SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 25 October 2018

doi: 10.2903/j.efsa.2018.5482

Evaluation of the safety and efficacy of the organic acids lactic and acetic acids to reduce microbiological surface contamination on pork carcasses and pork cuts

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Abstract

Studies evaluating the safety and efficacy of lactic and acetic acids to reduce microbiological surface contamination on pork carcasses pre-chill and pork meat cuts post-chill were assessed. Lactic acid treatments consisted of 2–5% solutions at temperatures of up to 80°C applied to carcasses by spraying or up to 55°C applied on cuts by spraying or dipping. Acetic acid treatments consisted of 2–4% solutions at temperatures of up to 40°C applied on carcasses by spraying or on cuts by spraying or dipping. The maximum treatment duration was 30 s. The Panel concluded that: [1] the treatments are of no safety concern, provided that the substances comply with the European Union specifications for food additives; [2] spraying of pork carcasses pre-chill with lactic acid was efficacious compared to untreated control, but based on the available data, the Panel could not conclude whether lactic acid was more efficacious than water treatment when spraying of pork carcasses pre-chill or pork meat cuts post-chill. The Panel concluded that dipping of pork meat cuts post-chill in lactic acid was more efficacious than water treatment. However, it could not conclude on the efficacy of acetic acid treatment of pork carcasses pre-chill and/or pork meat cuts post-chill; [3] the potential selection and emergence of bacteria with reduced susceptibility to biocides and/or resistance to therapeutic antimicrobials linked to the use of the substances is unlikely as long as Good Hygienic Practices are implemented; and [4] the release of both organic acids is not of concern for the environment, assuming that wastewaters released by the slaughterhouses are treated, if necessary, to counter the potentially low pH caused by lactic or acetic acid, in compliance with local rules.

Keywords: lactic acid, acetic acid, pork carcasses and cuts, toxicological safety, efficacy, antimicrobial resistance, environmental impact

Requestor: European Commission

Question number: EFSA-Q-2017-00666

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Acknowledgements: The CEP Panel wishes to thank the Working Group on the evaluation of substances used to remove microbial contamination from product of animal origin of the former EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) for the preparatory work on this scientific opinion, in particular Fernando Aguilar, Paul Fowler, Konstantinos Koutsoumanis and John Threlfall. In addition, the CEP Panel wishes to thank the members of the Panel on Biological Hazards (BIOHAZ): Ana Allende, Avelino Alvarez-Ordóñez, Marianne Chemaly, Robert Davies, Alessandra De Cesare, Lieve Herman, Friederike Hilbert, Roland Lindqvist, Maarten Nauta, Giuseppe Ru, Marion Simmons and Elisabetta Suffredini for the preparatory work on this scientific opinion. Finally the CEP Panel wishes to thank the hearing expert Fidel Toldrà and Giorgia Vianello for the support provided to this scientific output.

Suggested citation: EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Silano V, Barat Baviera JM, Bolognesi C, Brüschweiler BJ, Chesson A, Cocconcelli PS, Crebelli R, Gott DM, Grob K, Lampi E, Riviere G, Steffensen I-L, Tlustos C, Van Loveren H, Vernis L, Zorn H, Bolton D, Bover-Cid S, de Knecht J, Peixe L, Skandamis P, Baù A, Martino C, Messens W, Sarno E, Tomcikova D and Mortensen A, 2018. Scientific Opinion on the evaluation of the safety and efficacy of lactic and acetic acids to reduce microbiological surface contamination on pork carcasses and pork cuts. EFSA Journal 2018;16(12):5482, 76 pp. https://doi.org/10.2903/j.efsa.2018.5482

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Summary

Following a request from the European Commission, the European Food Safety Authority (EFSA) was asked to deliver a scientific opinion on a technical dossier submitted by the National Pork Producers Council (United States) for the approval of lactic and acetic acid solutions used individually by food business operators (FBOs) during processing to reduce microbial surface contamination on pork carcasses and cuts. The approval was sought for treatments using either lactic acid solutions with concentrations from 2% to 5% or acetic acid solutions with concentrations from 2% to 4%. Lactic acid solutions are to be applied at temperatures of up to 80°C on pork carcasses by spraying or up to 55°C on pork meat cuts by spraying or dipping. Acetic acid solutions are to be applied at a temperature of up to 40°C on pork carcasses by spraying or on pork meat cuts by spraying or dipping. For both organic acids, the maximum duration treatment is 30 s.

The primary purpose of the proposed treatment is to reduce the incidence of food-borne illness in consumers by reducing the prevalence and/or abundance of human pathogens on pork products. The target pathogens on pork products identified by the applicant are: *Salmonella* Enteritidis, *Salmonella* Typhimurium, *Campylobacter* spp., *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Yersinia enterocolitica*, *Aeromonas hydrophilia* and *Staphylococcus aureus*. The proposed treatments will also target other non-pathogenic members of the Enterobacteriaceae family, which are considered hygiene indicators.

EFSA was requested to evaluate the safety and efficacy of lactic and acetic acids considering (i) the toxicological safety of the substances (Term of Reference (ToR) 1); (ii) the efficacy, i.e. does the use of these two substances significantly reduce the level of contamination of pathogens on carcasses and cuts from pork (ToR 2); (iii) the potential for the emergence of reduced susceptibility to biocides and/or resistance to therapeutic antimicrobials linked to the use of the substances (ToR 3); and (iv) the risk related to the release of the processing plant effluents, linked to the use of the substances, into the environment (ToR 4).

The questions as specified in the ToRs 1, 3 and 4 have been addressed by evaluating the information provided by the applicant, supplemented with relevant studies identified by the Panel, and based on the EFSA guidance document: '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 BIOHAZ Panel, 2010a). For the question about the efficacy of lactic and acetic acids, as specified in ToR 2, a systematic, stepwise approach was applied.

Concerning the human toxicological safety of lactic and acetic acids (answer to ToR 1), no safety concerns are foreseen, provided that the substances used comply with the European Union (EU) specifications for food additives. This conclusion is based on the fact that both substances are authorised food additives in the EU at *quantum satis* and their intakes from selected components of the typical diet far outweigh the exposure from the intended uses as decontamination treatments.

Twelve records were included in the efficacy assessment based on predefined eligibility criteria (answer to ToR 2). These yielded 19 eligible experiments (16 for lactic acid and 3 for acetic acid) providing 71 comparisons or $\log_{10}$ reduction estimates (67 for lactic acid and 4 for acetic acid). The experiments used a wide range of experimental designs and thus differed in relation to products, settings, method of application, acid concentration, use of controls, microorganisms studied, storage time after application, etc. All these parameters may have impacted the efficacy both within and between studies, but the present assessment did not attempt to differentiate efficacy based on potentially influencing factors.

The Panel concluded that:

- Spraying of pork carcasses pre-chill with lactic acid was efficacious compared to untreated control; the Panel could not conclude, based on the available data, whether spraying of pork carcasses pre-chill or pork meat cuts post-chill with lactic acid was more efficacious than water treatment. In 24/29 comparisons, lactic acid spraying of pork carcasses pre-chill or pork meat cuts post-chill was at least equally efficacious as water spraying, but delivered significantly higher mean $\log_{10}$ reductions in nine comparisons, depending on the conditions of application. The range of the statistically significant additional mean $\log_{10}$ reductions reported for carcasses and cuts were 1.30–1.82 and 1.10–2.50 $\log_{10}$, respectively.
- Dipping of pork meat cuts post-chill in lactic acid was more efficacious than water treatment, as this delivered significantly higher $\log_{10}$ reductions than dipping in water. The range of the statistically significant mean $\log_{10}$ reductions was 0.73–4.01 $\log_{10}$. In the experiments where evidence was available, both immediately after treatment and during storage, the reductions were at least maintained throughout the duration of the experiments under chill storage.

- The Panel could not conclude on the efficacy of acetic acid on pork carcasses pre-chill and/or pork meat cuts post-chill, considering that only three eligible experiments, which in addition were also characterised as of medium strength of evidence, were available.

Concerning the potential for reduced susceptibility to biocides and/or resistance to therapeutic antimicrobials linked to the use of the substances (answer to ToR 3), the Panel concluded that there is no evidence suggesting the promotion of a horizontally transferable reduced susceptibility to lactic or acetic acid or resistance to therapeutic antimicrobials as a result of exposure to lactic or acetic acid. Considering the extensive natural presence of lactic and acetic acid, including in feed and food, the possibility of development of resistance to therapeutic antimicrobials is unlikely to be a significant issue. There is some evidence that repeated exposure to lactic acid can select for reduced susceptibility to the same substance. However, under Good Hygienic Practices (GHP), the Panel did not consider this a significant issue.

Regarding the environmental toxicity of lactic and acetic acids (answer to ToR 4), the Panel concluded that the release of both substances is of no concern for the environment, assuming the wastewaters released by the slaughterhouses are treated, if necessary, to counter the potentially low pH caused by lactic or acetic acid.

Additional studies are required to assess the efficacy of acetic acid on pork carcass and pork meat cuts, the potential of treatments to induce acid adaptation and/or select acid resistant bacteria, or cross-/co-resistance to biocides and antibiotics. To prevent acid adaptation and increased resistance in pathogenic organisms, the treatments with organic acids (lactic and acetic acids), subject to authorisation, should be sufficient to inactivate the target bacteria. Adherence to GHP, within the Hazard Analysis Critical Control Point (HACCP) framework, is considered essential for various reasons. Sublethal stress exposure of pathogens may lead to acid adaptation and potentially reduced susceptibility to the acid treatment. For use as a dip, the operator would be required to write into their HACCP plans their flow rate for replacement of dipping solutions, along with testing programmes to assure that the dipping solution maintains effective conditions of application and microbial testing of the product post-application to assure effectiveness. The latter is also recommended for spray applications. In addition, the dipping treatment should be performed in such way that minimises the likelihood of cross-contamination of treated meat cuts by pathogens accumulated in the dipping tank through consecutive meat treatments, should there be viable pathogens in the treatment solution.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

1.1.1. Background

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 (GHP) and hazard control measures at each step of the food chain.

According to EU scientific advice,[1] 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[2] 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, other legitimate factors and the precautionary principle.

1.1.2. Terms of Reference

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002[3], EFSA is requested to evaluate the safety and efficacy of two organic acids, lactic and acetic acid, intended to be used individually by food business operators during processing to reduce microbiological surface contamination on carcasses and cuts from pork. In particular the EFSA shall assess:

- The toxicological safety of the two substances (ToR 1);
- The efficacy, i.e. does the individual use of these two substances significantly reduce the level of contamination of pathogens on carcasses and cuts from pork (ToR 2);
- The potential emergence of reduced susceptibility to biocides and/or resistance to therapeutic antimicrobials linked to the use of these two substances (ToR 3);
- The risk related to the release of the processing plant effluents, following the use of these substances, into the environment (ToR 4).

1.2. Information on existing authorisation and/or evaluations from other authorities

In the European Union (EU), Commission Regulation (EU) No 101/2013[4] authorises the use of lactic acid to reduce microbiological surface contamination on bovine carcasses or half carcasses or quarters at the level of the slaughterhouse in compliance with the conditions set out in the Annex to this Regulation.

In the United States, USDA FSIS regulation 7120.1[5] authorises the use of pathogen reduction treatments on meats. Both lactic acid and acetic acid are included.

[1] 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/fs/sc/scv/out63en.pdf. EFSA Journal 388, p. 1–19.

[2] Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin. OJ L 139, 30.4.2004, p. 55–205.

[3] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L31, 1.2.2002, p. 1–24.

[4] Regulation (EU) No 101/2013 of 4 February 2013 concerning the use of lactic acid to reduce microbiological surface contamination on bovine carcases. OJ L 34, 5.2.2013, p. 1–3.

[5] FSIS Directive 7120.1, Revision 46, Safe and Suitable Ingredients Used in the Production of Meat, Poultry, and Egg Products, 19/3/18.

In Canada, Health Canada issues Letters of No Objection for antimicrobial processing aids, among which both lactic acid and acetic acid are listed.[6]

FAO/WHO issued in 2016 the 'Guidelines for the control of non-typhoidal *Salmonella* spp. in beef and pork meat'[7] and concluded that organic acid treatments, such as lactic and acetic acids washes can significantly reduce *Salmonella* prevalence on carcasses. The experts considered that the realistic reductions to be possibly achieved would not exceed 1 $\log_{10}$ CFU/cm^2.

In the EU, lactic acid (E 270) and acetic acid (E 260) are also authorised food additives, according to Annex II and Annex III to Regulation (EC) No 1333/2008, belonging to group I additives. Their use is permitted in several food categories mainly at *quantum satis*. Currently, their re-evaluation as food additives, as foreseen in Regulation (EC) No 257/2010[8], is still ongoing.[9]

According to Regulation (EC) No 1333/2008, both food additives are authorised for use in meat preparations with the following restriction: 'only prepacked preparations of fresh minced meat and meat preparations to which other ingredients than additives or salt have been added'. Moreover for lactic acid, only the L-(+)-isomer can be used in food for infants and young children as specified in category 13.1 under Regulation (EU) No 1333/2008.

In addition in the EU, lactic and acetic acids are authorised as food flavourings, according to Commission Regulation (EU) No 1334/2008 (i.e. FL-no: 08.004 and 08.002 respectively).

In 1974, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) issued an opinion on lactic acid and several of its salts as well as on acetic acid and its potassium and sodium salts, allocating an acceptable daily intake (ADI) of 'not limited' (JECFA, 1974, 1998). In 1991, this ADI was also supported by the Scientific Committee of Food (SCF) for lactic and acetic acids and their salts when used as food additives (SCF, 1991).

1.3. Additional information

1.3.1. Additional background information

1.3.1.1. Introduction

As indicated in the application dossier, the primary purpose of the proposed treatment is to reduce the incidence of food-borne illness in consumers by reducing the prevalence and/or abundance of human pathogens on pork products. The target pathogens on pork products identified by the applicant are: *Salmonella* Enteritidis, *Salmonella* Typhimurium, *Campylobacter* spp., *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Yersinia enterocolitica*, *Aeromonas hydrophilia* and *Staphylococcus aureus*. The proposed treatments will also target other non-pathogenic members of the Enterobacteriaceae family, which are considered hygiene indicators.

The second purpose of the treatment is to reduce spoilage bacteria,[10] measured by total viable counts or by enumerating specific spoilage organisms, on products, extending suitable storage periods, both under proper refrigerated storage and under temperature abuse. This can contribute to the reduction of food waste.

Approval was sought for lactic or acetic acid treatments on fresh hot/warm carcasses (referred to in the Scientific Opinion as pork carcass pre-chill) by spray and on chilled sections or cuts during fabrication, including pieces before retail packaging (referred to in the Scientific Opinion as pork meat cuts post-chill) by spray or dip.

1.3.1.2. Conditions of use and mode of application

The applicant submitted the following information in relation to the parameters for treatment application:

[6] Health Canada, 'Antimicrobial Processing Aids for which Health Canada has Issued a Letter of No Objection (LONO) or an interim Letter of No Objection (iLONO)', 17 December 2015.
[7] Codex Alimentarius, 'Guidelines for the control of non-typhoidal Salmonella spp. in beef and pork meat' CAC/GL 87-2016.
[8] Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives. OJ L 80, 26.3.2010, p. 19–27.
[9] EFSA-Q-2011-00596: re-evaluation of E270, lactic acid; EFSA-Q-2011-00592: re-evaluation of acetic acid, ethanoic acid.
[10] This was not assessed in this Scientific Opinion as it is outside the Terms of Reference of the mandate.

- Application type: either organic acid may be applied by spraying onto the surfaces of the meat carcasses or by either spraying or dipping during the fabrication process of meat cuts. The use of both organic acids is foreseen at any step in the production process after bleeding of the carcasses up to just prior to retail packaging. The size of the meat that may be sprayed ranges from whole carcasses immediately before chilling to retail meat cuts on the conveyor belt in the processing hall.
- Type of application in the processing line:

  Hot carcasses – pre-chill

  The organic acids may be applied by spraying onto the surfaces of the carcass. This would occur in any wash, applied in the final carcass wash or separately as a dedicated acid treatment. The spray may be carried out using a manual spray for smaller operations or an organic acid system cabinet.

  Meat cuts – post-chill

  The organic acids may be applied to cuts either by spray or dipping during the fabrication process, including immediately before bulk or retail packaging.
- Concentrations and conditions of use: the proposed concentration and the conditions of use may vary between the following limits:

  Lactic acid

  — 2–5% lactic acid at temperatures of up to 80°C on pork carcasses pre-chill by spray;
  — 2–5% lactic acid at temperatures of up to 55°C on pork meat cuts post-chill by spray or dip.

  Acetic acid

  — 2–4% acetic acid at temperatures of up to 40°C on pork carcasses pre-chill by spray;
  — 2–4% acetic acid at temperatures of up to 40°C on pork meat cuts post-chill by spray or dip.

'Pre-chilled carcass' refers to a carcass that is chilled before fabrication (boning/cutting in the boning hall). The different terminology stems from two different types of processing. In some operations, carcasses are chilled before fabrication. In other facilities, fabrication is conducted before chilling (i.e. hot-boned pork).
The applicant specified that concentrations of lactic or acetic acid higher than the limits specified above can, at least temporarily, change the appearance of meat cuts. Therefore, the maximum concentration of the acids used may depend on the final purpose of the cuts.

- Duration of exposure: The duration of either spray or dip would be sufficient to coat the surface, typically 5–10 s, and up to 30 s. This may be followed by a drip time that increases the effective treatment time. While not considered necessary, treatment may be followed by a subsequent spray or wash treatment, after sufficient drip time.
  A second treatment of individual cuts from a carcass that had a prior treatment is possible. While the first treatment would reduce pathogenic bacteria transferred to the carcass during slaughter and dressing, the second treatment would target cross-contamination from the processing environment.
  Volume to apply: The volume to be applied should be 'sufficient to coat the surface'. It depends upon the size of the piece being treated, i.e. a carcass requires a much greater volume than an individual retail cut.
- Subsequent removal conditions: No washing after treatment is foreseen. According to the applicant, as there is evidence that leaving a residue of the organic acids on meat surfaces helps inhibit bacterial growth, for example through recontamination, non-rinse is an important option to consider.
- Recycling: The recycling of organic acids solutions is not supported. For use as a dip, the operator would be required to write into their HACCP plans the frequency of replacing the liquid (batch system) or the flow rate for replacement of dipping solution (continuous system), along with testing programmes to assure that the dipping solution remains effective (temperature and concentration). Moreover, the product should be tested to ensure that the bacterial reduction expected by the acid treatment is actually being achieved on a consistent basis.

2. Data and methodologies

2.1. Data

The present evaluation is based on the data on lactic acid and acetic acid used for the reduction of pathogens on pork carcasses and cuts provided by the applicant in a dossier submitted in support of its application (see Documentation provided to EFSA n. 1).

Additional information was sought from the applicant during the assessment process in response to a request from EFSA sent on 22 February 2018 and was consequently provided (see Documentation provided to EFSA n. 2).

A second request for additional information was sent by EFSA on 22 June 2018; however, the applicant did not provide the additional data (see Documentation provided to EFSA n. 3). Consequently, the Panel concluded this assessment on the basis of the available data.

2.2. Methodologies

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 BIOHAZ Panel, 2010a). The assessment was conducted in line with the principles described in this guidance document.

2.2.1. Toxicological safety, potential emergence of resistance to biocides and/or to therapeutic antimicrobials and environmental risk assessment (ToRs 1, 3 and 4)

The questions as specified in the ToRs 1, 3 and 4 have been addressed by evaluating the information provided by the applicant supplemented with relevant studies identified by the Working Group and Panel members through a literature review.

2.2.2. Efficacy (ToR 2)

The question as specified in ToR 2 (efficacy) has been addressed by applying a systematic, stepwise approach, as follows:

- Formulation of the question under assessment and definition of the eligibility criteria for selecting experiments relevant to answer the question;
- Ascertainment of the comprehensiveness and relevance of the evidence provided by the applicant;
- Data extraction from the included experiments, using predefined data extraction forms;
- Appraisal of individual experiments included in the assessment, using a predefined critical appraisal tool (CAT) for the reliability evaluation;
- Data synthesis and interpretation of results in the light of the identified uncertainties.

2.2.2.1. Formulation of the question under assessment and eligibility criteria for study selection

The question under assessment is if individual application of lactic or acetic acid can achieve a significant reduction in the surface concentration of bacterial pathogens on pork carcasses pre-chill or pork meat cuts post-chill. The pathogens considered include: *S.* Enteritidis, *S.* Typhimurium, *Campylobacter* spp., *L. monocytogenes*, *E. coli* 0157:H7, *Y. enterocolitica*, *A. hydrophilia* and *St. aureus*. The proposed treatments will also target other non-pathogenic members of the Enterobacteriaceae family which are considered hygiene indicators.

In the EFSA guidance document (EFSA BIOHAZ Panel, 2010a) the use of decontaminating agents in a formulated product, under defined conditions, will be regarded efficacious 'when a reduction of the prevalence and/or numbers of pathogenic target microorganisms set according to determined criteria, is statistically significant[11] when compared to a non-treated control group (considering both a control group treated with potable water and a control group not treated at all). The achieved reduction in

[11] The extent of reduction is a risk management decision.

contamination should be expected to provide benefits to public health. This could be supported by reference to existing scientific data, such as epidemiological studies or risk assessments demonstrating public health benefits associated with similar reductions in extent of microbiological contamination'. The benefits to public health will be assessed in this opinion but the satisfactory level of this benefit is a risk management decision. In this assessment, the comparison was made with the untreated control if the water treatment control was not included in the experimental design. The EFSA guidance document on the assessment of the biological relevance of data in scientific assessments provides a general framework for establishing the biological relevance of observations at various stages of the assessment (EFSA Scientific Committee, 2017).

