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SCIENTIFIC OPINION

Scientific Opinion on the evaluation of the safety and efficacy of peroxyacetic acid solutions for reduction of pathogens on poultry carcasses and meat[1]

EFSA Panel on Biological Hazards (BIOHAZ)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

This scientific output, published on 13 June 2014, replaces the earlier version published on 26 March 2014*.

ABSTRACT

Studies evaluating the safety and efficacy of solutions, containing peroxyacetic acid (PAA) as the active ingredient, in mixtures with acetic acid, hydrogen peroxide, and 1-hydroxyethylidene-1,1-diphosphonic acid (HEDP) and possibly octanoic acid and peroxyoctanoic acid, for reduction of pathogens on poultry carcasses and meat were assessed. Treatments at ambient temperature consisted of dipping in short term baths, in chiller baths or spraying. On the basis of the previous EFSA exposure scenarios including short term baths that were not evaluated previously, no toxicity concerns were identified with regard to residues of peroxyacids, to HEDP and to possible reaction products of hydrogen peroxide and peroxyacids with lipids and proteins of the poultry carcasses. A relevant reduction of PAA treatment on *E. coli* and coliforms was demonstrated by dipping warm carcasses, but few data were available for pathogens (*Salmonella* and *Campylobacter*). Spraying appeared to be less effective than dipping in reducing indicator organisms than dipping. When dipping chilled carcasses, reduction of indicator organisms and pathogens was evident, although only in low or medium strength of evidence studies. In chiller bath application, there was a relevant impact on *E. coli,* but less effect on coliforms, and little data was available on reduction of pathogens. The emergence of acquired reduced susceptibility to biocides and/or resistance to therapeutic antimicrobials following the use of PAA was considered unlikely. There were no concerns for environmental risk of peroxyacids, acetic acid and octanoic acid. On the basis of a conservative preliminary guideline for surface water quality, the emission of HEDP from a poultry plant into the environment could not be considered safe *a priori*. It was recommended that HACCP plans should include monitoring of the concentration of HEDP and of the decontaminating substance in the working solution and post-marketing surveillance for resistance in both pathogenic and commensal bacteria.

[1] On request from the European Commission, Question No EFSA-Q-2013-00601, adopted on 6 March 2014.

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[3] Acknowledgement: The BIOHAZ Panel wishes to thank the members of the Working Group on safety and efficacy of peroxyacetic acid for reduction of pathogens on poultry: Dorte Lau Baggesen, Arie Havelaar, John Threlfall, Birgit Noerrung, the CEF experts Patrick Van Beelen and Leon Brimer and the members of the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF): Ulla Beckman Sundh, Mona-Lise Binderup, Claudia Bolognesi, Leon Brimer, Laurence Castle, Alessandro Di Domenico, Karl-Heinz Engel, Roland Franz, Nathalie Gontard, Rainer Gürtler, Trine Husøy, Klaus-Dieter Jany, Martine Kolf-Clauw, Wim Mennes, Maria Rosaria Milana, Iona Pratt (deceased), Kettil Svensson, Maria de Fatima Tavares Poças, Fidel Toldra and Detlef Wölfle for the preparatory work on this scientific opinion; and the EFSA staff: Alessandro Broglia, Maria Luisa Escudero Hernandez, George Kass, Mariano Lopez Romano, Winy Messens and Liese Van Gompel for the support provided to this scientific opinion.

* Minor edit made on page 1: The acronym 'BIOHAZ' was added after 'EFSA Panel on Biological Hazards'.

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KEYWORDS

decontamination, poultry, peroxyacetic acid, 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) were asked by the European Food Safety Authority (EFSA) to deliver a Scientific Opinion on an application dossier submitted by the U. S. Department of Agriculture (USDA) for the approval of peroxyacetic acid solutions intended to be used by food business operators during processing for the reduction of pathogens on poultry carcasses and meat.

EFSA was requested to evaluate the safety and efficacy of peroxyacetic acid solution intended to be used by food business operators during processing for the reduction of pathogens on poultry carcasses and meat, 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 pathogens on poultry carcasses and meat; iii) the potential emergence of reduced susceptibility to biocides and/or resistance to therapeutic antimicrobials linked to the use of the substance; iv) the risk related to the release of the processing plant effluents, linked to the use of the substance, into the environment.

Approval was sought for reduction of surface contamination of raw poultry carcasses and poultry meat by the use of an aqueous solution containing peroxyacetic acid (PAA) as the active ingredient. The solution also contains acetic acid and hydrogen peroxide, and 1-hydroxyethylidene-1,1-diphosphonic acid (HEDP) as a product stabilizer. In some mixtures, octanoic acid is added, functioning as a surfactant.

The Applicant applied for PAA being used 1) on warm eviscerated carcasses or parts (pre-chill); (2) on carcasses in chiller baths (chill); (3) on chilled carcasses or parts (post-chill). PAA can be applied as spray washing or dipping depending on the step in the processing line. The in-use concentration of the active ingredient is not to exceed 2 000 ppm in the short term baths (3 minutes), and up to 230 ppm in the long duration chiller baths (duration of exposure during chilling can be 1-2 h). The concentration in spray washes is typically 400-700 ppm, applied for 10 seconds. The maximum temperature is ambient temperature and pH of a 600 ppm solution is approximately 2.5. It is not intended to subsequently remove the PAA solution from the poultry carcasses or poultry meat. PAA is highly reactive and, when used in the presence of organic compounds, dissociates very rapidly and loses antimicrobial properties. PAA breaks down to acetic acid and water and the mixtures are not recycled.

Concerning the toxicological safety, on the basis of the previous EFSA exposure scenarios, which included all uses described in the present application, except for the short term bath (< 3 minutes), no toxicity concerns were identified with regard to residues of peroxyacids. This is due to the described high instability of the compound, including the use of the short term high concentration bath. No concerns are indicated with respect to residues of acetic acid and octanoic acid, respectively, again including the short term use of a high concentration bath. With regard to the product stabilizer HEDP, no safety concern was identified with regard to the high concentration bath since for HEDP, a margin of safety ranging from 3 420 to 43 103 can be calculated against a No Observed Adverse Effect Level (NOAEL) of 50 mg/kg bw/day obtained in rat and rabbit reproductive toxicity studies, although there is some uncertainty as to the validity of the NOAEL used. Regarding the question of the safety of possible reaction products of hydrogen peroxide and peroxyacids with lipids and proteins/amino acids of the poultry carcasses, it was concluded that no risk was expected because of the low amino acid content in the carcass surface, including the short term treatment at higher peroxide concentrations. With regard to lipid peroxidation, no by-products were identified in producer experiments referred to in the previous risk assessment, when using immersion for 60 minutes in 200 mg/L total peroxyacetic acid. On this basis, the short term high concentration bath scenario included in the present application is not expected to cause measurable lipid peroxidation.

The application dossier included eight peer-reviewed published papers, one conference proceeding and 15 reports with data of in-house studies for consideration in evaluating the efficacy of PAA solution in

poultry meat decontamination. All papers, except the conference proceeding and four in-house studies were considered in the evaluation of the efficacy. The studies submitted by the Applicant used a wide range of experimental designs and thus differed in relation to products, settings, method of application, PAA concentration, use of controls, microorganisms studied, time of analysis after application, etc. All these parameters impacted on the observed efficacy. Comparison beyond treatment groups was therefore not possible. Studies were classified as of high or medium strength of evidence if they used naturally-contaminated samples on industrial or pilot scale, respectively.

Reduction of bacterial counts was considered relevant if the confidence interval of the mean decimal reduction and of the relative prevalence reduction did not include 0 (statistically significant), or, following expert judgement (when confidence intervals were not available), if the mean decimal reduction was greater than 0.5 log-units. There was consistent evidence for a relevant impact (1-3 log-units over untreated controls) of PAA treatment on *E. coli* and coliforms when treating warm carcasses by dipping. There were few data on reduction of pathogens for this treatment. Spraying of warm carcasses appears to be less effective in reducing indicator organisms than dipping (0.5-1.5 log-units). There is consistent evidence for a relevant reduction (0.5-2 log-units) of indicator organisms and pathogens when treating chilled carcasses or parts by dipping, but the studies were of low or medium strength of evidence.

When adding PAA to chiller baths, a relevant impact of PAA treatment on *E. coli* (0.5-2 log-units) was registered, whereas the effects on coliform bacteria were less consistent. There were few data on reduction of the number of pathogens for this treatment. The *Salmonella* prevalence was reduced in 4 out of 5 studies of high strength of evidence. The efficacy of PAA treatment after storage was only investigated in two studies with naturally-contaminated samples, and these gave conflicting results. Such studies are required in the EFSA guidelines to evaluate whether micro-organisms are truly inactivated or only sublethally injured.

On the basis of the history of safe usage information provided by the Applicant, it was concluded that the emergence of acquired reduced susceptibility to biocides and / or resistance to therapeutic antimicrobials following the use of PAA is unlikely.

There is no concern about environmental toxicity of acetic acid and octanoic acid which are effectively neutralized before discharge of wastewater. Likewise, tests regarding development and dissemination of acquired reduced susceptibility of environmental microorganisms are therefore not considered necessary. On the basis of a conservative preliminary guideline for surface water quality from a literature review, the emission of HEDP from a poultry plant including *via* a wastewater treatment system into the freshwater environment cannot be considered safe *a priori*. Site-specific considerations related to dilution factors and improved efficiency of wastewater treatment plants can mitigate the possible environmental risk associated with the emission of HEDP from individual poultry plants using PAA solutions for decontamination treatment.

It is recommended that HACCP plans should include: i) monitoring of the concentration of HEDP in the working PAA solution in order to control residues of HEDP on poultry carcasses (a method for the determination of HEDP residues on poultry carcases should be developed and validated); ii) monitoring of the concentration of the decontaminating substance in the working PAA solution; iii) post-marketing surveillance for resistance in both pathogenic and commensal bacteria if PAA is applied for decontamination of poultry carcasses. Laboratory studies should be undertaken to confirm that reduced susceptibility to biocides and/or resistance to therapeutic antimicrobials following the use of PAA does not occur. Furthermore, in order to support the assessment of efficacy, treated carcasses should also be examined at the end of shelf life, to ensure that the level of contamination remains low.

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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. This 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[4], 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 (2) of Regulation (EC) No 853/2004 provides a legal basis to approve, and therefore authorise, 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, are also a matter of concern the potential emergence of reduced susceptibility to biocides and/or resistance to therapeutic antimicrobials and 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 results of a risk assessment based on the available scientific evidence and undertaken in an independent, objective and transparent manner.

EFSA GUIDANCE AS PROVIDED BY THE EUROPEAN COMMISSION

On 14 April 2010, the European Food Safety Authority (EFSA) issued a revision of a guidance document (EFSA Panel on Biological Hazards (BIOHAZ), 2010) 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 AS PROVIDED BY THE EUROPEAN COMMISSION

On 15 May 2013, the Commission received an application dossier from the U. S. Department of Agriculture (USDA) for the approval of peroxyacetic acid solution intended to be used by food business operators during processing for the reduction of pathogens on poultry carcasses and meat.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

EFSA is requested to evaluate the safety and efficacy of peroxyacetic acid solution intended to be used by food business operators during processing for the reduction of pathogens on poultry carcasses and meat, considering:

- the toxicological safety of the substance;
- the efficacy, i.e. does the use of the substance significantly reduce the level of contamination of pathogens on poultry carcasses and meat;
- the potential emergence of reduced susceptibility to biocides and/or resistance to therapeutic antimicrobials linked to the use of the substance;

[4] 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_).

- the risk related to the release of the 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 Panel on Biological Hazards (BIOHAZ), 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 peroxyacetic acid solution for use in the reduction of pathogens on poultry carcasses and meat.

After having received this request from the European Commission, EFSA assigned the mandate to the Panel on Biological Hazards (BIOHAZ Panel; leading Panel) and the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel). Chapters 2 and 5, and the respective conclusions were endorsed by the CEF Panel by written procedure on 28 February 2014.

The term "poultry carcasses and meat" is defined as carcasses and/or skin-on parts from poultry, including chicken.

ASSESSMENT

1. Introduction

Approval was sought for reduction of surface contamination of raw poultry carcasses and poultry meat by the use of an aqueous solution containing peroxyacetic acid as the active ingredient. The solution also contains acetic acid and hydrogen peroxide, and 1-hydroxyethylidene-1,1-diphosphonic acid (HEDP). The latter is added to prevent the breakdown of peroxyacetic acid and hydrogen peroxide by chelating metal ions. In some cases octanoic acid is added, functioning as a surfactant, and peroxyoctanoic acid is formed. The mixture will be referred to as **PAA stock solution,** no matter whether or not octanoic acid is present. Typical compositions of the mixtures are given in Table 1.

The PAA stock solution is prepared by mixing acetic acid, hydrogen peroxide, water, and octanoic acid if applicable. The reaction is allowed to continue for up to 10 days in order to increase product yield.

Table 1: Composition by weight (%) of peroxyacid mixtures, as provided by the Applicant

Component	Formula 1	Formula 2	Formula 3
Acetic acid	40.6	45	35
Peroxyacetic acid	12.0	20	15
Hydrogen peroxide	6.2	6.0	10
Water	36.6	29	39
1-hydroxyethylidene-1,1-diphosphonic acid (HEDP)	0.8	0.1	< 1.0
Octanoic acid	3.2		
Peroxyoctanoic acid	1.4		

Depending on the mode of application, the PAA stock solution is to be diluted on-site to a concentration of peroxyacetic acid in potable tap water for use as a decontaminating treatment for raw poultry carcasses or poultry meat.

Relative to the purpose of the treatment, the dossier indicates: *"PAA will be used to reduce the incidence of foodborne illness by decreasing the numbers of human pathogens on poultry carcasses or parts provided to consumers. While not a primary objective, the use of PAA may also reduce the numbers of spoilage organisms and may increase the storage life of chilled poultry carcasses and parts"*. A description is given about the presence of *Salmonella* and *Campylobacter* on broiler carcasses in the EU and the disease burden of human salmonellosis and campylobacteriosis.

1.1. Parameters for treatment application

The Applicant includes the following information in relation to the parameters for treatment application in the dossier:

- Where in processing line: PAA can be used at three steps in poultry processing: (1) on warm eviscerated carcasses or parts (**pre-chill**); (2) on carcasses in chiller baths (**chill**); (3) on chilled carcasses or parts (**post-chill**). PAA is typically added to water in equipment already present in the processing line.

- Application: PAA can be applied as spray washing or dipping depending on the step in the processing line: (1) the pre-chill treatment is to be carried out by either spray washing or short-duration dip treatment; (2) the chill treatment is to be carried out in chiller baths, either during an entire chill or in one or more stages of multi-stage chiller baths; (3) the post-chill treatment is to be carried out in short-duration dip treatment.

- Concentration: The concentrated stock PAA solution is diluted with potable water, to reach a concentration of the active ingredient, the peroxyacetic acid, not to exceed 2 000 ppm in the

short term baths, and up to 230 ppm in the long duration chiller baths. The concentration in spray washes is typically 400-700 ppm. Minimum concentration levels are not regulated in the US. Suppliers of PAA report that it can be effective at 25-30 ppm. The concentration a poultry production facility applies will be a function of its performance objective and integrated into its HACCP plan, layout, and operating environment. The primary active ingredient is the peroxyacetic acid. The ratios of acetic acid and hydrogen peroxide to PAA vary somewhat in the products provided by different manufacturers.

- Conditions of use: Deliberately elevated temperatures are not intended (the maximum temperature is ambient temperature). The pH of the diluted PAA solution is not to be adjusted and varies by the concentration and the water hardness. The pH of a 600 ppm solution is approximately 2.5.

- Exposure time:

  - For the short term baths a maximum duration is specified (3 minutes), but the minimum duration has not been specified.

  - The duration of exposure during chilling can be 1-2 h at lower concentrations (typically in the U.S., around 90 ppm). PAA may also be used for less than the entire chill time (e.g. in one segment of a multiple-section chill tank system).

  - For the spray washing treatment (pre chill), the exposure times are short, typically less than 10 seconds being sprayed in a commercial inside-outside bird washer, with a wetted time ranging between 30 seconds to a few minutes before entering a subsequent processing step.

- Volume to apply: Spray pressures and volumes are specific to the washers utilized by a processor. For typical commercially available washers, during the spray process the poultry carcass may receive 680-950 ml of water mostly delivered at about 900 kPa. The washing process often has two parts, with a final rinse pressure at about 300 kPa. About 1/8th of the water is used in the final rinse. Washing time is about 6-9 seconds per carcass. The PAA solution may also remain active during the drip time.

- Subsequent removal conditions: It is not intended to subsequently remove the PAA solution from the poultry carcasses or poultry meat. The presence of PAA on the carcasses may provide some protection against recontamination during processing. PAA is highly reactive and, when used in the presence of organic compounds, should dissociate very rapidly and lose antimicrobial properties.

- Information has not been provided on the impact of washing and/or immersion steps after the application of the PAA, e.g. immersion cooling after the spraying of warm carcasses.

- Recycling: PAA breaks down to acetic acid and water and is not recycled. As noted in the dossier, overflow from post-chill high PAA concentration tanks may run into the lower concentration chiller tanks in some facilities.

1.2. Previous EFSA assessment in relation to PAA

EFSA has assessed the toxicological risks to public health from possible reaction products of four substances when applied on poultry carcasses, among which were peroxyacids (EFSA, 2005). Based on the available data and taking into account that processing of poultry carcasses (washing, cooking) would take place before consumption, EFSA concluded that treatment with peroxyacid solutions, under the described conditions of use, would not be of toxicological safety concern although efficacy may also be reduced. It was noted that spraying of poultry carcasses with antimicrobials, by comparison to dipping and immersion treatments, will reduce the exposure to residues and by-products

that might arise. It was stressed that the use of antimicrobial solutions should not replace the need for good hygienic practices during processing of poultry carcasses, particularly during handling. The need to replace regularly the water of chiller baths was also stressed.

