EUROPEAN COMMISSION

Health & Consumer Protection
Directorate-General

Scientific Committee on Health and Environmental Risks
SCHER

Scientific Committee on Emerging and Newly Identified Health Risks
SCENIHR

Environmental impact and effect on antimicrobial resistance of four substances used for the removal of microbial surface contamination of poultry carcasses

Scientific Committees
on consumer products
on emerging and newly identified health risks
on health and environmental risks

The SCHER adopted this opinion at its 22nd plenary on 12 March 2008

The SCENIHR adopted this opinion at its 23rd plenary on 02 April 2008

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Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

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SCHER

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In particular, the Committee addresses questions related to new and existing chemicals, the restriction and marketing of dangerous substances, biocides, waste, environmental contaminants, plastic and other materials used for water pipe work (e.g. new organics substances), drinking water, indoor and ambient air quality. It addresses questions relating to human exposure to mixtures of chemicals, sensitisation and identification of endocrine disrupters.

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SCENIHR

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Keywords: SCHER, SCENIHR, scientific opinion, antimicrobial resistance, poultry carcasses, chlorine dioxide, trisodium phosphate, acidified sodium chlorite, peroxyacids.

Opinion to be cited as:

SCHER/SCENIHR scientific opinion on the environmental impact and effect on antimicrobial resistance of four substances used for the removal of microbial surface contamination poultry carcasses, April 2008

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1. BACKGROUND

In a report prepared by the Scientific Committee on Veterinary Measures relating to Public Health (SCVPH) issued on 30 October 1998[1], it was stated that antimicrobial substances should only be permitted for use if a fully integrated control programme is applied throughout the entire food chain. The SCVPH opinion issued on 14-15 April 2003[2] on the evaluation of antimicrobial treatments for poultry carcasses concluded that decontamination can constitute a useful tool in further reducing the number of pathogens. Both documents stressed that antimicrobial substances shall be assessed thoroughly before their use is authorised.

With the adoption of the hygiene package in 2004 and the introduction of the Hazard Analysis and Critical Control Points (HACCP) principles in the entire food chain, establishments will be obliged to improve their hygiene and processing procedures. In addition, Regulation (EC) No 2160/2003[3] will force Member States to initiate implementing salmonella control programmes for poultry and pigs at farm level. Under such conditions the use of substances for the removal of microbial surface contamination from food of animal origin could be considered.

Article 3(2) of Regulation (EC) No 853/2004[4] of the European Parliament and of the Council laying down specific hygiene rules for food of animal origin, provides a legal basis to permit "the use of a substance other than potable water" to remove surface contamination from products of animal origin.

In light of the preparation of implementing measures resulting from Regulation (EC) No 853/2004, permission for use should be preceded by a thorough scientific evaluation of all risks involved. A number of scientific evaluations have taken place on the general aspects of antimicrobial treatment of food of animal origin and on the safety and toxicological aspects of four specific substances that are considered for approval (see references).

A draft implementing measure has been proposed to allow the use of four specified substances (chlorine dioxide, trisodium phosphate, acidified sodium chlorite and peroxyacids) for the removal of surface contamination of poultry carcasses. The draft Commission Regulation will lay down detailed specifications for the use of the four substances including conditions of use (see appendix to this annex).

Recently the Commission has prepared a request to SCENIHR for an overall assessment of the antibiotic resistance effects of biocides. Furthermore, EFSA has initiated a self-tasking project on antimicrobial resistance.

In the light of these initiatives it is necessary to perform an assessment of the impact on the environment of the four substances mentioned above. Moreover, it is necessary to investigate if it is possible that the use of the four substances could lead to antimicrobial resistance in the micro-organisms.

2. TERMS OF REFERENCE

SCHER is requested:

To assess the possible environmental impact of chlorine dioxide, acidified sodium chlorite, trisodium phosphate and peroxyacids when used according to the proposed conditions of use as a substance to remove microbial surface contamination from poultry carcasses

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[1] Report by SCVPH on "Benefits and limitations of antimicrobial treatments for poultry carcasses" (1998).

[2] Opinion of the SCVPH on the "Evaluation of antimicrobial treatments for poultry carcasses" (2003).

[3] OJ L 325, 12.12.2003, p. 1.

[4] OJ L 139, 30.04.2004, p. 47-55

SCENIHR is requested:

To assess the possible effect on the emergence of antimicrobial resistance in case chlorine dioxide, acidified sodium chlorite, trisodium phosphate and peroxyacids were applied according to the proposed conditions of use as a substance to remove microbial surface contamination from poultry carcasses.

3. OPINION

3.1 Background documents

The information presented in this opinion draws on from previous opinions and reports from EU Scientific Committees and Panels, in particular:

- Report of the Scientific Committee on Veterinary Measures Relating to Public Health (SCVPH) on benefits and limitation of antimicrobial treatments for poultry carcasses (SCVPH, 1998).
- Opinion of the SCVPH on the Evaluation of Antimicrobial Treatment for Poultry Carcasses (SCVPH, 2003).
- Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food of the European Food Safety Authority (EFSA) on the treatment of poultry carcasses with chlorine dioxide, acidified sodium chlorite, trisodium phosphate and peroxyacids (EFSA, 2005a).
- Opinion of the Scientific Panel on Biological Hazards of EFSA on the evaluation of the efficacy of peroxyacids for use as an antimicrobial substance applied on poultry carcasses (EFSA, 2005b).
- ECOLAB Supporting documents

3.2 Introduction to use patterns and resistance concerns

3.2.1 Introduction

There is no accepted treatment for poultry carcasses except to rinse with potable water in the European Union (EU). This rinsing is performed in the slaughterhouses to avoid cross contamination of carcasses during the process. The European quality assurance system is based on adherence to the HACCP system during breeding, transportation and slaughtering of poultry. Thus poultry carcasses are only rinsed with potable water (with a residual disinfectant concentration of around 0.1mg/l of chlorine) in order to eliminate incidental microbiological contamination during the different steps encountered in slaughterhouse before packaging and transportation under refrigerated conditions, always according to the HACCP system.

For the use of disinfectants on poultry carcasses to be acceptable satisfactory answers are required to questions on the objective, the conditions of application, the potentially negative effects due to the promotion of resistant micro-organisms, the chemical reactions with the meat, and the impact on the environment.

Within the scope of the mandate our proposition is to limit the definition of "antimicrobials" to bacteria. The four substances concerned are used to remove microbial surface contamination of poultry carcasses. By "microbial contamination" we mean essentially the presence of *Salmonella* and *Campylobacter* on poultry carcasses.

Many chemical substances, with antimicrobial activity, are used in the production of food of animal origin. This includes the use of antibiotics for therapy, and the even larger amounts of chemicals used for disinfection or preservation. Antimicrobial compounds can exhibit a negative effect by: a) changing the flora towards intrinsically resistant more pathogen strains or b) selecting for acquired resistance in previously susceptible strains. Some mechanisms of acquired resistance might confer cross-resistance between chemically unrelated compounds or different mechanisms might be genetically linked on the same element. The use of one class of chemical might thereby select for resistance towards another class of therapeutically used biocides and/or antibiotics. Bacteria might also develop what is known as 'physiological resistance' through the creation of biofilms (McDonnell and Russel 1999).

In general, all use of antimicrobial chemicals has eventually led to the development of acquired resistance. Thus, any use of chemical substances should be kept to a minimum and they should only be used in cases where the benefit is large. It should also be noted that disinfectants act in a time-dependent manner. Thus, if the routine use of disinfectants in the food industry leads to a lack of other hygienic precautions, and thereby a higher degree of contamination, the use of disinfectants might not be sufficient to kill all pathogenic bacteria.

3.2.2 Situation of use of disinfectants on poultry carcasses and the new proposed conditions

In the EU antimicrobials have not been permitted for treating poultry carcasses, parts, or viscera since 1971 (EC, 1971). The EU meat hygiene regulations do not allow any method or product decontamination other than washing with potable water or applying steam. Regulators have argued that, if permitted, processors would use antimicrobials to mask unhygienic slaughtering or processing practices.

However, Regulation 853/2004 of the European Parliament and of the Council, laying down specific hygiene rules for food of animal origin (EC, 2004), applicable from 1 January 2006, provides a legal basis to permit the use of a substance other than potable water to remove surface contamination from products of animal origin.

In annex II to the draft regulation, the Commission introduced a provision aiming to authorize trisodium phosphate (TSP), acidified sodium chlorite (ASC), and chlorine dioxide as decontaminants for poultry carcasses (SANCO 55/2005, revision 3, Annex II). These chemicals are currently under review for final approval by EU authorities.

The European Food Safety Authority panel on food additives, flavourings, processing aids, and materials in contact with food has recently reported that poultry carcass decontamination with TSP, ASC, chlorine dioxide or peroxyacid solutions, under FDA-approved conditions of use, poses no toxicological risk to human health (EFSA, 2005a). It has been suggested, however, that the decontamination processes could render the surface of the carcass susceptible to preferential growth of dangerous bacteria (e.g., some serotypes or antibiotic-resistant strains), because of the removal of normal competitive microflora (Del Rio et al., 2006; FVE, 2005).

The Federation of Veterinarians of Europe (FVE, 2005) recommends that the decontamination of carcasses should not be allowed unless it has been demonstrated that such techniques are safe, taking into account the potential pathogenic microflora involved.

At slaughtering plants in North America, it is normal practice to subject carcasses to a variety of decontaminating treatments during the carcass-dressing process with the aim to reduce microbial loads. Many of these interventions are approved by the US Food and Drug Administration (FDA) in poultry processing plants as GRAS substances (generally recognized as safe), this being the case for 8 to 12 % TSP or 1.5 to 2.5 % organic acids, e.g. citric acid (CA). Other chemicals are considered processing aids and approved by the FDA as secondary direct food additives permitted in food for human consumption, including sodium chlorite at 500 to 1,200 ppm combined with a GRAS acid that achieves a pH between 2.3 and 2.9 in the solution (Castillo et al., 1999; Dinçer and Baysal, 2004; Oyarzabal, 2005).

