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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)

Table of contents

1 EXECUTIVE SUMMARY

In addition to the SCVPH opinion of 1998 and in consideration of the provided documentation and the published scientific evidence it is concluded that:

- The level of pathogens on poultry carcasses may be controlled by applying an integrated control strategy throughout the entire food chain. Provided this strategy has been followed, decontamination can constitute a useful element in further reducing the number of pathogens. In the case of a high bacterial load, the decontamination procedures under consideration will not meaningfully reduce the risk for the consumer.

- The documentation provided demonstrates that chlorine dioxide, acidified sodium chlorite, or trisodium phosphate are efficient against spoilage and pathogenic bacteria present on poultry carcasses in terms of reducing the pathogen load, albeit not in eliminating it. Data on the efficacy of peroxyacids are limited.

- Combined or consecutive decontamination steps may further reduce microbial levels. However, studies covering this issue have not been published. Hence the toxicological effects of several antimicrobial agents used simultaneously or consecutively cannot be evaluated.

- The evidence on microflora changes are inconclusive. However, it cannot be excluded that eliminating competitive flora may favour the growth of pathogens.

- The sensory effects of these products (chlorine dioxide, acidified sodium chlorite, peroxyacids, trisodium phosphate) on poultry are negligible.

- The toxicological risk for the consumer of poultry decontaminated with either chlorine dioxide, or acidified sodium chlorite, or peroxyacids, or trisodium phosphate, resulting from residues of these agents, is negligible.

- Reactive agents like chlorine dioxide, acidified sodium chlorite and peroxyacids may induce chemical changes in poultry carcasses. However, reaction products have not been identified and consequently a toxicological evaluation is not possible.

2 BACKGROUND

The Scientific Committee on Veterinary Measures relating to Public Health (SCVPH) has, on 30 October 1998, adopted a report on 'benefits and limitations of antimicrobial treatments for poultry carcasses'. In the report, a framework is established for the assessment of decontamination compounds. The Committee recommended that:

- antimicrobial treatment should only be used as part of an overall strategy for pathogen control throughout the whole production chain;

- before any decontamination compound or technique is authorised for use it should be fully assessed;

- the person/company proposing such a decontamination compound or decontamination technique must demonstrate that all aspects considered in the 4th section of the report[1] are covered;

- the person/organisation using a decontamination compound or decontamination technique must demonstrate that effective control of parameters critical for efficacy and safe use are in place and that good practice and appropriate HACCP plans are implemented.

In the framework of the Veterinary Agreement between the EU and the US, the Commission is required to carry out a scientific review of the use of antimicrobial techniques in the US. The US have recently submitted dossiers on the use of 4 different antimicrobial agents[2], for evaluation by the Commission.

Council Directive 71/118/EEC on health problems affecting trade in fresh poultry meat, as last amended by Directive 97/79/EC, currently prohibits the use of antimicrobial treatment of poultry carcasses. A new draft Regulation laying down specific hygiene rules for food of animal origin is under discussion in the Council and the European Parliament. This Regulation includes a procedure for the authorisation of the use of substances for antimicrobial treatment of carcasses in the EU.

[1] Overall efficacy, microflora changes and implications, potential for introducing other food safety hazards, potential for occupational hazards, impact on the environment, effects on sensory properties and quality of the product, feasibility and effectiveness of control under commercial conditions, consumer perception.

[2] Chlorine dioxide, trisodium phosphate, acidified sodium chlorite, peroxyacids.

3 TERMS OF REFERENCE

The Scientific Committee on Veterinary Measures relating to Public Health (SCVPH) is asked to evaluate the risks to public health resulting from the use of chlorine dioxide, trisodium phosphate, acidified sodium chlorite and peroxyacids (individually and in combination), for the decontamination of poultry carcasses on the basis of :

- its previous opinion issued in October 1998 (and in particular the relevant criteria of the 4th section of the report[3])
- the data provided by the US and the proposed conditions of use
- other available scientific knowledge.

4 INTRODUCTION

Foodborne pathogens pose a significant risk to public health throughout the world (EC, 2002; SCVPH, 2000; WHO/FAO, 2002).

Poultry is frequently incriminated in foodborne disease caused by *Salmonella* spp., and thermophilic *Campylobacter* ssp..

Therefore, decreasing the prevalence of zoonotic pathogens in poultry will help to reduce the burden of foodborne disease. For example the WHO/FAO risk characterisation of the salmonellae in broilers stated that the relationship between the carcass prevalence of *Salmonella* spp. and the attributable risk of human salmonellosis was essentially linear (WHO/FAO, 2002).

Successful control of foodborne human pathogens will require a farm to fork approach involving risk management interventions applied at producers of feed, breeding and production animals, slaughterhouses, processors, distributors, retailers, caterers and consumers. In the case of poultry meat production, the aim must be to establish an integrated control programme throughout the poultry supply chain.

A logical strategy for pathogen reduction in poultry is to ensure the lowest possible prevalence of pathogens in the production pyramid, starting from the top and moving downwards, i.e. from primary breeders (Grand Parent and Parent flocks) throughout the chain to production flocks.

Hygienic measures applied on the farm (including feed control), during transport, and in the slaughter- and processing plant will help to reduce microbial load of poultry.

It should be emphasised that the mechanisms of carcass contamination and hence the distribution of micro-organisms over a poultry carcass is rather specific (Thomas *et al.*, 1987): Microbial contamination of poultry carcasses during processing initially involves retention of bacteria in a liquid film in the skin. From that film

[3] Excluding the potential for occupational hazards and impact on the environment (environmental assessment) as these are not part of the SPS agreement between the EU and US.

organisms may become more closely associated with the skin, becoming entrapped in inaccessible sites. Spray rinsing at several points along the processing line is an effective means of minimising the level of skin contamination. Rinsing is less effective in reducing contamination levels in exposed areas of connective tissue such as the neck flap, which is normally the most heavily contaminated part of the carcass.

Generally, decontamination can be an element of a risk management but should not be used as the primary, or only, pathogen reduction measure. It may not suffice to use decontamination as a substitute for appropriate preventive measures at the production level or at the slaughterhouse. This is partly because most decontamination techniques result in a relative reduction, not elimination of pathogens, depending directly on the initial level of contamination. Hence, if the initial pathogen load is high, decontamination may not reduce the prevalence of positive carcasses.

Therefore, if decontamination treatments are used as a substitute for good hygienic practice, they may even be counter-productive to food safety.

HACCP-based production of poultry is increasingly being introduced within the EU and non-member states with positive effects on food safety (SCVPH, 2000).

Apart from chlorine dioxide, acidified sodium chlorite, trisodium phosphate and peroxyacids, other decontamination procedures have been proposed, used and evaluated. Some of these have been addressed in the Committee's report of 1998 (SCVPH, 1998).

Table 1: Antimicrobial substances considered in previous SCVPH opinion of 1998

chlorine dioxide
trisodium phosphate
acidified sodium chlorite
peroxyacids
organic acid
chlorine
ozone
ionising radiation
cetylpyridinium chloride

In addition to the points addressed below in more detail, the impact of chemical decontamination procedures on the environment in general and in the vicinity of the processing plant have to be considered in an overall assessment of any process.

In particular, oxidative chlorine containing agents such as chlorine dioxide, sodium chlorite and chlorate can have considerable effects on the local and global environment. WHO stated that there are insufficient data to conduct an environmental risk assessment of chlorine dioxide and its major decomposition

products. The few ecotoxicity data available show that chlorine dioxide, chlorite and chlorate can be highly toxic to aquatic organisms (WHO, 2002). Also, substances considered non-toxic such as trisodium phosphate may- if released, be an additional burden on the local environment, because of the high pH, with considerable effects on the wastewater treatment plants.

Occupational safety is not a major topic in this report but it has to be taken into consideration that the chemicals intended for decontamination are aggressive substances which certainly pose a health risk to the personnel handling and applying these.

Acute health hazards such as dermal, mucosal and respiratory irritation can be caused by reactive oxidising agents (chlorine dioxide, peroxyacid or chloric acid) or strong alkaline (trisodium phosphate). Long-term effects may include an increased risk for asthmatic symptoms, (Olin *et al.*, 2002) especially for workers in poultry-slaughterhouses, where an increased risk of respiratory symptoms including asthma have been reported (Zuskin *et al.*, 1995; Perfetti *et al.*, 1997).

These procedures of decontamination of carcasses has to be considered in a general context of actions led within the framework of the American rules at the level of the breeding and of poultry slaughterhouses notably by the statutory implementation of the HACCP systems for controlling potential food safety problems. In these conditions the systems of washing and water cooling of carcasses are recognized as critical points and the use of antimicrobial agents in these points are an integral part of the HACCP approach recognized by the US government. So, it is clearly stated that the main objective of the addition of antimicrobial agents to the chilling water is not to kill the microoganisms adhering on carcasses, but simply to help to limit the presence of pathogenic bacteria in the water to avoid cross-contamination: "Antimicrobial agents use in this application is not intended to mask poor hygiene, but as an aid in preventing cross-contamination". There are various means to limit the cross-contamination during the operations of cooling of poultry carcasses: including use of another mode of cooling such as ventilated air preventing contact between carcasses, or setting up up an effective technology of water cooling using systems with counter-current flows and sufficient quantities of water, both for the previous wash and during the dipping.

In the following the Committee evaluated the public health effects that might result from the use of chlorine dioxide, acidified sodium chlorite, trisodium phosphate and peroxyacids in decontamination procedures of poultry carcasses from both, the microbiological and the toxicological perspective.

5 OVERALL EFFICACY

Generally, the efficacy of these products is based on a destruction of the cellular walls of the bacteria. This action results in a significant decrease in numbers of the various types of bacterial flora. The predictable consequence will be an improvement of the microbiological quality of products by a reduction in the number of pathogenic bacteria and, an improved shelf-life of products in the refrigerated state (0 - 4°C) because of destruction of a part of the spoilage bacteria.

Nevertheless, it seems that the efficacy of these antimicrobial agents is influenced by exposure time, temperature, pH and hardness of the water. Furthermore, the

ability of bacteria to attach to the product and to produce a biofilm can influence the efficacy. Other factors affecting this efficacy, such as the presence of fat or organic material (blood, faeces), have been described.