Assessment of the efficacy of peroxyacids as an antimicrobial substance applied to poultry carcasses was also carried out by EFSA in 2005 (EFSA, 2005). Particularly, EFSA was asked to assess the efficacy of the peroxyacids on the growth and/or prevalence of some microorganisms and pathogens on poultry carcasses. The information provided was not sufficient to allow assessment of the efficacy of peroxyacids, for various reasons. In short, the protocols used were not always fully explained, and the processing conditions did not reflect the conditions and practices in Europe. In the trial on commercial processing lines, *Salmonella* was the only pathogen considered (not *Campylobacter* spp.). The two adequately described experiments were on a laboratory scale and were not sufficient to demonstrate the efficacy of peroxyacids under commercial conditions.

In 2008 EFSA assessed the possible effect of four antimicrobial treatment substances, including peroxyacids, on the emergence of antimicrobial resistance, when such substances were applied for poultry carcass decontamination (EFSA, 2008). The conclusion was that despite a long history of use, there were no published data that the application of peroxyacids to remove microbial contamination of poultry carcasses at the proposed conditions of use has led to either the occurrence of acquired reduced susceptibility to peroxyacids, or to development of resistance to therapeutic antimicrobials.

1.3. Approved uses of PAA

In the EU, use of PAA as a sanitizer and disinfectant is permitted under Directive 98/8/EC (updated and replaced by Regulation (EU) No 528/2012)[5] and Regulation No 1451/2007[6]. The uses for PAA solutions include: human hygiene biocidal products; private area and public health area disinfectants; veterinary hygiene biocidal products; food and feed area disinfectants, drinking water disinfectants; in-can preservatives (non-food); preservatives used in liquid-cooling and processing systems; and slimicides. In Europe, no post-marketing surveillance data are collected / available.

As specified in the dossier, in the USA, peroxyacids have been widely used as sanitizers and disinfectants, including: agricultural premises and equipment (e.g. poultry barns and cages between flocks); food handling premises; commercial, institutional, and industrial premises; residential and public access premises; medical premises and equipment. In addition, peroxyacids are permitted for use at various stages in the processing of red meat and poultry products.

1.4. Aim of this assessment

The aim of the present Scientific Opinion is to assess the safety and efficacy of PAA solution intended to be used by food business operators during processing for the reduction of pathogens on poultry carcasses and poultry meat, 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 pathogens on poultry carcasses and poultry meat?, (3) the potential emergence of reduced susceptibility to biocides and / or resistance to therapeutic antimicrobials linked to the use of the substance, and (4) the risk related to the release of the processing plant effluents, linked to the use of the substance, into the environment. Each of these assessments is described.

[5] Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing of biocidal products on the market. OJ L 123, 24.4.1998, p. 1-63.

[6] Commission Regulation (EC) No 1451/2007 of 4 December 2007 on the second phase of the 10-year work programme referred to in Article 16(2) of Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market. OJ L 325, 11.12.2007, p. 3-65.

2. The toxicological safety of the substance to humans

2.1. Evaluation

2.1.1. Technical data

2.1.1.1. Identity of the substances and specifications

As specified in Table 1 the composition by weight % of the stock solution may for each of the different components except water vary between: acetic acid 35-45, peroxyacetic acid 12-20, hydrogen peroxide 6-10, octanoic acid 0-3.2, peroxyoctanoic acid 0-1.4 and HEDP 0.1-1.0.

Acetic acid

Synonyms: ethanoic acid

CAS Registry number: 64-19-7

EC number: 200-580-7

Chemical formula: $C_2H_4O_2$

Molecular weight: 60.05

Acetic acid is authorized as a food additive E 260 with no intake limit (Commission regulation N° 1129/2011[7]).

Peroxyacetic acid

Synonyms: Peracetic acid, PAA, Ethaneperoxoic acid

CAS Registry number: 79-21-0

Chemical formula: $C_2H_4O_3$

Molecular weight: 76.05

Hydrogen peroxide

Synonyms: dihydrogen dioxide

CAS Registry number: 7722-84-1

EC number: 231-765-0

Chemical formula: H_2O_2

Molecular weight: 34.01

Octanoic acid

Synonyms: Caprylic acid

CAS Registry number: 124-07-2

[7] Commission Regulation (EU) No 1129/2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives. OJ 12.11.2011, L 295/1

EC number: 204-677-5

Chemical formula: $C_8H_{16}O_2$

Molecular weight: 144.21

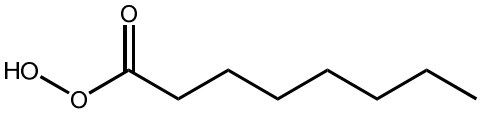
Peroxyoctanoic acid

Synonyms: Peroctanoic acid, peroxycaprylic acid, percaprylic acid

CAS Registry number: 33734-57-5

Chemical formula: $C_8H_{16}O_3$

Molecular weight: 160.21

Structural formula: 

1-hydroxyethylidene-1,1-diphosphonic acid (HEDP)

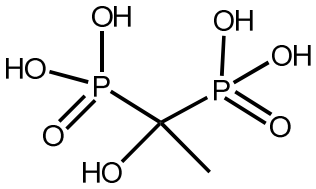
Synonyms: ehdp, HEDP, HEDPA, ETIDRONIC ACID

CAS Registry number: 2809-21-4

EC number: 220-552-8

Chemical formula: $C_2H_8O_7P_2$

Molecular weight: 206.03

Structural formula: 

No information is given on the purities of the different components in the commercial stock solutions for which authorisation is applied. Quality specifications for HEDP as a food additive have been published by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2004).

The PAA stock solution can be diluted on-site with potable tap water to the desired concentration of peroxyacetic acid for use as decontaminating treatment for raw poultry carcasses or poultry meat. When the concentrated stock PAA are diluted to the levels used for the decontamination of poultry carcasses and meat, the solutions are not considered to be a safety hazard.

The stock solutions described in the application are produced from acetic acid, hydrogen peroxide, octanoic acid and HEDP. While acetic acid and hydrogen peroxide are known to have antimicrobial effects, their effects within these solutions are minimal. Acetic acid reacts with hydrogen peroxide to generate peroxyacetic acid with which it is in equilibrium. Therefore the amount and presence of acetic acid and hydrogen peroxide is critical for the concentration of the peroxyacetic acid and therefore the antimicrobial effect. Octanoic acid functions as a surfactant, wetting hydrophobic surfaces, particularly on meat. The presence of peroxyoctanoic acid in the solution is a consequence of

the reaction of octanoic acid with hydrogen peroxide. HEDP has no antimicrobial effects, it functions as a stabilizer in these solutions by preventing metal ions from catalyzing the breakdown of peroxyacetic acid and hydrogen peroxide (JECFA, 2005, 2006).

From the information on the stock solution and the work solution provided by the Applicant the amount ranges of the different components are shown in Table 2.

Table 2: Ranges for the different components depending on the formulation and type of solution.

Component	Stock solution (% w/w)	Work solution (after dilution) (ppm, mg/L)
Peroxyacetic acid	12-20	230-2000
Acetic acid	35-45	518-6 767
Hydrogen peroxide	6-10	69-1 533
Octanoic acid	0-3.2	0-533
Peroxyoctanoic acid	0-1.4	0-233
HEDP	0.1-1.0	1.2-133

According to the Applicant, the levels of total peroxyacids can be determined by an iodine-sodium thiosulfate titration method for which commercial kits are available. Other analytical techniques for PAA in dilute solutions also exist (ECETOC, 2001; PAR/Cefic). For the quantification of HEDP levels in solutions, a titration technique is provided by JECFA (JECFA, 2004). Ion-exchange HPLC based methods for the quantification of HEDP in water samples are described in the literature (Ma et al., 2007; Nowack, 1997). The Applicant did not supply details of any technique for the determination of HEDP in food matrices, including poultry meat and no information could be found in the literature.

2.1.2. Consumer exposure assessment

JECFA estimated the intake of each peroxyacid solution component on the basis of the residual amounts anticipated to be present on treated food (meat and vegetables) at the time of consumption JECFA (JECFA, 2005, 2006). Due to the instability of hydrogen peroxide, peroxyacetic acid, or peroxyoctanoic acid, no residues were anticipated to be present on foods that have been treated with these solutions. In contrast, residues of acetic and octanoic acids were expected to remain on treated foods that are not washed or further processed after treatment. The highly conservative estimate of the exposure to octanoic acid resulting from the use of the antimicrobial solutions was 1.9 mg/day and the mean intake of octanoic acid from foods consumed as part of the diet in the USA was estimated to be approximately 200 mg/day. Intake of acetic acid was not determined; its use as vinegar in and on foods would result in a greater exposure than that from the use of peroxyacid antimicrobial solutions. HEDP was expected to remain on treated foods not further washed, processed, or cooked. The highest estimate of intake of HEDP was 3.6 μg/kg bw per day for the upper-bound estimate using a model for vegetables with a high surface area. The value was obtained from JECFA using national estimates of intake from the Czech Republic (2.2 μg/kg bw per day), the USA (2.2 to 4.7 μg/kg bw per day), and the United Kingdom (1.8 to 3.3 μg/kg bw per day).

In Europe, acetic acid is authorized as a food additive as *quantum satis* and with an acceptable daily intake (ADI) not specified (SCF 1986), In the USA octanoic acid is on the FDA Generally Recognized As Safe (GRAS) list as multipurpose ingredients in food, with a maximum use levels under Good Manufacturing Practice ranging from 10 mg/kg for various foods, up to 160 mg/kg in snack foods[8]. The GRAS status recognition was issued through experience based on common use in food and considering that the substance was used in food prior to January 1, 1958.

EFSA (2005) on the basis of the draft EU concise food consumption database (EFSA, 2005), which at that time included France, Sweden and Italy, found that the average daily consumption of meat and

[8] 21CFR184.1025 (last update April 2013): http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=184.1025

meat products was estimated to be between 120 and 151 g/day for adults. Assuming that the concentration of the substances in the edible part of meat was identical to the concentration in the carcass the exposure of a 60 kg individual to peroxyacetic acid and hydrogen peroxide was estimated to be 0.63 µg/kg bw/day at the mean and 1.08 and 1.46 µg/kg bw/day at the 95th and 99th percentile of meat consumption, respectively. The exposure of a 60 kg individual to HEDP was 0.43 µg/kg bw/day at the mean and 0.74 and 0.99 µg/kg bw/day at the 95th and 99th percentile of meat consumption, respectively (EFSA, 2005).

In the JECFA scenario, the inclusion of vegetables with a great surface to volume ratio in addition to meat for the estimation of exposure explains the higher value (3.6 µg/kg bw/day) as compared to the EFSA estimation (0.74 µg/kg bw/day) in which only meat and meat products are considered.

When using the most recent food consumption figures and body weight at the individual level available in the EFSA Comprehensive European Food Consumption Database (EFSA, 2011) which comprises 28 different dietary surveys carried out in 17 different European countries, the mean and high (95th percentile) poultry consumption for adults (≥ 18 years to < 65 years old) in Europe ranges from 0.1 to 0.6 g/kg bw/day and 0.5 to 2.2 g/kg bw/day, respectively. The mean and high (95th percentile) poultry consumption for toddlers (≥ 12 months to < 36 months old) in Europe ranges from 0.2 to 2.5 g/kg bw/day, and from 1.1 to 7.8 g/kg bw/day, respectively.

The highest concentration of up to 2 000 mg/kg (short term bath i.e. <3 min) of peroxyacetic acid (see Table 2) which is about 10 fold higher than the value used in previous exposure assessments (EFSA, 2005, JECFA 2005, 2006) leads to a residue level of 1883 µg HEDP/kg poultry[9]. The residue of HEDP on chicken carcasses that resulted from the low-concentration short-term treatment were only estimated by correction of the residue observed after a short-time low / concentration treatment for the difference in HEDP concentrations between the low- and high-concentration treatment. It was not investigated whether treatment time would greatly affect the residue level. Nevertheless, if it is assumed that HEDP is not absorbed by the carcass, this is a reasonable approach. If, however, HEDP is absorbed by the carcass, then this assumption is conservative, since the low-concentration treatment last longer than the high-concentration treatment and has therefore a higher potential for concentration-build-up in the poultry meat.

Assuming that there is no loss of HEDP during the processing, the maximum residue level of 1 883 µg HEDP/kg poultry has been used in the exposure calculations. On this basis, for adults the mean and high (95th percentile) poultry exposure to HEDP ranged from 0.18 to 1.16 µg/kg bw/day and 0.90 to 4.18 µg/kg bw/day, respectively. For toddlers the mean and high (95th percentile) poultry exposure to HEDP ranged from 0.34 to 4.76 µg/kg bw/day and 2.07 to 14.62 µg/kg bw/day, respectively.

2.1.3. Toxicological assessment

FDA, EFSA (section 1.2) and JECFA have already evaluated products containing peroxyacids. 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 stock solutions evaluated in this opinion (see Table 2) are, with regard to their components, qualitatively identical to the solutions formerly evaluated and accepted as potential antimicrobial washing products for carcasses (EFSA, 2005). Formulations assessed by EFSA in 2005 contained peroxyacetic acid (<15 %), peroxyoctanoic acid (<2 %), hydrogen peroxide <10 %) and HEDP at levels lower than 1 %. The solutions were intended to be used at a maximum concentration of total peroxyacetic acid, of 220 mg/L, a maximum concentration of hydrogen peroxide of 110 mg/L, and a

[9] Quotation from the memorandum dated April 3, 2009 referring to FCN00880 (FDA, 2009): "The maximum HEDP concentration in solutions applied to poultry carcasses in the current FCN is 10.46 times higher than the concentration of solutions applied to poultry in testing used to support FAP 1A4728 (136 ppm vs. 13 ppm). Therefore, the quantity of HEDP from this use would be 180 ppb x 10.46, or 1883 µg/kg poultry."

maximum concentration of HEDP of 13 mg/L (EFSA, 2005). JECFA (2005, 2006) considered the safety of antimicrobial solutions for which the concentrations of total peroxyacid(s) before use ranged from 80 to 200 mg/kg solution. These solutions, as per the current evaluation, were prepared from acetic acid, octanoic acid (singly or in combination), hydrogen peroxide, and HEDP as stabilizer (EFSA Panel on Biological Hazards (BIOHAZ), 2011b; JECFA, 2005, 2006).

In general, due to the instability of the peroxy-compounds, after dilution and application to the target (a carcass) mainly acetic acid, and octanoic acid will remain together with HEDP (Azanza, 2004). JECFA also indicates in their conclusion in 2005 that due to the reactivity of the peroxy compounds, only octanoic acid, acetic acid and HEDP will remain in food that are treated with the antimicrobial solution and that are not further washed, processed or cooked.

The EFSA assessment from 2005 quotes experiments made to establish residues of peroxyacetic and peroxyoctanoic acids and HEDP (EFSA 2005). In those experiments the residues of peroxyacids and hydrogen peroxide in chicken carcasses after 2, 5 and 10 min of spraying peroxyacids (200 mg/L) and immersing them for 60 min at less than 4°C were below the detection limit of 1 mg/L. Because of the low levels of peroxy compounds observed and the chemical instability/reactivity these substances are not likely to remain in the poultry carcasses and therefore there is no need to perform a safety assessment for these substances. Concerning HEDP, six chicken carcasses were treated with two different solutions. Solution 1 contained 200 mg/L of peroxyacids (as peroxyacetic acid) and 10 mg/L of HEDP and solution 2 contained 30 mg/L of peroxyacids and 1.5 mg/L of HEDP. All chicken carcasses were sprayed 15 s with solution 1 at ambient temperature. Three of the chicken carcasses were then immersed for 60 min in a bath at 3 ºC with solution 1 and the other three chicken carcasses were immersed for 60 min in a bath at 2 ºC with solution 2. Chicken carcasses treated with solution 1 in the bath gave a residual amount of 120-170 µg HEDP per kg carcass. In the case of solution 2 in the bath, the residual amount was 40-50 µg HEDP per kg carcass (close to the LOD).

From the above information it can be concluded that only acetic acid, octanoic acid and HEDP will remain in the carcasses after treatment without further washing or processing.

A number of amino acids and amino acid-derived compounds such as peptides and proteins may be oxidized by the peroxyacids present in the PAA solution (EFSA, 2005). Cystine can, for example, be oxidised to cysteic acid and methionine to methionine sulphoxide or methionine sulphone (Slump and Schreuder, 1973; Strange, 1984). Although there are several possibilities for the oxidation of amino acids, it was concluded that "no significant levels of amino acids by-products will be produced after treatment with peroxyacids since free amino acids levels in poultry meat, just before ageing, are very low" (EFSA, 2008). The application of peroxyacids solution could also cause oxidation of lipids from fatty acids with one or more double bonds (EFSA, 2008; Rhee et al., 1989). In this respect, in 2005 EFSA concluded that no significant differences in the TBARS (thiobarbituric acid reactive substances) values or the fatty acids profiles were observed when comparing treated samples with either raw or cooked samples (EFSA, 2005).

Both risk assessments performed by JECFA (2005, 2006) and EFSA (2005) conclude that the use of the evaluated solutions are of no health concern.

According to the risk assessment of JECFA (2005, 2006) several studies (human, rat, rabbits, dogs and monkeys) on the disposition of HEDP after oral administration have been performed. Collectively, the data indicated that absorption of HEDP from the gastrointestinal tract is very limited and that its metabolism is negligible. Some accumulation was seen in the bones, with a half-life in rats of about12 days (JECFA, 2005, 2006).

HEDP did not induce mutations in a bacterial gene mutation assay (Ames test) nor in an in vitro mammalian cell gene mutation test in L5178Y TK+/- mouse lymphoma cells (EC 2000, JECFA 2006). The unpublished study reports were, however, not available for re-evaluation.

Data on toxicity of HEDP have been provided by the Applicant from several studies referred to in the assessments by JECFA (2005, 2006), EFSA (2005) and FDA (2009):

- Two 90-day studies with rats: NOAEL 500 mg/kg bw/day
- One 90- day study with dogs: NOAEL 250 mg/kg bw/day
- One study with two generations of rats: no teratogenic effects at 50 or 250 mg/kg bw/day with a NOAEL at 50 mg/kg bw/day (embryotoxicity found at 250 mg/kg bw/day)
- One reproductive study with rabbits: NOAEL 50 mg/kg bw/day
- One study of the treatment of human Paget disease (bone growth disorder): the prescribed treatment is up to 5 mg/kg bw/day for up to six month periods; this may be followed by additional treatments after rest period.
- One 1-2 year subcutaneous (SC) study in dogs: NOAEL: 5 mg/kg bw/day (actual dose 0.01 mg/kg bw/day via SC injection; the NOAEL mentioned here is the oral equivalent of the SC dose after adjustment for gut absorption.

