results

For this statement the following literature was assessed (in addition to former submitted publications):

Authors	year	title
Mogoa E et al.	2011	Cellular response of the amoeba Acanthamoeba castellanii to chlorine, chlorine dioxide, and monochloramine treatments.
Finnegan M et al.	2010	Mode of action of hydrogen peroxide and other oxidizing agents: differences between liquid and gas forms.
Berg JD et al.	1986	Effect of chlorine dioxide on selected membrane functions of Escherichia coli.
Roller et al.	1980	Mode of bacterial inactivation by chlorine dioxide
Kim JS et al.	2014	Direct effect of chlorine dioxide, zinc chloride and chlorhexidine solution on the gaseous volatile sulfur compounds.

In general, the main mechanism of action of chlorine dioxide (gas) comprises the oxidation of cellular constituents. At high concentrations the ClO_2 alternates the cell membrane and at low concentrations the permeability of cell membranes is deranged (WHO, 2002) which leads to cell penetration and the protein synthesis termination (Young & Setlow, 2003; EFSA, 2005).

Chlorine dioxide gas (ClO_2) represents a promising technology for the inactivation and control of pathogenic and spoilage organisms. ClO_2 is a strong oxidizing agent with a broad antimicrobial spectrum. It reacts by taking electrons from several cellular constituents, breaking molecular bonds, and causing the death of microorganisms (Trinetta et al. 2013).

Several mechanisms of action have been proposed, but the most accepted theory of microbial inactivation by ClO_2 is the damage of protein synthesis.

Other research groups come to a similar conclusion that ClO_2 was shown to have a strong effect on complex protein degradation and enzyme inhibition. Benarde et al. (1967) hypothesized that the primary mechanism for inactivation of the E. coli cell was therefore disruption of the protein synthesis pathway by inhibition of enzymes or interference with nucleic acid–amino acid complexes.

However, a study by Roller et al. (1980) reported that the inhibition of protein synthesis may not be the primary inactivation mechanism as the percentage of E. *coli* cells killed was higher than the percentage of protein synthesis inhibited. This study also found that dehydrogenase activity was quickly inhibited by addition of ClO_2, leading Roller et al. to suggest that ClO_2 lethality was due to disruption of many cellular processes by irreversible damage to proteins.

Especially focussing gaseous chlorine dioxide it could be stated that the gaseous form might have higher kinetic energies and are uncharged, so that they can surround and penetrate the three-dimensional protein structures more easily, oxidizing, buried cysteine residues and breaking vulnerable bonds between subunits. In contrast, fully dissolved (liquid) biocides might not be able to penetrate three-dimensional structures, although this may be facilitated by formulation effects (Finnegan et al 2010). Regarding the mode of action of chlorine dioxide gas the authors concluded that ClO_2 has a strong effect on complex protein degradation and enzyme inhibition, which confirms some results of an early investigation into the physiological effects of ClO_2 by Benarde et al. (1967).

Berg et al (1986) assessed the effect of chlorine dioxide on selected membrane functions of E. *coli*. Therefore outer membrane permeability to macromolecules and potassium, and effects on respiration were observed. The results indicate that gross cellular damage involving significant leakage of intracellular macromolecules does not occur. There was a substantial efflux of potassium, however, and respiration was inhibited even at sublethal doses. It was concluded that the inhibition of respiration, which could be due to the damage to the cell envelope, was not the primary lethal event. Observations of the efflux of K+ strongly implicate the loss of permeability control as the primary lethal event at the physiological level, with nonspecific oxidative damage to the outer membrane leading to the destruction of the trans-membrane ionic gradient.

The effect of chlorine dioxide treatment on eukaryotic cells was published by Magoa et al. (2011). The group assessed the effect of different sanitizing substances on cell permeability of eukaryotic cells. For this purpose the free living amoebe Acanthamoeba castellanii was studied.

After treatment with compound the amoebe was stained and analyzed by flow cytometry in order to determine the morphology and the permeability of the cells.
The decrease of PI staining after chlorine dioxide treatment suggests that DNA might be partly destroyed. An electron microscopy of the cells showed some vacuolated areas within the cytoplasm. The underlying mechanism for this effect could be due to loss of membrane integrity.

Kim et al. (2014) additionally studied the effect of chlorine dioxide on gaseous volatile sulphur compounds (VSC) produced by bacteria. The VSC are the main source of oral malodour. The authors assumed that chlorine dioxide can reduce VSC production through their sulphur affinity to react with and oxidize the thiol-group of amino acids and VSC precursors. Consequently the mode of action of chlorine dioxide is an oxidation of the thiol-group of amino acids of the target organisms.