In the last several years investigations have been carried out to determine the relative resistance to chemical decontaminants of different spoilage and pathogenic microbial genera and species (Gilbert and McBain, 2003; Del Rio et al., 2007). However, the variation in resistance among strains of the same species has not so far been studied.

Intra-species resistance variation is of importance, as care has to be taken to choose the appropriate target strain for testing the effect of a decontamination procedure.

An association between resistance to antibiotics and chemical biocides (other than poultry decontaminants) has been previously demonstrated in *Salmonella* strains (Braoudaki and Hilton, 2004; Meyer, 2006; Potenski et al., 2003; Russell, 2003.). It has been hypothesized that some biocides and antibiotics could share a common target or targets in bacteria cells (Braoudaki and Hilton, 2004). The *Salmonella* serotype characteristics have also been shown to influence biocide resistance (Bacon et al., 2003). However, chemical decontaminants for poultry have not so far been tested in this regard.

3.2.3 Potential use goals and patterns

The use of disinfectants depends on the intended goals. Basically, two options should be considered:

- The treatment target is the potential surface microbial contamination produced during the last operations in slaughterhouse. Thus the process could introduce to the use of a residual disinfectant in order to avoid the proliferation of microorganisms during the distribution of water in networks. For this purpose it is accepted worldwide to consider for this operation the C.T concept, where C is the concentration of disinfectant and T the time period during which this concentration is applied. There are internationally accepted tables for C.T values to be applied for the protection against bacteria, or more resistant organisms like viruses or moulds and fungi.
- The treatment target is the elimination of a fixed indigenous flora linked to contamination during production or transportation. In these circumstances the concentration and contact-time of the disinfectant have to be higher. This use has been considered as unacceptable because it is not considered to be an efficient way for controlling the microbial quality of the carcasses; it might also be used to avoid some quality control measures during production and transportation.

  Thus this opinion considers exclusively the first option.

General requirements of use:

(a) there is no simultaneous or consecutive application of more than one approved substance;

(b) the poultry carcass is rinsed with potable water, at a point in the production process following the application of the approved substance, to ensure that the substance is intentionally removed to such an extent that it does not have a technological effect on the final product;

(c) an inside rinsing of the eviscerated poultry carcass is performed following the application of the approved substance;

(d) application of the approved substance and rinsing is performed in the slaughter room just before the poultry carcasses enter the chilling or refrigerating roam;

(e) where an approved substance is used in a pre-chiller or chiller tank, the solution containing that substance is replaced at regular intervals in order to maintain the prescribed concentration of the approved substance;

(f) where an approved substance is used more than once during the production process, the total contact time must not exceed the periods set out in the specific requirements of use.

Specific requirements of use:

I. Chlorine dioxide:

(a) at a maximum concentration of 3 mg/kg of residual chlorine dioxide in water at those points of the production process where the substance is approved for use;

(b) in the case of continuous, counter flow, immersion chilling, the contact time shall be proportionate to the size of the poultry carcasses and shall be assessed in connection with the concentration of the approved substance used.

2. Acidified sodium chlorite:

(a) in poultry processing waters applied as pre-chiller or chiller solutions at concentrations between 50 and 150 mg/kg of sodium chlorite combined with an acid permitted for food use that achieves a pH between 2.8 - 3.2 in the solution; the solution shall be applied as an immersion dip for up to 5-8 seconds;

(b) in poultry processing waters applied as spray solutions at concentrations between 500 and 1200 mg/kg of sodium chlorite combined with an acid permitted for food use that achieves a pH between 2.3 - 2.9 in the solution; the solution shall be applied as a spray for up to 15 seconds in total.

3. Trisodium phosphate:

(a) in poultry processing waters at concentrations from 80 g/kg to 120 g/kg. The solution shall be applied by dipping or spraying of carcasses, which have not been cooled, for up to 15 seconds in total.

4. Peroxyacids:

(a) in poultry processing waters, a mixture of peroxyacetic acid, octanoic acid, acetic acid, hydrogen peroxide, peroxyoctanoic acid and 1-hydroxyethylidene-1,1-diphosphonic acid (HEDP) may be used at a maximum concentration of 220 mg/kg peroxyacetic acid, 110 mg/kg hydrogen peroxide and 13 mg/kg HEPD for up to 15 seconds when applied as a spray or dip.

3.2.4 Knowledge about resistance or naturally insensitive / tolerant bacterial populations, knowledge about increased levels of tolerance

A large number of different substances with antimicrobial activity are used in the production of food animals. This includes metal and disinfectants in the primary production and a large number of disinfectants and preservatives during slaughter or in the final food product. This usage might select for intrinsically resistant pathogens or co-select for acquired resistance to antibiotics used for therapy.

There is currently insufficient knowledge on the potential negative effects of using the different biocides. In the present evaluation only four of a large number of substances are evaluated and for all four insufficient knowledge is available.

There is a need for research into the mechanisms by which bacteria might develop resistance of acquired reduced susceptibility to disinfectants, preservatives or metals and the importance for selection of pathogenic bacteria in the food chain as well as co-selection of antibiotic resistance. The research should focus on the biocides of most importance for the food industry and it ought to also include cost-benefit considerations.

The use of one or more of these four compounds (chlorine dioxide, acidified sodium chlorite, trisodium phosphate and peroxyacids and their subsequent derivatives obtained during exposure) can induce an oxidative chemical stress to bacterial cells. The stress response comprises various different and sophisticated regulatory cascades. Among them is the *sox* regulon which is linked to the superoxide response (e.g. H_2O_2, paraquat) a contributor to the multidrugresistance (MDR) activation in Gram-negative bacteria (Griffith et al, 2004; Pomposiello et al, 2001). In addition, these compounds can also trigger the SOS response which is involved in the genetic variability (mutation, movement/exchange of mobile elements) (Walsh, 2006; Hasting et al, 2004; Foster, 2007; Erill et al, 2007).

Consequently, we may mention different possible effects of the substances on bacterial flora (normal or colonizing bacteria):

- if a bacteria agent exhibits *sox* – SOS activated cascades involving the presence of active resistance mechanism, it may escape from the treatment (depending of the dose, time, etc.)
- an heterogeneous population of microbes (activated and non-activated), may promote an efficient selection of resistant bacteria during the subsequent steps of food processing
- the use of these compounds may change the microflora by eliminating the more intrinsic susceptible bacteria.

It should be noted that the treatment may only concern surface and available sites during the bath. The sensitivity of bacterial biofilm and spores are also relevant considerations.

3.3 Identification of the substances

The mandate covers four different substances or group of substances: chlorine dioxide, acidified sodium chlorite, trisodium phosphate and peroxyacids. A generic description is presented here (Based on SCVPH, 2003, and EFSA, 2005a).

3.3.1 Chlorine dioxide (CD)

Chemical name:	Chlorine peroxide
Synonyms:	Chloroperoxyl; chlorine (IV) oxide
CAS registry number:	10049-04-4
Chemical formula:	ClO_2
Description:	Greenish yellow to orange gas with a pungent odour

Formulation

Chlorine dioxide (ClO_2; CD) is water soluble, and solutions are quite stable if kept cool and dark.

A typical immersion bath used in poultry processing contains initially 20-50 mg CD per L (20-50 ppm), which rapidly decomposes to chlorite (ClO_2^-) and chlorate (ClO_3^-) (in a 7:3 ratio) due to the high content of organic matter in the immersion bath. This leaves tipically some 5% of the initial CD concentration, the resulting concentrations in poultry process water are thus 2.5 mg chlorine dioxide per L (2.5 ppm), 33 mg chlorite per L (33.25 ppm) and 14 mg chlorate per L (14.25 ppm) (USDA, 2002a).

Toxicity

The International Programme on Chemical Safety (IPCS, 2000) derived a tolerable daily intake (TDI) value for CD (expressed as chlorine) of 0.03 mg/kg bw/day, while US-EPA designated the same level (0.03 mg chlorite/kg bw/day) as a reference dose (RfD; SCVPH, 2003). The WHO guidelines for drinking-water quality set a guideline value of 5 mg/L for chlorine based on a TDI of 0.15 mg/kg bw/day, allocating 100 % of this TDI to water and assuming a 60 kg bw individual consumes 2 liters of water per day (WHO, 2004).

Ecotoxity

EU Risk Assessment Reports (RAR) on Chlorine and Sodium Hypochlorite are available and offers the following summary of toxicity data on aquatic organisms and PNEC derivation for freely available chlorine:

"For the derivation of the freshwater PNEC the TGD states that a factor of 10 applied to the lowest of three NOECs across different trophic levels is considered sufficient "only if the species tested can be considered to represent one of the more sensitive groups". From the available NOEC dataset, the most sensitive group is algae which has a NOEC (7d NOEC = 2.1 µgFAC/l) very close to the acutely toxic concentration to Daphnia (24h LC50 = 5 µgFAC/l). In addition for the primary consumer trophic level, long-term data are available only for bivalve molluscs, which play an important ecological role but are not among the most sensitive species. This holds true also for sodium hypochlorite. In fact, long-term mortality data for freshwater clams and a NOEC value for marine molluscs indicate that this group is markedly less sensitive than crustaceans (Daphnia). The same can be observed by comparing short-term toxicity data for the two groups. In a mesocosm study, zooplankton was affected at a concentration much lower than that necessary to reduce algae population. In conclusion it appears that the most sensitive group, i.e. crustacean (Daphnia) is not represented in the dataset.