From the studies mentioned above, the Panel considered the two-year study in dogs inappropriate to be used for the safety assessment of HEDP, since this study included relatively small numbers of animals, it only addressed skeletal effects and it used a parenteral route of exposure, which creates additional uncertainty in the extrapolation. A chronic feeding study in rats provided a NOAEL of 105 mg/kg bw/day, but this study was not available for evaluation. Lower NOAELs have been reported in reproductive toxicity studies in rats and rabbits (50 mg/kg bw/day). None of these studies was available for evaluation but based on FDA data, from these studies a NOAEL of 50 mg/kg bw/day emerges, also taking into account that in the study in rabbits a LOAEL of 100 mg/kg bw/day was reported. Assuming that the NOAEL of 50 mg/kg/bw/day was found in adequate studies, this NOAEL will be used for the safety assessment of HEDP.

For adults mean and high (95th percentile) poultry exposure estimates to HEDP from the use of the application as outlined in this opinion are up to 1.16 µg/kg/bw/day and 4.18 µg/kg/bw/day, respectively. Margin of Safety (MoS) for HEPD was calculated by dividing the NOAEL value of 50 000 µg/kg bw/day by the mean or high exposure estimates resulting in MoSs of 43 103 and 11 961, respectively.

For toddlers mean and high (95th percentile) poultry exposure estimates to HEDP are up to 4.76 µg/kg/bw/day and 14.62 µg/kg/bw/day, respectively. MoSs for HEDP were calculated for the mean and the high exposure as described above resulting in MoSs of 10 504 and 3 420 respectively.

Based on the assumption that the data available provide an adequate NOAEL for HEDP, these MoS values, calculated for adults and toddlers do not indicate a safety concern. The Panel notes, however, that the respective studies from which NOAEL was derived, were not available to assess their reliability.

2.2. Conclusions

Accepting the previous EFSA exposure scenarios (EFSA, 2005), which included all uses described by the present application, except for the short term bath (< 3 minutes) using a ten times higher concentration than previously evaluated by the JECFA (2005, 2006) and EFSA (2005), no toxicity concerns were identified with regard to residues of peroxyacids due to the described high instability, including the use of the short term high concentration bath. No concerns are indicated with respect to residues of acetic acid and octanoic acid, respectively, again including the short term use of a high concentration bath.

With regard to the product stabilizer HEDP no safety concern was identified with regard to the high concentration bath since for HEDP MoSs ranging from 3 420 to 43 103 can be calculated against a

NOAEL of 50 mg/kg bw/day obtained in rat and rabbit reproductive toxicity studies. The Panel noted that since the studies from which these NOAELs were derived were not available to be evaluated there is some uncertainty as to the validity of the NOAEL used. In addition, this conclusion is only applicable for working solutions of PAA containing up to 130 mg HEDP/L in combination with immersion times of 3 minutes. When longer contact times are applied, the HEDP concentrations should be reduced accordingly.

Regarding the question of the safety of possible reaction products of hydrogen peroxide and peroxyacids with lipids and proteins/amino acids of the poultry carcasses, the low amino acid content in the carcass surface used as argument for the former EFSA (2005) conclusion that no risk was expected, is still valid, including the short term treatment at higher peroxide concentrations.

With regard to lipid peroxidation, no by-products were identified in producer experiments referred to in the previous risk assessment, when using immersion for 60 minutes in 200 mg/L total peroxyacetic acid. On this basis short term high concentration bath scenario included in the present application is not expected to cause measurable lipid peroxidation.

2.3. Recommendations

To control residues of HEDP on poultry carcasses, monitoring of the concentration of HEDP in the working PAA solution should be considered in the HACCP plans.

A method for the determination of HEDP residues in poultry carcasses, poultry meat and poultry meat products should be developed and validated, to further inform the risk assessment.

3. The efficacy, i.e. does the use of the substance significantly reduce the level of contamination of pathogens on poultry carcasses and poultry meat

3.1. Introduction

According to the EFSA guidance document (EFSA Panel on Biological Hazards (BIOHAZ), 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 Panel on Biological Hazards (BIOHAZ), 2010).

A risk assessment study on *Campylobacter* on broiler carcasses pre-chill (EFSA Panel on Biological Hazards (BIOHAZ), 2011a) has shown that even 0.5 log unit microbial reductions may reduce consumer risks to a significant extent. In addition, 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 PAA solutions, containing < 2 000 ppm or < 230 ppm of peroxyacetic acid, the active ingredient, was petitioned for approval as a decontaminant treatment in raw poultry carcasses and poultry meat. The process and results of the evaluation of the studies included in the dossier for the efficacy of PAA as a decontamination agent for raw poultry carcasses and poultry meat are evaluated in this section.

3.2.1. Criteria used for inclusion or exclusion of submitted studies

The following criteria were used in the selection of studies to be used in the evaluation of decontamination efficacy by PAA:

- The studies considered were only those with criteria following within the conditions used as provided by the Applicant and described in section 1.1.
  - Treatment group 1: studies on warm carcasses with spray treatment:
    - ✓ Product treated: carcasses pre-chill;
    - ✓ Application: spray washing (in a commercial inside-outside bird washer);
    - ✓ PAA concentration: 400-700 ppm;
    - ✓ Maximum temperature: ambient;
    - ✓ Maximum duration of treatment: typically less than 10 seconds, with a wetted time ranging between 30 seconds to a few minutes before entering a subsequent processing step.
  - Treatment group 2: studies on warm carcasses or parts with dip treatment:
    - ✓ Product treated: carcasses pre-chill;
    - ✓ Application: short-duration dip treatment;
    - ✓ Maximum temperature: ambient;
    - ✓ Maximum PAA concentration: 2 000 ppm;
    - ✓ Maximum duration of treatment: 3 minutes.
  - Treatment group 3: studies on effects in chiller baths:

- ✓ Product treated: carcasses pre-chill;
- ✓ Application: in chiller baths, either during an entire chill or in one or more stages of multi-stage chiller baths;
- ✓ Maximum temperature: temperatures currently used in chilling baths;
- ✓ Maximum PAA concentration: 230 ppm;
- ✓ Maximum duration of treatment: 1-2 h at lower concentrations (in the US this is typically around 90 ppm). PAA may also be used for less than the entire chill time (e.g. in one segment of a multiple-section chill tank system).

– Treatment group 4: studies on chilled carcasses or parts with dip treatment:

- ✓ Product treated: carcasses or parts post-chill;
- ✓ Application: short-duration dip treatment;
- ✓ Maximum temperature: ambient;
- ✓ Maximum PAA concentration: 2 000 ppm;
- ✓ Maximum duration of treatment: 3 minutes.

- The studies selected for evaluation should involve application on poultry carcasses, poultry skin, or skin-on poultry parts.

- The studies on visibly contaminated poultry carcasses and poultry meat were excluded from the assessment. This is because decontamination treatments must not affect the food business operator's duty to comply with the requirements of EU legislation on food hygiene, as laid down in Regulation (EC) No 853/2004 Annex III, Section I, Chapter IV, point 10[10] and should in no way be considered as a substitution for good hygienic slaughtering practices and operating procedures or as an alternative to comply with the requirements of those Regulations. The Annex of Reg. 101/2013, concerning the use of lactic acid to reduce microbiological surface contamination on bovine carcasses, stipulates that *"lactic acid solutions must not be applied to carcasses with visible faecal contamination"*.

- The evaluation of the efficacy will focus on PAA treated samples *versus* water treated samples, or *versus* untreated controls. In the absence of a proper water treated control, data from solutions with a low chlorine concentration (around 30 ppm) as control were also used as these would lead to a conservative estimate of the overall efficacy.

- The targets applied for by the Applicant are poultry-borne organisms regarded as important human pathogens. The evaluation of the efficacy will focus on *Campylobacter* spp., *Salmonella* spp. and *Escherichia coli*, including strains pathogenic to humans. This is based on the most relevant biological hazards that were identified in the context of meat inspection of poultry, i.e. thermophilic *Campylobacter* spp., *Salmonella* spp. and ESBL/AmpC gene-carrying *E. coli* (EFSA Panel on Biological Hazards (BIOHAZ), 2012a). The evaluation will also take into account information on relevant indicator organisms, i.e. *Escherichia coli*, coliforms and Enterobacteriaceae. Reduction of spoilage organisms is regarded as a secondary objective since it is not expected to have any impact on the target pathogens, which do not grow on chilled poultry meat.

- The studies in which inoculation of the microorganisms was done after the PAA treatment were excluded from the assessment as these were not considered to represent practical applications.

[10] Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for on the hygiene of foodstuffs, Official Journal of the European Union 30.4.2004, L 139/55.

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

The body of evidence selected (see below) from the studies submitted in the dossier was evaluated, taking into account whether the studies were done in the laboratory, under pilot plant conditions or in a slaughterhouse (industrial scale), and whether they used inoculated or naturally-contaminated poultry samples. Table 2 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 have been used in three previous EFSA Opinions (EFSA Panel on Biological Hazards (BIOHAZ), 2011a, 2011b, 2012b) and were developed on the basis of 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 3 and 4.

Table 3: Relative strength of the contribution of study data to the general body of evidence, based on study type

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

(a): Includes studies where the meat surface was inoculated with pathogens in pure culture prior to the decontamination treatment.
(b): Experiments using industrial equipment in non-industrial settings.
(c): If the pilot process is representative of the industrial process; otherwise, evidence makes a "medium" contribution to the body of evidence.
(d): Data would not be sufficient to inform a quantitative microbial risk assessment or to allow definitive conclusions on risk reduction.
(e) 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 eight peer-reviewed published papers and one conference proceeding dealing with testing of PAA solution for decontamination (Table 4). All but one (the conference proceeding) were selected for consideration in evaluating the efficacy of PAA solution in poultry meat decontamination. The papers totalled ten studies, of which two were industrial, two pilot, and six laboratory level studies. One study was conducted on short-duration dip treatment pre-chill (treatment group 2 in Table 4), four in chiller baths (treatment group 3 in Table 4), and five by short-duration dip treatment post-chill (treatment group 4 in Table 4).

- Of the peer-reviewed studies that were included, three were of high strength of evidence, three of medium strength and five of low strength (Table 4).

- The Applicant also included in the application dossier 15 reports with data of in-house studies in support of the application for approval of PAA solution for use in the decontamination of fresh poultry products (Table 4). All but four of these reports were considered in the evaluation of the efficacy of PAA solution against microbial contamination on fresh poultry; rejection was because either only total viable bacteria were tested, the duration of the treatment was outside the range of the application, the concentration used was above the treatment limit, or no warm carcasses were used. The 11 reports that were included totalled 15 studies.

- Twelve of the in-house studies were conducted on poultry carcasses (Table 4) with natural contamination, ten were of industrial scale, two of pilot scale, and three laboratory scale. Seven studies were conducted by spray-washing of carcasses or parts pre-chill (treatment group 1 in Table 4), two on short-duration dip treatment pre-chill (treatment group 2 in Table 4), four in chiller baths (treatment group 3 in Table 4), and two by short-duration dip treatment

post-chill (treatment group 4 in Table 4). The PAA solution was not removed or rinsed in all studies.

- Ten of the in-house studies were classified as of high strength of evidence, two of medium, and three of low strength (Table 4).

Table 4: Studies submitted by the Applicant and the reasons for inclusion/exclusion from the assessment

Paper number	Type of paper/study (a)	Reference	Include in assessment	Reason for exclusion	Industrial/ pilot/lab	Natural/ inoculated	Microorganisms	Product group	Strength of evidence[h]
Treatment group 1: studies on warm carcasses with spray treatment									
1	IHS	(Abraham et al., 2006)	YES		Industrial	Natural	*Salmonella* (b), *E. coli*, coliforms	Broiler carcass	High
2	IHS	(Abraham et al., 2007)	YES		Industrial	Natural	*Salmonella* (b), *E. coli*, coliforms	Poultry carcass	High
9	IHS	(Dankert, 2011)	YES		Industrial	Natural	*Salmonella* (b), *E. coli*	Poultry carcass	High
13	IHS	(FMC, 2009)	YES		Industrial	Natural	*Salmonella* (b), *E. coli*, coliforms	Poultry carcass	High
14	IHS	(FSIS et al., 2012)	YES		Industrial	Natural	*Salmonella* (b), *E. coli*	Broiler carcass	High
18	IHS	(Rodrigues and Howarth, 2010)	YES		Industrial	Natural	*Salmonella* (b), *E. coli*	Broiler carcass	High
12	IHS	(Ecolab, 2001)	YES		Pilot	Natural	*E. coli*, coliforms	Poultry carcass	Medium
15	IHS	(Hochmuth, 2000)	NO	Not warm carcasses used					
Treatment group 2: studies on warm carcasses or parts with short-duration dip treatment									
3	IHS	(Abraham et al., 2010)	YES		Industrial	Natural	*Salmonella* (b), *E. coli*, coliforms	Broiler carcass	High
4	IHS	(Abraham, et al., 2011)	YES		Industrial	Natural	*Salmonella* (b), *E. coli*, coliforms	Broiler carcass	High
24	IHS	(Verkaar, 2006)	NO	Only total viable bacteria included					
16	PR	(Mehyar et al., 2005)	YES		Laboratory	Inoculated	*Salmonella* (e), *C. jejuni*, *E. coli* O157:H7	Chicken wing parts	Low
Treatment group 3: studies on effects in chiller baths									
6	PR	(Bauermeister et al., 2008b)	YES		Industrial	Natural	*Salmonella*, *Campylobacter* (b)	Broiler carcass	High
9	IHS	(Dankert, 2011)	YES		Industrial	Natural	*Salmonella*, *E. coli*	Broiler carcass and chicken parts [(c)]	High

Paper number	Type of paper/study (a)	Reference	Include in assessment	Reason for exclusion	Industrial/ pilot/lab	Natural/ inoculated	Microorganisms	Product group	Strength of evidence[h]
13	IHS	(FMC, 2009)	YES		Industrial	Natural	*Salmonella* (b), *Campylobacter*, *E. coli*, coliforms	Poultry carcass. Study in a turkey processing plant	High
21	IHS	(Thompson et al., 2009) (c)	NO	Concentration used exceeds limit of chilling baths (230 ppm)					
23	PR	(Vadhanasin et al., 2004)	YES		Industrial	Natural	*Salmonella* (b)	Broiler carcass	High
5	PR	(Bauermeister et al., 2008a)	YES		Pilot	Natural	*E. coli*, coliforms	Broiler carcass	High
12	IHS	(Ecolab, 2001)	YES		Pilot	Natural	*E. coli*, coliforms	Poultry carcass	Medium
5	PR	(Bauermeister et al., 2008a)	YES		Laboratory	Inoculated	*S.* Typhimurium, *C. jejuni*	Broiler carcass	Low
15	IHS	(Hochmuth, 2000)	YES		Laboratory	Inoculated	*S.* Typhimurium, *E. coli* O157:H7	Chicken wings	Low
22	CP (c)	(Trevanich et al., 2003)	NO	Not full details available					
Treatment group 4: studies on chilled carcasses or parts with short-duration dip treatment									
10	PR	(Del Rio et al., 2007a)[g]	YES		Laboratory	Natural	*Enterobacteriaceae*, coliforms	Chicken legs	Medium
17	PR	(Nagel et al., 2013)	YES		Pilot	Inoculated	*S.* Typhimurium, *C. jejuni*	Broiler carcass	Medium
7	PR	(Chantarapanont et al., 2004)[g]	YES		Laboratory	Inoculated	*C. jejuni*	Chicken skin	Low
8	IHS	(Dankert, 2010)[g]	YES		Laboratory	Inoculated	*Salmonella*, *E. coli*	Chicken part and carcass	Low
11	PR	(Del Rio et al., 2007b)[g]	YES		Laboratory	Inoculated	*S.* Enteritidis, *E. coli*	Chicken legs	Low
16	PR	(Mehyar et al., 2005)	YES		Laboratory	Inoculated	*Salmonella* (e), *C. jejuni*, *E. coli* O157:H7	Chicken wing parts (f)	Low

Paper number	Type of paper/study (a)	Reference	Include in assessment	Reason for exclusion	Industrial/ pilot/lab	Natural/ inoculated	Microorganisms	Product group	Strength of evidence[h]
19	IHS	(Rodrigues and Howarth, 2011)	NO	Duration of the treatment was 5 min					
20	IHS	(Rodrigues et al., 2011)	YES		Laboratory	Inoculated	*S.* Typhimurium, *C. jejuni*	Broiler carcass	Low

(a): PR=peer reviewed paper; IHS=in-house study.
(b): Prevalence study.
(c): Poster of FMC corporation presented at annual meeting of International Association for Food Protection.
(d): Washed is given in paper (assumed is dipping).
(e): Cocktail of two strains of *Salmonella* Typhimurium and one strain of *S.* Heidelberg.
(f): Obtained from retail.
(g): Studies classified by the Applicant under treatment group 2, but reclassified under group 4 because warm carcasses were not used.
(h): Strength of evidence assigned as presented in Table 2.

3.4. Statistical significance and statistical methods used

In this section the statistical methods used in the studies included in the assessment are discussed. The statistical methods used for analysing experimental data were reported in varying levels of detail, and in many papers were not fully documented. Several papers applied appropriate methods, but for other papers the appropriateness cannot be fully evaluated or can be questioned.

Papers 1 and 2 provided detailed descriptions of the statistical methods, including testing for normal distribution of count data. If data were not normally distributed, log-transformation does not appear to have been attempted but the non-parametric Mann-Whitney test has been applied. This test is appropriate for non-normally distributed data but less powerful than parametric tests on log-transformed data. In papers 3 and 4 (by the same first author), log transformation of count data was reported before applying Analysis of Variance (ANOVA).