The eligibility criteria for selecting studies for inclusion in the assessment are outlined in Table 1. They have been defined based on the conditions of use and mode of application as provided by the applicant (see Section 1.3.1.2) and were applied for assessing the relevance of the studies. The outcome of interest was a change in numbers (log_{10} reduction) and/or in the presence of *Salmonella* spp., *Campylobacter* spp., *Listeria* spp., shiga toxin-producing *Escherichia coli* (STEC)/verocytotoxigenic *Escherichia coli* (VTEC), *Yersinia* spp., *Aeromonas* spp., *Staphylococcus* spp., Enterobacteriaceae, coliforms and/or *E. coli* on the treated carcass/meat at any time point after the treatment.

Table 1: Eligibility criteria for study selection

Does lactic or acetic acid significantly reduce the level of contamination of *Salmonella* Enteritidis, *Salmonella* Typhimurium, *Campylobacter* spp., *Listeria monocytogenes*, *E. coli* 0157:H7, *Yersinia enterocolitica*, *Aeromonas hydrophilia*, *Staphylococcus aureus* and Enterobacteriaceae on carcasses and cuts from pork?		
Criteria related to study characteristics		
Population	In	Pork carcasses before chilling (pork carcasses pre-chill) or carcass pieces or primals post-chilling, and post-chilling cuts including retail cuts (pork meat cuts post-chill)
Intervention	In	Lactic acid used at a concentration of 2–5% and at a temperature of up to 80°C (in case of pork carcasses pre-chill) or up to 55°C (in case of pork meat cuts post-chill) for a duration of up to 30 s. Concentration, temperatures and duration of treatment needed to be reported/available to assess this
	In	Acetic acid used with a concentration of 2–4% and at a temperature of up to 40°C (in case of pork carcasses pre-chill and pork meat cuts post-chill) for a duration up to 30 s. Concentration, temperature and duration of treatment needed to be reported/available to assess this
Comparator	In	Water (or other solution) treated or untreated controls[(a)]
Outcome of interest	In	The change in numbers (log_{10} reduction) and/or in presence of *Salmonella* spp., *Campylobacter* spp., *Listeria* spp., STEC/VTEC, *Yersinia* spp., *Aeromonas* spp., *Staphylococcus* spp., Enterobacteriaceae, coliforms and/or *E. coli* on the treated carcass/meat at any time point after the treatment (e.g. immediately after treatment, during storage, or at the end of shelf-life)
Study design and setting	In	Experimental controlled studies were included (studies without a control group were excluded). These may have been undertaken in the laboratory, pilot-scale plant or in an industrial (commercial) setting
Criteria related to report characteristics		
Language of the full text	In	English
Time	In	From database inception to 14 February 2018
Publication type	In	Primary research studies (i.e. studies generating new data)
	Out	Systematic reviews Narrative reviews[(b)] Expert opinions, editorials and letters to the editors

(a): No treatment applied. These carcasses or cuts were left as they were without applying the organic acids or water or any other solution.

(b): Narrative reviews were collected for the purposes of reviewing the reference list but did not contribute to the final number of studies considered eligible unless they also contained original data.

2.2.2.2. Ascertainment of the comprehensiveness and relevance of the evidence provided by the applicant

Search for studies

The applicant stated that appropriate studies were identified by two literature searches (O'Connor, 2015; Bassett, 2016) and by additional searches using literature search engines (AGRICOLA) at the USDA National Agricultural Library. Studies that contained pertinent data (i.e. relevant records identified by the applicant) or that appeared to contain pertinent data (i.e. potentially relevant records identified by the applicant) were listed by the applicant. No study identified in the searches was omitted. The applicant stated that 'While it is probable that there are additional studies relevant to the efficacy of these organic acids, the references assembled here should represent a fairly complete compilation of the available studies'.

The applicant identified 41 relevant records, of which 29 considered lactic acid, 8 considered acetic acid and 4 included data on the individual treatment with both substances. In addition, 21 potentially relevant records were also included in the submission.

The search by Bassett (2016) was carried out in July 2016 and considered surface decontamination of meat of various species using various substances, including lactic acid, but not acetic acid. The search by O'Connor (2015) was carried out in January 2015 and considered surface decontamination of pork meat using various substances, such as lactic and acetic acid. The search strategy used in the literature searches provided by the applicant (e.g. search strings used for each information source, dates of the searches, search limits, etc.) was considered appropriate for the assessment. Therefore the *comprehensiveness* of the evidence provided was considered appropriate.

As the search by O'Connor (2015) was performed in January 2015, the Working Group and the Panel decided to search the literature for more recent relevant studies. The bibliographic databases Web of Science and CABI were searched on 14 February 2018, using the strategy reported in Table 2, adjusted from O'Connor (2015) by removing the search terms related to other decontamination treatments (e.g. peroxyacetic acid or steam). The language was restricted to English and the time span established was from 2015 onwards. Review papers were excluded, but used for hand-searching their reference lists. In total, 160 unique records were retrieved this way (97 retrieved using Web of Science and 137 using CABI).

Table 2: Details of the search strings used for literature search in Web of Science and CABI

Set number	Search
5	#3 OR #4
4	TITLE = ((DECONTAMINAT* OR CONTAMINAT*) AND CARCASS*)
3	#1 AND #2
2	TOPIC = (pathogen near/4 reduc*) OR (prt) OR (wash or washes or washing or washed or rinse or rinses or rinsing or rinsed) OR (spray or sprays or spraying or sprayed) OR (Organic NEAR/5 (decontaminat* or saniti*)) OR (ACETIC OR LACTIC) OR ((ACID) NEAR/5 (spray* or decontaminat* or saniti* or wash*)) OR NONACID OR ((hot or cold or warm) NEAR/3 water) OR 'water treatment$' OR ((Prevent* or reduc*) near/4 contaminat*) or TS = decontaminat*
1	TOPIC = ((pork or swine or pig or pigs or hog or hogs or boar or boars or sow or sows) near/7 (carcass* OR slaughter* or abattoir* or bellies))

These records were complemented with 14 records on efficacy prescreened for relevance by hand-searching the reference lists of three relevant review papers published since 2015 (Belluco et al., 2015; Totton et al., 2016; Young et al., 2016). The reference list of the review paper by Totton et al. (2016) included another review paper by Loretz et al. (2011) that did not contain additional records.

Applying the eligibility criteria illustrated in Table 1, the records were screened using the software DistillerSR® for relevance to the review question in two steps:

Study selection process and identification of relevant experiments

Applying the eligibility criteria illustrated in Table 1, the records were screened using the software DistillerSR for relevance to the review question in two steps:

- **Screening of titles and abstracts**

  Screening of title and abstracts was done to identify records containing:

  a) obviously irrelevant studies, to be excluded from the assessment;
  b) potentially relevant studies or studies with unclear scope, to be moved to full-text screening.

  The screening was done based on the following criteria: (1) primary research study, (2) at least one surface decontamination experiment on pork carcasses or cuts, and (3) individual application of lactic acid or acetic acid. If negative for one of these criteria, the record was excluded.
  This was done by two reviewers and, in case of doubts or divergences, the full article was screened.

- **Screening of full-text documents**

  Screening of full text documents was done in two steps:

  Step I: further identification of records to be excluded: not primary research study, not in English or full text not available
  Step II: identification of experiments within each record and evaluation of their relevance to the question under assessment. Experiments were differentiated based on their objective and the relevant experimental design (e.g. experimental setting, type of contamination, decontamination substance, application method, product category and product subcategory).

  Each experiment was classified with a Ref_ID number and a brief description stating the objective of each experiment. Then, each experiment was screened for relevance and validated. Possible divergences were solved by discussion.

The results are reported in the opinion using a flow chart, as recommended in the PRISMA statement on preferred reporting items for systematic reviews and meta-analyses (Moher et al., 2010). Reasons for exclusion are reported.

2.2.2.3. Data extraction from included experiments

The list of parameters to be extracted from the records included in the assessment was predefined using DistillerSR® software. Information expressed only in graphs/curves/figures was identified as such. Numerical values were used with the unit as presented in the records. The data set was created for all time points investigated in the studies selected and was checked by a different reviewer to correct any mistakes. Three hierarchical Distiller forms were used for data extraction. The data extracted can be found in Annex 1.

- The overarching form contained the experiment-defining variables, i.e. the experimental setting (laboratory scale, pilot-scale representative of industrial process and industrial scale), the type of contamination (natural or artificial), the substance (lactic acid or acetic acid), the application method (spraying or dipping), the product category (pork carcasses pre-chill or pork meat cuts post-chill) and the product subcategory (product as described in the record)
- The subform captured information related to:
  - the treatment characteristics: the concentration, temperature and pH of the decontamination solution, the duration of treatment and pressure of the application
  - the contamination characteristics: the bacterial group (*Salmonella* spp., *Campylobacter* spp., *Listeria* spp., STEC/VTEC, *Yersinia* spp., *Aeromonas* spp., *Staphylococcus* spp., Enterobacteriaceae, coliforms and/or *E. coli*) and subgroup (when provided). In case of artificial contamination, it was captured, when available, the origin of the strain(s), the pool of strains used, the stress status of the strains and the growth phase of the culture
  - the analytical methods: the analytical method used for monitoring the presence/absence or for enumeration, the method of meat sampling and the limit of quantification for enumeration (to be reported when one of the counts is below the limit)
- The subform captured information related to:
  - The treatment and storage characteristics: the treatment of samples, i.e. water, untreated, decontamination solution and both decontamination solution and control (when only $\log_{10}$ reductions were reported), the timing of sampling (i.e. before treatment,

immediately after treatment, first point after storage (when immediately after treatment is not available), end of storage) and, if storage, the storage characteristics (i.e. temperature, duration, conditions)

— The outcome extraction: the number of samples and/or trials, the concentration (central measure, dispersion measure and unit) when enumeration was performed for samples that have been treated with water or the decontamination solution or are left untreated, the number of positive samples and number of samples tested or proportion of positive samples when presence/absence testing was performed.

The Working Group (WG) applied a series of transformations to the data in order to harmonise the measurement unit used in the various experiments. For the comparison and the evaluation of experiments, results were reported as the mean $\log_{10}$ reduction in all graphs and tables. The $\log_{10}$ reduction is the difference between the means of the $\log_{10}$ concentration of control group and treated group. When the levels of reported values were below the detection or quantification limit, that limit was used (i.e. the most conservative estimate). The R code for statistical analysis can be found at https://doi.org/10.5281/zenodo.1479671.

There are three situations for reporting the enumeration outcomes:

- **Case 1:** The mean concentration is reported for both the treatment group and control group. The data extracted referred to control group as the water treated group or the untreated control group. In case both controls were reported, the water treated group was taken as control. The mean value of the $\log_{10}$ reduction was calculated as the difference between the mean value of the $\log_{10}$ concentration for the control group (M_c) and the mean value of the $\log_{10}$ concentration for the treatment group (M_T), where concentrations are expressed in the record for each group per g or cm^2 of product depending on how it was reported in the records. The mean $\log_{10}$ reductions were calculated based on the mean concentrations of the control group and treatment group, immediately after treatment and, whenever storage trials were included in the studies, at the last available data point of storage. The corresponding 95% confidence interval (95% CI) of the $\log_{10}$ reduction was calculated when the standard deviation (SD; SD_C, SD_T), or standard error of the mean (SEM) or CIs and the number of samples for both treatment (n_T) and control group (n_c) were known. When it was unclear whether the measurement dispersion represented the SD or SEM, it was assumed that the SEM was reported as that would result in the largest CI, in order to be more conservative. For some experiments this information for calculating the CI was not reported and therefore the CI could not be calculated. The $\log_{10}$ reduction (difference of means ΔM) and the 95% CI were calculated as follows:

$$\Delta M = M_c - M_t$$

$$CI = \Delta M - T_{0.975;nu} \times \sqrt{D},$$

where

$$D = \frac{SD_T^2}{nt} + \frac{SD_C^2}{nc} \text{ and}$$

$$nu = \frac{D^2}{\frac{SD_T^4}{nt^2(nt-1)} + \frac{SD_C^4}{nc^2(nc-1)}},$$

- **Case 2:** Only the $\log_{10}$ reduction (ΔM) is directly reported comparing the treatment group and control group with a number of samples (n). No further calculation was necessary. Also here, the data extracted referred to control group as the water treated group or the untreated control group. In case both controls were reported, the water treated group was taken as control. The corresponding 95% CI is either calculated, directly reported or this information is missing. The 95% CI was calculated as follows:

$$CI = \Delta M \pm T_{0.975;number\ of\ trials} \times \sqrt{2\frac{SD\ dif}{n}}$$

When variation was expressed as standard error, the SD was calculated as follows:

$$SD = SE\sqrt{n}$$

- **Case 3:** The following two values for mean $\log_{10}$ reductions were reported: the $\log_{10}$ concentration for the untreated control group minus the $\log_{10}$ concentration for the treatment group and the $\log_{10}$ concentration for the untreated control group minus the $\log_{10}$ concentration for the water treated group. The $\log_{10}$ reduction was calculated as the difference of the mean value of the $\log_{10}$ reductions of the first and the latter. It was assumed that both untreated control groups have the same concentrations. The corresponding 95% CI for the final value of the $\log_{10}$ reduction was calculated when the SD, or SEM, or CIs and the number of experiments for both treatment and control group were known. When it was unclear whether the measurement dispersion represented the SD or SEM, it was assumed that the SEM was reported as that would result in the largest CI. For some experiments this information for calculating the CI was not reported and therefore the CI could not be calculated.

2.2.2.4. Appraisal of individual experiments

The strength of each experiment included in the assessment was appraised taking into consideration elements related to relevance and reliability.

- Data **relevance** refers to the appropriateness of the data for the intended purpose of the assessment (adapted from Klimisch et al., 1997; Vermeire et al., 2013). Relevance refers to the extent to which available data address the objectives of the assessment (e.g. the right target population, hazard of concern, geographical area, etc.) (EFSA, 2015).
- **Reliability** refers to: (i) precision, i.e. the extent to which random error is minimised and the outcome of the process is reproducible over time (IPCS, 2009); and (ii) accuracy, i.e. the extent to which systematic error (bias) is minimised. Risk of bias (RoB) refers to the extent to which the design and conduct of a study are likely to have prevented bias, i.e. systematic error (Higgins and Green, 2011).

In previous EFSA opinions (EFSA BIOHAZ Panel, 2011a, 2014; EFSA BIOHAZ Panel and EFSA CEF Panel, 2012), specific criteria were used to define the 'strength of evidence' on a scale High/Medium/Low based on the experimental setting and inoculation type. These criteria were originally presented in the FAO/WHO report on Benefits and Risks of the Use of Chlorine-containing Disinfectants in Food Production and Food Processing (FAO/WHO, 2008). A similar approach was used for assessing the **relevance** of each experiment in this Opinion as shown in Table 3.

Table 3: Strength of evidence 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 demonstrate a disinfectant effect, reproducible in practice, but would not be sufficient to derive a quantitative microbial risk assessment or to allow conclusions on risk reduction.
(e): Data are indicative of a disinfectant effect that may be reproducible in practice, but are inconclusive on risk reduction.

Each experiment underwent a **reliability** appraisal. This was done through a CAT considering four elements: (a) Comparability of control and treated groups, (b) Inoculation procedure of the target organism and coverage of the meat surface with the substance, (c) Detection and enumeration method of the target organism, and (d) Statistical analysis and reproducibility (Appendix A). The rating scale was applied for each element individually and ranged from 4 to 1. For each element listed in the CAT, experts' judgement was translated into the rating scale shown in Table 4.

Table 4: Proposed rating scale for appraising the reliability of the experiments

Rating	Risk of bias	Precision
4	Definitively low risk of bias	Definitively appropriate
3	Probably low risk of bias	Probably appropriate
2	Probably high risk of bias	Probably not appropriate
1	Definitively high risk of bias	Definitively not appropriate

The CAT was converted into a DistillerSR® form to allow web-based appraisal of the studies. This was done independently by two reviewers and possible discrepancies were solved through discussion.

2.2.2.5. Data synthesis and interpretation of results in light of identified uncertainties

Each individual experiment included in the assessment was reported in tabular format in the scientific opinion. Each table illustrated the experiment characteristics, population, methods, intervention, outcome(s) and the appraised reliability. The data were summarised using plots of the $\log_{10}$ reductions of each bacterial group by application of either lactic or acetic acid on pork carcasses pre-chill by spray and on pork meat cuts post-chill by spray or dip. Tables were used to present the proportion of positive samples. An overview of the potential sources of uncertainty identified in the efficacy assessment and the impact that these uncertainties could have on the direction of the effect on prevalence reduction and $\log_{10}$ reductions is provided in tabular format.

3. Assessment

3.1. Toxicological safety of lactic acid to humans (ToR 1)

3.1.1. Identity of the substance

Lactic acid (2-hydroxypropanoic acid, C3H6O3) is a colourless to slightly yellow, nearly odourless, syrupy liquid to solid, soluble in water and water-miscible organic solvents. It is a weak acid (pK_a = 3.9 at 25°C) and largely dissociated at biologically relevant pH. There are two optical isomers: L-(+)- or (*S*)-lactic acid and D-(-)- or (*R*)-lactic acid, both of which occur naturally, although the L-(+)-isomer is the most abundant in all vertebrates, including humans. The structural formula of lactic acid is shown in Figure 1.

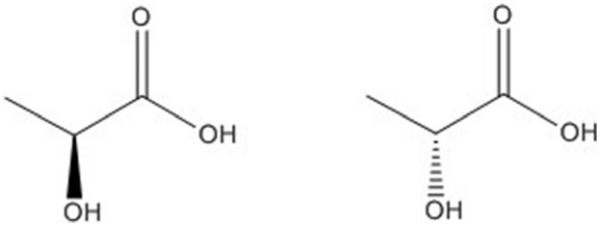


Figure 1: Structural formula of L-(+)- and D-(-)-lactic acid

Commercial lactic acid is produced either by fermentation of carbohydrates, such as glucose, sucrose or lactose, or by a chemical synthesis through the formation of lactonitrile from acetaldehyde and hydrogen cyanide, followed by hydrolysis. For food applications, normally L-(+) lactic acid made by fermentation is used, but also synthetic lactic acid is accepted, provided the specifications are met (see Table 5).

3.1.2. Specifications

According to the applicant, food-grade L-(+) lactic acid obtained by fermentation is used for pathogen reduction treatments, which is expected to meet the purity specifications of Commission Regulation (EU) No 231/2012[12] (Table 5).

[12] Regulation (EU) No 231/2012 of the European Parliament and of the Council of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008. OJ L 83, 22.3.2012, p. 1–280.

Table 5: Specifications for solid and aqueous forms of lactic acid (E 270) according to Commission Regulation (EU) No 231/2012

Definition	Consists of a mixture of lactic acid ($C_3H_6O_3$) and lactic acid lactate ($C_6H_{10}O_5$). It is obtained by the lactic fermentation of sugars or is prepared synthetically. Lactic acid is hygroscopic and when concentrated by boiling, it condenses to form lactic acid
Einecs	200-018-0
Chemical name	Lactic acid; 2-Hydroxypropionic acid; 1-Hydroxyethane-1-carboxylic acid
Chemical formula	$C_3H_6O_3$
Molecular weight	90.08
Assay	Content not less than 76%
Description	Colourless or yellowish, nearly odourless, syrupy liquid to solid
Identification	
Test for lactate	Passes test
Purity	
Sulphated ash	Not more than 0.1%
Chloride	Not more than 0.2%
Sulphate	Not more than 0.25%
Iron	Not more than 10 mg/kg
Arsenic	Not more than 3 mg/kg
Lead	Not more than 2 mg/kg
Mercury	Not more than 1 mg/kg

Note: This specification refers to 80% aqueous solution; for weaker aqueous solutions, calculate values corresponding to their lactic acid content.

3.1.3. Analytical method

According to the applicant, the concentration of lactic acid in solutions may be determined by titration. No method was provided for the determination of lactic acid in the pork meat. The Panel noted that nowadays lactic acid is commonly analysed chromatographically.

3.1.4. Reaction products

The decontamination treatment with a lactic acid solution temporarily reduces the pH of the meat surface. Shortly afterwards, the pH returns to near former levels due to the buffering capacity of the meat (Grajales-Lagunes et al., 2012).

The Panel noted that no chemical reaction by-products resulting from the decontamination treatment with lactic acid are expected that would not normally occur in meat.

The effect of sanitising solutions containing lactic acid may result in sensory defects in the treated carcasses or meat cuts such as discoloration of the surface and drip loss and cooking loss due to changes in the water-holding capacity of proteins when the pH is near to the isoelectric point (Pipek et al., 2005; Grajales-Lagunes et al., 2012; Mani-Lopez et al., 2012).

Lactic acid is typically purchased as an 80–88% solution and diluted to the desired concentration with potable water. The Panel noted that such water may contain residual chlorine (primarily as hypochlorus acid). This is, however, a very minor amount compared to the lactic acid used, and no reaction with lactic acid is expected.

3.1.5. Dietary exposure

Exposure to lactic acid from the intended use was based on consumption of pork and pork products reported in the Comprehensive European Food Consumption Database (see Appendix B), and in the absence of specific information from the applicant on analytically determined residual levels of lactic acid in pork meat, concentration data reported in the literature (Rose et al., 2004) was used.

Based on Rose et al. (2004), 12.5 g of beef meat pieces treated with 2.5% lactic acid wash (i.e. 30 g lactic acid/L) had an uptake of 0.6 mg of lactic acid from the wash treatment, equivalent to 48 mg/kg meat. The corresponding concentration in meat resulting from use of 5% (vol/vol) lactic acid was extrapolated, based on reported increased uptake between dose intervals (i.e. 0.38 mg/dL from

0.125% to 2% and 0.27 mg/dL from 2% to 2.5% wash water concentration), and estimated to be 99.2 mg/kg meat.