Summary of literature search on the mode of action (MoA) of chlorine dioxide gas

In general, the main mechanism of action of chlorine dioxide (gas) comprises the oxidation of cellular constituents by taking electrons from several cellular constituent and breaking molecular bonds.

This influences many cellular processes e.g. the permeability of cell membranes might be deranged which leads to cell penetration, protein synthesis termination and enzyme inhibition.

The loss of permeability control might lead to the destruction of the trans-membrane ionic gradient which was concluded by several authors as the primary lethal event at the physiological level of ClO_2 on bacterial cells.

The underlying mechanism for this effect could be due to loss of membrane integrity.

4) Literature search on newly published literature on residues of chlorine dioxide gas in/on food.

Search Strategy

The following keywords and combinations were searched on the literature databases "Pubmed" and "Scopus":

- Chlorine dioxide gas
- Chlorine dioxide gas + food
- Chlorine dioxide gas + residues
- Chlorine dioxide gas + food + residue
- Chlorine dioxide gas + food + by-products

The results from this search were sorted in different categories:

- Microbicidal efficacy data
- Use in different application areas
- Residues in /on food

Since this post submission mainly focusses on current findings concerning food residues of chlorine dioxide treatment and the mode of action of chlorine dioxide, the search results on efficacy data and search results from different application areas were excluded.

Search term	**Results (Pubmed/Scopus)**	**Number excluded* (Pubmed/Scopus)**	**Number included (Pubmed/Scopus)**
Chlorine dioxide gas	55 / 98	46 / 87	9 / 11
Chlorine dioxide gas + food	29 / 66	19 / 53	10 / 13
Chlorine dioxide gas + residues	4 / 12	1 / 7	3 / 5
Chlorine dioxide gas + food + residue	4 / 11	1 / 6	3 / 5
Chlorine dioxide gas + food + by-products	0 / 16	- / 11	- / 5

* Already cited in the original dossier, ClO_2 gas treatment only discussed in another field of use (e.g. hospital disinfection efficiency etc.), ClO_2 discussed only in the focus of microbial efficacy; report on combination treatments, doubled search results

Since an extensive literature search was already conducted at dossier submission (2012), the most interesting papers for this re-evaluation are the current years (2012 – 2015). But in addition also older and already cited literature is used in this statement.

For this statement the following literature was (newly) assessed:

Authors	**year**	**title**
Arango, et al.	2014	In situ quantification of chlorine dioxide gas consumption by fresh produce using UV-visible spectroscopy
Han et al.	2004	Decontamination of strawberries using batch and continuous chlorine dioxide gas treatments
Lee, Y. et al.	2015	Reaction and diffusion of chlorine dioxide gas under dark and light conditions at different temperatures
Novak, J et al.	2008	Novel chemical processes: Ozone, Supercritical CO2, electrolyzed oxidizing water, and chlorine dioxide gas
Rubino, M. et al.	2009	Effect of chlorine dioxide gas on physical, thermal, mechanical, and barrier properties of polymeric packaging materials
Smith, D.J. et al.	2014	Distribution and chemical fate of 36Cl-chlorine dioxide gas during the fumigation of tomatoes and cantaloupe
Trinetta, V. et al.	2011a	Chlorine Dioxide Gas Residues on Selected Food Produce
Vaid, R., et al.	2010	Comparison of inactivation of Listeria monocytogenes within a biofilm matrix using chlorine dioxide gas, aqueous chlorine dioxide and sodium hypochlorite treatments
Vandekinderen, I. et al.	2008	Effect of decontamination agents on the microbial population, sensorial quality, and nutrient content of grated carrots (Daucus carota L.)
Vandekinderen, I. et al.	2009a	Evaluation of the use of decontamination agents during fresh-cut leek processing and quantification of their effect on its total quality by means of a multidisciplinary approach
Vandekinderen, I. et al.	2009b	Moderate and high doses of sodium hypochlorite, neutral electrolyzed oxidizing water, peroxyacetic acid, and gaseous chlorine dioxide did not affect the nutritional and sensory qualities of fresh-cut iceberg lettuce (Lactuca sativa Var. capitata L.) after washing
Vandekinderen, I. et al.	2009c	Effect of decontamination on the microbial load, the sensory quality and the nutrient retention of ready-to-eat white cabbage
Trinetta, V., et al.	2011b	A comparative study on the effectiveness of chlorine dioxide gas, ozone gas and e-beam irradiation treatments for inactivation of pathogens inoculated onto tomato, cantaloupe and lettuce seeds

In general according to several authors chlorine dioxide acts primarily as an oxidant rather than as a chlorinating agent. Therefore, most of the initial chlorine dioxide will be reduced to chlorite and chlorate (Tsai et al., 1995; USDA, 2002a, EFSA Journal (2005), SCVPH, 2003, WHO 2008). Thus, chlorite and chlorate are the primary products resulting from the use of chlorine dioxide.

