



Benefits and Risks of the Use of Chlorine-containing Disinfectants in Food Production and Food Processing

**Report of a Joint FAO/WHO
Expert Meeting**

Ann Arbor, MI, USA

27-30 May 2008



**Food and Agriculture
Organization of
the United Nations**



**World Health
Organization**

World Health Organization

CONSULTATIONS AND WORKSHOPS

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EXECUTIVE SUMMARY

Background

The Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) expert meeting on the use of chlorine-containing disinfectants¹ in food production and food processing was held on 27–30 May 2008 in Ann Arbor, Michigan, United States of America. The meeting was supported by NSF International, WHO Collaborating Centre for Food and Water Safety and Indoor Environment.

The meeting was organized to provide scientific advice in response to a request made by the Codex Alimentarius Commission based on proposed terms of reference prepared by the thirty-seventh session of the Codex Committee on Food Additives and Contaminants and the thirty-seventh session of the Codex Committee on Food Hygiene on the safety and benefits of the use of “active chlorine” in food processing.

The primary intended benefits of disinfection processes are the reduction of microbial foodborne disease risk and the reduction of spoilage by control of contamination by pathogenic and non-pathogenic microorganisms. Control can be through direct treatment of foods and through management of cross-contamination from processing water and food contact surfaces. Disinfection treatment may lead to residues of disinfectants and disinfection by-products, which need to be considered in a risk–benefit assessment. The control of spoilage bacteria by disinfection, which increases the shelf life and stability of foods, was not considered by the expert meeting, as it has no direct impact on health risks.

Results

The expert meeting considered all available data related to the benefits and risks for human health of the use of disinfection processes in the food production and food processing industry. Emphasis was placed on chlorine-containing compounds, but alternative substances and methods used for disinfection of food and food contact surfaces were also considered.

The main goal of the meeting was to compare the health risk of chemical residues in food products following disinfection during food production and processing (including handling) with the benefit of lowering the risk of microbial hazards. The efficacy of chlorine treatment was considered, taking into account different treatment scenarios, different chlorine-containing substances and different combinations of pathogens and food commodities. These considerations focused on the most common current practices in various food sectors, as well as taking into account certain proposed new practices. Consideration was given to the efficacy and feasibility of potential alternative treatments to replace chlorine use. Unintended consequences, such as the potential for development of tolerance to microorganisms and effects on nutritional and organoleptic qualities, were also reviewed.

The main categories considered in food production and processing (including handling) were:

- meat and poultry;

¹ Chlorine-containing disinfectants include hypochlorous acid and its conjugate base, hypochlorite ion; chlorous acid and its conjugate base, chlorite ion; chlorine gas; and chlorine dioxide. Chloramines, chloramine-T and dichloroisocyanurate were included only where of relevance to the food processing industry.

- fish and fishery products;
- fresh produce (including hydroponics and sprouts);
- food contact surfaces.

Previous work and assessments carried out on national/regional and international levels formed the primary basis for the assessment, but additional information submitted in response to an open call for information was considered, as well as publicly available scientific studies and other information.

The approach taken was to identify the most common disinfection practices for the food categories described above; identify possible chemical residues in foods resulting from these treatments; estimate dietary exposure to these residues; estimate the potential risk to health from exposure to these chemical residues in foods; evaluate the efficacy of treatment in reducing the prevalence and numbers of pathogenic microorganisms on food; and estimate the potential resulting decreased health risk. The strength of the evidence was evaluated in all cases. Potential health risk from chemical exposure was then compared with the potential benefits of decreased health risk from reduced pathogen exposure in a systematic and stepwise approach.

A number of key use scenarios for each food category were described. Sodium hypochlorite is the most widely used disinfectant, in particular in the production and processing of poultry meat, leafy greens, sprouts, hydroponics and seafood, whereas its use in red meat processing is less common. Acidified sodium chlorite solutions are commonly used as an alternative to sodium hypochlorite in specific poultry processing steps. The use of chlorine-containing compounds in the fish and fishery products industry is focused mainly on disinfection prior to distribution, and the use on edible portions of fish and shellfish is limited. Non-chlorine-based chemical alternatives included peroxyacetic acid in poultry production and organic acids in meat production. Physical treatments were not considered.

A number of chlorine-containing disinfectants and their disinfection by-products as well as disinfectant alternatives can lead to residues in foods and hence to possible health risk. The toxicology of these substances was reviewed and compared with estimated dietary intakes. The identified residues of chlorine-containing disinfectants and disinfection by-products did not raise health concerns based on estimated dietary exposures. However, the evidence for health concerns associated with hypochlorite use in poultry, fish and shellfish was weak, owing to a lack of qualitative and quantitative information on the formation and presence of trihalomethanes (which are disinfection by-products) on the food. It was noted that although generally conservative estimates were used, there was a high degree of uncertainty in the dietary exposure assessments, as data on by-products were available primarily for drinking-water, and these data would have limited applicability to food. However, chlorine-containing chemicals are unstable, and it was concluded that there is a low potential for the presence of by-products in foods as consumed.

Microbiological risk assessments were performed for the key use scenarios, based on available studies and available risk assessments. It was concluded that the antimicrobial effects of disinfectants in food production may be overestimated by a lack of industrial-scale studies and a lack of inclusion of controls for the physical effects of water alone. In contrast, the effects may be underestimated by studying processes in isolation in industries where disinfectants have already been applied in previous steps. There was evidence for a reduction of pathogens on poultry carcasses and red meats by application of acidified sodium chlorite and chlorine dioxide and in smoked fish by application of sodium hypochlorite. There was also evidence that no pathogen reduction is achieved by application of sodium hypochlorite on poultry carcasses and red meats. Limited data provided evidence for reduction of cross-contamination by the application of disinfectants (in particular, sodium hypochlorite) in wash

and flume waters. Effective disinfection of food contact surfaces is an important means of reducing human exposure to pathogens in food.

Regarding unintended consequences of disinfection practices, the changes in nutrient content are low relative to the normal dietary intake of these nutrients. There is also no evidence to indicate that the use of chlorine-containing disinfectants and their alternatives is associated with acquired antimicrobial resistance to therapeutic agents.

Risk–benefit assessment integrates the results of two separate activities, risk assessment and benefit assessment, which can be done in a qualitative or quantitative way. Owing to a lack of data that would allow a quantitative assessment, the meeting developed a stepwise approach to risk–benefit assessment of chlorine-containing disinfectants and other alternatives to allow for a systematic comparison in a qualitative manner. Where scientific data were available, an assessment of risk and/or benefit was undertaken. The meeting categorized the use scenarios per food commodity in one of the following four categories:

- 1) No health concern identified; no benefits identified.
- 2) No health concern identified; benefits identified.
- 3) Health concern identified; no benefits identified.
- 4) Health concern identified; benefits identified.

The meeting identified several disinfectant use scenarios where there were no health concerns identified but for which there was a benefit. Only use scenarios for which it was concluded that there are both health concerns and benefits were considered to need further evaluation. However, the meeting did not identify any use scenarios that were of this type (i.e. both health concerns and benefits identified). The level of evidence supporting these conclusions as well as the uncertainties are discussed in the report.

Recommendations

The meeting identified important gaps in the available data. These data gaps constrained the scope of the risk–benefit assessments. Consequently, the meeting agreed on a number of recommendations for further scientific studies and the development of standardized practices.

The meeting emphasized that disinfectant treatment of water used in food processing must not be used to mask poor hygienic practices. The meeting recommended that disinfectants be used within the framework of good hygienic practice, with a system based on hazard analysis and critical control points where applicable and with adequate process controls in place.

INTRODUCTION

The Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) expert meeting on the use of chlorine-containing disinfectants¹ in food production and food processing was held on 27–30 May 2008 in Ann Arbor, Michigan, United States of America, at NSF International, WHO Collaborating Centre for Food and Water Safety and Indoor Environment.

The meeting was organized to provide scientific advice in response to a request made by the Codex Alimentarius Commission (FAO/WHO, 2006) based on proposed terms of reference prepared by the thirty-seventh session of the Codex Committee on Food Additives and Contaminants (FAO/WHO, 2005a) and the thirty-seventh session of the Codex Committee on Food Hygiene (FAO/WHO, 2005b) on the safety and benefits of the use of “active chlorine” in food processing.

The primary intended benefit of disinfection processes is the reduction of foodborne disease risk by control of contamination by pathogenic and non-pathogenic microorganisms through the direct treatment of foods and the elimination or management of cross-contamination from processing water and food contact surfaces. Such treatment may lead to residues of chemical by-products, which need to be considered in a risk–benefit assessment.

The expert meeting considered all available data related to the benefits and risks for human health associated with the use of disinfectants in the food production and food processing industry. Emphasis was placed on chlorine-containing compounds, but alternative substances and methods used for disinfection of food and food contact surfaces were also considered.

The main goal of the meeting was to compare the health risk of chemical residues in food products following the use of chlorine for disinfection purposes during food production and processing (including handling) with the benefit of lowering the risk of microbial hazards, taking into consideration the relevance and feasibility of potential alternative approaches (i.e. to replace chlorine use). The efficacy of chlorine treatment was considered, taking into account different treatment scenarios, different chlorine-containing substances and different combinations of pathogens and food commodities. These considerations were based on current practices in various food sectors, as well as taking into account certain proposed new practices. Unintended consequences, such as the potential for development of tolerance to microorganisms and effects on nutritional and organoleptic qualities, were also reviewed.

The main categories considered in food production and processing (including handling) were:

- meat and poultry;
- fish and fishery products;
- fresh produce (including hydroponics and sprouts);
- food contact surfaces.

Previous work and assessments carried out on national/regional and international levels formed the primary basis for the assessment, but additional information submitted in

¹ Chlorine-containing disinfectants include hypochlorous acid and its conjugate base, hypochlorite ion; chlorous acid and its conjugate base, chlorite ion; chlorine gas; and chlorine dioxide. Chloramines, chloramine-T and dichloroisocyanurate were included if of relevance to the food processing industry.

response to an open call for information was considered, as well as publicly available scientific studies and other information.

The experts invited to the meeting had expertise in many different disciplines essential for the complex topic of the assessment of the benefits and risks of the use of disinfectants in food production and food processing: food technology and food processing, chemistry, food microbiology, toxicology, dietary exposure assessment, epidemiology and risk–benefit assessment in the field of diet and human health. The list of invited experts is provided in Annex 1. Professor Gabriel Adegoke, Mr John Fawell, Dr Emma Hartnett, Dr Jean-Charles Leblanc, Professor Mark Nieuwenhuijsen and Mr Alan Reilly were not able to participate in the meeting.

Declaration of interests

The participating experts completed the WHO form on Declaration of Interests and a confidentiality undertaking. Mr Scott L. Burnett and Dr Michael Graz declared interests, as they are or had recently been employed by a relevant industry. The meeting considered that this could constitute a potential conflict of interest. It was decided that the expertise of Mr Burnett and Dr Graz would be very valuable for the discussion on the current uses of disinfectants, but that they could not participate in the discussion and decisions regarding conclusions and recommendations of the meeting. These participants therefore left the meeting at that point.

Preparatory work

FAO and WHO issued an open call for experts and data in March 2007. In consideration of the complexity of the request for scientific advice, it was decided to invite a core group of experts with expertise in the various areas to be covered to a meeting, held at the FAO Headquarters in Rome, Italy, on 7–9 November 2007. The invited members of the core group were Dr Bassam Annous, Dr Diane Benford, Dr Joseph Cotruvo, Dr Steve Crossley, Dr Joseph Frank, Dr Arie Havelaar, Professor Mark Nieuwenhuijsen, Mr Alan Reilly and Dr Inger-Lise Steffensen. The aim of this core group meeting was to provide input on the scope of the project, to outline and prepare the background documentation for the expert meeting and to identify potential experts for the drafting of these documents. The core group of experts also served as coordinators for the preparatory work for this expert meeting. The following outline of the background documentation was agreed to, and this outline was also followed in the report from this meeting:

- Chapter 1. Description of current processes
- Chapter 2. Chemistry of the compounds used
- Chapter 3. Chemical risk assessment
 - Toxicology and exposure assessment
 - Epidemiology
- Chapter 4. Microbiological risk assessment
- Chapter 5. Unintended consequences
- Chapter 6. Risk–benefit assessment
- Chapter 7. Conclusions and recommendations

The list of drafting experts is provided in Annex 2. FAO and WHO decided that it was not necessary to invite some of the experts drafting parts of the background document on current uses.