Papers 5, 7, 9, 10, 11, 12 also used ANOVA on log-transformed data to evaluate differences between factors in the experiments. Paper 17 used generalised linear models. Duncan's multiple range test was used to accommodate for multiple comparisons in papers 10 and 11. These methods are considered adequate for the purpose of this Scientific Opinion, although testing for normal distributions or log-transformations were not explicitly reported in papers 7 and 9.

Papers 18 and 20 used t-tests assuming unequal variances, which may be appropriate. Details on data transformation were lacking, as well as results for tests of equal variance, so that the statistical methods cannot be fully evaluated.

Handling zero counts has either not been reported by the authors, or follows difference conventions, replacing zeros by 1, 0.9 or 0.5 times the limit of quantification. The impact of these differences cannot be evaluated because the number of zero counts relative to the total number of counts has not been reported in any of the papers.

In papers 1, 2 and 9, presence-absence data were appropriately evaluated by contingency tables and X^2 tests, or Fischer's exact test to account for low prevalences. Paper 6 reported only presence/absence data, which were analysed by ANOVA. This is surprising as ANOVA is typically used for count data.

Statistical methods could not be evaluated for paper 16 (only use of SAS software is mentioned). No statistical analysis was reported in papers 8, 13, 14 and 23.

3.5. Evaluation of studies

The studies in the papers evaluated encompassed a wide range of experimental designs and thus differed in relation to products, settings, method of application, PAA concentration applied, temperature of application, types of controls used, microorganisms studied and the microbial load of the surface, microbiological methods used, storage time after application, etc. All of these parameters impacted on the PAA decontaminating efficacy both within and between studies. Given this wide range of application conditions, the assessment did not attempt to identify the contribution of differences among factors, such as PAA concentration and application temperature on the bactericidal effect.

In this section, a brief summary of the experimental set-up and main results and conclusions of each chapter are provided per treatment group. Then, the combined data for each treatment group are presented in forest plots, separately for enumeration and prevalence studies. For enumeration studies, decimal reduction values are presented with confidence intervals calculated by EFSA when the mean, standard deviation and sample size were known and /or raw data were available in the studies as provided by the Applicant and if variances between groups are equal as:

$$\text{Mean decimal reduction (MDR)} = \mu\ \text{bacterial counts}_C - \mu\ \text{bacterial counts}_T$$

$$\text{Confidence intervals (CI) of MDR} = \text{MDR} \pm 1.96\sqrt{\left(\frac{SD_C}{n_C}\right)^2 + \left(\frac{SD_T}{n_T}\right)^2}$$

Where:

- μ = Mean bacterial counts of control ($\mu_{\text{bacterial counts, C}}$) or treatment ($\mu_{\text{bacterial counts, T}}$) group
- SD = Standard deviation of mean bacterial counts of control (SD_C) or treatment (SD_T) group
- n = Sample size control (n_C) or treatment (n_T) group

For prevalence studies, the relative prevalence reduction (RPR) and confidence intervals (if the absolute number of positive samples in the controls were >5) were calculated as:

$$\text{Relative Prevalence Reduction (RPR)} = 1 - \frac{\frac{T_+}{n_T}}{\frac{C_+}{n_C}}$$

$$\text{Confidence intervals (CI) of RPR} = 1 - e^{\ln\left(\frac{\frac{T_+}{n_T}}{\frac{C_+}{n_C}}\right) \pm 1.96\sqrt{\left(\frac{1}{C_+}\right) + \left(\frac{1}{T_+}\right) - \left(\frac{1}{n_C}\right) - \left(\frac{1}{n_T}\right)}}$$

Where:

- C_+ = Positive samples in the control group
- T_+ = Positive samples in the treatment group
- n = Sample size control (n_C) or treatment (n_T) group

If absolute number of positive samples in treated group were <5 then CI were calculated by using the asymptotic method by Miettinen and Nurminen (robust approximation) (Miettinen and Nurminen, 1985; Newcombe, 1998).

$$\left\{\theta: |\hat{\theta} - \theta| \leqslant z\sqrt{\left[\lambda\left\{\frac{(\psi_\theta + \theta/2)(1 - \psi_\theta - \theta/2)}{m} + \frac{(\psi_\theta - \theta/2)(1 - \psi_\theta + \theta/2)}{n}\right\}\right]}\right\}$$

Where:

- $\theta = C_+/n_C - T_+/n_T$
- $\psi = (C_+/n_C + T_+/n_T)/2$
- $\lambda = (n_C+n_T)/(n_C+n_T -1)$
- z = 1.96
- m = sample size of control group
- n = sample size of treatment group

Finally, summary tables of results in different treatment groups are presented with conclusions. In these summary tables, results are presented separately depending on the availability of confidence intervals. For enumeration studies, results are considered relevant if the CI did not include 0 or, if CI were not available, following expert judgement, if the decimal reduction value was more than 0.5 log-units higher or lower than zero. For prevalence studies, CI were computed with the above formula if the absolute number of positive samples in the controls were >5, otherwise the trials were excluded from the summary graphs. The results were considered significant if the CI of the relative prevalence reduction did not include 0.

3.5.1. Treatment group 1: studies on warm carcasses with spray treatment

3.5.1.1. High strength of evidence studies on warm carcasses with spray treatment

Paper 1 (Abraham et al., 2006)

Set up: This is an online study where 25 ppm PAA was used in an online reprocessing (OLR) system, on 20 clean carcasses compared to 20 untreated clean carcasses. The OLR system is a spray cabinet set at a flow rate of 12 gallons per min. OLR sprays are typically 2-3 sec spray time followed by 30-60 sec drip time. All carcasses were tested for coliforms and *E. coli* counts, and *Salmonella* prevalence.

Results and conclusions: The *E. coli* and coliform counts were 0.72 and 0.59 log units lower on clean treated carcasses compared to untreated carcasses respectively. *Salmonella* was not detected on clean untreated carcasses and therefore *Salmonella* reduction could not be evaluated.

Paper 2 (Abraham et al., 2007)

Set up: In this study on-line reprocessing PAA treatments (from 50-180 ppm, 120-180 ppm, and 105 ppm) were tested in three different commercial facilities. Sampling and testing of carcasses were performed after cleaning and inspection. Only samples from visibly clean carcasses were considered. Twenty samples from each category (treated and untreated) were sampled for *E. coli*, coliforms (quantitatively) and *Salmonella* (qualitatively) at five, three and four occasions respectively at the three plants.

Results and conclusions: The *E. coli* reduction in treated versus untreated visually clean carcasses was 1.2, 0 and 1.0 log units in the three plants. Coliforms in treated *versus* untreated visually clean carcasses were reduced by 1.3, 0.1 and 1.0 log units in the three plants. *Salmonella* samples were only obtained from plant 1 and 2. In plant 1 a 87 % relative prevalence reduction of *Salmonella* was found in visibly clean carcasses (proportion positive carcasses reduced from 23/100 to 3/100). In plant 2 a

40 % relative prevalence reduction (proportion positive carcasses reduced from 5/60 to 3/60) was found in visible clean carcasses.

Paper 9 (Dankert, 2011)

Setup: The study represented an industrial evaluation of pathogen reduction by spraying with 40-95 ppm PAA (estimated average was about 73 ppm PAA) for 2-3 seconds and a total wetted time at 30 to 45 seconds. Three groups of 60 naturally-contaminated but visibly clean carcasses were quantitatively analysed for *E. coli* and qualitatively for *Salmonella* before and after treatment.

Results and conclusions: The study showed a reduction from 2.79 to 1.99 log units/g of *E. coli*. *Salmonella* prevalence was reduced from 50.5 % to 36.1 %.

Paper 13 (FMC, 2009)

Setup: This study (trial 2) represented an industrial investigation of the reduction of naturally contaminated carcasses by spray treatment in OLR Cabinet and Chiller – four different sites in two production lines (line 1: 90-105 ppm PAA; line 2: 135 ± 15 ppm PAA). For each treatment, samples for quantitative analysis for *E. coli* and coliforms and qualitative analysis for *Salmonella* were collected after evisceration (pre-treatment) and after the last washing cabinet (post-treatment). 20 carcasses were investigated at each point.

Results and conclusions: The results showed a log reduction of coliforms (line1/line2) of 1.33 and 0.95 log units/g and for *E. coli* (line1/line2) of 1.34 and 0.76 log $_{10}$ units /g. At the first line *Salmonella* was not reduced by the treatment (75 % prevalence both pre- and post-treatment), whereas at the second line the relative prevalence reduction was 70 %; prevalence declined from 50 % to 15 %.

Paper 14 (FSIS et al., 2012)

Set up: Summary report from commercial facilities tests of the efficacy of on-line reprocessing (OLR) systems using antimicrobial sprays. Six of the 11 data sets included in the study used PAA. Concentrations of PAA and specific conditions in each plant were not provided, although all but one included a measurement of both clean and contaminated carcasses. A clean poultry carcass was inspected by Federal inspection personnel and did not require reprocessing. A dirty or contaminated poultry carcass was inspected and required reprocessing because of visible digestive tract contamination in the carcass cavity. Given current industry practice, most of the OLR treatments were most probably in the range of 150 to 200 ppm PAA.

APC and *E. coli* abundance and *Salmonella* prevalence were measured in all plants

Results and conclusions:

Data generated from the in-plant trials demonstrated that the technologies used in the studies yielded definite improvements.

- Both OLR and off-line reprocessing (OFLR) in-plant trials demonstrated an average log reduction for APC, *E. coli* and coliforms. Both OLR and OFLR demonstrated a percent positive reduction for *Salmonella*.
- OLR sample size range: 823 – 1990.
- OFLR sample size range: 205 – 210.
- Although OFLR demonstrated a larger average log reduction for APC, *E. coli* and coliforms than OLR, the larger sample size for the OLR studies demonstrated the higher confidence with which OLR systems would achieve a definitive improvement for average log reduction.

- OLR had a better *Salmonella*-positive reduction then OFLR.

The results showed a log reduction of *E. coli* (plant 1, 2, 3, 4, 5) of 1.24, 0.30, 0.01, 1.30 log units and as small increase (0.72 log units in plant 6). Relative prevalence reductions in plants 1, 2, 3 and 5 were 68.0 % (decline from 68.8 % to 22 %), 60.9 % (decline from 23.0 % to 9.0 %), 19.2 % (decline from 5.0 % to 4.0 %) and 0 % (prevalence remained 5.0 %).

Paper 18 (Rodrigues and Howarth, 2010)

Setup: Processing system in commercial facility. 100 ± 7 ppm PAA in spray cabinet. 2-5 seconds spray, 20 ± 5 seconds contact time at ambient temperatures. 109 or 110 carcasses sampled before and after spraying for both carcasses considered clean (passing inspection) and carcasses considered marginal (small amounts of visible ingesta or faecal material)

Carcasses washed before sampling in an inside-outside bird washer with approximately 15 ppm PAA in the water (normal plant operation). Study conducted over 11 sampling days. Abundance of *E. coli,* APC and prevalence of *Salmonella* were measured.

Results and conclusions: Low reduction by PAA treatment (< 1.5 log units) for *E. coli*; high (68 %) relative prevalence reduction of *Salmonella* (*Salmonella* prevalence reduced from 68.2 % to 21.8).

3.5.1.2. Medium strength of evidence studies on warm carcasses with spray treatment

Paper 12 (Ecolab, 2001)

Set up: Pilot study with naturally-contaminated samples that were analysed by quantification of coliforms and *E. coli*. The objective of the study was to evaluate the effect of PAA in relation to reduction of spoilage or decay caused by bacteria, but in this context the occurrence of coliforms and *E. coli* were evaluated as indicators for pathogen contamination. The effect of spraying carcasses with 200 ± 5 ppm PAA for 15 seconds at ambient temperature was evaluated by comparing to spraying with water.

Results and conclusions: The results showed a reduction of contamination level of coliforms at 0.64 log units after spraying with PAA compared to a reduction at 0.33 log units by spraying with water. For *E. coli* the reduction was 0.84 log units after spraying with PAA and 0.46 log units after spraying with water.

3.5.1.3. Conclusions of studies on warm carcasses with spray treatment

Forest plot of results obtained with spray treatment on warm carcasses are shown in Figures 1 and 2.

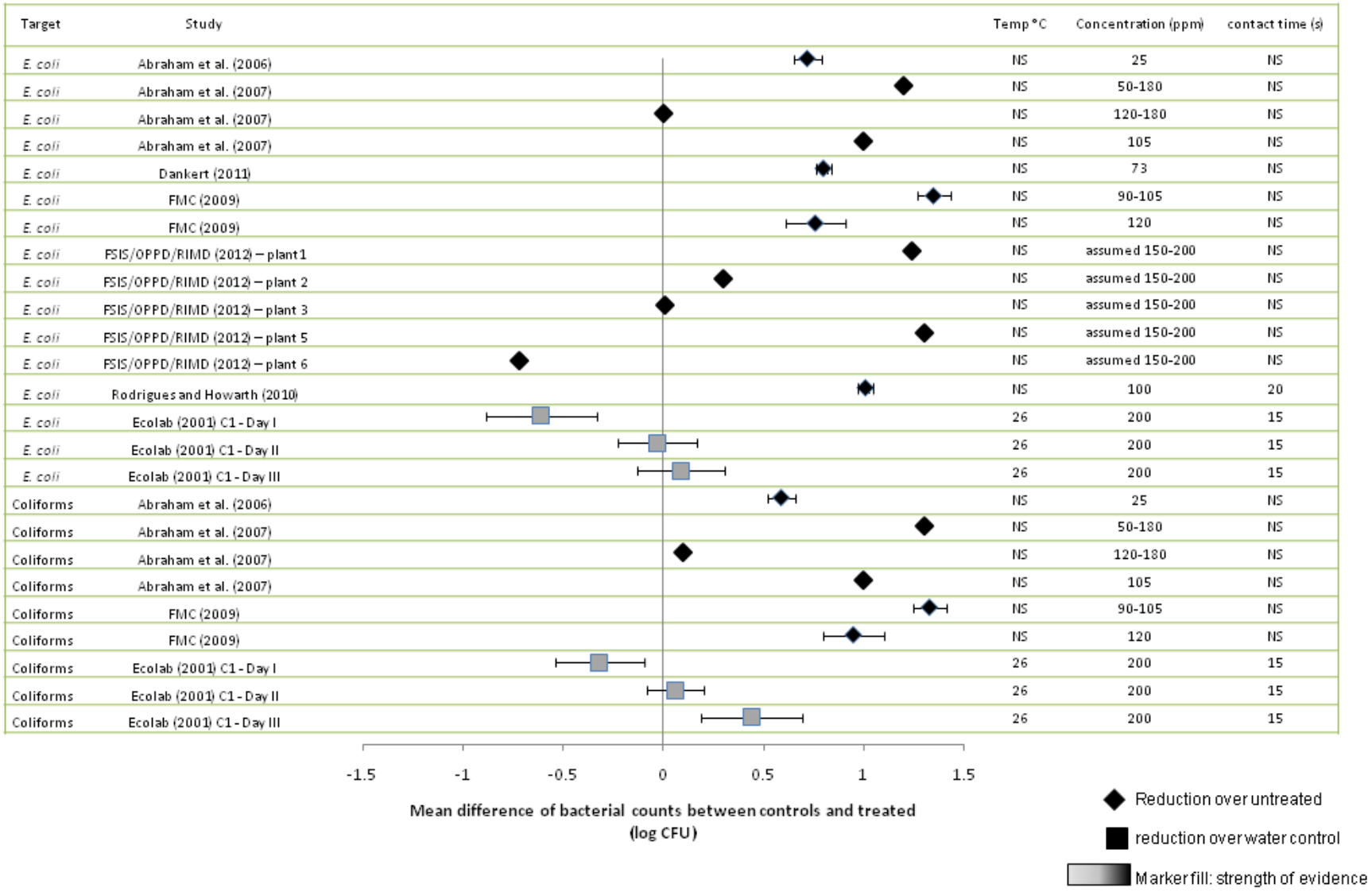


Figure 1: Forest plot of results obtained with spray treatment on warm carcasses (mean difference of bacterial counts; NS: not stated).