To assess the impact of decontamination on public health the following need to be considered:

- the prevalence of pathogens on carcasses,
- distributions of numbers of pathogens on each carcass,
- the detection limit for the microbiological procedure which may be a fixed number or a probability distribution,
- the infectious dose which may also be a fixed number of a probability distribution,
- the conditions along the food chain determining growth and survival/death of bacteria after decontamination,
- the type of pathogen.

(see also considerations outlined in Annex II)

It may be deduced from these considerations that:

(1) A reduction by, for example, 2 $\log_{10}$ in numbers of pathogens per carcass would have little impact on the prevalence of carcass contamination if the initial number of pathogens is high. However, if the infectious dose is high compared to the detection limit and the conditions after decontamination do not enable growth of the microorganisms, then decontamination could have a noteworthy public health effect.

(2) On the other hand, a 2 $\log_{10}$ reduction of numbers would have a large impact on the prevalence if the initial number of pathogens are around the detection limit. However, if the conditions after decontamination enable growth and cross-contamination, the effects on public health might be nullified.

(3) If the average numbers of pathogens per carcass are very low and under the detection limit no impact on the prevalence would be observed. Nevertheless, if the pathogens could grow thereafter and the decontamination actually eradicates the pathogens from a fraction of the poultry carcasses, it might result in a reduction of the public health risk.

It appears that the level of contamination i.e., the number of pathogens before de-contamination as well as the conditions thereafter along the food chain until consumption are critical parameters when assessing the public health impact of decontamination procedures.

A proper assessment should be in the context of a quantitative risk assessment and in particular the impact on the risk characterisation. For salmonellae in broilers, the risk characterisation was published by WHO/FAO in 2002; it was suggested that the relationship between the prevalence of *Salmonella* spp. in broiler carcasses and the

incidence of human *Salmonella* spp. infections caused by contaminated broiler meat was essentially linear. Moreover, it was stated that a percentage change in *Salmonella* spp. prevalence in broiler carcasses should result in the same percentage change in risk for human salmonellosis attributable to broiler meat.

All in-plant trials presented in the dossiers were realised according to the American Good Manufacturing Practices (NCC, 1992), that is for example, by using chlorinated water on one hand and without taking into account some European technological criteria (e.g. time of passage in the chilling tank, quantity of added water). This could have consequences on the doses of products which would be used to obtain the same efficacy.

5.1 Chlorine dioxide (CD)

Chlorine dioxide kills microorganisms by breaking the cellular membrane and by oxidation of cellular constituents. It seems that the antimicrobial efficacy of chlorine dioxide is not affected by the pH and is more effective than ozone and chlorine in higher organic loading.

The dossier on chlorine dioxide presents many studies demonstrating the efficacy of this product in slaughter plants against a broad spectrum of bacteria and specially *Salmonella* spp., *Campylobacter* spp. and *Escherichia coli.* Results showed a reduction of the level of contamination in the chilling water and on carcasses after chilling (Lillard 1979, 1980; Thiessen *et al.*, 1984).

Approved use:

The approved use is maximum 3 ppm of residual chlorine dioxide for poultry processing water.

Chlorine dioxide is used both at on-line reprocessing applications and chiller baths in poultry processing facilities. The most important effect appears to be the reduction of cross-contamination.

Factors that influence chlorine dioxide include concentration, exposure time, temperature, pH of the water, hardness, how firmly the bacteria are attached to the product (biofilm is much more resistant to decontamination), presence of fat or organic material in the process water.

It appears that the effect is greater on concentrations in water than on carcasses. Thus the greatest benefits could be reduced cross-contaminations during chilling.

In general, 1 to 2 decimal reductions of microbes on carcasses appear to be the common findings (Lillard 1979, 1980; Thiessen *et al*., 1984~~).~~

Specific reductions:

The bactericidal activity of this process was demonstrated several years ago (Thiessen *et al.*, 1984) in a commercial poultry plant, with a very strong efficiency against numerous pathogenic microorganisms (*Salmonella* spp., *Campylobacter* spp., *E. coli*).

Salmonella spp.: Residual concentrations of 0.5 to 1.3 ppm of chlorine dioxide completely eliminate detectable levels of salmonellae in poultry chiller water (Lillard, 1979). So, chlorine dioxide at levels lower than 3 ppm in the chilling water achieves 2 or 3 decimal reductions of these bacteria. The National Chicken Council (NCC, 2002) found a reduction of the prevalence of positive carcasses from 88% to 63% and on turkey carcasses (NTF, 2002) where the pre chill prevalence was around 7% the prevalence was more than halved.

E.coli: Foschino *et al.* (1998) found 5 decimal reductions in water after 30 sec in 1.4 ppm.

Campylobacter spp.: In two commercial broiler processing plants, Doyle and Waldroup (1996) found reductions of 90% of the level of *Campylobacter* spp. in the chill water. These results were confirmed by Marsh (1996).

However, Doyle and Waldroup (1996) conceded that the incidence of *Salmonella* spp. and *Listeria* spp. was not consistently reduced by immersion of carcasses in 1 to 3 ppm of residual chlorine dioxide and that the efficacy is affected by the amount of organic material present in the chill water.

5.2 Acidified sodium chlorite (ASC)

The antimicrobial action of ASC is derived from chlorous acid which kills microorganisms by direct action on the cellular membrane and by oxidation of cellular constituents. Under commercial operating conditions, 150 to 1200 ppm acidified sodium chlorite at a pH of 2.3 to 3.2 can achieve 1 to 2 decimal microbial reductions on poultry carcasses. Solutions of ASC at concentrations higher than 150 ppm may be used for spraying or dipping of carcasses before cooling or cuttings.

***Approved use**:*

(1) In poultry processing waters applied as pre chiller or chiller solutions at concentrations between 50-150 ppm of sodium chlorite combined with a GRAS acid that achieves a pH between 2.8-3.2 in the solution.

(2) In poultry processing waters applied as spray or dip solutions at concentrations between 500-1200 ppm, combined with a GRAS acid that achieves a pH between 2.3-2.9 in the solution.

(3) Common uses include a 5-8 second immersion dip or 15 seconds spray, either pre-chilling or on-line reprocessing application.

The presented trials show the antimicrobial efficacy of ASC products on a number of microorganisms. Indeed, in laboratory experiments the values of a decimal reduction are, in the majority of the cases in the order of 3 seconds for use of the product at 1200 ppm, at pH 2.5 and 20°C.

The application described under (3) above, resulted in reductions of pathogen counts, *Campylobacter* spp. or *Salmonella* spp. were all larger than 2 $\log_{10}$ or 99% (Alcide, 2002).

It was concluded that under commercial conditions acidified sodium chlorite can achieve microbial counts reductions of 1-2 $\log_{10}$ on poultry carcasses (Alcide, 2002;

Kemp 2000, 2001; Warf and Kemp 2002; Schneider *et al.*, 2002; Mullerat *et al.*, 1994, 1995).

These results have been published in international peer-reviewed journals and recognised analytical methodologies were used in these experiments. However, it was noted that all these studies were sponsored by manufacturers of the decontaminant.

Specific reductions:

Salmonella spp.: Mullerat *et al.* (1994) demonstrated reductions of 57-85% of *Salmonella* Typhimurium on drumsticks (i.e. $\log_{10}$ 0.7-0.9).

E.coli: Dipping for 5 seconds followed by 30 seconds residence time allowed reductions to residue levels of less than 1 $\log_{10}$ (10 c.f.u. per ml of liquid rinse of carcass) (Alcide, 2002). Microbial levels prior to dipping were not stated in that report.

Kemp *et al.* (2000, 2001) and Schneider *et al.* (2002) found reductions on faecal contaminated carcasses of 1.7 $\log_{10}$, if the treatment was preceded by fresh water carcass washing, the total effect was a 2.3 $\log_{10}$ reduction.

Campylobacter spp.: Kemp *et al.* (2000, 2001) and Schneider *et al.* (2002) found on faecal contaminated carcasses reductions of 1.5 $\log_{10}$. If the treatment was preceded by fresh water carcass washing, the total effect was a 2.6 $\log_{10}$ reduction.

5.3 Trisodium phosphate (TSP)

Trisodium phosphate is used in numerous food industries and allows to reduce the bacterial contamination of these products, in particular poultry carcasses.

The mechanism of action is not completely understood, but based at the cell-membrane level at a high pH (pH 12) helping to remove fat films and to have surfactant or detergent effect (Capita *et al.,* 2002). Sampathkumar *et al.* (2003), demonstrated a loss of cell viability and membrane integrity and a disruption of cytoplasmic and outer membranes of *S.* Enteritidis strains treated by different concentrations of TSP at pH 10 to 11.

Numerous trials were realized, both at the laboratory level and on industrial sites. At the industrial level, it seems that the ideal use corresponds to a spraying or a dipping of uncooled carcasses (temperature 20-30°C) in a TSP solution at concentrations from 8 to 12 %. In the majority of the trials, a decrease of the bacterial population, mainly pathogenic microorganisms, of 1 to 2 $\log_{10}$ was achieved.

Approved use:

In poultry processing plants, solutions at concentrations of 8-12 % at a temperature of 7-13 °C, are applied by dipping or spraying carcasses for up to 15 seconds. It is critical that the concentration is maintained above 8% for keeping the treatment effective.

On line reprocessing produces a reduction of microbial counts of around one $\log_{10}$ (NCC, 2001).

Specific reductions:

Salmonella spp.: Reductions of *S.* Typhimurium were found in post chill applications from 1.2 to 1.8 $\log_{10}$ (Tamblyn *et al.*, 1993). Dickens and Whittemore (1993) reported reductions of *Salmonella* spp. of 56% (of average numbers) or prevalence. *Salmonella* spp. prevalences were reduced from 25% to less than 1% (Rhone Poulenc, 1994) and from 19% to less than 0.5% (Rhodia, 2001) by pre-chill applications and on-line pre-chill processing respectively. For loosely attached cells a reduction of 1.3 $\log_{10}$ was found while for firmly attached cells a reduction of 1.8 $\log_{10}$ were found by (Tamblyn *et al.*, 1997). Salvat *et al.* (1997) found 2 decimal reductions of *Salmonella* spp.