Definitions for the purpose of this meeting

For the purpose of this meeting, the following definitions were adopted:

- *Disinfectants*: Substances used in aqueous solutions in food production and processing to eliminate or reduce the number of microorganisms on the food in washing, chilling and other processes. In some countries, a distinction is made between disinfection and sanitization, but for the purpose of this document, no such distinction is made.
- *Disinfection by-products*: Chemical compounds formed during disinfection processes, other than the original substances introduced in the aqueous solution used for disinfection.

References

FAO/WHO (2005a). Terms of reference for the FAO/WHO joint expert consultation to conduct a comprehensive assessment of use of active chlorine (aspects relevant to CCFAC). In: *Report of the thirty-seventh session of the Codex Committee on Food Additives and Contaminants, The Hague, 25–29 April 2005*. Rome, Food and Agriculture Organization of the United Nations, Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission (ALINORM 05/28/12, Appendix XV; ftp://ftp.fao.org/ag/agn/food/Chlorine_ToR_CCFAC_en.pdf).

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1. USE OF CHLORINE-CONTAINING COMPOUNDS IN FOOD PROCESSING

1.1 Introduction

The purpose of this chapter is to describe current practices in use of chlorine-containing compounds and their alternatives in food processing. The chapter is not meant to be a complete literature review of the subject, but rather is a summary of widely used and accepted current practices. Proposed alternatives to chlorine that do not have widespread current use within the industry are not within the scope of this chapter.

Current use information is presented in tabular form according to commodity, the unit process that uses chlorine compounds and the type of chlorine chemistry employed in each unit process. The tables present a summary of information obtained from multiple industrial and government sources and reflect common usage in countries where such uses are allowed. This information was obtained from responses to a request for information by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) and through direct contact with government agencies and suppliers. Sources of the current use information include the Codex Alimentarius Commission Codes of Practice, Food Standards Australia New Zealand, United States Department of Agriculture and personal communications with industrial suppliers to the food industry. Specific sources of information for the current use tables are not further specified, as information from all sources was compiled in the summary tables. Some information on efficacy is provided in this chapter as it relates to current uses, but a more comprehensive evaluation of the beneficial effects associated with the use of chlorine-containing compounds and their alternatives in food processing is provided in chapter 4 as a basis for the risk–benefit assessment in chapter 6.

Many food processes require water to wash, cool or transport the product. Potable water must be used for these processes. This document is concerned with the addition of chlorine compounds to process water in excess of what is required to ensure potability. This excess chlorine is required because active chlorine molecules readily react with organic matter on the product surface and from product exudates, resulting in loss of antimicrobial activity. The maintenance of antimicrobial activity in process water can have multiple functions, depending on the specific process. These functions include preventing the transfer of pathogenic and spoilage microorganisms between product items within a batch, preventing the transfer of pathogenic and spoilage microorganisms between batches of product, inhibiting biofilm formation by spoilage and pathogenic microorganisms on equipment surfaces during processing, and inactivating a portion of pathogenic and spoilage microorganisms that are attached to the food tissue. If the process is adequately controlled, the net result is a safer food product with a longer shelf life.

It should be noted that chlorine-containing disinfectants or their alternatives can be used at any food processing stage. In practice, these compounds may sometimes be used sequentially in several food processing stages (e.g. pre-chill spray or dip, chiller water immersion, post-chill spray or dip). In most cases, however, the treatments described in this chapter are not normally used in sequential combinations, and it is therefore reasonable to evaluate these treatments as stand-alone processes. It should also be noted that the use of chlorine in product wash water does not disinfect product surfaces, as insufficient pathogen reductions are obtained to make a contaminated product safe to consume without additional

treatment (i.e. cooking). Currently used chlorine alternatives also do not disinfect product surfaces.

Food processors recognize the public health benefits of chlorine by including chlorination of process water as a critical control point in their hazard analysis and critical control point (HACCP) plan (Rushing, Angulo & Beuchat, 1996). As HACCP plans are developed to control specific health hazards, the purpose of the chlorine treatment is usually specified as controlling specific pathogens associated with the product. These pathogens are referred to in this chapter as the “target” microorganisms. Although use of chlorine often provides control of spoilage microorganisms, HACCP plans are not directed towards spoilage, and therefore spoilage microorganisms are not usually recognized as a target of the process. As a result, there are few data on the effectiveness of chlorine and chlorine alternatives against spoilage microorganisms. The use of chlorinated water in food processing has limitations, which include inactivation of active agents by organic matter, loss of product quality if levels are excessive, dependence on appropriate pH for activity, metal corrosion at low pH or if excessive concentrations are used, and generation of chlorine gas at low pH. Most processors have controls in place to ensure that excessive concentrations of chlorine compounds are not used and that pH levels are controlled so that product sensory quality, worker safety and equipment surfaces are not compromised. The impact of high chlorine levels on sensory properties of food is manifest as discoloration or unacceptable flavours. These adverse sensory impacts probably limit consumer exposure to chlorination by-products through accidental overuse.

The previously mentioned limitations to the effectiveness of chlorine have led to the development of alternative treatments for process water disinfection. These alternatives are process and product specific, as processes differ in the expected functionality of chlorine use. Alternatives must be adopted with caution, because all the functional aspects of chlorine use may not be adequately realized. For example, if a product must be washed to remove field dirt or extraneous matter, the water may still need to be treated with chlorine (or other antimicrobial chemical), even if a chlorine-free antimicrobial treatment of the product occurs later in the process. In addition, active chlorine compounds rapidly inactivate suspended vegetative cells, with low levels of hypochlorous acid providing 90% inactivation in less than 10 s and contact times for effectiveness anywhere from 1.5 to 100 s (Marriott, 1999). A chlorine alternative that requires a longer contact time to achieve disinfection may not be practical or may require a redesign of the process.

Because the active chlorine content of process water is considered a critical control point in many HACCP plans, the ability to monitor the process is critical for maintaining process integrity. Chlorine monitoring equipment and test kits are readily available and widely used. If process water is treated with alternative chemicals, the processor must have the ability to monitor the level of the alternative active agent. Alternative agents that are not amenable to in-process monitoring may not provide the degree of confidence in process integrity required by the food processor for adequate HACCP implementation.

The effectiveness of chlorine and non-chlorine treatments for process water is dependent on various process factors, including temperature, pH, amount of organic matter, type of organic matter, product surface topography and contact time. The need for these treatments to reduce microorganisms on product surfaces and rapidly inactivate microorganisms suspended in process water makes selection of an appropriate biocidal agent a complex process. Treatments that show promise in laboratory- or pilot-scale trials often fail in commercial situations. Therefore, this chapter addresses primarily alternative treatments that have been proven in commercial application.

1.2 Poultry processing

Modern poultry processing is a complex, highly automated process that starts with a live animal transported to a slaughter facility and ends with a fully processed eviscerated and chilled carcass. Further processing includes activities that occur after the whole carcass has been chilled. These can include, but are not limited to, carcass cut up, bone removal, skin removal, marinating, breading, battering and cooking. This section focuses on chicken processing. However, the processes discussed also apply to other poultry species.

1.2.1 Initial loads of bacteria upon entry to processing

When a broiler chicken arrives at the processing plant, it has a substantial number of bacteria associated with it. These organisms are found both on the outer surfaces of skin, feet and feathers and in the alimentary tract, including the crop, colon, caeca and cloaca (Berrang, Buhr & Cason, 2000). Although some bacteria are resident on the skin and feathers of a market-age broiler, much of the external contamination is found in faeces or results from faecal contamination during production. Regardless of the source of the bacteria, poultry processing is designed to reduce the numbers of bacteria on the outer and inner surfaces of the carcass in order to produce a high-quality, safe and wholesome product. Overall, modern broiler processing is effective at providing the consumer with a product having low levels of pathogenic and spoilage bacteria, considering the product is a raw carcass with skin (Izat et al., 1988; Waldroup et al., 1992; Berrang & Dickens, 2000).

1.2.2 Cross-contamination of carcasses during processing

During poultry processing, most procedures lower the number of bacteria found on carcasses. However, feather removal is a notable exception (Izat et al., 1988; Berrang & Dickens, 2000). Cross-contamination occurs when equipment surfaces become contaminated with bacteria of concern, such as *Salmonella* or *Campylobacter*. This concept was demonstrated by study of commercial Belgian processing plants wherein carcasses from a *Salmonella*-negative flock became contaminated with *Salmonella* that was present on slaughter line equipment (Rasschaert, Houf & DeZutter, 2006). A detailed study of the potential for cross-contamination indicated that it can occur at multiple sites throughout transport, slaughter and processing. Mead, Hudson & Hinton (1994) found that cross-contamination could be demonstrated during transport in cages, by the automatic killing knife, during feather removal, in the head puller, on the transfer belt, in the vent opener and in the water chiller. Using an antimicrobial-resistant strain of *Escherichia coli*, Mead, Hudson & Hinton (1994) demonstrated that an inoculated knife in an automatic killer spread contamination to at least 500 carcasses; using a chlorinated spray (10 mg/l) resulted in 250–400 carcasses being contaminated at levels from 0.4 to 1.3 log lower than with the unwashed knife. Similar results were seen with the head puller, which spread contamination to 500 carcasses, but a chlorinated spray (25 mg/l) stopped the spread after only 25–100 carcasses. Inclusion of chlorine in chiller water at 18–30 mg/l made no difference in the spread of the antimicrobial-resistant *E. coli* to carcasses adjacent to inoculated carcasses (Mead, Hudson & Hinton, 1994). Although use of a chlorinated water spray or inclusion of chlorine in the chiller water did not eliminate cross-contamination, it did help to reduce it at each point (Mead, Hudson & Hinton, 1994). Therefore, part of the reason that chemicals such as chlorine are used in poultry processing is to limit the likelihood of cross-contamination by sanitizing equipment and food contact surfaces. The use of chlorine during air chill may not

be as helpful; spraying carcasses with chlorine at 50 mg/l did not prevent cross-contamination during air chilling of poultry (Mead et al., 2000).

1.2.3 Control of contamination during processing

1.2.3.1 Physical

Non-chemical approaches can be applied in poultry processing to limit the effects of cross-contamination. These include, but are not limited to, scheduled or logistic slaughter, counter-current scald, counter-current immersion chill, brush washers of carcasses or equipment, and hot water spray or immersion.

1.2.3.2 Chemical

In many countries, antimicrobial chemicals are used to disinfect process water and equipment surfaces to control cross-contamination and to reduce the numbers of bacteria on carcass surfaces. Chlorine may be added to carcass washers, equipment wash water, immersion chiller water and pre-chiller water. Online reprocessing is an extra wash step that, when instituted, is applied to every carcass on the shackle line to control faecal contamination prior to the chill tank. These online reprocessing systems may incorporate a chemical treatment. Chemicals used to treat poultry throughout the process include acidified sodium chlorite (ASC), calcium hypochlorite, sodium hypochlorite, cetylpyridinium chloride (CPC), chlorine gas, chlorine dioxide, 1,3-dibromo-5,5-dimethylhydantoin, electrolytically generated hypochlorous acid, citric acid with hydrochloric acid with or without phosphoric acid, ethyl lauroyl arginine, ozone, peroxyacetic acid, octanoic acid, acetic acid, peroxyoctanoic acid, 1-hydroxyethylidene-1,1-diphosphonic acid, sodium metasilicate and trisodium phosphate (TSP) (USDA, 2007). The chemicals used most commonly or on which most research has been conducted are discussed further in the remaining chapters of this report.

1.2.4 Effectiveness of control measures

1.2.4.1 Evaluating the literature

It is difficult to evaluate published literature relative to the effectiveness of chemicals for reducing cross-contamination and reducing levels of pathogens on poultry skin. It is not always clear if test conditions and logistics provided adequate experimental design, including the use of proper controls. For example, it may be possible that a non-chlorinated wash step may be just as effective as a chlorinated wash step to lower bacterial numbers on carcasses, but these types of controls are not always available when working in poultry processing plants. Therefore, laboratory and pilot plant research can be useful, especially with the experimental nature of some chemicals studied.

However, laboratory and pilot plant studies can be problematic as well, because many of them are conducted using inoculated skin or carcasses. Under these circumstances, the inoculated bacteria may not be adapted to the chicken skin environment, which could affect attachment, survival and the likelihood of detection after treatment. Under ideal circumstances, chemical efficacy research would utilize naturally occurring bacterial populations.

Another concern is failure to inactivate an antimicrobial chemical following treatment and before bacterial culture. Some studies may overestimate the effectiveness of a chemical treatment because the activity of the chemical was not neutralized prior to bacterial

culture. This can result in the chemical continuing to kill bacteria after the treatment is over, during the time when the number of viable cells remaining is being estimated.

