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SCIENTIFIC OPINION

Scientific Opinion on the evaluation of the safety and efficacy of Cecure® for the removal of microbial surface contamination of raw poultry products[1]

EFSA Panel on Biological Hazards (BIOHAZ)[2]

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)[3, 4]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

On request from the European Commission, results of studies submitted with an application for potential approval of Cecure® to be used for the removal of microbial surface contamination of raw poultry products were assessed to evaluate its safety and efficacy. The proposed treatment consisted of an aqueous solution containing cetylpyridinium chloride (CPC) as the active ingredient at a concentration not to exceed 1% and propylene glycol (PG), applied by drenching on whole chicken carcasses and recycled after use. Based on the available evidence, there is no concern for genotoxicity of CPC. Taking into account the estimated margins of safety and the conservative exposure estimates used to assess CPC exposure from consumption of poultry carcasses, there are no safety concerns for humans from the proposed use of Cecure®. Based on the information provided by the applicant, both Cecure® and CPC were found to be efficacious in reducing contamination with pathogenic microorganisms on fresh broiler carcasses. The efficacy of the treatment appeared to be influenced more by the concentration of the active ingredient (within the range of 0.2% to 0.5%), than by the volume of solution applied, flow rate, spraying pressure, rate of carcass processing, and time of exposure. The data about the potential emergence and selection of isolates with reduced susceptibility to biocides and/or resistance to therapeutic antimicrobials linked to the use of CPC under the conditions of application, in the recycled solution and in the wastewater, were not provided or not considered sufficient for the assessment. Based on the available limited data, the intended use of CPC in poultry slaughterhouses would pose risks for the environmental compartments surface water, sediment and soil. No risks for the function of sewage treatment plants are expected and there are no safety concerns regarding secondary poisoning for birds and mammals, and for humans indirectly exposed via the environment.

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[1] On request from the European Commission, Question No EFSA-Q-2011-00305, adopted on 8 March 2012 by the BIOHAZ Panel and No EFSA-Q-2011-01018, adopted on 21 March 2012 by the CEF Panel.

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[4] Acknowledgement: The CEF and BIOHAZ Panels wish to thank the members of the respective Working Groups on the evaluation of the safety and efficacy of Cecure for the removal of microbial surface contamination of raw poultry products: Fernando Aguilar, Jan Ahlers, Leon Brimer, Wilfried Bursch, Riccardo Crebelli, Joop de Knecht, Fidel Toldrà, Marco Vighi (CEF) and Arie Havelaar, Birgit Nørrung, John Sofos, John Threlfall, Luisa Peixe (BIOHAZ) for the preparatory work on this scientific opinion and EFSA staff: Cristina Croera, Anne Theobald (CEF) and Alessandro Broglia, Maria Teresa Da Silva Felício (BIOHAZ) for the support provided to this scientific opinion.

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KEY WORDS

Decontamination, poultry, Cecure®, cetylpyridinium chloride, efficacy, toxicological safety assessment, antimicrobial resistance, environmental impact

SUMMARY

Following a request from the European Commission, the Panel on Biological Hazards (BIOHAZ Panel) and the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel) of the European Food Safety Authority (EFSA) were asked to deliver a Scientific Opinion on an application dossier submitted by the company Safe Foods Corporation for approval of the use of the preparation Cecure® for removal of microbial surface contamination on raw poultry products.

More specifically, the approval was sought for treatments using an aqueous solution of Cecure® consisting of cetylpyridinium chloride (CPC) as the active ingredient and food-grade propylene glycol (PG). Cecure® should be applied by drenching at a concentration of less than 1.0% CPC, at room temperature, and the solution should be recycled for reuse.

The Commission asked EFSA to issue a Scientific Opinion on the assessment of the safety and efficacy of Cecure® when used to reduce microbial surface contamination on raw poultry products (defined as skin-on whole chicken carcasses or parts). Specifically, the task was to consider the toxicological safety of the substance, its antimicrobial efficacy, the potential emergence of reduced microbial susceptibility to biocides and/or resistance to therapeutic antimicrobials linked to the use of the substance, and any risk related to the release of effluents containing the substance from the slaughterhouse and/or processing plant into the environment. The assessment was based on the guidance document on the submission of data for the evaluation of the safety and efficacy of substances for the removal of microbial surface contamination of foods of animal origin intended for human consumption published by EFSA[5].

The available data indicate that CPC, formulated as a diluted solution in Cecure®, is not mutagenic in bacteria and not clastogenic in cultured mammalian cells. Negative results were also obtained in a gene mutation assay with CPC in mouse lymphoma cells and in limited tests in *Aspergillus*, *Tradescantia* and *Drosophila*. The CEF Panel also noted that, in addition to these consistently negative results, the substance does not contain structural alerts for genotoxicity. Thus, based on the available evidence, the CEF Panel considered that there is no concern for genotoxicity.

The CEF Panel had access to information on subchronic toxicity studies on CPC. From a recent 90-day toxicity study in Sprague-Dawley rats, the CEF Panel could identify a No-Observable-Adverse-Effect-Level (NOAEL) of 18 mg/kg bw/day in rats, based on increased caecum weights noted in males. The CEF Panel considered the increase in caecum weight as relevant for risk characterisation of Cecure® since CPC has been suggested to decrease the total number of microorganisms in the caecal contents of rats of both sexes. This led to an increase in caecum to body weight ratios, which was positively correlated with dietary levels of CPC. The CEF Panel considered thus that a potential similar effect of CPC on human gastrointestinal microflora should not be disregarded.

The data presented by the applicant allowed the CEF Panel to perform a very conservative risk assessment. The potential exposure to CPC was estimated to be up to 5.7 µg/kg bw/day at the mean and 17.8 µg/kg bw/day at the 95th percentile of poultry consumption. The potential exposure to PG by mean and high level consumers, such as young children would be up to 0.5 µg/kg bw/day at the mean and 1.4 µg/kg bw/day at the 95th percentile of treated poultry consumption. These exposure estimates are worst cases since they assumed that all poultry carcasses which are going to be consumed have been treated with Cecure®.

[5] EFSA Journal 2010;8(4):1544

Taking into account the highest calculated potential conservative exposure estimates to CPC from treated poultry consumption, the margins of safety for CPC would be more than 3000 at the mean and more than 1000 at the 95th percentile, when compared to the NOAEL of 18 mg CPC/kg bw/day, identified by the CEF Panel in a 13-week toxicity study in Sprague-Dawley rats. For PG, the margins of safety would be 22000 at the mean and 7000 at the 95th percentile, when compared to the Acceptable Daily Intake (ADI) of 0 - 10 mg/kg bw/day allocated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Furthermore, they would be 3500 times below the TDI of 5 mg/kg bw/day established by the Scientific Committee on Food (SCF) for PG.

Therefore based on the toxicological data available, the estimated margins of safety (going from three orders of magnitude for CPC to four orders of magnitude for PG) and the conservative exposure estimates used to assess CPC exposure from consumption of poultry carcasses, the CEF Panel considers that there are no safety concerns for humans from the proposed use of Cecure® for removal of microbial surface contamination from raw poultry under the usage conditions specified in this opinion.

A total of 15 peer reviewed published papers and 13 in-house studies dealing with testing of CPC or Cecure® for decontamination efficacy were submitted. Based on selection criteria, five peer-reviewed published papers and eleven in-house studies were selected and used in the assessment of the efficacy of Cecure® by the BIOHAZ Panel.

The selected studies were classified as high, medium or low strength of evidence, based on the experiment setting (industrial scale, pilot plant or laboratory) and on the type of microbial contamination of the analysed samples (naturally contaminated or inoculated).

Based on results published in peer-reviewed papers and in-house conducted studies, mostly performed at industrial scale and on naturally contaminated samples, both Cecure® and its active ingredient CPC were found to be efficacious in reducing contamination with pathogenic microorganisms on fresh broiler carcasses or chicken skin. The microbial reductions achieved on pre- and post-chill treated samples were in the range of <1.0 to 5.0 log units over untreated and water-treated controls. The lower reductions were generally associated with lower concentrations of CPC (e.g., 0.1% or 1 mg/ml CPC) applied to samples of low initial contamination, while the higher reductions were achieved with inoculated samples.

The BIOHAZ Panel further concluded that the efficacy appeared to be influenced more by the concentration of the active ingredient than by the volume of solution applied, flow rate, spraying pressure, rate of carcass processing, and time of exposure within the ranges examined. Since the Cecure® solution is to be recycled after use, the BIOHAZ Panel assessed the level of efficacy of the recycled solution, and concluded that there is inadequate evidence to support that the recycled Cecure® solution is as efficacious as the fresh solution and that it does not accumulate resistant bacterial cells and/or spores.

Data to address the issue of the potential emergence and selection of isolates with reduced susceptibility to biocides and/or resistance to therapeutic antimicrobials linked to the use of CPC were not provided. Moreover, no tests were undertaken by the applicant to test for the potential development of resistance of the target organisms either under the conditions of use, or in wastewater. The data provided were not considered useful to support the absence of antimicrobial activity of CPC in organic material. Further, evidence was not provided about testing potential microbial contamination of Cecure® solution in the recycling process for all bacterial species.

Concerning the risk related to the release of CPC into the environment as a result of use of Cecure®, basic data necessary for an assessment of the environmental compartments surface water, sediment and soil, as well as for evaluation of the function of sewage treatment plants, were not submitted or could not be validated. Therefore, data on the toxicity, fate and behaviour of CPC found in the open literature were used to perform a preliminary risk assessment.

The predicted environmental concentrations (PECs) and predicted no-effect concentrations (PNECs) for protection of the function of sewage treatment plants (STP) for surface water, as well as for sediment and soil, were derived using the software system EUSES 2.1.1 which has been developed for quantitative assessment of the risks of biocides and industrial chemicals to man and the environment. This system is fully based on the EU Technical Guidance Documents (TGD) for the risk assessment of these chemicals.

A comparison of PECs with the PNECs suggests that the use of CPC in poultry slaughterhouses does not pose a risk for the function of sewage treatment plants. As there are no indications of a high bioaccumulation potential, no risk for birds and mammals in the environment via indirect exposure through the food-chain (secondary poisoning) has to be expected. In view of the low vapour pressure, low bioaccumulation potential and the high adsorptive properties of CPC, indirect exposure of man via groundwater (as a source for drinking water), air and fish is expected to be negligible.
Despite the fact that it is assumed that a large proportion of the active ingredient is recycled and that the product is assumed to be used in a specific application system claimed by the applicant, risks for the environmental compartments surface water, sediment and soil are apparent.

It is recommended that, as requested in the EFSA guidance (EFSA, 2010), data addressing the potential emergence of and selection for reduced susceptibility to biocides and or resistance to therapeutic antimicrobials linked to the use of CPC should be provided by the applicant. Moreover the minimum CPC concentration applied for should be specified, and data about possible accumulation of bacterial spores, as well as data to support continuous efficacy of the recycled solution should be collected.

In order to improve the robustness and reduce the uncertainty of the assessment, the CEF Panel recommends to the applicant to provide more reliable data on the environmental fate and behaviour of CPC and to provide (long-term) tests relevant for the compartments surface water, sediment and soil. However, considering the high level of potential risk indicated by the present assessment, it is the opinion of the CEF Panel that the attainment of safe environmental levels would be highly unlikely without suitable measures to reduce environmental emissions. An option would be to reduce exposure by achieving a high proportion of recycling during treatment in poultry slaughterhouses.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The EU food hygiene legislation is aimed at protecting consumers against potential risks to health and maintaining a high level of consumer protection at all stages of the food chain. That objective must be achieved by applying the appropriate measures, including good hygiene practices and hazard control measures at each step of the food chain.

According to EU scientific advice[6], decontamination practices can constitute a useful tool in further reducing the number of pathogenic microorganisms but the use of substances intended to remove microbial surface contamination should only be permitted if a fully integrated control programme is applied throughout the entire food chain. Those substances shall be assessed thoroughly before their use is authorised.

Article 3 (23 of Regulation (EC) No 853/2004 provides a legal basis to approve the use of substances other than potable water to remove surface contamination from products of animal origin.

In addition to the safety of the substance, the potential emergency of reduced susceptibility to biocides and/or resistance to therapeutic antimicrobials is also a matter of concern as well as the impact of the substance or its by-products on the environment.

Therefore, before taking any risk management decisions on their approval, a risk analysis process should be carried out taking into account the result of a risk assessment based on the available scientific evidence and undertaken in an independent and transparent manner.

EFSA GUIDANCE

On 14 April 2010, the European Food Safety Authority (EFSA) issued a revision of a guidance document[7] on the submission of data for the evaluation of the safety and efficacy of substances for the removal of microbial surface contamination of foods of animal origin intended for human consumption.

APPLICATION FOR APPROVAL

On 14 March 2011, the Commission received an application dossier from the company Safe Foods Corporation for the approval of the substance Cecure® for uses to reduce microbial contamination of raw poultry products. According to the dossier, Cecure® is an aqueous solution containing cetylpyridinium chloride as the active ingredient, and food-grade propylene glycol.

TERMS OF REFERENCE AS PROVIDED BY EUROPEAN COMMISSION

EFSA is requested to evaluate the safety and efficacy of Cecure® to remove microbial surface contamination of raw poultry products, considering:

1. the toxicological safety of the substance;

2. the efficacy, i.e. does the use of the substance significantly reduce the level of contamination of pathogenic micro-organisms;

3. the potential emergence of reduced susceptibility to biocides and/or resistance to therapeutic antimicrobials linked to the use of the substance;

[6] SCVPH (Scientific Committee On Veterinary Measures Relating To Public Health), 1998. Report on the benefits and limitations of antimicrobial treatments for poultry carcasses, 30 October 1998. SCVPH (2003) Opinion on the evaluation of antimicrobial treatments for poultry carcasses (http://ec.europa.eu/food/fs/sc/scv/out14_en.pdf_).

[7] EFSA Journal 2010;8(4):1544

4. The risk related to the release of the slaughterhouse and/or processing plant effluents, linked to the use of the substance, into the environment.

APPROACH TAKEN TO ANSWER THE TERMS OF REFERENCE

In order to assist in assessing the safety and efficacy of a proposed decontaminating agent of foods of animal origin, EFSA issued in 2010 a revised guidance document titled "Revision of the joint AFC/BIOHAZ guidance document on the submission of data for the evaluation of the safety and efficacy of substances for the removal of microbial surface contamination of foods of animal origin intended for human consumption" (EFSA, 2010). The document presents the major components and data that an application dossier should contain. These guidelines, terminology and procedure have been used in this Scientific Opinion for the assessment of Cecure® for use in the decontamination of raw poultry products.

After having received this request from the European Commission, EFSA assigned the mandate to the Panel on Biological Hazards (BIOHAZ Panel) and the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel). Chapters 3 and 4 were adopted by the BIOHAZ Panel on 8th March 2012. Chapter 2 and 5 and the respective conclusions were adopted by CEF Panel on 21st March 2012.

The term "raw poultry products" is defined as whole chicken carcasses, as referred in the dossier and in the studies provided by the applicant. In one chapter of the dossier, turkey carcasses are also mentioned (page 158 of the dossier), but that study concerns the general slaughtering process, and is not related to the use of Cecure® for decontamination. Only one study (Baker et al., 2010) evaluated the shelf-life of chicken parts, boneless skinless breast meat, thighs, wings, split breasts and leg quarters, derived from treated carcasses. The laboratory studies by Arritt et al. (2002) and Breen et al. (1997) evaluated chicken skin samples and the ability of cetylpyridinium chloride (CPC) to inactivate *Campylobacter jejuni* and *Salmonella typhimurium*, respectively.

ASSESSMENT

1. Introduction

Approval was sought for removal of microbial surface contamination from raw poultry products by the use of an aqueous solution containing CPC as the active ingredient, and food-grade propylene glycol ("PG"), with the function to maintain the solubility and stability of the solution. The mixture has the trade name Cecure®. As proposed, Cecure® is to be diluted to < 1% concentration of the active ingredient in potable tap water for use as a decontaminant treatment for raw poultry in a drenching application cabinet which is part of the Cecure® application system that "captures and recycles virtually all solution".

Relative to the purpose of the treatment, the dossier indicates: Cecure® will be used as a food processing aid to control the following organisms on raw poultry carcasses and skin-on poultry parts: *Salmonella*, *Campylobacter*, *Listeria*, *Staphylococcus aureus*, *Escherichia coli* (including O157:H7), *Pseudomonas*, total coliforms, viruses, and other naturally-occurring microorganisms on raw poultry carcasses (page 12 of the dossier).

1.1. Parameters for treatment application

The applicant includes the following information in the dossier (pages 16-21):

- Where in processing line: Cecure® can be used to treat the inner and outer surfaces of raw pre-chill, skin-on poultry carcasses after the last inside/outside bird washer. Optionally, it can be applied post-chill to skin-on whole poultry carcasses or to skin-on parts.

- Concentration: The concentrated solution Cecure® is diluted on-site with potable water to reach a concentration, for which the approval is sought, not to exceed 1.0% CPC (10 mg/ml) in the final solution. It is stated that a processor is likely to apply a solution with a CPC concentration ranging from 0.2% to 0.5% (2.0 to 5.0 mg/ml CPC) depending on point of application.

- Conditions of use: neutral pH, at ambient temperature.

- Application: as a drench in a cabinet equipped with spray nozzles and a booster pump allowing application of the liquid at a constant volume in litres per minute (Figure 2, page 19 in submitted dossier). Drenching is different from spraying applications because drenching uses larger nozzles sizes, which produce thicker jets, and the larger volumes of liquid floods and soaks the carcasses.

- Exposure time: only a few seconds, typically (not limited to) ≤ 5 seconds for whole carcasses or up to (but not limited to) 20 seconds for skin-on parts.

- Volume to apply: the treatment volume will be in the range, but not limited to, approximately 1.0 to 2.0 litres per carcass.

- Subsequent removal conditions: the carcasses are treated with a potable water rinse (if carcasses are not immersion chilled); in the dossier it is indicated that typically a "very low" volume rinse is applied.

- Recycling: a recycling step is foreseen in the application. According to the dossier, the CPC concentration in the recycled solution is monitored in the Cecure® application system. It is stated that there is no decrease in efficacy when using Cecure® solution consisting entirely of drip recycled from treated carcasses. Bacterial contamination of Cecure® usage solution is reported in the dossier not to occur under the proposed conditions of use.

1.2. Cecure® application as related to carcass chilling

The applicant also provides the following details in the dossier (pages 160-162):

- After inspection and viscera removal, the carcasses are washed inside and outside as they pass through three or four inside/outside bird washers (IOBWs). These stainless steel cabinets are automated washing stations for the carcasses. Several gallons (litres) of water are used to clean each individual carcass – inside and out. All of the water used in these wash cabinets is directed to the offal line.

- Following processing through the IOBWs, carcasses may be treated with Cecure® just prior to chilling (pre-chill treatment). The carcasses then move via the overhead shackle line to the chilling phase of the process.

- Immersion Chilling. In immersion chilling, the carcasses are dropped automatically from the shackle line into a huge tank of water called the pre-chiller. This tank typically contains 30,240 litres of water held at 10° to 13°C. The carcasses typically remain in the pre-chiller for about 15 minutes.

- From the pre-chiller tank, the carcasses move automatically into the chiller tank. This tank is larger, containing 94,500 litres of colder water, usually 0° to 1°C, and the carcasses stay in this tank for about 45 minutes. USDA/FSIS, as well as the regulatory agencies in most other countries, require that the carcasses exiting the final chiller have an internal muscle temperature of ≤ 4.4°C.

- Air Chilling. During air-chilling, carcasses move on shackles through a cold room at 0° to 1°C for 90 to 130 minutes. The carcasses are misted with water prior to and periodically during the air-chilling process. As in immersion chilling, the internal temperature of the muscle must be ≤ 4.4°C at the exit of the air-chilling room.

- After immersion or air-chilling, carcasses are transported to other areas of the plant. They may move to a whole carcass packaging station, may go to a separate part of the plant for cut-up or deboning, or may be shipped to a different plant for further processing or cooking.

- Application of Cecure®: As mentioned above, the current clearance for CPC in the U.S. permits its use on pre-chill poultry carcasses or on post-chill poultry carcasses or skin-on poultry parts. Each application (pre- or post-chill) is discussed below in terms of environmental impact.

    - **Pre-chill use:** For pre-chill use of Cecure® as described in the dossier, i.e., ≤ 1.0% for whole skin-on carcasses that will be immersion or air-chilled, the Cecure® drench application cabinet is positioned just after the last IOBW. In plants that utilize air-chilling, Cecure®-treated carcasses are rinsed with potable water before entering the air-chilling room as they travel at the processing line speed in place at the plant. The carcasses then continue along the processing line to the air-chilling room.

    - **Post-chill use:** For post-chill application of Cecure®, carcasses are treated after removal from the final immersion chiller tank or after they exit the air-chilling room. The carcasses then receive a potable water rinse (regardless of chilling method, i.e., immersion or air-chilling), again travelling at the processing line speed in place at the plant. The carcasses then continue along the line for further processing and/or packaging.

The aim of the present opinion is to assess the safety and efficacy of Cecure® to reduce microbial surface contamination on raw poultry products considering (i) the toxicological safety of the substance, (ii) the efficacy, i.e. does the use of the substance significantly reduce the level of contamination of pathogenic microorganisms, (iii) the potential emergence of reduced susceptibility to biocides and/or resistance to therapeutic antimicrobials linked to the use of the substance, and (iv) the risk related to the release of the slaughterhouse and/or processing plant effluents, linked to the use of the substance, into the environment. Each of these assessments is described subsequently.

2. The toxicological safety of the formulated product to humans

2.1. Evaluation

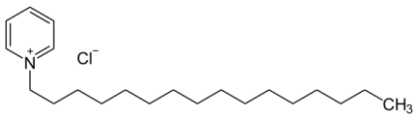
2.1.1. Technical data

2.1.1.1 Identity of the substance and specifications

Cetylpyridinium chloride (CPC)

Synonyms:	1-palmitylpyridinium chloride, C16-alkylpyridinium chloride, Acetoquat CPC, Aktivex, Ammonyx CPC, Cepacol, Ceprim, Cetafilm, Halest, Ipanol, Medilave, Mercocet, Merothol, and Pionin B.
Common names:	Ceepryn chloride, Cepacol chloride, Cetamium, Dobendan, Pristacin, and Pyrisept.
Chemical name:	1-hexadecyl pyridinium chloride
CAS Registry Number:	123-03-5 (anhydrous) and 6004-24-6 (monohydrate)
EC number:	204-593-9
Chemical formula:	$C_{21}H_{38}NCl$
Molecular weight:	340 g/mol

Structural formula:



CPC is typically present in the monohydrate form $C_{21}H_{38}NCl{:}H_2O$ with a molecular weight of 358 g/mol. The calculated elemental content is C: 70.45%, H: 11.26%, Cl: 9.90%, O: 4.47%, and N: 3.91%.