The estimated exposure to of lactic acid from use as a decontamination agent on pork meat derived in this opinion was compared to other dietary sources of lactic acid.

3.1.5.1. Exposure assessment methodology

For each individual, the lactic acid concentration data was combined with the average daily consumption of pork and pork products. Food groups containing pork were, where necessary, adjusted for pork content (see Appendix B – Table B.1). The resulting exposure was then summed in order to obtain total chronic exposure at individual level from all food sources. The mean and the higher percentile (i.e. 95th percentile) of the individual exposures were calculated for each dietary survey and each age class separately.

3.1.5.2. Exposure to lactic acid

Table 6 shows that exposure to lactic acid from pork and pork-based dishes involving decontamination with 5% lactic acid ranges on average from 0.1 to 13.9 mg/person per day and from 0.1 to 34.7 mg/person per day, at the 95th percentile across all population groups.

Table 6: Exposure to lactic acid from consumption of pork and pork-based dishes involving decontamination with 5% lactic acid

Population group	Mean			95th percentile		
	n	Min (mg/person per day)	Max (mg/person per day)	n	Min (mg/person per day)	Max (mg/person per day)
Infants	11	0.1	4.5	10	0.1	13.0
Toddlers	14	1.0	4.8	12	4.8	12.9
Other children	19	2.2	8.0	19	6.7	16.7
Adolescents	18	4.3	11.1	17	10.4	23.3
Adults	19	4.2	13.4	19	12.1	34.7
Elderly	18	3.2	10.9	18	8.6	27.2
Very elderly	15	2.6	13.9	10	7.9	21.1

n = number of food consumption surveys.

The Panel decided to put the above-derived figures into context by comparing them with naturally occurring lactic acid in pork meat.

Lactic acid is a natural component of meat, produced by glycolysis of glycogen and glucose in muscle. It is responsible for the pH decrease from around 7.1–7.3 to 5.4–5.7 during early *post-mortem* (Greaser, 1986) The amount of generated lactic acid is quite variable since it depends on different factors (e.g. resting of the animal before death, content of glycogen in muscle, animal species and age, feeding, level of preslaughter stress and exercise, etc.). It can be calculated that a decline in pH from 7.0 to 5.5 requires a formation of 60 to 80 mmol lactic acid/kg muscle tissue (i.e. 7.2 g lactic acid/kg muscle tissue), depending on animal species and muscle type, which corresponds to a natural content of approximately 0.7% of lactic acid in meat (Puolanne et al., 2002).

Based on the above and assuming a natural content of 0.7% (i.e. 7 g/kg meat) lactic acid in meat (Puolanne et al., 2002), the intake of endogenous lactic acid from consumption of pork and pork based products can be estimated (see Table 7).

Table 7: Estimated intake of endogenous lactic acid from consumption of pork and pork-based products

Population group	Mean			95th percentile		
	n	Min (mg/person per day)	Max (mg/person per day)	n	Min (mg/person per day)	Max (mg/person per day)
Infants	11	6	319	10	6	916
Toddlers	14	73	339	12	341	910
Other children	19	159	565	19	474	1,176
Adolescents	18	305	784	17	734	1,645
Adults	19	299	943	19	851	2,449
Elderly	18	226	768	18	608	1,921
Very elderly	15	184	980	10	560	1,491

n = number of food consumption surveys.

The intake of lactic acid naturally present in pork meat ranged from 6 to 980 mg/person per day at the mean and from 6 to 2,449 mg/person per day at the 95th percentile, and was found to far exceed the exposure to lactic acid resulting from its use as a decontamination agent on pork meat. Since no other sources of naturally occurring lactic acid were considered (e.g. fermented milk products), total exposure from all natural dietary sources is likely to be even higher. Since intake of lactic acid from endogenously present lactic acid already far exceeds exposure from use of lactic acid as decontamination agent, the Panel considered it not necessary to estimate intake of naturally present lactic acid from all dietary sources.

3.1.5.3. Uncertainty analysis

In accordance with the guidance provided in the EFSA Opinion related to uncertainties in dietary exposure assessment (EFSA Scientific Committee, 2007), the following sources of uncertainties have been considered and are summarised in Table 8.

Table 8: Qualitative evaluation of the influence of uncertainties on the dietary exposure estimate

Sources of uncertainties	Direction of impact[a]	
	Exposure to lactic acid from use as a decontamination treatment in pork meat	Exposure to naturally occurring lactic acid
Model input data		
Assumption that pork carcasses and cuts are only treated once with the decontaminating solution of 5% lactic acid	–	NA
Assumption that all pork consumed always contains residual lactic acid as a result of treatment with the 5% decontaminating solution	+	NA
Use of data from food consumption surveys of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+	+
Possible national differences in categorisation and classification of food	+/–	+/–
Model assumptions and factors		
Use of literature data on use of lactic acid on beef meat (i.e. Rose et al. 2004) to calculate the exposure to lactic acid used to decontaminate pork meat	+/–	NA
Use of literature data on use of lactic acid on beef meat (i.e. Rose et al. 2004) to extrapolate residual lactic acid following use of a 5% solution.	+/–	NA

Sources of uncertainties	Direction of impact[(a)]	
	Exposure to lactic acid from use as a decontamination treatment in pork meat	Exposure to naturally occurring lactic acid
Exclusion of other natural dietary sources of lactic acid, other than pork meat (e.g. milk fermentation products, fermented fruit- or vegetable-based foods, etc.), in the calculation of the natural dietary intake of lactic acid	NA	–
Adjustment for pork content of food groups containing pork (see Appendix B – Table B.1 'Pork fraction of food groups')	+/–	+/–

(a): + means that the (real) exposure is possibly overestimated, – means that the (real) exposure is possibly underestimated.

In the applied exposure model, the Panel assumed that all pork carcasses and cuts were treated only once, whereas the applicant indicated that in some cases a repeat treatment may take place (e.g. treatment of a carcass followed by treatment of an individual cut), which may lead to an underestimation of exposure. The Panel further assumed that all pork and pork products consumed always contained residual lactic acid as a result of treatment with the decontamination agent, which would lead to a considerable overestimation of exposure. The concentration data applied was extrapolated from data reported in the literature for beef, and the influence of this uncertainty on the exposure assessment cannot be estimated.

Given these observations, the Panel considered overall that the uncertainties identified would, in general, result in an overestimation of the exposure to lactic acid from its use as a decontamination agent in European countries considered in the EFSA European database.

3.1.6. Toxicological assessment

No toxicological data were provided by the applicant in view that, according to Commission Regulation (EC) No 1333/2008 on food additives, lactic acid is an authorised food additive that may be used *quantum satis* in a variety of foods other than meat and food intended for infants and young children without limitations. The use of lactic acid is also authorised in Europe to reduce microbiological surface contamination on bovine carcasses, according to Regulation (EU) No 101/2013[13].

Lactic acid is an endogenous substance. It is an intermediate of carbohydrate and amino acid metabolism. It is produced by almost all human tissues during anaerobic metabolism and in smaller amount by carbohydrate-fermenting bacteria normally present in the gastrointestinal tract. Normal lactate concentrations in human blood are within the approximate range of 0.5–2 mmol/L (Ewaschuk et al., 2005). The L-(+)-isomer is the major physiological enantiomer present in the human body. D-(–) lactate is also present in human blood but usually only at 100 times lower concentration of L-(+)-lactate (i.e. 5–20 μmol/L) (Talasniemi et al., 2008).

It has been shown that infants in their first three months of life have difficulties in metabolising the D-(–)-isomer and therefore they are more susceptible to D-lactic acidosis than adults (Petersen, 2005). The capacity to metabolise the D-(–)-isomer increases with age (Whittakers et al., 1974; Christie and Cranwell, 1976). For this reason, the authorisation of lactic acid as a food additive is restricted to the L-(+)-form in food specially prepared for infants and young children. Considering that pork meat is not consumed by infants in their first 3 months of life, the Panel noted that this restriction is not relevant in the context of the present assessment.

The Panel also noted that D-lactic acidosis, defined as plasma D-lactate > 3.0 mmol/L in association with metabolic acidosis (blood pH < 7.35) (Uribarri et al., 1998), is one of the many metabolic disorders that can occur in patients with short-bowel syndrome, a rare complication which follows surgical resection of more than half the length of the small intestine. However, only a massive increase in plasma D-(–)-lactic acid concentration of more than 2.5–3.0 mmol/L (i.e. > 225.2–270.2 mg/L) would lead to the development of D-lactic acidosis and symptomatic effects (Ewaschuk et al., 2005). Therefore, even if all the lactic acid used in the decontamination treatment consisted of the D-(–)-isomer, the maximum expected intake of D-(–)-lactic acid from the consumption of pork meat would

[13] Commission Regulation (EU) No 101/2013 of 4 February 2013 concerning the use of lactic acid to reduce microbiological surface contamination on bovine carcases.

only amount to 34.7 mg/person per day (see Section 3.1.5.2), not enough to trigger D-lactic acidosis in patients with short-bowel syndrome.

Based on the above considerations, and noting that exposure to lactic acid from endogenous sources far outweighs exposure from the intended uses (i.e. up to 70-fold), the Panel concluded that the use of lactic acid on pork carcasses and cuts is of no safety concern with respect to toxicity.

3.2. Toxicological safety of acetic acid to humans (ToR 1)

3.2.1. Identity of the substance

Acetic acid (ethanoic acid, CH_3COOH) is a colourless liquid with a pungent odour. Its concentrated form is often referred to as glacial acetic acid. It is a weak acid (pK_a = 4.76 at 25°C) and will predominantly be dissociated in meat.

It is the main ingredient of vinegar. Acetic acid is obtained by aerobic fermentation of wine, beer, fruits and grain mashes, but the major amount is produced from chemical synthesis, mainly from oxidation of ethylene or the reaction of methanol with carbon monoxide.

3.2.2. Specifications

For pathogen reduction treatments, food-grade acetic acid is used that is expected to meet the purity specifications for the food additive acetic acid (E 260), as provided by Regulation (EU) No 231/2012[12] (Table 9).

Table 9: Specifications for acetic acid according to Commission Regulation (EU) No 231/2012

Definition	
Einecs	200-580-7
Chemical name	Acetic acid; Ethanoic acid
Chemical formula	$C_2H_4O_2$
Molecular weight	60.05
Assay	Content not less than 99.8%
Description	Clear, colourless liquid having a pungent, characteristic odour
Identification	
Boiling point	118°C at 760 mm pressure (of mercury)
Specific gravity	About 1.049
Test for acetate	A one in three solution gives positive tests for acetate
Solidification point	Not lower than 14.5°C
Purity	
Non-volatile residue	Not more than 100 mg/kg
Formic acid, formats and other oxidisable substances	Not more than 1,000 mg/kg expressed as formic acid
Readily oxidisable substances	Dilute 2 mL of the sample in a glass-stoppered container with 10 mL of water and add 0.1 mL of 0.1 N potassium permanganate. The pink colour does not change to brown within 30 min
Arsenic	Not more than 1 mg/kg
Lead	Not more than 0.5 mg/kg
Mercury	Not more than 1 mg/kg

3.2.3. Acetic acid – analytical method

The concentration of acetic acid in solution may be determined by titration. No information was provided by the applicant on the determination of acetic acid in the pork meat. It would commonly be analysed chromatographically.

3.2.4. Reaction products

Acetic acid is a component of many foods at concentrations up to those used in pathogen reduction treatments.

No reaction product is expected that would not be formed normally in foods. At high concentrations and long treatment, acetic acid may cause discoloration of meat surfaces (Mani-Lopez et al., 2012).

The treatment reduces the pH of the meat surface, but shortly afterwards, the pH returns close to the former level due to the buffering capacity of meat (Goli et al., 2012).

Considering that food containing acetic acid may be subject to heat treatment, the Panel noted that cooking of meat treated with acetic acid can reasonably be expected not to contain any compounds not typically encountered in other acetic acid containing heat treated foods.

Acetic acid is typically purchased as glacial acetic acid (> 99.8%) and diluted to the desired concentration with potable water. The Panel noted that such water may contain residual chlorine (primarily as hypochlorus acid), which may react with acetic acid. This is, however, a very minor amount compared to the acetic acid used, and no reaction with acetic acid is expected.

3.2.5. Dietary exposure

Exposure to acetic acid from the intended use was based on consumption of pork and pork products reported in the Comprehensive European Food Consumption Database (see Appendix B), and, in the absence of specific information from the applicant on analytically determined residual levels of acetic acid in pork meat, concentration data reported in the literature (Anderson et al., 1980) was used.

Based on Anderson et al. (1980), 100 kg of beef meat took up 300 g of washing water containing 3% (v/v) acetic acid (31.5 g acetic acid/L, given the density of acetic acid, i.e. 1.049 g/mL), corresponding to 94.5 mg/kg meat. This level of residue does not take into account any volatile losses of acetic acid during storage or cooking and hence presents a conservative estimate.

Assuming the same mode of uptake as reported for the 3% wash, treatment with 4% acetic acid would result in a final concentration of 125.8 mg/kg meat.

Acetic acid has historically been known from the aerobic fermentation of wine or beer. It may also be produced by fermentation of other fruit and grain mashes. In vinegar, the acetic acid content can range from 4% to 5% for rice vinegars, 6–9% in wine vinegars and up to 6–15% in balsamic vinegars (Plessi, 2003). Vinegar has long been used worldwide as a basic seasoning in the preparation and cooking of certain foods; it is used in many condiments (e.g. ketchup, mustard, salad dressings) and is used as a preservative for both domestic use and in industrial food production. Such applications include preservation or pickling of a wide variety of foods, such as vegetables, meat, fish products, etc. (Plessi, 2003). Vinegar also features in many recommendations as to how to tenderise meat prior to cooking.

The estimated exposure to acetic acid from use as a decontamination treatment derived in this opinion was compared to other dietary sources of acetic acid.

3.2.5.1. Exposure assessment methodology

For each individual, the acetic acid concentration data was combined with the average daily consumption of pork and pork products. Food groups containing pork were, where necessary, adjusted for pork content (see Appendix B – Table B.1). The resulting exposure was then summed in order to obtain total chronic exposure at individual level from all food sources. The mean and the higher percentile (i.e. 95th percentile) of the individual exposures were calculated for each dietary survey and each age class separately.

The Panel assumed that pork carcasses and cuts were only treated once, which may lead to an underestimation of exposure, since repeat treatment may be applied (same methodology as described in Section 3.1.5.1).

3.2.5.2. Exposure to acetic acid

Table 10 shows that exposure to 4% acetic acid used as decontamination treatment from consumption of pork and pork based dishes ranged on average from 0.1 to 17.6 mg/person per day and from 0.1 to 44.0 mg/person per day, at the 95th percentile across, all population groups.

Table 10: Exposure to 4% acetic acid from consumption of pork and pork based dishes

Population group	Mean			95th percentile		
	n	Min (mg/person per day)	Max (mg/person per day)	n	Min (mg/person per day)	Max (mg/person per day)
Infants	11	0.1	5.7	10	0.1	16.5
Toddlers	14	1.3	6.1	12	4.8	16.4
Other children	19	2.9	10.1	19	6.7	21.1
Adolescents	18	5.5	14.1	17	10.4	29.6
Adults	19	5.4	16.9	19	12.1	44.0
Elderly	18	4.1	13.8	18	8.6	34.5
Very elderly	15	3.3	17.6	10	7.9	26.8

n = number of food consumption surveys.

In order to put the exposure to acetic acid from use as a decontamination agent on pork into context with acetic acid intake obtained via the typical diet, the Panel decided to assess the intake of acetic acid from consumption of vinegar as reported in the Comprehensive Database (see Table 11). An acetic acid content of 6% (w/v) in vinegar was assumed.

Table 11: Estimated intake of acetic acid (6% w/v) from consumption of vinegar

Population group	Mean			95th percentile		
	n	Min (mg/person per day)	Max (mg/person per day)	n	Min (mg/person per day)	Max (mg/person per day)
Infants	11	0	2.8	10	0	18.0
Toddlers	14	0	42.9	12	0	180.0
Other children	19	0	76.1	19	0	262.5
Adolescents	18	0	104.5	17	0	375.0
Adults	19	3.2	102.8	19	0	480.0
Elderly	18	2.3	123.3	18	0	480.0
Very elderly	15	0	84.9	10	0	360.0

n = number of food consumption surveys.

The intake of acetic acid from consumption of vinegar (6% w/v) ranged from 0 to 123.3 mg/person per day at the mean and from 0 to 480 mg/person per day at the 95th percentile, and was found to far exceed the exposure to acetic acid as a result from its use as a decontamination agent on pork meat. Also, no other natural dietary sources of acetic acid were taken into account, and therefore, the assessment is likely to present an underestimation of intake of acetic acid from all naturally occurring dietary sources. However, since intake of acetic acid from vinegar alone already far exceeds the exposure to acetic acid from use as a decontamination agent, the Panel considered it not necessary to estimate total intake of acetic acid from all natural sources.

3.2.5.3. Uncertainty analysis

In accordance with the guidance provided in the EFSA Opinion related to uncertainties in dietary exposure assessment (EFSA Scientific Committee, 2007), the following sources of uncertainties have been considered and are summarised in Table 12.

Table 12: Qualitative evaluation of the influence of uncertainties on the dietary exposure estimate

Sources of uncertainties	Direction of impact[(a)]	
	Exposure to acetic acid from use as a decontamination treatment in pork meat	**Exposure to naturally occurring acetic acid**
Model input data		
Assumption that pork carcasses and cuts are only treated once with the decontaminating solution of 4% acetic acid	–	NA
Assumption that all pork consumed always contains residual acetic acid as a result of treatment with the 4% decontaminating solution	+	NA
Use of data from food consumption surveys of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+	+
Possible national differences in categorisation and classification of food	+/–	+/–
Model assumptions and factors		
Use of literature data on use of acetic acid on beef meat (i.e. Anderson et al., 1980) to calculate the exposure to acetic acid used to decontaminate pork meat	+/–	NA
Extrapolation from residual acetic acid from use of 3% acid to 4% acid	+/–	NA
Use of single concentration point of acetic acid in wine vinegar in the calculation of the dietary intake of acetic acid from consumption of vinegar	NA	+/–
Inclusion of only one product category as natural source for acetic acid (i.e. vinegar) excluding other natural dietary sources for acetic acid (e.g. ketchup, mustard, salad dressings, etc.)	NA	–
Volatile losses of acetic acid during storage or cooking are not taken into account	+/–	NA

(a): + means that the (real) exposure is possibly overestimated, – means that the (real) exposure is possibly underestimated.

In the applied exposure model, it was assumed that all pork carcasses and cuts were treated only once, whereas the applicant indicated that in some cases a repeat treatment may take place (e.g. treatment of a carcass followed by treatment of an individual cut), which may lead to an underestimation of exposure. It was further assumed that that all pork and pork products consumed always contained residual acetic acid as a result of treatment with the decontamination agent, which would lead to a considerable overestimation of exposure. The concentration data applied was extrapolated from data reported in the literature, and the influence of this uncertainty on the exposure assessment cannot be estimated.

Given these observations, the Panel considered overall that the uncertainties identified would, in general, result in an overestimation of the exposure to acetic acid from its use as a decontamination agent in European countries considered in the EFSA European database.

3.2.6. Toxicological assessment

No toxicological data were provided by the applicant.

In view that acetic acid is an authorised food additive that may be used in a variety of foods at *quantum satis* (Commission Regulation (EC) No 1333/2008 on food additives), that it is a normal component of the human diet and that it is an intermediate in the metabolism of carbohydrates and fatty acids, the Panel considered that no further information on toxicity was required.

Based on the above considerations, and noting that acetic acid intake from vinegar outweighs exposure from the intended uses (i.e. up to 10-fold), the Panel concluded that the use of acetic acid on pork carcasses and cuts is of no safety concern with respect to toxicity.

3.3. The efficacy of reducing pathogens on pork carcasses and pork cuts (ToR 2)

3.3.1. Introduction

As mentioned in Section 2.2.2.1 and in line with the EFSA guidance document (EFSA BIOHAZ Panel, 2010a), the use of lactic and acetic acid solutions as decontaminating agents will be regarded efficacious when a reduction of the prevalence and/or numbers of pathogenic target microorganisms set according to determined criteria, is statistically significant when compared to a control group. The achieved reduction should be expected to provide benefits to public health but the satisfactory level of this benefit is a risk management decision.

Efficacy has been previously demonstrated to depend on a range of factors, such as the concentration of the decontaminating agent, the microbial pathogen and its load on the surface, contact time, temperature, mode of application (i.e. spraying or dipping) and other conditions of use, see for example EFSA BIOHAZ Panel (2011a) and EFSA BIOHAZ Panel and EFSA CEF Panel (2012).

When applying organic acids as dipping treatments, e.g. for decontamination of meat cuts, efficacy may be compromised by: (i) the dilution of concentration or 'quenching' of the organic acid activity in the treatment solution (e.g. due to pH increase, or entrapment of acid molecules) by the organic matter released from meat surfaces, which may enable bacterial survival in the treatment solution and (ii) the potential recontamination of treated surfaces with microbial populations accumulated in the treatment tank via consecutive meat immersions. Therefore, care should be taken to address these limitations, while applying organic acid decontamination, by proper equipment design, selection and monitoring of application conditions (e.g. dipping frequency, tank capacity, volumes, concentrations and temperature of decontamination solution and application of GHP).

3.3.2. Study selection and identification of relevant experiments

The studies on efficacy included records provided by the applicant which were complemented with studies on efficacy retrieved by searching two bibliographic databases for recent records (since 2015) and by prescreening the reference lists of three review papers for relevance (Belluco et al., 2015; Totton et al., 2016; Young et al., 2016).