1.2.4.2 Chlorine-based chemicals (Tables 1.1 and 1.2)

Tables 1.1 and 1.2 present summaries of information received on the current use of chlorine-based chemicals in the poultry industry. Aerobic microbial counts recovered from broiler carcasses have been shown to be reduced by about 1 log colony-forming units (cfu) per square centimetre from the use of chlorine at 50 mg/l in the final washer compared with unchlorinated water spray controls (Sanders & Blackshear, 1971). A 1976 study (Thomson, Cox & Bailey, 1976) reported that chlorine at 50 mg/l in an immersion pre-chill treatment at 45 °C was effective at preventing cross-contamination from carcasses inoculated with *Salmonella* to uninoculated carcasses. Thomson, Cox & Bailey (1976), however, also noted that in order to lessen numbers of *Salmonella* on inoculated carcasses, the chlorinated chiller water required the addition of acid for pH adjustment. Chlorine is most effective at neutral or lower pH; therefore, effectiveness can be optimized by careful control of pH in an immersion chill or pre-chill tank. Bailey et al. (1986) found that using chlorine at 40 mg/l in wash water to combat bacteria in a chicken fat matrix on stainless steel reduced numbers of *Salmonella* by 96% compared with a 50% reduction by using an unchlorinated water spray.

Table 1.1. Summary information for chlorine-based interventions in poultry processing: raw product

Process application	Use level (mg/l)	Exposure time
Hypochlorous acid/hypochlorite, calcium hypochlorite, chlorine gas and electrically generated hypochlorous acid (target microorganisms: <i>Salmonella</i> and <i>Campylobacter</i>)		
Pre-chill carcass spray	<50 or 3–5 free chlorine	5 s
Carcass rinse	200	60 s
Reprocessing eviscerated carcasses – pre-chill	20–50	NA
Chiller water	<50	45–60 min
Immersion chill	3–5 free chlorine	10–120 min
Recycling water	5	NA
Acidified chlorite/chlorous acid (target microorganisms: <i>Salmonella</i> and <i>Campylobacter</i>)		
Pre-chill carcass spray or dip	500–1200	10–15 s
Pre-chill or chiller water	50–150	35–60 min
Post-chill spray or dip	500–1200	NA
Chlorine dioxide generated at >90% efficiency (target microorganisms: <i>Salmonella</i>, <i>Campylobacter</i>, <i>E. coli</i>)		
Pre-chill spray	1–3 residual on carcass	15–20 s
Chill or pre-chill immersion	1–3 residual on carcass	40–60 min
Post-chill spray or dip	1–3 residual	15–20 s
Chlorite/chlorous acid (III) (target microorganisms: <i>Salmonella</i> and <i>Campylobacter</i>)		
Pre-chill spray or dip	500–1200	15–20 s
Pre-chill or chill tank	50–150	60 min
Post-chill spray or dip	500–1200	1–20 s

NA, data not available

Table 1.2. Summary information for chlorine-based interventions in poultry processing: ready-to-eat product

Process application	Use level (mg/l)	Exposure time
Chlorite/chlorous acid (III) (target microorganisms: <i>Salmonella</i> and <i>Campylobacter</i>)		
Product spray	500	15 s

Waldroup et al. (1992) demonstrated the effectiveness of a combined treatment that included physical methods (counter-current scalding and chilling) with chemical methods (chlorine at 20 mg/l in bird washers in the picking room, transfer belt and final washer and chlorine at 1–5 mg/l in the immersion chiller water). In that study, *Salmonella* was significantly lessened in two of five plants by an estimated 0.5 log cfu/ml carcass rinse; *Campylobacter* was lessened in four of five plants by 0.4–0.8 log cfu/ml. Bashor et al. (2004) found that carcass washers with chlorine at 25–35 mg/l reduced the numbers of *Campylobacter* by 0.5 log, but the design did not include a wash step without chlorine for comparison.

Northcutt et al. (2005) reported that adding chlorine at 50 mg/l to the water in a broiler inside–outside bird spray wash station did not have any effect on the numbers of *E. coli*, *Salmonella* or *Campylobacter* compared with an unchlorinated control; the conclusion was that physical removal from washing may be as important as chemical inactivation for these bacteria.

Berrang et al. (2007) found that use of a chlorinated spray before evisceration did not affect post-chill numbers of *Campylobacter* in commercial processing plants; however, chlorination in the immersion chill tank did result in lower numbers of *Campylobacter* on fully processed carcasses. Stopforth et al. (2007) examined numbers of bacteria before and after various processing steps in commercial poultry plants. They found that chlorine at 20–50 mg/l in carcass wash steps was not effective at significantly lowering numbers of bacteria, although most of these washes did lessen the incidence of *Salmonella*. The opposite was true for chilling treatments. Chlorine (20–50 mg/l) with ASC (sodium chlorite at 50–150 mg/l acidified to pH 2.8–3.2 by citric acid) in the chill tank was effective for lowering numbers of total bacteria by 1.2 log and *E. coli* by 0.8 log, but not for lessening the incidence of *Salmonella*.

Chlorinated water can be made by running an electric current through pure water with sodium chloride added. The result is referred to as electrolysed oxidizing water or electrically generated hypochlorous acid (Table 1.1). Kim, Hung & Brackett (2000) found that electrolysed oxidizing water was effective against various pathogens associated with meat and poultry foods. When applied in a poultry washing system, electrolysed oxidizing water at 50 mg/l resulted in a 1.7–1.9 log decrease in inoculated *Campylobacter* compared with water-sprayed controls (Park, Hung & Brackett, 2002).

ASC (Table 1.1) can be used as a carcass treatment during online reprocessing or carcass chilling. Addition of ASC to an online reprocessing system to remove faecal contamination reduced the numbers of *Campylobacter* by 99.2%, which represented a significant improvement over the 84.5% seen in the plant's standard online reprocessing system (Kemp & Schneider, 2002). This difference, however, was no longer evident after carcasses proceeded through an immersion chill tank. Addition of ASC to carcass washers was found to increase the effectiveness above that seen with chlorine at 25–35 mg/l by an additional 1.26 log decrease in numbers of *Campylobacter* (Bashor et al., 2004). Application of ASC after the chilling process may hold promise. A decrease of 0.9–1.2 log was noted when whole carcasses were dipped in ASC immediately following immersion chill (Oyarzabal et al., 2004).

1.2.4.3 Non-chlorine-based alternatives (Tables 1.3 and 1.4)

There are various alternatives to chlorine-based chemicals for reducing pathogen levels on poultry carcasses. Many alternatives that have been approved for use or made available have not been widely studied or evaluated in peer-reviewed research. The current use of alternatives to chlorine-based chemicals is presented in Tables 1.3 and 1.4.

Table 1.3. Summary information for non-chlorine-based alternative interventions in poultry processing: raw product

Process application	Use level (mg/l) ^a	Exposure time
Peroxyacetic acid/hydrogen peroxide (POA), hydrogen peroxide (HP), 1-hydroxyethylidene-1,1-diphosphonic acid (HEDP), sodium metasilicate (SM), ethyl lauroyl arginate (LAE), 1,3-dibromo-5,5-dimethylhydantoin (DBDMH), ozone, cetylpyridinium chloride (CPC), trisodium phosphate (TSP) (target microorganisms: <i>Salmonella</i> and <i>Campylobacter</i>)		
Spray or dip carcasses, parts, trim and organs	220–230 POA, 110–165 HP, 13–14 HEDP	NA
Scald tank dip	230 POA, 165 HP, 14 HEDP	30–120 s
Carcass chill tank	230 POA, 165 HP, 14 HEDP, <50 POA	60 min
Inside–outside bird washer	<80 POA	5 s
Online reprocessing	<200	15 s
Marinades	<2% SM by weight	NA
Inside–outside bird washer	1.1–6% SM	15 s
Carcass spray, pre-chill, fresh cut pieces	<200 (LAE)	5 s
Carcass spray, pre-chill	<200 DBDMH	5 s
Fresh cut raw or ready to eat	<200 DBDMH	NA
Chill tank immersion	100 DBDMH	60 min
Chiller water (ozone)	NA	NA
Raw whole carcass spray prior to chill	0.7 g CPC/kg product	NA
Raw whole carcass spray post-chill	0.7 g CPC/kg product	NA
Raw whole carcass spray or dip pre- or post-chill	8–12% TSP in water with chlorine at 20 mg/l	30 s

NA, data not available

^a Unless otherwise specified.

Table 1.4. Summary information for non-chlorine-based alternative interventions in poultry processing: ready-to-eat product

Process application	Use level (mg/l) ^a	Exposure time
Ozone, octanoic acid, citric acid, ethyl lauroyl arginate (LAE), lactic acid (target microorganism: <i>Listeria monocytogenes</i>)		
Product spray	2–3 ozone	30 s
Product spray	<400 octanoic acid	5 s
Product in casing prior to slicing (bologna)	10% citric acid	5 s
Product in casing prior to casing removal	3% citric acid	5 s
Product prior to casing removal	200 LAE	5 s
Prior to final packing	85 000–95 000 lactic acid	20–30 s

^a Unless otherwise specified.

CPC is considered an alternative to chlorine-based chemicals, as the chloride portion of the molecule is non-functional. CPC and TSP (Table 1.3) have been thoroughly evaluated for use in poultry processing. In a study of inoculated skin samples, 0.1% CPC applied as a 15 °C spray was effective at lowering numbers of *Salmonella* by 0.9–1.7 log compared with a water spray control. The decrease was larger when the CPC was sprayed at an elevated temperature (Kim & Slavik, 1996). Other studies conducted with 0.1% CPC resulted in a 1.6 log reduction in inoculated *Salmonella* on pre-chill chicken carcasses (Li et al., 1997) and up to a 1 log decrease in *Salmonella* numbers on chicken skin compared with a water control (Wang et al., 1997). Xiong et al. (1998) found that CPC applied as a spray at 0.1% and 0.5% lowered numbers of *Salmonella* by 0.5 and 0.9 log cfu/ml, respectively, compared with water-washed controls.

TSP can be applied to the carcass as a dip or a spray. The pH of TSP is very high (11–12); such alkaline conditions are by nature antibacterial. In much of the published literature evaluating the use of TSP to lower bacteria associated with poultry, it is unclear if the high pH was neutralized prior to bacterial culture, which could cause an inflated sense of efficacy. In addition, this high pH can cause waste disposal problems.

When broiler carcasses were dipped for 15 s in 10% TSP at pH 12, Whyte et al. (2001) were unable to detect *Salmonella* from the neck skin of TSP-treated carcasses compared with 1.04 log cfu/g detected on water control samples. Furthermore, they found a 1.1 log reduction in the numbers of *Campylobacter*. Application of 10% TSP as a spray has been tested under experimental conditions as well. Wang et al. (1997) found that, compared with water controls, TSP under these conditions resulted in up to a 1 log decrease in *Salmonella* inoculated on chicken skin. Compared with unsprayed controls, Xiong et al. (1998) found that 10% TSP resulted in a decrease of 0.9 log in numbers of inoculated *Salmonella* compared with water spray controls. Addition of TSP to a carcass washer in a processing plant increased the effectiveness by reducing numbers of *Campylobacter* an additional 1.0 log beyond that achieved using chlorine at 25–35 mg/l (Bashor et al., 2004).

An interesting series of studies conducted by Bourassa et al. (2004, 2005) evaluated the use of TSP to lower the recovery of *Salmonella* from broiler carcasses. In the first study (Bourassa et al., 2004), a 5 s dip in 10% TSP prior to chill significantly lowered the recovery of *Salmonella* from individually chilled carcasses (46% for controls, 26% for treated carcasses). This difference was maintained through 7 days of storage at 4 °C; 20% of control carcasses were positive for *Salmonella*, whereas only 4% of treated carcasses were positive. However, the authors noted that the TSP treatment resulted in significantly higher pH of the carcass rinses. In the later study (Bourassa et al., 2005), the authors adjusted the pH of the culture medium and found no difference in *Salmonella* prevalence between control and TSP-treated carcasses. This suggests that TSP may serve to wash some bacteria off and prevent outgrowth in culture media, but may not kill the pathogens of interest outright.