Based on these considerations a factor of 10 for the derivation of the PNEC is judged not sufficiently protective and we suggest to apply a factor of 50 to the lowest NOEC: PNEC = 3 µgTRC/l/50 = 0.06 µgTRC/l corresponding to 2.1 µgFAC/l/50 = 0.04 µgFAC/l."

Regarding the PNEC for microbial communities in the WWTP, no value is proposed in the RARs due to the following arguments:

"Despite the strong antimicrobial potential of hypochlorite, concern for inhibition effects on biological sewage treatment at the current hypochlorite utilization pattern is undue. Activated sludge flocs are not very sensitive to NaClO, probably due to protection by their glycocalix made out of polysaccharides.PNEC is not derived for hypochlorite used in waste water treatment applications, as reactions with the organic matter present in sewage will rapidly reduce the hypochlorite concentration to values as low as 10-32 g/l of free available chlorine (defined as the sum of dissolved chlorine gas (Cl2), hypochlorous acid (HOCl), and hypochlorite (OCl-) present in the test solution)."

Degradation

Decomposition, i.e., the oxidation and reduction reactions occurring during the decontamination process upon contact of CD with the surface of a poultry carcass, and occurring anyway in time, ultimately results in chloride (Cl^-), which is a normal constituent of all living organisms and waters, and is not expected to represent an environmental impact at the expected concentrations.

Semicarbazide was also considerer a potential reaction product (Hoenicke et al., 2004). However, since CD is a less aggressive oxidant than ASC, is used in lower concentrations, and it has not been detected in ASC treatments, it is unlikely that CD has the potential to form significant amounts of semicarbazide (EFSA, 2005a).

3.3.2 Acidified sodium chlorite (ASC)

Definition:	Acidified sodium chlorite is a combination of sodium chlorite and any acid generally approved in food
Chemical names:	Sodium chlorite; chlorous acid, sodium salt
Synonym:	Acidified chlorite
CAS registry number:	7758-19-2
Chemical formula:	$NaClO_2$
Description:	Clear, colourless liquid

Formulation

Sodium chlorite ($NaClO_2$; ASC), when mixed prior to use in the formulated product, results in a chemical equilibrium containing chlorous acid ($HClO_2$), which in turn degrades to CD and to a lesser extent to sodium chlorate ($NaClO_3$). The chlorate (ClO_3^-) eventually degrades to CD and chloride (Cl^-).

Chlorous acid and CD are responsible for the microbicidal action of the product.

A typical immersion bath used in poultry processing contains maximally 1.2 g/L sodium chlorite (1200 ppm) as an acidified aqueous solution, while chiller water may contain up to 0.15 g/L (150 ppm) of ASC (USDA, 2002b).

Toxicity

IPCS (2000) derived a TDI of 0.03 mg/kg bw/day for chlorite, while US-EPA designated the same level (0.03 mg/kg bw/day) as a RfD (SCVPH, 2003). The WHO guidelines for drinking-water quality set a guideline value of 0.7 mg/L for chlorite in drinking-water, based on a TDI of 0.03 mg/kg bw/day for chlorite (WHO, 2004).

Ecotoxity

EU Risk Assessment Reports on Chlorine and Sodium Hypochlorite are available and offers the following summary of toxicity data on aquatic organisms and PNEC derivation for freely available chlorine:

"*For the derivation of the freshwater PNEC the TGD states that a factor of 10 applied to the lowest of three NOECs across different trophic levels is considered sufficient "only if the species tested can be considered to represent one of the more sensitive groups". From the available NOEC dataset, the most sensitive group is algae which has a NOEC (7d NOEC = 2.1 µgFAC/l) very close to the acutely toxic concentration to Daphnia (24h LC50 = 5 µgFAC/l). In addition for the primary consumer trophic level, long-term data are available only for bivalve molluscs, which play an important ecological role but are not among the most sensitive species. This holds true also for sodium hypochlorite. In fact, long-term mortality data for freshwater clams and a NOEC value for marine molluscs indicate that this group is markedly less sensitive than crustaceans (Daphnia). The same can be observed by comparing short-term toxicity data for the two groups. In a mesocosm study, zooplankton was affected at a concentration much lower than that necessary to reduce algae population. In conclusion it appears that the most sensitive group, i.e. crustacean (Daphnia) is not represented in the dataset.*

Based on these considerations a factor of 10 for the derivation of the PNEC is judged not sufficiently protective and we suggest to apply a factor of 50 to the lowest NOEC: PNEC = 3 µgTRC/l/50 = 0.06 µgTRC/l corresponding to 2.1 µgFAC/l/50 = 0.04 µgFAC/l."

Regarding the PNEC for microbial communities in the WWTP, no value is proposed in the RARs due to the following arguments:

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Degradation

Decomposition, i.e., the oxidation and reduction reactions occurring during the decontamination process upon contact of ASC with the surface of a poultry carcass, and occurring anyway in time, ultimately results in chloride (Cl^-), which is a normal constituent of all living organisms and waters, and is not expected to represent an environmental impact at the expected concentrations.

Semicarbazide was also considered a potential reaction product (Hoenicke et al., 2004). However, EFSA (2005a) noted that the initial health concerns about semicarbazide, are no longer relevant. Firstly, as new data showed that semicarbazide is not genotoxic *in vivo* (EFSA, 2005c). Secondly, worst-case laboratory experiments using ASC did not form any detectable semicarbazide: potential semicarbazide levels from ASC treatment were below the limit of quantification of the analytical method (≤ 1 µg/kg) and would therefore be of no safety concern.

3.3.3 Trisodium phosphate (TSP)

Chemical name:	Trisodium orthophosphate
Synonym:	Trisodium monophosphate
CAS registry number:	7601-54-9
Chemical formula:	Na_3PO_4
Description:	Colourless or white crystals

Formulation

Trisodium phosphate (Na_3PO_4; TSP) is used in poultry processing applications as an 8 - 12 % aqueous solution, and applied by spraying or dipping carcasses for up to 15 seconds at a temperature of 7 - 13 ºC (FDA, 2002). Since 1994, interim approval has also been granted for the purpose of reducing micro-organisms when applied as an 8 - 12 % aqueous solution by dipping or spraying raw, unchilled carcasses for up to 15 seconds and raw, unchilled poultry giblets for up to 30 seconds, at 18 - 30 ºC (USDA, 2002d).

Toxicity

TSP is a permitted food additive in Europe identified as E 339 (iii) and authorized in several processed foods, including meat products (EC, 1995).

In the USA, sodium phosphates (mono-, di-, and tri-) are considered GRAS as multipurpose ingredients in food (FDA, 2002). This GRAS status recognition was issued through experience based on common use in food and considering that the substance was used in food prior to January 1, 1958.

A maximum tolerable daily intake (MTDI) of 70 mg/kg body weight for phosphates was established by the Joint FAO/WHO Expert Committee on Food Additives and Contaminants (JECFA; WHO, 1982).

The SCF (1991) confirmed the MTDI value estimated by the JECFA for phosphates used as food additives. Both evaluations concluded that the main risk, related to the ingestion of these additives, was their potential effect on the calcium-phosphorus-magnesium body balance.

Ecotoxity

Trisodium phosphate is ionised in water generating Na^+ and PO_4^{3-} ions. The toxicity of both ions is very low. However, phosphates are nutrients for algae and plants and may contribute to the eutrophication phenomena.

Degradation

TSP does not degrade or decompose in the proper sense of the word. In aqueous solution it readily dissociates into its ionic components, Na^+ and PO_4^{3-}. These ions are normal constituents of all living organisms and natural waters.

3.3.4 Peroxyacids

Definition:	Formulation of peroxyacetic acid (<15%), peroxyoctanoic acid (<2%), and hydrogen peroxide <10%)
Chemical names:	Ethaneperoxoic acid; octaneperoxoic acid, and hydrogen dioxide
Synonyms:	Peroxyacids; acetyl peroxide; acetyl hydroperoxide
CAS registry numbers:	79-21-0, 33734-57-5 and 7722-84-1, respectively
Chemical formulas:	CH_3COOOH, $C_7H_{15}COOOH$ and H_2O_2, respectively
Description:	Clear, colourless liquid

Formulation

To generate a peroxyacid mixture, suitable for use as an antimicrobial agent in poultry processing, acetic acid (AA; 100%) is mixed with (in this order) deionised water, 1-hydroxyethylidene-1,1-diphosphonic acid (HEDP; synonym etidronic acid; 60%), octanoic acid (OA; 100%) and hydrogen peroxide (HP; 35%) at approximately 25 ºC in the proportion of 55:10:1:4:30 (w/w), and allowed to equilibrate for some 7 to 10 days. In this process, HP oxidises AA and OA to peroxyacetic acid (PAA) and peroxyoctanoic acid (POA), respectively. HEDP is added as a stabiliser because of its metal chelating activity: metals catalyse the reduction of HP, PAA and POA.

The FDA (2001) approved the use of peroxyacid mixtures as antimicrobial agents in poultry processing at maximum concentrations of 220 mg of PAA per L, 110 mg of HP per L, and 13 mg of HEDP per L.

The concentration of HP can rather easily be determined, but analytical measurements to differentiate between PAA and POA are relatively complex, time-consuming and expensive. For practical purposes the concentration of the peroxyacids is measured as the sum of PAA and POA, corrected for the different molecular weights of PAA and POA, and expressed as PAA[5]). In order not to exceed the maximally allowed PAA concentration of 220 mg/L, the PAA concentration in poultry process water is generally aimed at 200 mg PAA per L, thus allowing for 10% variation in target peroxyacid composition. Over a period of 6 months the total peroxyacid composition will decrease by about 4%; peroxyacids containing process water has a shelf life of 12 months (USDA, 2002c).