A total of 227 records were screened at title/abstract level and further screened for eligibility based on the full text (part I), with 43 records being eligible. In total, 99 experiments were defined. Of these, 12 records and 19 experiments fulfilled the criteria for inclusion (see Figure 2 for PRISMA flow chart). Of these, 71 comparisons (reduction estimates) were derived. Considering the substance, lactic acid was included in 11 records, 16 experiments and 67 comparisons; acetic acid was included in 2 records, 3 experiments and 4 comparisons. A detailed analysis of the number of studies screened, and of the reasons for exclusion is reported in Annex 2.

The applicant provided 41 records that they considered as relevant (**group 1**), of which one was a duplicate (Frederick, 1993; Frederick et al., 1994) and excluded at full-text level step I. According to this assessment, 28 records did not include any eligible experiment because the studies described were outside the scope for which the applicant is seeking approval. More specifically, at least the concentration of the lactic or acetic acid solution used was outside the range applied for in seven records, i.e. 1% lactic acid solution was used for treatment of pork carcasses pre-chill in Prasai et al. (1992) while Fu et al. (1994) used either 1.5% lactic or acetic acid for treatment of pork carcasses pre-chill. In addition, 0.2 M, 0.2% and 1.25% lactic acid solution were used for treatment of pork meat cuts post-chill in Nissen et al. (2001), Woolthuis et al. (1984) and Wan et al. (2007), respectively. The concentration of the acetic acid solution used was 0.1 N for treatment of pork carcasses pre-chill in Biemuller et al. (1973) and 1% for treatment of pork meat cuts post-chill in Mendonca et al. (1989). Seven records were excluded because the duration of the treatment was greater than 30 s, i.e. 1 min in Choi et al. (2009), 30 min in DeGeer et al. (2016), 1, 3, 5, 7 min in Rahman et al. (2013), 120 s in van Netten et al. (1998), 1 min in Mabesa et al. (1986), 75 s in Castelo et al. (2001b) and 2 min in Tibru et al. (2009). One record dealing with pork carcasses pre-chill treated with lactic acid (Le Roux et al., 2008) was excluded because there was no proper control group used. Six records were excluded because they did not report on a change in numbers and/or the presence of any of the target bacteria under consideration (i.e. *Salmonella* spp., *Campylobacter* spp., *Listeria* spp., STEC, *Yersinia* spp., *Aeromonas* spp., *Staphylococcus* spp., Enterobacteriaceae, coliforms, *E. coli*) on the treated carcass/meat, two dealing with pork carcasses pre-chill treated with lactic acid (Pipek et al.,

2004, 2006), one dealing with pork meat cuts post-chill treated with lactic acid (Shrestha and Min, 2006), two dealing with pork meat cuts post-chill treated with acetic acid (Cacciarelli et al., 1983; Lin and Chuang, 2001) and one dealing with pork meat cuts post-chill treated with either lactic or acetic acid (Anthappan et al., unspecified). Another seven records were excluded because either the temperature of the lactic acid or acetic acid solution and/or the duration of treatment were not reported (Snijders et al., 1985; Epling et al., 1993; Clayton, 2002; Eggenberger-Solorzano et al., 2002; Fabrizio and Cutter, 2004; Reynolds, 2005; Dan et al., 2007).

The 21 records considered potentially relevant by the applicant (**group 2**) were either excluded at the title/abstract screening or at the full text part I screening, and thus did not result in an eligible experiment. This was also the case for four out of five unique records identified through hand-searching the reference list of recent review papers (**group 3**). One record described an experiment in which pork meat cuts were treated post-chill with lactic acid, but the concentration of the lactic acid solution used was outside the range applied for (Morild et al., 2011). This reinforces that the comprehensiveness of the evidence provided by the applicant was appropriate.

Of the 160 recent records identified through literature search (**group 4**), two records were assessed for eligibility Part II. One record was excluded because the duration of the treatment was 90 s, and thus above 30 s, for the treatment of pork meat cuts post-chill (Dan et al., 2017a). The other record was excluded because both the temperature of the lactic acid and acetic acid solutions used and the duration of treatment were not reported (Dan et al., 2017b).

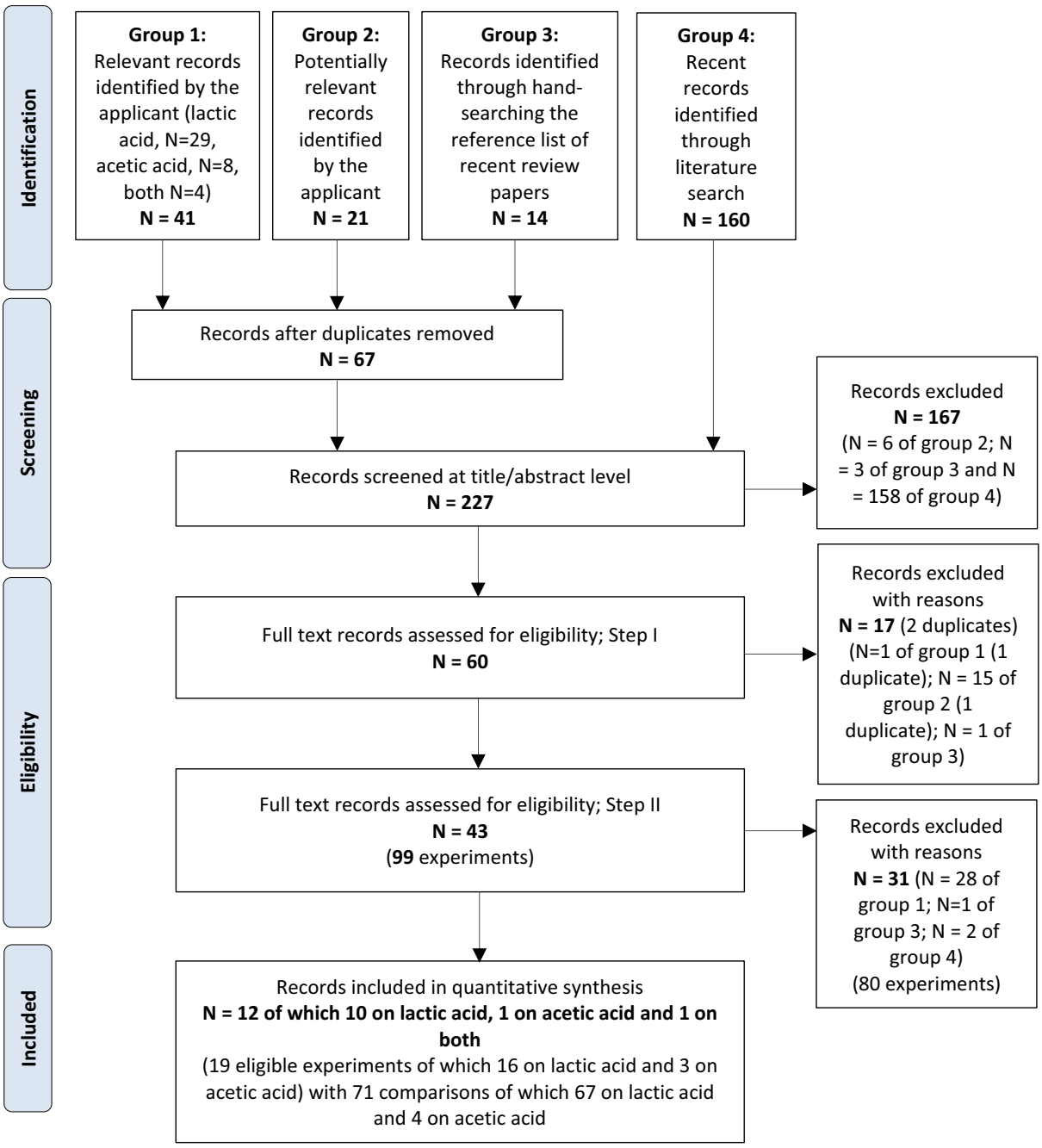


Figure 2: PRISMA flow chart (adapted from Moher et al., 2010)

3.3.3. Description of the eligible experiments

Finally, 19 eligible experiments from 12 records were considered eligible. All of these experiments came from group 1, i.e. relevant records identified by the applicant. A summary of the number of experiments by decontamination substance and product treated is given in Table 13. It also considers the method of application and the strength of evidence of the experiment.

An overview of the eligible experiments including a description, (experimental setting, type of contamination and application method), is provided in Table 14. A further description of the various outcomes in the experiments considering the treatment characteristics (concentration and temperature of the decontamination solution, duration of treatment and pressure of the application) and the bacterial group, is given in Tables 15–18.

Table 13: Number of eligible experiments by product category and application method for each substance

Product category	Decontamination substance with way of application				
	Lactic acid by spraying	Lactic acid by dipping	Acetic acid by spraying	Acetic acid by dipping	
Pork carcass pre-chill	7 (4/2/1)[(a)]	NA[(b)]	1 (1/0/0)	NA[(b)]	8 (5/2/1)
Pork meat cuts post-chill	7 (1/4/2)	2 (0/0/2)	2 (0/1/1)	0	11 (1/5/5)
	14 (5/6/3)	2 (0/0/2)	3 (1/1/1)	0	19 (6/7/6)

(a): In brackets the number of high/medium/low strength of evidence experiments as defined in Table 3.
(b): NA: not applicable.

Table 14: Overview of the 19 eligible experiments in alphabetical order of the authors (record)

Record	Experiment no/total no	Ref_ ID	Experiment description	Experimental setting[a]	Type of contamination[b]	Strength of evidence[c]	Decontamination substance[d]	Application method[e]	Product category	Product subcategory
Brustolin et al. (2014)	1/6	1	Preliminary: To examine the effect of water pressure (2, 3, 4 bars) and lactic acid concentrations (0%, 1%, 2%) on mesophilic and Enterobacteriaceae counts of uncontaminated pig carcasses	Ind	Nat	High	LA	Spray	Pork carcasses pre-chill	
Brustolin et al. (2014)	2/6	240	Preliminary: To examine the effect of water pressure (2, 3, 4 bars) and lactic acid concentrations (0%, 1%, 2%) on mesophilic and Enterobacteriaceae counts of faecally (intentionally) contaminated pig carcasses	Ind	Nat	High	LA	Spray	Pork carcasses pre-chill	

Record	Experiment no/total no	Ref_ ID	Experiment description	Experimental setting[a]	Type of contamination[b]	Strength of evidence[c]	Decontamination substance[d]	Application method[e]	Product category	Product subcategory
(Carpenter et al., 2011)	1/2	2	To examine the effect of water (control) and lactic acid (2%) single spray treatments (20s, 20 psi, 55.4°C) on the immediate reduction and growth of *Salmonella*, during storage at 8°C of vacuum packaged pork bellies	PNRI	Art	Medium	LA	Spray	Pork meat cuts post-chill	Pork bellies
(Castelo et al., 2001a)	1/3	3	Optimisation experiments: To examine the effect of application time (0–120 s) of lactic acid spray (2%), hot water and hot air on total coliform counts on lean pork trims	PRI	Nat	High	LA	Spray	Pork meat cuts post-chill	Lean pork trims

Record	Experiment no/total no	Ref_ ID	Experiment description	Experimental setting[a]	Type of contamination[b]	Strength of evidence[c]	Decontamination substance[d]	Application method[e]	Product category	Product subcategory
Christiansen et al. (2009)	1/2	32	To evaluate the microbial reductions (*Escherichia coli*, *Salmonella* Typhimurium and *Yersinia enterocolitica*) achieved with hot (80°C) water and 1% and 2.5% lactic acid (55°C and 80°C) applied for 0, 5 and 15 s on the skin surface of pork jowl	Lab	Art	Low	LA	Spray[f]	Pork carcasses pre-chill	Fresh pork jowls before chilling
Christiansen et al. (2009)	2/2	301	To evaluate the microbial reductions (*Escherichia coli*, *Salmonella* Typhimurium and *Yersinia enterocolitica*) achieved with hot (80°C) water and 1% and 2.5% lactic acid (55°C and 80°C) applied for 0, 5 and 15 s on the meat surface of pork jowl	PRI	Art	Medium	LA	Spray[f]	Pork carcasses pre-chill	Fresh pork jowls immediately before chilling

Record	Experiment no/total no	Ref_ ID	Experiment description	Experimental setting[a]	Type of contamination[b]	Strength of evidence[c]	Decontamination substance[d]	Application method[e]	Product category	Product subcategory
Frederick et al. (1994)	3/3	294	Phase II: To determine the effect of acetic acid (2%) and temperature on *Salmonella*, total viable counts and total coliform counts on pork cheek meat at industrial scale	Ind	Nat	High	AA	Spray	Pork carcasses pre-chill	Pork cheek meat
Greer and Dilts (1995)	1/2	9	To examine the effect of immersion in 3% lactic acid (15 s, 55°C) vs. water (as control) on the immediate reduction and growth of *Pseudomonas fragi*, *Brochothrix thermosphacta*, *Listeria monocytogenes*, *Yersinia enterocolitica* and *Aeromonas hydrophila* during aerobic storage of pork fat discs at 4°C for 15 days	Lab	Art	Low	LA	Dip	Pork meat cuts post-chill	Pork fat discs

Record	Experiment no/total no	Ref_ ID	Experiment description	Experimental setting[a]	Type of contamination[b]	Strength of evidence[c]	Decontamination substance[d]	Application method[e]	Product category	Product subcategory
Greer and Dilts (1995)	2/2	278	To examine the effect of immersion in 3% lactic acid (15 s, 55°C) vs. water (as control) on the immediate reduction and growth of *Pseudomonas fragi*, *Brochothrix thermosphacta*, *Listeria monocytogenes*, *Yersinia enterocolitica* and *Aeromonas hydrophila* during aerobic storage of pork lean discs at 4°C for 15 days	Lab	Art	Low	LA	Dip	Pork meat cuts post-chill	Pork lean discs
Kang et al. (2003)	1/4	10	To examine the effect of lactic acid (0.5%, 1%, 1.5%, 2%) hand spray (15 s, 30°C) vs water (control) on the immediate reduction and changes of total plate counts and coliforms of fresh pork loins during aerobic storage at 4°C for 14 days	Lab	Nat	Medium	LA	Spray	Pork meat cuts post-chill	Pork loins

Record	Experiment no/total no	Ref_ ID	Experiment description	Experimental setting[a]	Type of contamination[b]	Strength of evidence[c]	Decontamination substance[d]	Application method[e]	Product category	Product subcategory
Kang et al. (2003)	2/4	279	To examine the effect of lactic acid (0.5%, 1%, 1.5%, 2%) hand spray (15 s, 30°C) vs water (control) on the immediate reduction and changes of *Salmonella* Typhimurium population on fresh pork loins during aerobic storage at 4°C for 24 h	Lab	Art	Low	LA	Spray	Pork meat cuts post-chill	Pork loins
Kang et al. (2003)	3/4	280	To examine the effect of acetic acid (0.5%, 1%, 1.5%, 2%) hand spray (15 s, 30°C) vs water (control) on the immediate reduction and changes of total plate counts and coliforms of fresh pork loins during aerobic storage at 4°C for 14 days	Lab	Nat	Medium	AA	Spray	Pork meat cuts post-chill	Pork loins

Record	Experiment no/total no	Ref_ ID	Experiment description	Experimental setting[a]	Type of contamination[b]	Strength of evidence[c]	Decontamination substance[d]	Application method[e]	Product category	Product subcategory
Kang et al. (2003)	4/4	281	To examine the effect of acetic acid (0.5%, 1%, 1.5%, 2%) hand spray (15 s, 30°C) vs water (control) on the immediate reduction and changes of *Salmonella* Typhimurium population on fresh pork loins during aerobic storage at 4°C for 24 h	Lab	Art	Low	AA	Spray	Pork meat cuts post-chill	Pork loins
King et al. (2012)	1/1	11	To examine the effect of lactic acid spray (2%, 10 s, 40–50°C) as 2nd treatment in sequence with water wash chilling (4°C) and freezing (-15°C) on the reduction of *Salmonella* Hadar, *Campylobacter coli* and *Yersinia enterocolitica* immediately after treatment and during frozen (−15°C) storage of pork variety meats (heart, stomach, liver and intestine)	PRI	Art	Medium	LA	Spray	Pork meat cuts post-chill	Pork variety meats[g]

Record	Experiment no/total no	Ref_ ID	Experiment description	Experimental setting[a]	Type of contamination[b]	Strength of evidence[c]	Decontamination substance[d]	Application method[e]	Product category	Product subcategory
Rodriguez et al. (2004)	1/1	36	To develop and validate (total viable counts, total coliforms *and E. coli*) a carcass sanitising spray system (lactic acid at 55°C for 20 s) for pork and beef slaughterhouses	PRI	Nat	High	LA	Spray	Pork carcasses pre-chill	Half carcasses
van Netten et al. (1994)	2/4	298	To examine the effect of lactic acid (1%, 1.5%, 2%) spray (for 15, 30, 90 sec) on the immediate reduction of Enterobacteriaceae in pork belly	Lab	Nat	Medium	LA	Spray	Pork meat cuts post-chill	Pork bellies
van Netten et al. (1994)	4/4	299	To examine the effect of 2% lactic acid spray (for 30, 90 sec) on the immediate reduction of *Campylobacter*, *Salmonella* and *Listeria* in artificially contaminated pork belly	Lab	Art	Low	LA	Spray	Pork meat cuts post-chill	Pork bellies

Record	Experiment no/total no	Ref_ ID	Experiment description	Experimental setting[a]	Type of contamination[b]	Strength of evidence[c]	Decontamination substance[d]	Application method[e]	Product category	Product subcategory
van Netten et al. (1995)	2/2	259	To examine the effect of lactic acid (2% or 5%) spray (55°C) on the immediate reduction of *Salmonella* in artificially contaminated pork carcasses	PRI	Art	Medium	LA	Spray	Pork carcasses pre-chill	Carcass
van Netten et al. (1997)	1/1	21	To examine the effect of lactic acid (1%, 2%, 5%) spray (55°C) on the immediate reduction and the change during storage at 4°C of aerobic plate counts, total gram negative counts, total gram positive counts, mesophilic Enterobacteriaceae, and *Lactobacillus* counts in pork carcasses	PRI	Nat	High	LA	Spray	Pork carcasses pre-chill	Split, dressed slaughter-warm pork carcass

(a): Ind: industrial scale; Lab: laboratory scale; PNRI: pilot-scale not representative of industrial process; PRI: pilot-scale representative of industrial process.
(b): Art: Artificial; Nat: Natural.
(c): High/medium/low strength of evidence as defined in Table 3.
(d): AA: acetic acid; LA: lactic acid.
(e): Dip: dipping; Spray: spraying.
(f): Pork samples were placed vertically and poured with the decontamination solution by a watering device in order to imitate in-line cabinet hot water carcass decontamination with water sheets.
(g): Livers, intestines, hearts and stomachs.

Table 15: Description of eligible experiments with the treatment characteristics for treatment of pork carcasses pre-chill with lactic acid by spraying

Record	Experiment no/no of experiments	Ref_ ID	Experimental setting[a]	Type of contamination[b]	Product subcategory	Outcome nb	Concen-tration	Temperature	Duration	Pressure[c]	Bacterial group
Brustolin et al. (2014)	1/6	1	Ind	Nat		OUTC_01	2%	22.5°C	15 s	2 bar	Enter-obacteriaceae
						OUTC_02	2%	22.5°C	15 s	4 bar	Enter-obacteriaceae
Brustolin et al. (2014)	2/6	240	Ind	Nat		OUTC_01	2%	22.5°C	15 s	2 bar	Enter-obacteriaceae
						OUTC_02	2%	22.5°C	15 s	4 bar	Enter-obacteriaceae
Christiansen et al. (2009)	1/2	32	Lab	Art	Fresh pork jowls before chilling	OUTC_01	2.5%	55°C	5 s	NP	*Salmonella* spp.
						OUTC_02	2.5%	55°C	5 s	NP	*Escherichia coli*
						OUTC_03	2.5%	55°C	5 s	NP	*Yersinia* spp.
						OUTC_04	2.5%	80°C	5 s	NP	*Salmonella* spp.
						OUTC_05	2.5%	80°C	5 s	NP	*Escherichia coli*
						OUTC_06	2.5%	80°C	5 s	NP	*Yersinia* spp.
						OUTC_07	2.5%	55°C	15 s	NP	*Salmonella* spp.
						OUTC_08	2.5%	55°C	15 s	NP	*Escherichia* coli
						OUTC_09	2.5%	55°C	15 s	NP	*Yersinia* spp.
						OUTC_10	2.5%	80°C	15 s	NP	*Salmonella* spp.
						OUTC_11	2.5%	80°C	15 s	NP	*Escherichia coli*
						OUTC_12	2.5%	80°C	15 s	NP	*Yersinia* spp.