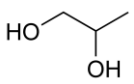
Description:

CPC is a white powder, with a melting point of 77°C in anhydrous form and 83°C in its monohydrate form. It is freely soluble in water, alcohol and chloroform, but is hardly soluble in benzene and ether. The log octanol-water partition coefficient (log $K_{o/w}$) is 1.71.

Propylene glycol (PG)

Synonyms:	α-propylene glycol, 1,2-propanediol, 1,2-Dihydroxypropane, methyl ethyl glycol ("MEG"), methylethylene glycol, PG, Sirlene.
Chemical name:	Propane-1,2-diol
CAS Registry Number:	57-55-6
EC number:	200-338-0
Chemical formula:	$C_3H_8O_2$
Molecular weight:	76.09 g/mol

Structural formula:



PG is authorised as a food additive E 1520 (Commission Regulation N° 1129/2011[8]).

Description:

PG is a colourless, clear, hygroscopic, viscous liquid. PG has a melting point of -59°C, a boiling point of 188.2°C, and is freely soluble in water, ethanol and acetone.

Other specifications of CPC

Purity: The applicant does not manufacture CPC. A manufacturer provided the US Pharmacopeia-grade CPC to the applicant with a certificate of analysis in compliance with the specifications. The quality of CPC used for the preparation of Cecure® may contain very low levels of pyridine (70-120 mg/kg CPC as stated by the applicant) as a residual starting reactant from the synthesis of CPC. At the requested use level as proposed by the applicant, pyridine cannot be detected (at a detection level of 1 mg/L). No other known impurities, by-products, contaminants or reaction products of concern have been reported by the applicant in CPC by using liquid chromatography-mass spectrometry (LC-MS).

Stability: The applicant assayed the stability of CPC solutions, from the commercial Cecure® solution of known concentration in water, by determining pyridine as the potential breakdown product. The solutions were heated to 95°C for 10, 20, 30, 60, or 90 minutes. After heat treatment, the solutions were analyzed for free pyridine using LC/MS with a sensitivity of 1 ng/mL. Results demonstrated there was no release of free pyridine in the heated solutions and no other reaction products were found. The applicant also analysed CPC for hexadecene and cetyl chloride during storage for several years and no detectable amount of either compound was found with methodology sensitive to 0.05%.

Description of the product to be used, conditions of storage and shelf-life: Cecure® is a food processing aid, supplied as a concentrate solution of CPC dissolved in an aqueous solution with PG. Cecure® concentrate solution is recommended by the applicant to be stored at temperatures above 18ºC to avoid crystallization of CPC in the solution. However, if crystallization occurs, it is claimed that the solution can be heated to restore its viscosity without degradation of the Cecure® solution. The stability of CPC under normal storage conditions (4°C, 20°C, and 48°C, as well as after freezing and thawing) was ensured by the applicant through analytical data on production batches. The results showed acceptable CPC assay, melting range, and moisture content for five years from the date of manufacture.

Description of chemical reactivity of the substance under intended conditions of use: The carbon-nitrogen (C-N) bond attaching the aliphatic carbon tail to the pyridine ring in CPC is very strong and would require strong oxidants, not routinely used within poultry processing plants, to disrupt the bonds. The aliphatic carbon tail is saturated and thus contains a uniform electron distribution that would greatly hinder nucleophilic attack by chemicals typically present in poultry processing and rendering plants.

Previous evaluations and authorizations: Cecure® is approved for use as decontaminant treatment for raw poultry carcasses and skin-on poultry parts in the USA by the US Food and Drug Administration (FDA) and by the United States Department of Agriculture/Food Safety Inspection Service (USDA/FSIS). Cecure® is also approved in other countries, including Canada, Mexico, Panama, Costa Rica, Colombia, Russia, South Africa, Saudi Arabia, and Jordan. CPC is a cationic quaternary ammonium compound found in many types and brands of worldwide, commercially available products such as mouthwash, toothpaste, lozenges, throat sprays and anti-snore throat sprays, as well as baby teething gels and baby wipes.

[8] Commission Regulation (EU) No 1129/2011 of 11 November 2011. Official Journal of the European Union L295/177

2.1.1.2 Manufacturing process

The substance is not manufactured by the applicant. It is available commercially from different suppliers.
According to the applicant, CPC can be prepared by the interaction of cetyl chloride and pyridine under pressure at an elevated temperature. In aqueous solution, CPC is synthesized by alkylation of pyridine with cetyl chloride to yield the monohydrate of the quaternary salt of pyridine and cetyl chloride. The product is supplied with a certificate of analysis of the manufacturer.

2.1.1.3 Reactions and fate of the decontaminating agents of the formulated product on the treated foods of animal origin

Quantification of residual levels of the substance in the treated food: The applicant assayed 5 trials, for pre or post-chill treatments, with or without use of brushes, with a variety of CPC concentrations in the application solution (0.05% to 2% CPC) and varying volumes of treatment solutions (1.2 to 7.6 L per bird) followed by potable water rinsing. The amount of CPC that was absorbed in the skin of chicken carcasses after those treatments with typical Cecure® solutions at different final concentrations of use was reported by the applicant to be within the range 2.9 – 25.9 mg/kg poultry skin, the higher concentrations being reported for the post-chill treatments. It was reported that no CPC residues could be detected in the meat (with a detection limit of 0.19 µg/g).

The residues of propylene glycol (PG) in the carcass were also assayed by the applicant. The PG residue on pre- and post-chill treated carcasses ranged from 0.9 mg/kg to 2.1 mg/kg, including both samples that were rinsed and samples that were not rinsed before analysis. It was reported that no PG residues could be detected in the meat.

Degradation products of the substance: The applicant reports that due to its structural nature, CPC is resistant to breakdown and subsequent generation of degradation products, as a result of operational steps performed routinely within poultry processing and rendering plants. The application of 95°C for up to 60 minutes or indirect steam to treated chicken samples did not alter the HPLC results. Typical cooking procedures, like baking and frying, were also tested by the applicant. Nine poultry drumsticks were baked at 190.6ºC in an oven for 45 min and 9 other poultry drumsticks were fried in vegetable oil heated to 175ºC for 20 min. Based on the similar CPC recovery and the respective chromatograms for both cooking procedures, it can be concluded that no degradation or reaction products were formed. In the case of PG, the applicant reports that it does not break down on the treated food or in the processing environment.

Description of any reaction by-products resulting from potential reactions with natural compounds in the food during and after treatment: As described above, degradation of CPC is not expected as a result of the intended use. Considering the chemical nature of CPC, no oxidative or acid catalysed reactions with lipids, proteins or carbohydrates are to be expected.

2.1.1.4 Methods of analysis

The analytical method to detect residues of CPC in poultry is based on HPLC with UV detection while the analysis of PG is based on Gas Chromatography. The limit of detection for CPC is reported by the applicant to be 0.19 µg/g. The CEF Panel notes that the analytical methods for CPC and PG have been validated with spiked samples and described in detail by the applicant. The analytical methodology to detect pyridine in the study of heat treatment stability of CPC is based on LC-MS and the limit of detection for pyridine is reported by the applicant to be approximately 1 ng/mL.

2.1.2. Dietary exposure assessment of CPC resulting from use as a decontaminating agent

Information on estimated residue levels of CPC and PG in the skin of chicken carcasses was provided in the dossier. The amount of CPC that can be absorbed in the skin of chicken carcasses from different treatment conditions has been reported to be within the range 2.9 – 25.9 mg/kg poultry skin.

To estimate the skin weight as a percentage of the carcass weight, the applicant used data from 5 trials reporting skin weight versus average carcass weight and average live weight for birds processed in the USA in 2005. The average carcass weight for each trial was calculated by the applicant as the multiplication of the average live weight for that trial by the estimated percent yield for carcasses of that size, as provided by the management personnel in the processing plants where the trials were conducted. The applicant provides an estimate of 8.8 % of skin in relation to total weight of the carcass. Taking into account 25.9 mg/kg skin as the worst case of CPC residue content in the skin, the average concentration of CPC per kg of poultry which is consumed with the skin on would be 2.3 mg/kg poultry.

The applicant provided an estimation of the consumption of poultry in the European Union based on the UK, as the country consuming the largest amounts of poultry within the EU, and dividing such consumption by the total UK population. This is not considered as representative of worse case consumption because not all the population consumes poultry and, in addition, high consumers of poultry were not taken into consideration. So, a new consumer exposure assessment was performed based on the EFSA European food consumption databases (EFSA, 2011b). The consumption of poultry for mean and high level consumers (at the 95th percentile), such as young children in an EU country with high poultry consumption like Bulgaria, was 2.5 and 7.8 g/kg bw/day, respectively (EFSA, 2011b).

On this basis, the potential exposure to CPC would be up to 5.7 µg/kg bw/day at the mean and 17.8 µg/kg bw/day at the 95th percentile of treated poultry consumption. In the case of PG, the residual values that can be absorbed in the skin of chicken carcasses are within the range 0.9 mg/kg to 2.1 mg/kg. Taking the last value as the worst case, the residual PG content for the full carcass would be 0.2 mg/kg poultry. The potential exposure to PG by mean and high level consumers, such as young children in Bulgaria, as described above, would be up to 0.5 µg/kg bw/day at the mean and 1.4 µg/kg bw/day at the 95th percentile of treated poultry consumption.

2.1.3. Toxicological data

This section deals with the evaluation of the safety of Cecure® commercial product, containing CPC as active ingredient for the removal of microbial surface contamination from raw poultry carcasses, under the usage conditions specified in this opinion.
From the information available, it can be concluded that CPC has not been evaluated previously as a food ingredient, although CPC has been evaluated as part of pharmaceutical formulations or oral cavity drug products as described in the following section.
The EFSA guidance for the evaluation of the safety and efficacy of substances for the removal of microbial surface contamination of foods of animal origin intended for human consumption (EFSA, 2010) does not stipulate a fixed set of toxicological studies. The full reports of the following studies were provided by the applicant:

- A bacterial mutagenicity assay on Cecure® commercial product
- An *in vitro* Chromosome Aberration in Chinese Hamster Ovary Cells for Liquids on CPC
- A 14-day palatability study of CPC in Sprague-Dawley rats
- A 28-day short-term toxicity feeding study of CPC in Sprague-Dawley rats
- A 28-day toxicity feeding study of CPC in Beagle dogs
- A 90-day toxicity feeding study of CPC in Sprague-Dawley Rats
- A 90-day toxicity feeding study of CPC in Beagle Dogs

In addition, the applicant provided bibliographic references or summaries of a series of toxicological studies, reports and scientific papers addressing several endpoints: genotoxicity, acute oral toxicity, short-term and subchronic toxicity, chronic toxicity and carcinogenicity, reproductive and developmental toxicity of Cecure® or CPC. The Panel is aware that additional data on the toxicology of CPC (including genotoxicity and reproductive toxicity) have been generated within the context of data requirements for biocides. However, these data were not available to EFSA.

A thorough evaluation of the scientific reliability and robustness of all the studies provided was done and is described below.

2.1.3.1 Acute oral toxicity

LD_{50} values of 200 to 681 mg CPC/kg bw in rats, of 125 mg/kg bw in mice and of 400 to 500 mg/kg bw in rabbits have been reported (BIBRA, 1988; Genco, 1995). LD_{50} values of 99 to 159 mg/kg bw were reported for male mice whereas values of 286-406 mg/kg bw for rats were reported in other reports (USAEH-HT, 1969). An LD_{50} value of 400 mg/kg bw in rats (males and females combined) has also been reported (Zeeland Chemicals Inc., 1995). In a study involving groups of 3 or 10 or 13 Sprague-Dawley male rats given solutions containing 100, 200, 250, 300, 350, 400 and 450 mg CPC/kg bw a LD_{50} of 200 mg/kg bw was reported (Nelson & Lyster, 1946).

Effects such as limb paralysis were reported at high (unspecified) doses of CPC, while doses of 400 mg/kg bw caused diarrhoea (BIBRA, 1988). More pronounced effects on the central nervous system (CNS) were reported following CPC administration via intravenous or intraperitoneal routes but these routes of administration do not appear relevant to the intended uses in the application.

Safety profile sheets on CPC monohydrate in the application dossier mention that the compound is poisonous by ingestion, intraperitoneal, intravenous and subcutaneous routes (Lewis, 1996).

One publication mentions that fatal doses for quaternary ammonium salts in humans by ingestion can be estimated to be between 1 and 3 grams. The principal manifestations of human poisoning from ingestion of quaternary ammonium salts are vomiting, collapse and coma due to caustic effects (Arena & Drew, 1986).

2.1.3.2 Short-term and subchronic toxicity

Orally administered CPC to rats and dogs at doses of 5 to 500 mg/kg bw/day were reported to cause morbidity and death at 125, 250, and 500 mg/kg bw/day (no more details available). At lower doses (50 mg/kg bw/day) gastric irritation was reported (Genco, 1995).

One subchronic toxicity study done in the 1940's on CPC is summarised in a report document submitted to FDA's over-the-counter (OTC) Review Panel on Oral Cavity Drug Products (Procter & Gamble, 1979). Groups of 6 or 10 or 12 rabbits were administered orally 0, 10 or 100 mg/kg bw of CPC for 28 days. No overall effects on body weight gain were reported, although the animals that lost weight showed temporary diarrhoea (no more details). No evidence of gross pathological changes was reported. Histological examination reported varying degrees of diffuse vacuolisation of the cytoplasm of liver cells in both control and treated groups. Similar findings were reported in the cytoplasm of kidney cells lining the tubules in all groups, more pronounced in the high-dose group. The authors considered these findings as not toxicologically significant.

In a 14-day palatability study, groups of 5 Sprague Dawley rats of both sexes were administered 0, 100, 500, 1000, 1500 and 2000 ppm of CPC orally in the diet, equivalent to 0, 5, 25, 50, 75 and 100 mg/kg bw/day (Redfield Laboratories, 2001a). The study was done under GLP conditions according to international guidelines. Regular observations included clinical parameters, body weights and feed consumption. Thinness was observed in one female from the highest dose group (100 mg/kg bw/day), the effect correlated with lower feed consumption and was considered treatment related. No other adverse findings were reported upon clinical observations. Overall, there was a treatment-related decrease in body weight gains in male and female rats, starting in animals in the 50 mg/kg bw/day group for the males and in the 75 mg/kg bw/day group for the females. Over the duration of the study a treatment-related decrease in feed consumption beginning in males and females from the 25 mg/kg bw/day group was reported. The effect was statistically significant starting at the 50 mg/kg bw/day group animals only, although a clear trend in decreased feed intake was observed at all doses when compared to controls.

In a 28-day study, groups of 10 Sprague Dawley rats of both sexes were administered 0, 125, 250, 375, 500, 750 and 1000 ppm of CPC orally in the diet, equivalent to 0, 6.25, 12.5, 18.7, 25, 37.5 and 50 mg/kg bw/day (Redfield Laboratories, 2001b). The study was done under GLP conditions according to international guidelines. Observations included body weights and feed consumptions measured weekly. Haematology, clinical chemistry and urinalysis were evaluated at termination. All animals underwent gross necropsy and specific tissues underwent histopathology examination. No adverse clinical observations were reported. Body weights and body weight gains were significantly lower in males and females 37.5 and 50 mg/kg bw/day groups. These effects were considered treatment-related and were attributed to a direct effect on feed consumption. Similar findings were reported in animals from the remaining treated groups but they were inconsistent. A dose-related decrease in feed consumption was reported in males and females from 37.5 and 50 mg/kg bw/day groups. Feed consumption was also reduced in females from the 18.7 and 25 mg/kg bw/day groups. No ophthalmological abnormalities were reported. There were some changes in the haematology, clinical chemistry, including lower total bilirubin concentration at all doses in females and higher glucose and higher aspartate aminotransferase activity in males from the 50 mg/kg bw/day mg/kg bw/day group. Urinalysis parameters examined showed inconsistent changes across sexes. Several differences in absolute and relative organ weights in males and females were reported without histopathology changes. In males, statistically significant increases in weight of adrenal glands, brain, caecum and testes, relative to body weight, was observed in the 50 mg/kg bw/day group. In the case of caecum and testes, there was a consistent dose-related increase in weight, relative to body weight, in males, although it only became significant from the 37.5 and the 50 mg/kg bw/day groups, respectively. In females, statistically significant increase organ weight, relative to body weight, were observed in adrenal glands (25 mg/kg bw/day group), brain (18.7 and 50 mg/kg bw/day groups), kidneys (37.5 and 50 mg/kg bw/day groups) and caecum (18.7, 37.5 and 50 mg/kg bw/day groups). For caecum relative weights, there was a consistent dose-related increase, although not statistically significant, at the 25 mg/kg bw/day dose.

Groups of 15 or 20 Weanling rats per sex were administered 0, 5, 10 or 20 ml of a solution containing CPC and domiphen bromide at a ratio of 9:1 (Procter & Gamble, 1979). Relative liver weights were reported as significantly higher in all treated male rats than in the water control animals. The females from the middle and high dosage groups were also reported to show significantly higher relative liver, kidney, and adrenal glands weights. The authors concluded that these changes could not be attributed to the active ingredients, based on the finding that the values for these parameters were not significantly different to those in the ethanol control animals. No other treatment related effects on haematological parameters or urinalysis where reported. Significant decreases in serum alkaline phosphatase and albumin serum levels were reported in all treated male rats, these were reported as not dose-related. No gross and histological findings in tissues were reported as treatment-related. The relevance of these findings in the evaluation of the safety of CPC is questionable, given that it was administered as a mixture with another compound.

Groups of six rats (strains not specified) of both sexes were administered in the diet 0, 125, 300, 800, 2000, 5000, 10000 ppm CPC (equivalent to approximately 0, 6.25, 15, 40, 100, 250 and 500 mg/kg bw/day) for 90 days (USAEH-HT, 1969). All animals administered 250 mg/kg bw/day and 500 mg/kg bw/day died within three weeks after initiation of the test. An significant increase in caecum to body weight ratios, when compared to controls, was reported in female rats in the ~15 mg/kg bw/day, 40 mg/kg bw/day and 100 mg/kg bw/day groups, as well as in males in the 40 and 100 mg/kg bw/day groups. It was noted that, as the concentration of CPC increased, the total number of microorganisms in the caecal contents decreased, in both sexes. A positive correlation was noted between dietary levels of CPC and increases in caecum to body weight ratio. Unspecified differences in body weight gain, liver and kidney to body weight ratios and food utilisation of male and female rats in the 100 mg/kg bw/day group were reported. Gross and microscopic examination of liver, kidneys, lung, spleen, caecum and testis from any of the administered groups were reported to show no appreciable differences compared to controls.

BIBRA summarised two studies done on rats and rabbits with CPC (BIBRA, 1988), one of these studies presumably was the same as that described above. BIBRA described these studies as follows:

Groups of six rats of both sexes (strains not identified) were given diets containing up to 1.0 % CPC for 90 days, equivalent to approximately 500 mg/kg bw/day. All animals given 0.5 % (~250 mg/kg bw/day) and above, died within 3 weeks. It is reported that no gross effects or changes in organ weights were seen in the group administered approximately 6.25 mg/kg bw/day (0.0125%). Increased caecum weights were reported in females administered 0.03 % (~15 mg/kg bw/day) and above and in males administered higher than 0.06 %. (~30 mg/kg bw/day). Adverse effects on growth and (unspecified) changes in liver and kidney weights were seen at 0.2 % (~100 mg/kg bw/day). The liver, kidneys, lungs, spleen, caecum and testes were normal upon microscopic examination.

Groups of 10 to 12 rabbits administered 10 to 100 mg/kg bw/day for 4 weeks (presumably by gavage) showed no gross treatment-related abnormalities. Upon microscopic examination, there was limited evidence of mild effects in the kidney and liver at all doses, although similar less severe findings in controls (unspecified) led the investigators to express doubts that they were treatment-related.

Groups of two or four dogs (breed not identified) were administered by gavage single daily doses of a pharmaceutical formulation containing CPC, benzyl alcohol, liquid glucose and sucrose for 30 days (Scientific Laboratories, 1969a). The groups were described as control (distilled water), group II dosed 1 ml/kg bw/day, group III 3 ml/kg bw/day and group IV 10 ml/kg bw/day. It is reported elsewhere that the pharmaceutical formulation contains per dosing 1.47 mg cetylpyridinium, 6.5 mg benzyl alcohol, 1.1 g liquid glucose and 1.2 g sucrose (Cepacol ®). In the absence of specific information, it is assumed that the same concentrations per ml were tested in this study. The animals underwent clinical laboratory determinations as well as complete necropsy and histopathological examinations. According to the authors' conclusion the animals tolerated the pharmaceutical formulation up to the highest dose tested. Microscopic examinations were done in 2 male and 2 female dogs in the control group, in 1 male and 1 female dogs in the group I, in 1 male and 1 female dog in the group II and in 2 male and 2 female dogs in the group III. The only adverse effects reported were salivation and occasional vomiting. It is reported that one high dose female lost weight during the first week of treatment but recovered and that one high dose male exhibited a slight anaemia terminally. Since apparently these symptoms were mild or cleared before the end of the study, the authors considered that the formulation was non toxic to dogs under the conditions of the study. It is observed however by the CEF Panel that only one or two animals per sex were subjected to examination in the high dose group formulation. The relevance of these findings for the evaluation of the safety of CPC is questionable, given that it was administered as a mixture with another compound. The same report describes a study done on groups of 20 Sprague-Dawley rats of both sexes administered the same doses of the pharmaceutical formulation Cepacol® as in the previous study with dogs for 30 days (Scientific Laboratories, 1969b). Some rats exhibited signs of mild respiratory disease during the study. All other effects appeared to be incidental, except for deaths which were numerous in the high dose group but attributed to the intubation protocol, the drug concentrations (without details) as well as dosage volume. Food consumption and body weight gains were not reported affected. Clinical laboratory and urine examinations were reported as not showing effects. Gross necropsy findings (such as organ weight determinations) and microscopic examinations were reported as not showing effects related to the treatment. The authors considered that the formulation was non toxic to rats under the conditions of the study. The relevance of these findings for the evaluation of the safety of CPC is questionable, given that it was administered as a mixture with another compound.