E. coli: a reduction of prevalences from 78% to ~ 0% (Rhone-Poulenc, 1994) and on visibly soiled carcasses from 88% to 2% (Rhodia, 2001) was observed. A prechill wash with TSP produced more than 2 decimal reductions (NCC, 2002).

Campylobacter spp.: 4 decimal reductions and reduction of prevalence from ~ 100% to 30%, were observed (Rhone- Poulenc, 1994).

Nevertheless it seems that Gram positive bacteria (*Brochothrix thermosphacta* and *Listeria monocytogenes*) are more resistant to TSP treatment (5%) than Gram negative such as *Pseudomonas* spp., *E. coli* and *Salmonella* Enteritidis (Somers *et al.*, 1994; Korber *et al.*, 2002).

Many studies were published in international peer-reviewed journals on the efficacy of TSP against many microorganisms (Capita *et al.*, 2002). These trials were done not only at a laboratory level but also at *in plant* level and concerned poultry and many other foods. In general the decimal reduction was between 1 and 2.

5.4 Peroxyacids (PA)

Peroxyacid used in poultry decontamination is a mixture of peroxyacetic acid, octanoic acid, acetic acid, hydrogen peroxide and peroxyoctanoic acid and 1-hydroxyethylidene-1,1-diphosphonic acid (HEDP). The most powerful mechanism of antimicrobial action is the strong oxidising function of peroxyacetic acid, hydrogen peroxide and peroxyoctanoic acid that disrupts cell membrane permeability and will penetrate bacterial cell walls to disrupt protein synthesis by virtue of reactions with sulfhydryl, sulfide, and disulfide-containing amino-acids and nucleotides (Ecolab, 2001).

The second, possibly subordinate, antimicrobial mechanism is the acidification of the carcass surface thereby decreasing the biological activity as a result of pH changes in the cell's environment; also, undissociated acid will penetrate the bacterial wall and, upon dissociation, acidify the cell interior and hence interfere with the bacterial cell's metabolism (Smulders, 1995)

Approved use:

A mixture of peroxyacetic acid, octanoic acid, acetic acid, hydrogen peroxide and peroxyoctanoic acid and HEDP can be used on poultry carcasses, parts and organs at a maximum concentration of 220 ppm peroxyacetic acids.

Peroxyacetic acid, peroxyoctanoic acid, acetic acid and hydrogen peroxide are suggested to be the main antimicrobials, HEDP is a stabilizer, octanoic acid functions as a surfactant, while acetic acid's primary function is that of an acidifier.

Specific reduction:

The provided data on the antimicrobial effects of peroxyacids reflect the action of both, the oxidative and acidifying mechanisms combined.

Whereas a lot of information on the effects of organic acids for purposes of decontaminating carcass surfaces and cuts - both on the laboratory and in-plant scale - is available (e.g. reviews of Smulders, 1995, Smulders and Greer, 1998, Paulsen and Smulders, 2003), information is scarce for peroxyacids. Yet, in view of the bacterial attachment characteristics and the very distinct associated bacterial topography on poultry (Thomas *et al*., 1987), conclusions cannot be extrapolated from studies on other species but must be based on trials with poultry.

Specific information on the efficacy of peroxyacids for poultry decontamination is not published and therefore major conclusions are drawn from the data of the two studies, one lab-scale one in-plant study, comprising both spray and immersion tests, which were specifically commissioned.

In interpreting the results of these trials the following needs to be considered. When applied as spray (depending on the applied pressure) at least a portion of the prevalent microorganisms are removed by a mere mechanical effect (see Smulders, 1995). Hence, the decontaminating performance that is strictly associated with the chemical agent can be estimated by subtracting the (mechanical) performance of spray treatment with water only from the overall performance of peroxyacid sprays.

The overall reduction in bacterial counts (in-plant tests) (Ecolab, 2001)

Indicators comprised total aerobic counts and total coliforms reduction (in both these cases reductions ranged from 0.6-1-3 $\log_{10}$ units) and generic *E.coli* (reduction 0.8-1.4 $\log_{10}$ units).

Sensitivity of important bacterial pathogens (Ecolab, 2001)

These laboratory trials relate to the sensitivity to peroxyacids (spray or immersion) of three indicator organisms, i.e. *S.* Typhimurium*, L. monocytogenes* and *E. coli* O157:H7.

The reductions achieved with spray treatment of carcasses were::

S. Typhimurium: 2.01 (peroxyacid spray) - 1.26 (mechanic effect of water)= 0.75 $\log_{10}$ units

L. monocytogenes: 3.24 (peroxyacid spray) - 1.13 (mechanic effect of water)= 2.11 $\log_{10}$ units

E.coli: 3.27 (peroxyacid spray) - 0.10 (mechanic effect of water)= 3.17 $\log_{10}$ units

The reductions achieved by immersion treatment of chicken wings and liver were:

S. Typhimurium: 0.32 (wings) - 0.48 (liver) $\log_{10}$ units

L. monocytogenesis: 0.60 (liver) - 1.25 (wings) $\log_{10}$ units

The reductions in the *E. coli* counts are rather substantial and significant in comparison with the very limited reductions usually found after organic acid (lactic or acetic acid) treatment, to which *E. coli* appears rather resistant.

6 MICROFLORA CHANGES AND IMPLICATIONS

The implementation of a process of decontamination of carcasses affects all bacteria present, although certain agents are known to more or less target Gram positive or Gram negative bacteria. This imbalance of the bacterial flora may have 3 consequences:

- The unhampered development of certain potentially pathogenic microorganisms present initially;
- The faster development of the spoilage flora, though possibly subjected to initial stress of the treatment, but subsequently experiencing more favourable conditions because of the absence of competitions;
- Acquired tolerance of bacteria against the agent.

In general, for all agents, the documented evidence about potential effects on the bacterial ecology of poultry carcasses and cuts is circumstantial. A specific study on the 'bacterial association' (including spoilage flora) would indicate if there should be concern about a potential flora shift. However, this information has not been provided specifically for the various agents. With regard to the ecological changes following exposure to low pH conditions (as relevant in the case of peroxyacids) the literature suggests that the flora shift is considered beneficial (Paulsen and Smulders, 2003).

There is an increase of the shelf life depending on the dose of chlorine dioxide (Lillard, 1980, Thiessen *et al.*, 1984). Nevertheless, there are no data on the possible acquired tolerance and the bacterial changes especially for pathogenic bacteria by using this product. There is some evidence of *Salmonella* spp. strains acquiring tolerance to hypochlorous acid (Mokgatla *et al.*, 2002).

The use of acidified sodium chlorite shows also an increase in shelf life of the poultry carcasses (Alcide, 2002), but there are no data on the microflora change, neither on spoilage not on pathogenic bacteria.

The use of trisodium phosphate notably increases the shelf life of poultry carcasses. Some studies showed a modification of the psychrotrophic bacteria. It seems that Gram positive bacteria (*B. thermosphacta* and *L. monocytogenes*) are more resistant to TSP treatment (5%) than Gram negative such as *Pseudomonas* spp., *E. coli* and *Salmonella* Enteritidis (Somers *et al.*, 1994, Korber *et al.*, 2002). Nevertheless, the use of TSP treatment prioritise the growth of spoilage bacteria such as *B.x thermosphacta* before the emergence of other Gram positive bacteria such as *L. monocytogenes* (Salvat *et al.*, 1997).

While reports on reduced susceptibility of bacteria towards certain disinfective agents have been published (Willingham *et al*., 1996; Sander *et al.,* 2002), no data

are available on bacterial tolerance towards these specific antimicrobial treatments, which appears especially possible by a repeated or continued use of these agents.

7 TOXICOLOGICAL FOOD SAFETY HAZARDS

The food safety hazards arising from decontamination of poultry carcasses result from possible residues of the decontaminants used and their decomposition products. Following the Terms of Reference these hazards will be considered for the consumer only. Consequently, the hazards for people occupationally involved in decontaminating poultry carcasses and the environmental hazards will be left aside.

7.1 Formulations

7.1.1 Chlorine dioxide (CD) and acidified sodium chlorite (ASC)

Chlorine dioxide (ClO_2 [gas]; CD) is water soluble, and solutions are quite stable if kept cool and dark. It is marketed and transported as a stabilised aqueous solution generally less than 1% v/v (as more concentrated solutions are explosive). Free CD in air is explosive when its concentration exceeds 10% v/v.

A typical immersion bath used in poultry processing contains initially 20-50 mg CD per L (20-50 ppm), which rapidly decomposes to chlorite and chlorate (in a 7:3 ratio) due to the high content of organic matter in the immersion bath, leaving generally 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). For the terminology of the different chlorine oxides and acids see the Annex.

Sodium chlorite ($NaClO_2$), 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 chlorine dioxide and chloride (Cl^-); see the Annex. Chlorous acid and chlorine dioxide 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 acidified sodium chlorite (ASC) (USDA, 2002b).

7.1.2 Trisodium phosphate (TSP)

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).

7.1.3 Peroxyacids

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) deionized 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 [4]). 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).

7.2 Toxicity

7.2.1 Chlorine dioxide (CD) and sodium chlorite (ASC)

As outlined in the previous paragraph, in considering the toxicity of CD and ASC three substances have to be evaluated: chlorine dioxide (ClO_2), chlorous acid / chlorite ion ($HClO_2$ / ClO_2^-), and chlorate ion (ClO_3^-). Formally also the chloride ion (Cl^-) should be considered, but Cl^- is hardly toxic at all (it is an endogenous constituent of body water of animals including man), and is anyway much less toxic than the various chlorine oxides and acids. Hence, in considering the toxicity of chlorine derivatives to be used as decontaminants for poultry carcasses the toxicity of Cl^- is not relevant.

Chlorine dioxide, chlorite and chlorate were evaluated in 1996 by the EMEA (EMEA, 1996a) and the IPCS (2000). The IARC (1991), the WHO (1996), the EPA (2000) and the ATSDR (2002) evaluated chlorine dioxide and chlorite. The WHO evaluated chlorine dioxide in 2002 (WHO, 2002). The following is an excerpt of these evaluations.