Record	Experiment no/no of experiments	Ref_ ID	Experimental setting[a]	Type of contamination[b]	Product subcategory	Outcome nb	Concen-tration	Temperature	Duration	Pressure[c]	Bacterial group
Christiansen et al. (2009)	2/2	301	PRI	Art	Fresh pork jowls immediately before chilling	OUTC_01	2.5%	55°C	5 s	NP	*Salmonella* spp.
						OUTC_02	2.5%	55°C	5 s	NP	*Escherichia coli*
						OUTC_03	2.5%	55°C	5 s	NP	*Yersinia* spp.
						OUTC_04	2.5%	80°C	5 s	NP	*Salmonella* spp.
						OUTC_05	2.5%	80°C	5 s	NP	*Escherichia coli*
						OUTC_06	2.5%	80°C	5 s	NP	*Yersinia* spp.
						OUTC_07	2.5%	55°C	15 s	NP	*Salmonella* spp.
						OUTC_08	2.5%	55°C	15 s	NP	*Escherichia coli*
						OUTC_09	2.5%	55°C	15 s	NP	*Yersinia* spp.
						OUTC_10	2.5%	80°C	15 s	NP	*Salmonella* spp.
						OUTC_11	2.5%	80°C	15 s	NP	*Escherichia coli*
						OUTC_12	2.5%	80°C	15 s	NP	*Yersinia* spp.
Rodriguez et al. (2004)	1/1	36	PRI	Nat	Half carcasses	OUTC_01	2%	55°C	20 s	40 psi	Coliforms
						OUTC_02	2%	55°C	20 s	40 psi	*Escherichia coli*
						OUTC_03	2%	55°C	20 s	40 psi	Coliforms
						OUTC_04	2%	55°C	20 s	40 psi	*Escherichia coli*
						OUTC_05	2%	55°C	20 s	40 psi	Coliforms
						OUTC_06	2%	55°C	20 s	40 psi	*Escherichia coli*
						OUTC_07	2%	55°C	20 s	40 psi	Coliforms

Record	Experiment no/no of experiments	Ref_ ID	Experimental setting[a]	Type of contamination[b]	Product subcategory	Outcome nb	Concen-tration	Temperature	Duration	Pressure[c]	Bacterial group
						OUTC_08	2%	55°C	20 s	40 psi	*Escherichia coli*
van Netten et al. (1995)	2/2	259	PRI	Art	Carcass	OUTC_01	2%	55°C	30 s	NP	*Salmonella* spp.
						OUTC_02	5%	55°C	30 s	NP	*Salmonella* spp.
van Netten et al. (1997)	1/1	21	PRI	Nat	Split, dressed slaughter-warm pork carcass	OUTC_01	2%	55°C	30 s	NP	Enter-obacteriaceae
						OUTC_02	5%	55°C	30 s	NP	Enter-obacteriaceae

(a): Ind: industrial scale; Lab: laboratory scale; PRI: Pilot-scale representative of industrial process.
(b): Art: Artificial; Nat: Natural.
(c): NP: not provided.

Table 16: Description of eligible experiments with the treatment characteristics for treatment of pork meat cuts post-chill with lactic acid by spraying or dipping

Record	Experiment no/no of experiments	Ref_ ID	Experimental setting[a]	Type of contamination[b]	Application method[c]	Product sub-category	Outcome nb	Concen-tration	Temper-ature	Duration	Pressure[e]	Bacterial group
Carpenter et al. (2011)	1/2	2	PNRI	Art	Spray	Pork bellies	OUTC_01	2%	55.4°C	20 sec	20 psi	*Salmonella* spp.
Castelo et al. (2001a)	1/3	3	PRI	Nat	Spray	Lean pork trims	OUTC_01	2%	15°C	30 sec	35 lb/in^2	Coliforms
Greer and Dilts (1995)	1/2	9	Lab	Art	Dip	Pork fat discs	OUTC_01	3%	55°C	15 sec	NP	*Listeria* spp.
							OUTC_02	3%	55°C	15 sec	NP	*Yersinia* spp.
							OUTC_03	3%	55°C	15 sec	NP	*Aeromonas* spp.
Greer and Dilts (1995)	2/2	278	Lab	Art	Dip	Pork lean discs	OUTC_01	3%	55°C	15 sec	NP	*Listeria* spp.
							OUTC_02	3%	55°C	15 sec	NP	*Yersinia* spp.
							OUTC_03	3%	55°C	15 sec	NP	*Aeromonas* spp.
Kang et al. (2003)	1/4	10	Lab	Nat	Spray	Pork loins	OUTC_01	2%	30°C	15 sec	NP	Coliforms
Kang et al. (2003)	2/4	279	Lab	Art	Spray	Pork loins	OUTC_01	2%	30°C	15 sec	NP	*Salmonella* spp.
King et al. (2012)	1/1	11	PRI	Art	Spray	Pork variety meats[d]	OUTC_01	2%	45°C	10 sec	NP	*Salmonella* spp.
							OUTC_02	2%	45°C	10 sec	NP	*Yersinia* spp.
							OUTC_03	2%	45°C	10 sec	NP	*Campylobacter* spp.
van Netten et al. (1994)	2/4	298	Lab	Nat	Spray	Pork bellies	OUTC_01	2%	21°C	30 sec	NP	Enterobacteriaceae
							OUTC_02	2%	36°C	15 sec	NP	Enterobacteriaceae
							OUTC_03	2%	52°C	15 sec	NP	Enterobacteriaceae

Record	Experiment no/no of experiments	Ref_ ID	Experimental setting[a]	Type of contamination[b]	Application method[c]	Product sub-category	Outcome nb	Concen-tration	Temper-ature	Duration	Pressure[e]	Bacterial group
van Netten et al. (1994)	4/4	299	Lab	Art	Spray	Pork bellies	OUTC_01	2%	21°C	30 sec	NP	*Campylobacter* spp.
							OUTC_02	2%	21°C	30 sec	NP	*Salmonella* spp.
							OUTC_03	2%	21°C	30 sec	NP	*Listeria* spp.

(a): Ind: industrial scale; Lab: laboratory scale; PNRI: Pilot-scale not representative of industrial process; PRI: Pilot-scale representative of industrial process.
(b): Art: artificial; Nat: natural.
(c): Dip: dipping; Spray: spraying.
(d): Livers, intestines, hearts and stomachs.
(e): NP: not provided.

Table 17: Description of eligible experiments with the treatment characteristics for treatment of pork carcasses pre-chill with acetic acid by spraying

Record	Experiment no/no of experiments	Ref_ ID	Experimental setting[a]	Type of contamination[b]	Product subcategory	Outcome nb	Concentration	Temperature	Duration	Pressure[c]	Bacterial group
Frederick et al. (1994)	3/3	294	Ind	Nat	Pork cheek meat	OUTC_01	2%	25°C	5 sec	NP	*Salmonella* spp.
						OUTC_02	2%	25°C	5 sec	NP	Coliforms

(a): Ind: industrial scale.
(b): Nat: natural.
(c): NP: not provided.

Table 18: Description of eligible experiments with the treatment characteristics for treatment of pork meat cuts post-chill with acetic acid by spraying or dipping

Record	Experiment nb/nb of experiments	Ref_ ID	Experimental setting[(a)]	Type of contamination[(b)]	Application method[(c)]	Product subcategory	Outcome nb	Concen-tration	Tempe-rature	Duration	Pressure[(d)]	Bacterial group
Kang et al. (2003)	3/4	280	Lab	Nat	Spray	Pork loins	OUTC_01	2%	30°C	15 sec	NP	Coliforms
Kang et al. (2003)	4/4	281	Lab	Art	Spray	Pork loins	OUTC_01	2%	30°C	15 sec	NP	*Salmonella* spp.

(a): Lab: laboratory scale.
(b): Art: artificial; Nat: natural.
(c): Spray: spraying.
(d): NP: not provided.

3.3.4. Synthesis of the results of the eligible experiments

3.3.4.1. Outcome expressed as proportion of positive samples

In only two experiments was the efficacy to reduce the level of contamination of pathogens on carcasses and cuts from pork expressed as a proportion of positive samples remaining after the decontamination treatment was completed.

In the first experiment (Table 19), pork carcasses in an abattoir were artificially contaminated with *S.* Typhimurium in faecal suspensions at a level of 1.7 $\log_{10}$ CFU/cm^2 and then sprayed for 30 s with 2% (pH 2.3) or 5% (pH 1.9) lactic acid at 55°C. The inoculum was allowed to adhere to the meat surface for 20 min before spraying. All 15 pork carcasses sampled were *Salmonella* positive before treatment. When compared with water treated carcasses, the number of positive carcasses was reduced by 60% and 80% when using 2% and 5% lactic acid, respectively (Ref_ID 259).

In the second experiment (Table 20), pork cheek meat was sprayed with a commercial slaughter facility carcass wash with 2% acetic acid at 25°C and compared to control (non-treated) cheeks. The incidence of *Salmonella* decreased by 67% on acid-treated cheeks (from three to one out of ten samples being positive) (Ref_ID 294).

Table 19: Proportion of positive samples in eligible studies on pork carcasses pre-chill sprayed with lactic acid

Ref_ ID	Strength of evidence	Product sub-category	Outcome number	Concen-tration	Tempe-rature	Duration	Pressure	Bacterial group	Storage time	Storage tempera-ture	Appraisal score[d]	No of positive samples/no of samples tested		Relative prevalence reduction (%)[f]
												Treatment group	Control group	
259	Medium[a]	Carcass	OUTC_01	2%	55°C	30 s	NP[b]	*S.* Typhimurium	NA[c]	NA[c]	1/3/4/3	6/15	15/15[e]	**60**
			OUTC_02	5%	55°C	30 s	NP[b]	*S.* Typhimurium	NA[c]	NA[c]	1/3/4/3	3/15	15/15[e]	**80**

(a): Artificial contamination and pilot-scale representative of industrial process.
(b): NP: not provided.
(c): NA: not applicable.
(d): The appraisal score for the questions: Comparability of control and treated groups/Inoculation procedure of the target organism and coverage of meat surface with substance/Detection and enumeration method of the target microorganism/Statistical analysis and reproducibility.
(e): Water-treated control group.
(f): Relative prevalence reduction = $(P_{control}-P_{treated})/P_{control}$ with P = prevalence. This reduction is shown in bold when significance has been demonstrated.

Table 20: Proportion of positive samples in eligible studies on pork carcasses pre-chill sprayed with acetic acid

Ref_ ID	Strength of evidence	Product sub-category	Outcome number	Concen-tration	Tempe-rature	Duration	Pressure[b]	Bacterial group	Storage time	Storage tempera-ture	Appraisal score[d]	No of positive samples/no of samples tested		Relative prevalence reduction (%)[f]
												Treatment group	Control group	
294	High[a]	Pork cheek meat	OUTC_01	2%	25°C	5 s	NP[b]	*Salmonella* spp.	NA[c]	NA[c]	3/4/3/3	1/10	3/10[e]	67

(a): Natural contamination and industrial process.
(b): NP: not provided.
(c): NA: not applicable.
(d): The appraisal score for the questions: Comparability of control and treated groups/Inoculation procedure of the target organism and coverage of meat surface with substance/Detection and enumeration method of the target microorganism/Statistical analysis and reproducibility.
(e): Untreated control group.
(f): Relative prevalence reduction = $(P_{control} - P_{treated})/P_{control}$ with P = prevalence. This reduction is shown in bold when significance has been demonstrated.

3.3.4.2. Outcome expressed as log_{10} reductions

In Table 21, the number of comparisons by decontamination substance, product category, timing of sampling and bacterial group at different sampling times is provided giving an idea of the evidence available. Reduction estimates are expressed as log_{10} reductions, calculated as described in Section 2.2.2.3. As such, a positive reduction number over water controls, immediately or shortly after treatment, represents the reductions caused by lactic or acetic acid, additionally to the physical removal of contamination caused by water alone. Positive numbers at the end of storage suggest either that the observed differences between control and treated samples after treatment are also maintained during storage, or that the target organisms in treated samples grew slower and/or their levels were further reduced during storage compared to those on water controls. When negative reductions are reported, it is suggested that the reductions of the target organisms in the control (caused by water wash), immediately after treatment, were higher than those observed in organic acid treated samples.

The mean range of lactic and acetic acid efficacies (expressed as log_{10} reductions) for different conditions in each eligible experiment is shown in Tables 22–25 and Figures 3–6. The appraisal score (rating as green: 4; yellow: 3; orange: 2; red: 1; where green is the best rating) for each experiment and outcome can also be seen from these figures where the scoring from left to right is related to: (a) Comparability of control and treated groups, (b) Inoculation procedure of the target organism and coverage of the meat surface with the substance, (c) Detection and enumeration method of the target organism, and (d) Statistical analysis and reproducibility.

For lactic acid treatment of pork carcasses pre-chill, 40 comparisons were derived from 6 experiments. For lactic acid treatment of pork meat cuts post-chill, 27 comparisons (15 by application of spraying and 12 by application of dipping) were derived from 9 experiments. In total, 2 comparisons of treated carcasses and 10 of pork meat cuts post-chill refer to post-storage results of lactic acid efficacy. The remaining (majority) comparisons represent the efficacy assessed immediately or shortly after treatment. The two carcass-related comparisons present microbial reductions observed after storage at 4°C.

For acetic acid treatment, four comparisons were derived: one using pork carcasses pre-chill and three using pork meat cuts post-chill by spraying. One comparison of pork meat cuts post-chill was made after storage, the others referring to comparisons made immediately or shortly after treatment.

As expected, in general, the comparisons detailed below, between lactic or acetic acid treated samples and those left untreated, showed higher reductions than the comparisons between acid treated and water treated samples.

Microbial reductions, through spray application of lactic acid solutions on pork carcasses pre-chill, estimated immediately or shortly after treatment were as follows:

- Microbial reductions varied across different comparisons, in the ranges detailed below.
- Mean *Salmonella* reductions as compared to water control samples, based on medium and low strength of evidence experiments, ranged from 1.68 to 1.95 log_{10} units (Ref_ID 301) and 0.31 to 2.56 log_{10} units (Ref_ID 32), respectively. Mean *Salmonella* reductions as compared to untreated control samples, based on medium and low strength of evidence experiments, ranged from 1.71 to 1.84 log_{10} units (Ref_ID 301) and 1.52 to 2.34 log_{10} units (Ref_ID 32), respectively.
- Mean *Yersinia* reductions over water control samples, based on medium and low strength of evidence experiments, ranged from 1.3 to 1.39 log_{10} units (Ref_ID 301) and 0.31 to 2.64 log_{10} units (Ref_ID 32), respectively. Mean *Yersinia* reductions over untreated control samples, based on medium and low strength of evidence experiments, ranged from 1.65 to 1.77 log_{10} units (Ref_ID 301) and 1.63 to 2.72 log_{10} units (Ref_ID 32), respectively.
- Mean Enterobacteriaceae reductions over water control samples, based on high strength of evidence experiments were variable, ranging from 2.36 log_{10} units less than those achieved with water treatment (as in Ref_ID 1) to 1.82 log_{10} units higher than those obtained with water (Ref_ID 1, 21, 240).
- Mean coliforms reductions over untreated control samples, based on high strength of evidence experiments, ranged from 0.9 to 1.2 log_{10} units (Ref_ID 36).
- Mean reductions of *E. coli* over water control samples, based on medium and low strength of evidence experiments, ranged from 1.66 to 1.77 log_{10} units (Ref_ID 301) and −0.37 to 1.87 log_{10} units (Ref_ID 32), respectively. The reductions of *E. coli* over untreated samples, based on high, medium and low strength of evidence experiments ranged from 0.5 to 1 (Ref_ID 36),

2.33 to 2.4 (Ref_ID 301) and 2.56 to 3.37 $\log_{10}$ units, respectively (Ref_ID 32). Nonetheless, the 4 comparisons of Ref_ID 301 and 32, showing the highest magnitude of *E. coli* reductions over both untreated or water-treated samples are also associated with high uncertainty (Figure 3), depicted by large error bars and low appraisal scores for the criterion 4, the latter being associated with lack of or non-specified independent trials.
- There was no evidence available for *Campylobacter* spp., *Listeria* spp. STEC/VTEC, *Aeromonas* spp. and *Staphylococcus* spp.

Regarding the statistical significance of the 18 $\log_{10}$ reduction estimates through spray application of lactic acid solutions on pork carcasses pre-chill, immediately or shortly after treatment, compared to water treated control as shown in Table 22:

- Six $\log_{10}$ reduction estimates (33%) were statistically significant so providing evidence that lactic acid treatment was more efficacious than water treatment
- Nine $\log_{10}$ reduction estimates (50%) were not statistically significant, so it could not be shown that lactic acid treatment was more efficacious than water treatment
- Three comparisons (17%) suggests that the treatment with water was more efficacious than lactic acid treatment

In the same table, all 20 $\log_{10}$ reduction estimates (100%) on lactic acid spray treated pork carcasses were statistically significant compared to untreated control.

Mean microbial reductions of Enterobacteriaceae after a 10 days chilled storage of carcasses treated with 2% and 5% lactic acid at 55°C, over those of water-treated carcasses were 0.8 and 1.5 $\log_{10}$ units, which is of similar magnitude (i.e. 0.5 and 1.6 $\log_{10}$ units) to those reductions recorded immediately after treatment (Ref_ID 21).

Microbial reductions, through spray application of lactic acid solutions on pork meat cuts post-chill, estimated immediately or shortly after treatment, were as follows:

- Mean *Salmonella* reductions over water control samples, based on medium and low strength of evidence experiments, were 0.6 $\log_{10}$ units (Ref_ID 2, 11) and 0.6–1.44 $\log_{10}$ units (Ref_ID 279, 299), respectively.
- Mean *Campylobacter* reductions over water control samples, based on a low strength of evidence experiment, were 2.5 $\log_{10}$ units (Ref_ID 299).
- Similar reductions were obtained in *Listeria* counts with the lactic acid treatment and the water (control) treatment based on one low strength of evidence experiment (Ref_ID 299).
- Mean Enterobacteriaceae, coliforms and/or *E. coli* reductions as compared to water control samples, based on high (Ref_ID 3) and medium (Ref_ID 10, 298) strength of evidence experiments, ranged from little or no difference to up to 0.7 $\log_{10}$ unit reductions with the lactic acid treatment.
- No evidence is available for *Yersinia*, STEC/VTEC, *Aeromonas* spp. and *Staphylococcus* spp.

Regarding the statistical significance of the 11 $\log_{10}$ reduction estimates through spray application of pork meat cuts post-chill, immediately or shortly after treatment, compared to water treated control as shown in Table 23:

- Three $\log_{10}$ reduction estimates (27%) were statistically significant so providing evidence that lactic acid treatment was more efficacious than water treatment.
- Six $\log_{10}$ reduction estimates (55%) were not statistically significant, so it could not be shown that lactic acid treatment was more efficacious than water treatment.
- Two comparisons (18%) suggest that the treatment with water was more efficacious than lactic acid treatment.

The mean differences between $\log_{10}$ counts of coliforms on meat cuts treated with 2% lactic acid at 30°C and those on water treated cuts, after 14 days of chilled storage was 3.6 $\log_{10}$ units, which is higher than the 1.1 $\log_{10}$ unit reductions recorded after 24 h storage (Ref_ID 10). Some comparisons from one experiment were only available at the last point of storage (Ref_ID 11) giving rise to reductions of 0.4, 1 and 0.8 $\log_{10}$ units over water control samples in *S.* Hadar, *C. coli* and *Yersinia*, respectively.

Microbial reductions through dip application of lactic acid solutions on pork meat cuts post-chill estimated immediately or shortly after treatment were as follows:

- Mean *Listeria* reductions over water control samples, based on low strength of evidence experiments, ranged from 1.02 to 2.9 $\log_{10}$ units (Ref_ID 9, 278)

- Mean *Yersinia* reductions over water control samples, based on low strength of evidence experiments, ranged from 0.73 to 1.85 $\log_{10}$ units (Ref_ID 9, 278)
- Mean *Aeromonas* reductions over water control samples, based on low strength of evidence experiments, ranged from 3.42 to 4.01 $\log_{10}$ units (Ref_ID 9, 278)
- No evidence is available for *Salmonella*, *Campylobacter*, STEC/VTEC, *Staphylococcus* spp., Enterobacteriaceae, coliforms and/or *E. coli*

Regarding the statistical significance, all six $\log_{10}$ reduction estimates through dip application of lactic acid solutions on pork meat cuts post-chill, immediately or shortly after treatment, compared to water treated control were significant (Table 23) and thus provided evidence that lactic acid treatment was more efficacious than water treatment.

The differences between levels of *L. monocytogenes*, *Y. enterocolitica*, and *A. hydrophila* on lactic acid treated pork meat cuts after storage were higher than those observed immediately or shortly after treatment.

Microbial reductions, through spray application of acetic acid solutions on pork carcasses pre-chill were as follows:

- Enterobacteriaceae, coliforms and/or *E. coli* reductions over untreated control samples based on high strength of evidence studies, were estimated as 1.41 $\log_{10}$ units (Ref ID 294).
- No evidence is available for *Campylobacter* spp., *Listeria* spp., STEC/VTEC, *Yersinia* spp., *Aeromonas* spp., and *Staphylococcus* spp.

Microbial reductions through spray application of acetic acid solutions on pork meat cuts post-chill were as follows:

- Mean *Salmonella* reductions over water control samples, based on a low strength of evidence experiment, were 1.52 $\log_{10}$ units (Ref ID 281)
- For the Enterobacteriaceae, coliforms and/or *E. coli* group, reductions over water control samples, based on an experiment with medium strength of evidence using 2% acetic acid at 30°C, were estimated as 1.27 $\log_{10}$ units. Mean differences between $\log_{10}$ counts of control and treated samples after 14 days of chilled storage increased to 3.61 $\log_{10}$ units (Ref ID 280)
- No evidence is available for *Campylobacter* spp., *Listeria* spp., STEC/VTEC, *Yersinia* spp., *Aeromonas* spp. and *Staphylococcus* spp.

No evidence was available for microbial reductions through dip application of acetic acid solutions on pork meat cuts post-chill.

3.3.4.3. Uncertainty assessment

EFSA's Scientific Committee has developed a guidance document on how to characterise, document and explain all types of uncertainty arising in EFSA's scientific assessments. The document (EFSA Scientific Committee, 2018) provides a framework and principles for uncertainty analysis, with the flexibility for assessors to select different methods to suit the needs of each assessment. Attention was given to identifying sources of uncertainty and their impact on the outcome of the assessment.