1.2.5 Conclusions

Chlorine-containing compounds are useful and effective in poultry processing for controlling cross-contamination and limiting the presence and numbers of pathogenic and spoilage bacteria on the product. Non-chlorine-containing alternatives have been developed for reducing pathogen levels on poultry carcasses, and their efficacy has been determined at laboratory or pilot scale. Little information is available on the ability of alternatives to chlorine-based chemicals to prevent cross-contamination or reduce biofilm formation on equipment surfaces. Some data on alternative chemicals may be misleading because of a lack of chemical inactivation before bacterial culture. This has been particularly evident in some of the TSP studies, where the efficacy of TSP to actually lower numbers of bacteria by killing

is questionable. At this point, chlorine-containing compounds remain the most common and effective choice for controlling bacterial contamination during poultry processing.

1.3 Red meat processing

The red meat processing industry can be divided into primary and secondary (further) processing. These are in most cases independent of each other and often conducted by separate companies. This is unlike the case in the poultry industry, where these processes are often conducted contiguously by the same company. Primary processing entails the slaughter of animals and all processes up to the dispatch of whole animal parts, trim and by-products. Further processing entails the conversion of a variety of cuts of meat into products, such as sausages and sliced meat products, through various processing steps, including grinding, curing, cooking and slicing. In most cases, further processing also involves the addition and use of other ingredients, such as spices, brines and binders. The degree of automation varies substantially between primary and secondary processing; the former is a labour-intensive process, whereas the later is often highly automated. As a result of differences between primary and further processing, the microbiological issues also differ, resulting in concomitant differences in the use of chlorine-containing compounds. The primary and further red meat processing sectors are therefore considered separately in this section.

1.3.1 Primary red meat processing

Whereas the muscle tissues of healthy animals are considered sterile before slaughter, the hide, gastrointestinal tract and lymph nodes are sources of a diversity of microbiological contaminants. Specifically, contact of hides with the carcasses during hide removal and the puncturing of the gastrointestinal tract and the spilling of its contents onto carcasses result in the majority of visible and microbiological contamination and cross-contamination during primary meat processing. Contamination of carcasses with microorganisms originating in the processing environment is possible, but of lesser importance (Sofos, 1994).

The main microorganisms of concern with respect to primary meat processing are *E. coli*, as an indicator of hygiene, and pathogenic *E. coli*, especially the O157 serotype. A number of outbreaks of disease associated with *E. coli* O157 and other pathogenic Shiga toxin-producing strains have been reported (Erickson & Doyle, 2007). These pathogens contaminate the meat directly from either the hides or the gastrointestinal passage of incoming animals. Cross-contamination from individual animals shedding high numbers of *E. coli* O157 is substantial as a result of the high throughput and degree of manual handling in processing facilities (Fegan et al., 2005a). *Salmonella* is another pathogen of concern during primary meat processing, and its presence is also linked to cross-contamination from individual animals, although patterns of transmission may differ from that of *E. coli* (Fegan et al., 2005b). The microorganisms of concern and issues related to processing for different animal species are similar, with some minor variations. In all cases, however, microbial contamination of carcasses with pathogens can occur to some degree, and intervention strategies to control contamination are useful for mitigating this risk.

The meat processing industry puts substantial effort into controlling microbial contamination of carcasses. The control measures applied are largely physical or process control in nature and may entail pre-slaughter animal washes, dehairing and mechanical methods to prevent rupture of the gastrointestinal tract (Sofos & Smith, 1998). Many of these methods are effective at removing visible contamination, but ineffective or only marginally effective at removing microbiological contamination (Gill, 2004). For these reasons, carcass

washing with online sprays or other interventions (e.g. steam) are widely used to control microbiological contamination. In many countries, including the United States of America (USA), additives to the water used in these washes are permitted and even required. In other countries, regulations on the use of additives are stricter and currently prevent the use of many additives in these washes.

1.3.1.1 Effectiveness of chlorine-based control measures (Tables 1.5 and 1.6)

Information on the use of chlorine compounds in red meat processing is presented in Tables 1.5 and 1.6. Chlorine was one of the first chemical treatments to be used for microbial control in the red meat industry. Significant reductions in microbial counts on carcasses have been achieved, although inconsistently, using water chlorinated at 200–500 mg/l. For example, water chlorinated to 200 mg/l gave 1.5–2.3 log cfu/cm² reductions in total aerobic bacteria on beef carcasses, depending on temperature and pH (Kotula et al., 1974). Similarly, Emswiler, Pierson & Kotula (1976) reported that chlorine in water at levels from 100 to 400 mg/l resulted in reductions of 1.4–1.8 log cfu/cm² in total aerobic bacteria on beef. By contrast, Stevenson, Merkel & Lee (1978) reported no reduction in coliforms and total aerobic bacteria on beef carcasses after treatment with chlorine at 200 mg/l. More recently, sprays containing chlorine at 50, 100, 250, 500 and 900 mg/l were found to be only marginally (<1 log/cm²) effective in reducing numbers of two strains of *E. coli* O157 that had attached to the surface of beef carcasses and lean fat. Delmore et al. (2000) and Kalchayanand et al. (2008) found that chlorine was largely ineffective at reducing levels of *E. coli* and total aerobic bacteria on various meat types. Reasons for these discrepancies may be related to pH effects and levels of contamination on carcasses. The degree of efficacy of chlorine treatments is often less for naturally contaminated carcasses or meat than it is for inoculated carcasses or meat.

Table 1.5. Summary information for chlorine-based interventions in red meat processing: raw product

Process application	Use level (mg/l)	Exposure time (s)
Hypochlorous acid/hypochlorite, calcium hypochlorite, chlorine gas and electrically generated hypochlorous acid (target microorganisms: <i>E. coli</i> O157:H7 and <i>Salmonella</i>)		
Carcass spray	50	3–5
Primal cut spray	20–50	3–5
Pre-hide removal spray	50	3–5

Chlorine dioxide has been considered as an alternative to traditional chlorine, as it has a pH-independent activity. Cutter & Dorsa (1995) observed that the use of chlorine dioxide at 20 mg/l resulted in little or no difference in numbers of total aerobic bacteria on beef compared with using potable water.

ASC has been applied as a microbial control treatment in the primary processing of meat. Harris et al. (2006) demonstrated that ASC at 1200 mg/l resulted in a reduction of 1.5–2.5 log cfu/g for *Salmonella* Typhimurium and *E. coli* O157:H7 on beef trim and ground beef. Castillo et al. (1999), in contrast, showed that phosphoric acid- and citric acid-activated ASC showed a reduction of *E. coli* O157:H7 and *Salmonella* Typhimurium of up to 4.6 log cfu/cm² on inoculated beef carcasses. ASC at 1600 mg/l sprayed onto naturally contaminated chilled beef carcasses was found to be ineffective in reducing aerobes, coliforms and *E. coli* in some cases (Gill & Badoni, 2004). In other cases, ASC was less effective than acetic acid in reducing numbers of these pathogens.

Table 1.6. Summary information for chlorine-based interventions in red meat processing: raw product and further processed meat

Process application	Use level (mg/l)	Exposure time (s)
Acidified chlorite/chlorous acid (target microorganisms: <i>E. coli</i> O157:H7 and <i>Salmonella</i> for raw product, <i>Listeria monocytogenes</i> for further processed product)		
Carcass and part spray	500–1200	15–20
Carcass and part immersion	500–1200	15–30
Trim decontamination	500–1200	15–30
Further processed meat	500–1200	15
Chlorine dioxide generated at >90% efficiency (target microorganisms: <i>E. coli</i> O157:H7 and <i>Salmonella</i> for raw product, <i>Listeria monocytogenes</i> for further processed product)		
Carcass and part spray	<3 residual	10–20
Trim decontamination	<3 residual	10–20

1.3.1.2 Effectiveness of non-chlorine-based alternatives (Table 1.7)

Non-chlorine-based alternatives for decontamination of meat during primary processing are widely used, probably more frequently than chlorine-based products (Table 1.7). Most typically, these are organic acid-based products, although ozone, peroxyacetic acid, 0.5% CPC and TSP, among others, have also been evaluated and may be used. Many of the recent studies previously discussed make direct comparisons of the efficacy of chlorine-based and non-chlorine-based compounds.

Table 1.7. Summary information for non-chlorine-based alternative interventions in red meat processing: raw product

Process application	Use level	Exposure time (s)
Hydrogen peroxide/ peroxyacetic acid mixture (POA), ozone, lactic acid (target microorganisms: <i>E. coli</i> O157:H7 and <i>Salmonella</i>)		
Carcass and part spray	POA at 220 mg/l	15–25
Trim decontamination	POA at 220 mg/l	15–25
Carcass and part spray	Ozone at 2–3 mg/l	15–30
Carcass and part spray	5% lactic acid	1–3
Subprimal and trim	2–5% lactic acid	1–3

Lactic acid is the most widely used compound in washes for primary processing of red meat. In the study by Harris et al. (2006), for example, the use of 2% acetic acid and the use of 4% lactic acid were compared with the use of ASC at 1200 mg/l, and no differences were found in the ability of any of the methods to reduce *Salmonella* Typhimurium and *E. coli* O157:H7 on trim and ground beef. Delmore et al. (2000), in contrast, demonstrated that 2% acetic acid and 2% lactic acid were more effective than chlorine-based compounds at reducing levels of *E. coli* and total aerobic bacteria on beef carcasses.

Peroxyacetic acid at 180 mg/l reduced *E. coli* O157:H7 inoculated onto carcass surfaces of beef and veal by 3.6 log cfu/cm² (Penney et al., 2007). Using ozone at 95 mg/l in water, the reduction of *E. coli* O157:H7 and *Salmonella* Typhimurium was similar to that of water alone (Castillo et al., 2003). CPC at a concentration of 0.5% resulted in a 2.50 log cfu/cm² reduction in *E. coli* O157 on fresh beef. Cutter & Rivera-Betancourt (2000) observed reductions of *E. coli* O157:H7 and *Salmonella* Typhimurium of >3 log cfu/cm² by treating beef carcasses with TSP; however, they did not counter the effects of the TSP by neutralizing the growth medium.

1.3.2 Further red meat processing

1.3.2.1 Sources, types and control of contamination

Survival of bacteria associated with further processed meats may occur if process control is lost, but the risk is low if adequate cooking and/or curing steps are followed (Doyle et al., 2001). Products may be contaminated throughout post-lethality processing before final packaging (Farber & Peterkin, 1991).

Listeria monocytogenes is the pathogen of greatest concern with respect to ready-to-eat cooked meat and meat products, such as pâté, sausages, hotdogs, bologna, ham and luncheon meats. These products often have high water activities and pH values that are favourable to the growth of this pathogen (Farber & Peterkin, 1991). Furthermore, they are frequently stored under refrigerated conditions that inhibit the growth of many competing spoilage bacteria, but allow the growth of *L. monocytogenes*, often to high numbers (Dykes, 2003). In the case of fermented meat products, such as salami, the survival and subsequent growth of pathogens such as *E. coli* O157 in products produced under conditions that are not strictly controlled are of substantial public health concern, and a number of outbreaks of disease have been associated with these products (Tilden et al., 1996). In general, all the above bacteria contaminate further processed meat at low initial levels and subsequently become a problem after growth on the product (Doyle et al., 2001).

Control of pathogens and spoilage microorganisms in further processing largely entails the application of hygiene and the HACCP system during processing. As contamination can occur during slicing and other equipment contact, effective cleaning of surfaces is critical (Farber & Peterkin, 1991). Whereas these processes may be effective in reducing numbers, they are not capable of eliminating pathogens on further processed meats. The inclusion of preservatives in processed meats to prevent the growth of pathogens is widely applied throughout the industry. Although chlorine-based compounds are often used on processing surfaces during further processing of red meat, these compounds do not usually have direct product application (with the exception of ASC). Therefore, issues related to chlorine use are not as prevalent in further red meat processing as in primary processing.

1.3.2.2 Effectiveness of chlorine-based control measures

One of the few reported studies of the use of chlorine compounds in further processing of meats demonstrated that solutions of ASC at 250, 500, 750 and 1000 mg/l sprayed onto cooked roast beef resulted in up to a 2.5 log cfu/g reduction of *L. monocytogenes* on this product (Beverly, Janes & Oliver, 2006). ASC is used for this purpose in some countries (see Table 1.6).

1.3.2.3 Effectiveness of non-chlorine-based alternatives

Non-chlorine-based chemical compounds as well as physical preservative methods such as in-pack pasteurization are widely used to control *L. monocytogenes* on further processed meats. The compounds listed in Table 1.4 for poultry products are also used for further processed red meat products. Additional alternatives are presented in Table 1.7.