All compounds of interest (ClO_2, ClO_2^- and ClO_3^-) were shown to be well absorbed by the oral route as demonstrated in studies with radiolabelled compounds

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

administered to rats. Absorbed ClO_2 rapidly decomposed, the primary compounds found in plasma were ClO_2^- (20%) and Cl^- (80%). Plasma elimination half-lives of ClO_2^- and ClO_3^- ranged from 35 to 44 h. The main route of excretion was via the kidneys, predominantly as Cl^-, with some ClO_2^- and a little ClO_3^-. No radioactivity was detected in expired air.

Repeated-dose toxicity tests with the compounds of interest in various animal species (rat, mouse, rabbit, and African green monkey) revealed for all compounds a similar pattern of toxicity. The main effect was on haematological parameters (reduced glutathione concentration, reduced erythrocyte counts, reduced packed cell volume, reduced haemoglobin concentration, reduced osmotic fragility of erythrocytes, haemolysis, methaemoglobin formation), observed in rats and mice. ClO_2 dissolved in drinking water and administered to African green monkeys resulted in a dose-related and reversible inhibition of thyroid hormone synthesis (LOAEL and NOAEL were 9 and 3 mg/kg bw/day, respectively). ClO_2^- and ClO_3^- in dosages up to 60 mg/kg bw/day during 30-60 days had no effect on thyroid function.

Reproductive and developmental toxicity of doubtful relevance was seen with ClO_2 and ClO_2^- only at relatively high doses (100 mg/L of drinking water, equivalent with approximately 7 mg/kg bw/day), but at this dose although maternal toxicity was observed.

In teratogenicity studies with the three compounds no effects were found.

Mutagenicity testing resulted in conflicting results. Ames tests with ClO_2 revealed positive effects in only (TA100) out of the six strains tested. It was negative in an *in vitro* chromosome aberration test in Chinese hamster fibroblasts. *In vivo* two mouse micronucleus assays were conducted, resulting in one positive and one negative result. It did not induce sperm head abnormalities in mice.

ClO_2^- was positive in two separate Ames tests, an *in vitro* chromosomal aberration test, and an *in vivo* mouse micronucleus test after intraperitoneal administration. However, it was negative in a mouse micronucleus test when administered orally, and did not induce sperm-head abnormalities in mice.

ClO_3^- was positive in two separate Ames tests with metabolic activation, and in a Drosophila recessive lethal mutation test. It was negative in two mouse micronucleus assays and in a bone marrow chromosomal aberration test.

Although classical carcinogenicity studies have not been performed, long-term experiments with mice (80 weeks, administration of ClO_2^- in drinking water, dose estimates approx. 40 and 80 mg/kg bw/day) and rats (85 weeks, administration of ClO_2^- in drinking water, dose estimates approx. 20 and 40 mg/kg bw/day) did not show significant differences in survival rate or tumour incidences between control and treated groups.

The EPA (2000) classified both chlorine dioxide and chlorite as *'not classifiable as to human carcinogenicity'* (class D) because of inadequate data in humans and animals. The EPA did not classify chlorate. The IARC (1991) classified sodium chlorite as *'not classifiable as to its carcinogenicity to humans'* (group 3) because of inadequate evidence of carcinogenicity in experimental animals and no data on the carcinogenicity in humans. IARC did not classify chlorine dioxide and chlorate.

Various (inter)national organisation derived toxicological limit values for the intake of chlorine dioxide and sodium chlorite. These limit values are summarised in Table 2. For chlorate, however, such limit values were not derived.

Table 2. Toxicological limit values for chlorine dioxide, chlorite and chlorate

Substance	Toxicological limit value (mg/kg bw/day)		Reference
Chlorine dioxide	NOAEL: 3.0 [1])	TDI: 0.03 [1])	IPCS, 2000
	NOAEL: 3.0 [2])	RfD: 0.03 [2])	EPA, 2000
Chlorite	NOAEL: 3.0	RfD: 0.03	EPA, 2000
	NOAEL: 2.9	TDI: 0.1 [3])	ATSDR, 2002
	NOAEL: 1.0	TDI: 0.01	WHO, 1996
	NOAEL: 3.0	TDI: 0.03	IPCS, 2000
Chlorate	NOAEL: 100 [4])	-	IPCS, 2000

For abbreviations see the Annex.

[1]) Based on data on chlorite.

[2]) Expressed as chlorite.

[3]) Oral TDI for intermediate exposure.

[4]) This is the NOAEL from a 90-days study in rats with chlorate in the drinking water, as reviewed by IPCS (2000). This NOAEL is based on pituitary and thyroid gland lesions. A margin of safety of 300 appears advisable (composed of UFs of 10 for intra- and 10 for interspecies variation, and an additional UF of 3 for the lack of chronic data). IPCS (2000) refers to an 'ongoing long-term study in progress' with chlorate.

7.2.2 Trisodium phosphate (TSP)

The ionisation products of TSP, Na^+ and PO_4^{3-}, are normal constituents of living organisms and do not pose a health hazard unless they occur at abnormally high levels.

Sodium phosphates have a very low acute toxicity: for disodium hydrogenphosphate (Na_2HPO_4) the oral LD_{50} in rats is 13 grams per kg bw (NTP, 2001).

In 1975 the JECFA recommended a maximum tolerable daily intake (MTDI) for phosphorus from all sources of 70 mg/kg bw/day (as P; JECFA, 1974), which would equal 13.2 mg TSP per kg bw per day if other sources of phosphorous are ignored. This limit value was the result of considerations involving a LOAEL for nephro-calcinosis of approximately 0.3 - 1.0 gram phosphorous per kg bw per day (equalling approx. 50 - 200 mg TSP per kg bw per day) in a repeated-dose experiment with rats and extrapolation to man based on comparing the energy intakes of rat and humans, and the required intake of phosphorous being an essential nutrient. This MTDI was confirmed in 1982 (JECFA, 1982). More recent data are not available.

7.2.3 Peroxyacids

In considering the toxicity of peroxyacid mixtures 6 substances have to be evaluated: AA, OA, PAA, POA, HP, and HEDP. AA and OA, however, are considered non-

toxic; ADIs/TDIs were not established because the intake of these compounds at the current levels are considered not to be of any safety concern (JECFA, 1997).

7.2.3.1 Peroxyacetic acid (PAA)

PAA as a vapour or liquid is corrosive to the eyes and skin, and to mucous membranes. Pain, irritation and ulceration of tissues may occur after contact. Ingestion may cause difficult swallowing, nausea, vomiting, and oral, oesophageal, and gastrointestinal tract burns, followed by circulatory collapse and shock (ECETOC, 2001a).

The acute toxicity of PAA differs substantially between species: LD_{50} oral rat: 1540 mg/kg; LD_{50} oral guinea pig: 10 mg/kg; LD_{50} skin rabbit: 1410 mg/kg (NTP, 1990).

The only well-conducted repeated-dose study is an oral 4-weeks study with rats reported by Veger *et al.* (1977), in which the animals were administered PAA via the drinking water, with daily freshly prepared solutions. Concentrations were 0, 1, 10, and 50 mg PAA per L in distilled water. Already at the lowest dose (1 mg/L, equivalent to 0.13-0.15 mg/kg bw/day) increased spleen weights and increased hemosiderin in spleen red matter were found (hemosiderin is an iron-containing pigment resulting from excessive breakdown of erythrocytes). In the 10 and 50 mg/L dose groups also spleen abnormalities (cloudy swelling of white pulp), liver (cloudy swelling) and kidney (congestion of medulla) were seen in the majority of the treated animals. Except the spleen, other organ weights (lungs, heart, liver, kidneys, adrenals, stomach) of treated animals were not different from controls. The LOAEL with regard to haematological changes was thus 0.13 mg/kg bw/day (ECETOC, 2001a).

Relevant and reliable data on reproductive and developmental toxicity are not available (ECETOC, 2001a).

Information on the genotoxic and mutagenic potential of PAA is scarce. Its cytotoxicity hampered the evaluation of bacterial test. Two DNA repair tests in human foetal lung cells did not indicate genotoxicity, and neither did an *in vivo / ex vivo* UDS assay in rats. With an *in vitro* chromosome aberration tests positive results were only seen at cytotoxic concentrations. A well-conducted *in vivo* micronucleus test (mice) was negative.

Chronic toxicity or carcinogenicity studies have not been conducted, and the only available initiation-promotion study (mouse skin, applying 40% PAA) suffers from several experimental and reporting deficiencies. The positive effects observed are likely due to a secondary effect following severe local skin irritation (ECETOC, 2001a).

7.2.3.2 Peroxyoctanoic acid (POA)

Data on the toxicity of POA are not available. It is generally accepted that the toxicity of POA will be similar (qualitatively as well as quantitatively) to that of PAA (FDA, 2001).

7.2.3.3 Hydrogen peroxide (HP)

HP is formed intracellularly as a result of oxidative enzymatic reactions. Estimations show that the human liver produces 150-270 mg HP per h, which is rapidly metabolised by catalase. The steady-state concentration in liver is approximately 30 ng/kg (EMEA, 1996b).

Concentrated solutions are toxic to humans if ingested and are corrosive to the skin, eyes and mucous membranes.

Repeated oral exposure in drinking water caused a decrease in body weight gain in most studies and resulted in mortality of rats and mice at concentrations greater than 1%.

Oral administration to catalase-deficient mice for 90 days produced mucosal hyperplasia in the duodenum that was completely reversible after a 6-week recovery period. No effects on reproductive organs were noted. The NOAEL in this study was 100 ppm, corresponding to 26 mg/kg bw/day for males and 37 mg/kg bw/day for females. The NOAEL from a rat gavage study was 30 mg/kg bw/day (EMEA, 1996b; SCTEE, 2001).

Data on the teratogenic potential and reproductive toxicity are limited, and a complete evaluation is not possible. In mice, male fertility was not affected following a dose of 1% in drinking water for 21 days. Rats exposed to 0.005 - 50 mg HP per kg bw per day by gavage for 6 months showed decreased sperm motility at the highest dose. Only 3 out of 9 high-dose females produced litters of pups, the body weight of these pups was decreased. In a further but inadequate study with Wistar rats, foetotoxicity and skeletal hypoplasia, but no teratogenicity was observed at a maternal toxic dose (10% in feed) (EMEA, 1996b; SCTEE, 2001).