An overview of the potential sources of uncertainty identified in the efficacy assessment and the impact that these uncertainties could have on the direction of the effect on prevalence reduction and $\log_{10}$ reductions is presented in Table 26.

3.3.4.4. Impact on public health

The impact of reducing *Salmonella* on pork carcasses on the reduction of human salmonellosis cases has been previously estimated using available Quantitative Microbiological Risk Assessments (QMRA). In 2010, the EFSA BIOHAZ Panel concluded that a reduction of 2 $\log_{10}$ units (99%) of *Salmonella* numbers on contaminated carcasses pre-chill would result in a more than 90% reduction of the number of human salmonellosis cases attributable to pig meat consumption in all EU MSs. A reduction of 1 $\log_{10}$ unit (90%) would result in a more than 80% reduction of human cases (EFSA BIOHAZ Panel, 2010b). This was supported by a QMRA model developed by Hill et al. (2010) to assess the impact of hypothetical reductions of slaughter-pig prevalence and the impact of control measures on the risk of human *Salmonella* infection. A key consideration during the QMRA development was the characterisation of the variability between EU MSs, and therefore, a generic MS model was developed that accounts for differences in pig production, slaughterhouse practices, and consumption patterns. Consumption of one of three product types (pork cuts, minced meat, and fermented ready-to-eat

sausages) was considered by Snary et al. (2016). Such type of information is not available for the other pathogens. In this opinion, variability in $\log_{10}$ reductions is not taken into account. According to Duarte et al. (2016), this variability in $\log_{10}$ reductions, together with the variation in initial carcass contamination and magnitude (i.e. the mean) of $\log_{10}$ reductions, have been shown to markedly affect the relative risk of salmonellosis as compared to non-application of decontamination treatment. In particular, the lower the achieved reduction, i.e. up to 2 $\log_{10}$ units the more relevant is to consider variation in the efficacy of the treatment and for such low magnitude of reductions, the higher the variation the lower the overall risk reduction (Duarte et al., 2016).

3.3.5. Concluding remarks

- The use of lactic and acetic acid solutions as decontaminating agents will be regarded efficacious when a reduction of the prevalence and/or numbers of pathogenic target microorganisms set according to determined criteria, is statistically significant when compared to a control group. The achieved reduction should be expected to provide benefits to public health but the satisfactory level of this benefit is a risk management decision.
- Twelve records were included in the efficacy assessment of both substances as decontaminating agents for pork carcasses pre-chill and pork meat cuts post-chill based on predefined eligibility criteria. These yielded 19 eligible experiments (16 for lactic acid and 3 for acetic acid) providing 71 comparisons or $\log_{10}$ reduction estimates (67 for lactic acid and 4 for acetic acid).
- The experiments used a wide range of experimental designs and thus differed in relation to products, settings, method of application, lactic and acetic acid concentration, use of controls, microorganisms studied, storage time after application, etc. All these parameters may have impacted the efficacy both within and between studies but the assessment did not attempt to differentiate efficacy based on potentially influencing factors.
- The statistical significance could be demonstrated for all comparisons over untreated control using lactic acid spraying of pork carcasses pre-chill.
- In 24/29 comparisons, lactic acid spraying of pork carcasses pre-chill or pork meat cuts post-chill was at least equally efficacious as water spraying, though it could deliver significantly higher mean $\log_{10}$ reductions in nine comparisons depending on the conditions of application. The range of the statistically significant additional mean $\log_{10}$ reductions reported for carcasses and cuts were 1.30–1.82 and 1.10–2.50 $\log_{10}$, respectively.
- Lactic acid dipping of pork meat cuts post-chill delivered significantly higher $\log_{10}$ reductions than dipping in water. The range of the statistically significant mean $\log_{10}$ reductions was 0.73–4.01 $\log_{10}$.
- In the experiments where evidence was available, both immediately after treatment and during storage, the reductions were at least maintained throughout the duration of the experiments under chill storage.
- The Panel could not conclude on the efficacy of acetic acid on pork carcasses pre-chill and/or pork meat cuts post-chill, considering that only three eligible experiments, which in addition were also characterised as of medium strength of evidence, were available.

Table 21: Number of comparisons (log_{10} reduction estimates) by decontamination substance, product category and bacterial group at different sampling times

Substance	Product category	Application	Timing of sampling	*Salmonella* spp.	*Campylobacter* spp.	*Listeria* spp.	*Yersinia* spp.	*Aeromonas* spp.	Enterobacteriaceae	Coliforms	*E. coli*
Lactic acid	Pork carcasses pre-chill	Spraying	Immediately after treatment	8	0	0	8	0	6	4	12
Lactic acid	Pork carcasses pre-chill	Spraying	First point during storage	0	0	0	0	0	0	0	0
Lactic acid	Pork carcasses pre-chill	Spraying	Last point during storage	0	0	0	0	0	2	0	0
Lactic acid	Pork meat cuts post-chill	Spraying	Immediately after treatment	1	1	1	0	0	3	2	0
Lactic acid	Pork meat cuts post-chill	Spraying	First point during storage	2	0	0	0	0	0	1	0
Lactic acid	Pork meat cuts post-chill	Spraying	Last point during storage	1	1	0	1	0	0	1	0
Lactic acid	Pork meat cuts post-chill	Dipping	Immediately after treatment	0	0	2	2	2	0	0	0
Lactic acid	Pork meat cuts post-chill	Dipping	First point during storage	0	0	0	0	0	0	0	0
Lactic acid	Pork meat cuts post-chill	Dipping	Last point during storage	0	0	2	2	2	0	0	0
Acetic acid	Pork carcasses pre-chill	Spraying	Immediately after treatment	0	0	0	0	0	0	1	0
Acetic acid	Pork carcasses pre-chill	Spraying	First point during storage	0	0	0	0	0	0	0	0
Acetic acid	Pork carcasses pre-chill	Spraying	Last point during storage	0	0	0	0	0	0	0	0
Acetic acid	Pork meat cuts post-chill	Spraying	Immediately after treatment	0	0	0	0	0	0	0	0

Substance	Product category	Application	Timing of sampling	*Salmonella* spp.	*Campylobacter* spp.	*Listeria* spp.	*Yersinia* spp.	*Aeromonas* spp.	Enterobacteriaceae	Coliforms	*E. coli*
Acetic acid	Pork meat cuts post-chill	Spraying	First point during storage	1	0	0	0	0	0	1	0
Acetic acid	Pork meat cuts post-chill	Spraying	Last point during storage	0	0	0	0	0	0	1	0
Acetic acid	Pork meat cuts post-chill	Dipping	Immediately after treatment	0	0	0	0	0	0	0	0
Acetic acid	Pork meat cuts post-chill	Dipping	First point during storage	0	0	0	0	0	0	0	0
Acetic acid	Pork meat cuts post-chill	Dipping	Last point during storage	0	0	0	0	0	0	0	0

Table 22: Comparisons (log_{10} reduction estimates) in eligible studies on pork carcasses pre-chill treated with lactic acid by spraying

Ref_ID	Bacterial group	Concentration	Temperature	Duration	Storage	Timing of sampling	Strength of evidence	Control group	Appraisal score[(a)]	Mean log_{10} reduction[(c)]	CI_low	CI_up
301	*S.* Typhimurium	2.5%	80°C	5 s	No	Immediately after treatment	M	Water	4/4/4/2	**1.68**	0.32	3.04
301	*S.* Typhimurium	2.5%	80°C	15 s	No	Immediately after treatment	M	Water	4/4/4/2	1.95	−1.35	5.25
32	*S.* Typhimurium	2.5%	80°C	5 s	No	Immediately after treatment	L	Water	4/4/4/2	0.31	−2.07	2.69
32	*S.* Typhimurium	2.5%	80°C	15 s	No	Immediately after treatment	L	Water	4/4/4/2	2.56	−0.53	5.65
301	*Yersinia* spp.	2.5%	80°C	5 s	No	Immediately after treatment	M	Water	4/4/4/2	**1.30**	0.01	2.59
301	*Yersinia* spp.	2.5%	80°C	15 s	No	Immediately after treatment	M	Water	4/4/4/2	**1.39**	0.30	2.48
32	*Yersinia* spp.	2.5%	80°C	5 s	No	Immediately after treatment	L	Water	4/4/4/2	0.31	−1.05	1.67
32	*Yersinia* spp.	2.5%	80°C	15 s	No	Immediately after treatment	L	Water	4/4/4/2	2.64	−0.79	6.07
1	Enterobacteriaceae	2%	22.5°C	15 s	No	Immediately after treatment	H	Water	4/4/3/3	−**2.35**		

Ref_ID	Bacterial group	Concentration	Temperature	Duration	Storage	Timing of sampling	Strength of evidence	Control group	Appraisal score[a]	Mean log_{10} reduction[c]	CI_low	CI_up
1	Enterobacteriaceae	2%	22.5°C	15 s	No	Immediately after treatment	H	Water	4/4/3/3	**−0.46**		
240	Enterobacteriaceae	2%	22.5°C	15 s	No	Immediately after treatment	H	Water	4/4/3/3	0.66		
240	Enterobacteriaceae	2%	22.5°C	15 s	No	Immediately after treatment	H	Water	4/4/3/3	**1.82**		
21	Enterobacteriaceae	2%	55°C	30 s	No	Immediately after treatment	H	Water	4/3/4/3	0.50	−0.19	1.19
21	Enterobacteriaceae	2%	55°C	30 s	Yes[b]	Last point during storage	H	Water	4/3/4/3	**0.80**	0.01	1.59
21	Enterobacteriaceae	5%	55°C	30 s	No	Immediately after treatment	H	Water	4/3/4/3	≥ 1.60		
21	Enterobacteriaceae	5%	55°C	30 s	Yes[b]	Last point during storage	H	Water	4/3/4/3	≥ 1.50		
301	*E coli*	2.5%	80°C	5 s	No	Immediately after treatment	M	Water	4/4/3/2	**1.77**	0.03	3.51
301	*E coli*	2.5%	80°C	15 s	No	Immediately after treatment	M	Water	4/4/3/2	**1.66**	0.35	2.97
32	*E coli*	2.5%	80°C	5 s	No	Immediately after treatment	L	Water	4/4/3/2	−0.37	−2.50	1.76
32	*E coli*	2.5%	80°C	15 s	No	Immediately after treatment	L	Water	4/4/3/2	1.87	−2.15	5.89
301	*S.* Typhimurium	2.5%	55°C	5 s	No	Immediately after treatment	M	Untreated	3/4/4/2	**1.71**	1.02	2.40
301	*S.* Typhimurium	2.5%	55°C	15 s	No	Immediately after treatment	M	Untreated	3/4/4/2	**1.84**	0.94	2.74
32	*S.* Typhimurium	2.5%	55°C	5 s	No	Immediately after treatment	L	Untreated	3/4/4/2	**1.52**	0.94	2.10
32	*S.* Typhimurium	2.5%	55°C	15 s	No	Immediately after treatment	L	Untreated	3/4/4/2	**2.34**	1.27	3.41
301	*Yersinia* spp.	2.5%	55°C	5 s	No	Immediately after treatment	M	Untreated	3/4/4/2	**1.65**	0.83	2.47
301	*Yersinia* spp.	2.5%	55°C	15 s	No	Immediately after treatment	M	Untreated	3/4/4/2	**1.77**	0.99	2.55

Ref_ID	Bacterial group	Concentration	Temperature	Duration	Storage	Timing of sampling	Strength of evidence	Control group	Appraisal score[(a)]	Mean log_{10} reduction[(c)]	CI_ low	CI_up
32	*Yersinia* spp.	2.5%	55°C	5 s	No	Immediately after treatment	L	Untreated	3/4/4/2	**1.63**	0.66	2.60
32	*Yersinia* spp.	2.5%	55°C	15 s	No	Immediately after treatment	L	Untreated	3/4/4/2	**2.72**	1.75	3.69
36	Coliforms	2%	55°C	20 s	No	Immediately after treatment	H	Untreated	3/4/3/3	≥ **0.90**	0.45	1.35
36	Coliforms	2%	55°C	20 s	No	Immediately after treatment	H	Untreated	3/4/3/3	≥ **1.00**	0.59	1.41
36	Coliforms	2%	55°C	20 s	No	Immediately after treatment	H	Untreated	3/4/3/3	≥ **1.00**	0.63	1.37
36	Coliforms	2%	55°C	20 s	No	Immediately after treatment	H	Untreated	3/4/3/3	≥ **1.20**	0.76	1.64
36	*E coli*	2%	55°C	20 s	No	Immediately after treatment	H	Untreated	3/4/3/3	**0.50**	0.17	0.83
36	*E coli*	2%	55°C	20 s	No	Immediately after treatment	H	Untreated	3/4/3/3	**0.70**	0.35	1.05
36	*E coli*	2%	55°C	20 s	No	Immediately after treatment	H	Untreated	3/4/3/3	**0.70**	0.34	1.06
36	*E coli*	2%	55°C	20 s	No	Immediately after treatment	H	Untreated	3/4/3/3	**1.00**	0.61	1.39
301	*E coli*	2.5%	55°C	5 s	No	Immediately after treatment	M	Untreated	3/4/3/2	**2.33**	1.26	3.40
301	*E coli*	2.5%	55°C	15 s	No	Immediately after treatment	M	Untreated	3/4/3/2	**2.40**	1.58	3.22
32	*E coli*	2.5%	55°C	5 s	No	Immediately after treatment	L	Untreated	3/4/3/2	**2.56**	1.74	3.38
32	*E coli*	2.5%	55°C	15 s	No	Immediately after treatment	L	Untreated	3/4/3/2	**3.37**	2.59	4.15

(a): The appraisal score for the questions: Comparability of control and treated groups/Inoculation procedure of the target organism and coverage of meat surface with substance/Detection and enumeration method of the target microorganism/Statistical analysis and reproducibility.
(b): Aerobic storage at 4°C for 10 days.
(c): The mean reduction is shown in bold when significance was demonstrated. With `≥' it is meant that the mean reduction will be equal or above the figure as the numbers were below the detection limit after treatment.

Table 23: Comparisons (log_{10} reduction estimates) in eligible studies on pork meat cuts post-chill treated with lactic acid by spraying or dipping

Ref_ID	Bacterial group	Application	Concen-tration	Tempe-rature	Duration	Storage	Timing of sampling	Strength of evidence	Control group	Appraisal score[a]	Mean log_{10} reduction[g]	CI_low	CI_up
2	*S.* Enteritidis	Spraying	2%	55.4°C	20 s	Yes[b]	First point during storage	M	Water	4/4/3/3	0.60		
299	*S.* Typhimurium	Spraying	2%	21°C	30 s	No	Immediately after treatment	L	Water	4/3/4/2	0.60	−0.82	2.02
279	*S.* Typhimurium	Spraying	2%	30°C	15 s	Yes[c]	First point during storage	L	Water	3/3/3/3	**1.44**		
11	*S.* Hadar	Spraying	2%	45°C	10 s	Yes[d]	Last point during storage	M	Water	3/4/3/4	**0.40**		
299	*C. jejuni*	Spraying	2%	21°C	30 s	No	Immediately after treatment	L	Water	4/3/4/2	**2.50**	1.08	3.92
11	*C. coli*	Spraying	2%	45°C	10 s	Yes[d]	Last point during storage	M	Water	3/4/4/4	**1.00**		
299	*L. monocytogenes*	Spraying	2%	21°C	30 s	No	Immediately after treatment	L	Water	4/3/4/2	0		
11	*Yersinia* spp.	Spraying	2%	45°C	10 s	Yes[d]	Last point during storage	M	Water	3/4/3/4	**0.80**		
298	Enterobacteriaceae	Spraying	2%	36°C	15 s	No	Immediately after treatment	M	Water	4/3/4/2	0.30	−0.86	1.46
298	Enterobacteriaceae	Spraying	2%	21°C	30 s	No	Immediately after treatment	M	Water	4/3/4/2	0.40	−0.76	1.56
298	Enterobacteriaceae	Spraying	2%	52°C	15 s	No	Immediately after treatment	M	Water	4/3/4/2	0.70	−0.46	1.86
3	Coliforms	Spraying	2%	15°C	30 s	No	Immediately after treatment	H	Water	1/4/3/3	−0.49	−0.95	−0.03
3	Coliforms	Spraying	2%	15°C	30 s	No	Immediately after treatment	H	Water	1/4/3/3	−0.56	−1.20	0.08
10	Coliforms	Spraying	2%	30°C	15 s	Yes[c]	First point during storage	M	Water	3/4/3/3	**1.10**		
10	Coliforms	Spraying	2%	30°C	15 s	Yes[f]	Last point during storage	M	Water	3/4/3/3	≥ **3.60**		
9	*L. monocytogenes*	Dipping	3%	55°C	15 s	No	Immediately after treatment	L	Water	4/4/3/2	**2.90**	1.96	3.84

Ref_ID	Bacterial group	Application	Concen-tration	Tempe-rature	Duration	Storage	Timing of sampling	Strength of evidence	Control group	Appraisal score[a]	Mean log_{10} reduction[g]	CI_low	CI_up
9	*L. monocytogenes*	Dipping	3%	55°C	15 s	Yes[e]	Last point during storage	L	Water	4/4/3/2	≥ **7.01**	6.65	7.37
278	*L. monocytogenes*	Dipping	3%	55°C	15 s	No	Immediately after treatment	L	Water	4/4/3/2	**1.02**	0.66	1.38
278	*L. monocytogenes*	Dipping	3%	55°C	15 s	Yes[e]	Last point during storage	L	Water	4/4/3/2	**1.64**	1.10	2.18
9	*Y. enterocolitica*	Dipping	3%	55°C	15 s	No	Immediately after treatment	L	Water	4/4/3/2	**1.85**	1.23	2.47
9	*Y. enterocolitica*	Dipping	3%	55°C	15 s	Yes[e]	Last point during storage	L	Water	4/4/3/2	≥ **6.97**	6.45	7.49
278	*Y. enterocolitica*	Dipping	3%	55°C	15 s	No	Immediately after treatment	L	Water	4/4/3/2	**0.73**	0.43	1.03
278	*Y. enterocolitica*	Dipping	3%	55°C	15 s	Yes[e]	Last point during storage	L	Water	4/4/3/2	**4.83**	3.78	5.88
9	*A. hydrophilia*	Dipping	3%	55°C	15 s	No	Immediately after treatment	L	Water	4/4/3/2	≥ **4.01**	3.73	4.29
9	*A. hydrophilia*	Dipping	3%	55°C	15 s	Yes[e]	Last point during storage	L	Water	4/4/3/2	≥ 3.41	−0.69	7.51
278	*A. hydrophilia*	Dipping	3%	55°C	15 s	No	Immediately after treatment	L	Water	4/4/3/2	≥ **3.42**	2.54	4.30
278	*A. hydrophilia*	Dipping	3%	55°C	15 s	Yes[e]	Last point during storage	L	Water	4/4/3/2	≥ 0.16	−0.73	1.05

(a): The appraisal score for the questions: Comparability of control and treated groups/Inoculation procedure of the target organism and coverage of meat surface with substance/Detection and enumeration method of the target microorganism/Statistical analysis and reproducibility.
(b): Vacuum packed storage at 4°C for 10 days.
(c): Aerobic storage at 4°C for 10 days.
(d): Packed/bagged storage at −15°C.
(e): Aerobic storage at 4°C for 15 days.
(f): Aerobic storage at 4°C for 14 days.
(g): The mean reduction is shown in bold when significance was demonstrated. With `≥' it is meant that the mean reduction will be equal or above the figure as the numbers were below the detection limit after treatment.

Table 24: Comparisons (log_{10} reduction estimates) in eligible studies on pork carcasses pre-chill treated with acetic acid by spraying

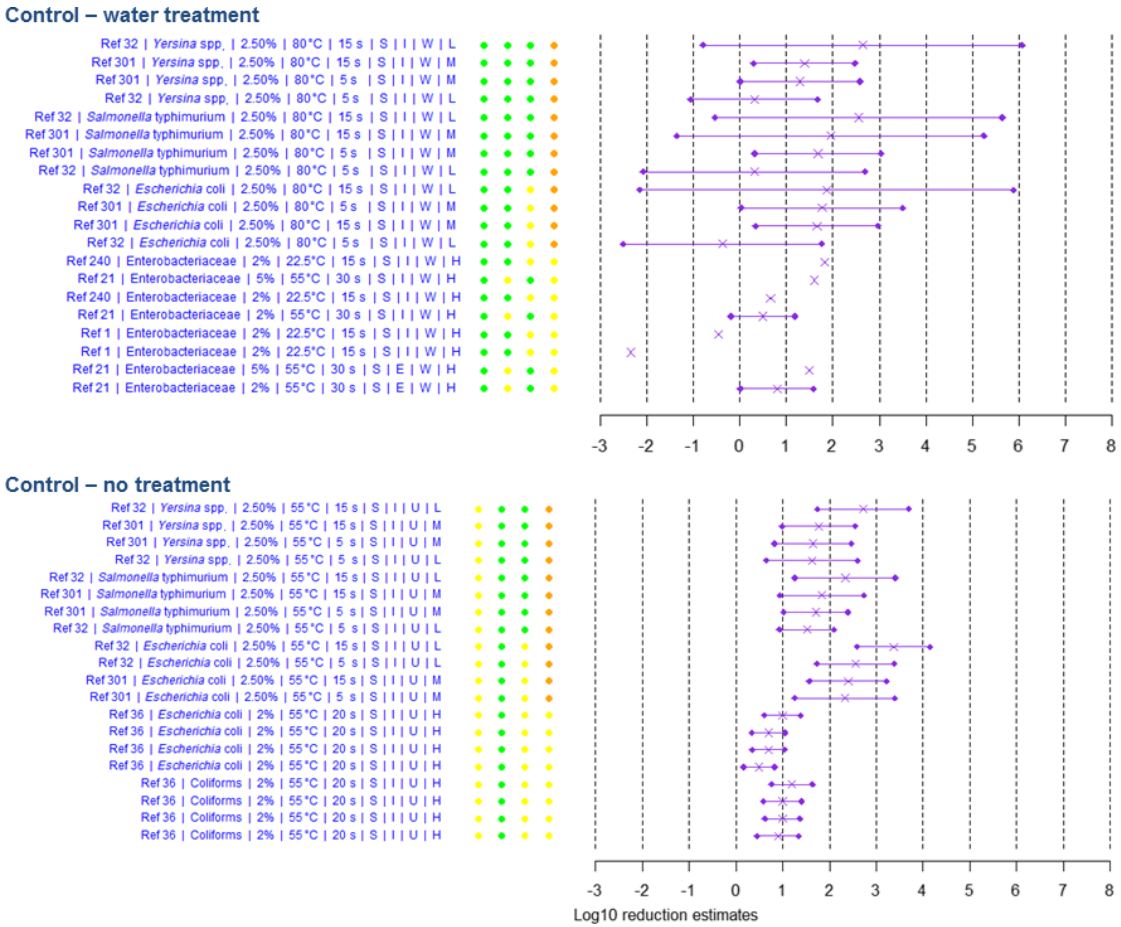
Ref_ID	Bacterial group	Concentration	Temperature	Duration	Storage	Timing of sampling	Strength of evidence	Control group	Appraisal score[a]	Mean log_{10} reduction[b]	CI_low	CI_up
294	Coliforms	2%	25°C	5 s	No	Immediately after treatment	H	Untreated	3/4/3/3	**1.41**		

(a): The appraisal score for the questions: Comparability of control and treated groups/Inoculation procedure of the target organism and coverage of meat surface with substance/Detection and enumeration method of the target microorganism/Statistical analysis and reproducibility.
(b): The mean reduction is shown in bold when significance was demonstrated.