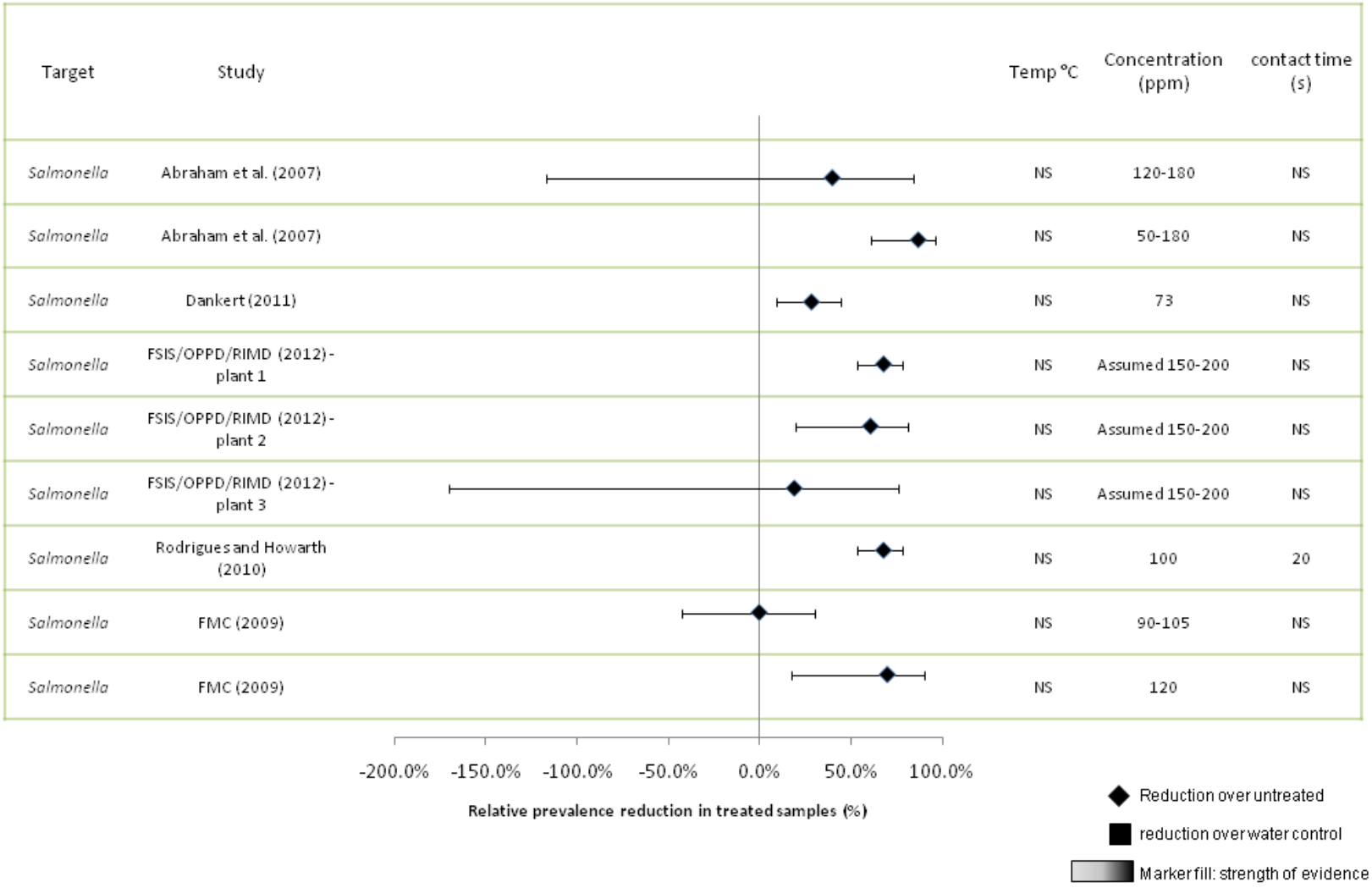


Figure 2: Forest plot of results obtained with spray treatment on warm carcasses (relative prevalence reduction)

Table 5 shows a summary of individual data sets from each study. In enumeration studies, there was a positive+ effect on *E. coli* (i.e. either a significant reduction of the log CFU or a mean decimal reduction > 0.5 log-units if CI were not provided) in 9/16 datasets; the mean decimal reduction ranged between 0.72 and 1.35 log-units. For coliforms, there was a positive effect in 6/9 studies, with mean decimal reduction ranging between 0.5 and 1.3 log-units. There was one dataset that indicated a significant increase of coliforms counts; two that indicated a significant increase of *E. coli* counts. All studies with indicator organisms were of high or medium strength of evidence. No enumeration data on pathogens were provided.

Nine prevalence studies were included in the summary graphs (with the number of positive samples in the control group > 5) and were provided only for *Salmonella*. The *Salmonella* prevalence was significantly reduced in 6/9 datasets; the mean relative prevalence reduction was >50 % in 5/6 of those datasets. 3/9 studies showed a non-significant *Salmonella* prevalence reduction. All prevalence studies were of high strength of evidence and all showed reduction over untreated control samples.

Table 5: Summary of results of studies on warm carcasses with spray treatment

Decimal reduction	*E. coli*	Coliforms	*Salmonella*	*Campylobacter*
Enumeration studies, confidence interval provided				
Statistically significant reduction	5(0*)	4(1*)	0	0
No significant effect	2*	1*	0	0
Significant increase	1*	1*	0	0
Enumeration studies, confidence interval not provided				
MDR > 0.5 log	4(0*)	2(0*)	0	0
-0.5<MDR<0.5 log	3(0*)	1(0*)	0	0
MDR < -0.5 log	1(0*)	0	0	0
Prevalence studies				
Statistically significant reduction	0	0	6(0*)	0
No effect	0	0	3(0*)	0

* number of studies out of the total where water control was applied

3.5.2. Treatment group 2: studies on warm carcasses or parts with dip treatment

3.5.2.1. High strength of evidence studies on warm carcasses or parts with dip treatment

Paper 3 (Abraham et al., 2010)

Set up: Clean and dirty carcasses were treated on line in a commercial facility with 100, 200, 1000 and 1 200 ppm PAA. Samples were taken after evisceration, washing, and inspection and after treatment with PAA. Each concentration was tested (submerged time 25 seconds) on a single day and flock. Twenty carcasses were sampled for each condition. All carcasses were treated and the purpose was to demonstrate that visible contaminated carcasses post treatment were microbiological equivalent to visibly clean birds before treatment. Only the efficacy on visibly clean carcasses was considered.

Results and conclusions: The *E. coli* reduction in treated versus untreated (pre- *versus* post-treatment) visually clean carcasses ranged from 1.24 to > 2.5 log unit reduction. The reductions in coliforms ranged from 1.16 to above 2.94 log units. The log reduction increased with the PAA concentration used. The relative prevalence reduction in *Salmonella* was between 50-100 % in visibly clean carcasses.

Paper 4 (Abraham, et al., 2011)

Set up: Clean and dirty carcasses were treated on line in a commercial facility with 100, 500, 1000 and 2 000 ppm PAA. Samples were taken after evisceration, washing, and inspection and after treatment with PAA. Each concentration was tested (submerged time 25 seconds) on a single day and flock. Twenty carcasses were sampled for each condition. All carcasses were treated and the purpose was to

demonstrate that visible contaminated carcasses post treatment are microbiological equivalent to visible clean birds before treatment. Only the efficacy on visible clean carcasses was considered.

Results and conclusions: The *E. coli* reduction in treated versus untreated (pre- versus post-treatment) visually clean carcasses was from 1.94 to 2.91 log unit reduction (zero values were assigned a value equal to the detection limit. The log reduction increased with the PAA concentration used. The reduction in *Salmonella* prevalence was between 71-100 % in visible clean carcasses. In all cases where 1 000 and 2 000 ppm PAA were used the reduction in *Salmonella* prevalence was 100 %.

3.5.2.2. Low strength of evidence studies on warm carcasses or parts with dip treatment

Paper 16 (Mehyar et al., 2005)

Setup: Chicken wing parts (drumettes) obtained from commercial processing plant after defeathering inoculated with strains of *E. coli* O157:H7, *C. jejuni*, and three strains of *S. enterica* by dipping into culture for 15 seconds then allowed to drain for 15 minutes. Treatment with 200 ppm PAA (formula containing octanoic acid) by dipping for 1 minute in treatment or water followed by 30 second drain time. Unchilled samples; internal temperatures, 38 to 40°C. Treatment solution at ambient temperature. In some tests application of antimicrobial made one minute before application of bacterial inoculation. Storage study after treatment with 1minute dip and storage. Storage study at 7°C; measurement of naturally occurring pseudomonades and psychrotrophs. Measurement of log reductions on log units/g relative to water-dip control on unchilled samples treated by dipping for 1 minute into 200 ppm PAA after inoculation.

Results and conclusions: log unit reductions of 0.04 (*Salmonella*), 0.32 (*Campylobacter*) 0.63 (*E. coli* O157) over control.

3.5.2.3. Conclusions of studies on warm carcasses or parts with dip treatment

Forest plots of results obtained with dip treatment of warm carcasses or parts are shown in Figures 3 and 4.

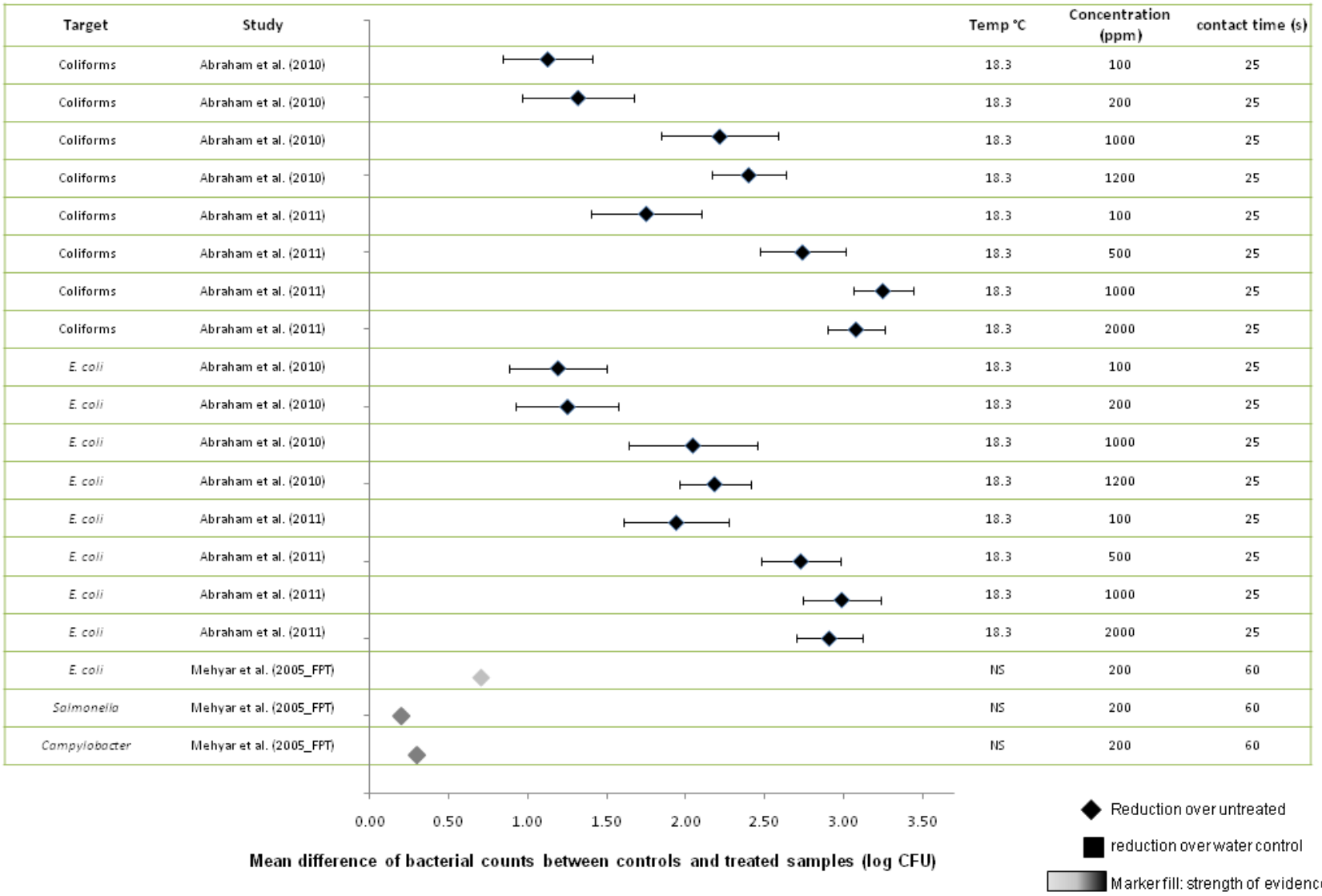


Figure 3: Forest plots of results of mean difference of bacterial counts obtained with dip treatment of warm carcasses or parts

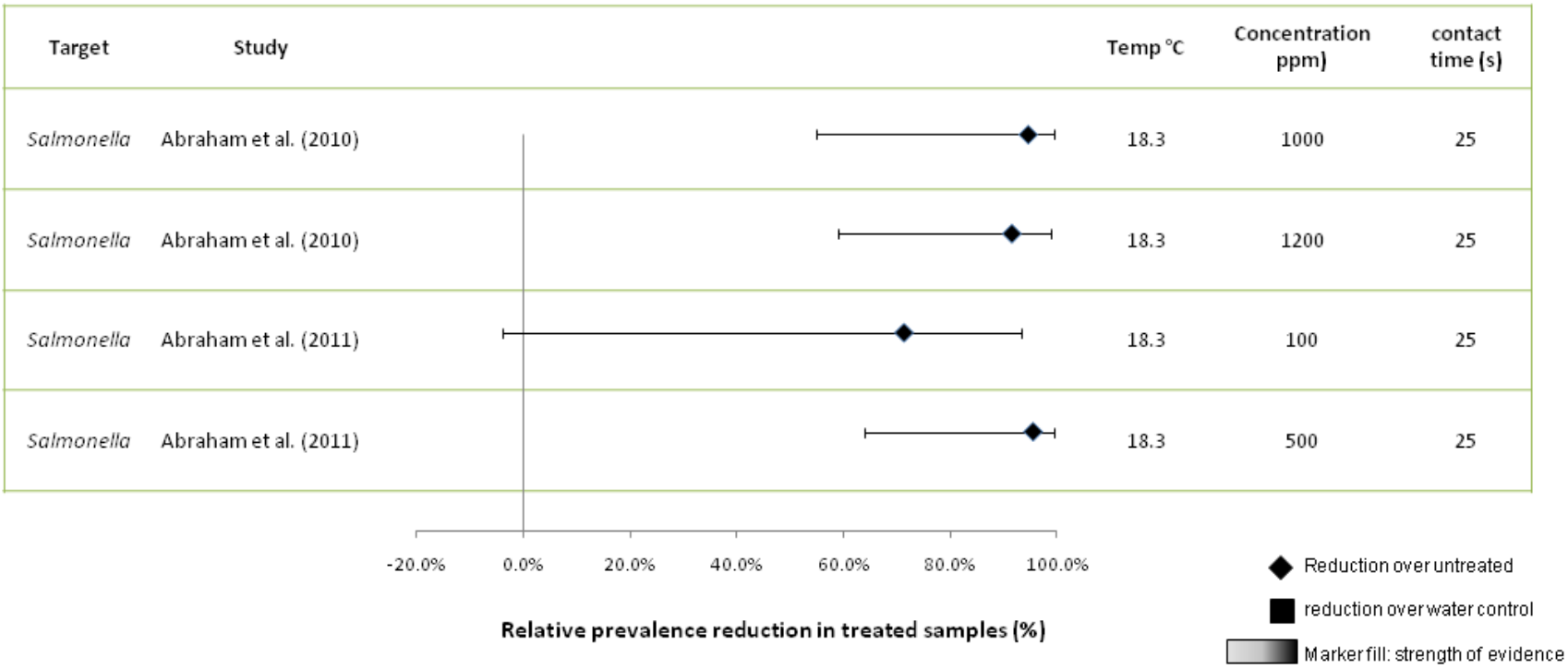


Figure 4: Forest plots of results of relative prevalence reduction obtained with dip treatment of warm carcasses or parts

Table 5 shows a summary of individual data sets from each study. In enumeration studies, there was a positive effect on *E. coli* (i.e. either a significant reduction of the log CFU or a reduction > 0.5 log-units if CI were not provided) in 9/9 datasets; the mean decimal reduction (MDR) ranged between 0.7 and 3.0 log-units. For coliforms, there was a positive effect in 8/8 studies with decimal reduction ranging between 1.12 and 3.25 log-units. All studies with indicator organisms, except one with *E. coli* were of high strength of evidence. No enumeration data on pathogens were provided. One study with low strength of evidence showed mean decimal reduction of *Salmonella* and *Campylobacte*r of <0.5 log-units; in this study the *E. coli* reduction was also less than in all other studies. All studies showed reduction over untreated control samples.

Four prevalence studies were included in the summary graphs (with number of positive samples in the controls >5) and were provided only for *Salmonella.* The *Salmonella* prevalence was significantly reduced in 3/4 datasets; the relative prevalence reduction was >50 % in those datasets.. All prevalence studies were of high strength of evidence and all showed reduction over untreated control samples.

Table 6: Summary of results of studies on warm carcasses parts with dip treatment

Decimal reduction	Coliforms	*E. coli*	*Salmonella*	*Campylobacter*
Enumeration studies, confidence interval provided				
Statistically significant reduction	8(0*)	8(0*)	0	0
No significant effect	0	0	0	0
Significant increase	0	0	0	0
Enumeration studies, confidence interval not provided				
MDR > 0.5	0	1(0*)	0	0
-0.5<MDR<0.5	0	0	1(0*)	1(0*)
MDR < -0.5	0	0	0	0
Prevalence studies				
Statistically significant reduction	0	0	3(0*)	0
No effect	0	0	1(0*)	0

* number of studies out of the total where water control was applied

3.5.3. Treatment group 3: studies on effects in chiller baths

3.5.3.1. High strength of evidence studies on effects in chiller baths

Paper 6 (Bauermeister et al., 2008b)

Set up: 85 ppm PAA was evaluated for effectiveness compared with the 30-ppm chlorine treatment in a commercial setting. In this trial, 100 broiler carcasses were sampled for *Salmonella* and *Campylobacter* spp. prior to chilling and 100 carcasses were sampled after chilling. In all, 400 carcasses were sampled using 85 ppm of PAA in the chiller and 400 carcasses were sampled using the chlorine treatment.

Results and conclusions: PAA at 85 ppm reduced *Salmonella*-positive carcasses from 30.5 % to 2.5 % on exiting the chiller, i.e. a 91.8 % relative prevalence reduction. Treatment with 30 ppm of chlorine resulted in a 57 % relative prevalence reduction. Additionally, PAA gave a relative prevalence reduction of *Campylobacter*–positive carcasses exiting the chiller of 43 % while chlorine resulted in a 13 % relative prevalence reduction.

Paper 9 (Dankert, 2011)

Set up: The study represented an industrial investigation of reduction of natural contamination by supplementing chilling bath in three production lines with 8 to 30 ppm PAA for 45 to 90 minutes. A total of 60 pre-chill carcasses for each of the lines were investigated and compared to 60 post-chill carcasses by quantitatively analysed for *E. coli* and qualitatively for *Salmonella*.

Results and conclusions: The study demonstrated a reduction from 2.05 to 0.63 log units/g *E. coli*. The prevalence of *Salmonella* was reduced from 37.2 % to 1.6 %, corresponding to a 95.5 % relative prevalence reduction.

Paper 13 (FMC, 2009)

Setup: Industrial study separated in 4 trials - #1; #3,-#5 for investigation of the reduction of natural contamination of pathogens (*Salmonella* and *Campylobacter*) or indicator organisms as coliforms and *E. coli*. Treatments were applied by adding PAA to the chilling tanks in variable concentrations. Different chilling tanks placed in different positions at the slaughter line were included. Sampling at identical point on days without treatment served as controls.