Genotoxicity and mutations have been induced *in vitro* in bacteria, yeast and mammalian cells (Chinese hamster V79, mouse lymphoma cells). Chromosomal aberrations and sister chromatid exchanges were observed in human and other mammalian cells. Addition of S-9 mix or catalase abolished or markedly reduced the genotoxic responses. *In vivo* micronucleus assays in mice after single intraperitoneal or 14 day oral administration were all negative, as well as an *ex vivo* UDS test in rat hepatocytes after intravenous infusion of HP. Also a *Drosophila* sex-linked recessive lethal test and two host mediated assays in mice were negative. According to IARC, HP did not induce chromosomal aberrations in the bone-marrow cells of exposed rats (EMEA, 1996b; IARC, 1999; SCTEE, 2001).

HP was tested for carcinogenicity in mice by oral, dermal and subcutaneous administration, in rats by oral administration and in hamsters by topical application to oral mucosa. In catalase-deficient mice, adenomas and carcinomas of the duodenum were found following oral administration in the drinking water at a dose level of approximately 300 mg/kg bw/day. In rats, no increase in the incidence of tumours was observed. The other studies in rats, mice and the study in hamsters are inadequate for evaluation. One study in mice and one study in hamsters showed no promoting activity of HP (IARC, 1999; SCTEE, 2001).

IARC (1999) concluded that there is *inadequate evidence* in humans for the carcinogenicity of HP, and that there is *limited evidence* for its carcinogenicity in

experimental animals. HP was classified in Group 3 *(not classifiable as to its carcinogenicity to humans)*.

7.2.3.4 1-Hydroxyethylidene-1,1-diphosphonic acid (HEDP)

HEDP exhibits low acute oral and dermal toxicity. It is moderately irritating to the skin and is corrosive to the eyes (Ecolab, 2001).

In a 90 days feeding study rats were given a diet containing 0.2, 1.0 or 5.0 % HEDP, approximately equivalent to 250, 500 and 2500 mg/kg bw/day. The group fed 5% HEDP in the diet showed severe mortality and weight loss, and was terminated after 1 week; histopathological lesions included gastrointestinal erosion. The two other groups were necropsied at the end of the study. No significant histopathological lesions or alterations of blood parameters were reported. The NOAEL in this study can thus be concluded to be 500 mg/kg bw/day (Nixon *et al*., 1971).

Developmental studies have been conducted with rats given 0.1 or 0.5 % HEDP in the diet, either during lifetime, or only during days 2-16 of pregnancy through two generations. Consistent or dose-related effects on any reproductive parameter or increases in malformations were not observed. The same authors reported a teratogenicity study with rabbits exposed to 25, 50 or 100 mg HEDP per kg bw per day via the diet, or 100 mg/kg bw/day by gavage during days 2-16 of pregnancy. Adverse effects on any reproductive parameter or increases in malformations were not seen (Nolen and Buehler, 1970).

Hence the available subchronic study with HEDP allows the conclusion of a NOAEL for serious effects including mortality of 500 mg/kg bw/day.

HEDP was not mutagenic in five *Salmonella* Typhimurium tester strains and in L5178Y TK mouse lymphoma cells, with or without metabolic activation. Nothing in the available literature suggests any indication of HEDP being genotoxic and/or carcinogenic (Ecolab, 2001). Toxicological limit values

The toxicological limit values for the various components of peroxyacid mixtures are presented in Table 3.

Table 3. Toxicological limit values of peroxyacid components

Substance	**LOAEL/NOAEL (mg/kg bw/day)**	**Reference**
Peroxyacids (as peroxyacetic acid)	LOAEL: 0.13 [1])	Veger *et al.* 1977, ECETOC, 2001a
Hydrogen peroxide	NOAEL: 26 [2])	EMEA, 1996; SCTEE, 2001
HEDP	NOAEL: 500 [3])	Nixon *et al.*, 1971

For abbreviations see the Annex.

[1]) minor effects.

[2]) effects observed in catalase-deficient mice.

[3]) serious effects including mortality.

7.3 Exposure assessment

7.3.1 Chlorine dioxide (CD)

In a typical decontamination of poultry carcasses with CD, the carcass is immersed in a solution containing initially 20-50 mg CD per L (20-50 ppm). Generally, this is done in the chiller bath that follows evisceration, in order to lower the internal temperature of the carcass from about 40ºC to at least 4.4ºC. The residence time in the chiller bath is approximately 1 h, but can be as long as 3 h (USDA, 2002a). As outlined above, a working decontaminant solution of CD contains at equilibrium 2.5 mg chlorine dioxide, 33 mg chlorite and 14 mg chlorate per L. Studies on the residue formation in poultry carcasses after decontamination with CD are not available. However, it is reasonable to assume a behaviour of the decontaminant chemicals similar to the decontamination process applying ASC as summarised below. Hence a decontamination process of 1 h applying CD would result in maximum residue levels of 0.13 mg chlorite and 0.06 mg chlorate per kg carcass (0.4% of 33 mg chlorite and 14 mg chlorate, respectively), 10 min after finalising the chilling and decontamination procedure. The CD present would only add at most 0.01 mg additional residue in the form of chlorite. Again ignoring the expected decreases in the levels of these residues after processing (including cooking), and assuming an adult individual (with a body weight of 65 kg) consuming 1 kg poultry daily, this would result in an average (worst-case) intake of 0.002 mg chlorite and 0.0009 mg chlorate per kg bw per day.

7.3.2 Acidified sodium chlorite (ASC)

A typical decontamination treatment of poultry carcasses with ASC implies dipping of the carcass in a solution of (maximally) 1.2 g $NaClO_2$ per L (1200 ppm) at a pH of 2.5, firstly the so-called pre-chill immersion dip for 5 minutes, secondly the 1 hour immersion at $\leq$ 5º in a solution of up to 0.15 g $NaClO_2$ per L (150 ppm) (USDA, 2002b).

In its international registration dossier the Alcide Corporation (the manufacturer of ASC) reported several studies regarding the residues of chlorite and other oxychlorine compounds on poultry carcasses decontaminated with ASC applying various treatment regimens (Alcide, 2002). These studies are summarised in Table 4.

The results of these studies showed that immersion of the carcass for one hour in a decontamination bath with 0.15 g $NaClO_2$ per L (at pH 2.8 and 5 ºC) followed by 5 min drip, left relatively the highest residue if analysed immediately after 5 min dripping and 5 min immersion in tap water: 0.54 mg chlorite per kg carcass. This residue concentration rapidly declined in time: already more than 5-fold after 10 min, and below the limit of detection (LOD; 16 µg/kg) after 2 h. The chlorate residue was already below the LOD (19 µg/kg) immediately after dripping. In this study the decrease of residual chlorite in the poultry carcass appears to follow a first-order kinetic process.

From this, the chlorite residue concentration immediately after the 1 h immersion (before dripping) can be estimated to have been 1.7 mg per kg carcass. Since the chlorite concentration in the immersion bath was 150 mg/L, apparently 1.1 % penetrates a 1 kg carcass during a 1 h immersion. This percentage obviously

decreases to approximately 0.4 % after 5 min dripping followed by 5 min immersion in tap water.

Applying a shorter immersion (5 min) in a decontamination bath with a higher concentration of $NaClO_2$ (1.2 g/L at pH 2.5 and unreported but probably ambient temperature) results in residues of chlorite and chlorate measured immediately after 5 min dripping that were both below the LOD (9 and 11 μg per kg carcass, respectively).

Spraying poultry carcasses with a $NaClO_2$ solution containing 1.2 g/L during 15 sec resulted in residues below the LODs, albeit that in these experiments the LODs were relatively high: 100 μg per kg carcass.

A worst-case intake scenario for chlorite and chlorate residues can be evaluated taking the highest residue levels observed in these studies. These were chlorite (92 μg/kg, 10 min after decontamination, dripping and rinsing with tap water) and chlorate (< 19 μg/kg), both found in the study in which carcasses were immersed during 1 h in a decontamination bath containing 0.15 gram ASC per L (Table 4, experiment 2). However, since ASC is permitted in concentrations up to 1.2 g/L, a maximum residue level of 14.4 mg chlorite per kg carcass (1.2 % of 1.2 g/L) and <0.5 mg chlorate can be estimated as being the uppermost level of residue conceivable immediately after a 1 h immersion bath. This will have decreased to approximately 5 mg of chlorite and <0.18 mg of chlorate per kg carcass if this carcass is allowed to drip for 5 min and subsequently is immersed in tap water for another 5 min. Ignoring the expected decreases in the levels of these residues after processing (including cooking), and assuming an adult individual (with a body weight of 65 kg) consuming 1 kg poultry daily, this would result in an average (worst-case) intake of 0.077 mg chlorite and <0.003 mg chlorate per kg bw per day.

Table 4. Chlorite and chlorate residues on poultry carcasses after decontamination with acidified sodium chlorite (data from Alcide, 2002)

Exp.	n	Poultry	Treatment 1	Treatment 2	Assay	Results
1	2	Post-slaughter carcass, 32 ºC	Immersion ASC [1]) 1.2 g/L, 5 min, pH 2.5, ambient temp.	Drip 5 min; immersion tap water 5 min, 5 ºC	carcass at t=0	ClO_2^- : < 9 µg/kg ClO_3^- : < 11 µg/kg
2	2	Post-slaughter carcass, 32 ºC	Immersion ASC 0.15 g/L, 1 h, pH 2.8, 5 ºC	Drip 5 min	carcass at t=0, 10 min; 1, 2, 4, and 20 hr	ClO_2^- : 540 µg/kg [2]) ClO_3^- : < 19 µg/kg [3])
3	5	Post-slaughter carcass, 32 ºC	Spray ASC 1.2 g/L, 15 sec, pH 2.5	Hydrocooling 45 min; deboning and breast removal after 24 h	uncooked breast	ClO_2^- and ClO_3^- : < 100 µg/kg
	5				cooked breast	ClO_2^- and ClO_3^- : < 100 µg/kg
4	5	Post-slaughter carcass, 32 ºC	Spray ASC 1.2 g/L, 15 sec, pH 2.5	Air-chilling, 2 h, 2-3 ºC	uncooked carcass	ClO_2^- and ClO_3^- : < 100 µg/kg
	5				cooked carcass	ClO_2^- and ClO_3^- : < 100 µg/kg

[1]) ASC: acidified sodium chlorite.