As with primary processed meats, many of the control methods used for further processed products are based on organic acids. A study that investigated the combined effects of antimicrobials on frankfurters and hotdogs (Samelis et al., 2002) concluded that post-processing contamination by *L. monocytogenes* on these cured meats may be controlled by 1.8% sodium lactate (which is lower than the 3% permitted by the USA) in combination with

permissible levels (0.25%) of sodium acetate, sodium diacetate or glucono-delta-lactone in the formulation. Islam et al. (2002) also found that higher concentrations of generally recognized as safe (GRAS) chemicals were required if the product was sprayed than if it was immersed in the preservative. Schlyter et al. (1993) found that antilisterial activity was enhanced in treatments containing sodium lactate (2.5%) and sodium diacetate (0.1%) compared with similar treatments containing sodium diacetate or sodium lactate alone. Bacteriocins (which are antibacterial toxins produced by bacteria) have also been applied as antimicrobials during red meat processing. The data indicate that bacteriocins reduce but often do not stop growth or prevent survival of *L. monocytogenes* on food (Katla et al., 2001). Furthermore, the initial reduction in viable numbers is often followed by regrowth of the microorganism, probably due to the presence of a subpopulation of bacteriocin-resistant cells (Gravesen et al., 2002). Although data on chlorine-based compounds are limited, it seems clear that options for control of bacteria on further processed meats using non-chlorine-based compounds are substantial.

1.3.3 Conclusions

Overall, the use of chlorine-based compounds in the red meat industry is less than that in many other food industries, such as poultry and fresh produce processing. An issue that casts some doubt on their usefulness and requires further consideration is the apparent lower efficacy of chlorine-based compounds against natural compared with inoculated contamination. In addition, there are a substantial number of other compounds available for most processing applications that appear to be at least as effective as, or often more effective than, the chlorine-based ones. However, chlorine-based compounds are still used for controlling microbial contamination, particularly during primary processing of carcasses.

1.4 Fish and fishery product processing

Fish and fishery products cover a variety of products derived from finfish, which are any of the cold-blooded aquatic vertebrates, and shellfish, which are those species of aquatic molluscs and crustaceans that are commonly used for food that may be processed for fresh or frozen distribution. The source of the products can be either from the capture of wild stock or from aquaculture and can be either marine or freshwater in nature.

Freshly harvested finfish contain a diverse natural microflora, whose levels may range from 2 to 7 log cfu/cm² (Liston, 1980). Furthermore, the presence of large amounts of non-protein nitrogen in fish tissue and the near-neutral pH (>6.0) make fish tissue an ideal medium for growth of bacteria (Gram & Huss, 1996). Shellfish contain similar groups of microorganisms but may also contain the microbial pathogens in the waters in which they grow, as molluscs are filter feeders and concentrate these within themselves. Therefore, processing needs to include steps to reduce the microbial load on the surface of the fish and keep the surfaces that come in contact with fish clean to prevent cross-contamination. Successful use of chlorine in water disinfection for over a century has provided the background for use of chlorinated water in washing fish and cleaning processing surfaces and containers, among others.

The fishing industry includes a large number of small and medium-sized industries that are mechanized to varying degrees, from artisanal to fully automated processes. Furthermore, an important feature of the fish processing industry is the diversity of fish species handled (several hundred different species in the European Union alone), each with different intrinsic characteristics with respect to microbial load and microbial hazards; and

the large diversity in the products, ranging from raw whole fish to ready-to-eat products with widely ranging quality and safety requirements.

As indicated previously, the source of fish and fishery products is divided between wild-caught and aquaculture. In the wild-caught industry, the pre-harvest microbial hazards include organisms naturally occurring in the aquatic environment (e.g. *Vibrio parahaemolyticus*, *V. vulnificus*, *V. cholerae*), whereas post-harvest, they include those present in the general environment (e.g. *Listeria monocytogenes*) and those introduced as contaminants during handling (e.g. *Salmonella*) (Huss, Ababouch & Gram, 2003). In the aquaculture industry, especially in non-maricultural systems, owing to the high densities of biomass of fish in a limited area and the level of human intervention, pathogens such as *Salmonella* and *L. monocytogenes* could be associated with the fish and fishery products pre-harvest (Angulo, 1999). In both cases, this does not include any microorganisms that may contaminate the product through cross-contamination.

1.4.1 Types of chlorine compounds used in fish processing

Many countries provided data on the use of chlorine in the fish processing sector at the national level (Reilly, 2000). Examples of industry practices in the whitefish industry from South Africa are shown in Table 1.8. Although practices differ, most countries follow the WHO drinking-water guidelines (WHO, 2008) for potable water used in processing and the Codex Alimentarius Commission Code of Practice for Fish and Fishery Products for the level of chlorine in water that comes in contact with fish and fishery products (FAO/WHO, 2008c). Uses of chlorine in the fish and fishery product industry are summarized in Table 1.9.

The most commonly used forms of chlorine in the fish processing industry are calcium hypochlorite (granular or powdered form) and sodium hypochlorite (liquid form). The most common procedure is to utilize tanks to produce a solution from calcium hypochlorite salt or from concentrated sodium hypochlorite solutions; this solution is then pumped and mixed with a large tank containing the final chlorinated water. Alternatively, constant input of a high-concentration solution of hypochlorite is provided in the incoming water flow through automatic and semiautomatic devices like a flow metering pump. In most simple systems, the input of the solution is adjusted to the water input flow. However, in the more sophisticated control systems, the free chlorine content is adjusted automatically, through continuous amperometric analysers.

Chlorine dioxide has been shown to be effective in eliminating large populations of microorganisms and to extend the storage time of many foods, including fishery products (Richardson et al., 1998). Some of the reported advantages of chlorine dioxide over aqueous chlorine as a disinfection agent are that it is 7 times more potent than hypochlorite in killing bacteria, the bactericidal activity of chlorine dioxide is not affected by alkaline conditions and/or the presence of high levels of organic matter (Lin et al., 1996) and chlorine dioxide treatment produces very little or no trihalomethanes (THMs) in treated water (Kim et al., 1999). The use of chlorine dioxide resulted in up to 4.8 log reductions in the pathogenic population on fish (Kim et al., 1999; Andrews et al., 2002; Shin, Chang & Kang, 2004). Chlorine dioxide has also been used for treatment of seawater and ice-water slurry for storage of fish during the sorting process (Table 1.8).

Chlorine dioxide gas is unstable, and the hazards involved in handling and transportation are factors contributing to its limited application. Industrially, it could be prepared via the reduction of sodium chlorate by sulfur dioxide in aqueous solution.

Table 1.8. Application of chlorine-based sanitizers in the whitefish industry^a

Chemical	Medium	Application	Concentration used (mg/l)	Temperature (°C)	pH	Time	Where applied
Sodium hypochlorite	Liquid	End-point sanitizing of food contact surfaces with post-sanitization rinse	25	Ambient	5.5–7	15 min	Land based and on fishing vessels
		End-point sanitizing and cleaning of non-food contact surfaces	50–100	8–22			
Calcium hypochlorite	Powder	End-point sanitizing and cleaning of non-food contact surfaces, both smooth and rough	50–100	Ambient		10–15 min	Land based for drains and floors and on fishing vessels
		End-point sanitizing of food contact and non-food contact areas after potential contamination of surfaces with post-sanitization rinse	200	8–22			Land based and on fishing vessels
Chlorine dioxide	Gas dissolved in water	Treatment of raw water	5	4–22		Up to 6 h	Land-based treatment of seawater (where used)
		General processing	2.5	0–4		Up to 30 min ^b	Storage of fish during sorting process prior to draining of water from ice-water slurry
Chlorine gas	Gas	Treatment of raw water	50–100	8–12			Land-based treatment of seawater (where used)

^a From Graz (2008).

^b Time depends on the period taken to sort fish into specific weight ranges using an automated grader. After 30 min, a tub is replaced. Water containing chlorine dioxide is drained 10 min after completion of the grading, and the fish is left on ice.

Table 1.9. Summary information for chlorine-based interventions in fish and fishery product processing

Process application	Use level (mg/l)	Exposure time
Sodium/calcium hypochlorite (target microorganisms: <i>Vibrio</i> , <i>Salmonella</i> for raw product and <i>Listeria monocytogenes</i> for further processed and ready-to-eat product)		
Post-harvest rinse of whole or headed and gutted finfish	10	NA
Washing of slaughtered fish pre-processing (salmon)	200	Up to 8 h if transport
Immersion of headless shell on shrimp	50	NA
Treatment of water for depuration of shellfish	5	NA
Chlorine dioxide generated at >90% efficiency (target microorganisms: <i>Vibrio</i> , <i>Salmonella</i> for raw product and <i>Listeria monocytogenes</i> for further processed and ready-to-eat product)		
Ice to cool fish	100	NA
Dipping of fillets	5	NA
Acidified sodium chlorite (target microorganisms: <i>Vibrio</i> , <i>Salmonella</i> for raw product and <i>Listeria monocytogenes</i> for further processed and ready-to-eat product)		
Washing of salmon	50	1 min
Storage of salmon fillets on ice	50	7 days

NA, data not available

1.4.2 Industry practices

Fish and fishery products can be exposed to chlorine-containing compounds by dipping in baths, either in batches or in continuous processing, or by sprays (with or without pressure). In some cases, washings can be conducted in association with other operations, such as de-scaling inside rotating horizontal washers. Washing in batches usually takes a longer time than the other types of processes and could be associated with other operations, such as thawing or incorporation of additives (e.g. polyphosphates or sulfites). Washing by immersion in belts or by spray usually takes a few minutes (an exception could be fish thawing), and speed can usually be adjusted by adjusting belt speed or rotating speed and lean angle, in the case of rotating washers.

By far the largest exposure of fish could be to free chlorine in the water from melting ice. Time on ice since capture or harvest (from aquaculture) depends on the shelf life of the specific fish (e.g. for a fish with a 12- to 14-day maximum shelf life in ice, 7–9 days could be the maximum storage period on board before landing). There are two possible scenarios with respect to melting ice. First, chlorine dioxide may be added to the ice during the ice-making process, notwithstanding the potential corrosive effect on the ice bunker. This would suggest that the fish may be exposed to an excess of chlorine. This practice, however, affects the natural microflora on the surface of the fish, which, if present, reduce the attachment of cross-contaminating organisms by competitive exclusion and may, if not properly controlled, lead to the creation of an environment conducive to the contamination of the fish. Second, ice is produced with potable water having low free chlorine (sometimes water is dechlorinated to produce ice by filtering through charcoal), to avoid corrosion problems in ice machines. Therefore, the practical challenge in icing fish could be the lack of chlorine rather than an excess.

The number of times that fish and fishery products are exposed to chlorine between landing/harvest and the plant could vary according to distribution and marketing chains; in many cases, there is a washing/de-icing step followed by a re-icing after weighing at the time of the first sale or delivery to a proprietary processing plant. Fresh water is normally utilized

for this step, but in some locations, treated seawater is utilized. Free chlorine levels in the fresh water at this point are low, usually corresponding to the normal concentrations in public drinking-water. Free chlorine in the treated seawater may be elevated if chlorine is used as the disinfectant to treat the seawater. In the salmon industry, the use of solutions of free chlorine at 200 mg/l has been described for rinsing/washing steps of the whole slaughtered fish prior to processing or for transport of slaughtered fish from the growing centres to the processing plants.

The number of exposures to chlorine throughout the process depends on the conditions of the raw material, the type of final product to be processed and the type of technology utilized in the plant. At reception, there could be a de-icing step, which includes a rinse by immersion, followed by icing for storage before processing (and after weighing and coding). There could be a de-icing step as described previously before the fish enter the processing lines. The current practice of rapid processing to produce frozen fillets and fillet derivatives from whole fish may include 2–4 immersions in water, which may be chlorinated. The three initial washing/rinsing steps are after the heading and gutting (which may also be performed on the vessels at sea, depending on the process used by the capturing vessel), between the “dirty” and “clean” zones in a plant, and potentially after filleting, skinning and trimming. Further rinses could be used, but this is process dependent (e.g. addition of polyphosphates). If the time between the initial de-heading process and freezing is too long, there could be intermediate immersions in chlorine-containing ice-water slurries in the so-called “chillers”, aimed at reducing the temperature of fillets or intermediate products (the cooling effect can be achieved by icing the intermediate product, but in this case, a bath to de-ice would be necessary). The current tendency is towards “cleaner production”, which includes the reduction of the amount of water utilized in food and fish processing, thereby reducing the number of multiple immersions to reduce fish temperature.