Another subchronic toxicity study was done in groups of male and female beagle dogs, administered during 30 days a pharmaceutical formulation containing dextromethorphane HBr (5.0 mg), doxylamine succinate (32.0 mg), CPC (1:1500), benzyl alcohol (0.3 %), menthol (1.1 mg), horchound flavour compound (9.16 mg), glycerin (22 mg) and sucrose, glucose, C. Yellow No.5, Blue No. 1 and Red No. 2 (Cepa-Tuss Teoches) (Scientific Laboratories, 1965). Twelve animals were divided in four groups dosed with 0 (4 dogs, 2 males & 2 females), 1 (2 dogs, 1 male & 1 female), 3 (2 dogs, 1 male & 1 female) and 10 (4 dogs, 2 males & 2 females) ml/kg bw/day of the formulation. No particular

effect related to the treatment was reported. Occasional vomiting in the high dose group and some increased salivation of all treated animals was noticed. Slight reductions in haemoglobin, hematocrit and erythrocyte counts were reported in the high dose group, but overall haematology parameters were either within normal ranges or were explained by reactions to parasite infections. Biological chemistry and urinalysis were normal. Gross necropsy and microscopic findings were reported as not related to the treatment. For example, mild to moderate pulmonary granulomatosous pneumonia and granulomas in liver were attributed to a rare nematode parasites infection (*Filarioides milksi*), whether focal cyatitis in the urinary bladder was related to catheterisation manipulation. The authors concluded that dogs tolerated well 10 ml/kg bw/day or less of the pharmaceutical formulation.

The same report describes a study done in four groups of 20 Wistar-Morini albino rats (10 per sexes), administered during 30 days a pharmaceutical formulation containing benzocaine 0.2 mg, dibucaine 0.03 mg and CPC 0.05 mg for 18 days (Scientific Laboratories, 1965). Groups were dosed with 0, 1, 3 and 10 ml/kg bw/day of the formulation. Almost all animals in the high dose group died during the first 8 days of treatment, at the time the animals were being intubated. The increased mortality was attributed to the greater dose volume of formulation and concentration of the compound and thus dosage was reduced for all groups afterwards. A few rats showed signs of mild respiratory dysfunction. No changes in food consumption and body weights were reported. Haematology, biological chemistry and urinary parameters were reported as normal. Gross necropsy and microscopic findings were reported as not related to the treatment (for example sporadic liver and lungs abscess, oedemas, lymphoid infiltrations, haemorrhages). Organ weights were not changed. The authors concluded that rats tolerated well 10 ml/kg bw/day or less of the pharmaceutical formulation.

The relevance of these studies for the evaluation of the safety of CPC is questionable, given that it was administered as a mixture with another compound.

In a more recent 13-week study, groups of 20 Sprague-Dawley albino rats of both sexes were administered 0, 125, 250, 500, and 1000 ppm of CPC orally in the diet, corresponding to an average consumption of approximately 0, 9, 18, 35 and 70 mg CPC/kg bw/day for males, and to 0, 11, 22, 42 and 84 mg CPC/kg bw/day for females, respectively (Charles River Laboratories, 2006a). The study was done under GLP conditions according to international guidelines. Regular observations included clinical parameters, body weights and feed consumptions, ophthalmology and neurological examinations such as functional observation tests performed on all animals (passive home cage, interactive cage behaviour, response to handling, etc); no open field assessments were performed. Further observations included haematology and coagulation parameters, serum chemistry, urinalysis, organ weights, histopathology on all tissues from all animals in groups 0 and 1000 ppm, in all early death animals and on all gross lesions. There was only one death during the study, which was not considered by the authors to be related to the treatment (carditis). In males and females, from the 1000 ppm group, mean body weights were significantly lower than in controls and were related to decreased feed consumption. No ophthalmological abnormalities were reported. Neurological examinations did not show changes related to the treatment. Haematological examination showed statistically significant increases in haematocrit values in females from the 1000 ppm group, whereas mean corpuscular haemoglobin concentration in those animals was decreased consistently but did not reach statistical significance at the end of the study. Red blood cell counts were consistently increased in females from the 1000 ppm group throughout the study and were considered treatment related by the authors. However since males did not show changes they were considered by the authors as not biologically significant or adverse. No other significant change in haematology parameters was reported. Serum chemistry showed significant lower levels of alkaline phosphatase and creatinine levels in males from the 1000 ppm groups. In females creatinine levels were also significantly decreased in the 1000 ppm group. The authors considered this effect as treatment related but not to be biologically significant or adverse since there were no similar changes in females. No other significant change was reported in serum chemistry. Urinalysis did not report treatment related changes. In males caecum weights relative to body weight in the 500 and 1000 ppm groups were statistically significantly increased. Weights of brain, heart and testis, relative to body weight, were also statistically increased in males from the 1000 ppm group. In females weights of adrenals glands, brain, caecum, heart, kidney, liver,

lung, ovary and spleen, relative to body weight, were significantly increased in the 1000 ppm group. The authors did not considered them to be adverse because they were not associated with any other effects (not precisely identified) or with histopathological lesions. According to the CEF Panel, taking into account the increase in caecum weights in males in the 500 ppm group, a No-Observable-Adverse-Effect-Level (NOAEL) of 250 ppm (18 mg/kg bw/day) can be identified.

In a 28-day study, groups of one female and one male Beagle dogs were administered 0, 250, 500, 1000, and 1500 ppm of CPC orally in the diet, corresponding to an average consumption of approximately 0, 8, 8, 16 and 20 mg CPC/kg bw/day for males, and to 0, 7, 11, 15 and 11 mg CPC/kg bw/day for females, respectively (Charles River Laboratories, 2006b). Observations performed included clinical observations, body weights and feed consumptions, haematology and coagulation parameters, clinical chemistry, urinalysis, organ weights and histopathology. In both sexes administration of 1500 ppm CPC resulted in abnormal stool (soft or watery). In this dose group animal body weights and feed consumption decreased significantly throughout the study duration. Due to these effects, the average consumption of CPC by animals in the 1500 ppm group, more pronounced in females, was comparable to the animals allocated to the 500 ppm group. In males the average consumption of CPC did not differ amongst animals allocated to the 1000 and 1500 ppm groups or amongst animals allocated to the 250 and 500 ppm groups. These findings suggest that a full dose-dependent exposure could not be achieved in this study. No haematological or coagulation changes related to the treatment were reported. Alanine aminotransferase activity was increased in males from the 250 and 1000 ppm group and females showed an increased dose-dependent trend in ALT activity from the 250 ppm group onwards. These changes were considered as not adverse by the authors since no histopathological lesions in the liver were associated with this change. No changes in urinalysis parameters and organ weights were reported. Upon histopathology examination, bilateral vacuolation of the tubular epithelial cells of the straight collecting ducts in the medullar rays was reported in females from the 1500 ppm group. The vacuolated cells contained multiple clear cytoplasm vacuoles varying in size from 2 to 8 microns in diameter approximately. Male animals in the same dose group did not show these lesions nor did the animals, females and males, from the other dose groups. The authors considered the vacuolisation effect in females as an incidental background finding based on the absence of a similar finding in males from the same group and in the animals from the 250 ppm group The CEF Panel considered however that no conclusions can be drawn from this study since as a result of treatment with CPC feed intake was strongly diminished, not allowing establishment of a dose-response. Furthermore, the number of animals was insufficient to allow characterisation of the observed effects.

In a 90-day study, groups of four Beagle dogs of both sexes were administered 0, 250, 375 and 1000/500 ppm of CPC orally in the diet, corresponding to an average consumption of approximately 0, 8, 12, 14 and 17 mg CPC/kg bw/day for males, and to 0, 8, 11, 17 and 17 mg CPC/kg bw/day for females, respectively (Charles River Laboratories, 2006c). Observations performed included clinical observations, physical examinations body weights and feed consumptions, ophthalmic examinations, cardiology evaluations, neurological examinations (home cage behaviour, out of cage behaviour, reflex activity, and postural reactions). Further observations included haematology and coagulation parameters, serum chemistry, urinalysis, organ weights and histopathology on control animals and animals from the 1000 ppm group. Body weight gains and feed consumption decreased in male animals from the 375, 500 and 1000 ppm groups as compared to controls. Thin body conditions were observed in one male in each group starting at a dose of 375 and onwards and in females from the 100/500 ppm group. Because of these effects in male animals from the 1000 ppm group administration of CPC was stopped from study day 29 to study days 42/41 (males/females). After this period dosing with CPC at 500 ppm was continued until the end of the study. Body weights were no longer statistically significant different from the controls at this dose. No effect on body weights were reported in female animals. Across all groups administration of CPC resulted in abnormal stools (soft, watery or mucoid aspect) in both sexes. The average amount of CPC consumed by males and females animals from the 500 and 1000 ppm doses were similar (14 vs. 17 mg/kg bw/day and 17 vs. 17 mg/kg bw/day, respectively). No physical, ophthalmology or neurological changes related to the treatment were reported. Upon cardiology evaluations no differences were noted for heart rate, RR interval, PR

interval, QRS duration, QT interval or QTc, except on study day 86. On study day 86 RR interval was statistically significant longer in males from the 375 ppm group and in females from the 375, 500 and 1000 ppm groups as compared to controls. The mean RR interval differences ranged from 14 to 22 milliseconds. These changes were not considered toxicologically significant by the authors. Haematological examination showed dose-dependent significant decreases in red blood cell counts, haemoglobin and haematocrit concentrations of male animals. Platelet counts were also decreased in males across all treated groups although not reaching statistical significance. Activated partial thrombin time was significantly decreased in females from almost all treated groups and reticulocyte levels were consistently increased in those animals. Haemoglobin levels and hematocrit values were significantly decreased in females from the 1000 ppm group. Monocytes were decreased in females from the 375, 500 and 1000 ppm groups. Some of the changes were considered by the authors as incidental and not related to CPC administration because they were not dose-dependent, were gender specific and inversely related. The presence of reticulocytes however was considered by the authors as suggesting a dose-dependent regenerative anaemia, most likely related to decreased feed consumption. The CEF Panel considered that taken together the haematology findings reported in male and female animals starting from the 375 ppm group suggest a treatment related effect on the blood homeostasis which is directly or indirectly related to CPC treatment. Cholesterol levels in males from the 250 ppm and onward groups were decreased although changes became significant at different study days. Other changes on urea nitrogen, aspartate aminotransferase, sorbitol dehydrogenase, creatinine and inorganic phosphorus were also reported in those animals. There were no changes reported as treatment related in the urinalysis parameters measured. No abnormal organ weights were reported in females. In males from the 375 ppm group and onwards reduced absolute or relative weights on epididymis, livers and thymus were reported. The authors considered these changes as not dose-dependent, and not supported by histopathology findings and thus they were not treatment related. Upon histopathology examination none of the few findings reported were considered by the authors as related to CPC administration (infiltration of mononuclear cells in the brain, haemorrhage and neutrophilic infiltration in the rectum, hyperplasia and haemorrhage of the lymph nodes, cysts presence in some animals, etc). However, the CEF Panel noted that the average amount of CPC consumed by males and females animals from the 500 and 1000 ppm doses was similar and thus a dose-dependency of effect on these two groups cannot be expected. Furthermore, the CEF Panel noted that the lack of histopathology findings to discard the relevance of the reduced weights of some organs identified in this study cannot be argued since histopathological examinations were only performed on animals from the control and the 1000 ppm groups. Taking into account the lack of full dose-response and the palatability issue, the CEF Panel considered that, the study was not suitable for the derivation of a NOAEL. In summary, the CEF Panel had access to information on subchronic toxicity studies on CPC. The most recent information submitted by the applicant consisted of a 14-day palatability study, a 28-day and a 90-day toxicity studies in Sprague-Dawley rats, as well as a 28-day and a 90-day toxicity studies in Beagle dogs. Other available subchronic studies were either insufficiently described or tested mixtures of CPC with other compounds, which did not allow clear conclusions on the safety of CPC to be drawn.

From the more recent 90-day toxicity study in Sprague-Dawley rats, the CEF Panel could identify a NOAEL of 18 mg/kg bw/day in rats, based on increased caecum weights noted in males. The CEF Panel considered the increase in caecum weight as relevant for risk characterisation of Cecure®. An increase in caecum to body weight ratios has been consistently positively correlated with increased dietary levels of CPC in sub-chronic rat studies (Redfield Laboratories, 2001b; Procter & Gamble, 1979; USAEH-HT, 1969; BIBRA, 1988; Charles River Laboratories, 2006a). Furthermore, in one of these studies it was noted that as the concentration of CPC chloride increased, the total number of microorganisms in the caecal contents decreased in both sexes (USAEH-HT, 1969). An increase in caecum weight in animals has also been associated elsewhere with modification on the composition of the intestinal microbiota (Licht et al., 2006) and therefore the CEF Panel considered that the possibility of a potential similar effect of CPC occurring in the gastrointestinal microflora of humans should not be disregarded.

2.1.3.3 Chronic toxicity and carcinogenicity

No studies specifically done with Cecure® were available to the CEF Panel addressing this parameter. Two chronic toxicity studies of 6 months and 1 year in which doses from 5 to 75 mg/kg bw/day of CPC were administered by gavage to animals (species not described) were reported (Genco, 1995). Significant decreases in body weight and body weight gain at doses of 40 and 75 mg/kg bw/day were reported in animals of both sexes and in some animals at 15 mg/kg bw/day dosage. At necropsy, gastrointestinal irritation was observed, manifested as thickening of the stomach mucosa.
BIBRA summarised a carcinogenicity study done on rats with CPC (BIBRA, 1988) as follows:

Groups of 10 rats of both sexes (species not identified) were administered CPC incorporated in a vinyl-copolymer (no more details) at dietary levels providing 7 or 35 mg/kg bw/day of CPC in the diet for one year. No clinical effects (unspecified) or blood changes were not reported nor were there any microscopic abnormalities in the major tissues analysed (unspecified). BIBRA mentions the limited utility of this study to assess carcinogenicity given the small number of animals and tissues examined.

2.1.3.4 Reproductive and developmental toxicity

No studies specifically done with Cecure® or CPC were available to the CEF Panel addressing this parameter.
BIBRA describes a reproductive/developmental toxicity study done on rats (strains not identified) fed CPC incorporated in a vinyl-copolymer (no more details), as follows:

Groups of 4 female rats (strain not described) were fed 7 or 35 mg/kg bw/day CPC for 3 months prior to mating and throughout pregnancy and lactation (BIBRA, 1988). At weaning, offspring were given the same diet as their mothers for 3 months prior to mating and throughout pregnancy and lactation. Third-generation offspring were again fed the CPC diet and mated after 3 months. Fertility and the incidence of malformations were within normal limits in each generation.

Groups of 15 pregnant New Zealand SPF rabbits were gavaged with 0, 2.5, 12.0, or 100 mg/kg bw CPC containing 1/9 domiphen bromide (0, 0.28, 1.33, and 11.08 mg/kg bw respectively), from day 7 to day 18 of gestation (Procter & Gamble, 1979). Most of the dams in the 100 mg/kg bw/day group died. The authors decided to create two new groups in which the six remaining untreated dams for each of the two high-dose groups were given CPC at 25 mg/kg bw or a combination of 25 mg/kg bw CPC and 2.8 mg/kg bw domiphen bromide daily from day 7 to day 18 of gestation. Two more dams from the 12.0 mg/kg bw/day and 25 mg/kg bw/day CPC died at the end of the study. Necropsy examination of these dams revealed severe irritation of the gastrointestinal tract, accompanied by diarrhoea and gastric ulceration. Weight losses were reported in animals from the new two groups associated with observed anorexia. There were aborted foetuses in the 2.5 mg/kg bw/day (one), 12.0 mg/kg bw/day (two), and 25 mg/kg bw CPC and 2.8 mg/kg bw domiphen bromide (two) groups. The foetus losses were associated with maternal toxicity which included (no more details) anorexia and weight loss. No differences were reported by the authors in the average numbers of corpora lutea or resorptions, but the high dose group (25 mg/kg bw/day) without domiphen bromide showed a higher incidence of resorptions associated by the authors to the maternal toxicity. Also differences in the number of implants were reported in the 25 mg/kg bw/day group and from the high-dose group containing domiphen bromide, but they were considered by the authors as not treatment-related since exposure occurred after implantation. In the dams of these two groups there were also reduced numbers of live foetuses reported but they were associated with maternal toxicity. Female foetal weights were significantly lower than controls in the high-dose CPC group. No differences were reported in the average numbers of foetuses of both sexes in the remaining lower concentration groups, nor were there reported differences in the foetal soft tissue or skeletal abnormalities. The authors conclude that the test materials were increasingly toxic to the dams and indirectly to the embryos or foetuses as the doses were increased, but none of the toxic effects were considered developmental. The dose of 25 mg/kg bw/day was considered by the authors as a non-effect dose for developmental effects.

Several deviations and adjustments in this study to compensate for the initial death rates at the highest dose administered and the high rate of maternal toxicity reported do not allow valid conclusions from this study to be drawn with respect to reproductive toxicity. Furthermore, the relevance of this publication to evaluate the safety of CPC is questionable, given that it was administered as a mixture with another compound.

2.1.3.5 Genotoxicity

Information on Cecure® genotoxicity is provided in two unpublished studies submitted by the applicant. In these studies Cecure® solution, as described in paragraph 1.1, was evaluated for the induction of reverse mutations in bacteria and chromosomal aberrations in cultured mammalian cells. Both studies were performed in compliance with Good Laboratory Practice, following the most recent OECD Test Guidelines.

In the bacterial reversion assay (Next Century Inc., 2002), Cecure® solution was tested in the *Salmonella typhimurium* strains TA1535, TA97a, TA98, TA100 and with *Escherichia coli* WP2 uvrA pKM101 in a plate incorporation assay, with and without metabolic activation by Aroclor-induced rat liver S9 at the following concentrations: 5, 10, 50, 100, 500, 1000, 2500 and 5000 μg Cecure®/plate (equivalent respectively to 0.05, 0.1, 0.5, 1, 5, 25, 50 μg CPC/plate). Deionised water was used as solvent. Based on the toxicity observed in the first trial, in the repeat test the following concentrations were evaluated: 5, 10, 50, 100 and 500 μg Cecure®/plate (equivalent respectively to 0.05, 0.1, 0.5, 1 and 5 μg CPC/plate) without S9; 100, 500, 1000, 2500, 5000 μg Cecure®/plate (equivalent respectively to 1, 5, 10, 25 and 50 μg CPC/plate) with S9. In both trials, all concentrations were tested in triplicate. Treatment with Cecure® was toxic in the absence of S9 at concentrations ≥ 1000 μg Cecure®/plate (equivalent to 10 μg CPC/plate). No treatment related increase of revertant colonies was observed with or without S9 in any tester strain.

The cytogenetic assay was performed with Chinese hamster ovary cells (CHO-K_1) (Next Century Inc., 2001). Cecure® was tested for clastogenicity using duplicate cultures and scoring structural chromosomal aberrations in one hundred metaphases per culture. The following dose levels were selected for microscopic analysis: 100, 500 and 1000 μg /ml (equivalent to 1, 5 and 10 μg CPC/ml) in the first experiment with 3 h treatment ± S9 and harvest at 20 h; 50, 100 and 250 μg/ml (equivalent to 0.5, 1 and 2.5 μg CPC/ml) in the second experiment with continuous (20 h) treatment without S9, and 250, 500 and 1000 μg/ml (equivalent to 2.5, 5 and 10 μg CPC/ml) in the repeat test with S9. Higher doses resulted in excessive toxicity, assessed as percent confluence. In both experiments, treatment with Cecure® did not increase the frequency of aberrant cells or structural chromosomal aberrations. No increase in polyploid cells was observed either.

Supplementary information on the genotoxicity of CPC is provided by the following published studies:

In a screening of pharmaceutical drugs, negative results were obtained with a CPC preparation (Cepacol) in tests for mitotic non-disjunction and crossing-over in *Aspergillus nidulans* (Bignami et al., 1974). No further details are given.

Negative results with Cepacol were reported in a screening of 140 health-related agents in the *Tradescantia*-micronucleus assay. The test material was applied by liquid absorption through the stem at the dose of 0.5 – 1 tablet (Ma et al., 1984). No further details are available from this study.

Negative results were also obtained with a xerox reprographic toner containing 2 % of CPC in the following battery of genotoxicity tests: i) bacterial reversion (Ames) test with *Salmonella typhimurium* TA1535, TA1537, TA1538, TA98, TA100 (from 0.5 to 1,000 μg toner/plate, with and without S9); ii), mouse lymphoma forward mutation test at the *tk* locus (from 31.1 to 400 μg toner/ml, with and without S9); iii) sister chromatid exchanges in CHO cells (from 0.16 to 100 μg toner/ml, with and without S9); iv) micronucleus in bone marrow of rats (10 males and 9 females) exposed by inhalation to the toner dust at 1343 mg/m^3 6h/day, 5 days/week for 13 weeks (Lin, 1999).

The Cepacol® mouthwash (0.05% CPC) tested positive in the *Drosophila* Wing-spot test, where increased mitotic recombination was observed in larvae feed with dry medium rehydrated with 75% and 100% Cepacol®. However, this result was attributed to the ethanol content in the mouthwash (16.8%), as pure CPC at the same concentration present in the mouthwash produced negative results (Rodriguez et al., 2007)

No relevant information is provided by the other published studies submitted by the applicant: in the study by Yamaguchi and Yamashita (1979) the effects of various detergents, including CPC, on the mutagenicity of autoxidised linoleic acid was evaluated in the *Salmonella typhimurium* reversion test. However, no data on CPC alone are presented. In the study by Smith and Lofty (1955), aberrant anaphase divisions, which are of questionable relevance for mammalian cells, were observed in meristems of *Vicia faba* grown in presence of CPC (0.001 – 0.02%).

2.1.3.6 Allergenicity, hypersensitivity and intolerance

No information on oral allergenicity was available. Skin irritation test in animals and humans have demonstrated that CPC is an irritant compound (Watanabe et al, 2002), also when inhaled.