[2]) Concentrations (µg/kg) of ClO_2^- were 540 at t=0, 92 at t=10 min, 21 at t=1 hr, and <16 at t=2, 4 and 20 hr.

3) According to the study report (Alcide, 2002), ClO_3^- -concentrations after t=0 have been measured, but were not reported.

7.3.3 Trisodium phosphate (TSP)

Studies on the residues in poultry after being decontaminated with TSP are not available.

However, as estimated in the paragraph on ASC, immersion during 1 h of a poultry carcass in a decontamination solution of 120 grams of TSP per L (which is a worst-case scenario because the standard decontamination treatment with TSP involves a 30 sec immersion, see 7.1.2) would theoretically incorporate at most 120,000 × 0.004 = 480 mg TSP in 1 kg of this carcass after 5 min dripping and 5 min rinsing in tap water. An individual (65 kg bw) consuming 1 kg of this chicken would thus be exposed to 480 mg of TSP, which is equivalent to 7.4 mg/kg bw.

7.3.4 Peroxyacids

For the exposure assessment of peroxyacids only PAA, POA, HP and HEDP are relevant, since the other components of a peroxyacid mixture are considered non-toxic.

7.3.4.1 Peroxyacetic acid (PAA), peroxyoctanoic acid (POA) and hydrogen peroxide (HP)

Upon contact with the surface of a poultry carcass, PAA, POA and HP rapidly decompose to the corresponding acids (AA and OA) and water, respectively.

In a study on residues of PAA, POA and HP conducted by the manufacturer of peroxyacids (Ecolab, 2001) six chicken carcasses (weighing 1164 to 1697 grams) were treated in a manner comparable to the routine decontamination treatment following slaughter. The carcasses were sprayed with a peroxyacid mixture containing 220 mg total peroxyacids (as PAA) per L and 110 mg HP per L during 15 sec at ambient temperature, followed by an immersion for 60 min at 4ºC in a similar mixture containing 200 mg total peroxyacids (as PAA) per L and 100 mg HP per L. Carcasses were then removed from the immersion bath and shaken gently for 10 sec, after which each of them was put in a clean polypropylene bag. Two, five and ten min after removal from the immersion bath 400 ml of deionised water was added to each of two bags, shaken for approximately half a minute, and samples were taken for analysis of residues.

Residue concentrations in the rinse water were measured by iodine - sodium thiosulfate redox titration, with or without a prior oxidation step. This resulted in (1) a total peroxyacid + HP concentration and (2) a total peroxyacid concentration only, respectively. From these the concentration of HP was determined by subtracting (2) from (1).

At all time intervals the concentrations of peroxyacids and HP were below the detection limit of 1 mg/L. The weights of the two carcasses analysed after 2 min were 1649 and 1616 grams, so it can be concluded that already two min after the decontamination treatment the residues were < 400/1000 mg peroxyacids as well as HP per 1616 grams of chicken carcass, equivalent with < 0.25 mg per kg carcass.

This means that an adult individual weighing 65 kg and consuming 1 kg chicken (uncooked weight) is at most (worst-case) exposed to 0.25 mg peroxyacids and 0.25 mg HP, equal to 0.0038 mg of each of the two substances per kg bw at most.

7.3.4.2 1-Hydroxyethylidene-1,1-diphosphonic acid (HEDP)

In a study on the residues of HEDP conducted by the manufacturer of peroxyacids (Ecolab, 2001) six chicken carcasses were treated with two different peroxyacid solutions. Solution 1 contained 200 mg peroxyacids (as PAA), 100 mg HP, 655 mg AA, 52 mg OA, and 10 mg HEDP, all per L. Solution 2 contained 30 mg peroxyacids (as PAA), 15 mg HP, 98 mg AA, 8 mg OA, and 1.5 mg HEDP, all per L. All six carcasses were firstly treated by spraying them during 15 sec with solution 1 at ambient temperature. Secondly the carcasses were submersed in a chiller bath at 2 - 4 ºC, three of them in a bath with solution 1, the other three in a bath with solution 2. After this, carcasses were pulled out of the chiller bath and shaken gently during 30 sec. From each carcass the legs were removed and rinsed in nitric acid (30 mM HNO_3). Legs were then dissected and the weights of bone and meat were determined. HEDP was analysed in the nitric acid rinses. This resulted in residues of 120-170 µg HEDP per kg meat of the carcasses treated in the chiller bath with solution 1, and approximately 40-50 µg HEDP per kg meat of the carcasses treated in the chiller bath with solution 2 (the latter value is quite close to the detection limit of the HEDP analysis).

An adult individual weighing 65 kg and consuming 1 kg of chicken meat is thus exposed to at most 0.17 mg HEDP, equal to 0.0026 mg HEDP per kg bw at most.

7.3.5 Other chlorinated and oxidised residues and by-products

The formation of chlorinated organic substances following decontamination with ASC was investigated by immersion of chicken wings in a solution of 2.5 g chlorite per L (which is actually twice the permitted concentration) during 5 min at pH 2.8 and ambient temperature. After rinsing with deionised water the wings were left soaking overnight in hexane; the resulting hexane was analysed gas-chromatographically. Even at these worst-case conditions chlorinated lipids were not detected; the LOD was substantiated to be 2.5 µg per kg carcass. Similar experiments aiming at detecting potential formation of chlorinated amino acids and/or proteins were negative, i.e., also these residues were not found (Alcide, 2002).

Oxidative changes in poultry carcasses were studied by decontamination treatments applying various treatments with ASC and analysing meat samples for malondialdehyde oxidation products by the thiobarbituric acid assay. Increases in the formation of these products were observed in the skin but not in the muscle of treated samples. However, the increases were small compared with the increases observed after storage and/or cooking. Moreover, the increases in the formation of oxidation products following storage and/or processing (such as cooking) were independent of the decontamination treatments (and were the same as in non-decontaminated skin and meat samples). Although the malondialdehyde – thiobarbituric acid assay is probably not the most sensitive method to detect potentially hazardous oxidation products, the observation that the increase in formation of these substances following storage and processing are larger (also in untreated carcasses) than after decontamination treatment is significant. Hence, the formation of oxidation products due to decontamination with ASC is not relevant in view of the formation of these products during storage and processing (Alcide, 2002).

While no residues of the antimicrobial agents have been detected it is known that chlorine dioxide and chlorite do react quickly with organic matter, such as certain amino acids including cystein, tryptophan, histidine, tyrosin, proline, hydroxyproline and peptides and proteins. Phenols are oxidised to benzoquinones and chlorobenzoquinones. These reactions are obvious in the reported bleaching of poultry skin.

Hydroxyproline and peptides reacted with chlorine dioxide to mutagenic products as detected in *Salmonella* spp. mutagenicity tests (Tan *et al.*, 1987, Owusu-Yaw *et al*., 1991). No studies have been performed on the formation of these mutagenic substance in chlorine dioxide treated poultry.

Mutagenic activity was detected in chiller water containing chlorine dioxide. Although mutagenicity was less compared to chlorinated chiller water. Mutagenic agents formed have neither been identified nor have further genotoxicity studies been reported (Tsai *et al.*, 1997). Mutagenic activity in chlorine dioxide treated poultry meat was not investigated. Monarca *et al.* reported on unidentified mutagenic agents formed by reaction of peroxyacetic acid with organic matter. (Monarca *et al.*, 2002).

Toxicological assessments for chlorine dioxide, sodium chlorite and peroxyacids by international bodies have been performed for water disinfection. Influences of organic matter and reaction products have received less attention.

The Committee on the mutagenicity of chemicals in food in the UK (Department of Health 1998) concluded that there was no difference in mutagenic potential between chlorine dioxide treated and control flour.

Data on oxidative changes in poultry carcasses due to decontamination with peroxyacids are not available and upon application of CD or ASC for decontamination purposes oxidation of lipids was not detected. The formation of oxidised amino acids or proteins was not investigated. Since possible oxidation products have not been identified a toxicological risk assessment of these products is not possible.

7.4 Risk assessment

For a proper risk assessment the exposure scenario involving a daily consumption of 1 kg poultry, including the assumption that it is always decontaminated with one particular substance, is extremely worst case. The average consumption of poultry meat in the EU is estimated to be in the order of magnitude of 32 grams (uncooked weight) per person per day (ECETOC, 2001b). A scenario involving the daily consumption of 100 grams of poultry is therefore considered to be a more realistic worst case approach.

The maximum intakes based on the daily consumption of 100 grams of poultry meat (assumed to be always decontaminated with one particular substance) are summarised in Table 5 and compared with the toxicological limit values as reported in 7.2.

An estimated intake of a particular residue below the TDI indicates no adverse health risks, even at a lifelong daily exposure to this residue.

For residues without an established TDI the intake must be evaluated applying the so-called MOS (margin of safety) approach. The MOS is the quotient of NOAEL and actual intake. If toxicity data are complete and reliable, in general a MOS of 100 is considered to indicate a safe level of exposure, in other words, an intake to which an individual can be lifelong exposed daily without experiencing negative health effects. In the case of peroxyacid mixture substances, a MOS of 1,000 would be recommendable in view of the limited databases of the toxicity of these substances.

The MOSs of the estimated intakes (reasonable worst case) of residues resulting from the consumption of poultry decontaminated with CD, ASC, TSP and peroxyacids (except PAA) indicate a negligible risk.

The MOS for PAA, however, appears to be rather small. But in evaluating this risk it must be recognised that preparing poultry for consumption (cooking, frying, etc.) will inevitably reduce the amount of residues significantly, particularly residues of unstable substances like PAA, HP and chlorite. In the above risk assessments this reduction has not been taken into account. In other words, poultry ready for consumption will generally contain (much) less residues of unstable decontaminants than the worst case levels estimated above.