Toxicity

The most recent evaluation of peroxyacid solutions has been performed by JECFA (WHO, 2005). Whereas HEDP is stable in solution, the peroxyacids rapidly breaks down to AA, OA, water and oxygen upon contact with organic matter.

Food containing residues of AA and OA, arising from the use of peroxyacid antimicrobial solutions, has previously been considered as safe for human consumption (SCVPH, 2003; WHO, 2005).

For the peroxyacids (as peroxyacetic acid), SCVPH (2003) cites a LOAEL of 0.13 mg peroxyacetic acid/kg bw/day based on increased spleen weight and increased hemosiderin in spleen red mater in rats receiving the compound via drinking water for four weeks.

For hydrogen peroxide a NOAEL of 26 mg/kg bw/day for males and 37 mg/kg bw/day for females was identified in a 90-day oral study using catalase-deficient mice. The NOAEL from a rat gavage study was 30 mg/kg bw per day (SCVPH, 2003).

For HEDP a NOAEL of 500 mg/kg bw/day was identified from two 90-days feeding studies in rats (WHO, 2005). Notably, histopathological lesions, including gastrointestinal erosion, were observed upon HEDP treatment at the higher doses tested (SCVPH, 2003; WHO, 2005). When tested in a 90-day study in dogs at oral dose levels up to 250 mg HEDP/kg bw/day no adverse effects were reported (WHO, 2005), whereas a NOAEL of 50 mg HEDP/kg bw/day, via the diet, was found in a combined two-generation study of reproductive toxicity and teratogenicity in rats. No evidence of teratogenicity was found, but HEDP was embryotoxic at 250 mg/kg bw/day. HEDP was not teratogenic in rabbits but a similar NOAEL of 50 mg HEDP/kg bw/day was found for embryotoxicity (WHO, 2005). In humans, an oral starting dose of 5 mg HEDP/kg bw/day, for not longer than 6 months, is used to treat Paget disease (WHO, 2005).

Ecotoxity

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[5]) Total peroxyacid concentration = [weight% PAA] + [weight% POA × 76/160], in weight% PAA equivalents.

The compilation presented in IUCLID suggests that, as expected, the toxicity of acetic acid to aquatic organisms is basically associated with the pH reduction.

No ecotoxicity information on the peroxyacids has been submitted. Henkel has presented a report with some information on hydrogen peroxide, which cannot be validated.

An EU Risk Assessment Report on Hydrogen Peroxide is available and offers the following summary of toxicity data on aquatic organisms and PNEC derivation:

"There is a complete "base-set" of acute toxicity data for hydrogen peroxide. From the three base-set species tested, algae seem to be the most sensitive species for the aquatic compartment with an EC50 of 1.6 mg/l. The lowest EC50 for daphnia (2.3 mg/l) is of the same order.

There is one freshwater algae study (72-hour), where a NOEC value can be regarded as long-term toxicity value. A long-term study on zebra mussel which represents the same trophic level as daphnids is available and can be taken into account when determining the assessment factor.

However, as there are no long-term data available on fish, an assessment factor of 50 should be used. Using the result from the algae test (NOEC = 0.1 mg/l) and the assessment factor of 50 the PNECaquatic would be 2 µg/l."

The Henkel report also offers a table with toxicity data on HEDP suggesting low acute toxicity to fish, daphnia and bacteria (based on in-house Henkel reports). However, as the original studies have not been presented, the Committee cannot validate this information. It should be noted that no information on toxicity to algae is reported, thus the data set is in any case insufficient for a proper assessment.

Regarding the activity of microbial communities in the WWTP, the following estimation is done in the RAR:

"The EC50 value of the activated sludge respiration test has been used in the calculation of PNEC.

According to the TGD the EC50 value from the OECD 209 guideline (466 mg/l) is divided by an assessment factor of 100.

PNECmicroorganisms = 4.66 mg/l.

However, it is well known that waste water treatment plants, especially adapted industrial WWTPs, are able to tolerate much higher concentrations without adverse effects on the functioning of the WWTPs"

Degradation

Decomposition, i.e., the oxidation and reduction reactions occurring during the decontamination process upon contact of peroxyacids (HP, PAA and POA) with the surface of a poultry carcass, and occurring anyway in time, results in the formation of H_2O, AA and OA, respectively.

3.4. Expected use patterns and release estimations

The use of these substances is currently not allowed in the EU. The approved use patterns in the USA and the conditions described by the companies in the submitted information should be used to estimate the potential environmental releases if the use of these substances is approved in the EU.

3.4.1. Use conditions at the slaughterhouse

With this method the contaminated surface is either immersed (as in the case of small objects) in a sterile fluid, or the fluid brought into contact with the surface being treated. This may require acetic removal of part of the surface into the diluents. During 2-3 minutes the carcasses will be exposed to a solution of 1-2 ppm acid. In larger operation it is anticipated that a spray treatment is most likely to be the primary mode of application. The spray will be applied via pressurised spray nozzles, for varying exposures times up to about 30 seconds before the product exits the enclosure.

In order to minimize the potential for possible off-gassing into the immediate worker environment, the semi-enclosed spray enclosure will be negatively pressurized via an aspirating air hose venting to the outside of the building. This should ensure the removal of excess gaseous materials, while a dedicated drainpipe will route excess fluids to an enclosed drain for removal.

In smaller operations, where the application of products is expected to typically hand-held “on/off” applicators, the volume of product used is reduced compared to larger commercial systems.

In some facilities ASC products will typically be applied both pre- and postchill.

3.4.2. Release estimations

The release estimations of the different chemicals from the slaughterhouse production were calculated using the following scenario.

The slaughterhouse identify in this scenario processes 50 tons /day of meat. This value is the threshold designated by the IPPC Directive. The EPER database indicates that just a few slaughterhouses in the EU are above this limit. The very large facilities, exceeding this production level, have specific environmental controls through the IPPC Directive. However, since the large majority of slaughterhouses in the EU are below this limit, the 50 tons meat per day limit is consider appropriate for a generic assessment. It is assumed that slaughterhouses not covered by the IPPC may discharge waste water from the production directly to the municipal WWTP without pre-treatment at the production site.

Following standard use conditions in USA, for the proposed treatment of carcasses 50 l of disinfectant solution are employed per 1000 lb carcasses corresponding to 454 kg meat.

It has previous been identified that the optimal initial concentrations in the disinfectant solution is:

[chlorine dioxide] = 50 ppm

[sodium chlorite] = 1200 ppm

[phosphate] = 8-12 % corresponding to 80 – 120 g / l

[PAA] = 220 ppm

[H_2O_2] = 110 ppm

The ecotoxicity data presented in the RARs refers to free chlorine. As the conditions in the effluent are unknown, a precautionary worst case approach has been selected, based on the maximum theoretical amount of free chlorine that could be produced by the treatments with chlorine dioxide and acidified sodium chlorite. Regarding the peroxy acids, the RAR presents a PNEC for hydrogen peroxide; the toxicity of the PAA is estimated through its potential to release hydrogen peroxide.

The considered default WWTP has a size of 10.000 PE as outlined in the TGD. Such a WWTP treats 2.000.000 litre of waste water (house hold plus domestic waste water). In this scenario it is anticipated that the WWTP treats 835.000 litre of waste water from the slaughterhouse that already is included in the total treatment of 2.000.000 litres.

The total amount of waste water produced per carcass corresponds to 16.7 l/kg carcass.

Producing 50 tons/day of carcasses demands 5506 litres of disinfectant treatment solution.

For this assessment, the following three PEC/PNEC ratios are estimated:

Table 1 - The estimated PEC/PNEC ratios

Scenario 1	Scenario 2	Scenario 3
PEC water: waste water from slaughterhouse discharged to the environment, using the TGD default dilution factor of 10 PNEC: for aquatic organisms	PEC WWTP: waste water from slaughterhouse discharged to 10.000 PE WWTP (influent concentration) PNEC: for WWTP microbial community	PEC water: waste water from slaughterhouse discharged from the 10. 000 PE WWTP, using the TGD default dilution factor of 10 PNEC: for aquatic organisms

For the treatment of carcasses the following concentrations of the chemicals chlorine, phosphate, PAA and H_2O_2 are assumed 9.24; 0.27; 220 and 110 ppm, respectively.

Based on the usages concentration of the chemicals, the results are calculated in table 2.

Table 2 - Preliminary risk estimations for the three scenarios. Scenario 1: direct discharge of the slaughterhouse waste water into aquatic bodies. Scenario 2: risk for the municipal WWTP receiving the slaughterhouse waste water. Scenario 3: tentative risk assessment for the slaughterhouse waste water discharged through a default municipal WWTP.

Compound	Treatment level mg/l	Amount g/day	Waste water level g/l	PNEC WWTP µg/l	PNEC water µg/l	Scenario 1 PECwater µg/l	Scenario 2 PEC WWTP µg/l	Scenario 3 PECwater µg/l
Free Chlorine	50	147,9	1,76E-04	?	0,04	17,60	74	7
Free Chlorine	1200	6607	7.86E-03	NA	0.04	306	1281	128
phosphates	10000	550661	1.77E-06	NA	NA	65515.3	275574	27557.4
PAA	220	1211.5	1.44E-03	9320	4	144.13	604	60
H_2O_2	110	605.7	7.21E-04	4660	2	72.07	302	30

Table 3 - PEC/PNEC ratio for the three scenarios

Compound	Scenario (1)	Scenario (2)	Scenario (3)*
Free Chlorine	440-7643	NA	184-3203
Phosphate	Corresponds to 520 PE exposure		
PAA	36	0.065	15
H_2O_2	36	0.065	15

*Note: This scenario does not consider the degradation within the WWTP

Results show that for scenario 1 concentrations of 0.78 mg/l, 65.5mg/l, 0.14 mg/l and 0.07 mg/l for Chlorite, phosphate, PAA and H_2O_2, respectively is predicted for direct discharges of the slaughterhouse waste water into water bodies using the default TGD dilution factor of 10. These estimations results in PEC/PNEC ratios of a range between 440-7643, 36 and 36 for discharging the waste water into the environment.