Generally, rregarding the chemistry of chlorine dioxide gas it can be summarized, that chlorine dioxide reacts as an electron acceptor, and hydrogen atoms present in activated organic C–H or N–H structures are thereby not substituted by chlorine (Hoigne & Bader, 1994). Furthermore chlorine dioxide is likely to be less reactive and produces fewer by-products than chlorine in the reaction during food processing. Due to the higher selectivity it enters in only few side reactions. If chlorine dioxide is pure, it does not chlorinate organic material and therefore does not form trihalomethans (THM) and other chlorinated disinfection by-products.

Besides this more general overview of chlorine dioxide reactions and by-products, in this statement additionally literature is assessed, addressing the reaction of chlorine dioxide gas in/on food.

Regarding the microbial efficacy and nutritional findings of chlorine dioxide (gas), Vaid et al. (2010) compared different sanitizing treatments (Chlorine dioxide gas, aqueous chlorine dioxide and sodium hypochlorite) on the inactivation of Listeria monocytogenes.
Therefore four day old biofilms were developed and treated with 0.3 mg/l (109 ppm) chlorine dioxide gas. The efficiency of the different methods leads to > log 3 reduction in cell count after 10 minutes treatment. In this experiment no residues or by-products were measured.

The use of chlorine dioxide in different application areas was described by Novak et al. (2008). The authors summarized that chlorine dioxide (ClO_2) is a strong oxidant, less corrosive and toxic than chlorine, with a broad and high biocidal effectiveness against bacteria, fungi, viruses, bacterial spores, and algae. It is used as antimicrobial agent for decontamination of food and food-contact surfaces. ClO_2 gas is highly effective (achieving more than 5-log reductions) in reducing foodborne pathogens (Salmonella spp., L. monocytogenes, and E. coli O157:H7) on produce surfaces.

The effect of different decontamination agents on the microbial population, sensorial quality, and nutrient content of grated carrots (Daucus carota L.) was assessed by Vanderkinderen et al. (2008). Several decontamination agents including chlorine dioxide gas were tested for their effectiveness to reduce the natural microflora on grated carrots. The microbial reduction of the total aerobic count for chlorine dioxide gas was 3.0 log colony-forming units (cfu)/g. To maintain the nutritional value, the influence of the decontamination agents on carotenoid content, R-tocopherol content, total phenols, and antioxidant capacity was studied. The nutrient losses caused by adding sanitizers were rather limited. After the treatment of chlorine dioxide gas a slight loss in carotenoid content was observed (-9%). The authors finally concluded that the microbial quality of fresh cut carrots could be improved by using decontamination agents without negatively influencing their sensory quality and nutrient content.

In a further study Vandekinderen et al. (2009a) evaluated the impact of decontamination agents on the microbial and sensory quality of fresh-cut leek. This includes a quantification of the effect on the total quality (e.g. nutrient content). The researchers found out that contact with 1.59 mg/L chlorine dioxide gas reduced the native microflora with 1.48 log cfu/g. The treatment had

no effect on the sensory quality of the raw fresh-cut leek. Apart from the effect of leaching of nutrients into the wash water, the supplementary effect on nutrient content caused by adding a decontamination agent was limited with the exception of some isolated cases such as the losses of vitamin C (23%) after a treatment with chlorine dioxide.
A significantly reduced total aerobic plate count of cut lettuce using chlorine dioxide gas (1.54 mg/ L) could additionally be observed (Vanderkinderen et al. 2009b). The treatment does not affect the sensory quality of the lettuce significantly, although small color changes were observed after colorimetric measurements. From a nutritional point of view water rinsing significantly decreased the vitamin C (maximum 35%) and phenol (maximum 17%) contents, but did not affect the carotenoid and R-tocopherol contents. Additional effects caused by adding a sanitizer to the wash water were not observed for vitamin C and phenols. The use of gaseous chlorine dioxide had a slide impact on the lutein content (-18%).