There may be specific requirements in certain industries to reduce the load of high-risk pathogens. Overchlorinated water with chlorine levels of 200 mg/l has been used to control raw materials contaminated with pathogenic bacteria. It must, however, be noted that at free chlorine levels above 200 mg/l, sensory changes are induced in fish fillets (Castell, 1947). The most discussed situation is the handling of raw fish intended for raw consumption or the preparation of ready-to-eat products, in particular, cold-smoked salmon. In this specific case, the hazard is *Listeria monocytogenes*, and usual handling practices, including potable water wash and icing, do not reduce the pathogen load (Huss, Jorgensen & Vogel, 2000; Gram, 2001). Bremer & Osborne (1998) reported that wash regimes with water containing chlorine at 200 mg/l could eliminate over 99% of *L. monocytogenes* artificially inoculated on the surface of gilled and gutted king salmon (*Oncorhynchus tshawytscha*), but could not ensure a *Listeria*-free product. The current practice in the smoked salmon industry is to use free chlorine at 50–200 mg/l to dip fillets. This process has also been encountered in the processing of fresh whitefish fillets prior to air transport. A similar practice has been reported for control of *L. monocytogenes* in shrimp, probably intended for the sushi and sashimi market (FAO/WHO, 2008a,b). Although the previously described process has been criticized on different grounds, attempts to solve the problem of *L. monocytogenes* contamination using chlorinated water have continued. It is recognized that for the cold-smoked fish industry, it is vital that there be a control step to eliminate possible *Listeria* contamination on the external surface of fish prior to filleting and skinning (Bremer, Fletcher & Osborne, 2003).

Dinesh (1991) noted about 2 log reductions in counts of pathogens such as *Vibrio cholerae*, *V. parahaemolyticus*, *V. vulnificus* and *Salmonella* in laboratory-contaminated shrimp following washing with water containing chlorine at 10 mg/l or iodophor at 1 mg/l. Thampuran, Sreeranga & Surendran (2006) reported that chlorine at 4 mg/l could completely eliminate 10^3 *V. cholerae*/g in shrimp meat in 10 min, whereas in headless shell-on shrimp,

7 mg/l was required to achieve this reduction. Ice containing chlorine dioxide at 100 mg/l caused 4.8, 2.6 and 3.3 log reductions in numbers of *Escherichia coli* O157:H7, *S. Typhimurium* and *L. monocytogenes* on fish (mackerel) skin (Shin, Chang & Kang, 2004). Reduction in microbial levels up to 3 log units in shrimp and 1 log unit in crawfish was obtained after pressure washing with chlorine dioxide at ≥ 30 mg/l (Andrews et al., 2002). The dipping/washing of shrimp in solutions of hypochlorite at 50 mg/l has also been described, both in practice as well as in laboratory studies (FAO/WHO, 2008a,b; see Table 1.9). ASC wash of salmon fillets resulted in a 0.5 log unit reduction of *L. monocytogenes*, and the antimicrobial activity of ASC was enhanced when salmon was washed in ASC and stored in ASC ice (Su & Morrissey, 2003).

Bivalve molluscs from waters subject to microbiological contamination can be made safer by relaying in a suitable area or by using a depuration process, which may be done in water chlorinated to 5 mg/l as free chlorine to reduce the level of pathogenic bacteria in the water. This latter step can be performed only with low concentrations of chlorine, as higher concentrations would be toxic to the bivalves.

Norovirus infections must be considered an emerging infectious disease, with contaminated bivalve molluscs playing a major role in foodborne transmission. Other viral infections with regard to bivalve molluscs, particularly hepatitis A, must also be considered. Noroviruses serve as a model for other enteric viruses, such as hepatitis A virus, hepatitis E virus and the enteroviruses (FAO/WHO, 2006).

No evidence has been found that chlorine-based compounds are applied directly to fish and fishery products for the specific reduction of viruses.

1.4.2.1 Chlorine-based solutions for non-product contact situations

Chlorinated water not in contact with fish and fish products is utilized in the fish industry for different purposes, such as to clean facilities and equipment, to clean utensils (e.g. knives, cutting boards), to clean garments (boots, gloves, aprons), to wash empty packages (if necessary), for hand sanitation after hand washing and to cool sterilized products (e.g. cans, jars and pouches taken out from retorting).

In the case of cleaning of facilities and equipment, chlorinated waters are utilized in the rinsing and disinfection steps. Rinsing is usually performed with low chlorine concentration water (e.g. normal tap water); disinfection is performed with high chlorine concentrations (see Table 1.8). Whereas products other than chlorine have been suggested for disinfection and are utilized, chlorinated water (with and without the addition of acids or other disinfecting substances) is widely utilized in the fish industry, in both developed and developing countries. In a recent study on the general microbial ecology of fish processing plants, Bagge-Ravn et al. (2003) observed that in four different fish industries (two of cold-smoked salmon, semipreserved herring and caviar), disinfection was carried out with hypochlorite in three of them (alone or in association with other products); only in one was the disinfecting agent peroxyacetic acid.

Cleaning is usually performed by hand and/or utilizing some movable equipment, such as low-pressure foam cleaners. In more advanced and mechanized fish processing plants, a part of the line could be covered by the clean-in-place procedure.

The number of cleaning steps is variable, but in general they encompass the following: 1) cleaning of the fish processing plant for the removal of debris (by brush or scraping); 2) washing with clean water and appropriate detergents; 3) intermediate rinsing to eliminate remaining detergent; 4) disinfection (e.g. with chlorinated water); 5) rinsing to eliminate the excess of disinfecting agent; and 6) draining and drying with filtered hot air

(drying without rinsing is not recommended because of the possible production of chlorine gas).

Drying with hot air to obtain a clean, dry plant is a tendency in modern food and fish processing; however, it is not a step followed in all the fish industry around the world yet. Formerly, it was recommended that the disinfecting solution be left overnight and that rinsing be performed before restarting production the following day (Clucas & Ward, 1996). Today, however, it is preferred to dry the plant after disinfection, because chlorine-depleted water could become a vector for cross-contamination.

The disinfection of garments (e.g. gloves before entering the processing room) and plastic and rubber items such as boots and aprons is performed with a chlorine solution of 50 mg/l. Cleaning procedures for crates, boxes and plastic containers usually include a disinfection step with 50 mg/l. These are then rinsed in normal tap water (up to 5 mg/l as free chlorine).

Water for cooling canned products just after retorting is chlorinated, because at that stage, the sealing compound in the double seams is still molten; therefore, the vacuum forming in the headspace could pull micro-drops and bacteria through the double seams. Common chlorination levels taken at the drain point are usually in the order of 5–20 mg/l as residual chlorine (Clucas & Ward, 1996). This is because cans are usually somewhat contaminated on their exterior with organic material, such as oil, sauce and fish debris, all of which will consume chlorine, depleting the cooling solution and therefore increasing the risk of cross-contamination.

1.4.2.2 Non-chlorine-based alternatives

There are very few studies on alternatives to chlorine in fish processing. Whereas Rice, Graham & Lowe (2002) reported ozone as a microbiocidal agent reducing bacterial numbers in various types of foods, including fish, Vaz-Velho et al. (2006) noted that ozone treatment had no significant effect on *Listeria* counts in salmon-trout. The ineffectiveness of ozone treatment in reducing bacterial numbers in fish has been reported by several investigators (Haragushi, Simudu & Aiso, 1969; Ravesi, Licciardello & Racicot, 1987; Da Silva, Gibbs & Kirby, 1998). However, Gelman et al. (2005) noted that ozone pretreatment of tilapia extended the storage life when stored at 5 °C. Similarly, Campos et al. (2006) reported an extension of shelf life of farmed turbot in ozone-slurry ice. Thus, the results are not consistent, and it should be pointed out that ozone treatment is much more expensive than chlorine treatment.

Other alternatives to chlorine in fish processing that have been applied or trialled in the industry include quaternary ammonium compounds (QACs), which have been successfully used for the sanitation of hard surfaces (especially in areas sensitive to corrosion). Peroxyacetic acid and phosphoric acid have been trialled successfully for end-point disinfection (South African Deep Sea Trawling Industry Association Whitefish Technical Committee, personal communication, 2007) and in the wash steps prior to processing, but have not been implemented.

1.4.3 Summary

The use of chlorine-based compounds in the fish and fishery product industry is mainly focused on the end-point disinfection of product contact and non-product contact surfaces. Chlorine-based products, especially hypochlorites, are used because of their high antimicrobial efficacy and their relatively low cost, notwithstanding the corrosive nature of the products.

The application of chlorine-based compounds directly to the edible portions of fish and shellfish is limited to wash or rinse steps on whole fish and the dipping of fillets for pathogen reduction. Chlorine may also be used to treat water for depuration of bivalve molluscs.

Whereas chlorine is effective against the viruses associated with foodborne disease in, especially, shellfish, the application of chlorine directly to the product to reduce virus levels has not been reported to date.

1.5 Fresh fruits and vegetables

1.5.1 Leafy greens

Fresh fruits and vegetables are often washed to cool the product and remove field dirt before distribution. Water used to wash fresh produce is often treated with chemical disinfectants to prevent cross-contamination and reduce microbial growth on equipment surfaces. The washing process may also reduce microbial populations on produce surfaces. Leafy greens are discussed in this section as a representative of fresh fruits and vegetables. Leafy greens present a large surface to volume ratio, thereby incurring a greater exposure of edible tissue to the disinfectant compared with other fresh produce. In addition, the washing of leafy greens in water disinfected with chlorine is widely practised in countries where it is allowed.

The processing of fresh leafy greens has changed dramatically during the past 20 years. Products such as head lettuce are washed and bagged in the field. The production of bagged salad mixes is highly mechanized. Produce harvested into bins at the field is placed into refrigerated trucks. The leafy greens are moved to a refrigerated warehouse/processing facility and subsequently blended by being dumped onto a conveyor, which may carry the greens through a shaker to remove foreign material; the greens are then washed and sanitized in a water flume and centrifuged to remove excess water. The greens are then ready to be packaged.

1.5.1.1 Initial load of bacteria upon entry to processing

Leafy greens are a raw agricultural commodity and, as such, carry a robust bacterial load prior to entering the processing facility. The total aerobic bacterial levels can range from 5 to 6 log cfu/g on leafy greens (Johnston et al., 2006). These organisms may come from the soil, irrigation water, fertilizers, pesticides or human contact. Although coliforms may be present in substantial numbers, reaching 5 log cfu/g, they are generally not a human health concern. Pathogenic microbes are rarely found in association with field-harvested leafy greens; when they are present, levels are generally extremely low. The leafy green processing practices are designed to prevent cross-contamination, reduce the levels of microbes on the surface of the product and minimize any increase in microbial population associated with processing (Parish et al., 2003). The use of novel technology and maintenance of a cold chain from field to retail has been effective in limiting the growth of microorganisms with fresh and minimally processed leafy greens. The processing environment and particularly equipment (conveyor belts, bins and centrifuge) may have substantial microbial loads depending on the microbial load of the commodity handled and the cleaning and sanitizing programme in place (see section 1.6). Pathogens of concern, including *Escherichia coli* O157:H7, *Salmonella* and *Listeria monocytogenes*, and the predominant spoilage contaminant, *Pseudomonas* spp., can survive for extended periods on food contact surfaces (Wilks, Michels & Keevil, 2006).

1.5.1.2 Control of contamination during processing

Chemical control

Microorganisms associated with produce processing facilities and leafy greens are typically controlled through the use of disinfectants. Chlorine is perhaps the most universal disinfecting agent used. Chlorine is used to sanitize equipment and to control microbial populations in wash waters and on commodities. Disinfectants used to treat water for washing leafy greens include chlorine gas, ASC, calcium and sodium hypochlorite, chlorine dioxide, hydrogen peroxide, iodine, ozone, peroxyacetic acid and TSP. Information on the use of these disinfectants is presented in Tables 1.10 and 1.11.

Table 1.10. Summary information for chlorine-based interventions in uncut leafy green processing

Process application	Use level (mg/l)	Exposure time
Hypochlorous acid/hypochlorite (target microorganisms: enteric pathogenic bacteria and viruses and spoilage microorganisms)		
Whole product spray, at harvest, pre-cooling	50–200	2–10 s
Whole product dip or spray, post-harvest	25	2 min
Flume water for transport of leafy greens	10–50	30 s – 5 min
Flume water for whole fruits and vegetables prior to final wash	3	15 min
Pre-package spray or dip	200	5–10 s
Chlorite/chlorous acid (target microorganisms: enteric pathogenic bacteria and viruses and spoilage microorganisms)		
Processing water leafy greens	500–1200	15 s – 2 min
Generated chlorine dioxide (target microorganisms: enteric pathogenic bacteria and viruses and spoilage microorganisms)		
Flume water	3	NA
Whole fruits/vegetables	3	NA
Lettuce wash with spray	3	30 s – 5 min

NA, data not available. See text for activity under non-commercial conditions.