2.2. Conclusions on toxicological studies

The available data indicate that CPC, tested as a working diluted solution in Cecure®, is not mutagenic in bacteria and not clastogenic in cultured mammalian cells. Negative results were also obtained in a gene mutation assay with CPC in mouse lymphoma cells and in limited tests in *Aspergillus*, *Tradescantia* and *Drosophila*. The CEF Panel noted that relatively low doses of the active component CPC were applied in the genotoxicity studies carried out on Cecure® solution; on the other hand, given the toxicity elicited by the test material, which can reasonably be attributed to the CPC content, testing of higher doses was not feasible. The CEF Panel also noted that the highest toxicity of the test material was elicited in the bacterial assay (without S9), as expected in view of the antimicrobial properties of CPC. Whilst this fact may to some extent limit the relevance of the bacterial assay for the assessment of the mutagenic potential of CPC, supporting information regarding this end-point are provided by the negative results in the mouse lymphoma assay reported in the study by Lin (1999). The Panel also noted that in addition to these consistently negative results the substance does not contain structural alerts for genotoxicity. Thus, based on the available evidence, the CEF Panel considered that there is no concern for genotoxicity.

The CEF Panel was not able to derive a toxicological reference value for CPC on the basis of the data provided by the applicant. There were no data on long term studies available nor were data available on reproductive and developmental toxicity on CPC. The only combined reproductive and developmental study with CPC for this evaluation was a rat study for which the data available to the CEF Panel was a summary reporting no effects on fertility or malformation of offspring up to the third generation. Other long term or repro/developmental toxicity studies available were either insufficiently described or tested CPC in combination with other compounds, although they showed negative results overall. The CEF Panel noted however that none of the findings reported on reproductive organs in sub-chronic toxicity studies were related to the treatment with CPC.
However, based on the data provided by the applicant the CEF Panel could establish a point of departure to assess the safety of Cecure® based on the NOAEL of 18 mg/kg bw/day derived from the 13-week toxicity study in Sprague-Dawley rats, in which an increase in caecum weights in male rats was observed. The CEF Panel considered the increase in caecum weight as relevant for risk characterisation of Cecure®. An increase in caecum to body weight ratios has been consistently positively correlated with increased dietary levels of CPC in sub-chronic rat studies (Redfield Laboratories, 2001b; Procter & Gamble, 1979; USAEH-HT, 1969; BIBRA, 1988; Charles River Laboratories, 2006a). Furthermore, in one of these studies it was noted that as the concentration of CPC increased, the total number of microorganisms in the caecal contents decreased in both sexes (USAEH-HT, 1969). An increase in caecum weight in animals has also been associated elsewhere with modification on the composition of the intestinal microbiota (Licht et al., 2006) and therefore, the

CEF Panel considered that the possibility of a potential similar effect of CPC occurring in the gastrointestinal microflora of humans should not be disregarded.

Taking into account the highest calculated potential exposure to CPC of up to 5.7 µg/kg bw/day at the mean and 17. 8 µg/kg bw/day at the 95th percentile of treated poultry consumption, the conservative margins of safety would be more than 3000 at the mean and more than 1000 at the 95th percentile, respectively, when compared to the NOAEL of 18 mg/kg bw/day identified by the CEF Panel in a 13-week toxicity study in Sprague-Dawley rats. The CEF Panel noted that these exposure estimates are worst cases since they assumed that all poultry carcasses, which are going to be consumed, have been treated with Cecure®. Concerning PG exposure arising from the use of Cecure® as processing aids, worst case exposure estimations are 0.5 µg/kg bw/day at the mean and 1.4 µg/kg bw/day at the 95th percentile. This estimated daily intake of PG is more than 22000 and 7000 times, respectively, below the Acceptable Daily Intake (ADI) of 0 - 10 mg/kg bw/day allocated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (WHO Technical report series No. 14, 1980) and 3500 times below the TDI of 5 mg/kg bw/day established by the Scientific Committee on Food (SCF) for PG.

Therefore, based on the toxicological data available, the estimated margins of safety (going from three orders of magnitude for CPC to four orders of magnitude for PG) and the conservative exposure estimates used to assess CPC exposure from consumption of poultry carcasses, the CEF Panel considers that there are no safety concerns for humans from the proposed use of Cecure® for removal of microbial surface contamination from raw poultry under the usage conditions specified in this opinion. The Panel was not able to assess total exposure of CPC from other potential dietary sources and non dietary sources.

3. The efficacy, i.e. does the use of the formulated product significantly reduce the level of contamination of pathogenic microorganisms

3.1. Introduction

According to the EFSA guidance document (EFSA, 2010), the use of substance(s) as decontaminating treatments will be regarded efficacious when any reduction of the prevalence and/or numbers of pathogenic target microorganisms is statistically significant as compared to the control (e.g. water) and, at the same time, this reduction has a positive impact on reduction of human illness cases (EFSA, 2010). Risk assessment studies on pathogenic microbial species (EFSA, 2011a, 2011b) have shown that mean reductions in microbial counts by even a 0.5 $\log_{10}$ unit may reduce consumer risks to a significant extent. In addition, the data show that there is a linear correlation between reductions in prevalence and reductions of consumer risks. Efficacy depends on a range of factors such as concentration of the decontaminating agent, contact time, temperature, mode of application, the microbial load of the surface, and other conditions of application.

3.2. Selection of studies for evaluation

As indicated, use of potable water solutions of Cecure®, containing < 1% CPC, the active ingredient, in combination with food-grade polypropylene glycol, was petitioned for approval as a decontaminant treatment in raw poultry meat. The process and results of the evaluation by the BIOHAZ Panel of the studies included in the dossier for the efficacy of Cecure® as a decontamination agent for raw poultry meat are as follows:

3.2.1. Criteria used by the BIOHAZ Panel for inclusion or exclusion of submitted studies

The following criteria were used by the BIOHAZ Panel in the selection of studies to be used in the evaluation of decontamination efficacy by Cecure®:

- The studies selected for evaluation should involve application on broiler carcasses, chicken skin, or skin-on chicken parts.
- The evaluation of the efficacy should focus on Cecure® treated samples *versus* water washed samples, or *versus* untreated controls.
- Target microbial groups to be considered are those listed in the petition by the applicant, which included "*Salmonella*, *Campylobacter*, *Listeria*, *Staphylococcus aureus*, *Escherichia coli* (including O157:H7), *Pseudomonas*, total coliforms, viruses, and other naturally occurring microorganisms on raw poultry carcasses."

3.2.2. Determination of the strength of evidence of selected for evaluation studies

The body of evidence selected (see below) from the studies submitted in the dossier was evaluated by the EFSA BIOHAZ Panel, taking into account whether the studies were done in the laboratory, in a slaughterhouse (industrial scale) or under pilot plant conditions, and whether they used inoculated or naturally contaminated poultry samples. Table 1 summarizes the weight given to the data from naturally contaminated *versus* inoculated samples and industrial-scale *versus* pilot-scale *versus* laboratory-scale studies. These criteria were used in two previous EFSA Opinions (EFSA, 2011b, 2011c) and were developed in the FAO/WHO report on Benefits and Risks of the Use of Chlorine-containing Disinfectants in Food Production and Food Processing (FAO/WHO, 2008). The results of this evaluation are shown in Tables 2 and 3.

Table 1: Relative strength of the contribution of study data to the general body of evidence, based on study type

Study type	Natural contamination	Inoculated studies
Industrial	High	Not applicable
Pilot-scale[a]	High[b]/medium	Medium[c]
Laboratory	Medium[c]	Low[d]

[a] Experiments using industrial equipment in non-industrial settings.
[b] If the pilot process is representative of the industrial process; otherwise, evidence makes a "medium" contribution to the body of evidence.
[c] Data would not be sufficient to inform a quantitative microbial risk assessment or to allow definitive conclusions on risk reduction.
[d] Data are indicative of a disinfectant effect that may be reproducible in practice, but individually do not allow definitive conclusions on risk reduction.

3.3. Results of the selection of studies for evaluation

- The application dossier included 15 peer reviewed published papers dealing with testing of CPC or Cecure® for decontamination (Table 2). Of the 15 peer reviewed papers submitted by the applicant for consideration; five were selected for consideration in evaluating the efficacy of CPC or Cecure® in poultry meat decontamination. Four papers were excluded because they did not evaluate poultry meat, two did not test CPC nor Cecure®, two used chicken skin as a model to evaluate bacterial attachment effects, one determined minimum inhibitory concentrations (MIC), one evaluated boneless/skinless poultry meat (whereas the application is for skin-on products), and one is a review of published literature with no additional data. Therefore, the peer-reviewed published papers selected and evaluated included two industrial, one pilot, and two laboratory level studies.

- Of the included peer reviewed studies, two were of high strength of evidence, one of medium strength and two of low strength of evidence (Table 2).

- The applicant also included in the application dossier 13 reports with data of in-house studies in support of the application for approval of Cecure® for use in the decontamination of fresh poultry products (Table 3). All, but two of these studies were considered in the evaluation of the efficacy of Cecure® against microbial contamination on fresh poultry; rejection of the two papers was based on the fact that contamination levels in controls were too low to allow quantification of decontaminating effects. One of the studies (No. 100901) considered potential sub-lethal injury of microorganisms due to treatment with the decontamination agent as it evaluated counts of inoculated microorganisms on thighs immediately after treatment and after 7 days of refrigerated storage.

- Eight of the in-house studies considered by the BIOHAZ Panel were conducted on broiler carcasses (Table 3) with natural contamination (except for study No. 060607 which involved inoculation with *E. coli* isolated from a carcass rinse, and study No. 060613 which involved inoculation with 6 $\log_{10}$ units of *Salmonella* and *E. coli*). Seven of the studies (Nos. 060302, 060401, 060407, 060510, 061010, 070414, and Waldroup et al., 1999) were of industrial scale, and three of pilot scale (Nos. 060607 and 060613, and Waldroup, 2000a); three studies were conducted on carcasses pre-chill (Nos. 061010, 070414, and Waldroup et al., 2000a), six on post-chill carcasses (Nos. 060407, 060510, 060607, 060613, 100901, and Waldroup et al., 1999), and two on pre- and post-chill carcasses (Nos. 060302 and 060401). For the studies reporting it, treatment temperature was at 21°-35°C. Treatment application involved: drenching in studies No. 060302, No. 060401, No. 060407, No. 060510, No. 060607, No. 060613, No. 0 61010, and No. 070414; spraying in studies Waldroup et al. (1999; 2000b); and, misting and dipping in Waldroup et al. (2000a). The antimicrobial was removed or rinsed in all studies, except for Waldroup et al. (1999), which involved 3 min dripping after treatment, and sampling by whole carcass rinsing.

- Seven of the in-house studies were classified as of high strength of evidence (Nos. 060401, 060407, 060302, 060510, 061010, 070414, and Waldroup et al., 1999), one of high/medium (Waldroup et al., 2000a), two of medium (Nos. 060607, 060613), and one of low strength of evidence (No. 100901) (Table 3).

- Studies evaluating the active ingredient (CPC) as well as the formulated product/preparation (Cecure®) were considered in the evaluation. The active ingredient, CPC, instead of the preparation, Cecure®, was tested in the published studies by Breen et al. (1997), Arritt et al. (2002) and Li et al. (1997), and in the in-house studies at Plant-1 by Waldroup et al. (1999) and by (Waldroup et al., 2000a); the plant-2 study of (Waldroup et al., 1999), and all other in-house studies evaluated Cecure®. In these studies it was not always clear whether the concentrations reported were for CPC or Cecure®.

- The studies submitted by the applicant and selected for evaluation by the BIOHAZ Panel included data for the following microorganisms: *Salmonella enterica* serovars, *Campylobacter* spp., *E. coli* (specific data on serotype O157:H7 were not included in the studies evaluated), *Pseudomonas*, and total coliforms, while most studies also evaluated changes in aerobic plate counts (APC).

Table 2: Peer reviewed papers submitted by the applicant and the reasons for exclusion/inclusion from the assessment

Reference	Inclusion in the assessment	Reason for exclusion	Industrial /pilot/ lab	Natural /inoculated	Microorganisms	Product group	Strength of evidence
Baker et al. (2010)	**YES**		**Industrial**	**Natural**	**APC**[9]	**Broiler carcass**	**High**
Beers et al. (2006)	**YES**		**Industrial**	**Natural**	**APC, *E. coli*, Coliforms, *Salmonella*, *Campylobacter***	**Broiler carcass**	**High**
Bereswill et al. (1999)	NO	Determined MIC					
Breen et al. (1995)	NO	Bacterial attachment to chicken skin and MIC					
Breen et al. (1997)	**YES***		**Lab**	**Inoculated**	***Salmonella* Typhimurium**	**Chicken skin**	**Low**
Arritt et al. (2002)	**YES****		**Lab**	**Inoculated**	***Campylobacter***	**Chicken skin**	**Low**
Pohlman et al. (2002)	NO	Not about poultry					
Singh et al. (2005a)	NO	Not about poultry					
Singh et al. (2005b)	NO	Not about poultry					
Singh et al. (2005c)	NO	Not about poultry					
Slavik et al. (1995)	NO	Not about CPC					
Waldroup et al. (2010)	NO	Review of previous results					
Bai et al. (2007)	NO	Boneless / skinless meat					
Li et al. (1997)	**YES**		**Pilot**	**Inoculated**	***Salmonella* Typhimurium**	**Broiler carcass**	**Medium**
Waldroup, 1992	NO	Not about CPC					

*on irradiated skin **skin model

[9] Although the application of the formulated product is intended to reduce the prevalence and/or numbers of target pathogenic microorganisms, data on the counts of non-pathogenic microorganisms, such as indicator microorganisms and total viable counts, should be provided and may also assist in the assessment of the overall efficacy of the proposed application.

Table 3: In-house studies submitted by the applicant and the reasons for exclusion/inclusion from the assessment

Reference	Inclusion in assessment	Reason for exclusion	Industrial /pilot/lab	Natural/ inoculated	Product group	Microorganisms	Strength of evidence
No. 060302	**YES**		**Industrial**	**Natural**	**Broiler carcasses**	**APC, Coliforms, *E. coli***	**High**
No. 060401	**YES**		**Industrial**	**Natural**	**Broiler carcasses**	**APC, *Salmonella*, *E. coli***	**High**
No. 060407	**YES**		**Industrial**	**Natural**	**Broiler carcasses**	**APC, Coliforms, *E. coli***	**High**
No. 060510	**YES**		**Industrial**	**Natural**	**Broiler carcasses**	**APC**[10]	**High**
No. 060607	**YES**		**Pilot**	**Inoculated**	**Broiler carcasses**	**APC, Coliforms, *E. coli***	**Medium**
No. 060613	**YES**		**Pilot**	**Inoculated**	**Broiler carcasses**	**APC, *Salmonella*, *E. coli***	**Medium**
No. 061010	**YES**		**Industrial**	**Natural**	**Broiler carcasses**	**APC, Enterobacteriaceae, *Pseudomonas*, Coliforms, *E. coli*, *Campylobacter***	**High**
No. 070414	**YES**		**Industrial**	**Natural**	**Broiler carcasses**	***E. coli***	**High**
No. 100901	**YES**		**Laboratory**	**Inoculated**	**Broiler thighs**	***S.* Typhimurium, *Campylobacter*, *E. coli***	**Low**
(Waldroup et al., 1992)	NO	Control contamination level too low to allow quantification of reductions	Industrial	Natural	Broiler skin	*Listeria monocytogenes*	
Waldroup et al. (1999)	**YES**		**Industrial**	**Natural**	**Broiler carcasses**	**APC, Coliforms, *E. coli*, *Campylobacter***	**High**
(Waldroup et al., 2000a)	**YES**		**Pilot**	**Natural**	**Broiler carcasses**	**APC, Coliforms, *E. coli*, *Campylobacter***	**High/ Medium**
(Waldroup et al., 2000b)	NO	Control contamination level too low to allow quantification of reductions					

[10] Although the application of the formulated product is intended to reduce the prevalence and/or numbers of target pathogenic microorganisms, data on the counts of non-pathogenic microorganisms, such as indicator microorganisms and total viable counts, should be provided and may also assist in the assessment of the overall efficacy of the proposed application.

3.4. Statistical significance and statistical methods used

Statistical analysis was performed in data of all experiments except for the in-house studies Nos. 060607 and 060613. Either analysis of variance (ANOVA) or generalised linear models (GLM) were used, followed by different methods for multiple comparisons (Newman-Keuls multiple range analysis, Duncan's multiple range test, Tukey-Kramer's test or means separation with least square means) to divide treatments into groups with significantly different means. These methods differ in their way to control for family-wise/experiment-wise error rates (i.e. incorrect rejection of the null hypothesis that all means are equal because of the inflated Type I error rate due to the multiple tests performed on the same set of data). Only the Tukey-Kramer's test is exact in this respect. An alternative choice would have been the use of Dunnett's test for pairwise comparisons with a defined control group (i.e. the untreated or water treated samples). Nevertheless, the log-reductions documented in the application are meaningful, and there is clear evidence of increasing effects with increasing concentrations of CPC. The BIOHAZ Panel therefore considers the documented reductions as biologically relevant.

3.5. Additional information provided by the applicant in the dossier

- The microbiological analytical methods used are variants of conventional culture methods. In addition a proprietary PCR method based on ribotyping (BAX, Dupont Qualicon) for *Salmonella* identification was used, which was stated as approved by AOAC. The applicant has extensively used Petrifilm® to enumerate coliforms and *E. coli*. These methods have been certified by AFNOR on the basis of EN ISO 16104.

- In one peer-reviewed study (Beers et al., 2006) the authors sampled visibly contaminated carcasses, although these carcasses would be trimmed according to EU Reg. 853/2004, Annex III, chapter IV, point 10 "The carcasses must not contain visible faecal contamination. Any visible contamination must be removed without delay by trimming or alternative means having an equivalent effect."

- Although quaternary ammonium compounds (QACs) such as CPC are bactericidal, the application dossier does not indicate the mode or mechanism of action.

- The Cecure® recycling system is designed to adjust automatically the concentration through the use of spectrophotometer. The dossier indicates that the recycled Cecure® solution has been demonstrated not to contain microbial contamination under the proposed conditions of use by referring to a study by Breen et al. (1995). Since in this report evidence is provided only for *S.* Typhimurium in pure cultures, this conclusion cannot be generalised to include other bacterial species or bacterial spores.

- It is stated in the dossier that there is no decrease in efficacy when using recycled Cecure® solution. This conclusion, however, is based on only one experiment conducted on ten carcasses. Moreover the test was performed not on the recycled solution but on the diluted stock solution of carcass drip and compared to CPC diluted in tap water; this may not represent the real situation and needs confirmation.

- Further, by analysing the results, it may be argued that the counts after treatment with Cecure® dissolved in carcass drip were more variable than the counts after treatment with Cecure® dissolved in water. Finally, the difference between the counts in the two groups was not tested for statistical significance.

3.6. Evaluation of studies

3.6.1. Peer reviewed papers

Two laboratory studies of low strength of evidence were conducted with chicken skin (Arritt et al., 2002; Breen et al., 1997). One involved inoculation of irradiated chicken skin with *Salmonella* Typhimurium (Breen et al., 1997) and found that 1% CPC for 1 min reduced counts by 0.6 $\log_{10}$

cfu/2.5 cm^2 of skin compared to the untreated control. In the other laboratory study (Arritt et al., 2002) chicken skin was inoculated with *Campylobacter jejuni*; sprayed at 8 psi, 21°C, with 0.1 and 0.5% CPC (1 and 5 mg/ml) for 1, 3 or 10 min, and stored for 10 min without rinsing. When treated for 1 min with 0.5% CPC, counts were reduced, compared to the untreated control, by 2.9 log_{10} cfu/ml.

One pilot scale study (Li et al., 1997) of medium strength of evidence evaluated, by spraying in a test chamber (30, 60, or 90 sec), CPC (0.1% at 22°C) on pre-chill broiler carcasses inoculated at 6 log_{10} units with *S.* Typhimurium; the carcasses were rinsed with water after treatment. Reductions achieved (15 carcasses and 3 replicates), in addition to water treatment, after a 30 sec treatment at 207, 345 and 827 kPa were 0.5, 0.5 and 0.9 log_{10} cfu/bird. After a 90 sec treatment, the corresponding reductions were 1, 1 and 1.6 log_{10} cfu/bird. Spraying pressure and time of exposure appeared to have no major influence on efficacy of CPC against *Salmonella*.

One industrial scale study (Baker et al., 2010) evaluated the effect of 0.3% (3 mg/ml) Cecure® under industrial conditions on whole carcasses post-chill and found that initial APC were reduced by 0.5 to >1.0 log_{10} units compared to the control. The other industrial scale peer-reviewed published study (Beers et al., 2006) considered in the evaluation, also examined broiler carcasses with natural contamination. The carcasses evaluated needed re-processing and were treated by spraying in a 4-linear foot cabinet after the last inside-outside bird washer and before the chiller with 0.5-0.7% Cecure® by spraying for 2-3 sec at line speeds of 11-70 birds/min in three plants. Samples were taken for analysis, after 45-60 sec of dripping, but prior to immersion chilling. Based on testing of 180-200 samples per treatment over a 12-week period, initial counts of APC, *E. coli*, coliforms and *Campylobacter* were 3.7-4.9, 1.8-3.1, 1.3-2.9 and 1.8-3.1 log_{10} cfu/ml, respectively. For the corresponding above microbial groups, mean (standard deviations were in the range 0.3-1.3) reductions by Cecure® compared to untreated controls, were 2.5-3.9, 1.6-2.9, 1.2-2.7 and 0.8-2.1 log_{10} units , respectively. Relative prevalence of *Salmonella* and *Campylobacter* were reduced by 50-95 and 90-97 %, respectively.

3.6.2. In-house studies

3.6.2.1. High strength of evidence

Pre-chill application. Of the four industrial scale studies with high strength of evidence conducted with broiler carcasses at the pre-chill level (Nos. 060302, 060401, 061010, and 070414), data of the pre-chill application component of study No. 060302, indicated that applying Cecure®, at 0.1 % (1 mg/ml CPC), reduced coliforms over untreated controls by 0.5 log_{10} cfu/ml. When applied at 0.9 and 3.8 litres/bird, corresponding reductions in *E. coli* were 0.5 and 0.7 and in APC 0.5 and 0.7 log_{10} cfu/ml respectively. In the same study, the Cecure® concentration of 0.6 % achieved reductions of 2.0 (coliforms, 0.9 litres/bird), 1.6 (coliforms, 3.8 litres/bird), 2.0 (*E. coli*, 0.9 litres/bird), 1.5 (*E. coli*, 3.8 litres/bird), 2.6 (APC, 0.9 litres/bird), and 2.1 (APC, 3.8 litres/bird) log_{10} cfu/ml. Volume applied per bird (0.9 *versus* 3.8 litres/bird) had no major influence, while the concentration of 0.6 % was more effective than the 0.1 %.