The same research group also analyses the effects of the food composition on the inactivation of foodborne microorganisms by chlorine dioxide (Vandekinderen 2009c).
Gram-negative and gram-positive bacteria, yeasts, mould spores and Bacillus cereus spores were tested for their susceptibility to 0.08 mg/L gaseous ClO_2 during 1 min. In this screening, the resistance of the microorganisms towards gaseous ClO_2 generally increased in the order Gram-negative bacteria, Gram-positive bacteria, yeasts and mould spores and Bacillus cereus spores.
With this treatment, reductions of microbial numbers between 0.1 and 3.5 log cfu/cm^2 could be achieved. In a second experiment the authors evaluated the effect of the food components starch, fat, protein and NaCl on the microbial efficacy of chlorine dioxide gas treatment. Soluble starch and NaCl did not have an effect on the antimicrobial efficiency of ClO_2. However, butter, corn oil or whey protein in the agar almost eliminated the antimicrobial effect of ClO_2.

Regarding the gas diffusion and elimination kinetics Lee et al. (2015) examined the reaction and diffusion of chlorine dioxide gas under dark and light conditions at different temperatures. The diffusion coefficient was found to be 0.129, 0.145, 0.173 cm^2 /s at 5, 23, and 40 °C, respectively. Degradation of ClO_2 gas under dark, UV-A and fluorescent lamp exposure was found to follow a first-order reaction.
Rubino et al. (2009) assessed the effects of gaseous chlorine dioxide (ClO_2) on properties and performance of 10 selected polymeric packaging materials (e.g. PE, PET etc.). Physical, mechanical and color properties as well as infrared (IR) spectra were assessed before and after polymer samples were exposed to 3600 ppmV (10mg/L) ClO_2 gas at 23°C for 24, 168, and 336h. The IR spectra of the ClO_2-treated samples revealed some changes in their chemical characteristics.

But compared to the release rates of Knick'n'clean® sticks (327 µg per day or 3.27µg/L assuming a 100 L refrigerator) the gas concentrations used in this experiment are about factor 3000 higher compared to the use concentrations of Knick'n'clean® sticks. So in consequence the findings on polymeric packaging materials will not be comparable to the use of Knick'n'clean® sticks.

The chlorine dioxide gas consumption by fresh produce was studied by Arango et al. (2014). The group developed an analytical method to quantify the chlorine dioxide gas consumption using UV–visible spectroscopy in -situ. Strawberries were selected as model fresh produce. It was found that strawberries consume ClO_2 gas rapidly and that longer exposure times (17 and 84 min) for continuous exposure resulted in around 45% ClO_2 absorption whereas shorter exposure times (7 min) consumed less than 20% of the injected ClO_2 because the latter did not reach steady state ClO_2 consumption prior to completion.
Since only ClO_2 gas consumption was measured in this experiment, it is unknown whether a higher gas consumption leads to increased amounts of residues on the surface of the strawberries. So this experiment does not show a correlation of ClO_2 gas consumption and the emergence of residues.

In a study of Trinetta et al. (2011a/b) the residues of chlorine dioxide gas treatment on fresh produce were evaluated. Therefore the residual ClO_2 amounts, chlorite, chlorate and chloride were analysed. Seven different foods (tomatoes, oranges, apples, strawberries, lettuce, alfalfa sprouts, and cantaloupe) were analyzed after ClO_2 treatment for surface residues. Very low residues were detectable for all the food products except lettuce and alfalfa sprouts, where the measured concentrations were significantly higher. But in general the residues are acceptable compared to the EPA drinking water levels. The researchers come to the final conclusion that chlorine dioxide technology leaves minimal to no detectable chemical residues in several food products, thus result in no significant risks to consumers.

Vandekinderen et al. (2009c) additionally analysed the by-products of chlorine dioxide gas in the selected food components. In corn oil–water emulsions treated with gaseous ClO_2 the peroxide value increased significantly, indicating the formation of primary oxidation products. Similarly, a treatment with ClO_2 increased the protein carbonyl content and induced the transformation of SH-groups to –S–S-groups in whey protein. The findings suggest that gaseous ClO_2 will be a highly effective decontaminating agent for carbohydrate-rich foods, but that it would be less effective for the decontamination of high-protein and fatty foods.