Table 1.11. Summary information for non-chlorine-based alternative interventions in uncut leafy green processing

Process application	Use level (mg/l)	Exposure time
Ozone (target microorganisms: enteric pathogenic bacteria and viruses and spoilage microorganisms)		
Flume water	2–3	30 s
Peroxyacetic acid (target microorganisms: enteric pathogenic bacteria and viruses and spoilage microorganisms)		
Flume water	20–30	1 min

Non-chemical control

The types of non-chemical methods that can be used to control microorganisms on leafy greens are few and include irradiation and ultraviolet (UV) light. The advantage of irradiation is that post-package (bagged) leafy greens can be treated, basically eliminating the potential for cross-contamination. Research suggests that human enteric pathogens may become internalized into leaf tissue (Solomon, Potenski & Matthews, 2002; Solomon, Yaron & Matthews, 2002; USFDA, 2009). Levels of internalized *Escherichia coli* O157:H7

associated with lettuce and spinach were reduced by 4 and 3 log, respectively, following irradiation by 1.0 kGy (Niemira, 2007). The D_{10} value (the radiation dose needed to inactivate 1 log of a target microorganism) obtained for *E. coli* O157:H7 in the study was 0.39 kGy, which is approximately 3- to 4-fold higher than that obtained for surface-inoculated *E. coli* on various types of leaf lettuce (Niemira, Somers & Fan, 2002). The United States Food and Drug Administration (USFDA) is reviewing the use of irradiation for prepackaged fresh produce, including leafy greens (USFDA, 2006). In 2008, the USFDA approved the use of irradiation for packaged iceberg lettuce and spinach. However, products to be treated by irradiation may still need to be washed to cool or remove field dirt. This wash water may require the addition of a chemical disinfectant.

1.5.1.3 Effectiveness of control measures

The efficacy of various disinfectants for the control of foodborne pathogens associated with leafy greens has been extensively studied under laboratory and pilot plant conditions. These conditions may not be accurate representations of commercial production. This research provides guidance with respect to the efficacy of washing treatments against a range of pathogenic and non-pathogenic microorganisms that could not be evaluated under commercial conditions.

When evaluating research presented in these papers, inoculation methods, sample size, sample processing and statistical analysis must all be considered. Many studies include conditions that would not be used or acceptable under commercial production practices. For example, commodity exposure times beyond 2 min or use of flume water temperatures above 4 °C are practices not currently used and would be difficult to implement, especially with respect to elevated flume water temperatures.

Chlorine-based interventions (Table 1.10)

The forms of chlorine commonly used to disinfect water used in the processing of leafy greens include chlorine gas, sodium hypochlorite and calcium hypochlorite (Table 1.10). The efficacy of chlorine in preventing cross-contamination and reducing the microbial load by dipping or spraying the commodity depends on the amount of free available chlorine in the solution, the pH and the amount of organic matter. There are many benefits to the use of chlorine, including cost and ease of implementation, and numerous studies demonstrate a 1–2 log reduction in microbial populations on the product as a result of washing in disinfected water (García, Mount & Davidson, 2003; Kim, Ryu & Beuchat, 2006). High loads of organic matter in the wash water likely play a significant role in limiting the effectiveness of chlorine. Most studies investigating the efficacy of chlorine compounds in reducing the microbial load on lettuce have used lettuce pieces. The action of cutting the lettuce tissue releases exudates that can significantly reduce chlorine availability. This can also lead to reduced effectiveness of the disinfectant in eliminating cross-contamination.

Bacteria adhere to a greater extent to cut than to uncut fresh lettuce, resulting in significant differences in the effectiveness of wash treatments. Seymour et al. (2002) showed approximately a 1 log greater reduction in *Salmonella* Typhimurium on uncut lettuce compared with cut lettuce following washing in potable water. The researchers achieved a 0.72 log reduction in *Salmonella* on cut lettuce following exposure to a solution of free chlorine at 100 mg/l (pH 7.0) for 10 min. Washing in potable water resulted in a 0.38 log reduction of *Salmonella*. Other studies using fresh-cut lettuce have shown no significant decrease in *E. coli* O157:H7 levels following treatment with chlorine at 20 mg/l (Li et al., 2001).

The use of chlorine dioxide in reducing levels of microorganisms on fresh vegetables has received considerable attention, as the activity of this compound is not substantially diminished in the presence of organic matter, and it does not react with ammonia to form chloramines (Huang et al., 2006; Mahmoud & Linton, 2008). Populations of *E. coli* O157:H7 and *Salmonella enterica* on lettuce leaves were reduced more than 5 log following exposure to chlorine gas at 5 mg/l for 14.5 and 19.0 min, respectively. However, the processing conditions had a negative impact on the visual quality of the product. Exposure of lettuce leaves for 30 min to chlorine dioxide at 4.3 mg/l decreased the levels of *E. coli* O157:H7, *Listeria monocytogenes* and *Salmonella* Typhimurium by 3.4, 5.0 and 4.3 log units, respectively (Lee, Costello & Kang, 2004).

Non-chlorine-based alternatives (Table 1.11)

Ozone and peroxyacetic acid are the main alternatives to chlorine for disinfection of water used in processing of leafy greens (see Table 1.11). Bacteria are killed very quickly in ozonated water; however, the efficacy of ozone gas in the inactivation of microorganisms associated with fresh uncut leafy greens is variable. The efficacy of ozone is influenced by the surface properties of the commodity, concentration, exposure time, relative humidity and microbial load. Again, studies reported in the literature have been conducted using fresh lettuce leaves cut into smaller sections. Ozone reduced the aerobic plate count of iceberg lettuce by about 1.0 log when it was treated with ozone at 7.5 mg/l for 10 min (García, Mount & Davidson, 2003). The reduction was comparable to treatment with chlorine at 200 mg/l. Similarly, exposure of lettuce to ozone at 5 mg/l for 5 min at ambient temperature resulted in a 1.4 log cfu/g reduction in aerobic plate count (Koseki & Isobe, 2006). Peroxyacetic acid in wash water reduced the level of *Listeria monocytogenes* on cut pieces of iceberg lettuce by 1.7 log units, which was significantly greater than the 1.0 log reduction achieved by using chlorine rinse (Hellstrom et al., 2006). Beuchat, Adler & Lang (2004) observed similar reductions in *L. monocytogenes* for these treatments when using iceberg lettuce pieces, but reductions were less for shredded lettuce and romaine lettuce pieces.

1.5.1.4 Summary

Chlorine-containing compounds are widely used throughout the fresh produce industry. Depending on the operation, whole intact leafy greens may be sprayed with or immersed into water containing elevated levels of chlorine. Chlorine is effective at reducing cross-contamination due to wash water, but minimal reduction in microbial load of the commodity is reported. Non-chlorine-based alternatives added to wash water have been evaluated for reducing pathogenic microorganisms on uncut leafy greens. The efficacy of these treatments is generally similar to that of chlorine.

1.5.2 Hydroponic fresh produce

The principal fresh fruits and vegetables produced hydroponically are sweet peppers, tomatoes, cucumbers, eggplants and lettuce. As hydroponic production generally requires a high initial financial investment, advanced technology and large material input, most hydroponic production is in developed countries, such as the Netherlands, Spain and France in Europe, Canada and the USA in North America, and Japan and the Republic of Korea in Asia. Because of stringent environmental policies and water shortages, increasing numbers of hydroponic greenhouse operations have started recycling their irrigation water. For example, a survey conducted by Richard, Zheng & Dixon (2006) reported that 58% of the hydroponic vegetable greenhouse area in Ontario, which has the most acreage of hydroponic production

in Canada, recycles its nutrient solutions. One of the biggest risks of recycling nutrient solution in hydroponic systems is the spread of plant diseases. To prevent disease spread in hydroponic systems, an array of water treatment technologies are being utilized. The most common technologies are heat treatment (pasteurization), UV radiation, ozonation and slow sand filtration. Although not common, chlorination as well as copper ionization (Zheng, Wang & Dixon, 2005), hydrogen peroxide treatment, ultrafiltration and iodine treatment are also used in some hydroponic systems for irrigation solution treatment.

The major target microorganisms for hydroponic water disinfection treatment include waterborne fungal pathogens (e.g. *Pythium*, *Phytophthora*, *Verticillium* and *Fusarium* spp.), bacterial pathogens (e.g. *Erwinia*, *Xanthomonas*, *Pseudomonas* spp.) and viral pathogens (e.g. cucumber green mottle mosaic virus, tomato mosaic virus). The most commonly used chlorine compounds are sodium hypochlorite, calcium hypochlorite and chlorine gas. For water disinfection, chlorine compounds are either injected into irrigation lines or injected/dissolved (for calcium hypochlorite) into water holding tanks. In most cases, water temperature is maintained around 20 °C; however, it often ranges from 15 °C to 30 °C. The recommended pH of the nutrient solution for hydroponic vegetable production ranges from 5.2 to 6.5. However, the pH during disinfection can range from 5.0 to 7.5, depending on individual situations.

There is little published information on the effectiveness of using chlorination in disinfecting the irrigation water or nutrient solution in commercial hydroponic systems. The information on the effectiveness of chlorination is mostly generated from small-scale research settings. For example, research conducted in a small-scale hydroponic tomato production system showed that free chlorine at 3 mg/l was as effective as a UV treatment and reduced the total counts of bacteria by 80% (Ewart & Chrimes, 1980). Hong et al. (2003) and Hong & Richardson (2004) reported that free chlorine at 2 mg/l at pH 6 provided complete control of zoospores of 15 isolates of *Pythium* and 8 isolates (7 species) of *Phytophthora* and concluded that free chlorine at 2 mg/l at discharge points (e.g. sprinklers) could effectively control zoospores of *Pythium* and *Phytophthora* species in irrigation water. Cayanan et al. (2009a) reported that free chlorine at 0.3–1 mg/l could kill zoospores or sporangia of two *Phytophthora* species with a 3 to 6 min contact time; free chlorine at 2 mg/l could kill zoospores of *Pythium aphanidermatum* with a contact time of 3 min; however, chlorine concentrations of 14 and 12 mg/l were required to control *Fusarium oxysporum* conidia and *Rhizoctonia solani* mycelia with a 10 or 6 min contact time, respectively. These experiments were conducted at room temperature with a nutrient solution of pH 6.5–7.0. Cayanan et al. (2009b) also found that free chlorine at 2.4 mg/l with a contact time of 5 min killed *Fusarium* sp., *Phytophthora* sp., *Pythium* sp. and *Verticillium dahliae* that were present in the irrigation water at a southern Canadian commercial nursery operation. Most of the aforementioned research used sodium hypochlorite.

Regardless of which chlorine compound is used, the main limitation is the risk of phytotoxicity due to high concentration of the free chlorine. Ewart & Chrimes (1980) reported damage in roots of hydroponic tomatoes when a free chlorine concentration of 3 mg/l was used in the hydroponic system. Cayanan et al. (2009b) reported that 8 out of 22 plant species investigated showed negative chlorine effects when overhead irrigation solution contained free chlorine at 2.4 mg/l. Use of each chlorine compound has its advantages and disadvantages. Whereas chlorine gas is easily injected into an irrigation solution and without any adverse effects on the hydroponic system, the initial investment is very expensive. Also, safety and security are major issues, whereby users are required to build special facilities to secure chlorine gas. Although sodium hypochlorite is readily soluble, cheap and easy to use and the initial investment in equipment is more economical than that of chlorine gas, the potential for having high concentrations of sodium ion in the hydroponic system is not

desirable. Calcium hypochlorite is much safer to handle compared with both chlorine gas and sodium hypochlorite; however, calcium deposition may cause clogging of the irrigation lines.

1.5.3 Sprouts and sprouting seeds

1.5.3.1 Chlorine-based interventions

The use of chlorine to minimize microbial risks associated with sprout production varies considerably, depending on regulatory policies related to chlorine use in different countries, whether particular types of sprouts are traditionally consumed raw or cooked, and other factors.