#1 treatment in final chiller bath with PAA concentration between 60 and 90 ppm. Samples were investigated qualitatively for *Salmonella* and *Campylobacter*.

#3 treatment in two final chillers (between 45 – 60 ppm) performed on two lines over three days. Samples were investigated quantitatively for coliforms and *E. coli* and qualitatively for *Salmonella*.

#4 treatment in the pre-chiller tank (105 ppm) performed at over two days. Ten carcasses were selected for investigation before entering the pre-chill tank and 10 carcasses were selected after exit from the final chiller. At day two 8 of 10 carcasses selected post-treatment were identical with those selected pre-treatment. Samples were investigated quantitatively for coliforms and qualitatively for *Salmonella* and *Campylobacter*.

#5 treatment in a series of three chillers; 50 ppm PAA was add to the last chiller – turkey plant. Eight samples were collected randomly before entering the chiller and 10 samples after chilling. Similar samples, collected from chilling system without added PAA, served as control. Samples were investigated quantitatively for coliforms and *E. col*i and qualitatively for *Salmonella.*

Results and conclusions:

#1 The results showed a relative prevalence reduction by PAA treatment of *Salmonella* at 97.5 % (from 62.1 % to 1.6 %) and *Campylobacter* at 96.6 % (from 79.3 % to 3.1 %). When compared to the reduction obtained during chilling without PAA at 33 % and 19 % respectively, the relative prevalence reduction was 98 % and 96 %.

#3 The results showed a reduction of coliforms (line1/line2) from 2.22 and 0.85 log units/g to below the detection limit and of *E. coli* from 1.97 and 0.53 log unit/g to below the detection limit. At the first line *Salmonella* was reduced from a prevalence of 26.7 % to 3.3 % (87.5 % relative prevalence reduction) by the treatment whereas at the second line the prevalence declined from 32.7 % to 1.2 % (96.4 % relative prevalence reduction).

#4 The contamination level of coliforms before treatment was 0.87 and 1.24 log unit/g at day 1 and 2, respectively. After treatment the level was 0.89 and 0.67 log units/g, respectively, which documented a growth of coliforms at day 1 and a log reduction at 0.57 at day 2. Also the level of *Salmonella* increased at day 1 whereas it declined at day 2 from a prevalence of 40 % to a prevalence of 20 % (relative prevalence reduction of 50 %). The relative prevalence reduction for *Campylobacter* was 83 % (from 60 % to 10 %) at day 1 and 100 % (from 10 % to zero) at day 2.

#5 The results showed a reduction of coliforms from 2.87 log unit/ml before chilling to below the detection limit after chilling. Similarly, the level of *E. coli* decline from 2.54 log units/ml before chilling to below detection limit after chilling. The study also demonstrated a reduction by chilling without treatment with PAA. By including these data the efficiency of PAA over the control was 2.87 and 2.54 log units/ml for coliforms and *E. coli* respectively.

The prevalence of *Salmonella* was reduced from 75 % to 10 % (87 % relative prevalence reduction) by treatment of PAA whereas there was a reduction from 100 % to 20 % (80 % relative prevalence reduction) in the control system.

Paper 23 (Vadhanasin et al., 2004)

This study is included assuming that a 250 ppm concentration can be considered as in line with 230 ppm threshold.

Set up: In an experimental intervention in commercial poultry plants, 250 ppm PAA was added to chiller water; results were compared with historical controls with chiller water with unspecified (low*) levels of chlorine. Chiller temperature was 4 to 15 °C, and chiller duration 45 to 50 minutes. 25 g of "meat" from carcasses was sampled and analysed for *Salmonella* by presence/absence testing.

Results and conclusions: *Salmonella* prevalence in carcasses from chillers with water with "unspecified low levels of chlorine" was 22.7 %, although sampled only after treatment. Therefore no relative prevalence reduction could be calculated; this was reduced to 5 % by addition of PAA. Statistical analysis was stated to be by t-test, although this test can be applied to count data, but not to presence/absence data. Medium strength of evidence studies on effects in chiller baths.

3.5.3.2. Medium strength of evidence studies on effects in chiller baths

Paper 5 (Bauermeister et al., 2008a)

Set up: This was a storage study in a simulated chiller. Five hundred naturally contaminated carcasses were collected after slaughter and processing. These carcasses (100 for each treatment) were treated with 100 ppm, 150 ppm, 200 ppm PAA, 30 ppm chlorine and untreated water for 2 hours at chill, 4°C. Carcasses were not inoculated and counts of *E. coli* and coliforms were measured at day 1, 7, 10 and 15 after treatment.

Results and conclusions: By day 1, the number of *E. coli* was only lower, in samples treated with 150 and 200 ppm PAA compared to water treated samples. Thus the *E. coli* counts (log CFU) were 0. 57 log units lower (for 150 ppm) and 1.11 log units lower (for 200 ppm). Also for coliforms only samples treated with 150 ppm and 200 ppm had lower counts than was seen in water treated samples. Thus the coliform counts were 0.46 log and 1.14 log units lower than the counts seen in water treated samples. By day 7, there were no differences noted in the *E. coli* or coliforms among any of the treatments tested.

Comment: This result raises the question as whether Gram-negative bacteria are only sublethally injured by PAA under the conditions used in the experiment.

Paper 12 (Ecolab, 2001)

Set up: Pilot study with naturally-contaminated samples that were analysed by quantification of coliforms and *E. coli*. The objective was to evaluate the effect of PAA in relation to reduction of spoilage or decay caused by bacteria, but in this context the occurrence of coliforms and *E. coli* were evaluated as indicators for pathogen contamination. The effect of chilling with 30 ppm PAA and the combination of chilling and spraying, as described above, were evaluated by comparing to chilling/spraying with water.

Results and conclusions: The results, showed for chilling only, a reduction of contamination level of coliforms at 1.27 log units after treatment with PAA compared to a reduction at 0.60 log units after chilling with water. For *E. coli* the reduction was 1.37 log after chilling with PAA and 0.56 log units after spraying with water. The improved efficacy of PAA over water was 0.67 log units for coliforms and 0.81 log units for *E. coli*.

After combination of chilling and spraying, the results showed a reduction of contamination level of coliforms at 1.31 log units after treatment with PAA compared to a reduction at 0.78 log units after treatment with water. For *E. coli* the reduction was 1.44 log units after treatment with PAA and 0.85 log units after treatment with water. The improved efficacy of PAA compared to water was 0.53 log units for coliforms and 0.59 log units for *E. coli*.

3.5.3.3. Low strength of evidence studies on effects in chiller baths

Paper 5 (Bauermeister et al., 2008a)

Setup: The ability of different PAA levels to reduce *Salmonella* and *Campylobacter* on inoculated samples has been compared to levels of chlorine. 100 broiler carcasses were obtained from commercial processing facility pre-chiller. 40 were inoculated with *S.* Typhimurium and 40 with *C. jejuni* followed by 10 minute attachment time. Carcasses were placed in the assigned treatment. Treatments were 25, 100, 200 ppm PAA, or 30 ppm chlorine for one hour at 4 C.

Results and conclusions: The study showed that all PAA levels reduced the CFU/sample of *Salmonella* more than the reduction obtained by using 30 ppm chlorine. The reduction in *Salmonella* counts at 25 ppm, 100 ppm and 200 ppm compared to chlorine treated samples was 0.9, 1.2 and 1.3 log units, respectively. The reduction in *Campylobacter* counts at 25 ppm, 100 ppm and 200 ppm compared to chlorine treated samples was 0.4, 0.3 and 0.8 log units, respectively. Thus only high levels (200 ppm) of PAA reduced the *Campylobacter* count significantly more than did 30 ppm chlorine.

Paper 15 (Hochmuth, 2000)

Set up: Previously frozen chicken wings (livers are not considered) were inoculated with *Salmonella* Typhimurium or *E. coli* by dipping for 5 sec followed by 5 minutes attachment time. Five replicates were used for each condition. These samples were treated with 30 ppm PAA or water at 4 ± 2 °C for 60 min.

Results and conclusions: The *Salmonella* and *E. coli* counts on chicken wings were 0.32 and 1.20 log units lower on PAA treated carcasses compared to water treated samples.

3.5.3.4. Conclusions of studies on effects in chiller baths

Forest plot of results obtained with chiller baths are shown in Figures 5 and 6.

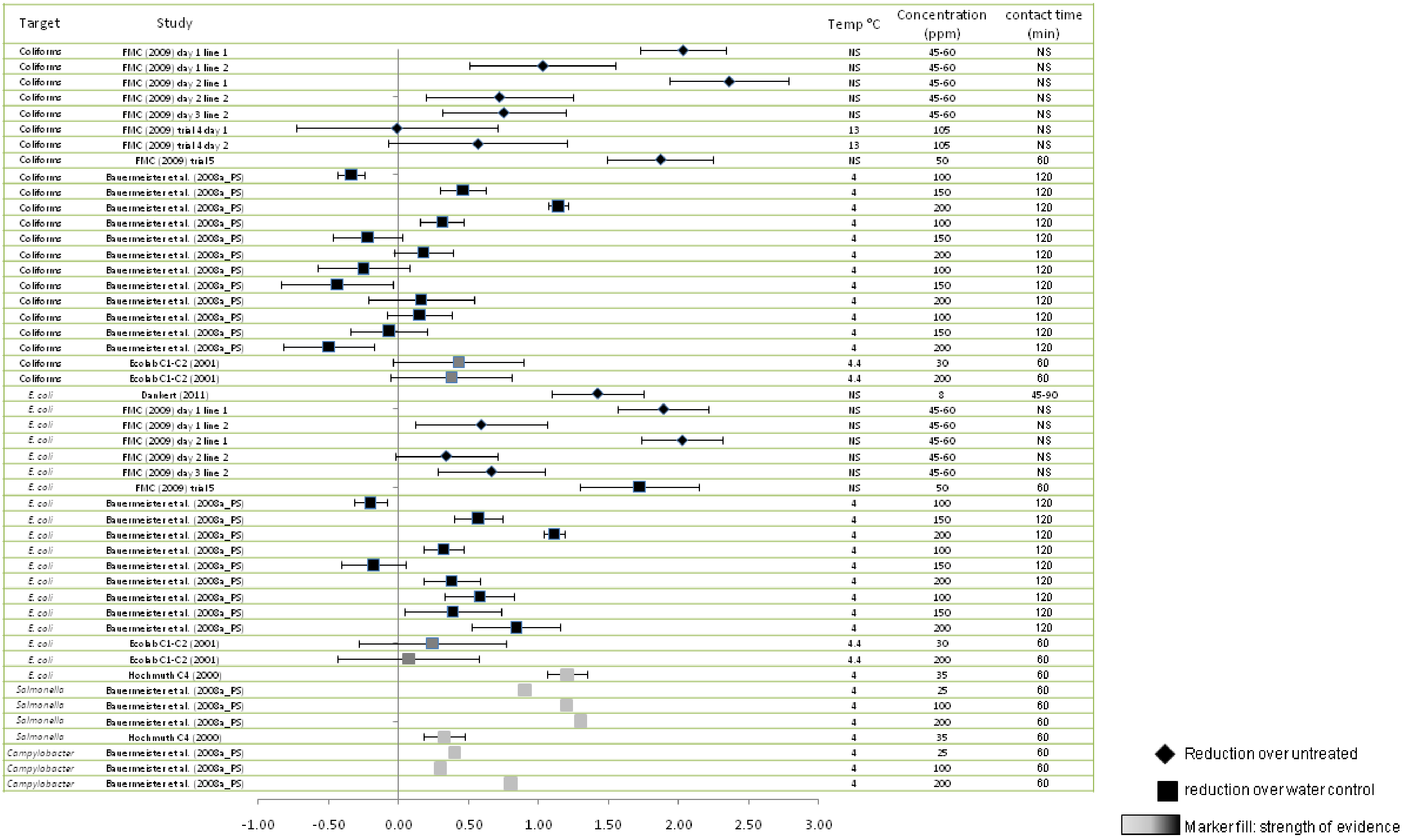


Figure 5: Forest plots of results of mean difference of bacterial counts obtained with chiller baths.

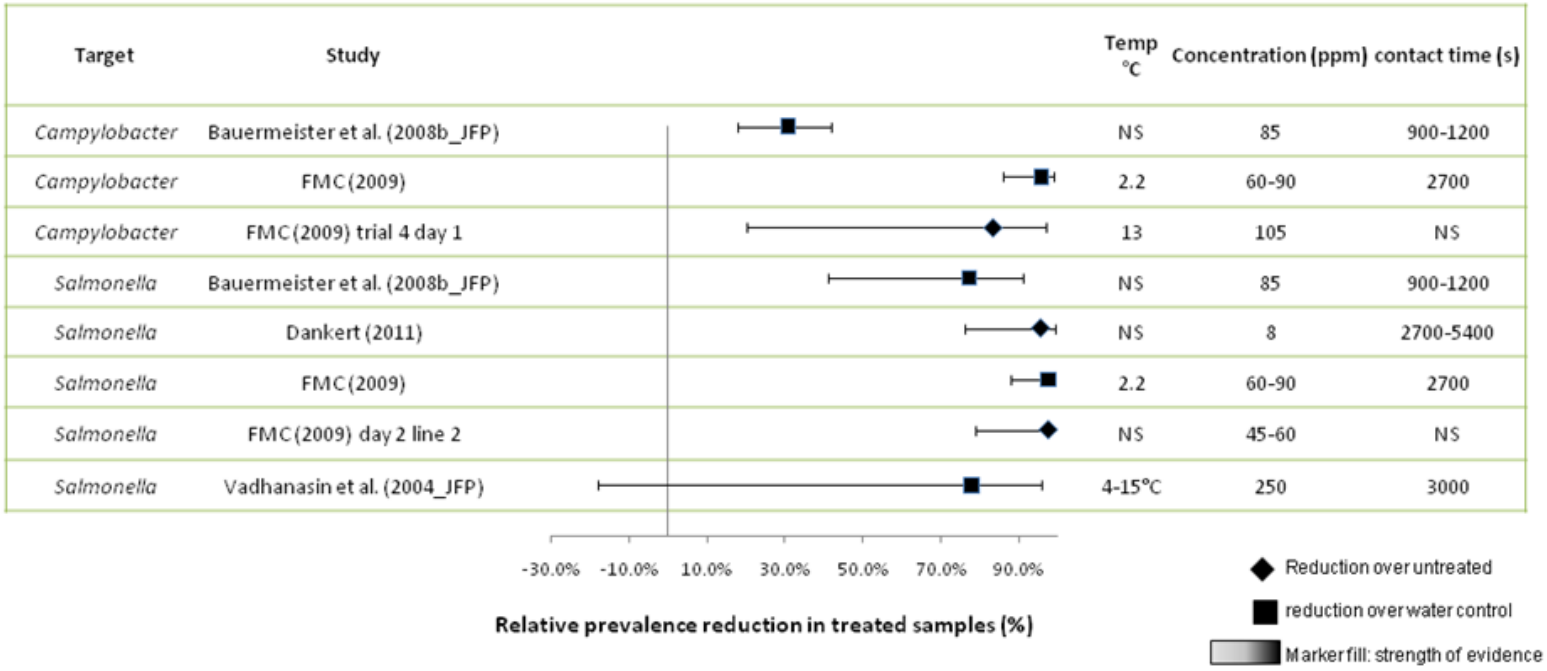


Figure 6: Forest plots of results of relative prevalence reduction obtained with chiller baths

Table 7 shows a summary of individual data sets from each study. In enumeration studies, there were positive effects on *E. coli* (i.e. either a significant reduction of the log CFU or a reduction > 0.5 log-units if CI were not provided) in 14/19 datasets; the mean decimal reduction (MD) ranged between 0.4 and 2.0 log-units. For coliforms, there was a positive effect in 9/22 studies with decimal reduction ranging between 0.3 and 2.4 log-units. All studies with indicator organisms were of high strength of evidence except four of medium strength and one of low strength, twelve studies showed significant reduction over water treated control samples. For *Salmonella*, positive effects were observed in 3/4 datasets with mean decimal reduction ranging between 0.3 and 1.3 log-units. For *Campylobacter*, positive effects were observed in 1/3 datasets, even though all datasets indicated mean decimal reduction values ranging between 0.3 and 0.8 log-units. All pathogen studies were of low strength of evidence, five studies showed significant reduction over water treated control samples.

Eight prevalence studies were included in the summary graphs (with number of positive samples in the controls >5) and five were provided for *Salmonella* and three for *Campylobacter*. The *Salmonella* prevalence was significantly reduced in 4/5 datasets; the relative prevalence reduction was >50 % in all these datasets. All prevalence studies were of high strength of evidence and all but three showed reduction over water treated control samples.

Table 7: Summary of results of studies with chiller baths

Decimal reduction	*E. coli*	Coliforms	*Salmonella*	*Campylobacter*
Enumeration studies, confidence interval provided				
Statistically significant reduction	14 (9*)	9(3*)	1*	0
No significant effect	4(3*)	10(8*)	0	0
Significant increase	1*	3*	0	0
Enumeration studies, confidence interval not provided				
MDR> 0.5	0	0	3*	1*
-0.5<MD<0.5	0	0	0	2*
MDR< -0.5	0	0	0	0
Prevalence studies				
Statistically significant reduction	0	0	4(2*)	3(2*)
No effect	0	0	1*	0

* number of studies out of the total where water control was applied.

3.5.4. Treatment group 4: studies on chilled carcasses or parts with dip treatment

3.5.4.1. Medium strength of evidence studies on chilled carcasses or parts with dip treatment

Paper 17 (Nagel et al., 2013)

Set up: 20 broiler carcasses per treatment conventionally processed at pilot scale facility. Carcasses chilled on ice for 24 hours before inoculation of each carcass with *Salmonella* and *Campylobacter* cultures and allowed to stand for 20 minutes before treatment. Treatment in commercial post-chill dip tank at 4 ± 2°C with a 20 second dwell time. Two PAA treatments of 400 and 1 000 ppm and water only treatment.

Results and conclusions: log reductions of 1.3/1.4 for *Salmonella*, and 1.25/1.35 for *Campylobacter* over water control, dependent on concentration of PAA (400/1 000 ppm respectively.