In study No. 060401, based on treatment with 2.2 litres/bird, reductions of APC and *E. coli*, at 35 birds/min treated with 0.05% Cecure® (0.5 mg/ml CPC) were 1.7 and 1.5, and 2.2 and 0.8 log_{10} cfu/ml over controls and water treatment, respectively, while *Salmonella* was present in 60% and 0% of water and Cecure® treated samples, respectively. Corresponding reductions at 70 birds/min were 1.0 and 0.8, and 1.5 and 0.2 log_{10} cfu/ml. APC and *E. coli* reductions (cfu/ml) at the concentration of 0.6 % (6 mg/ml CPC) were 3.5 and 3.3, and 2.6 and 1.3 (at 35 birds/min), 3.5 and 3.3, and 2.6 and 1.3 (for 70 birds/min); no *Salmonella* were recovered. The rate of carcass processing pre-chill (35 *versus* 70 per min) had no major influence on extent of microbial reductions.

In study No.061010, Cecure® at concentrations of 0.2 and 1.0 % (2 and 10 mg/ml CPC), applied at 3.8 litres/bird as a 60 sec drench at a rate of 70 birds/min, on broiler carcasses pre-chill, reduced over water-treated controls, APC, *Enterobacteriaceae*, *Pseudomonas*, coliforms, *E. coli* and *Campylobacter*

by 3 and 3.4, 1.7 and 1.8, 0.8 and 1.1, 1.6 and 1.7, 1.5 and 1.6, and 0.7 and 0.7 $\log_{10}$ units, respectively. Based on these data, under the conditions evaluated, Cecure® had similar efficacy against most of the microbial groups examined and at both concentrations tested; it was more effective against APC.

In study No. 070414 conducted at industrial level, Cecure® applied pre-chill on naturally contaminated broiler carcasses by drenching at 3.8 litres/bird and at a concentration of 0.6 % (6 mg/ml CPC) leads to a reduction of *E. coli* over control by 1.0 $\log_{10}$ cfu/ml.

Post-chill application. Five (Nos. 060302, 060401, 060407 and 060510, and Waldroup et al., 1999) industrial scale studies of high strength of evidence were conducted with broiler carcasses post-chill.

In the post-chill component of study No. 060302, Cecure® treatment applied at 0.1% (1 mg/ml CPC) reduced APC, coliforms and *E. coli* over untreated controls by 1.4 and 2.1, 0.5 and 0.9 and 0.4 and 0.8 $\log_{10}$ cfu/ml when applied at 0.9 and 3.8 litres/bird, respectively. In the same study, the concentration of 0.6% achieved reductions of 2.1 (APC, 9 litres/bird), 2.3 (APC, 3.8 litres/bird), 1.0 (coliforms, 0.9 litres/bird), 1.1 (coliforms, 3.8 litres/bird), 0.8 (*E. coli*, 0.9 litres/bird), and 0.9 $\log_{10}$ cfu/ml (*E. coli*, 3.8 litres/bird). Thus, application volumes of 0.9 and 3.8 litres/bird had no major effect on extent of reductions, which were generally higher at the concentration of 0.6 % compared to 0.1 %.

In the post-chill component of study No. 060401 reductions of APC and *E. coli*, on samples treated with 0.05 % Cecure® (0.5 mg/ml CPC) at 35 birds/min, were 3.0 and 2.4, and 1.9 and 1.5 $\log_{10}$ cfu/ml over controls and water treatment, respectively, while samples were *Salmonella* positives at rates of 20% and 0% in control and Cecure® treated products, respectively; corresponding reductions at 70 birds/min were 2.4 and 1.7, and 1.5 and 0.1 $\log_{10}$ cfu/ml, and treated samples were 20% *Salmonella* positive. APC and *E. coli* reductions at the concentration of 0.6% (6 mg/ml CPC) were 3.2 and 2.6, and 1.9 and 1.5 (for 35 birds/min), and 3.2 and 2.6, and 1.9 and 1.5 (for 70 birds/min) $\log_{10}$ units; Samples were positive for *Salmonella* at rates of 0% for control and treated products. The rate of carcass processing post-chill (35 *versus* 70 per min) had no major influence on extent of microbial reductions.

Study No. 060407 involved use of 0.4% Cecure® (4 mg/ml CPC) on broiler carcasses, at 70 birds/min. Reductions of APC, coliforms and *E. coli* on samples treated with 2.2, 3.0, 4.9 and 5.7 litres/bird were 2.1, 2.4, 2.3 and 2.0, 1.3, 1.8, 2.1 and 1.0, and 1.0, 1.7, 2.0 and 0.9 $\log_{10}$ units, respectively. Thus, volumes applied in the range 2.2 to 5.7 litres/bird were similar in efficacy.

In study No. 060510, treatments of 0.1, 0.2, 0.3, and 0.4% (1, 2, 3, 4 mg/ml CPC), applied at flow rates of 176-465 litres/min and treatment volumes of 0.25-0.70 litres/bird, reduced APC over untreated controls on post-chill treated carcasses in two replicates by 1.9-2.5 $\log_{10}$ units, indicating no major effect of concentration, flow rate and treatment volume in the ranges tested.

Waldroup et al. (1999) evaluated the decontaminating activity of CPC and Cecure®, each in one of two plants. The studies evaluated post-chill naturally contaminated broiler carcasses by spraying at 40-60 psi, 0.25-0.4 % CPC equivalent, at 0.06-0.12 litres/bird, on 70-90 birds/min for 2-3 sec exposure time. Samples were analysed after 3 min of dripping following treatment, without rinsing. In the study evaluating CPC (Plant-1), $\log_{10}$ reductions of APC, coliforms, *E. coli* and *Campylobacter* over untreated controls at concentrations of 0.25 % and 0.4 % were 0.9 and 2.0, 0.7 and 0.8, 0.7 and 0.9, and 0.4 and >2.0; *Salmonella* positive samples were too low to allow comparisons. In the study evaluating Cecure® (Plant-2), the treatment reduced counts of APC, coliforms, *E. coli* and *Campylobacter* over control samples to below the detection level of 1 cfu/ml.

3.6.2.2. High/medium strength of evidence

Pre-chill application. Waldroup et al. (2000a) evaluated Cecure® on naturally contaminated broiler carcasses pre-chill in a pilot plant. Reductions of APC, coliforms, *E. coli* and *Campylobacter* over water-treated controls by misting for 3 sec at concentrations of 0.2 % and 0.5 % (2 and 5 mg/ml CPC)

were 1.1 and 1.6, 0.7 and 0.9, 0.3 and 0.3, and 1.0 and 1.5 log_{10} cfu/ml, respectively. Corresponding reductions achieved when 0.2 and 0.5 % was applied by dipping for 10 sec were 1.3 and 2.6, 0.8 and 1.3, 0.2 and 0.6, , 1.5 and 1.6 log_{10} cfu/ml. Dipping may have achieved somewhat higher reductions compared to misting.

3.6.2.3. Medium strength of evidence

Post-chill application. Pilot scale study No. 060607 involved 0.2, 0.4, 0.6, 0.8 and 1.0% Cecure® (2, 4, 6, 8 mg/ml CPC) applied post-chill on carcasses inoculated with *E. coli* isolated from a carcass rinsate under laboratory conditions. Treatments applied at 0.2 and 0.4 % (0.95 litres/bird and at 151 litres/min), achieved reductions of APC, coliforms and *E. coli* over control samples of 1.4 and 2.2, 1.7 and 2.7, and 1.6 and 2.7, log_{10} cfu/ml, respectively. Corresponding reductions on water treated samples were 0.7 and 1.6, 0.8 and 1.9, and 0.5 and 1.8 log_{10} cfu/ml. Reductions of APC, coliforms and *E. coli* over untreated control samples at 0.6 % concentration applied at 0.95 litres/bird were 4.3, 4.0 and 4.0 log_{10} units. Corresponding reductions at 0.8% (0.95 litres/bird) were 4.9, 4.5 and 4.5 log_{10} units. Reductions at 0.6 % concentration applied at 1.9 litres/bird were 4.4 and 3.5, and 4.4 and 3.7 log_{10} units. At 0.95 litres/bird and 0.8 %, reductions were 4.5, 4.4 and 4.4 log_{10} units. Reductions at 1.0 concentration were similar to those at 0.6-0.8%. It appears that reductions increased with Cecure® concentrations up to 0.6 %, while volume applied per bird (0.95 and 1.9 litres/bird) and flow rate (151 and 303 litres/min) had no major influence in the ranges tested.

Study No. 060613 was conducted on *E. coli* and *Salmonella* inoculated (6 log_{10} units) broiler carcasses treated with Cecure® in a laboratory. At 0.95 litres/bird, concentrations of 0.2, 0.4, 0.6, 0.8, and 1.0 % CPC (2, 4, 6, 8 and 10 mg/ml CPC) caused reductions, over water-treated samples, of 2.2 and 2.8, 4.6 and 3.8, 4.8 and 5.0, 4.8 and 5.0, and 4.8 and 5.0 log_{10} cfu/ml, respectively. Reductions at 1.9 litres/bird at concentrations of 0.6, 0.8 and 1.0 % CPC over water-treated controls were 4.8 and 5.0, 4.8 and 5.0, and 4.8 and 5.0 log_{10} cfu/ml. These data also indicate that concentrations of CPC above 0.6% do not increase reductions, which are not affected by volume applied per bird in the range examined.

3.6.2.4. Other studies

Study No. 100901[11] evaluated effects of 0.5 and 1.0% Cecure® (5 and 10 mg/ml CPC) on the counts of *S.* Typhimurium, *Campylobacter* and *E. coli* inoculated on skin-on broiler thighs at 0 time after treatment and after 7 days of refrigerated storage. The inoculated thighs were treated by misting or drenching. On day 7, APC of control samples reached 8.7 log_{10} cfu/ml, while APC of samples treated with 0.5% and 1.0% Cecure® reached 7.0-7.7 log_{10} cfu/ml. As expected, counts of *E. coli*, coliforms, and *Campylobacter*, regardless of inoculation level (low or high) or percent Cecure® (0.5% or 1.0%), did not increase during refrigerated storage. It was concluded that there is no sublethal recovery of potential human pathogens during refrigerated shelf life of raw poultry products that have been treated with Cecure® under the proposed conditions of use.

3.6.3. Efficacy of decontamination of different CPC concentrations: comparison of in-house studies

In order to compare the results regarding the efficacy of decontamination obtained in different in-house studies, as an example in Figure 1, the relative log_{10} reduction of *E. coli* contamination in pre- and post-chill carcasses (treated *vs* water rinsed samples) at different Cecure® concentration is presented.

The graph shown in Figure 1 may suggest that for studies Nos. 060613, 060401 and 60607, and Waldroup (2000a) there is an increasing decontamination effect with increasing concentrations of CPC, especially between 0.2% and 0.6%. In study No. 060613, where five different concentration levels were tested (ranging from 0.2% to 1%), beyond 0.6% concentration no more increase on the *E. coli* reduction was detected. Nevertheless, this finding should be confirmed by comparing data from

[11] Low strength of evidence, not included in the table A in Appendix

more studies conducted under similar conditions. In fact it can be observed that, at 0.6% CPC concentration, there was high variability in reduction levels among the studies of which the results are shown in Figure 1. This can be due to the variable experimental conditions in the different trials such as the initial level of contamination that may influence considerably the reduction efficacy.

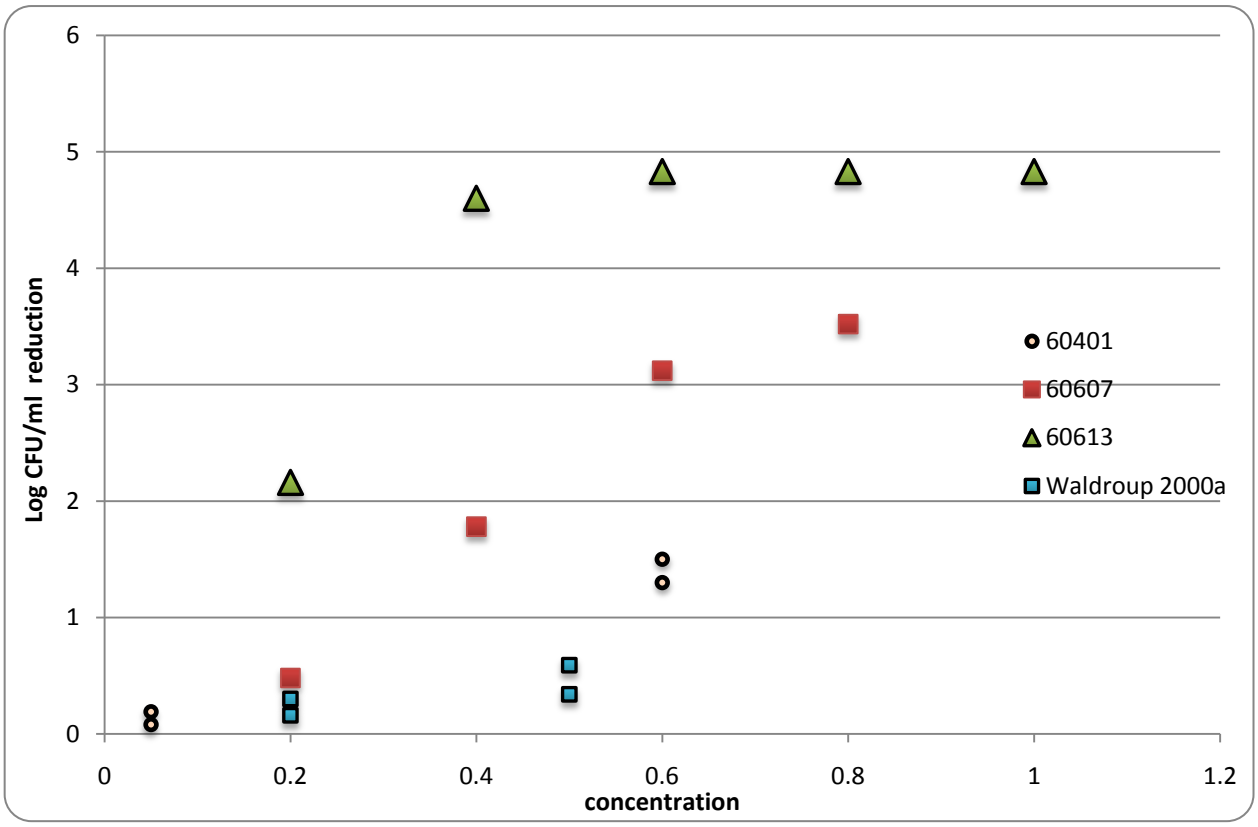


Figure 1: Reduction of *E. coli* contamination in pre- and post-chill carcasses (treated *versus* water rinsed samples) at different Cecure® concentrations in different in-house studies.

3.4. Conclusions

- Studies, considered in evaluating efficacy, included peer-reviewed published papers and in-house conducted studies. The studies considered were mostly of industrial scale with some of pilot and laboratory scale, and most evaluated naturally contaminated samples. Studies considered were classified as of high, high/medium, medium, and low strength of evidence.
- Both Cecure® and its active ingredient CPC were found efficacious in reducing pathogenic contamination on fresh broiler carcasses or chicken skin, when applied pre- and post-carcass chilling.
- Overall reductions of various pathogenic microorganisms on fresh broiler carcasses were in the range of <1.0 to 5.0 $\log_{10}$ units over untreated and water-treated controls. The lower reductions are generally associated with lower concentrations of CPC (e.g., 0.1% or 1 mg/ml CPC) applied to samples of low initial contamination, while the higher reductions were achieved with inoculated samples.
- Based on the results of a peer reviewed published paper:
    - Cecure®, applied as proposed in the application, reduced coliforms, *E. coli* and *Campylobacter* counts by 0.6-2.7 $\log_{10}$ units more than water treatment; *Salmonella* and *Campylobacter* relative prevalence was reduced by 50-95% and 90-97%, respectively.

- Based on data from in-house studies:
    - Most studies found Cecure® effective against APC, coliforms and *E. coli*, certain studies demonstrated activity against *Salmonella* and *Campylobacter*, while one study found it also effective against *Enterobacteriaceae* and *Pseudomonas*.
    - Evidence provided to support that the recycled Cecure® solution is as efficacious as the fresh solution was not adequate.
- Other conclusions
    - Considering the reduction of *E. coli* contamination in pre- and post-chill carcasses the results from four in-house studies may suggest that the concentration of 0.6% CPC was more effective than 0.1% CPC, while concentrations above 0.6 % CPC did not further increase microbial reductions. This should be confirmed by further experimental evidence.
    - Spraying pressure and time of exposure did not appear to have a major influence on efficacy of CPC against *Salmonella*.
    - Volume of solution applied (0.9-5.7 litres/bird) had no major influence on efficacy against coliforms and *E. coli.*
    - In the range tested (151 and 303 litres/min), flow rate had no major influence on efficacy.
    - The rate of carcass processing (35 *versus* 70 birds per min) had no major influence on extent of microbial reductions, both in pre- and post-chill Cecure® applications.

4. The potential emergence of reduced susceptibility to biocides and/or resistance to therapeutic antimicrobials linked to the use of the formulated product

4.1. Introduction

In relation to the potential emergence of reduced susceptibility to biocides and / or resistance to therapeutic antimicrobials linked to the use of the formulated product, the applicant has provided the following argumentations:

i. The potential human pathogenic organisms of concern on raw poultry would not be exposed to low levels of CPC in the production because:

   a. CPC is not utilized as a disinfectant or sanitizer in the production of live poultry;

   b. CPC binds so tightly to poultry offal that it is not bio-available to function as an antimicrobial, and, moreover, at EU level live poultry are not fed poultry meat and bone meal (as happens in the USA); and

   c. The concentrations to which potential pathogens will be exposed in the processing facility, for the purpose of microbial reduction, are very high. The applicant provides studies and trials showing that CPC residues in poultry by-product meat and bone meal is irreversibly tightly bound to these products in a biologically inactive form, which negates the concern for microbial resistance via this route (Table 25 and 26, page 154 of the dossier). Moreover, the applicant concluded from this observation that CPC bound to offal material has no antimicrobial activity (pg. 189).

ii. According to the EFSA guidance (EFSA, 2010), tests about development and dissemination of acquired reduced susceptibility to biocides and/or resistance to therapeutic antimicrobials in environmental microorganisms and a post-market evaluation are not required for Cecure®. This is because it is stated that the antimicrobial solution is neutralized prior to discharge of wastewater (as described in Section 7.1.3. and in the study entitled “Neutralization of Cetylpyridinium Chloride (CPC) with Activated Carbon” Section 2.5.2.7. of the dossier).

4.2. Comments on information provided

The active constituent of the formulated product is CPC. This substance is a cationic Quaternary Ammonium Compound (QAC), which is classified as an antiseptic agent, and has been used alone or in combination with other drugs for oral and health care. It is used also as a preservative in pharmaceutics and in cosmetics.

The following aspects of its proposed use as a decontamination agent in poultry in respect of the development of reduced susceptibility to biocides and / or resistance to therapeutic antimicrobials have not been taken into consideration by the applicants:

4.2.1. Development of resistance to biocides and therapeutic antimicrobials.

In a study of changes in antimicrobial susceptibility in *Pseudomonas stutzeri* following exposure to gradually increasing concentrations of chlorhexidine diacetate or CPC, strains were shown to develop stable resistance to these compounds and also reduced sensitivity to biocides such as triclosan and therapeutic antibiotics such as nalidixic acid, erythromycin and ampicillin (Tattawasart et al., 1999). A more recent study has demonstrated that exposure of a wild-type strain of *Serratia marcesens* to CPC resulted in the formation of a QAC-resistant mutant exhibiting 2- to 16- fold more resistance to biocides and antibiotics, including CPC, benzalkonium chloride, chlorhexidine gluconate, fluoroquinolones, tetracyclines and chloramphenicol than did wild-type strains (Maseda et al., 2009).

The mechanism of resistance involved mutational up-regulation of a multidrug efflux pump in the mutant strain.

As the primary mechanism of resistance development in relation to the use of CPC involves changes in efflux, it is unlikely that the use of this compound will result in the appearance and / or selection of microbes (both pathogens and non-pathogens) with new enzymatic-based resistance to therapeutic antibiotics. Nevertheless the possibility of mutational changes in global regulatory genes as a consequence of exposure to such compounds either at high concentrations or for long periods resulting in 'low level' resistance has not been fully considered. Horizontal transfer of such resistances from non-pathogens to pathogens is unlikely to occur by conjugation, which is for the most part confined to plasmids, transposons and integrons, but is theoretically possible, albeit at very low level, by natural genetic transformation of the mutated global regulatory genes (Courvalin, 2008; EFSA, 2010). Such considerations (i.e., changes in global regulatory genes) may also apply to the development of reduced susceptibility to biocides (Karatzas et al., 2008).

4.2.2. Selection for resistance to biocides and therapeutic antimicrobials.

An area of special concern is the potential for selection of bacteria carrying QAC determinants linked to therapeutic resistance determinants in mobile genetic elements.

QAC determinants are often associated with mobile genetic elements in different bacterial species, either in plasmids or class 1 integrons (Bjorland et al., 2003; Heir et al., 1998; Kazama et al., 1998; Machado et al., 2008; Paulsen et al., 1996; Poole, 2002). Class 1 integrons have been associated with antimicrobial resistance (AMR) in many Gram-negative organisms and are characterized by the presence of a 5′ conserved segment (5′-CS) containing an integrase gene (*intI1*), a 3′ conserved segment (3′-CS) containing *qacEΔ1* and *sul*1 genes, and a central *attI* recombination site which may contain a gene cassette(s) (Hall and Collis, 1998). For example, together with the *qacEΔ1* gene, the ampicillin, chloramphenicol/florfenicol, streptomycin, sulphonamides and tetracycline resistance genes in the well-characterised strain of *S.* Typhimurium definitive phage type (DT) 104 (=DT104) (Threlfall, 2000), reside within the 43-kb chromosomally-encoded *Salmonella* Genomic Island 1 (SGI-1). SGI-1 is transferable by mobilisation and transduction has become widely disseminated, both by the worldwide spread of DT104 and also by horizontal transfer, to at least 15 other serovars of *Salmonella* and also to *Proteus* spp. (Mulvey et al., 2006). Additionally, there are a number of examples of co-resistance to QAC and other antimicrobial agents by linkage on the same genetic unit such as plasmids, transposons or integrons, or on a combination of these (Antunes et al., 2007; Hegstad et al., 2010; Naas et al., 2001; Norman et al., 2008).