Table 25: Comparisons (log_{10} reduction estimates) in eligible studies on pork meat cuts post-chill treated with acetic acid by spraying or dipping

Ref_ID	Bacterial group	Application	Concentration	Tempe-rature	Duration	Storage	Timing of sampling	Strength of evidence	Control group	Appraisal score[a]	Mean log_{10} reduction[d]	CI_low	CI_up
281	*S.* Typhimurium	Spraying	2%	30°C	15 s	Yes[b]	First point during storage	L	Water	3/3/3/3	**1.52**		
280	Coliforms	Spraying	2%	30°C	15 s	Yes[b]	First point during storage	M	Water	3/4/3/3	1.27		
280	Coliforms	Spraying	2%	30°C	15 s	Yes[c]	Last point during storage	M	Water	3/4/3/3	≥ **3.61**		

(a): The appraisal score for the questions: Comparability of control and treated groups/Inoculation procedure of the target organism and coverage of meat surface with substance/Detection and enumeration method of the target microorganism/Statistical analysis and reproducibility.
(b): Aerobic storage at 4°C for 24 h.
(c): Aerobic storage at 4°C for 14 days.
(d): The mean reduction is shown in bold when significance was demonstrated. With `≥' it is meant that the mean reduction will be equal or above the figure as the numbers were below the detection limit after treatment.



The reduction is expressed as $\log_{10}$ reduction, i.e. the difference between the means of the $\log_{10}$ concentrations of control group and treated group (×) and corresponding 95% confidence interval (95% CI) (•—•) when this information was available. The following parameters are included: the reference identification (Ref_ID), the bacterial (sub)group, the concentration of the decontamination solution (either lactic or acetic acid), the temperature of the decontamination substance, the duration of treatment, the type of application (S Spraying, D Dipping), the sampling time (I immediately after treatment, F first point during storage (when immediately after treatment not available), E last point during storage), the type of control treatment (W: water, top graph, U: untreated, bottom graph), the strength of evidence (H high, M medium, L low) and coloured bullets reflect the appraisal score (rating as green: 4; yellow: 3; orange: 2; red: 1) for the questions from left to right being: (a) Comparability of control and treated groups, (b) Inoculation procedure of the target organism and coverage of meat surface with the substance, (c) Detection and enumeration method of the target organism, and (d) Statistical analysis and reproducibility.

Figure 3: Log_{10} reduction estimates of bacterial groups when applying lactic acid to pork carcasses pre-chill by spraying

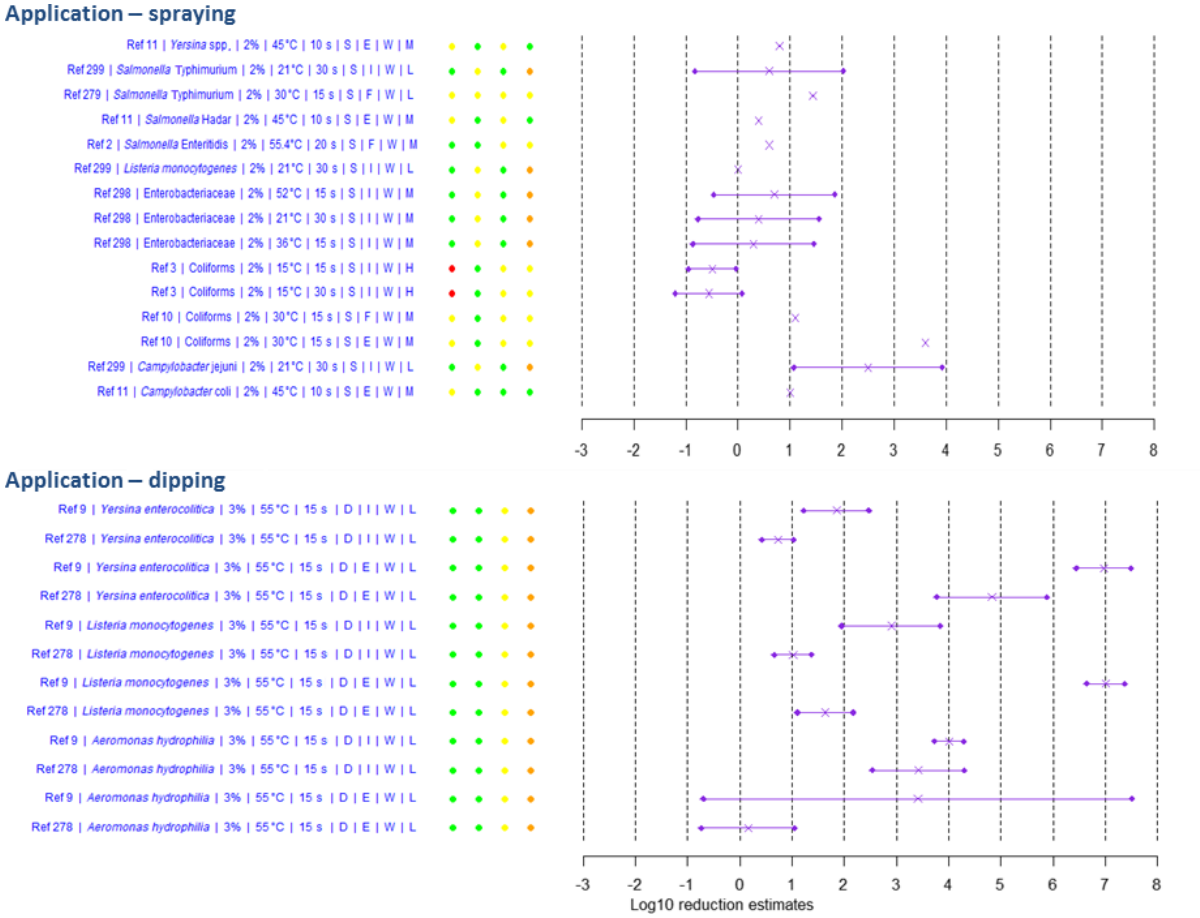


Figure 4: Log_{10} reduction estimates of bacterial groups when applying lactic acid to meat cuts post-chill by spraying (top graph) or dipping (bottom graph)

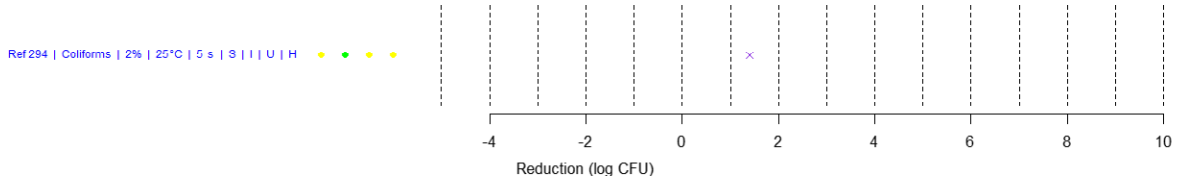


Figure 5: Log_{10} reduction estimates of bacterial groups when applying acetic acid to pork carcasses pre-chill by spraying

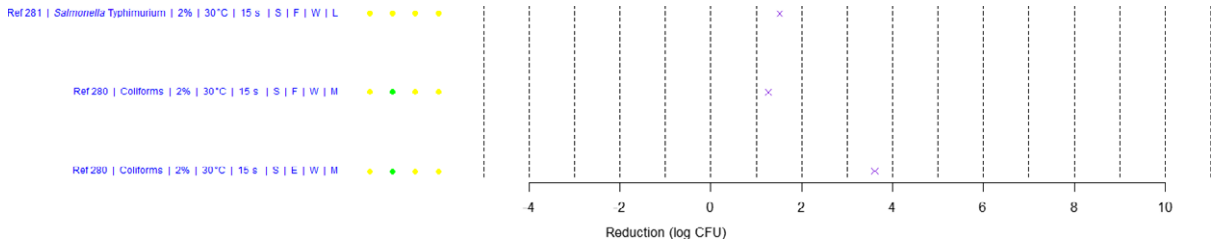


Figure 6: Log_{10} reduction estimates of bacterial groups when applying acetic acid to pork meat cuts post-chill by spraying or dipping

Table 26: Potential sources of uncertainty identified in the efficacy assessment and the impact that these uncertainties could have on the direction of the effect on log_{10} reductions and prevalence reduction

Source of uncertainty	How uncertainty has been addressed	Direction of the effect on log_{10} reduction and prevalence reduction −/+[(a)]
The application parameters including the concentration or temperature of the decontamination solution, duration of treatment, etc., are variable or not known	Studies not specifying application parameters or providing non eligible values based on specified conditions of applications were excluded from the assessment	+/−
The use of artificially (inoculated) samples	The strength of evidence has been assessed for each experiment based on study type (see Table 3)	+
Experiment conducted at laboratory scale that may overestimate observed reductions. For instance, the solution was freshly prepared and applied more accurately on the target surface than in a real commercial situation. Also, pathogens are more loosely attached on meat surfaces than in natural settings, where they may reside in various depths form the surface and/or sheltered by carcass surface roughness, within protective niches (e.g. topped by fat layers). The bacterial cell counts applied in experimental studies are much higher than naturally contaminated carcasses or meat cuts	The strength of evidence has been assessed for each experiment based on study type (see Table 3) and the appraisal score of AQ 2 (see below)	+
Experiment conducted at pilot-scale that is not representative of the industrial process	The strength of evidence has been assessed for each experiment based on study type (see Table 3)	+
The control group was left untreated	This was considered in the reliability of the experiment (AQ1) and, in addition, it was clearly indicated when presenting the outcomes that the control was left untreated	+
The treated and control group do not only differ in the presence or absence of the decontaminating substance but also in the method of application (e.g. duration or pressure) or other factors (e.g. procedure for inoculation with the target organism, temperature and conditions of storage, detection and/or enumeration method)	This was considered in the reliability of the experiment (AQ1) and the appraisal score is shown when presenting the outcomes	−/+
For artificial contamination: The inoculum was not evenly distributed over the meat surface	This was considered in the reliability of the experiment (AQ2) and the appraisal score is shown when presenting the outcomes	++/−

Source of uncertainty	How uncertainty has been addressed	Direction of the effect on log_{10} reduction and prevalence reduction −/+[(a)]
For artificial contamination: the time between inoculation of the target organism and treatment with the substance was not sufficient to allow attachment of the bacteria (e.g. at least 15 min)	This was considered in the reliability of the experiment (AQ2) and the appraisal score is shown when presenting the outcomes	+
The substance was not evenly distributed over the meat surface	This was considered in the reliability of the experiment (AQ2) and the appraisal score is shown when presenting the outcomes	−
The detection and enumeration method of the target organism does not maximise the recovery of the bacteria	This was considered in the reliability of the experiment (AQ3) and the appraisal score is shown when presenting the outcomes	+
The use of different carcass/meat cut sampling methods (excision, versus swab)	This was considered in the reliability of the experiment (AQ3) and the appraisal score is shown when presenting the outcomes	+/−
The method and/or the number of independent trials and representative samples (replicates) is not specified	This was considered in the reliability of the experiment (AQ4). The appraisal score and the CI of the estimate are shown when presenting the outcomes	++/−
Statistical significance of the reduction of prevalence or concentration is not specified, and in some cases it cannot be estimated from the reported information	This was considered in the reliability of the experiment (AQ4). The appraisal score and the calculated CI of the estimate are shown when presenting the outcomes	++/−

(a): + means that the (real) outcome/effect is possibly overestimated, − means that the (real) outcome/effect is possibly underestimated.

3.4. The potential emergence of reduced susceptibility to biocides and/or resistance to therapeutic antimicrobials linked to the use of lactic and acetic acids (ToR 3)

3.4.1. Information provided by the applicant

3.4.1.1. Promotion of resistance to therapeutic antibiotics

In the technical dossier (see Documentation provided to EFSA n. 1) the applicant reported: 'The evaluation that use of lactic or acetic acids is not a threat to the development of resistance to therapeutic antibiotics when used on pork is based upon the common presence of these substances in human diet, animal and plant tissues, and the environment in general. These two common food acids have been used for preservation of foods for millennia and for the entire history of therapeutic antibiotics. If they could have led to bacteria developing resistance to antibiotics, it would have already taken place. We believe the prior EFSA opinion on the matter is sufficient on this matter.

As summarized by EFSA BIOHAZ Panel (2011b) the antimicrobial activity of organic acids is based upon their ability to pass through cell membranes then to dissociate inside the cell, decreasing the intracellular pH, thereby causing a general disruption of cellular metabolic functions. Further, the organic acids appear to disrupt the cellular membrane of bacteria. This general disruption of cellular function is unlike the very specific modes of action of therapeutic antibiotics in blocking a particular metabolic pathway. Concerning lactic acid use on beef, EFSA BIOHAZ Panel (2011a) concluded that the possibility of mutational change resulting in development of resistance to therapeutic antimicrobials is unlikely to be a significant issue. With the natural occurrence of acetic acid, and its long use in foods and industry, the situation is similar for acetic acid. For either acid, their use as a wash step is extremely unlikely to provide any new selective pressures to create novel metabolic pathways in bacteria, much less a mechanism to confer resistance to an antibiotic'.

3.4.1.2. Development of bacterial resistance to the disinfectant action of organic acids

The applicant also reported: 'Exposure of bacteria to sub-lethal concentrations of acid through multiple generations can select for acid-adapted strains, raise their minimum inhibitory concentration, and reduce the adapted bacteria's sensitivity to an acid disinfectant (e.g. discussion in Mani-Lopez et al., 2012). van Netten et al. (1998) studied this effect for lactic acid. They found that the reduction in populations of acid-adapted strains of *Salmonella*, *Staphylococcus*, and *E. coli* O157:H7 was measurably less upon exposure to 2% lactic acid than non-adapted strains, though still significantly susceptible to the lactic acid.

It is unlikely that acid-adapted strains of bacteria would accompany pigs as they arrive at slaughter. However, the continued use of an organic acid in a slaughterhouse or fabrication facility could provide opportunity for acid-adapted strains to develop and re-contaminate meat if proper facility sanitation is not observed. Plant sanitation should encompass different disinfectants than the organic acids, and systematically alternate cleaning disinfectants so as to prevent the survival of bacterial colonies adapted to the disinfectants'.

As, in the technical dossier, in relation to the reduced susceptibility to biocides, few references have been provided, EFSA asked for additional information, such as experimental studies, and considering the pre-market and post-market evaluation and requested 'to undertake tests on a statistically-significant selection (if possible > 50) of isolates of a test organism such as *E. coli* following pre- and post-exposure to lactic acid to ensure that there are no changes in the inherent susceptibility of such isolates to the compound after exposure, under the conditions used by the applicant'.

The applicant informed that they are 'aware of no studies which monitor for changes in minimum inhibitory concentration of bacteria after a commercial treatment with an organic acid. Nor are we aware of any study which studied minimum inhibitory concentration (MIC) in bacteria in a single short-term exposure on surfaces which would simulate the commercial environment. As both lactic acid and acetic acid are commonly found in foods, bacteria would naturally have exposure to these and ample opportunity to develop resistance to these'.

3.4.2. Evaluation on the information provided

Generally, in some foods, the levels of the two organic acids are higher than those found on the carcasses/meat cuts after treatment. Nonetheless, treatment of carcasses/cuts may temporarily increase the levels of lactic and acetic acid on the meat surface (as estimated in Sections 3.1.6 and

3.2.5) compared to those naturally present in these products, or in the environment of the abattoir. A second lactic or acetic acid treatment of individual cuts from a carcass that had received a prior organic acid treatment may further contribute to the increased in organic acid levels on the treated surfaces. The potential of this situation for induction of acid adaptation and/or selection of strains that are less susceptible to the organic acids used are detailed in the next paragraphs, based on available studies.

The primary concerns about using organic acids as carcass or meat cut treatments are: (1) the emergence and/or selection of acid resistant strains with potentially reduced susceptibility to lactic or acetic acid, which may compromise the efficacy of the corresponding decontamination treatments and (2) promoting and/or enhancing antibiotic or biocide (e.g. cleaning agents) resistance in bacteria.

3.4.2.1. Emergence and/or selection of strains with reduced susceptibility to lactic or acetic acid

There is considerable evidence supporting the hypothesis that exposure to mild acidic conditions induces acid resistance in bacteria (van Netten et al., 1998; Uljas and Ingham, 1998; Berry and Cutter, 2000). Unintentional contact of water and acidic carcass run-off fluids in meat plants, e.g. due to improper GHP, may result in the formation of mildly acidic environmental conditions on the carcasses and in the abattoir environment. Pathogens residing on carcass surfaces and/or the meat processing environment, exposed (repetitively) to these sublethal acid stress conditions may become acid adapted, and thus, increase their tolerance to acid, or be selected for inherent reduced susceptibility to acid (Samelis et al., 2001, 2002; Samelis and Sofos, 2003). Also, as stated above, consecutive treatments of individual cuts from carcasses that had received a prior organic acid treatment may further contribute to the increased exposure of pathogens to the two acids. Studies by Skandamis et al. (2007) demonstrated that *E. coli* O157:H7 cells, for example, exposed to lactic acid washing solutions were able to grow at lower pH than non-exposed cells, i.e. cells that were not exposed to carcass run-off fluids of sublethal lactic-acid-based acidity. Most human enteric pathogens, such as pathogenic *E. coli* and *Salmonella* spp. have the capacity to adapt to acidic conditions. This involves a combination of constitutive and inducible strategies including: (1) the direct removal of protons from the cell using proton pumps; (2) changes in the composition of the cell membrane (e.g. by increasing the concentration of cyclopropane fatty acids and/or blocking outer membrane porins by binding polyphosphate or cadaverine); (3) the alkalisation of the external environment by switching metabolic systems so that less acid is produced (e.g. using ribose, arabinose and fructose as the carbon sources, all of which result in less acid production as compared to glucose metabolism); (4) the direct consumption of intracellular protons using the hydrogen-gas-producing formate hydrogen lyase (FHL) complex and the pyridoxal-5-phosphate (PLP)-dependent amino acid decarboxylase AR systems and [5] the production of general shock proteins and chaperones. Furthermore, Stopforth et al. (2007) assessed the acid tolerance response (ATR) of stationary phase, acid-adapted or non-acid-adapted *E. coli* O157:H7, grown individually or in a mixed culture, prior to inoculation of beef or meat decontamination runoff (washings) fluids (acidic [pH 4.95] or nonacidic [pH 7.01]). In this study, *E. coli* O157:H7 appeared to become more tolerant to acid following incubation in acidic washings of sublethal pH (4.89–5.22) compared to nonacidic washings (pH 6.97–7.41) at 4°C or in both types of washings incubated at 15°C. The ATR of the pathogen inoculated into washings was enhanced when cells were previously acid-adapted and incubated at 4°C. Similarly, the ATR on meat was increased by previous acid-adaptation of the inoculum in broth and enhanced by storage at 4°C. Although on day 0 there were no significant differences in ATR between acid-adapted and non-acid-adapted populations on meat, acid-adapted cells displayed consistently reduced susceptibility throughout the 6 days of the experiment. This suggests that acid-adapted *E. coli* O157:H7 introduced on meat may become resistant to subsequent lactic acid exposure and maintain such resistance during refrigerated storage (4°C). In conclusion, acid adaption or selection of strains with reduced susceptibility to the substances evaluated, following exposure to low pH has been demonstrated as a potential scenario that may occur on meat surfaces or in sites within a processing environment where organic acids are applied.