Paper 10 (Del Rio et al., 2007a)

Set up: Laboratory study with natural contaminated samples and quantitative analysis of Enterobacteriaceae and coliforms. As samples were transported from the plant to the laboratory in an ice chest and stored at 3 °C ± 1 °C for no longer than 1 h the study was recognized as an evaluation of the efficacy of PAA applied for treatment of chilled samples. Effect of dipping chicken legs for 15 min

in 220 ppm peroxyacids; dipping in tap water (water-dipped control). Samples were evaluated for microbiological quality, pH values, and hedonic scores, after 0, 1, 3 and 5 days of storage.

Results and conclusions: Compared to water treated samples, a reduction in Enterobacteriaceae as well as coliforms was identified both immediately after treatment and in the following days. The log reduction of Enterobacteriaceae was 0.25 immediately after treatment and increased to 2.21 after three days of storage; after five days the effect was declining showing 1.66 log reduction of Enterobacteriaceae . For coliforms the log reduction was 0.30 immediately after treatment and 1.94 after three days of storage. After five days a 1.20 log reduction was demonstrated for coliforms.

3.5.4.2. Low strength of evidence studies on chilled carcasses or parts with dip treatment

Paper 16 (Mehyar et al., 2005)

Set up: Chicken wing parts (drumettes) obtained from commercial processing plant after defeathering inoculated with strains of *Escherichia coli* O157:H7, *Campylobacter jejuni*, and a cocktail of three strains of *Salmonella enterica*, by two methods:. Firstly, by dipping into a culture for 15 seconds then allowing to drain for 15 minutes; secondly, by treatment with 200 ppm PAA (formula containing octanoic acid) by dipping for 1 minute in treatment or water followed by 30 second drain time. Unchilled samples; internal temperatures, 38 to 40°C. Treatment solution at ambient temperature. In some tests application of antimicrobial was made one minute before application of bacterial inoculation. Storage study after treatment with 1 minute dip and storage. Storage study at 7°C. Occurrence of naturally-occurring pseudomonades and psychrotrophs at 7, 24, 22 and 120 hours also assessed Measurement of reductions inn log units/g relative to water-dip control on unchilled samples treated by dipping for 1 minute into 200 ppm PAA after inoculation.

Results and conclusions: For comparisons of log reductions in cfu/g relative to water-dip control on unchilled samples treated by dipping for 1 minute into 200 ppm PAA after inoculation log unit reductions of 0.04 (*Salmonella*), 0.32 (*Campylobacter*) and 0.63 (*E. coli* O157) over control. were recorded. For experiments on samples treated by dipping for 1 minute into 200 ppm before inoculation reductions of 0.8, 0.3 and 0.5 were recorded for *Salmonella, Campylobacter* and *E. coli* O157 respectively; for application of PAA both before and after inoculation the reductions were 1.0 for *Salmonella*, and 0.9 for *Campylobacter* (no results for *E. coli* O157 were provided). Although not relevant for this assessment, log reductions of between 0.2 and 2.27 for psychotrophs and pseudomonads also recorded for different exposure times.

Paper 20 (Rodrigues et al., 2011)

Set up: Chicken carcasses were purchased at a local market and split into halves. Carcass halves were inoculated by spray with *S.* Typhimurium or *C. jejuni*. The level of inoculation was not clearly specified. Inoculated carcasses were let rest for 1 hour. 20 carcass halves were subjected to different treatments (10 carcasses on each of 2 days); control was city water (unspecified level of residual chlorine). Treatment was aimed at a PAA concentration of 500 ppm, measured residual after 2 and 5 min were 560 and 520 ppm PAA, respectively. Halves were placed in bins containing 10 litres of treatment solution with hand agitation for either 2 or 5 minute treatments, baths were kept chilled by use of icepacks. Only results after 2 minutes have been considered, as the application specifies a maximum duration of 3 minutes.

After the intended contact time, residual PAA was neutralised with a sodium thiosulphate solution and halved carcass were sampled using rinsing in city water. Rinsates were serially diluted for analysis by plate count on selective media without resuscitation.

Results and conclusions: Data generated from the laboratory experiments demonstrated 1.57 log units reduction of *S.* Typhimurium and 3.00 log units reduction of *C. jejuni* above the effect of water. Both effects were highly significant.

Paper 7 (Chantarapanont et al., 2004)

Set up: The objective was to determine the effect of chlorine, acidified sodium chlorite, and peracetic acid treatments on viable *Campylobacter jejuni* located at various depths within follicles or folds of chicken skin. Skin samples were inoculated with *C. jejuni*. Water control and two PAA treatments (40 and 100 ppm) were used for 2 and 15 min exposure. Exposure was at room temperature.

Results and conclusions: PAA treatment resulted in approximately a 1.05 log units decrease per square cm over the water control treatment when used at 100 ppm for 15 min and no significant decrease when used at 40 ppm for 2 min.

Paper 8 (Dankert, 2010)

Set up: In this laboratory study the efficacy of PAA against *E. coli* and *Salmonella* Typhimurium inoculated on parts or whole poultry carcasses obtained at retail. PAA concentrations were 0, 20, 40, 80 ppm respectively.

Results and conclusions: The reduction in surviving colonies of *E. coli* and *Salmonella* ranged from 33-90 % (0.17 to 0.98 log unit over control) for *E. coli* and 57-86 % (0.36 to 0.85 log unit over control) for *Salmonella*. The reductions increased with increased PAA concentration.

Paper 11 (Del Rio et al., 2007b)

Set up: Laboratory study with high level (6.93 and 6.82 log units/g, respectively) inoculation of samples with *Salmonella* Enteritidis and *Escherichia coli*. As samples were transported from the plant to the laboratory in an ice chest and stored at 3 °C ± 1 °C for no longer than 1 h the study was recognized as an evaluation of the efficacy of PAA applied for treatment of chilled samples. Effect of dipping chicken legs for 15 min in 220 ppm peroxyacids ; dipping in tap water (water-dipped control). Samples were evaluated for microbiological quality, pH values, and hedonic scores, after 0, 1, 3 and 5 days of storage.

Results and conclusions: Compared to water treated samples, a small log reduction of *S.* Enteritidis at 0.03 was observed immediately after treatment. The relative log reduction of this pathogen increased to 0.14; 1.11 and 1.51 during the following 1, 3 and 5 days of storage. For *E. coli* the immediately log reduction was 0.44 increasing to 0.59; 1.02 and 1.78 during the following 1, 3 and 5 days of storages.

3.5.4.3. Conclusions of studies on chilled carcasses or parts with dip treatment

Forest plot of results obtained with dip treatment of chilled carcasses or parts are shown in Figure 7.

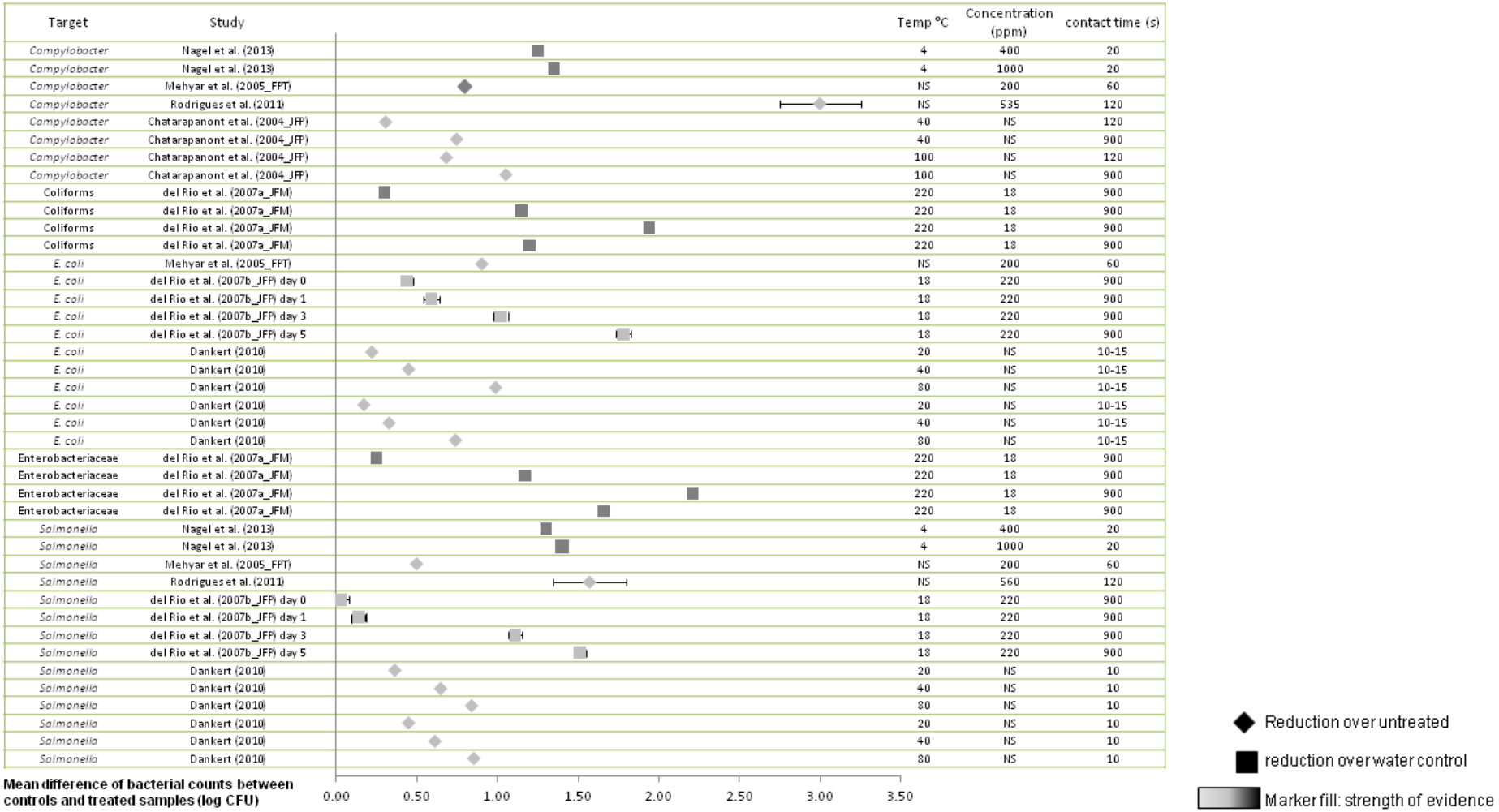


Figure 7: Forest plots of results of mean difference of bacterial counts obtained with dip treatment of chilled carcasses or parts.

Table 7 shows a summary of individual data sets from each study. In enumeration studies, there were positive effects on *E. coli* (i.e. either a significant reduction of the log CFU or a reduction > 0.5 log-units if CI were not provided) in 7/11 datasets; the mean decimal reduction (MD) ranged between 0.17 and 1.78 log-units. For coliforms, there was a positive effect in 3/4 studies with decimal reduction ranging between 0.30 and 1.94 log-units and for Enterobacteriaceae, 3/4 studies showed a positive effect ranging between 0.25 and 2.21 log-units.. All studies with indicator organisms were of low or medium strength of evidence, ten studies showed significant reduction over water treated control samples. For *Salmonella*, positive effects were observed in 10/14 datasets with mean decimal reduction ranging between 0.14 and 1.57 log-units. For *Campylobacter*, positive effects were observed in 7/8 datasets, with mean decimal reductions values ranging between 0.30 and 3.00 log-units. All pathogen studies, except one of medium, were of low strength of evidence. Seven studies showed significant reduction over water treated control samples.

No prevalence studies were provided for this treatment group.

Table 8: Summary of results of studies with dip treatment of chilled carcasses or parts

Decimal reduction	*E. coli*	Coliforms	*Salmonella*	Enterobacteriaceae	*Campylobacter*
Enumeration studies, confidence interval provided					
Statistically significant reduction	4*	0	4(3*)	0	1
No significant effect	0	0	1*	0	0
Significant increase	0	0	0	0	0
Enumeration studies, confidence interval not provided					
MDR > 0.5	3	3*	6(2*)	3*	6(2*)
-0.5<MDR<0.5	4	1*	3	1*	1
MDR < -0.5	0	0	0	0	0
Prevalence studies					
Statistically significant reduction	0	0	0	0	0
No effect	0	0	0	0	0

* number of studies out of the total where water control was applied.

3.6. Conclusions

- Evaluations could only be performed for meat classified as poultry (assumed to be chicken) and for chicken carcasses and parts. No data were provided for other poultry species, with the possible exception of a single study involving turkey meat.

- Studies were classified as of high or medium strength of evidence if they used naturally-contaminated samples on industrial or pilot scale, respectively; 6/17 studies provided data on the reduction of bacteria over water controls.

- The statistical analysis of the data in the published studies was of variable quality; where possible confidence intervals were calculated by the Biological Hazard Panel. The reduction of bacterial counts was considered relevant if the confidence interval did not include zero (statistically significant), or, following expert judgement (when confidence intervals were not available), if the mean decimal reduction was greater than 0.5 log-units.

- There was consistent evidence (16/17 data points) for substantial reductions (1-3 log-units over untreated controls) in the counts of *E. coli* and coliforms when treating warm carcasses by dipping into PAA solutions.

- Data on pathogen reduction following this treatment were limited; the prevalence reduction of *Salmonella* was statistically significant for 3/4 data points (the relative prevalence reduction ranged between 91 % to 95 %).

- Spraying of warm carcasses was less effective than dipping in reducing indicator organisms; 15/25 data points showed relevant reductions of indicator organisms (0.5-1.5 log-units). The prevalence reduction of *Salmonella* was statistically significant in 6/9 studies (the relative prevalence reduction ranged between 28 % to 87 %).

- There was evidence for a reduction of counts of *Salmonella* and *Campylobacter* and indicator organisms when treating chilled carcasses or parts by dipping; the effects were considered relevant for 30/41 data points. The studies provided were categorised as having low or medium strength of evidence. The effect of PAA treatment (0-2 log-units) was less than for warm carcasses.

- There was evidence for a reduction of *E. coli* (0.5-2 log-units) after the addition of PAA to chiller baths; the effects were considered relevant for 14/19 data points. The effects on coliform bacteria were less consistent; the effects were considered relevant for 9/22 data points. Data on reduction of the number of *Salmonella* and *Campylobacter* following this treatment was limited; the effects were considered relevant for 5/7 data points. The prevalence reduction of *Salmonella* and *Campylobacter* was statistically significant in 7/8 data points from studies of high strength of evidence (the relative prevalence reduction ranged between 30 % to 99 %).

- The study designs were heterogeneous. Further integration of data and evaluation of the effect of different processing parameters (PAA concentration, contact time, temperature, pH etc.) was therefore not possible.

- The efficacy of PAA treatment after storage of treated poultry carcasses and products was only investigated in two studies with naturally-contaminated samples, and these gave conflicting results. Such studies, are required in the EFSA guidelines to evaluate whether micro-organisms are truly inactivated or only sublethally injured.

Recommendations

- Further high strength of evidence studies with pathogens should be undertaken, particularly for *Campylobacter*.

- The effects of PAA treatment needs to be regularly evaluated in poultry processing plants using PAA solutions as a decontaminating agent.

- Monitoring of the concentration of the decontaminating substance in the working PAA solution should be included in HACCP plans.

- As mentioned in the EFSA guidelines (EFSA Panel on Biological Hazards (BIOHAZ), 2010), treated carcasses should also be examined at the end of shelf life to ensure that the level of contamination remains low.

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

4.1. Submission by the Applicant (1)

In relation to testing for the possibility of the development of resistance to the compound, or of resistance to therapeutic antibiotics, the Applicant does not appear to have undertaken any of the tasks outlined in sections 1.1. and 1.2. of the EFSA guidelines (EFSA Panel on Biological Hazards (BIOHAZ), 2010).

The key points of their submission in relation to the possibility of resistance development fall under the clause in the EFSA guidelines '*the Applicant may apply for approval based on the history of apparent safe use. If data are available from application of the product for uses other than removal of food surface contamination, they could be submitted for consideration'.*

4.1.1. Information provided in support of the above:

The Applicant has stated that: *'there appear to be no reports of bacterial populations developing resistance to PAA disinfectants'*, for the following reasons:

'PAA disrupts cellular activity by oxidizing the cell membrane and indiscriminately oxidizing cellular components. Its mode of action makes it difficult to conceive how resistance would develop due to the treatment of poultry carcasses. As discussed in the dossier, PAA is not like an antibiotic that blocks a specific metabolic pathway. PAA solutions are widely used disinfectants. If any resistance or hardening of bacteria to PAA solutions, it would have occurred in response to use as a surface disinfectant. We do not believe that any data or published studies exist that examines the possibility of resistance by bacteria to PAA solutions and are not aware of any study that indicates a reduction of effectiveness'. Because of its mode of action it seems very improbable that use of PAA would lead to transferable genetic components that would provide resistance to antibiotics'.

'There seems to be a very low probability that the use of PAA for pathogen reduction on poultry carcasses or parts would increase the risk of development of antimicrobial resistance to the disinfectant itself and an extremely low probability that it would contribute to the development of resistance to therapeutic antibiotics'.

'HEDP does not have antimicrobial properties so it is not expected to promote antimicrobial resistance'.

'Safe use in other domains'

'Our application for the approval of PAA in poultry production incorporates a range of poultry-carcass-contact uses, but the solutions have been used in many other domains for decades'. See Section 2 of the dossier and the references cited therein (ECETOC, 2001; EPA, 1993, 2009) and the EU Directive 98/8/EC[11] and EC Regulation 1451/2007[12].

The history of safe use of PAA is not only in other domains - the safety of PAA has been demonstrated on poultry in the U.S. and in other countries over the past decade. We also note that in 2008 the EU BIOHAZ panel: *"....concluded that despite a long history of use, there are currently no published data to conclude that the application of chlorine dioxide, acidified sodium chlorite, trisodium phosphate or peroxyacids to remove microbial contamination of poultry carcasses at the proposed conditions of use will lead to the occurrence of acquired reduced susceptibility to these*

[11] Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing of biocidal products on the market

[12] Commission Regulation (EC) No 1451/2007 of 4 December 2007 on the second phase of the 10-year work programme referred to in Article 16(2) of Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market

substances. Similarly, there are currently no published data to conclude that the application of chlorine dioxide, acidified sodium chlorite, trisodium phosphate or peroxyacids to remove microbial contamination of poultry carcasses at the proposed conditions of use will lead to resistance to therapeutic antimicrobials."