4.2.3. Target organisms

In the application it is stated that Cecure® will be used as a food processing aid to control the following organisms on raw poultry carcasses and skin-on poultry parts: *Salmonella*, *Campylobacter*, *Listeria*, *Staphylococcus aureus*, *Escherichia coli* (including O157:H7), *Pseudomonas*, total coliforms, viruses, and other naturally occurring microorganisms on raw poultry carcasses (page 12 of the dossier). Elsewhere in the document the applicant has stated that 'the potential human pathogenic organisms of concern on raw poultry, primarily *Salmonella* and *Campylobacter*, would not be exposed to low levels of CPC in the production' (section 6.2).

As far as can be observed from the dossier, there have been no tests undertaken to monitor the development of resistance to therapeutic antimicrobials and to biocides in the above organisms, either under the conditions of use or in wastewater.

4.2.4. Antimicrobial activity of CPC in organic material

The applicant concluded that CPC residues in poultry offal do not have antimicrobial activity; this negates the concern for the development of microbial resistance via these routes (Table 25 and 26 of the dossier). This opinion is supported in data from trials showing that when offal containing a certain concentration of CPC was added to a culture of one bacterial strain, the level of bacterial growth was

not different from that observed for the offal alone. The description of the experimental design is very concise and no specification on the CPC concentration tested is provided. Lack of details on the analytical method and strain source were also apparent. Samples with different amounts of CPC and several species with different MICs should have also been tested. In view of these shortcomings the results are not considered useful for supporting the absence of antimicrobial activity of CPC in offal material.

4.3. Conclusions

- Data to address the issue of the potential emergence of reduced susceptibility to biocides and/or resistance to therapeutic antimicrobials linked to the use of CPC have not been provided;
- Data to address the issue of the potential selection of isolates with reduced susceptibility to biocides and/or resistance to therapeutic antimicrobials linked to the use of CPC have not been provided;
- There are reports of the development/selection of resistance to biocides and therapeutic antibiotics in some organisms following exposure to CPC; the principal mechanism of resistance involves up-regulation of a multidrug efflux pump;
- In some pathogenic bacteria the dissemination of antibiotic resistance genes may be facilitated by the linkage between such genes and *qac* genes;
- The development of enzymatic resistance to biocides and/ therapeutic antimicrobials as a result of exposure to CPC is highly unlikely;
- As far as can be observed from the dossier, there have been no tests undertaken to monitor the development of resistance of the target organisms to biocides and/or therapeutic antimicrobials either under the conditions of use, or in wastewater;
- Evidence is not provided about testing potential contamination of Cecure® solution in the recycling process for all bacterial species;
- Data provided were not considered useful to support the absence of antimicrobial activity of CPC in organic material such as poultry offal.

5. The risk related to the release of the slaughterhouse and/or processing plant effluents, linked to the use of the formulated product, into the environment.

Basic data necessary for an assessment of the effect of Cecure® on the environmental compartments surface water, sediment and soil as well as for evaluation of the function of sewage treatment plants were either not provided by the applicant or could not be validated by the CEF Panel. The CEF Panel is aware that additional data relevant for the environmental risk assessment of Cecure® have been generated within the context of data requirements for biocides. However these data were not available to EFSA.

All the relevant data provided by the applicant were critically evaluated. When considered invalid or when data were lacking, additional information from the open literature was used to perform a preliminary environmental risk assessment for CPC from the use of Cecure®. The methodology of this assessment was based on the EU Technical Guidance Documents (TGD) for the risk assessment of biocides and industrial chemicals. The software system EUSES 2.1.1, developed for quantitative assessment of the risks of these chemicals to man and the environment, has been used to estimate the distribution of CPC in the environment and to calculate the predicted environmental concentrations (PECs). This is described in section 5.1. In section 5.2 the predicted no effect concentrations (PNECs) are presented, which in section 5.3 are compared with the PECs to characterise the risk for the different environmental compartments. The overall conclusions are presented in section 5.4.

5.1 Exposure assessment

5.1.1. Environmental releases

Cecure® can either be applied on pre-chilled raw poultry carcasses prior to immersion in a chiller or on post-chilled carcasses. In the pre-chilled application, after the carcasses exit the treatment cabinet, a drip tray running below the process line will collect liquid dripping from the carcasses and recycle it. CPC that drips from the carcasses for the remainder transit time to the chiller will not be captured and recycled. During immersion chilling, residues of CPC will be released into the rinse solution. The combination of CPC in the drip collected plus the mass of CPC found in the rinse water represents the mass of CPC that is not recycled.

In the post-chilled application after the carcasses exit the treatment cabinet, a drip tray running below the process line will collect liquid dripping from the carcasses and recycle it. The carcasses will then enter a potable water rinse cabinet, followed by an additional drip time before further processing or packaging. The combination of CPC in the rinse water plus CPC in the post-rinse drip represents the mass of CPC that is not recycled.

The applicant provided a study in which the mass of non-recycled material per carcass in both pre-chilled and post-chilled application at the concentration proposed in the dossier was determined. This total mass of CPC found in the drip and rinse solutions can be used to determine the environmental exposure of CPC from the application process. For the diluted Cecure® solution the total non-recycled mass was 7.8 and 8.9 mg per carcass for the pre-chilled and post-chilled, respectively.

To determine the total release from slaughterhouses, the same scenario is taken as in the Scientific Committee on Health and Environmental Risks (SCHER) opinion[12] by assuming that 50 tons of poultry meat is processed per day. This value is the threshold designated by the Integrated Pollution Prevention and Control (IPPC) Directive[13]. The European Pollutant Emission Register (EPER) database indicates that just a few slaughterhouses in the EU are above this limit. The very large

[12] Scientific Committee on Health and Environmental Risks (SCHER) and of Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR). Environmental impact and effect on antimicrobial resistance of four substances used for the removal of microbial surface contamination of poultry carcasses. The SCHER adopted this opinion at its 22nd plenary on 12 March 2008; The SCENIHR adopted this opinion at its 23rd plenary on 02 April 2008.

[13] Best Available Techniques in the Slaughterhouses and Animal By-products Industries, European IPPC Bureau, 2005

facilities, exceeding this production level, have specific environmental controls through the IPPC Directive. However, since the large majority of slaughterhouses in the EU are below this limit, the 50 tons meat per day limit is considered appropriate for a generic assessment.

Based on an average slaughter weight of a broiler of 1.2 kg, the number of carcasses processed per day is around 42000 carcasses per slaughterhouse. This would mean that around per day 0.33 and 0.37 kg CPC will not be recycled in pre-chilled and post-chilled application, respectively, and be disposed with the wastewater of the slaughterhouse.

Slaughterhouses in the European Union are divided between those that treat their waste water on-site and discharge directly to the local water course and those that discharge their waste water to the local municipal sewage treatment plants (STP) with the permission of their local sewerage company. The latter category carry out some pretreatment of the waste water on-site, usually to at least screen solid materials, although they may also undertake other treatments, like Dissolved Air Floatation (DAF).

The applicant did not provide data on the removal efficacy of the pre-treatment systems in the EU. In the FDA environmental risk assessment report (as provided by the applicant, publicly not available) it was assumed in all slaughterhouse waste water was treated in DAF generator with a removal efficiency of 99%. DAF uses very fine air bubbles to remove suspended solids. The suspended solids float to the top of the liquid and form foam, which is then skimmed off.

Based on the information given in the Refinery Best Available Technique Reference Document (IPPC, 2005) many large slaughterhouses in the EU use a DAF treatment plant to further treat their waste water prior to discharge to surface water. Alternatively, some large slaughterhouses have installed biological treatment plants which convert soluble and colloidal materials into biosolids. These are usually activated sludge plants which, depending on their capacity, may be preceded by sedimentation or DAF. At present insufficient information is available to validate the removal efficacy of an on-site DAF treatment plant or an alternative biological treatment plant. For this reason only the environmental risk related to discharge of waste water to the local municipal sewage treatment plants will be assessed.

5.1.2. Environmental fate and distribution

5.1.2.1. Degradation and transformation in the environment

No information on the degradability of CPC has been provided by the applicant. In a GDCh-Advisory Committee on Existing Chemicals report (BUA, 2003) it is mentioned that CPC is not readily biodegradable: in a closed bottle test (OECD 301D) 25 % was eliminated, based on the theoretical oxygen demand, in 28 days. Based on the chemical structure CPC is not expected to hydrolyse. In this preliminary risk assessment it is assumed that CPC is not readily biodegradable.

5.1.2.2. Adsorption

The applicant submitted an FDA assessment report (FAP 2A4736, environmental assessment) including data on the adsorption of CPC to DAF, sludge and soil, showing that the substance is very adsorptive. However, the description of the experimental setup is very limited and no specification on the DAF, sludge or soil type is given. Also the number of samples is very small. No details on the analytical method are given. Overall, it does not seem that any standard protocol is followed. As only one type of each matrix is examined the representativeness of the data is rather questionable. Furthermore, the results do not allow determination of a partitioning coefficient of CPC for any of the matrices. In view of these shortcomings the results are not considered useful for the environmental risk assessment.

Based on information found in the scientific literature, it appears that quaternary ammonium compounds (QACs) have a high affinity to adsorb on biosolids. Cumming *et al* (2011) investigated the sorption of QACs to humic acid and derived an adsorption coefficient (Kd) value of 52000 L/Kg for

CPC. According to the researchers the concentrations of the humic acid employed were comparable to suspended solids levels in the influent of STPs. The available information was however insufficient to validate the Kd value proposed by the applicant nor could the assumption be verified.

Based on an adsorption study with different sludge types with four QACs belonging to either monoalkonium or benzalkonium Ismail *et al*. (2010) concluded that the affinity depends mainly on the QAC structure rather than the sludge type and composition. QACs with a longer alkyl chain adsorb more than QACs with a shorter alkyl chain. The benzyl group further enhances the adsorption of QACs, but this effect diminishes as the alkyl chain length increases. The mechanism of QAC sorption on biosolids is complex and both hydrophobic and ionic interactions are probably in effect. For the most related compound to CPC, i.e. hexadecyl benzyl dimethyl ammonium chloride (C_{16}BDMA), an absorption capacity factor Kf of 20730 and 19000 L/kg determined in primary sludge and waste activated sludge, respectively (Ismail *et al*, 2010). These values are close to the Kd value of CPC determined with humic acid.

In the absence of better data, the Kf values found for C_{16}BDMA in primary and activated sludge can be used as first estimate of the solids-water partitioning coefficient in raw and activated sewage sludge of CPC to assess the fate in an STP. In order to predict the dissolved concentration in receiving waters, the solids-water partitioning coefficient for suspended matter is estimated to be 7000, based on the difference in organic carbon content between raw sludge and suspended matter.

5.1.2.3. Elimination in Municipal Sewage Treatment Plants

Based on the assumption that CPC is not volatile, not readily biodegradable and has a Kf value of 20730 and 19000 L/kg for primary sludge and waste activated sludge, respectively, the removal in a STP is determined by using the SimpleTreat module integrated in the EUSES model which is also used to determine the PECs in the environment (see section 5.1.3). The standard setting of the SimpleTreat module represents an STP with a primary settler (producing primary sludge), an aeration tank (containing activated sludge) and a solids liquid separator (recycling waste sludge back to aeration tank). The output indicates that the overall removal of CPC in a STP is approximately 81.5 % via adsorption to sludge. Approximately 18.5 % is expected to be released via the effluent to the water recipient

5.1.2.4. Bioaccumulation

No information has been provided on the bioaccumulation potential of CPC. Tolls et al. (1997) have published a critical review on the bioaccumulative properties of surfactants. It appears that the bioconcentration of cation surfactants is influenced by the headgroup structure. Possibly CPC is highly stabilised in the lipid bilayer of the membrane which slows down and limits the uptake rate in aquatic species. It is therefore concluded that CPC has a low potential to bioaccumulate and no assessment of secondary poisoning is deemed necessary. Knezovich et al. (1989) studied the bioaccumulation and tissue distrubution of hexadecylpyridinium bromide in the tadpole *Rana catesbeiana*, the fish *Pimephales promelas* and the clam *Corbicula fluminea*. Whole-body bioconcentrations factors (BCFs) were 13 ± 4, 22 ± 8, and 21 ± 7 (mean ± SD) for tadpoles, fish, and clams, respectively. Based on the findings, it can be concluded that the uptake of CPC in fish is limited. It is therefore concluded that CPC has a low potential to bioaccumulate and no assessment of secondary poisoning is deemed necessary. The low BCF values also indicate that CPC does not meet the B criterium of the PBT (Persistent, Bioaccumulative and Toxic) or vPvB (very persistent and very bioaccumulative) classification (Annex XIII of the REACH Regulation).

In view of the low vapour pressure, low bioaccumulation potential and the high adsorptive properties of CPC, indirect exposure of man via groundwater (as a source for drinking water), air and fish is expected to be negligible. Other exposure routes via uptake in plants, and transfer to milk and meat are difficult to assess as the current models used for quantitative estimation of these routes are driven by the octanol-water partition coefficient (K_{ow}), but this parameter cannot be regarded as characterizing the partitioning of ionic surfactants. It is however expected that also here a slow passage through the

membranes will strongly limit exposure of man via these routes (i.e. low absorption of CPC from soil pore water by plants and also low systemic absorption of CPC from the GI-tract in food production animals as well as in humans).

5.1.3. Predicted environmental concentration

The concentrations in the environment are calculated for pre-chilled or post-chilled application in a slaughterhouse without an on-site treatment, in which waste water is discharged to a municipal STP.

The fate and distribution of CPC in the municipal STP is modelled using the SimpleTreat model described in the European Union System for the Evaluation of Substances (EUSES, http://ecb.jrc.it/), which is also used for the exposure assessments of industrial chemicals, biocides and human drugs in the EU. The considered default STP has a size of 10000 inhabitants. Such a STP treats 2000$m^3$of waste water (house hold plus domestic waste water). In this scenario it is anticipated that the STP treats waste water from the slaughterhouse that already is included in the total treatment of 2000 m^3.

The other EUSES modules are used to determine the fate and distribution of CPC in the environment and to calculate the predicted environmental concentrations (PECs) in surface water and sediment, resulting from discharge of effluent (considering dilution and adsorption to suspended sediment), and in soil resulting from application of sludge to arable – and grassland. The PECs in effluent, surface water, sediment and soil resulting from the pre-chilled and post-chilled application are presented in Table 4. The effluent of the municipal STP will be discharged to a standard river with a flow rate of 18000 m^3/day, resulting in a dilution factor of 10.

Table 4: Calculation of $PEC_{effluent}$, PEC_{water}, $PEC_{sediment}$ and PEC_{soil} resulting from a pre-chilled and post-chilled application of CPC

Application	STP	PEC effluent (µg/L)	PEC surface water (µg/L)	PEC sediment (mg/kg_{dw})	PEC soil (mg/kg_{dw})
Pre-chilled	Municipal	30.5	2.76	19.3	5.66
Post-chilled	Municipal	34.2	3.09	21.7	46.4

5.2 Effect assessment

The aim of the effect assessment is to derive a predicted no effect concentration (PNEC) based on the available data. The fundamentals for derivation of PNEC are described in EU Technical Guidance Documents (TGD, 2003) as well as in REACH (REACH, 2008) and EFSA guidance (EFSA, 2010). For the aquatic compartment the minimal data requirements to characterise environmental hazard are acute toxicity tests performed with fish, daphnia and algae. The lowest concentration which causes mortality of 50% of the test organisms (LC_{50} value) or causes a measurable adverse effect in 50% of the test organisms (EC_{50} value) is divided by an assessment factor of 1000 in order to meet the uncertainties of extrapolation from mono-species laboratory tests to the aim to protect structure and function of the ecosystem. In principle, if the minimum data-set is not available, a PNEC cannot be calculated and the risk characterization cannot be performed.

5.2.1 Sewage treatment plant (STP)

5.2.1.1 Toxicity to microorganisms

According to TGD (2003) in assessing toxicity of the test substance to microorganisms, the aim is to ensure the function of the Sewage Treatment Plant and not the protection of individual bacterial strains.
Therefore tests with activated sludge are more relevant than those with single bacterial strains. The BUA report (BUA, 2003) states that at a concentration of 20 mg/L CPC the respiration rate was 50 % of

that shown by the control (IC_{50}) measured according to OECD 209. The non-biocidal concentration was 1.0 mg/L. This value is used for derivation of a PNECstp.

In addition a bacterial nitrification test showed that 15.2 mg/L of 1% Cecure® solution did not affect the nitrification. The test was performed in one sample tank only, and information on the density and the history of the inoculum is not given.The Panel therefore considered this information could not be used in the risk assessment

5.2.1.2 PNEC for microorganisms in STP

In principle a PNEC for micro-organisms in an STP cannot be derived as no reliable data have been provided by the applicant. Applying the non-biocidal concentration of 1.0 mg/L given in the report (BUA, 2003) and an assessment factor of 10 from TGD (2003), the following PNECstp is derived: PNECstp = NOEC / 10 = 1.0 mg/L / 10 = 0.1 mg CPC / L.

5.2.2 Aquatic compartment

5.2.2.1 Toxicity to algae

No data has been provided by the applicant on the toxicity of CPC to algae.

In the BUA report (BUA, 2003) on CPC an effect concentration (no further information) of 0.05 mg/L is presented, which may be used as an indication that algae are less sensitive than fish.

5.2.2.2 Toxicity to aquatic invertebrates

The applicant provided a table listing LC_{50} values for several shrimps and prawns (*Macrobrachium rosenbergii, Metapenaeus ensis, Panaeus japonicus, P. monodom, P. penicillatus, P. semisulcatus*), and one snail species (*Biomphalaria)*, The LC_{50} values, varying from 130 to 3100 µg/L, were all determined after short-term exposure duration (24-48 h). Furthermore, the raw data and methodological details were not provided. These data are also reported in the BUA report (BUA; 2003) as taken from a Japanese paper (written in Japanese with English summary). Therefore, the reliability cannot be assessed. The data therefore can be used only as a first indication that invertebrates may be less sensitive compared to vertebrates.

The table also contain LOEC values for C12-pyridinium for a clam (*Mercenaria mercenaria*) and an oyster (*Crassostrea virginica*) after an exposure period of 14 days, effects in the range of 10 – 50 µg/L. As the study report was not provided, the reliability of these data cannot be assessed. Furthermore, no argumentation is given how these data should be read across to CPC.

5.2.2.3 Toxicity to fish

No data has been provided by the applicant on the toxicity of CPC for fish. In the BUA report (BUA, 2003) a 96h LC_{50} value for carp of 10 µg/L is reported. In the same report a 96h LC30 value of 10 µg/L was described for the species *Catostomus* sp. and LC_0 values were given for goldfish, catfish bluegill sunfish as well as rainbow trout.

5.2.2.4 Toxicity to sediment organisms

No data has been provided by the applicant on the toxicity of CPC for sediment organisms.

5.2.2.5 PNEC for the aquatic compartment

In principle a PNEC for the aquatic compartment cannot derived as no reliable data has been provided by the applicant. Even with complementation by data found in the open literature, and assuming these data are reliable, the minimum data set is not complete. To make an estimation of the potential risk, a tentative PNEC of 10 ng/L is calculated based on the lowest LC_{50} value of 10 µg/L for carp applying an assessment factor of 1000.

5.2.2.6 PNEC for sediment-dwelling organisms

In the absence of data a tentative PNEC of 70 µg/kg dw is calculated using the Equilibrium Partitioning Method (EPM). In this screening procedure, described in TGD (2003) and in REACH guidance (2008) the concentration of CPC in pore-water (calculated from adsorption coefficients or log K_{ow}) is compared with the aquatic toxicity. In this approach it is assumed that only the substance dissolved in pore-water is bioavailable. As other routes of exposure (eg. ingestion of sediment) are neglected, in case of high adsorption to sediment (log K_{ow}>5) an additional assessment factor of 10 is added to the PEC/PNEC ratio. This is the case with CPC.

5.2.3 Terrestrial compartment

5.2.3.1 Predicted no effect concentration (PNEC) for soil-dwelling organism

No data on the toxicity of CPC to soil-dwelling organisms has been provided by the applicant, therefore a reliable PNEC cannot be derived. A 5d IC_{20} of 50 mg CPC/L has been described in the BUA report (BUA, 2003) for terrestrial nematodes. No standard tests are available. Therefore no valid PNEC can be derived. (Chronic) tests with soil organisms (preferably plants and soil microorganisms) should be performed.
Using the equilibrium partitioning approach described above for sediment-dwelling organisms, a tentative PNEC of 14 µg/kg dry weight (dw) is calculated**.**

5.3 Risk Characterisation

The PNECs for STP, surface water, sediment and soil are compared with the appropriate Predicted Environmental Concentrations (PEC), given in Table 4. If the PEC/PNEC ratio is > 1, a risk for the environment is apparent. If this is the case long-term tests should be performed, and on the basis of lower assessment factors (one chronic test: 100, two chronic tests: 50 and three chronic tests: 10) applied on the lowest NOEC from the long-term test the PNEC will be revised.

As the PNEC for sediment and soil is determined by the Equilibrium Partitioning approach, according to the REACH guidance for highly adsorbing / binding substances (log K_{oc} > 5) the PEC/PNEC ratios should be increased by a factor of 10 in order to take uptake via ingestion of sediment or soil into account. It should be borne in mind that this approach is considered only as a screen for assessing the level of risk to sediment/soil dwelling organisms. If with this method a PEC/PNEC ratio > 1 is derived, then (long-term) tests with sediment or soil organisms have to be conducted to support a refined risk assessment. In Table 5 the ratios between the PECs and PNECs in the different compartments are given.

Table 5: PEC/PNEC ratios for STP, surface water, sediment and soil, resulting from a pre-chilled and post-chilled application of CPC.

Application	Elimination	STP	Surface water	Sediment	Soil
Pre-chilled	Municipal	0.3	276	2760	4040
Post-chilled	Municipal	0.3	309	3090	4530

The results indicate that CPC poses no risk for municipal STP. However, for the environmental compartments surface water, sediment and soil, the PEC/PNEC ratios are ≥ 1. Therefore, based on the available data, the use of CPC in poultry slaughterhouses will pose a risk for the environment under the described processing conditions.

Due to the lack of data this risk assessment can only be seen as preliminary. It could be refined by revising the exposure data and / or by presenting valid effect tests for the compartments surface water, sediment and soil.