Table 5. Comparison of toxicological limit values, maximum intakes and margins of safety for residues following decontamination of poultry

Substance	**Toxicological limit values (mg/kg bw/day)**		**Estimated daily intakes (mg/kg bw/day)** [1])	**Margin of safety**
Chlorine dioxide	NOAEL: 3 [2])	TDI: 0.03 [2])	- [3])	- [3])
Chlorite	NOAEL: 3	TDI: 0.03	7.7×10^{-3}	- [3])
Chlorate	NOAEL: 100	-	$<0.3 \times 10^{-3}$	333,000
Trisodium phosphate	LOAEL: 50-200	TDI: 13	0.74	170 [4])
Peroxyacids (as peroxyacetic acid)	LOAEL: 0.13 [5])	-	0.38×10^{-3}	340 [6])
Hydrogen peroxide	NOAEL: 26	-	0.38×10^{-3}	68,400
HEDP	NOAEL: 500 [7])	-	0.26×10^{-3}	1,923,000

For abbreviations see the Annex.

[1]) Based on the daily consumption of 100 grams of poultry meat always treated with one particular decontaminant.

[2]) Based on chlorite.

[3]) No chlorine dioxide residue. Estimated exposure from consuming 100 grams of poultry meat decontaminated with CD: 0.2×10^{-3} mg chlorite and $0,09 \times 10^{-3}$ mg chlorate per kg bw per day, which is considerably less than the residue intake from poultry decontaminated with ASC.

[4]) Compared to mean LOAEL.

[5]) Minor effects.

[6]) Compared to LOAEL.

[7]) Serious effects including mortality.

In conclusion the risk for adverse health effects for an individual consuming approximately 100 grams of poultry per day decontaminated with chlorine dioxide, trisodium phosphate, or peroxyacids, appears to be negligible.

8 EFFECT ON THE SENSORY PROPERTIES AND QUALITY OF THE PRODUCT

8.1 Chlorine dioxide and acidified sodium chlorite

The taste and odour threshold for chlorine dioxide is 0.4 mg/L. Residue levels are certainly far below this level, so no direct sensory effects are to be expected.

In a study on chicken carcasses a colour change from normal pinkish-white to slight greyish-white in chicken breast skin treated with CD was reported (Thiessen *et al*., 1984). No off flavours or off aromas upon oven cooking were detected in ClO_2 treated broilers (Thiessen *et al.*, 1984). Bleaching of the skin was reported in turkey carcasses especially on the wings and breast (Villarreal *et al.*, 1990).

However, controlled scientific studies with consumers or expert panels on sensory properties and consumer perception are not available.

Increased lipid oxidation (measured by thiobarbituric assay) was observed in poultry carcasses samples treated with acidified sodium chlorite by spraying or in a chill tank as compared to water or chlorine treated carcasses. However these effects were small compared to lipid oxidation occurring during cooking (Alcide, 2002).

8.2 Trisodium phosphate

In a studies on sensory changes of chicken legs treated with TSP at different concentrations a detectable chemical off odour and a darker, brownish colour was observed for chicken legs treated with TSP at 10% or higher. (Kim and Marshall, 1999, Capita *et al*., 2000). In a study on chicken breasts and thighs treatment with 12% TSP did not affect consumer acceptance of raw and fried pieces (Hathcox *et al.*, 1995).

8.3 Peroxyacids

While there is no specific information on the sensory effects of peroxyacids, the sensory effects of acid rinses or immersion fluids on poultry can be summarised as follows (Smulders, 1995).

In general, carcass surfaces are whitened slightly. Unless applied in very high concentrations this discoloration tends to be reverted to normal after 24 h.

After treatment acids tend to accumulate in the skin. In effective microbicidal doses acids generally affect odour and flavour. Particularly broiler carcasses treated with acetic acid are reported to have a rather unpleasant vinegar-like odour.

8.4 Consumer perception

No information is published on consumer perception of antimicrobial treatment of poultry with these particular decontaminants, i.e. chloride dioxide, acidified sodium chlorite, trisodium phosphate and peroxyacids.

9 COMBINED AGENTS

Decontamination treatments do not sterilise the carcass but can only reduce the number of microorganisms on the surface. When carcasses are initially contaminated with a high level of pathogenic microorganisms an additional (different) treatment may result in a further reduction of bacterial counts (Pohlman *et al*., 2002). Some combinations are obviously not suitable such as acidic and alkaline treatment. Chlorine and hypochlorite may be formed when alkaline solution, like TSP, are applied on residues of ClO_2 or chlorite. Acidic treatment in combination with chlorine dioxide will result in excessive evaporation of chlorine dioxide gas into the air.

Efficacy

Little to no information is available with regard to the combined use of antimicrobial agents (Pohlman *et al*., 2002). Particularly, no studies are published investigating the toxicological potential of combinations. Therefore, simultaneous or consecutive use of several antimicrobial agents cannot be evaluated.

Other hazards

Water used to chill carcasses could contain chlorine (20 ppm for spray washing and between 20 and 50 ppm in the chilling tank) "in order to facilitate the reduction of total microbiological loads" (NCC, 1992). The amount of chlorine added to the intake water should be sufficient to achieve 1 to 5 ppm available chlorine at the chiller overflow. This practice is not authorized in the European food industries for which only potable drinking water must be used in the contact of foods (Council Directive 71/118/EC).

10 CONCLUSIONS

In addition to the SCVPH opinion of 1998 and in consideration of the provided documentation and the published scientific evidence it is concluded that:

- The level of pathogens on poultry carcasses may be controlled by applying an integrated control strategy throughout the entire food chain. Provided this strategy has been followed, decontamination can constitute a useful element in further reducing the number of pathogens. In the case of a high bacterial load, the decontamination procedures under consideration will not meaningfully reduce the risk for the consumer.

- The documentation provided demonstrates that chlorine dioxide, acidified sodium chlorite, or trisodium phosphate are efficient against spoilage and pathogenic bacteria present on poultry carcasses in terms of reducing the pathogen load, albeit not in eliminating it. Data on the efficacy of peroxyacids are limited .

- Combined or consecutive decontamination steps may further reduce microbial levels. However, studies covering this issue have not been published. Hence the toxicological effects of several antimicrobial agents used simultaneously or consecutively cannot be evaluated.

- The evidence on microflora changes are inconclusive. However, it cannot be excluded that eliminating competitive flora may favour the growth of pathogens.

- The sensory effects of these products (chlorine dioxide, acidified sodium chlorite, peroxyacids, trisodium phosphate) on poultry are negligible.

- The toxicological risk for the consumer of poultry decontaminated with either chlorine dioxide, or acidified sodium chlorite, or peroxyacids, or trisodium phosphate, resulting from residues of these agents, is negligible.

- Reactive agents like chlorine dioxide, acidified sodium chlorite and peroxyacids may induce chemical changes in poultry carcasses. However, reaction products have not been identified and consequently a toxicological evaluation is not possible.

11 GLOSSARY

AA: Acetic acid.

ASC: Acidified sodium chlorite.

ATSDR: Agency for Toxic Substances and Disease Registry, USA.

bw: Body weight.

CD: Chlorine dioxide.

EMEA: European Agency for the Evaluation of Medicinal Products.

EPA: United States Environmental Protection Agency.

FDA: United States Food and Drug Administration.

HEDP: 1-Hydroxyethylidene-1,1-diphosphonic acid.

HP: Hydrogen peroxide.

IARC: International Agency for Research on Cancer.

IPCS: International Programme on Chemical Safety of the World Health Organization.

LOD: Limit of detection.

LO(A)EL: Lowest observed (adverse) effect level - the lowest dose level in a study with experimental animals at which a(n) (adverse) health effect was observed.

MOS: Margin of safety.

NO(A)EL: No observed (adverse) effect level - the highest dose level in a study with experimental animals at which no (adverse) health effects were observed.

OA: Octanoic acid.

PAA: Peroxyacetic acid.

POA: Peroxyoctanoic acid.

RfC: Reference concentration - An estimate of the daily inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime (definition of the EPA).

RfD: Reference dose - An estimate of the daily oral exposure to the human population (including sensitive subgroups) that is likely to be without

an appreciable risk of deleterious effects during a lifetime (definition of the EPA).

SCTEE: Scientific Committee on Toxicity, Ecotoxicity and the Environment of the EC.

TCA: Tolerable concentration in air - An estimate of the concentration in air to which one can be exposed daily during lifetime without adverse health effects.

TDI: Tolerable daily intake - An estimate of the daily dose that can be taken daily during lifetime by the oral route without adverse health effects.

TSP: Trisodium phosphate.

UDS: Unscheduled DNA synthesis.

USDA: United States Department of Agriculture.

WHO: World Health Organization.

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14 ANNEX I– PROPERTIES OF DECONTAMINATION AGENTS

14.1 Chemical and physical properties

14.1.1 Chlorine dioxide and acidified sodium chlorite

Chlorine dioxide (ClO_2; CD) is a greenish to orange-yellow gas with a pungent odour. Its taste and odour threshold in water is approximately 0.4 mg/L. With respect to the use as a decontaminant its high water solubility is particularly noteworthy. Once in solution it does not hydrolyse to any appreciable extent.

Sodium chlorite ($NaClO_2$) is a white crystalline solid. In aqueous solution it has a pH of about 12. Acidified sodium chlorite (ASC) is a clear colourless liquid with a mild chlorine-like odour, which is produced by adding a weak acid to a solution of $NaClO_2$. The active ingredient of ASC at pH 2.3 - 3.2 consists mainly of chlorous acid ($HClO_2$) in equilibrium with chlorite ion (ClO_2^-) and H^+. These can only exist in solution. At a pH >7, ClO_2^- is the primary species present, which slowly decomposes into ClO_3^- and Cl^-, with no ClO_2 or $HClO_2$ being formed. At pH <2.2, however, $HClO_2$ is increasingly converted to ClO_2 which in turn (in the presence of H^+) is converted to additional ClO_2^-, continuing the cycle. An acidified $NaClO_2$ solution thus consists mainly of ClO_2^- ions (65-95% at pH 2.3-3.2, respectively), H^+ ions and $HClO_2$ (35-5% at pH 2.3-3.2, respectively).