Smith at al. (2014) analysed the chemical fate of ^{36}Cl-chlorine dioxide gas during the fumigation of tomatoes and cantaloupe. The group came to the conclusion that the most prominent by-products were chloride (> 80%) and chlorate (5 – 20%). Absent from tomatoes and cantaloupe were ^{36}Cl-chlorite. Only about 1% of the by-products was perchlorate. Follow-up studies have shown that chlorate and perchlorate formation can be completely eliminated by protecting fumigation chambers from light sources. Since this is the case for example in refrigerators were Knick'n'clean® is used, perchlorate will not be formed. The absence of chlorate could also be shown in a recently performed analytical measurement by Fraunhofer ITEM. Here chlorate was not detected in /on food matrices after chlorine dioxide treatment (see point 2).

The occurrence of possible emerging other by-products in/on food was already discussed in a previous post submission paper by Fraunhofer ITEM (13.07.2013). The main discussion points are summarized in the following:

The treatment of cut and peeled fruits and vegetables with dilute chlorine dioxide solution (approximately 10 mg/kg) did not result in the detection of any halocarbons (USFDA, 1994).
The use of a chlorine dioxide solution (approximately 3 mg/kg) on poultry did not affect the oxidation value of unsaturated fatty acids compared with untreated poultry. Treated beef did also not show differences in lipid oxidation (Jiménez-Villarreal et al., 2003).

No chlorine residuals were present following chlorine dioxide treatment (10–40 mg/l) of fish.
This is in accordance with the findings of the analytical evaluation by Fraunhofer ITEM on several model matrices.
In addition, total organic halogen analysis of shrimp and crawfish indicated that no chlorine by-products were produced from sanitizing treatment with chlorine dioxide (Kim et al., 1999).

No significant effects on the protein content of salmon and red grouper fillets were reported after treatment with chlorine dioxide (20–200 mg/l in brine solution for 5 min). Additionally no obvious change in the lipid content or fatty acid composition in both salmon and red grouper fillets after treatment was detected (Kim et al., 1998).

The lack of volatile halocarbons (i.e. chloroform) being formed in the treatment of fresh produce with chlorine dioxide also supports the absence of chlorination reactions (FAO/WHO, 2008).

The question of possible residues in/on food after the usage of chlorine dioxide gas (knick'n'clean® sticks) was also addressed in a previously performed study by Fraunhofer ITEM (see point 2). In that experiment it could be shown that chlorine dioxide gas does not produce chlorinated by-products in none of the surrogate matrices after exposure.
The occurrence of the main by-products chloride (Cl^-) and chlorite (ClO_2^-) described in literature could be confirmed. Additionally some mono- and di-oxidized species could be detected after treatment. The occurrence of chlorate was not detected probably due to that the measurements were conducted under realistic conditions, i.e. refrigerator like conditions (6°C in the dark). This is in accordance with the current literature (Smith et al. 2014).

In contrast to chlorine dioxide gas treatment the treatment using Cl_2 gas shows some chlorination of the model matrices tryptophan and 3-octenoic acid. This confirms the finding described in the literature (Fukayama 1986).

5) Overall summary on residues of chlorine dioxide treatment in/on food and mode of action (MoA)

In the submission dossier a list of possible emerging by-products after the use of chlorine dioxide (gas) was already submitted. In addition to this list some recently published literature was assessed on possible emerging by-products of chlorine dioxide (gas) treatment.

Since chlorine dioxide acts primarily as an oxidant rather than a chlorinating agent, the occurring by-products will be mostly oxidized organic compounds and inorganic chlorinated species. In consequence the most prominent and published by-products of chlorine dioxide treatment are chloride (Cl^-), chlorite (ClO_2^-). For these known residues the existing authorization limits can be applied. For chlorite an acceptable daily intake limit (ADI) is available.

The non-formation of chlorinated toxic by-products is one important advantage of ClO_2. As opposed to chlorine, which reacts via oxidation and electrophilic substitution, ClO_2 reacts only by oxidation; this explains why it does not produce organochlorine compounds (WHO, IPCS 2000).

Based on the newly performed analytical measurements and the recently performed literature study it could be concluded that the treatment using chlorine dioxide (gas)

- leads to only low amounts of by-products on several food matrices (mostly oxidative nature)
- does not produce chlorinated organic by-products
- mostly reacts to the most prominent inorganic by-products chloride (Cl) and chlorite (ClO_2^-)
- does not significantly effects the nutritional quality of the tested foods
- act as oxidizing agent (SH groups → S-S groups)
- chlorate does not occur under realistic refrigerator conditions (6°C in the dark)

6) References

The following document regarding the residues of chlorine dioxide (gas) in food is mainly based on:

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