For phosphate it may be concluded that 550 kg/day is used daily. Using the figure of 1.5 g phosphorous/person/ day, representing an additional discharge of 520 PE of phosphate daily in the STP (10.000 PE).

Considering the waste water to be discharged to a local STP (10.000 PE) no unacceptable risks for the microbial activity of the WWTP are estimated.

For scenario 3, worst case tentative PEC/PNEC values for the diluted waste water is computed to a range between 184-3203, 15 and 15 for Chlorite (as Cl), PAA and H_2O_2, respectively. However, free chlorine, PAA and H_2O_2 are highly reactive and will be largely dissipated in the WWTP minimizing their risk for the environment.

Nevertheless, as mentioned in the RARs some degradation products of chlorine may be of environmental concern. It should be also considered that in several EU MSs, waste waters from municipal WWTP undergo chlorination of the effluent at the facility.

3.5 Environmental impact assessment for each substance

The potential environmental impacts that should be considered when assessing the use of these chemicals as antimicrobial agents to treat poultry carcasses are:

- The chemical risk associated with the releases of each chemical into the aquatic environment or into WWTPs, which can be estimated through the comparison of PNEC for aquatic organisms and for WWTP microbial communities respectively, with the PEC.
- The nature, toxicity and predicted concentrations of any by-products resulting from the interaction of each biocide with aqueous media and with organic matter.
- The consequences of the effect of the biocide on micro-organisms, in particular the potential to produce resistant organisms and the implications of this.
- The contribution from the use of each biocide for carcass treatment to the total environmental load of biocides and antibiotics in waste water treatment facilities and the wider environment.

Based on the available information, preliminary assessments are presented below.

3.5.1 Chlorine dioxide (ClO_2)

Use patterns and mechanism of action

Chlorine dioxide is an oxidizing agent with a low redox potential. For use as an antimicrobial agent it is added to water in a concentration of up to 50 mg/L in order to maintain a residual concentration of 2.5 mg/L (USDA, 2002a).

Chlorine dioxide is reduced in water generating the chlorite ion:

$$ClO_2 + e^- \rightarrow ClO_2^-$$

Chlorite is reduced to chloride ion:

$$ClO_2^- + 4H^+ + 4e^- \rightarrow Cl^- + 2H_2O$$

In the absence of oxidisable substances in water and presence of alkali, chlorine dioxide gives chlorite and chlorate ions:

$$2ClO_2 + H_2O \rightarrow ClO_2^- + ClO_3^- + 2H^+$$

Environmental Impact Assessment

Two different scenarios should be considered, the impacts associated with the disposal of the chlorine dioxide solution at the treatment facility and the environmental consequences associated to the residues in poultry carcasses.

Impacts associated to the effluents at the slaughterhouse

The impact on the environment of the use of chlorine dioxide treatment in slaughterhouses premises has to be assessed in relation with the conditions of use and of effluent treatment.

Chlorine dioxide is very reactive and is rapidly transformed to chlorite and chlorate ions in a ratio of 7:3. Thus, the concentrations of chlorite and chlorate (starting from a concentration of 50 mg/L) would be 33 and 14 mg/L, respectively. Only 2.5 mg/L (about 5% of the initial content) is estimated to remain as chlorine dioxide.

The maximum concentration allowed for uses in processing water in chiller baths is 3 mg/L. So, we can assume that the final products are:

ClO_2^- = about 33 mg/L

ClO_3^- = about 14 mg/L

ClO_2 = about 3 mg/L

The available data on the treatment of poultry carcasses with chlorine dioxide does not indicate a safety concern. Further data would be helpful to confirm that chlorinated compounds are not generated to a significant extent.

The environment impact would depend on the treatment of these effluents at the slaughterhouse. The direct discharge of used solutions into water bodies may represent a significant impact. Even after dilution with the slaughterhouse waste waters the estimated PEC/PNEC (scenario 1) is 440, indicating a potential environmental risk even if less than 0.25% is presented as free chlorine.

The RAR does not consider risk for the biological processes of the WWTP. The discharge of the treatment solution through a WWTP (scenario 3), would represent a potential environmental risk if over 0.5% of the chlorine is still present as free chlorine.

The information available to the Committee is limited and the potential risk associated to the expected by-products, produced by the contact of chlorine with organic matter, cannot be assessed. Generic information on the potential impact of these by-products can be found in the EU RARs.

Impacts associated to the presence of residues in the carcasses.

According to SCVPH (2003), poultry carcasses would incorporate, after decontamination with chlorine dioxide for 1 hour, 0.13 mg chlorite and 0.06 mg chlorate per kg carcass. In addition, 0.01 mg chlorine dioxide, in the form of chlorite, per kg carcass would also be incorporated. Assuming a poultry consumption of European adults of about 50 g/day (based on the assumption that poultry meat represents one third of total meat consumption), the potential dietary consumption of chlorine dioxide would be up to 0.5 µg/day at the mean. In the case of chlorite and chlorate, potential consumption for a 60 kg individual would be up to 7 and 3 µg/day respectively, at the mean.

It follows that, by assuming a waste water production of about 200 L/day per capita, and that the total amount of this waste will be discharged (unrealistic worst-case assumption), the contribution of total chlorinated compounds to WWTP would be of the order of 0.03-0.07 µg/L. This concentration can be assumed as negligible, without any effect on the microbial processes of WWTP, as well as on the environment.

3.5.2 Acidified sodium chlorite ($NaClO_2$)

Use patterns and mechanism of action.

Sodium chlorite, at a concentration of 500-1200 mg/L, is activated with any acid approved for use in foods at levels sufficient to provide solutions with pH values in the range 2.3-2.9 for either a 15 second spraying or 5-8 second dipping. In the case of immersion in chilling water, the concentration is up to 150 mg/L at pH between 2.8 and

3.2. The mean residence time of poultry carcasses in the chiller is typically one hour but can be as long as 3 hours (USDA, 2002b).

The main active ingredient of acidified sodium chlorite (ASC) solution is chlorous acid which is a very strong oxidizing agent. The level of chlorous acid depends on the pH of the solution. So, 31% is formed at pH 2.3, near 10% at pH 2.9 and only 6% at pH 3.2. The potential formation of chlorine dioxide is limited, not exceeding 1-3 mg/L (International registration Dossier, 2003).

The addition of acid to sodium chlorite generates chlorous acid through the following reaction:

$NaClO_2 \rightarrow ClO_2^- + Na^+$

$ClO_2^- + H^+ \rightarrow HClO_2$

Other compounds like chlorine dioxide, chlorite ion and chlorate ion are generated, their proportion depending on the pH of the mixture. Below pH 4.0, chlorite ion reacts with extra acid to give chlorous acid but it is unstable and dissociates back to chlorite ion, reaching equilibrium:

$ClO_2^- + H^+ \rightarrow HClO_2$

Chloride ion may be formed from the oxidation/reduction of chlorous acid and chlorite ion via the following reactions:

$HClO_2 + 3H^+ + 4e^- \rightarrow Cl^- + 2H_2O$

$ClO_2^- + 4H^+ + 4e^- \rightarrow Cl^- + 2H_2O$

The usual application conditions are:

(a) in poultry processing waters applied as pre-chiller or chiller solutions at concentrations between 50 and 150 mg/L of sodium chlorite combined with an acid permitted for food use that achieves a pH between 2.8 – 3.2 in the solution; the solution shall be applied as an immersion dip for up to 5-8 seconds;

(b) in poultry processing waters applied as spray solutions at concentrations between 500 and 1200 mg/L of sodium chlorite combined with an acid permitted for food use that achieves a pH between 2.3 – 2.9 in the solution; the solution shall be applied as a spray for up to 15 seconds in total.

So, final products are:

$HClO_2$ = from 6 to 31 %

ClO_2^- = about 66% of the remaining

ClO_3^- = about 28% of the remaining

ClO_2 = about 6% of the remaining

Environmental Impact Assessment

Two different scenarios should be considered, the impacts associated to the disposal of the acidified sodium chlorite solutions at the treatment facility and the environmental consequences associated to the residues in poultry carcasses.

Impacts associated to the effluents at the slaughterhouse

The impact on the environment of the use of acidified sodium chlorite treatment in slaughtering premises has to be assessed in relation with the conditions of use and of effluent treatment.

By assuming, as a worst case, spray solutions at concentrations of 1200 mg/kg of sodium chlorite, concentrations of different products (at a pH of 2.9) should be:

$HClO_2$ = about 120 mg/L

ClO_2^- = about 700 mg/L

ClO_3^- = about 300 mg/L

ClO_2 = about 60 mg/L

For dipping batches the maximum concentrations are:

$HClO_2$ = about 15 mg/L

ClO_2^- = about 85 mg/L

ClO_3^- = about 40 mg/L

ClO_2 = about 10 mg/L

The environment impact would depend on the treatment of these effluents at the slaughterhouse. The direct discharge of used solutions into water bodies may represent a significant impact. Even after dilution with the slaughterhouse waste waters the estimated PEC/PNEC (scenario 1) is 7643, indicating a potential environmental risk even if less than 0.03% is presented as free chlorine.

The RAR does not consider risk for the biological processes of the WWTP.

The discharge of the treatment solution through a WWTP (scenario 3), would represent a potential environmental risk if over 0.1% of the chlorine is still present as free chlorine.

The information available to the Committee is limited and the potential risk associated to the expected by-products, produced by the contact of chlorine with organic matter, cannot be assessed. Generic information on the potential impact of these by-products can be found in the EU RARs.