For the sake of clarity the terminology of the various chlorine oxides, acids and salts is depicted in Table 8; the chemical-physical properties of CD and ASF are summarised in Table 9.

Table 8. Terminology of chlorine oxides, acids, and salts

Formula	Name	CAS no.	Mol. weight
Cl_2	Chlorine	7782-50-5	70.91
Cl_2O	Chlorine monoxide	7791-21-1	86.91
ClO_2	Chlorine dioxide	10049-04-4	67.45
HCl	Hydrochloric acid	7647-01-0	36.46
Cl^-	Chloride (ion)	16887-00-6	35.45
HClO	Hypochlorous acid	7790-92-3	52.46
ClO^-	Hypochlorite (ion)	14380-61-1	51.45
$HClO_2$	Chlorous acid	-	68.46
ClO_2^-	Chlorite (ion)	7758-19-2 [1])	67.46
$HClO_3$	Chloric acid	7790-93-4	84.46
ClO_3^-	Chlorate (ion)	-	83.46
$HClO_4$	Perchloric acid	7601-90-3	100.46
ClO_4^-	Perchlorate (ion)	-	99.46

[1]) CAS no. of sodium chlorite

14.1.2 Trisodium phosphate

Trisodium phosphate (Na_3PO_4; TSP) is a white or colourless crystalline solid. Aqueous solutions have a distinct sharp alkaline odour. It is used as a cleaning agent, as a water softener, and for preventing corrosion of pipes and tubes. Some chemical and physical properties of TSP are shown in Table 9.

In aqueous solution TSP readily dissociates into its ionic components, Na^+ and PO_4^{3-}. These ions are normal constituents of all living organisms.

14.1.3 Peroxyacids

Peroxyacid mixtures as used in the USA for antimicrobial treatment of poultry carcasses are generally composed of peroxyacetic acid (PAA), acetic acid (AA), peroxyoctanoic acid (POA), octanoic acid (OA, synonym caprylic acid), and hydrogen peroxide (HP), while 1-hydroxyethylidene-1,1-diphosphonic acid (HEDP; synonym etidronic acid) is added as a stabiliser (because of its metal chelating activity). Their use is approved at maximum concentrations of 220 mg of PAA per L, 110 mg of HP per L, and 13 mg of HEDP per L (FDA, 2001). In this mixture, HP, PAA and POA function as antimicrobial agents, AA is an acidifier, and OA is a surfactant and acts as a synergist.

Peroxyacid mixtures are clear, colourless liquids with a sharp, pungent vinegar-like odour. A 1% solution has a pH of 2.3 and a boiling point > 100 ºC. They are strong oxidising agents. The decomposition of peroxyacids is strongly exothermic, liberating oxygen gas. To prevent decomposition, commercially available formulations contain low concentrations of stabilisers.

The physical and chemical properties of the components of the peroxyacid mixture used as decontaminant are summarised in Table 9

Table 9. Physical-chemical properties of decontaminant constituents [1])

	Molecular formula	Mol. weight	CAS reg. no.	Melting point (ºC)	Boiling point (ºC)	Water solubility (g/L)	Density (g/ml)
Chlorine dioxide	ClO_2	67.45	10049-04-4	–59	11	3.01 [2])	1.64 [3])
Sodium chlorite	$NaClO_2$	90.45	7758-19-2	180-200	dec. [4])	390	2.47
Acetic acid	CH_3COOH	60.05	64-19-7	16.6	117.9	miscible	1.05
Peroxyacetic acid	CH_3COOOH	76.05	79-21-0	–0.2	105	miscible	1.23
Octanoic acid	$CH_3(CH_2)_6COOH$	144.21	124-07-2	16.7	239.7	0.068	0.91
Peroxyoctanoic acid	$CH_3(CH_2)_6COOOH$	160.21	33734-57-5	31	n.d. [5])	n.d.	n.d.
Hydrogen peroxide	H_2O_2	43.01	7722-84-1	–0.41	150.2	miscible	1.41
HEDP [6])	$CH_3C(OH)(PO(OH)_2)_2$	206	2809-21-4	n.d.	n.d.	miscible	1.45
Sodium triphosphate	Na_3PO_4	163.9	7601-54-9	75 (dec.)	-	8.8	2.5

[1]) Data mainly taken from references USDA, 2002a-d. Some entries, however, have been corrected due to inaccurate data in these references.
[2]) At 25 °C and 34.5 mm Hg.
[3]) At 0 °C (liquid).
[4]) Decomposes.
[5]) No data available.
[6]) 1-Hydroxyethylidene-1,1-diphosphonic acid; etidronic acid.

14.2 Mode of action

CD, ASC, PAA, POA and HP are oxidising agents, i.e., in chemical reactions they take up electrons. The ease by which a substance gains or loses electrons is expressed by its redox potential (measured in volts), which is the affinity of the substance for electrons (its electronegativity) compared with hydrogen, which is set at zero. Substances more strongly electronegative (that is, capable of oxidising) than hydrogen have a positive redox potential, substances less electronegative than hydrogen (that is, capable of reducing) have negative redox potentials. The redox potential is pH dependent.

The redox potentials of the compounds mentioned above, together with ozone and chlorine as reference agents, are summarised in Table 10. Since, e.g., PAA has a higher redox potential than CD, PAA will react with more compounds than CD.

The antimicrobial action of CD, ASF, and peroxyacid mixtures derives from the oxidising capacity of the constituents. These disrupt the permeability of the bacterial cell membrane, penetrate bacterial cells and disrupt protein synthesis (EPA, 1999).

The mechanism of action of TSP as an antimicrobial agent is assumed to result from its high alkalinity in aqueous solution. A solution of 10 g TSP per L has a pH of 12.1, which can help to remove fat fills to allow the chemical to contact more bacteria, as well as disrupt fatty molecules in the bacterial cell membrane causing the cell to leak intracellular fluid. In addition it is thought that TSP may help prevent attachment of bacteria to poultry skin and may act as a surfactant to facilitate the removal of bacteria from carcasses.

Table 10. Redox potentials of some oxidising agents

Name	Formula	Redox potential (volts)
Ozone	O_3	2.07
Peroxyacetic acid	CH_3COOOH	1.81
Hydrogen peroxide	H_2O_2	1.76
Peroxyoctanoic acid	$CH_3(CH_2)_6COOOH$	n.d. [1])
Chlorous acid	$HClO_2$	1.58
Chlorine	Cl_2	1.36
Chlorine dioxide	ClO_2	0.95
Chlorite (ion)	ClO_2^-	0.78

[1]) No data available.

14.3 Oxidizing reactions

Aqueous oxidation/reduction reactions of the various chlorine-derivatives result in the formation of chloride ions, as shown in Figures 1 and 2. Note that the oxidation/reduction of acidified sodium chlorite involves in fact two oxidising agents (chlorous acid and chlorite ion), and must thus be seen as two separate oxidising/reduction reactions. The oxidation/reduction of chlorine dioxide is a two-step reaction with the formation of chlorite ion (also an oxidising agent) as an active intermediate, resulting ultimately also in two separate oxidising/reduction reactions.

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

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

Fig. 1. Oxidation/reduction of chlorine dioxide

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

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

Fig. 2. Oxidation/reduction of acidified chlorite

Figures 3 and 4, finally, show the contents and decomposition products of chlorite and chlorine dioxide, respectively.

$$NaClO_2 \rightarrow ClO_2^- + Na^+ \qquad \text{(a)}$$

$$\begin{array}{l} \uparrow \rightarrow ClO_2^- + H^+ \leftrightarrow HClO_2 \rightarrow \downarrow \\ \quad \leftarrow H^+ + e + ClO_2 \leftarrow \end{array} \qquad \text{(a)}$$

$$ClO_2^- + H_2O \rightarrow ClO_3^- + 2H^+ + 2e \qquad \text{(b)}$$

$$2ClO_3^- + 8H^+ + 7e \rightarrow ClO_2 + Cl^- + 4H_2O \qquad \text{(b)}$$

Fig. 3. Contents (a) and degradation products (b) of an aqueous solution of sodium chlorite

$$2ClO_2 + 4\,H_2O \rightarrow ClO_2^- + ClO_3^- + 2H^+$$

Fig. 4. Decomposition of chlorine dioxide in water, in the absence of oxidisable substances and in the presence of alkali

Aqueous oxidation/reduction reactions of HP, PAA and POA result in the formation of H_2O, AA and OA, respectively (Figure 5).

$$H_2O_2 + 2H^+ + 2e \rightarrow 2H_2O$$

$$CH_3COOOH + 2H^+ + 2\,e \rightarrow CH_3COOH + H_2O$$

$$CH_3(CH_2)_6COOOH + 2H^+ + 2e \rightarrow CH_3(CH_2)_6COOH + H_2O$$

Fig. 5. Oxidation/reduction of hydrogen peroxide, peroxyacetic acid and peroxyoctanoic acid

Upon contact with the surface of a poultry carcass, PAA, POA and HP rapidly decompose according to the reactions shown in Figure 6.

$$R\text{-}COOOH \rightarrow R\text{-}COOH + H_2O_2$$

$$2R\text{-}COOO \rightarrow 2RCOOH + O_2$$

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Fig. 6. Decomposition of peroxyacetic acid, peroxyoctanoic acid and hydrogen peroxide [R= CH_3- (PAA) and R= $CH_3(CH_2)_6$- (POA), respectively]

15 ANNEX II - THE RELATIONSHIP BETWEEN THE NUMBER OF BACTERIA AND THE PREVALENCE

Pathogen reduction is either presented as $\log_{10}$ reduction, decimal reduction or a percentage reduction of number of pathogens on a carcass, or as reductions in prevalence often expressed as the fraction or percentage of contaminated carcasses.

These two concepts can be linked mathematically by the first moment of the Poisson distribution if we know the detection limit of the bacteriological procedure.

The probability of a test negative poultry carcass would then be:

$$P\ (\text{negative carcass}) = \exp(-\#\text{c.f.u/detection limit})$$

and the prevalence of positive carcasses would be

$$PV = 1 - \exp(-\#\text{c.f.u/detection limit})$$