3.4.2.2. Promoting and/or enhancing antibiotic or biocide resistance in bacteria

It is currently unclear as to whether or not exposure to sublethal concentrations of lactic or acetic acid may confer enhanced antibiotic or biocide resistance. Komora et al. (2017) reported a relationship between acid, osmotic stress and antibiotic resistance in *L. monocytogenes*, although further studies on an increased number of isolates would be required before a conclusion could be drawn. McMahon et al. (2007) reported increases in the MIC to amikacin, ceftriaxone and nalidixic acid (*E. coli*), gentamicin and

erythromycin (*St. aureus*) and amikacin, ceftriaxone and trimethoprim (*S.* Typhimurium) when isolates were exposed to pH stress in Mueller Hinton broth acidified with HCl. Such decreases in antibiotic susceptibility were mostly transient and maintained as long as the acid stress was present. The authors concluded that the above results suggest that increased use of bacteriostatic (sublethal), rather than bactericidal (lethal), food preservation systems may possibly contribute to the development and dissemination of antibiotic resistance (ABR) among important food-borne pathogens. Nevertheless, in this study, lactic acid or acetic acid was not assessed, a small set of isolates was tested and the MIC values were not presented. Thus, the clinical significance of any observed increase in MIC could not be assessed.

Based on the evidence discussed above, the Panel concluded that: (1) treatment of pork carcasses with lactic or acetic acid could enhance reduced susceptibility in bacteria potentially increasing their ability to survive exposure to the two substances in decontamination solutions. This can be minimised under GHP and ensuring that target application conditions of the decontamination treatments are maintained throughout processing; (2) there is insufficient evidence to support the hypothesis that exposure of bacteria to sublethal concentrations of organic acids may promote or enhance therapeutic antimicrobial or biocide resistance. In addition, even though the scenario of reduced efficacy of organic acids due to acid adaptation of targeted pathogens, or of small increases in their MIC to certain antibiotics cannot be excluded, their impact on public health is unknown. Considering the above and the natural presence of lactic and acetic acid, it cannot be deduced that exposure of food-borne pathogens on pork carcasses or cuts to the two substances presents any newly identified concern of reduced susceptibility to biocides, or resistance to therapeutic antimicrobials.

3.5. The risk related to the release of the processing plant effluents, linked to the use of lactic and acetic acids, into the environment (ToR 4)

Lactic and acetic acids are fully biodegradable. Assuming that the wastewaters released by the slaughterhouses are treated on-site, if necessary, to counter the potentially low pH caused by lactic or acetic acid, the Panel does not anticipate any adverse effects with respect to the release of lactic and acetic acids from this application into the environment.

4. Conclusions

4.1. Conclusions regarding ToR 1 on the toxicological safety of lactic and acetic acids

- The Panel concluded that treatment of pork carcasses or pork cuts with either lactic or acetic acid meeting the specifications of Regulation (EU) No 231/2012 on food additives does not raise any safety concern.

4.2. Conclusions regarding ToR 2 on the efficacy, i.e. does the individual use of these two substances significantly reduce the level of contamination of pathogens on carcasses and cuts from pork

- The Panel concluded that spraying of pork carcasses pre-chill with lactic acid was efficacious compared to untreated control. The Panel could not conclude based on the available data whether lactic acid was more efficacious than water treatment.
- The Panel could not conclude based on the available data whether spraying of pork meat cuts post-chill with lactic acid was more efficacious than water treatment.
- The Panel concluded that dipping of pork meat cuts post-chill in lactic acid was more efficacious than water treatment.
- Based on the limited available data, the Panel could not conclude on the efficacy of acetic acid treatment on pork carcasses pre-chill and/or pork meat cuts post-chill.

4.3. Conclusions regarding ToR 3 on the potential emergence of reduced susceptibility or resistance to therapeutic antimicrobials

- There is some evidence that repeated exposure to lactic acid can select for reduced susceptibility to the same substance. This may be favoured by an increased level of lactic acid on the meat surface compared to that naturally present and/or to the associated low pH of treated meat surfaces. However, under GHP the Panel did not consider this a significant issue.
- There is no evidence suggesting the promotion of a horizontally transferable reduced susceptibility to lactic or acetic acid or resistance to therapeutic antimicrobials as a result of exposure to lactic or acetic acid.
- Considering the extensive natural presence of lactic and acetic acid, including in feed and food, the possibility of development of resistance to therapeutic antimicrobials is also unlikely to be a significant issue.

4.4. Conclusions regarding ToR 4 on the risk related to the release of the processing plant effluents, linked to the use of lactic and acetic acids, into the environment

- The Panel concluded that the release of lactic and acetic acid is of no concerns for the environment, assuming that wastewaters released by the slaughterhouses are treated on-site, if necessary, to counter the potentially low pH caused by lactic or acetic acid, in compliance with local rules.

5. Recommendations

- Additional studies are required assessing the efficacy of acetic acid on pork carcass and pork meat cuts, the potential of treatments to induce acid adaptation and/or select acid-resistant bacteria, or cross-/co-resistance to biocides and antibiotics.
- To prevent acid adaptation and increased resistance in pathogenic organisms, the treatment of pork carcasses pre-chill and pork cuts post-chill with lactic or acetic acid, subject to authorisation, should be sufficient (in terms of application method, concentration, temperature, duration and pressure) to inactivate the target bacteria.
- Adherence to GHP, within the HACCP framework, is essential: (i) to minimise the probability and time of pathogens exposure to sub-lethal levels of organic acids as a result of unintentional mixing of water and organic acid solutions within a meat plant and (ii) to minimise the concentration of pathogens on the carcasses/meat, thereby enhancing the capacity of lactic or acetic acid to reduce surface microbial contamination. Sublethal (and repetitive) stress exposure of pathogens may lead to acid adaptation and potentially reduced susceptibility to the lactic acid and acetic acid treatment.
- For use as a dip, the operator would be required to write into their HACCP plans their flow rate for replacement of dipping lactic or acetic acid solution, along with testing programmes to assure that the dipping solution maintains effective conditions of application (e.g. temperature, concentration and duration) and microbial testing of product post-application to assure effectiveness. The latter is also recommended for spray applications.
- The dipping treatment should be performed in such way (e.g. adjusting dipping frequency, tank capacity, volumes, concentrations and temperature of decontamination solution in a GHP context) that minimises the likelihood of cross-contamination of treated meat cuts by pathogens accumulated in the dipping tank through consecutive meat treatments, should there be viable pathogens in the treatment solution. Pathogen survival in the treatment solution may be enhanced, e.g. by the buffering effect of organic matter released from meat surfaces to the treatment solution.

Documentation provided to EFSA

1) Submission for the authorization of use of food-grade lactic and acetic organic acids on pork carcasses and cuts for the purpose of reducing pathogens and microbial spoilage organisms. December 2017. Submitted by National Pork Producers Council (United States).

2) Rose SE, Belk KE, Sofos JN, Scanga JA, Tatum JD, Hossner KL and Smith GC, 2004. An Evaluation of Lactic Acid Treatment of Fresh Beef Trimmings on Microbiological, Chemical, and Sensory Properties.
3) Additional information, March 2018. Submitted by National Pork Producers Council (United States).
4) Response letter to EFSA's request for additional information, June 2018. Submitted by National Pork Producers Council (United States).

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Abbreviations

AABR	antibiotic resistance
ADI	acceptable daily intake
ANSES	French Agency for Food, Environmental and Occupational Health & Safety
ATR	acid tolerance response
BIOHAZ	EFSA Panel on Biological Hazards
CAT	critical appraisal tool
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
CFU	colony forming unit
CI	confidence interval
FAO	Food Agriculture Organization of the United Nations
FBO	Food Business Operator
FHL	formate hydrogen lyase
FSIS	Food Safety and Inspection Service
GHP	Good Hygienic Practices
HACCP	Hazard Analysis Critical Control Point
JECFA	Joint FAO/WHO Expert Committee on Food Additives
MIC	minimum inhibitory concentration
PLP	pyridoxal-5-phosphate
RoB	Risk of bias
QRMA	Quantitative Microbiological Risk Assessments
SCF	Scientific Committee of Food
SD	standard deviation
SEM	standard error of the mean
STEC	shiga toxin-producing *Escherichia coli*
ToR	Term of Reference
USDA	United States Department of Agriculture
VTEC	verocytotoxigenic *Escherichia coli*
WG	Working Group
WHO	World Health Organization

Appendix A – Critical appraisal tool (CAT) for appraising the reliability of each experiment

No	Question	Rating	Explanation for expert judgement
1	**Comparability of control and treated groups**	4	There is **direct** evidence that the only difference between the treated and control group is the presence or absence of the decontaminating substance and not the method of application or other factors (e.g. inoculated with the target organism using the same procedure, stored at the same temperature and under the same storage conditions, same detection and/or enumeration method used). The control treatment is identical to the treated sample, except for the substance
		3	There is **direct** evidence of the above (scored 4), except that the control group is left untreated (e.g. no water used) OR There is **indirect** evidence of the above (scored 4)
		2	There is **indirect** evidence that the treated and control group differ in other aspects than being untreated
		1	There is **direct** evidence of the above (scored 2)
2	**Inoculation procedure of the target organism and coverage of the meat surface with the substance**	4	(for artificial contamination) There is **direct** evidence that the inoculum was evenly distributed over the meat surface and that the time between inoculation of the target organism and treatment with the substance was sufficient to allow attachment of the bacteria (e.g. at least 15 min) and the substance was evenly distributed over the meat surface (for natural contamination) There is **direct** evidence the substance was evenly distributed over the meat surface
		3	There is **indirect** evidence of the above (scored 4)
		2	There is **indirect** evidence that the above (scored 4) does not apply
		1	There is **direct** evidence that the above (scored 4) does not apply
3	**Detection and enumeration method of the target organism**	4	There is **direct** evidence that a validated reference method or parts thereof, that maximises the recovery of the bacteria, has been used for the detection and enumeration of the target organism (e.g. FDA method, ISO method)
		3	There is **direct** evidence that an acceptable method other than a validated reference method (e.g. Petri film), that maximises the recovery of the bacteria, has been used for the detection and enumeration of the target organism
		2	There is **indirect** evidence of the above (scored 3 or 4)
		1	There is **direct** or **indirect** evidence that the detection and enumeration method contains errors (to be spelled out when scoring)
4	**Statistical analysis and reproducibility**	4	Definitively appropriate: There is **direct** evidence of statistical analysis (e.g. ANOVA, t-test, post-hoc test) and independent experimental trials using representative samples (replicates) have been used
		3	Probably appropriate: There is **direct** evidence of statistical analysis but the method and/or the number of independent trials and representative samples (replicates) are not specified
		2	Probably not appropriate: Independent trials (replicates) were used but there is **direct** evidence that a statistical analysis was not used
		1	Definitively not appropriate: A single trial is used (no replicates) and no or insufficient statistical analysis has been performed

Appendix B – Exposure Assessment

B.1. EFSA Comprehensive European Food Consumption Database

Since 2010, the Comprehensive Database has been populated with detailed national data on food consumption. Competent authorities in European countries provide EFSA with data regarding the level of food consumption by individual consumers, as taken from the most recent national dietary survey in their country (EFSA, 2011).

The food consumption data gathered by EFSA were collected using different methodologies and thus direct country-to-country comparisons should be interpreted with caution. Depending on the food category and the level of detail used in exposure calculations, uncertainties might be introduced owing to possible subjects' underreporting and/or misreporting of consumption amounts. Nevertheless, the EFSA Comprehensive Database represents the best available source of food consumption data across Europe.

Food consumption data from the following population groups: infants, toddlers, children, adolescents, adults and the elderly were used for the exposure assessment. For the present assessment, food consumption data were available from 33 different dietary surveys carried out in 19 European countries.

Chronic exposure was calculated based on summary statistics, excluding surveys with only one day per subject. High-level exposure should was calculated for only those population groups, in which the sample size was sufficiently large to allow calculation of the 95th percentile (i.e. n = 60 subjects) (EFSA, 2011).

Table B.1: Lactic acid and acetic acid concentration data, when used as decontamination treatment of pork carcasses and pork cuts, lactic acid naturally present in pork meat, acetic acid naturally present in vinegar and Foodex food groups Level 4 used in the exposure assessment model

FOODEX_L4_ Foodgroup	Foodex L4	Lactic acid as decontamination agent*	Acetic acid as decontamination agent*	Lactic acid naturally present in pork*	Acetic acid in vinegar	Pork fraction in food group
		mg/kg	mg/kg	mg/kg	mg/L	
Meat and meat products (including edible offal)	A.01.000727	99.2	125.8	7,000	0	1
Livestock meat	A.01.000728	99.2	125.8	7,000	0	1
Pork/piglet meat (*Sus scrofa*)	A.01.000731	99.2	125.8	7,000	0	1
Mixed meat	A.01.000760	49.6	62.9	3,500	0	0.5
Mixed beef and pork meat	A.01.000761	49.6	62.9	3,500	0	0.5
Mixed pork and mutton/lamb meat	A.01.000762	49.6	62.9	3,500	0	0.5
Edible offal, farmed animals	A.01.000766	99.2	125.8	7,000	0	1
Pork liver	A.01.000769	99.2	125.8	7,000	0	1
Pork kidney	A.01.000777	99.2	125.8	7,000	0	1
Tongue (beef, veal, mutton, lamb, pork)	A.01.000779	99.2	125.8	7,000	0	1
Heart (beef, veal, pork, mutton, lamb)	A.01.000780	99.2	125.8	7,000	0	1

FOODEX_L4_ Foodgroup	Foodex L4	Lactic acid as decontamination agent*	Acetic acid as decontamination agent*	Lactic acid naturally present in pork*	Acetic acid in vinegar	Pork fraction in food group
		mg/kg	mg/kg	mg/kg	mg/L	
Brain (veal, lamb, pork)	A.01.000781	99.2	125.8	7,000	0	1
Spleen (beef, pork)	A.01.000785	99.2	125.8	7,000	0	1
Tail (beef, pork, lamb)	A.01.000788	99.2	125.8	7,000	0	1
Totters and feet (calf, pork)	A.01.000789	99.2	125.8	7,000	0	1
Preserved meat	A.01.000795	99.2	125.8	7,000	0	1
Ham, pork	A.01.000796	99.2	125.8	7,000	0	1
Pork, dried	A.01.000799	99.2	125.8	7,000	0	1
Bacon	A.01.000802	99.2	125.8	7,000	0	1
Corned pork	A.01.000804	99.2	125.8	7,000	0	1
Pastrami, pork	A.01.000806	99.2	125.8	7,000	0	1
Luncheon meat	A.01.000809	99.2	125.8	7,000	0	1
Sausages	A.01.000811	79.36	100.64	5,600	0	0.8
Fresh and lightly cooked sausage	A.01.000812	79.36	100.64	5,600	0	0.8
Salsiccia	A.01.000813	89.28	113.22	6,300	0	0.9
Bratwurst	A.01.000814	89.28	113.22	6,300	0	0.9
Thuringer-Style Sausage	A.01.000815	79.36	100.64	5,600	0	0.8
Weisswurst	A.01.000816	89.28	113.22	6,300	0	0.9
Bockwurst	A.01.000817	89.28	113.22	6,300	0	0.9
Uncooked smoked sausage	A.01.000818	79.36	100.64	5,600	0	0.8
Mettwurst, sausage	A.01.000820	79.36	100.64	5,600	0	0.8
Kielbasa, sausage	A.01.000821	79.36	100.64	5,600	0	0.8
Cooked sausage	A.01.000822	79.36	100.64	5,600	0	0.8
Blood sausage	A.01.000823	79.36	100.64	5,600	0	0.8
Blood and tongue sausage	A.01.000824	79.36	100.64	5,600	0	0.8
Liver sausage, liverwurst	A.01.000825	79.36	100.64	5,600	0	0.8
Cooked smoked sausage	A.01.000826	79.36	100.64	5,600	0	0.8
Berliner-Style, Sausage	A.01.000827	79.36	100.64	5,600	0	0.8
Bologna, sausage	A.01.000828	79.36	100.64	5,600	0	0.8
Boterhamworst	A.01.000829	79.36	100.64	5,600	0	0.8
Frankfurters, sausage	A.01.000831	79.36	100.64	5,600	0	0.8
Knackwurst, sausage	A.01.000832	79.36	100.64	5,600	0	0.8
Wiener, sausage	A.01.000833	79.36	100.64	5,600	0	0.8
Semi-dry sausage	A.01.000834	89.28	113.22	6,300	0	0.9

FOODEX_L4_ Foodgroup	Foodex L4	Lactic acid as decontamination agent*	Acetic acid as decontamination agent*	Lactic acid naturally present in pork*	Acetic acid in vinegar	Pork fraction in food group
		mg/kg	mg/kg	mg/kg	mg/L	
Goteborg cervelat	A.01.000838	89.28	113.22	6,300	0	0.9
Landjaeger cervelat	A.01.000840	89.28	113.22	6,300	0	0.9
Mortadella	A.01.000842	99.2	125.8	7,000	0	1
Vienna Sausage	A.01.000843	79.36	100.64	5,600	0	0.8
Dry sausage	A.01.000844	89.28	113.22	6,300	0	0.9
Italian-type salami	A.01.000845	89.28	113.22	6,300	0	0.9
Arles	A.01.000846	99.2	125.8	7,000	0	1
Beerwurst	A.01.000847	89.28	113.22	6,300	0	0.9
Cooked salami	A.01.000849	89.28	113.22	6,300	0	0.9
German salami	A.01.000852	89.28	113.22	6,300	0	0.9
Hungarian-type salami	A.01.000853	89.28	113.22	6,300	0	0.9
Chorizo	A.01.000854	89.28	113.22	6,300	0	0.9
Cabanos	A.01.000856	89.28	113.22	6,300	0	0.9
Meat specialities	A.01.000857	49.6	62.9	3,500	0	0.5
Pork meat loaf	A.01.000858	59.52	75.48	4,200	0	0.6
Meat in aspic	A.01.000860	59.52	75.48	4,200	0	0.6
Ham and cheese loaf	A.01.000861	69.44	88.06	4,900	0	0.7
Liver cheese or liver loaf	A.01.000862	99.2	125.8	7,000	0	1
Head cheese (Brawn)	A.01.000865	99.2	125.8	7,000	0	1
Sulze	A.01.000866	59.52	75.48	4,200	0	0.6
Pastes, pâtés and terrines	A.01.000867	99.2	125.8	7,000	0	1
Meat paste	A.01.000868	49.6	62.9	3,500	0	0.5
Pate, pork liver	A.01.000871	99.2	125.8	7,000	0	1
Terrine	A.01.000872	59.52	75.48	4,200	0	0.6
Animal fat	A.01.001347	99.2	125.8	7,000	0	1
Pork lard (Schmaltz)	A.01.001351	99.2	125.8	7,000	0	1
Vinegar, wine	A.01.001653	0	0	0	60,000	0
Vinegar, apple	A.01.001654	0	0	0	60,000	0
Ready-to-eat meal for infants and young children	A.01.001733	49.6	62.9	3,500	0	0.5
Ready-to-eat meal for children, meat/fish-based	A.01.001736	49.6	62.9	3,500	0	0.5
Ready-to-eat meal for children, meat and vegetables	A.01.001737	49.6	62.9	3,500	0	0.5

FOODEX_L4_ Foodgroup	Foodex L4	Lactic acid as decontamination agent*	Acetic acid as decontamination agent*	Lactic acid naturally present in pork*	Acetic acid in vinegar	Pork fraction in food group
		mg/kg	mg/kg	mg/kg	mg/L	
Sandwich and sandwich-like meal	A.01.001791	19.84	25.16	1,400	0	0.2
Sandwich, meat filling	A.01.001793	19.84	25.16	1,400	0	0.2
Sandwich, meat and vegetable filling	A.01.001798	19.84	25.16	1,400	0	0.2
Pizza and pizza-like pies	A.01.001800	9.92	12.58	700	0	0.1
Pizza and pizza-like pies, meat, and vegetables	A.01.001804	9.92	12.58	700	0	0.1
Pizza and pizza-like pies, cheese, meat, and vegetables	A.01.001805	9.92	12.58	700	0	0.1
Pizza and pizza-like pies, cheese, meat, and mushrooms	A.01.001807	9.92	12.58	700	0	0.1
Pizza and pizza-like pies, cheese, meat, mushrooms, and vegetables	A.01.001808	9.92	12.58	700	0	0.1
Pasta, cooked	A.01.001809	49.6	62.9	3,500	0	0.5
Pasta, cooked, meat filling	A.01.001813	49.6	62.9	3,500	0	0.5
Pasta, cooked, meat and vegetable filling	A.01.001815	49.6	62.9	3,500	0	0.5
Rice-based meals	A.01.001816	49.6	62.9	3,500	0	0.5
Rice and meat meal	A.01.001818	49.6	62.9	3,500	0	0.5
Potato based dishes	A.01.001820	49.6	62.9	3,500	0	0.5
Potatoes and meat meal	A.01.001822	49.6	62.9	3,500	0	0.5
Potatoes, meat, and vegetables meal	A.01.001823	49.6	62.9	3,500	0	0.5
Beans-based meals	A.01.001825	49.6	62.9	3,500	0	0.5
Beans and meat meal	A.01.001826	49.6	62.9	3,500	0	0.5
Beans, meat, and vegetables meal	A.01.001828	49.6	62.9	3,500	0	0.5
Meat-based meals	A.01.001829	49.6	62.9	3,500	0	0.5
Meat burger	A.01.001830	59.52	75.48	4,200	0	0.6
Meat balls	A.01.001831	59.52	75.48	4,200	0	0.6

FOODEX_L4_ Foodgroup	Foodex L4	Lactic acid as decontamination agent*	Acetic acid as decontamination agent*	Lactic acid naturally present in pork*	Acetic acid in vinegar	Pork fraction in food group
		mg/kg	mg/kg	mg/kg	mg/L	
Goulash	A.01.001832	49.6	62.9	3,500	0	0.5
Meat stew	A.01.001833	49.6	62.9	3,500	0	0.5
Meat/poultry soup	A.01.001860	49.6	62.9	3,500	0	0.5

*: Concentration data reflects the pork fraction in the food group.