Impacts associated to the presence of residues in the carcasses

The levels of chlorite and chlorate in the carcasses were 0.54 mg and 19 µg per kg carcass, respectively. The levels of chlorite and chlorate were also determined in post-treated carcasses up to 20 hours after the treatment. The residual chlorite and chlorate levels in the poultry carcasses were 16 and 19 µg per kg carcass, respectively. This leads to a potential dietary assumption per capita to chlorite and chlorate of up to 0.8 and 1 µg/day, respectively.

In this case too, by assuming a waste water production of about 200 L/day per capita, the contribution of chlorinated compounds to WWTP would be lower than 0.03 µg/L. This concentration can be assumed as negligible.

3.5.3 Trisodium monophosphate (TSP)

Use patterns and mechanism of action.

Trisodium phosphate is typically used in aqueous solutions containing 8 to 12% with a high pH value (pH 12). The solution is kept at a temperature between 7 and 13ºC and applied by dipping or spraying the carcasses for up to 15 seconds. Carcass exposure time is controlled by process line speed and length of the time in application cabinet. It is critical that the concentration is maintained above 8% for keeping the treatment effective.

The mechanism of action involves a combination of high pH, ionic strength, and detergent effects. The high alkalinity in solution (pH 12.1) can disrupt cell membranes and remove fat films causing the cell to leak intracellular fluid. These combined actions result in cytoplasmic membrane disruption and an increase in the water solubility of DNA. It can also act as a surfactant contributing to elimination of bacteria not yet strongly adhered to the surface of poultry skin (SCVPH, 2003; Sampathkumar et al., 2003; EFSA, 2005a).

Environmental Impact Assessment

Trisodium phosphate is ionised in water generating Na^+ and PO_4^{3-} ions. The toxicity and ecotoxicity of both ions is very low. The environmental impact of sodium ion is associated with the increase in ionic strength, and is expected to be of low relevance. Thus, the main environmental impacts associated to the use of TSP are related to the increase in the anthropogenic emission of phosphate ions, and their potential contribution to the eutrophication process.

Two different scenarios should be considered, the impacts associated with the disposal of the TSP solution at the treatment facility and the environmental consequences associated to the increase of phosphate in the poultry.

Impacts associated to the effluents at the slaughterhouse

The impact on the environment of the use of TSP treatment in slaughtering premises has to be assessed in relation with the conditions of use and of effluent treatment.

The use of TSP results in release of orthophosphate. It can be found at high concentrations in the dipping / spraying solution and at low concentrations in the drainage from the carcasses after treatment.

The environmental impact would depend on the treatment of these effluents at the slaughterhouse, and the total amount of released phosphate.

The direct discharge of used dipping solutions into water bodies directly or through a WWTP will increase the anthropogenic contribution of phosphate. The relevance of this contribution would depend on the waste water management practices at the facility. It should be noticed that standard WWTP operations produce a minor (ca. 20%) reduction in phosphate retention, and that specific tertiary treatments for phosphate removal are required in order to obtain a high reduction in the overall emissions. An eutrophication risk assessment model has been developed by INIA and commented by the SCHER (SCHER, 2007), and after validation, could be used as a tool for quantitative estimations of the risk if required.

Impacts associated to the increase on phosphate in the carcasses.

According to previous estimations by the SCVPH (2003), the treatment of poultry carcasses with trisodium phosphate (TSP) would incorporate up to 480 mg TSP per kg carcass (worst case estimations). This amount will be released into the environment mostly through municipal WWTP, as results of washing processes and releases by humans after consumption of poultry.

A meat consumption figure of 150 g per person per day is assumed in this assessment. Assuming that poultry may represent about one third, the estimation should be about 24 mg of TSP per person and day (equivalent to 4.5 mgP/person and day). Considering that the contribution of phosphorus from human metabolism is about 1.5 gP per person per day as average, the maximum expected increase in phosphate releases to WWTP due to the use of STP for disinfection of poultry carcasses is 0.3 %.

3.5.4 Peroxyacids

Use patterns and mechanism of action

Peroxyacids consist of a mixture of peroxyacetic acid, octanoic acid, acetic acid, peroxyoctanoic acid, hydrogen peroxide, and HEDP (1-hydroxy-1-ethane-1,1-diphosphonic acid).

The solution is used at a maximum concentration of total peroxyacid, expressed as peroxyacetic acid, of 220 mg per L, a maximum concentration of hydrogen peroxide of 110 mg per L, and a maximum concentration of HEDP of 13 mg per L. This solution may be used either in on-line reprocessing (15 second sprays or washes) or up to 60 minute immersion in chiller baths to limit the potential for microbial cross-contamination. A combined amount of peroxyacids, expressed as peroxyacetic acid, is usually given due to the difficulties in the analytical differentiation between peroxyacetic and peroxyoctanoic acids.

Microorganisms are killed by oxidation of the outer cellular membrane. A secondary mechanism could be the acidification of the carcass surface.

Environmental Impact Assessment

Peroxyacids are highly reactive. Upon application to the carcasses, acetic acid, octanoic acid, water and oxygen are generated as natural breakdown products. Under normal practices negligible environmental releases of peroxyacetic acid, peroxyoctanoic acid or hydrogen peroxide are expected.

Several products have been identified after disinfection treatment of surface water with peroxyacetic acid. These compounds are 1-methoxy-4-methylbenzene, nonanal and decanal (Monarca *et al.* 2003; 2004).

Two different scenarios should be considered, the impacts associated to the disposal of used solutions at the treatment facility and the environmental consequences associated to the residues in poultry carcasses.

Impacts associated to the effluents at the slaughterhouse

The impact on the environment of the use of peroxyacids in slaughtering premises has to be assessed in relation with the conditions of use and of effluent treatment.

The use of peroxyacids results in solutions with high concentrations of acetic and octanoic acids in the dipping / spraying solution and at lower concentrations in the drainage from the carcasses after treatment.

The environment impact would depend on the treatment of these effluents at the slaughterhouse. The direct discharge of used solutions into water bodies may represent a significant impact. Even after dilution with the slaughterhouse waste waters the estimated PEC/PNEC (scenario 1) is 36, indicating a potential environmental risk even if less than 3% is presented as hydrogen peroxide.

A low risk for the biological processes of the WWTP (scenario 2) is expected.

The discharge of the treatment solution through a WWTP (scenario 3), would represent a potential environmental risk if over 10% of the added amount is still present as hydrogen peroxide.

The information available to the Committee is limited and the potential risk associated to the expected by-products cannot be assessed. Generic information on the potential impact of these by-products can be found in the EU RAR.

The risk associated to HEDP (1-hydroxy-1,1-diphosphonic acid) cannot be quantified as insufficient validable information is presented on the environmental fate and ecotoxicity of this component.

Impacts associated to the presence of residues in the carcasses.

The environmental consequences associated with the presence of residues in the poultry carcasses are expected to be negligible due to the low level of residues:

- < 0.25 mg per kg carcass for peroxyacids and hydrogen peroxide.
- 40-170 μg HEDP per kg meat of the carcasses treated in the chiller bath.

3.6 Assessment of possible resistance and by-product production following each proposed treatment.

3.6.1 Chlorine dioxide (ClO_2)

Chlorine compounds are highly active oxidising compounds and probably exhibit their action by destroying the functionality of various proteins (Bloomfield 1996). The activity of chlorine compounds is dependent on pH and has highest activity at low pH (McDonnel and Russel 1999). It is well known that bacteria have a natural protection against oxidants such as superoxide and hydrogen peroxide. In contrast there is limited knowledge on their protection mechanisms against other oxidants.

At this moment there is no published information indicating cross-resistance or co-resistance between chlorine dioxide and antibiotics. No studies have been published on the selection for antibiotic resistance by enhancing DNA uptake due to exposure to ClO_2. Mechanisms which could allow ClO_2 exposure to influence antibiotic resistance have not been reported. There is also no indication that the use of ClO_2 could support the spread of antibiotic resistance by direct selection, although it may be possible by indirect selection.

Tolerant strains to hydrochlorous acid (HOCl)) were reported for Salmonella by Mokgatla et al. (1998), or other species (Chesney et al., 1996). This phenomenon seems not yet been published for ClO_2, even the mode of action seems to be identical.

To investigate the possible protective mechanisms involved in the increased tolerance to HOCl, Mokgatla et al. (2002) have studied one resistant *Salmonella* strain and a sensitive one at a final active concentration of 28 mg/l of HOCl. The resistant *Salmonella* isolate differed from the sensitive one in a number of ways: production of catalase and decrease of the activity levels of hydroxyl radicals and singlet oxygen, moieties thought to be integrally involved in the antibacterial action of HOCl. Furthermore, the resistant strain did not display the same degree of DNA damage as did the sensitive one. Thus this mechanism of protection by decreasing the levels of species that could react with oxidants to generate toxic reactive oxygen radicals and by improved DNA damage repair mechanisms, demonstrated for HOCl, is probably efficient against ClO_2.

A single study has indicated that the production of glutathione can protect *Escherichia coli* against the activity of chlorine (Chesney et al*.*, 1996). There are however, no reports on naturally occurring chlorine resistant bacteria or cross-resistance. The only study found in the recent literature referring to the application of ClO_2 was performed by Jin and Lee (2007) on the combined effect of aqueous chlorine dioxide and modified atmosphere packaging on vegetables (mungbean sprouts). The total mesophilic microorganisms were not significantly reduced by treatment with water or ClO_2 (100 mg/l for 5 minutes at 5°C). However, when samples were packaged under vacuum or gas following treatment with ClO_2, the populations of total mesophilic microorganisms were significantly reduced during storage. During this research the antimicrobial sensitivity of *Salmonella typhimurium* and *Listeria monocytogenes* was not studied.