5.4 Conclusions of the environmental risk assessment

The data presented by the applicant are insufficient for a valid environmental risk assessment (ERA) to be carried out. Basic data necessary for an assessment of the environmental compartments surface water, sediment and soil as well as for evaluation of the function of sewage treatment plants were not provided or could not be validated.

Using data found in the open literature predicted no-effect concentrations (PNEC) for protection of the function of sewage treatment plants (STP), for surface water, as well as for sediment and soil, could be derived based on the risk assessment methodology applied to biocides and industrial chemicals (REACH / TGD).

A comparison of the predicted environmental concentrations (PEC) with the PNECs suggests that the use of CPC in poultry slaughterhouses does not pose a risk for the function of STP.

As there are no indications of a high bioaccumulation potential, no risk for birds and mammals in the environment via indirect exposure through the food-chain (secondary poisoning) has to be expected. In view of the low vapour pressure, low bioaccumulation potential and the high adsorptive properties of CPC, indirect exposure of man via groundwater (as a source for drinking water), air and fish is expected to be negligible.

Despite the fact that it is assumed that a large proportion of the active ingredient is recycled and that the product is assumed to be used in a specific application system claimed by the applicant, the risks for the environmental compartments surface water, sediment and soil are apparent.

More specific information on the fate and behaviour of CPC in on-site treatment plants is needed to determine the risk of CPC when used in slaughterhouses discharging their waste water direct to surface water via these treatment plants. Based on the current toxicity data, it is estimated that these plants need to reach a removal efficacy of more than 99.9% to exclude a risk for surface water.

In order to improve the robustness and reduce the uncertainty of the assessment, it is recommended to the applicant to provide more reliable data on the environmental fate and behaviour of CPC and to provide (long-term) tests relevant for the compartments surface water, sediment and soil. This additional information would reduce the level of uncertainty of the assessment. However, considering the high level of potential risk indicated by the present assessment, it is the opinion of the CEF Panel that the attainment of safe levels would be highly unlikely without suitable measures to reduce environmental emissions. An option would be to reduce exposure by achieving a high proportion of recycling during treatment in poultry slaughterhouses.

6. Conclusions and recommendations

6.1. Conclusions

ToR 1. The toxicological safety of the substance

- This opinion deals with the evaluation of the safety of Cecure® commercial product containing CPC as active ingredient, for removal of microbial surface contamination from raw poultry carcasses, under the usage conditions specified in this opinion. The safety of CPC when used as biocide is outside the remit of CEF Panel.

- The CEF Panel considered the data on identity, specifications of Cecure® and on stability of CPC under the intended conditions of use as sufficient.

- The available data indicate that CPC, tested as a working diluted solution in Cecure®, is not mutagenic in bacteria and not clastogenic in cultured mammalian cells. Negative results were also obtained in a gene mutation assay with CPC in mouse lymphoma cells and in limited tests in *Aspergillus*, *Tradescantia* and *Drosophila*. The Panel also noted that in addition to these consistently negative results, the substance does not contain structural alerts for genotoxicity. Thus, based on the available evidence, the CEF Panel considered that there is no concern for genotoxicity.

- The CEF Panel had access to information on subchronic toxicity studies on CPC. From a recent 90-day toxicity study in Sprague-Dawley rats, the CEF Panel could identify a NOAEL of 18 mg/kg bw/day in rats, based on increased caecum weights noted in males. The CEF Panel considered the increase in caecum weight as relevant for risk characterisation of Cecure® since CPC, the active component of Cecure®, has been suggested to decrease the total number of microorganisms in the caecal contents of rats of both sexes. This led to an increase in caecum to body weight ratios which was positively correlated with dietary levels of CPC. The CEF Panel considered that a potential similar effect of CPC on human gastrointestinal microflora should not be disregarded.

- The data presented by the applicant allowed the CEF Panel to perform a very conservative risk assessment. The potential exposure to CPC was estimated to be up to 5.7 µg/kg bw/day at the mean and 17.8 µg/kg bw/day at the 95th percentile of poultry consumption. The potential exposure to propylene glycol (PG) by mean and high level consumers such as young children would thus be up to 0.5 µg /kg bw/day at the mean and 1.4 µg/ kg bw/day at the 95th percentile of treated poultry consumption. These exposure estimates are worst cases since they assumed that all poultry carcasses which are going to be consumed have been treated with Cecure®. The Panel was not able to assess total exposure of CPC from other potential dietary sources and non dietary sources.

- Taking into account the highest calculated potential conservative exposure estimates to CPC from treated poultry consumption, the margins of safety for CPC would be more than 3000 at the mean and more than 1000 at the 95th percentile, when compared to the NOAEL of 18 mg CPC/kg bw/day, identified by the CEF Panel in a 13-week toxicity study in Sprague-Dawley rats. For PG, the margins of safety would be 22000 at the mean and 7000 at the 95th percentile, when compared to the Acceptable Daily Intake (ADI) of 0 - 10 mg/kg bw/day allocated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Furthermore, they would be 3500 times below the TDI of 5 mg/kg bw/day established by the Scientific Committee on Food (SCF) for PG.

- Therefore based on the toxicological data available, the estimated margins of safety (going from three orders of magnitude for CPC to four orders of magnitude for PG) and the conservative exposure estimates used to assess CPC exposure from consumption of poultry carcasses, the CEF Panel considers that there are no safety concerns for humans from the

proposed use of Cecure® for removal of microbial surface contamination from raw poultry under the usage conditions specified in this opinion.

- The CEF Panel is aware that additional data on the toxicology of CPC (including genotoxicity and reproductive toxicity) have been generated within the context of data requirements for biocides. However these data were not available to EFSA. If these data are made available to EFSA in the future, EFSA will take them into account.

ToR 2. The efficacy, i.e. does the use of the substance significantly reduce the level of contamination of pathogenic micro-organisms

- Both Cecure® and its active ingredient CPC were found efficacious in reducing contamination with pathogenic microorganisms on fresh broiler carcasses or chicken skin, when applied on pre- or post-chill.

- Overall microbial reductions achieved on pre- and post-chill treated samples were in the range of <1.0 to 5.0 log units over untreated and water-treated controls.

- Based on a peer reviewed published paper, reporting data from three industrial evaluations, Cecure®, applied as proposed in the application on carcasses needing reprocessing, reduced *Salmonella* and *Campylobacter* relative prevalence by 50-95% and 90-97 %, respectively.

- The documented microbial reductions by treatment with Cecure® are considered as biologically relevant.

- Additional data are needed to confirm that the concentration of 0.6% CPC was more effective than 0.1% CPC, while concentrations above 0.6 % CPC do not further increase microbial reductions. The minimum CPC concentration to be applied was not specified.

- Efficacy appeared not to be affected by volume of solution applied, flow rate, spraying pressure, rate of carcass processing, and time of exposure within the ranges examined.

- Evidence is inadequate to support that the recycled Cecure® solution is as efficacious as the fresh solution and does not accumulate resistant bacterial cells and/or spores.

- Data concerning possible accumulation of spores during recycling and reuse of solution were not provided.

ToR 3. The potential emergence of reduced susceptibility to biocides and/or resistance to therapeutic antimicrobials linked to the use of the substance

- Data to address the issue of the potential emergence of reduced susceptibility to biocides and/or resistance to therapeutic antimicrobials linked to the use of CPC have not been provided.

- Data to address the issue of the potential selection of isolates with reduced susceptibility to biocides and/or resistance to therapeutic antimicrobials linked to the use of CPC have not been provided.

- There are reports of the development/selection of resistance to biocides and therapeutic antibiotics in some organisms following exposure to CPC; the principal mechanism of resistance involves upregulation of a multidrug efflux pump.

- In some pathogenic bacteria the dissemination of antibiotic resistance genes may be facilitated by the linkage between such genes and the *qac* genes.

- The development of enzymatic resistance to biocides and/ therapeutic antimicrobials as a result of exposure to CPC is highly unlikely.

- Data provided were not considered useful to support the absence of antimicrobial activity of CPC in organic material such as poultry offal.

ToR 4. The risk related to the release of the slaughterhouse and/or processing plant effluents, linked to the intended use of the substance, into the environment

- Basic data necessary for an assessment of the risk of Cecure® for the environmental compartments surface water, sediment and soil, as well as for evaluation of the function of sewage treatment plants were not provided or could not be validated. Additional data relevant for the environmental risk assessment of Cecure® have been generated within the context of data requirements for biocides. However these data were not available to EFSA.

- Using data found in the open literature predicted no-effect concentrations (PNEC) for protection of the function of sewage treatment plants (STP), for surface water, as well as for sediment and soil, could be derived based on the risk assessment methodology applied to biocides and industrial chemicals (REACH/TGD).

- The PEC/PNEC ratios calculated indicate that the use of CPC in poultry slaughterhouses does not pose a risk for the function of sewage treatment plants. As there are no indications of a high bioaccumulation potential, no risk for birds and mammals in the environment via indirect exposure through the food-chain (secondary poisoning) has to be expected.

- In view of the low vapour pressure, low bioaccumulation potential and the high adsorptive properties of CPC, indirect exposure of man via groundwater (as a source for drinking water), air and fish is expected to be negligible.

- Despite the fact that it is assumed that a large proportion of the active ingredient is recycled and that the product is assumed to be used in a specific application system claimed by the applicant, risks for the environmental compartments surface water, sediment and soil are apparent.

6.2. Recommendations

- As requested in the guidance (EFSA, 2010), data addressing the potential emergence of and selection for reduced susceptibility to biocides and or resistance to therapeutic antimicrobials linked to the use of CPC should be provided by the applicant.

- The minimum CPC concentration applied for should be specified.

- To assess the safety and efficacy of recycling and reusing the substance, data about possible accumulation of bacterial spores, as well as data to support continuous efficacy of the recycled material should be collected.

- In order to improve the robustness and reduce the uncertainty of the environmental risk assessment, it is recommended that the applicant provides more reliable data on the fate and behaviour of CPC and provides (long-term) tests relevant for the compartments surface water, sediment and soil. However, considering the high level of potential risk indicated by the present assessment, it is the opinion of the CEF Panel that the attainment of safe levels would be highly unlikely without suitable measures to reduce environmental emissions. An option would be to reduce exposure by achieving a high proportion of recycling during treatment in poultry slaughterhouses.

7. Documentation provided to EFSA

- Dossier in Support of the Use of Cecure® as a Processing Aid for the Decontamination of Raw Poultry Products Pursuant to Art. 3(2) of Reg. 853/2004 of European Parliament and Council, March 2011 (revised in December 2011), submitted by SAFE FOODS CORPORATION
    - Annex A: Cecure® Approval Documentation
    - Annex B: Referenced journal articles and code of federal register
    - Annex C: Experimental protocols – reports from in-house studies
    - Annex D: Technical data sheets and attachments to environmental assessment
    - Annex E: Attachments to food additive petitions 2A4736 (FAP A) and 6A4767 (FAP B)
    - Toxicity studies, info on purity, impurities and dosage methods, on residues of CPC and updated data on consumer exposure assessment, submitted in October 2011

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APPENDICES

A. TABLE WITH DETAILED DATA OF CECURE® TREATMENT OF RAW POULTRY PRODUCTS OF THE IN-HOUSE STUDIES INCLUDED IN THE ASSESSMENT

Study	Target strains	Log_{10} red Treated/ untreated log CFU/ml	Log_{10} red Treated/ Water CFU/ml	Log_{10} red Water/ untreated CFU/ml	Statistical significance	Industrial/ pilot/lab	Natural contamination /inoculation	Meat products	Pre/post chill	% CPC	pressure	volume (litres / bird)	line speed (birds / min)
HIGH STRENGTH OF EVIDENCE													
60302	APC	0.45	NA	NA	Anova, Tukey's Kramer	industrial	natural	broiler carcass	Pre chill	0.1	NA	0.9	NA
60302	*E.coli*	0.51	NA	NA	Anova, Tukey's Kramer	industrial	natural	broiler carcass	Pre chill	0.1	NA	0.9	NA
60302	coliforms	0.45	NA	NA	Anova, Tukey's Kramer	industrial	natural	broiler carcass	Pre chill	0.1	NA	0.9	NA
60302	APC	0.68	NA	NA	Anova, Tukey's Kramer	industrial	natural	broiler carcass	Pre chill	0.1	NA	3.8	NA
60302	*E.coli*	0.51	NA	NA	Anova, Tukey's Kramer	industrial	natural	broiler carcass	Pre chill	0.1	NA	3.8	NA
60302	coliforms	0.45	NA	NA	Anova, Tukey's Kramer	industrial	natural	broiler carcass	Pre chill	0.1	NA	3.8	NA
60302	APC	2.59	NA	NA	Anova, Tukey's Kramer	industrial	natural	broiler carcass	Pre chill	0.6	NA	0.9	NA
60302	*E.coli*	2.04	NA	NA	Anova, Tukey's Kramer	industrial	natural	broiler carcass	Pre chill	0.6	NA	0.9	NA
60302	coliforms	1.99	NA	NA	Anova, Tukey's Kramer	industrial	natural	broiler carcass	Pre chill	0.6	NA	0.9	NA
60302	APC	2.15	NA	NA	Anova, Tukey's Kramer	industrial	natural	broiler carcass	Pre chill	0.6	NA	3.8	NA
60302	*E.coli*	1.54	NA	NA	Anova, Tukey's Kramer	industrial	natural	broiler carcass	Pre chill	0.6	NA	3.8	NA
60302	coliforms	1.61	NA	NA	Anova, Tukey's Kramer	industrial	natural	broiler carcass	Pre chill	0.6	NA	3.8	NA
60302	APC	1.4	NA	NA	Anova, Tukey's Kramer	industrial	natural	broiler carcass	post chill	0.1	NA	0.9	NA

Study	Target strains	Log_{10} red Treated/ untreated log CFU/ml	Log_{10} red Treated/ Water CFU/ml	Log_{10} red Water/ untreated CFU/ml	Statistical significance	Industrial/ pilot/lab	Natural contamination /inoculation	Meat products	Pre/post chill	% CPC	pressure	volume (litres / bird)	line speed (birds / min)
60302	*E.coli*	0.37	NA	NA	Anova, Tukey's Kramer	industrial	natural	broiler carcass	post chill	0.1	NA	0.9	NA
60302	coliforms	0.49	NA	NA	Anova, Tukey's Kramer	industrial	natural	broiler carcass	post chill	0.1	NA	0.9	NA
60302	APC	2.06	NA	NA	Anova, Tukey's Kramer	industrial	natural	broiler carcass	post chill	0.1	NA	3.8	NA
60302	*E.coli*	0.81	NA	NA	Anova, Tukey's Kramer	industrial	natural	broiler carcass	post chill	0.1	NA	3.8	NA
60302	coliforms	0.91	NA	NA	Anova, Tukey's Kramer	industrial	natural	broiler carcass	post chill	0.1	NA	3.8	NA
60302	APC	2.05	NA	NA	Anova, Tukey's Kramer	industrial	natural	broiler carcass	post chill	0.6	NA	0.9	NA
60302	*E.coli*	0.81	NA	NA	Anova, Tukey's Kramer	industrial	natural	broiler carcass	post chill	0.6	NA	0.9	NA
60302	coliforms	0.95	NA	NA	Anova, Tukey's Kramer	industrial	natural	broiler carcass	post chill	0.6	NA	0.9	NA
60302	APC	2.33	NA	NA	Anova, Tukey's Kramer	industrial	natural	broiler carcass	post chill	0.6	NA	3.8	NA
60302	*E.coli*	0.91	NA	NA	Anova, Tukey's Kramer	industrial	natural	broiler carcass	post chill	0.6	NA	3.8	NA
60302	coliforms	1.05	NA	NA	Anova, Tukey's Kramer	industrial	natural	broiler carcass	post chill	0.6	NA	3.8	NA
60401	APC	1.74	1.53	0.21	Anova, Tukey's Kramer	industrial	natural	broiler carcass	pre chill	0.05	NA	2.2	35
60401	*E.coli*	2.15	0.81	1.34	Anova, Tukey's Kramer	industrial	natural	broiler carcass	pre chill	0.05	NA	2.2	35
60401	APC	1.04	0.83	0.21	Anova, Tukey's Kramer	industrial	natural	broiler carcass	pre chill	0.05	NA	2.2	70
60401	*E.coli*	1.53	0.19	1.34	Anova, Tukey's Kramer	industrial	natural	broiler carcass	pre chill	0.05	NA	2.2	70
60401	APC	3.54	3.33	0.21	Anova, Tukey's Kramer	industrial	natural	broiler carcass	pre chill	0.6	NA	2.2	35
60401	*E.coli*	2.64	1.3	1.34	Anova, Tukey's Kramer	industrial	natural	broiler carcass	pre chill	0.6	NA	2.2	35

Study	Target strains	Log_{10} red Treated/ untreated log CFU/ml	Log_{10} red Treated/ Water CFU/ml	Log_{10} red Water/ untreated CFU/ml	Statistical significance	Industrial/ pilot/lab	Natural contamination /inoculation	Meat products	Pre/post chill	% CPC	pressure	volume (litres / bird)	line speed (birds / min)
60401	APC	3.54	3.33	0.21	Anova, Tukey's Kramer	industrial	natural	broiler carcass	pre chill	0.6	NA	2.2	70
60401	*E.coli*	2.64	1.3	1.34	Anova, Tukey's Kramer	industrial	natural	broiler carcass	pre chill	0.6	NA	2.2	70
60401	APC	2.98	2.37	0.61	Anova, Tukey's Kramer	industrial	natural	broiler carcass	post chill	0.05	NA	2.2	35
60401	*E.coli*	1.94	1.5	0.44	Anova, Tukey's Kramer	industrial	natural	broiler carcass	post chill	0.05	NA	2.2	35
60401	APC	2.36	1.75	0.61	Anova, Tukey's Kramer	industrial	natural	broiler carcass	post chill	0.05	NA	2.2	70
60401	*E.coli*	1.52	0.08	1.44	Anova, Tukey's Kramer	industrial	natural	broiler carcass	post chill	0.05	NA	2.2	70
60401	APC	3.23	2.62	0.61	Anova, Tukey's Kramer	industrial	natural	broiler carcass	post chill	0.6	NA	2.2	35
60401	*E.coli*	1.94	1.5	0.44	Anova, Tukey's Kramer	industrial	natural	broiler carcass	post chill	0.6	NA	2.2	35
60401	APC	3.23	2.62	0.61	Anova, Tukey's Kramer	industrial	natural	broiler carcass	post chill	0.6	NA	2.2	70
60401	*E.coli*	1.94	1.5	0.44	Anova, Tukey's Kramer	industrial	natural	broiler carcass	post chill	0.6	NA	2.2	70
60407	APC	2.14	NA	NA	Anova, Tukey's Kramer	industrial	natural	broiler carcass	post chill	0.4	NA	2.2	70
60407	*E.coli*	1	NA	NA	Anova, Tukey's Kramer	industrial	natural	broiler carcass	post chill	0.4	NA	2.2	70
60407	coliforms	1.3	NA	NA	Anova, Tukey's Kramer	industrial	natural	broiler carcass	post chill	0.4	NA	2.2	70
60407	APC	2.42	NA	NA	Anova, Tukey's Kramer	industrial	natural	broiler carcass	post chill	0.4	NA	3	70
60407	*E.coli*	1.67	NA	NA	Anova, Tukey's Kramer	industrial	natural	broiler carcass	post chill	0.4	NA	3	70
60407	coliforms	1.82	NA	NA	Anova, Tukey's Kramer	industrial	natural	broiler carcass	post chill	0.4	NA	3	70
60407	APC	2.3	NA	NA	Anova, Tukey's Kramer	industrial	natural	broiler carcass	post chill	0.4	NA	4.9	70

Study	Target strains	Log_{10} red Treated/ untreated log CFU/ml	Log_{10} red Treated/ Water CFU/ml	Log_{10} red Water/ untreated CFU/ml	Statistical significance	Industrial/ pilot/lab	Natural contamination /inoculation	Meat products	Pre/post chill	% CPC	pressure	volume (litres / bird)	line speed (birds / min)
60407	*E.coli*	1.99	NA	NA	Anova, Tukey's Kramer	industrial	natural	broiler carcass	post chill	0.4	NA	4.9	70
60407	coliforms	2.06	NA	NA	Anova, Tukey's Kramer	industrial	natural	broiler carcass	post chill	0.4	NA	4.9	70
60407	APC	2.01	NA	NA	Anova, Tukey's Kramer	industrial	natural	broiler carcass	post chill	0.4	NA	5.7	70
60407	*E.coli*	0.9	NA	NA	Anova, Tukey's Kramer	industrial	natural	broiler carcass	post chill	0.4	NA	5.7	70
60407	coliforms	0.95	NA	NA	Anova, Tukey's Kramer	industrial	natural	broiler carcass	post chill	0.4	NA	5.7	70
Waldroup 1999	APC	0.9	NA	NA	yes	industrial	natural	broiler carcass	post chill	0.25	40-60 psi	0.06-0.12	70-90
Waldroup 1999	*E.coli*	0.7	NA	NA	yes	industrial	natural	broiler carcass	post chill	0.25	40-60 psi	0.06-0.12	70-90
Waldroup 1999	coliforms	0.7	NA	NA	yes	industrial	natural	broiler carcass	post chill	0.25	40-60 psi	0.06-0.12	70-90
Waldroup 1999	*Campylobacter*	0.4	NA	NA	yes	industrial	natural	broiler carcass	post chill	0.4	40-60 psi	0.06-0.12	70-90
Waldroup 1999	APC	2	NA	NA	yes	industrial	natural	broiler carcass	post chill	0.4	40-60 psi	0.06-0.12	70-90
Waldroup 1999	*E.coli*	0.9	NA	NA	yes	industrial	natural	broiler carcass	post chill	0.4	40-60 psi	0.06-0.12	70-90
Waldroup 1999	coliforms	0.8	NA	NA	yes	industrial	natural	broiler carcass	post chill	0.4	40-60 psi	0.06-0.12	70-90
Waldroup 1999	*Campylobacter*	>2	NA	NA		industrial	natural	broiler carcass	post chill	0.4	40-60 psi	0.06-0.13	70-90
Waldroup 1999	APC	> 99%	NA	NA	yes	industrial	natural	broiler carcass	post chill	NA	40-60 psi	0.06-0.12	70-90
Waldroup 1999	*E.coli*	> 99%	NA	NA	yes	industrial	natural	broiler carcass	post chill	NA	40-60 psi	0.06-0.12	70-90
Waldroup 1999	coliforms	> 99%	NA	NA	yes	industrial	natural	broiler carcass	post chill	NA	40-60 psi	0.06-0.12	70-90