4.2. Submission by the Applicant (2)

A further recommendation in the EFSA guidelines is that:

'If the product is released into the environment without neutralisation, a post-market monitoring and evaluation is recommended to determine the long-term effects of using the formulated product on selection and dissemination of acquired reduced susceptibility to biocides and/or resistance to therapeutic antimicrobials'.

4.2.1. Information provided by the Applicant

Resistance: 'Several studies have been conducted that address the question of resistance, and we review five of these below. However, it is important to note that PAA's active ingredients do not persist.

'Some bacteria can develop higher minimum inhibitory (MIC) concentrations upon exposure to disinfectants. This may occur when disinfectants are used incorrectly at sub-lethal concentrations or applied before surfaces have been properly cleaned. Because of this, development of resistance is a significant consideration in the widespread use of surface disinfectants. However, the increases in MIC against disinfectants generally occur through mechanisms such as biofilm formation or biological pumps. These are more the result of external environmental conditions coupled with the microorganism's already existing genetic response, rather than an evolution of new intrinsic biological pathways. The development of increased resistance over time was studied directly by Alonso-Hernando et al. (2009). Even in the case where multiple generations of bacteria were grown in sub-lethal concentrations of PAA, under conditions that had the greatest chance for development of resistance, the MIC against PAA for the two bacterial strains studied was only raised about 10 %, far lower than values typically seen with antibiotic resistance, and MICs remained unaffected for the two other strains analyzed. These results, although relevant for repeated use of surface sanitizers on equipment, are not representative or equal to the conditions under which PAA is used on rinsing carcasses for meat safety'.

'Alonso-Hernando et al. (2009) also studied whether PAA and other disinfectants caused resistance to therapeutic antimicrobials (i.e. antibiotics). Again, multiple generations of bacteria were grown under sub-lethal concentrations, conditions specifically created to foster resistance. For PAA, this resulted in an increased MIC for some of the antibiotics tested, but the disinfectants were used repeatedly on the same surface area. This is representative of surface sanitizer use in a plant or hospital setting, but not use as a single rinse for carcasses. However, as noted in the paper, the mechanisms of resistance for microbes to antibiotics verses PAA (and the other disinfectants) differ greatly. This is important as the bacterial defence mechanisms exercised under these conditions are probably irrelevant to the development of intrinsic (genetic) mechanisms of bacterial resistance to antibiotic'.

'Geornaras et al. (2012) considered whether bacteria that are resistant to antibiotics are also less susceptible to disinfectants like PAA. If bacteria did utilize similar mechanisms for developing resistance to an antibiotic and PAA, then those same resistant bacteria should have reduced sensitivity to both PAA and the antibiotics. When this research was conducted, antibacterial resistant and non-resistant strains of bacteria did not exhibit increased resistance to disinfectants like PAA, suggesting different mechanisms are employed by bacteria for protection against disinfectants verses antibiotics'.

'In a recent review by Møretrø et al. (2012) there was no evidence for increased antimicrobial resistance of *Salmonella* to any of the currently used disinfectants in the poultry industry, and even when resistance was reported in wild type serovars, the MIC was still below recommended user

concentrations for all the disinfectants currently used in the industry including PAA. Even in human medicine the development of antimicrobial resistance to disinfectants is still poorly understood, especially for non-spore forming bacteria. Humphreys et al. (2013) found PAA to be a viable alternative to disinfection with chlorine bleach for disinfection in hospital settings despite its already widespread applications of PAA. There is no evidence to support an increased risk or incidence of the development of antimicrobial resistance to PAA in the meat industry since the first patent was obtained in 1950'.

4.3. Evaluation from EFSA Biological Hazards (BIOHAZ) Panel (3)

The Panel has assessed the information provided by the Applicant as summarised in sections 4.1. and 4.2. above.

4.3.1. Development of resistance

Although no direct experiments have been conducted to test for the potential emergence of acquired reduced susceptibility to biocides and/or resistance to therapeutic antimicrobials following the use of PAA, evidence provided as to the history of safe use of PAA is sufficient to meet the requirements of EFSA Guidelines of 2010 cited above, in that *'the applicant may apply for approval based on the history of apparent safe use'.*

4.3.2. Post-market evaluations

As with section 4.3.1. above, no direct post-market evaluations have been undertaken, Nevertheless the information provided by the Applicant, and in that '*PAA used by poultry production facilities is neutralized and does not reach the environment. PAA solutions degrade before discharge of wastewater'* is indicative that a targeted post-market evaluation of the potential persistence of PAA the environment may not be necessary.

4.4. Conclusions

- On the basis of the information provided by the Applicant, the emergence of acquired reduced susceptibility to biocides and/or resistance to therapeutic antimicrobials following the use of PAA is considered unlikely.

4.5. Recommendations

- Laboratory studies should be undertaken to confirm that reduced susceptibility to biocides and/or resistance to therapeutic antimicrobials following the use of PAA does not occur.

- Post-marketing surveillance for resistance in both pathogenic and commensal bacteria should be included in HACCP plans should PAA be applied for decontamination of poultry carcasses.

5. The risk related to the release of the processing plant effluents, linked to the use of the substance, into the environment

A typical poultry plant slaughters 200 000 chickens per day. In this process 5.8 million litres of wastewater is generated according to the Applicant. The daily amount of 378 litres of concentrated peroxyacetic acid solution contains: 67.4 kg peroxyacetic acid, 25.3 kg hydrogen peroxide, 235.8 kg acetic acid and 3.8 kg HEDP according to the Applicant. The first three compounds of this list are readily degradable in a sewage water treatment system of the poultry plant. Acetic acid and hydrogen peroxide are common metabolic intermediates and degrade quickly via the citric acid cycle or catalase activity. Peroxyacetic acid is in chemical equilibrium with hydrogen peroxide and acetic acid (ECETOC, 2001). Therefore, it will therefore decompose quickly in a sewage treatment system. The same holds true for octanoic acid. Hydrogen peroxide and peroxyacetic acid or peroxyoctanoic acid are highly reactive chemically unstable disinfectants and therefore the development of antibiotic resistance is not considered an issue. The reduction of these compounds by peroxidases is a common mechanism in aerobic organisms since it is vital for survival in the presence of oxygen. Catalase is a common enzyme, which can also remove hydrogen peroxide.

As acetic acid, peroxyacetic acid, octanoic acid, peroxyoctanoic acid and hydrogen peroxide are effectively neutralized before discharge of wastewater, tests regarding development and dissemination of acquired reduced susceptibility of environmental microorganisms are likewise not considered necessary. Similarly, on the surface of poultry carcasses themselves, the active ingredients of PAA have a measured lifetime of a few minutes, so there are no peroxyacids to measure on the product after leaving the processing plant.

In contrast, HEDP will not be biodegraded in a sewage treatment system. A concentration of 3800/5.81= 650 µg/L wastewater can flow into the sewage treatment system. HEDP can be partially removed in sewage treatment systems by sorption to the sludge. This depends on the specific conditions in the system and removal percentages of HEDP vary around 50 % (HERA, 2004). The wastewater dilutes when it flows into a river or a lake. Both big and small poultry plants produce the similar concentration of HEDP in their wastewater but the dilution factor will be larger when a small plant pollutes a big river. The default dilution factor for sewage from treatment plants is a factor 10. (ECB, 2003) This yields a final Predicted Environmental Concentration (PEC) of 650×10 %×50 %=32.5 µg/L in surface water.

A preliminary guideline for surface water quality (No Effect Concentration) was derived for HEDP in literature review (Oste et al., 2009) to be 1 µg/L. This follows from the No Observed Effect Concentration (NOEC) of a *Daphnia magna* reproduction test of 100 µg/litre using a safety factor of 100 (Oste et al., 2009). Preliminary surface water quality guidelines may be replaced in the future by a full-scale environmental risk assessment to be performed according to the European guidelines (ECB, 2003). HEDP can chelate metals and therefore reduce the availability of essential metals in a reproduction test. Nevertheless, the essential metals are added in excess in a reproduction test. The metal availability in a natural freshwater system, amended with water from a sewage treatment system, might be better or worse than that in a reproduction test. As a reasonable worst-case estimate, it is assumed that the metal availability in a freshwater system is similar to that in a reproduction test. This means that the relatively low NOEC of the *Daphnia magna* reproduction test can be used for the risk evaluation. Consequently, the PEC/NEC ratio is 32.5 indicating a risk for the environment of the use of HEDP in poultry plants. Preliminary guidelines are often overprotective because of lack of knowledge. HEDP is a high production volume chemical which is used in, e.g. soaps.

5.1. Conclusions

At this stage, the emission of HEDP from a poultry plant *via* a sewage treatment system into the freshwater environment cannot be considered safe *a priori*. Therefore, site-specific considerations are needed to evaluate the possible risk of the emission of HEDP in each poultry plant. A dedicated on-site sewage treatment system might be able to remove a larger percentage of HEDP. The dilution factor for sewage from treatment plants might be higher than the standard factor 10, when a small

poultry plant pollutes a big water body. The annual average dilution factor will not be appropriate since the susceptible crustaceans (e.g. *Daphnia magna*) reproduce in summertime when the flow of rivers and streams often is minimal.

CONCLUSIONS AND RECOMMENDATIONS

GENERAL CONCLUSIONS:

- This Opinion deals with the evaluation of the safety and efficacy of mixtures containing peroxyacetic acid (PAA) as active ingredient, for reduction of pathogens on poultry carcasses and meat, under the usage conditions as specified by the Applicant. These include (i) treatment of warm carcasses with spray treatment (PAA concentrations typically between 400-700 ppm and spray times up to 10 seconds); (ii) treatment of warm carcasses or parts with dip treatment (PAA concentrations up to 2 000 ppm and contact times up to 3 minutes); (iii) treatment of carcasses in chiller baths (PAA concentrations up to 230 ppm and contact times between 1-2 hours) and (iv) treatment of chilled carcasses or parts with dip treatment (PAA concentrations up to 2 000 ppm and contact times up to 3 minutes). In the mixtures, PAA is in chemical equilibrium with hydrogen peroxide and acetic acid. The mixtures also contain 1-hydroxyethylidene-1,1-diphosphonic acid (HEDP) and some mixtures also octanoic acid and its reaction product peroxyoctanoic acid.

- PAA has previously been evaluated by EFSA and other international bodies; these evaluations have been taken into account in this Opinion.

ToR 1.

The toxicological safety of the substance

- Accepting the previous EFSA exposure scenarios (EFSA, 2005), and supplemented with more recent information on consumption data of poultry meat within the EU (EFSA, 2011), there are no toxicity concerns with regard to residues of peroxyacids as these compounds are unstable and break down into acetic acid and water.

- Similarly there are no concerns in relation to residues of acetic acid and octanoic acid.

- There are also no concerns when applying PAA using dip treatments of no more than 3 minutes (short term) with the proposed higher concentration of the peroxyacetic acid solutions.

- There are no toxicity concerns for the product stabilizer HEDP with regard to the dip treatment, referring to the margin of safety of 43 103 as calculated from European intake scenario.

- Regarding the safety of possible reaction products of hydrogen peroxide and peroxyacids with lipids and proteins/amino acids of the poultry carcasses, no risk was anticipated due to the high instability of the peroxyacids.

- No lipid peroxidation was identified in producer experiments when using immersion for 60 minutes in 200 mg/L total peroxyacetic acid. The short term high concentration bath scenario included in the present application dossier should not cause measurable lipid peroxidation.

ToR 2. The efficacy, i.e. does the use of the substance significantly reduce the level of contamination of pathogens on poultry carcasses and poultry meat

- The studies submitted by the Applicant used a wide range of experimental designs and thus differed in relation to products, settings, method of application, PAA concentration, use of

controls, microorganisms studied, time of analysis after application, etc. All these parameters impacted on the observed efficacy. Comparison beyond treatment groups was not possible.

- Evaluation could only be performed for tests on chicken carcasses and parts, with one possible exception no data were provided on other poultry species.

- The statistical analysis of the data in the published studies was of variable quality, where possible confidence intervals were calculated by the Biological Hazard Panel. The reduction of bacterial counts was considered relevant if the confidence interval did not include zero (statistically significant), or, following expert judgement (when confidence intervals were not available), if the mean decimal reduction was greater than 0.5 log-units.

- There was consistent evidence for a relevant impact (1-3 log-units over untreated controls) of PAA treatment on *E. coli* and coliforms when treating warm carcasses by dipping. There were few data on reduction of *Salmonella* and *Campylobacter* for this treatment. There was evidence for statistically significant *Salmonella* prevalence reduction (the relative prevalence reduction ranged between 91% and 95%).

- Spraying of warm carcasses appeared to be less effective in reducing indicator organisms than dipping (0.5-1.5 log-units). There was evidence for statistically significant *Salmonella* prevalence reduction (the relative prevalence reduction ranged between 28% and 87%).

- There was consistent evidence for a relevant reduction (0.5-2 log-units) of indicator organisms and *Salmonella* and *Campylobacter* when treating chilled carcasses or parts by dipping, but the studies were of low or medium strength of evidence.

- There was consistent evidence for a relevant impact on *E. coli* when adding PAA to chiller baths (0.5-2 log-units). The effects on coliform bacteria were less consistent. There were few data on reduction of the number of *Salmonella* and *Campylobacter* for this treatment. There was evidence for statistically significant *Salmonella* and *Campylobacter* prevalence reduction (the relative prevalence reduction ranged between 30% and 99%).

- The efficacy of PAA treatment after storage of treated carcasses/products was only investigated in two studies with naturally-contaminated samples, and these gave conflicting results. Such studies are required in the EFSA guidelines to evaluate whether micro-organisms are truly inactivated or only sublethally injured.

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

- On the basis of the safe usage information provided by the Applicant, the emergence of acquired reduced susceptibility to biocides and/or resistance to therapeutic antimicrobials following the use of PAA is considered unlikely.

ToR 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.

- Acetic acid, peroxyacetic acid, octanoic acid, peroxyoctanoic acid and hydrogen peroxide are effectively neutralized before discharge of wastewater. There is therefore no concern about environmental toxicity of these compounds. Likewise, tests regarding the development of acquired reduced susceptibility in environmental microorganisms then subsequent dissemination are not considered necessary.

- On the basis of a conservative preliminary guideline for surface water quality from a literature review, the emission of HEDP from a poultry plant including *via* a wastewater treatment system into the freshwater environment cannot be considered safe *a priori*.

- Site-specific considerations related to dilution factors and improved efficiency of wastewater treatment plants, can mitigate the possible environmental risk associated with the emission of HEDP from individual poultry plants using PAA solutions for decontamination treatment.

RECOMMENDATIONS

ToR 1. The toxicological safety of the substance

- To control residues of HEDP on poultry carcasses, monitoring of the concentration of HEDP in the working PAA solution should be considered in the HACCP plans.

- A method for the determination of HEDP residues on poultry carcases, poultry meat and poultry meat products should be developed and validated, to further inform the risk assessment.

ToR 2. The efficacy, i.e. does the use of the substance significantly reduce the level of contamination of pathogens on poultry carcasses and poultry meat

- Further high strength of evidence studies with pathogens should be undertaken, in particular with *Campylobacter*.

- Monitoring of the concentration of the decontaminating substance in the working PAA solution should be considered in HACCP plans.

- As mentioned in the EFSA guidelines, treated carcasses should be examined at the end of shelf life to ensure that the level of contamination remains low.

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

- Laboratory studies should be undertaken to confirm that reduced susceptibility to biocides and/or resistance to therapeutic antimicrobials following the use of PAA does not occur.

- Post-marketing surveillance for resistance in both pathogenic and commensal bacteria should be considered in HACCP plans should PAA be applied for decontamination of poultry carcasses.

DOCUMENTATION PROVIDED TO EFSA

1. Letter Ref. Ares(2013)2190494 received on 17 June 2013 including the request from the Commission and application dossier in electronic copy from U.S. Department of Agriculture (USDA) "Submission of data for the authorization of peroxyacetic acid solutions for uses to reduce microbial contamination of poultry carcasses".

2. The mandate and technical/application dossier in electronic and paper copy from U.S. Department of Agriculture (USDA) "Submission of data for the authorization of peroxyacetic acid solutions for uses to reduce microbial contamination of poultry carcasses" received on 24 June 2013.

3. Reply to EFSA's request for missing information on 31 July 2013. Received by EFSA from U.S. Department of Agriculture (USDA) on 4 September 2013.

4. Reply to EFSA's request for additional data on 11 October 2013. Received by EFSA from U.S. Department of Agriculture (USDA) on 6 November 2013.

5. Reply to EFSA's request for additional data on 27 November 2013. Received by EFSA from U.S. Department of Agriculture (USDA) on 17 December 2013.

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ABBREVIATIONS

ADI	Acceptable Daily Intake
ADI	Acceptable Daily Intake
APC	Aerobic Plate Count
BOD	Biological Oxygen Demand
Bw	Body Weight
CAS	Chemical Abstracts Service
CFU	Colony Forming Unit
CI	Confidence Interval
EC	European Commission
ECB	European Chemical Bureau
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
EFSA	European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organisation of the United Nations
FDA	Food and Drug Administration
GHP	Good Hygienic Practices
GRAS	Generally Recognised As Safe
HACCP	Hazard Analysis Critical Control Point
HEDP	1-hydroxyethylidene-1,1-diphosphonic acid
HERA	Human and Environmental Risk Assessment
JECFA	Joint FAO/WHO Expert Committee on Food additives
LOD	Limit of Detection
MDR	Mean Decimal Reduction
MoS	Margin of Safety
NEC	No Effect Concentration
NOAEL	No Observed Adverse Effect Level
NOEC	No Observed Effect Concentration
PAA	Peroxyacetic acid
PEC	Predicted Environmental Concentration
RPR	Relative Prevalence Reduction
TBARS	Thiobarbituric Acid Reactive Substances
WHO	World Health Organization