3.6.2 Acidified sodium chlorite

The antimicrobial action of ASC is attributed to chlorous acid, which is derived from the conversion of chlorite ion into acid form under acidic conditions. Chlorous acid kills microorganisms by direct action on the cellular membrane and by oxidation of cellular constituents (Castillo et al., 1999; SCVMPH, 2003). The relationship between ASC and antibiotic resistance of Salmonella has not been tested so far. However, cross-resistances (mediated by a genetic linkage) to antibiotics and to chlorine biocides other than ASC have been previously reported (Langsrud et al., 2003; Russell, 2003).

3.6.3 Trisodium phosphate

Trisodium phosphate is an effective chemical for minimizing bacterial populations on carcass surfaces. Trisodium phosphate exhibits its action by a combination of high pH, ionic strength and detergent effects. A recent study has looked at the effects of trisodium phosphate adaptation of *Escherichia coli* 0157 (Yuk and Marshall 2006). The authors did not make any strong conclusions, but indicated that reduced susceptibility to trisodium phosphate was associated with increased susceptibility to acid and thereby perhaps less likelihood of passing the gastric barrier. The authors did not look at cross-resistance to other antibiotics. A similar result of increased acid sensitivity following trisodium phosphate adaptation was also found for *Salmonella Enteritidis* (Sampathkumar et al. 2003). This later study also found trisodium phosphate adaptation to induce termo-tolerance.

No studies on the comparative susceptibility of different bacterial species or cross-resistance to therapeutic antibiotics have to our knowledge been published.

3.6.4 Peroxyacids

Peroxyacids exhibit their action by the production of reactive oxygen species (ROS's), which are antimicrobial activity. These compounds are also produced during normal respiration under aerobic conditions. Bacteria and other respiratory cells have developed a number of mechanisms to cope with such substances. The best known are catalases and superoxide dismutase, which are found in all bacteria that can grow under aerobic conditions. When used for disinfection peroxyacids are used in such high concentrations that the normal defence mechanisms are unable to cope with them. It must be considered unlikely that bacteria will develop high-level resistance to ROS's.

Recently, high frequencies of hypermutable isolates (mutators) have been observed in natural, clinical and experimental bacterial populations. This has especially been detected for *Pseudomonas* obtained from chronically infected lungs, as shown by the applicants (Ciofu et al. 2005, Oliver et al. 2000). Evolution of multidrug resistant bacteria occurs very fast in mutators due to accumulation of multiple mutations occurring simultaneously. The most likely inducers of these hypermutable populations are reactive oxygen species, which induce oxidative damage to the bacterial DNA (Ciofu et al. 2005, Oliver et al. 2000). In addition, the stress response exhibited by ROS's might also induce the SOS-response, which has been shown to promote horizontal gene transfer of both antibiotic resistance genes (Beaber et al. 2003) and virulence genes (Ubeda et al. 2005), further showing the general importance of this response.

There is also a difference in the intrinsic susceptibility of bacteria to these compounds. Thus, the use might change the microflora. However, *Salmonella* bacteria were more susceptible to H202 than *Escherichia coli* and enterococci (Aarestrup and Hasman 2004).

4. CONCLUSIONS

The environmental impacts associated with the use of these four antimicrobial substances have been assessed for two main scenarios:

1. the disposal of used solutions and leaching waters, and
2. the environmental impacts associated to the presence of residues in the poultry carcasses and their further release under normal use practices.

The disposal of used solutions

The impact of the disposal of used solutions at the treatment facilities may be relevant and should be included. Not enough information is available to produce comprehensive quantitative assessments however some general conclusions can be presented:

- The preliminary assessments indicate that the direct discharge of chlorine dioxide and acidified sodium chlorite used solutions may represent a significant risk for the receiving water bodies even after dilution with the slaughterhouse waste waters. The discharge through a Waste Water Treatment Plant (WWTP) may contribute to the overall inflow of chlorine. According to the EU Risk Assessment report (RAR), no risk to the biological processes within the WWTP is expected. The risk for the waters receiving the WWTP effluent would depend on the amount of remaining free chlorine. The environmental relevance is also associated to the WWTP practices, as some facilities include chlorination processes of the effluents.

- In addition to free chlorine, chlorination by-products can be produced due to these treatments. The environmental risk of these by-products cannot be assessed on the basis of the available information.
- Trisodium phosphate used solutions should be managed in order to avoid releases of phosphate into the aquatic environment, with the associated risk of eutrophication. Eutrophication risk assessment models are now available and, once validated, could be used for quantitative estimation after calibration at the local level.
- Peroxyacids used solutions are composed principally of acetic and octanoic acids. The preliminary assessments indicates that the direct discharge of solutions, containing residual amounts of hydrogen peroxide and/or peroxyacids, may represents a significant risk for the receiving water bodies even after dilution with the slaughterhouse waste waters. For the discharge through a WWTP low risk for the biological processes within the WWTP is expected. The risk for the waters receiving the WWTP effluent depends on the amount of remaining hydrogen peroxide and/or peroxyacids. The effluent treatment in a well managed WWTP facility is considered sufficient to minimize the environmental impacts of these acids. The remaining issue is the potential risk of HEDP and potential by-products, which cannot be addressed based on the available information.

The presence of residues in the poultry carcasses

- The potential residues in the carcasses have been also evaluated, on the basis that the presence of chemicals in consumer products represents a diffuse source of environmental releases. A low environmental risk from this source has been estimated for the four assessed chemicals.

Antimicrobial resistance through the environment

- Limited specific evidence on the potential of these treatments to produce bacterial resistance, if used on poultry carcasses, is currently available. Nevertheless, the chemicals are able to select less susceptible strains of Salmonella and some other pathogens. There is insufficient data to determine whether they may cause cross resistance to antibiotics or the selection of specific microbial groups associated to resistance.
- Sufficient information on the conditions for application of the substances for the removal of the microbial surface contamination of poultry carcasses should be available for the evaluation of the efficacy and subsequently the potential emergence of acquired reduced susceptibility to these substances and/or resistance to therapeutic antimicrobials.
- Information about intrinsic and extrinsic factors influencing the efficacy of the substances is needed from the manufacturing company.
- There is an environmental concern about the possibility that resistant strains could be disseminated or selected in the waste waters and the general environment. In addition to the human health risk, the production of bacterial resistance is relevant for the environmental impact assessment. Additional information is needed for a proper assessment of these issues and the environmental consequences. This cannot be covered in this opinion due to the lack of available information.

5. REFERENCES

Aarestrup FM and Hasman H. Susceptibility of different bacterial species isolated from food animals to copper sulphate, zinc chloride and antimicrobial substances used for disinfection. Vet. Microbiol. 2004; 100(1-2):83-9

Bacon RT, Sofos JN, Kendall PA, Belk KE and Smith GC. Comparative analysis of acid resistance between susceptible and antimicrobial-resistant Salmonella strains cultured under stationary-phase acid tolerance-inducing and noninducing conditions. J. Food Prot. 2003; 66:732-40

Beaber JW, Hochhut B, Waldor MK. SOS response promotes horizontal dissemination of antibiotic resistance genes. Nature. 2003;427:72-4.

Bloomfield, S. F. 1996. Chlorine and iodine formulations. In: Ascenzi, J. M. (ed.). Handbook of disinfectants and antiseptics. Marcel Dekker Inc, New York, USA, pp 133-158.

Braudaki M and Hilton HC. Adaptative resistance to biocides in *Salmonella enterica* and *Escherichia coli* O157 and cross-resistance to antimicrobial agents. J. Clin. Microbiol. 2004; 42:73-8

Castillo A, Lucia LM, Kemp GK and Acuff GR. Reduction of Escherichia coli O157:H7 and Salmonella typhimurium on beef carcass surfaces using acidified sodium chlorite. J. Food Prot. 1999; 62:580-4

Chesney JA, Eaton JW and Mahoney, JR Jr. Bacterial glutathione: a sacrificial defense against chlorine compounds. *J. Bacteriol.* 1996; 178(7):2131-5.

Ciofu O, Riis B, Pressler T, Poulsen HE and Hoiby N Occurrence of hypermutable *Pseudomonas aeruginosa* in cystic fibrosis patients is associated with the ossidative stress caused by chronic lung inflammation. Antimicrob. Agents Chemother. 2005; 49: 2276-82

Del Rio E, Capita R, Prieto M and Alonso-Calleja C. Comparison of pathogenic and spoilage bacterial levels on refrigerated poultry parts following treatment with trisodium phosphate. Food Microbiol. 2006; 23:195-8

Del Rio E, Panizo-Moran M, Prieto, M Alonso-Calleja C and Capita R. Effects of various chemical decontamination treatments on natural microflora and sensory characteristic of poultry. Int J Food Microbiol 2007; 115:268-80

Dincer AH and Baysal T. Decontamination techniques of pathogen bacteria in meat and poultry. Crit. Rev. Microbiol. 2004; 30:197-204

EC 1971 Council Directive 71/118/EEC of 15 February 1971 Official Journal of the European Communities 8.3.1971, L55, p. 23–39

EC 1995, European Parliament and Council Directive 95/2/EC of 20 February 1995 (amended) Official Journal of the European Communities 18.3.1995 L61, p. 1–40

EC 2004, Regulation N° 853/2004 of the European Parliament and of the Council of 29 April 2004. Official Journal of the European Communities 30.04.2004, L139, p.47-55

EFSA Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food of the European Food Safety Authority on the treatment of poultry carcasses with chlorine dioxide, acidified sodium chlorite, trisodium phosphate and peroxyacids. The EFSA Journal 2005a; 297:1-27

EFSA Opinion of the Scientific Panel on Biological Hazards on the valuation of the efficacy of peroxyacids for use as an antimicrobial substance applied on poultry carcasses. The EFSA Journal 2005b; 306:1-10

EFSA Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food of the European Food Safety Authority on semicarbazide in food. The EFSA Journal 2005c; 219:1-36

Erill I, Campoy S, Barbé J. Aeons of distress: an evolutionary perspective on the bacterial SOS response. FEMS Microbiol Rev. 2007; 31(6):637-56.