Study	Target strains	Log_{10} red Treated/ untreated log CFU/ml	Log_{10} red Treated/ Water CFU/ml	Log_{10} red Water/ untreated CFU/ml	Statistical significance	Industrial/ pilot/lab	Natural contamination /inoculation	Meat products	Pre/post chill	% CPC	pressure	volume (litres / bird)	line speed (birds / min)
Waldroup 1999	*Campylobacter*		NA	NA	yes	industrial	natural	broiler carcass	post chill	NA	40-60 psi	0.06-0.12	70-90
61010	Enterobacteriaceae	2.03	1.71	0.32	Anova, Tukey's Kramer	industrial	natural	broiler carcass	pre chill	0.2	NA	3.8	70
61010	APC	3.35	3.01	0.34	Anova, Tukey's Kramer	industrial	natural	broiler carcass	pre chill	0.2	NA	3.8	70
61010	Pseudomonas	1.29	0.84	0.45	Anova, Tukey's Kramer	industrial	natural	broiler carcass	pre chill	0.2	NA	3.8	70
61010	coliforms	1.87	1.61	0.26	Anova, Tukey's Kramer	industrial	natural	broiler carcass	pre chill	0.2	NA	3.8	70
61010	*E.coli*	1.78	1.53	0.25	Anova, Tukey's Kramer	industrial	natural	broiler carcass	pre chill	0.2	NA	3.8	70
61010	*Campylobacter*	0.93	0.72	0.21	Anova, Tukey's Kramer	industrial	natural	broiler carcass	pre chill	0.2	NA	3.8	70
61010	Enterobacteriaceae	2.15	1.83	0.32	Anova, Tukey's Kramer	industrial	natural	broiler carcass	pre chill	1	NA	3.8	70
61010	APC	3.72	3.38	0.34	Anova, Tukey's Kramer	industrial	natural	broiler carcass	pre chill	1	NA	3.8	70
61010	Pseudomonas	1.51	1.06	0.45	Anova, Tukey's Kramer	industrial	natural	broiler carcass	pre chill	1	NA	3.8	70
61010	coliforms	1.96	1.7	0.26	Anova, Tukey's Kramer	industrial	natural	broiler carcass	pre chill	1	NA	3.8	70
61010	*E.coli*	1.85	1.6	0.25	Anova, Tukey's Kramer	industrial	natural	broiler carcass	pre chill	1	NA	3.8	70
61010	*Campylobacter*	0.93	0.72	0.21	Anova, Tukey's Kramer	industrial	natural	broiler carcass	pre chill	1	NA	3.8	70
70414	*E.coli*	0.97	NA	NA		industrial	natural	broiler carcass	pre chill	0.6	NA	NA	NA
060510	APC	2.02	1.41	NA	NA	industrial	natural	Broiler carcass	Post chill	0.1	NA	0.95	175
060510	APC	2.48	1.87	NA	NA	industrial	natural	Broiler carcass	Post chill	0.1	NA	1.5	175
060510	APC	2.19	1.58	NA	NA	industrial	natural	Broiler carcass	Post chill	0.1	NA	2.1	175

Study	Target strains	Log_{10} red Treated/ untreated log CFU/ml	Log_{10} red Treated/ Water CFU/ml	Log_{10} red Water/ untreated CFU/ml	Statistical significance	Industrial/ pilot/lab	Natural contamination /inoculation	Meat products	Pre/post chill	% CPC	pressure	volume (litres / bird)	line speed (birds / min)
060510	APC	2.23	1.61	NA	NA	industrial	natural	Broiler carcass	Post chill	0.1	NA	2.6	175
060510	APC	2.48	1.87	NA	NA	industrial	natural	Broiler carcass	Post chill	0.2	NA	0.95	175
060510	APC	2.48	1.87	NA	NA	industrial	natural	Broiler carcass	Post chill	0.2	NA	1.5	175
060510	APC	2.48	1.87	NA	NA	industrial	natural	Broiler carcass	Post chill	0.2	NA	2.1	175
060510	APC	2.42	1.81	NA	NA	industrial	natural	Broiler carcass	Post chill	0.2	NA	2.6	175
060510	APC	2.48	1.87	NA	NA	industrial	natural	Broiler carcass	Post chill	0.3	NA	0.95	175
060510	APC	2.48	1.87	NA	NA	industrial	natural	Broiler carcass	Post chill	0.3	NA	1.5	175
060510	APC	2.48	1.87	NA	NA	industrial	natural	Broiler carcass	Post chill	0.3	NA	2.1	175
060510	APC	2.48	1.87	NA	NA	industrial	natural	Broiler carcass	Post chill	0.3	NA	2.6	175
060510	APC	2.48	1.87	NA	NA	industrial	natural	Broiler carcass	Post chill	0.4	NA	0.95	175
060510	APC	2.48	1.87	NA	NA	industrial	natural	Broiler carcass	Post chill	0.4	NA	1.5	175
060510	APC	2.48	1.87	NA	NA	industrial	natural	Broiler carcass	Post chill	0.4	NA	2.1	175
060510	APC	2.48	1.87	NA	NA	industrial	natural	Broiler carcass	Post chill	0.4	NA	2.6	175
HIGH-MEDIUM STRENGTH OF EVIDENCE													
Contact time: 3 sec													
Waldroup 2000a	APC	1.36	1.09	0.27	General Linear Model	pilot	natural	broiler carcass	pre chill	0.2	NA	0.29	NA

Study	Target strains	Log_{10} red Treated/ untreated log CFU/ml	Log_{10} red Treated/ Water CFU/ml	Log_{10} red Water/ untreated CFU/ml	Statistical significance	Industrial/ pilot/lab	Natural contamination /inoculation	Meat products	Pre/post chill	% CPC	pressure	volume (litres / bird)	line speed (birds / min)
Waldroup 2000a	*E.coli*	0.68	0.3	0.38	General Linear Model	pilot	natural	broiler carcass	pre chill	0.2	NA	0.29	NA
Waldroup 2000a	coliforms	0.6	0.72	-0.12	General Linear Model	pilot	natural	broiler carcass	pre chill	0.2	NA	0.29	NA
Waldroup 2000a	*Campylobacter*	1.71	0.99	0.72	General Linear Model	pilot	natural	broiler carcass	pre chill	0.2	NA	0.29	NA
Waldroup 2000a	APC	1.91	1.64	0.27	General Linear Model	pilot	natural	broiler carcass	pre chill	0.5	NA	0.29	NA
Waldroup 2000a	*E.coli*	0.72	0.34	0.38	General Linear Model	pilot	natural	broiler carcass	pre chill	0.5	NA	0.29	NA
Waldroup 2000a	coliforms	0.76	0.88	-0.12	General Linear Model	pilot	natural	broiler carcass	pre chill	0.5	NA	0.29	NA
Waldroup 2000a	*Campylobacter*	2.25	1.53	0.72	General Linear Model	pilot	natural	broiler carcass	pre chill	0.5	NA	0.29	NA
Contact time: 10 sec													
Waldroup 2000a	APC	1.6	1.33	0.27	General Linear Model	pilot	natural	broiler carcass	pre chill	0.2	NA	0.29	NA
Waldroup 2000a	*E.coli*	0.54	0.16	0.38	General Linear Model	pilot	natural	broiler carcass	pre chill	0.2	NA	0.29	NA
Waldroup 2000a	coliforms	0.65	0.77	-0.12	General Linear Model	pilot	natural	broiler carcass	pre chill	0.2	NA	0.29	NA
Waldroup 2000a	*Campylobacter*	2.25	1.53	0.72	General Linear Model	pilot	natural	broiler carcass	pre chill	0.2	NA	0.29	NA
Waldroup 2000a	APC	2.83	2.56	0.27	General Linear Model	pilot	natural	broiler carcass	pre chill	0.5	NA	0.29	NA
Waldroup 2000a	*E.coli*	0.97	0.59	0.38	General Linear Model	pilot	natural	broiler carcass	pre chill	0.5	NA	0.29	NA
Waldroup 2000a	coliforms	1.18	1.3	-0.12	General Linear Model	pilot	natural	broiler carcass	pre chill	0.5	NA	0.29	NA
Waldroup 2000a	*Campylobacter*	2.34	1.62	0.72	General Linear Model	pilot	natural	broiler carcass	pre chill	0.5	NA	0.29	NA

Study	Target strains	Log_{10} red Treated/ untreated log CFU/ml	Log_{10} red Treated/ Water CFU/ml	Log_{10} red Water/ untreated CFU/ml	Statistical significance	Industrial/ pilot/lab	Natural contamination /inoculation	Meat products	Pre/post chill	% CPC	pressure	volume (litres / bird)	line speed (birds / min)
MEDIUM STRENGTH OF EVIDENCE													
60613	APC	2.27	1.98	0.29	NA	pilot	inoculated	broiler carcass	post chill	0.2	NA	0.95	160
60613	*E.coli*	2.47	2.16	0.31	NA	pilot	inoculated	broiler carcass	post chill	0.2	NA	0.95	160
60613	*Salmonella*	3.44	2.83	0.61	NA	pilot	inoculated	broiler carcass	post chill	0.2	NA	0.95	160
60613	APC	5.13	4.84	0.29	NA	pilot	inoculated	broiler carcass	post chill	0.4	NA	0.95	160
60613	*E.coli*	4.91	4.6	0.31	NA	pilot	inoculated	broiler carcass	post chill	0.4	NA	0.95	160
60613	*Salmonella*	4.4	3.79	0.61	NA	pilot	inoculated	broiler carcass	post chill	0.4	NA	0.95	160
60613	APC	6.01	5.72	0.29	NA	pilot	inoculated	broiler carcass	post chill	0.6	NA	0.95	160
60613	*E.coli*	5.14	4.83	0.31	NA	pilot	inoculated	broiler carcass	post chill	0.6	NA	0.95	160
60613	*Salmonella*	5.64	5.03	0.61	NA	pilot	inoculated	broiler carcass	post chill	0.6	NA	0.95	160
60613	APC	6.19	5.9	0.29	NA	pilot	inoculated	broiler carcass	post chill	0.6	NA	1.9	160
60613	*E.coli*	5.14	4.83	0.31	NA	pilot	inoculated	broiler carcass	post chill	0.6	NA	1.9	160
60613	*Salmonella*	5.64	5.03	0.61	NA	pilot	inoculated	broiler carcass	post chill	0.6	NA	1.9	160
60613	APC	6.22	5.93	0.29	NA	pilot	inoculated	broiler carcass	post chill	0.8	NA	0.95	160
60613	*E.coli*	5.14	4.83	0.31	NA	pilot	inoculated	broiler carcass	post chill	0.8	NA	0.95	160
60613	*Salmonella*	5.64	5.03	0.61	NA	pilot	inoculated	broiler carcass	post chill	0.8	NA	0.95	160

Study	Target strains	Log_{10} red Treated/ untreated log CFU/ml	Log_{10} red Treated/ Water CFU/ml	Log_{10} red Water/ untreated CFU/ml	Statistical significance	Industrial/ pilot/lab	Natural contamination /inoculation	Meat products	Pre/post chill	% CPC	pressure	volume (litres / bird)	line speed (birds / min)
60613	APC	6.36	6.07	0.29	NA	pilot	inoculated	broiler carcass	post chill	0.8	NA	1.9	160
60613	*E.coli*	5.14	4.83	0.31	NA	pilot	inoculated	broiler carcass	post chill	0.8	NA	1.9	160
60613	*Salmonella*	5.64	5.03	0.61	NA	pilot	inoculated	broiler carcass	post chill	0.8	NA	1.9	160
60613	APC	6.33	6.04	0.29	NA	pilot	inoculated	broiler carcass	post chill	1	NA	0.95	160
60613	*E.coli*	5.14	4.83	0.31	NA	pilot	inoculated	broiler carcass	post chill	1	NA	0.95	160
60613	*Salmonella*	5.64	5.03	0.61	NA	pilot	inoculated	broiler carcass	post chill	1	NA	0.95	160
60613	APC	6.3	6.01	0.29	NA	pilot	inoculated	broiler carcass	post chill	1	NA	1.9	160
60613	*E.coli*	5.11	4.8	0.31	NA	pilot	inoculated	broiler carcass	post chill	1	NA	1.9	160
60613	*Salmonella*	5.64	5.03	0.61	NA	pilot	inoculated	broiler carcass	post chill	1	NA	1.9	160
60607	APC	1.35	0.69	0.66	NA	pilot	inoculated	broiler carcass	post chill	0.2	NA	0.95	160
60607	*E.coli*	1.39	0.48	0.91	NA	pilot	inoculated	broiler carcass	post chill	0.2	NA	0.95	160
60607	coliforms	1.59	0.81	0.78	NA	pilot	inoculated	broiler carcass	post chill	0.2	NA	0.95	160
60607	APC	2.25	1.59	0.66	NA	pilot	inoculated	broiler carcass	post chill	0.4	NA	0.95	160
60607	*E.coli*	2.69	1.78	0.91	NA	pilot	inoculated	broiler carcass	post chill	0.4	NA	0.95	160
60607	coliforms	2.68	1.9	0.78	NA	pilot	inoculated	broiler carcass	post chill	0.4	NA	0.95	160
60607	APC	4.29	3.63	0.66	NA	pilot	inoculated	broiler carcass	post chill	0.6	NA	0.95	160
60607	*E.coli*	4.03	3.12	0.91	NA	pilot	inoculated	broiler carcass	post chill	0.6	NA	0.95	160

Study	Target strains	Log_{10} red Treated/ untreated log CFU/ml	Log_{10} red Treated/ Water CFU/ml	Log_{10} red Water/ untreated CFU/ml	Statistical significance	Industrial/ pilot/lab	Natural contamination /inoculation	Meat products	Pre/post chill	% CPC	pressure	volume (litres / bird)	line speed (birds / min)
60607	coliforms	4.02	3.24	0.78	NA	pilot	inoculated	broiler carcass	post chill	0.6	NA	0.95	160
60607	APC	4.51	3.85	0.66	NA	pilot	inoculated	broiler carcass	post chill	0.6	NA	1.9	160
60607	*E.coli*	4.43	3.52	0.91	NA	pilot	inoculated	broiler carcass	post chill	0.6	NA	1.9	160
60607	coliforms	4.43	3.65	0.78	NA	pilot	inoculated	broiler carcass	post chill	0.6	NA	1.9	160
60607	APC	4.92	4.26	0.66	NA	pilot	inoculated	broiler carcass	post chill	0.8	NA	0.95	160
60607	*E.coli*	4.48	3.57	0.91	NA	pilot	inoculated	broiler carcass	post chill	0.8	NA	0.95	160
60607	coliforms	4.52	3.74	0.78	NA	pilot	inoculated	broiler carcass	post chill	0.8	NA	0.95	160
60607	APC	4.85	4.19	0.66	NA	pilot	inoculated	broiler carcass	post chill	0.8	NA	1.9	160
60607	*E.coli*	4.45	3.54	0.91	NA	pilot	inoculated	broiler carcass	post chill	0.8	NA	1.9	160
60607	coliforms	4.49	3.71	0.78	NA	pilot	inoculated	broiler carcass	post chill	0.8	NA	1.9	160
60607	APC	5.04	4.38	0.66	NA	pilot	inoculated	broiler carcass	post chill	1	NA	0.95	160
60607	*E.coli*	4.65	3.74	0.91	NA	pilot	inoculated	broiler carcass	post chill	1	NA	0.95	160
60607	coliforms	4.7	3.92	0.78	NA	pilot	inoculated	broiler carcass	post chill	1	NA	0.95	160
60607	APC	4.58	3.92	0.66	NA	pilot	inoculated	broiler carcass	post chill	1	NA	1.9	160
60607	*E.coli*	4.6	3.69	0.91	NA	pilot	inoculated	broiler carcass	post chill	1	NA	1.9	160
60607	coliforms	4.56	3.78	0.78	NA	pilot	inoculated	broiler carcass	post chill	1	NA	1.9	160

B. TABLE WITH DETAILED DATA OF CECURE® TREATMENT OF RAW POLUTRY PRODUCTS OF THE PEER-REVIEWED PAPERS INCLUDED IN THE ASSESSMENT

Study	Target strains	Log_{10} red Treated/ untreated log CFU/ml	Log_{10} red Treated/ Water CFU/ml	Log_{10} red Water/ untreated CFU/ml	Stat signif	Industrial/ pilot/lab	Natural contam /inoculation	Meat products	Pre/post chill	% CPC	pressure	volume (litres / bird)	line speed (birds / min)
Baker et al. 2010	APC	0.5-1				Industrial		boneless skinless breast meat, thighs, wings, split breasts, leg quarters and whole carcasses	post chill	0.3	na	1.89	
Beers et al. 2006	APC	2.5	1.8	0.7	Yes	Industrial		broiler carcass (on-line reprocessing)	Pre-chill	0.5-0.7%		2 to 3.3 oz./pound	70
Beers et al. 2006	APC	3.4	3.8	0.4	Yes	Industrial		broiler carcass (on-line reprocessing)	Pre-chill	0.5-0.7%		3 to 3.3 oz./pound	11-52
Beers et al. 2006	APC	3.9	3.7	0.2	Yes	Industrial		broiler carcass (on-line reprocessing)	Pre-chill	0.5-0.7%		4 to 3.3 oz./pound	70
Beers et al. 2006	*E. coli*	1.6	1.1	0.5	Yes	Industrial		broiler carcass (on-line reprocessing)	Pre-chill	0.5-0.7%		5 to 3.3 oz./pound	70
Beers et al. 2006	*E. coli*	2.1	2.6	0.5	Yes	Industrial		broiler carcass (on-line reprocessing)	Pre-chill	0.5-0.7%		6 to 3.3 oz./pound	11-52
Beers et al. 2006	*E. coli*	2.9	2.7	0.2	Yes	Industrial		broiler carcass (on-line reprocessing)	Pre-chill	0.5-0.7%		7 to 3.3 oz./pound	70
Beers et al. 2006	Total coliforms	1.2	0.7	0.5	Yes	Industrial		broiler carcass (on-line reprocessing)	Pre-chill	0.5-0.7%		8 to 3.3 oz./pound	70
Beers et al. 2006	Total coliforms	1.8	2.3	0.5	Yes	Industrial		broiler carcass (on-line reprocessing)	Pre-chill	0.5-0.7%		9 to 3.3 oz./pound	11-52
Beers et al. 2006	Total coliforms	2.7	2.6	0.1	Yes	Industrial		broiler carcass (on-line reprocessing)	Pre-chill	0.5-0.7%		10 to 3.3 oz./pound	70
Beers et al. 2006	*Campylobacter*	1.2	0.6	0.6	Yes	Industrial		broiler carcass (on-line reprocessing)	Pre-chill	0.5-0.7%		11 to 3.3 oz./pound	70
Beers et al. 2006	*Campylobacter*	0.8	1.2	0.4	Yes	Industrial		broiler carcass (on-line reprocessing)	Pre-chill	0.5-0.7%		12 to 3.3 oz./pound	11-52
Beers et al. 2006	*Campylobacter*	2.1	2.1	0	Yes	Industrial		broiler carcass (on-line reprocessing)	Pre-chill	0.5-0.7%		13 to 3.3 oz./pound	70
Beers et al. 2006	*Salmonella*				Yes	Industrial		broiler carcass (on-line reprocessing)	Pre-chill	0.5-0.7%		14 to 3.3 oz./pound	70

Study	Target strains	Log_{10} red Treated/ untreated log CFU/ml	Log_{10} red Treated/ Water CFU/ml	Log_{10} red Water/ untreated CFU/ml	Stat signif	Industrial/ pilot/lab	Natural contam /inoculation	Meat products	Pre/post chill	% CPC	pressure	volume (litres / bird)	line speed (birds / min)
Beers et al. 2006	*Salmonella*				Yes	Industrial		broiler carcass (on-line reprocessing)	Pre-chill	0.5-0.7%		15 to 3.3 oz./pound	11-52
Beers et al. 2006	*Salmonella*				Yes	Industrial		broiler carcass (on-line reprocessing)	Pre-chill	0.5-0.7%		16 to 3.3 oz./pound	70
Breen et al 1997	*Salmonella* Typhimurium	0.59*			Anova	lab		Chicken skin		1, 2, 4, 8%		5 ml/6.25 cm2	
Arritt et al 2002	*Campylobacter*	2.89			Tukey's HSD	lab		Chicken skin		0.1% - 0.5%	8 psi		
Li et al. 1997	*Salmonella* Typhimurium		0.46**		Anova	Pilot		broiler carcass	Pre chill	0.10 %(30 sec)	207 kPa		
Li et al. 1997	*Salmonella* Typhimurium		0.5**		Anova	Pilot		broiler carcass	Pre chill	0.10 %(30 sec)	345 kPa		
Li et al. 1997	*Salmonella* Typhimurium		0.85**		Anova	Pilot		broiler carcass	Pre chill	0.10 %(30 sec)	827 kPa		
Li et al. 1997	*Salmonella* Typhimurium		0.97**		Anova	Pilot		broiler carcass	Pre chill	0.10 % (90 sec)	207 kPa		
Li et al. 1997	*Salmonella* Typhimurium		0.96**		Anova	Pilot		broiler carcass	Pre chill	0.10 % (90 sec)	345 kPa		
Li et al. 1997	*Salmonella* Typhimurium		1.63**		Anova	Pilot		broiler carcass	Pre chill	0.10 % (90 sec)	827 kPa		

*(cfu/2.5cm^2)
** cfu/bird

C. GLOSSARY AND ABBREVIATIONS

ADI	Acceptable Daily Intake
APC	Aerobic Plate Count
BOD	Biological Oxygen Demand
bw	Body weight
cfu	Colony Forming Units
DAF	Dissolved air flotation
EPM	Equilibrium partitioning method
GHP	Good Hygienic Practices
HACCP	Hazard Analysis Critical Control Point
Eb	*Enterobacteriaceae*
EC50	Effect concentration on 50% of the tested animals
Kd	(Solids-water) Adsorption coefficient
Kf	Adsorption capacity factor
LC_{50}	Lethal concentration to 50% of tested animals
log K_{oc}	Logarithm of the soil organic carbon partition coefficient
log K_{ow}	Logarithm of the octanol/water partition coefficient
PEC	Predicted environmental concentration
PNEC	Predicted no effect concentration
QAC	Quaternary ammonium compounds
Salm	*Salmonella*
STEC	Shigatoxin-producing *Escherichia coli*
STP	Sewage treatment plant
VTEC	Verotoxigenic *Escherichia coli*