FDA US Food and Drug Administration, Secondary Direct Food Additives Permitted in Food for Human Consumption. Federal Register 65/228 2001; 70660-1

FDA. US Food and Drug Administration, Department of Health and Human Services: Regulation 21CFR182.1778 title 21: Food and Drugs, Chapter 1, Subchapter B: Food for Human Consumption, Subpart B: Multiple Purpose GRAS Food Substances - Sodium Phosphate, 2002; p. 458

FVE, Federation of Veterinarians of Europe public Health. Implementing measures of the hygiene package, p4-6. Newsletter May 2006, Brussels

Foster KL, Stress-induced mutagenesis in bacteria. Crit. Rev. Biochem. Mol. Biol. 2007;42(5):373-97.

Gilbert P and McBain AJ. Potential increased use of biocides in consumer products on prevalence of antibiotic resistance. Clin. Microbiol.Rev. 2003; 16(2) 189-208

Griffith KL, Shah IM, Wolf RE Jr. Proteolytic degradation of Escherichia coli transcription activators SoxS and MarA as the mechanism for reversing the induction of the superoxide. (SoxRS) and multiple antibiotic resistance (Mar) regulons. Mol. Microbiol. 2004; 51(6):1801-16.

Hastings PJ, Rosemberg SM, Slack A. Antibiotic-induced lateral transfer of antibiotic resistance. Trends Microbiol. 2004; 12(9):401-4

Hoenicke K, Gatermann R, Hartig L, Mandix M, Otte S,. Formation of a semicarbazide (SEM) in food by hypochlorite treatment: is SEM a specific marker for nitrofurazone abuse? Food Add. Contam. 2004; 21:526-537

HYDROGEN PEROXIDE RISK ASSESSMENT REPORT. Available at the ESIS database http://ecb.jrc.it/esis/

IPCS. Disinfectants and disinfection by-products. Environmental Health Criteria 216, International Program on Chemical Safety, 2000. World Health Organization, Geneva, Switzerland.

IUCLID FILE ACETIC ACID. Available at the ESIS database http://ecb.jrc.it/esis/

Jin HH and Lee SY Combined effect of aqueus chlorine dioxide and modified atmosphere packaging on inhibiting *Salmonella typhimurium* and *Listeria monocytogenes* in mungbean sprouts. J. Food Science 2007; 72(9) 441-5

Langsrud S, Sidhu MS, Heir E and Holck AL. Bacterial disinfectant resistence – a challenge for the food industry. Int. biodet. Biodegr. 2003; 51:283-90

McDonnell, G and Russell AD Antiseptics and disinfectants: activity, action, and resistance. Clin. Microbiol. Rev. 1999; 12(1):147-79.

Meyer B. Does antimicrobial resistance to biocides create a hazard to food hygiene? Int. J. Food Microbiol. 2006; 112:275-9

Mokgatla RM, Brozel VS and Gouws PA. Isolation of Salmonella resistant to hypochlorous acid from a poultry abattoir. Letters Appl. Microbiol. 1998; 27:379-82.

Mokgatla RM, Gouws PA and Brozel VS Mechanisms contributing to hypochlorous acid resistance of Salmonella isolate from a poultry-processing plant. J. Appl. Microbiol. 2002; 92(3):566-73.

Monarca S, Rizzoni M, Gustavino B, Zani C, Alberti A, Feretti D, Zerbini I. Genotoxicity of surface water treated with different disinfectants using in situ plant tests. Environ. Mol. Mutagenesis 2003; 41:353-359.

Monarca S, Zani C, Richardson SD, Thruston AD Jr, Moretti M, Feretti D, Villarini M (2004) A new approach to evaluating the toxicity and genotoxicity of disinfected drinking water. Water Res. 38: 3809-3819.

Oliver A, Canton R, Campo P, Baquero F and Blazquez J High frequency of hypermutable *Pseudomonas aeruginosa* in cystic fibrosis lung infection. Science, 2000; 288:1251-4

Oyarzabal OA Reduction of Campilobacter spp. By commercial antimicrobials applied during the processing of broiler chickens: a review from the United State perspective. J. Food Prot. 2005; 68:1752-60

Pomposiello PJ, Bennik MH and Demple B Genome-wide transcriptional profiling of the *Escherichia coli* responses to superoxide stress and sodium salicylate. J. Bacteriol. 2001; 183(13):3890-902.

Potenski CJ, Gandhi M and Matthews KR Exposure of *Salmonella enteritis* to chlorine or food preservatives increases susceptibility to antibiotics. FEMS Microb. Letters. 2003; 220:181-6

Russell AD Biocide use and antibiotic resistance: the relevance of laboratory findings to clinical and environmental situations. Lancet Infect. Dis. 2003; 3:794-803

Sampathkumar B, Khachatourians GG, and Korber DR High pH during trisodium phosphate treatment causes membrane damage and destruction of Salmonella enterica serovar Enteritidis. Appl. Environ. Microbiol. 2003; 69:122–9

SCF European Commission, Report of the Scientific Committee on Food on a First Series of Food Additives of Various Technological Functions. Reports of the SCF, 1991; 25th series.

SCHER opinion on model implementation and quantification of the eutrophication risk associated with the use of phosphates in detergents (INIA report – Green Planet Report), 29 November 2007

SCVPH Report on benefits and limitations of antimicrobial treatments for poultry carcasses, 1998

SCVPH Opinion on the evaluation of antimicrobial treatments for poultry carcasses, 14-15 April, 2003

Ubeda C, Maiques E, Knecht E, Lasa I, Novick R P and Penades JR Antibiotic-induced SOS response promotes horizontal dissemination of pathogenicity island-encoded virulence factors in staphylococci. Mol. Microbiol. 2005; 56(3): 836-44.

USDA The use of chlorine dioxide as an antimicrobial agent on poultry processing in the United States; United States Department of Agriculture, Foreign Agricultural Service, and Food Safety and Inspection Service, Office of International Affairs; 2002a

USDA The use of acidified sodium chlorite as an antimicrobial agent on poultry processing in the United States; United States Department of Agriculture, Foreign Agricultural Service, and Food Safety and Inspection Service, Office of International Affairs; 2002b

USDA The use of peroxyacids as an antimicrobial agent on poultry processing in the United States; United States Department of Agriculture, Foreign Agricultural Service, and Food Safety and Inspection Service, Office of International Affairs; 2002c

USDA The use of trisodium phosphate as an antimicrobial agent in poultry processing in the United States; United States Department of Agriculture, Foreign Agricultural Service, and Food Safety and Inspection Service, Office of International Affairs; 2002d

Walsh TR Combinatorial genetic evolution of multiresistance. Curr. Opin. Microbiol. 2006; 9(5):476-82

WHO, Food additives series 17, 1982; World Health Organization, Geneva, Switzerland

WHO, Guidelines for drinking-water quality, 3rd edition, 2004; World Health Organization, Geneva, Switzerland

WHO, Sixty-third report of the Joint FAO/WHO Expert Committee on Food Additives and Contaminants. Technical Report Series 928, 2005; World Health Organization, Geneva, Switzerland

Yuk HG and Marshall DL Effect of trisodium phosphate adaptation on changes in membrane lipid composition, verotoxin secretion and acid resistance of *Escherichia coli* O157:H7 in simulated gastric fluid. Int. J. Food Microbiol. 2006; 106:39-44

4. LIST OF ABBREVIATIONS

AA	Acetic Acid
ASC	Acidified Sodium Chlorite
CD	Chlorine Dioxide
EFSA	European Food Safety Authority
EPER	European Pollutant Emission Register
GRAS	Generally Recognised As Safe
HACCP	Hazard Analysis and Critical Control Points
HEDP	Etidronic acid
HP	Hydrogen Peroxide
OA	Octanoic Acid
PAA	Peroxyacetic Acid
PE	Population Equivalents
PEC	Predicted Effect Concentration
PNEC	Predicted No Effect Concentration
POA	Peroxioctanoic Acid
RAR	Risk Assessment Report
SCENIHR	Scientific Committee on Emerging Newly Identified Health Risks
SCVPH	Scientific Committee on Veterinary Measures Relating to Public Health
STP	Sodium tri-polyphosphates
TGD	Technical Guidance Document
TSP	Tri Sodium Phosphate
WWTP	Waste Water Treatment Plant

5. SUPPORTING DOCUMENTS

1. Report of the Scientific Committee on Veterinary Measures relating to Public Health on "Benefits and limitations of antimicrobial treatments for poultry carcasses" (adopted on 30 October 1998).
2. Opinion of the Scientific Committee on Veterinary Measures relating to Public Health on the "Evaluation of antimicrobial treatments for poultry carcasses" (adopted on 14-15 April 2003).
3. Opinion of the Scientific Panel on Biological Hazards on the "Evaluation of the efficacy of peroxyacids for use as an antimicrobial substance applied on poultry carcasses" (EFSA-Q-2005-106A) adopted on 14-15 December 2005.
4. Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on "Treatment of poultry carcasses with chlorine dioxide, acidified sodium chlorite, trisodium phosphate and peroxyacids" (EFSA Q2005-002) adopted on 6 December 2005.
5. "Short report on the assessment of risks posed by use of disinfectants" prepared by the Community reference laboratory for antimicrobial resistance on 29 March 2007, 4 pp.
6. The proposed conditions for use of the four substances have been added as an annex.