Because of the microbial risks involved, sprout production is generally defined as a food process. As such, it may involve the use of chlorine or other sanitizers in ways similar to what could be expected in a wide range of food processing environments—that is, for food contact surfaces and, in some instances, at low concentrations as a final product rinse. Seed disinfection treatments using strong calcium hypochlorite solutions (e.g. 20 000 mg/l) are used by some sprout producers in order to be in compliance with the USFDA's guidance recommendations for minimizing microbial risks associated with sprouts (USFDA, 1999a,b).

The rationale for strong seed disinfection interventions is that seed has been determined to be a likely vehicle by which microbial contamination can get into sprouts (USFDA, 1999a,b). However, the research evidence in support of the use of such high levels of chlorine for seed sanitization has shown inconsistent results. Published reports on the efficacy of the use of 20 000 mg/l chlorine seed soaks mention pathogen reductions ranging from <1 to 8 log units (Montville & Schaffner, 2004, 2005). Possible factors for such a wide range of results include different properties of different seed types and individual seed lots (Charkowski, Sarreal & Mandrell, 2001), the use of inoculated samples rather than naturally contaminated seeds (Stewart et al., 2001), the “tailing effect” (Periago et al., 2002) and the lack of a standard protocol for carrying out seed sanitization studies (Beuchat et al., 2001).

1.5.3.2 Non-chlorine-based alternatives

There have been many investigations into alternatives to chlorine-based chemicals as a disinfection treatment in the production of sprouts (Beuchat, 1997; Beuchat & Taormina, 1999; Fett & Rajkowski, 2005). Several have shown effectiveness comparable to, or possibly greater than, that of chlorine seed treatments (Hu, Churey & Worobo, 2004; Fett & Rajkowski, 2005; Kumar et al., 2006; Bari et al., 2008).

Rapid immersion in hot water is used effectively for disinfecting mung beans (Bari et al., 2008), and dry heat over periods of several days or longer has also shown promising results with mung bean seed (Hu, Churey & Worobo, 2004). However, the variety of seed types (and sizes) being used for sprouts, plus variations in the condition of the seed coat, may require trial and error adjustments in heat settings and duration for each seed type, and possibly even for different seed lots within a given type.

Gamma irradiation (Thayer et al., 2003) and electron beam irradiation of seed have also been investigated. With both, there is some loss of yield with doses adequate for disinfection. With electron beam irradiation of alfalfa seed, some stunting of growth and curling of the sprouts were observed that might negatively affect value and customer acceptance (R. Sanderson, personal communication, 2008).

Regarding other possibly effective treatment options that may exist, as of May 2008, no alternatives to the 20 000 mg/l chlorine seed treatment have been acknowledged as being acceptable by the USFDA, and so many producers in the USA are reluctant to use them.

The use of a sanitization step, such as chlorine or any alternative, as a seed treatment is somewhat problematic, in that it is done at the start of the sprouting process and is therefore followed by 2–6 or more days of sprout growth in warm, moist conditions in a nutrient-rich environment, allowing for the possible recovery and proliferation of any treatment survivors (USFDA, 1999a,b). For this reason, it may make sense to consider treatment options other than the usual kill-step approach (Montville & Schaffner, 2004).

Research into the use of competitive exclusion as a pathogen control method in sprout production has shown promise (Matos & Garland, 2005; Fett, 2006). Further research is needed to determine whether a single organism or combination of organisms would be most effective in inhibiting or eliminating organisms of concern. One attractive aspect of the competitive exclusion approach is that the establishment and maintenance of benign microbial populations may inhibit growth of organisms inaccessible to treatment, as well as lessen vulnerability to cross-contamination that can result from a disinfection step, where commensal flora are reduced or eliminated.

1.6 Food contact surfaces

1.6.1 Disinfection of food contact surfaces using chlorine-based compounds

1.6.1.1 Function and target microorganisms

The function of the application of chlorine-containing compounds onto hard non-porous food contact surfaces prior to the beginning of a food processing shift is to reduce populations of disease- and spoilage-causing microorganisms that may be present on equipment or utensils after cleaning. The cleaning and disinfecting programmes associated with food production processes include multiple steps, generally beginning with a pre-rinse with potable water to remove large food soils and debris. This is followed by the application of a cleaner, which is selected by considering the nature of the soil to be removed, the characteristics of the water in the food processing facility, the material composition of the surface being cleaned, the method of application and the environmental impact that the chemistry may play in the waste stream. A post-rinse step typically follows cleaning to remove residual cleaning chemicals. Next, a disinfectant is applied and, in some cases, is followed again by a potable water rinse. The use of chemical disinfectants in food processing facilities is generally regulated by governmental bodies throughout the world. Routine application of disinfectants at concentrations of active biocide resulting in the reduction of populations of vegetative bacterial pathogens is in some countries referred to as “sanitization” and is not followed by a water rinse. This application is hereby referred to as “no-rinse disinfection”. In some instances, disinfectants are applied at relatively higher active biocide concentrations, which are followed by a water rinse. In the European Union and other parts of the world, the application of a disinfectant at any level to a food contact surface is required to be followed by a potable water rinse to remove residual chemicals.

Biocides are also used during operation to control the accumulation of microbial populations on the food contact surfaces associated with conveyor belts and slicers. The majority of these applications are located in fresh and ready-to-eat meat and poultry processing facilities. Conveyor belts are used to transfer product through processing and ultimately to packaging. Over the course of production, fat and protein soils accumulate on belt and slicer surfaces along with a population of microorganisms originating predominantly from the product being conveyed or processed in a slicer. Cross-contamination between the food contact surfaces and food product is therefore a concern to processors. Control of

pathogenic bacteria such as *Salmonella*, *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Campylobacter* and spoilage-causing bacteria is critical.

1.6.1.2 Active chlorine compounds used

There are six primary forms of chlorine-containing compounds used in food production and processing for the disinfection of food contact surfaces. The hypochlorites are most commonly used. ASC and chlorine dioxide are used primarily to disinfect process water; their use to disinfect surfaces is a secondary benefit in the process application. Working solutions with an operational pH of 2.3–3.2 exhibit a chemistry that is principally that of chlorous acid, which forms on acidification of chlorite (Rao, 2007).

To a lesser extent than the inorganic forms, organic chlorine compounds are used, particularly chloramine-T and dichloroisocyanurate. Chloramine-T contains approximately 25% available chlorine, whereas the sodium and potassium salt forms of dichloroisocyanurate contain 60% and 59% available chlorine, respectively (Dychdala, 2001).

1.6.1.3 Treatment conditions

Conditions for the treatment of food contact surfaces with chlorine-containing biocides are presented in Table 1.12. In practice, disinfectant solutions are applied to surfaces in a number of different ways. They can be sprayed or circulated through equipment, referred to commonly as “flooding the surface”. They may also be atomized or fogged into the air to help reduce airborne contamination. Key to their effectiveness is intimate contact of a proper disinfectant concentration with the target microbial cell. Therefore, adequate coverage and exposure time over a precleaned surface are required.

Table 1.12. Treatment conditions for chlorine-containing compounds applied to food contact surfaces prior to operation

Compound	Application	Exposure time (min)	Concentration (mg/kg) ^a	pH	Temperature (°C)
Hypochlorite/ hypochlorous acid (I)	No-rinse disinfection ^b	1	Up to 200	6–8	12–21
	Disinfection	10	600–1200	6–8	12–21
Chlorite/chlorous acid (III)	No-rinse disinfection	1	Up to 335	2–3	12–21
Mixed oxychlorine species (III/IV)	No-rinse disinfection	1	Up to 200 (chlorine dioxide)	2–7	12–21
Chlorine dioxide (IV)	No-rinse disinfection	1	Up to 200	5–7	12–21
Chloramine-T	No-rinse disinfection	1	Up to 200 (available chlorine)	8.5	12–21
	Disinfection	5	0.3–0.5% solution	8.5	12–21
Dichloroisocyanurate	No-rinse disinfection	1	Up to 200 (available chlorine)	5–7	12–21

^a Unless otherwise specified.

^b Limited to the application of United States Environmental Protection Agency–registered sanitizers for food contact surfaces.

In clean-in-place programmes, the disinfectant is applied as a separate step in the cleaning programme. Disinfectants may also be applied via a central disinfection system,

which consists of a centralized preparation and distribution system to carry the use solution to the point of use. The disinfectant is distributed via piping and drop hoses to the locations where it will be used. This significantly increases ease of use and helps to ensure that the disinfectant will be used.

The minimal time of exposure depends on regional governmental requirements as well as the surface and related food processing-specific conditions. In the USA, chlorine use solution concentrations of generally up to 200 mg/kg as free chlorine are not required to be rinsed off food contact surfaces prior to operation. After the no-rinse disinfectant is applied, it must be allowed to adequately drain from surfaces before contact with food. In other regions throughout the world, a potable water rinse is required.

To disinfect belts during processing operations, use solutions are generally sprayed onto the surface continuously throughout production. A spray nozzle manifold is fitted over the return side of the conveyor belt, and nozzles are configured on both sides of the belt to ensure adequate coverage. The use solution is sprayed over the surface via a low-flow, low-pressure application to minimize the potential for aerosolization of soils and microorganisms. The application is generally not followed by a potable water rinse. Sanitizer is allowed to drain from food contact surfaces as the belt travels through the return side, underneath the conveyor.

Disinfectants applied to slicers are generally applied intermittently (i.e. when food product is not actively being sliced or processed), and the application is typically not followed by a potable water rinse.

1.6.1.4 Effectiveness of the process

When used appropriately and in accordance with manufacturer recommendations, chlorine-containing biocides for food contact surfaces are generally effective in reducing populations of microorganisms in the food plant environment. In the USA, the United States Environmental Protection Agency (USEPA) requires no-rinse disinfectants for food contact surfaces to achieve a 5 log reduction in populations of suspended *Escherichia coli* and *Staphylococcus aureus* in 30 s at an exposure temperature of 25 °C. The antimicrobial activity of chlorine is dependent on environmental factors during exposure of microorganisms to the biocide. Factors such as pH, temperature, organic load and water hardness can all play significant roles in antimicrobial efficacy.

The influence of chlorine use solution pH on antimicrobial efficacy has been well characterized. Dychdala (2001) reviewed much of the early research in this area. Most commercial hypochlorite solutions produce a slightly alkaline pH at their use dilution. The antimicrobial properties of chlorine are not as favourable under slightly alkaline conditions; however, the stability of the solution is much improved. Other commercial products, however, are formulated to ensure a buffered pH of 6–7.5 to maximize efficacy (Stopforth et al., 2002). Chlorine is not affected by hard water salts unless they cause an upward drift in pH of the working use solution.

The effect of organic matter on the bactericidal efficacy of chlorine compounds is well documented (Kotula et al., 1997; Dychdala, 2001). The type of organic soil and the amount of this material present influence the extent to which efficacy is depressed (Hekmati & Bradley, 1979). Differences between soil types may contribute to the binding of free chlorine by amino groups in the proteinaceous lean versus lipid- or carbohydrate-based materials (Cords et al., 2005). The tenacity of bacterial cells within biofilms to resist inactivation or death by exposure to chlorine compounds has been repeatedly demonstrated (Joseph et al., 2001; Stopforth et al., 2002; Cords et al., 2005). Resistance is likely the result of impedance by the biofilm extracellular matrix of chlorine penetration by a reaction-

diffusion interaction (Chen & Stewart, 1996). Ronner & Wong (1993) demonstrated that recoverable cells within biofilm communities associated with rubber gasket material were reduced by less than 1–2 log following treatment with free chlorine at 100 mg/l. Their planktonic counterparts were completely killed following similar treatment in suspension.

The fungicidal activity of chlorine has not been as extensively reported as its bactericidal activity. Cheng & Levin (1970) studied the inactivation of *Aspergillus niger* conidiospores upon exposure to free chlorine at 1–20 mg/l. A comparison of their findings with those of other authors (Hays, Elliker & Sandine, 1967; Ito & Seeger, 1980) indicates that fungal spores are more resistant than vegetative bacteria. Ver Kuilen & Marth (1980) investigated the sporicidal effect of hypochlorite on *A. parasiticus*. Following treatment with chlorine at 3 mg/l for 15 min, the number of recovered conidia fell by 3.5 log units.

Factors affecting the efficacy of chlorine may not necessarily apply to chlorine dioxide, which does not form hypochlorous acid when dissolved in water, like other chlorine sources. For example, chlorine dioxide is more tolerant of organic material than chlorine.

Few published data are available regarding the effectiveness of hypochlorite or ASC in controlling the growth and/or accumulation of bacteria on belt and slicer surfaces during processing operations. Effectiveness can be inferred through studies evaluating the impact of organic material on chlorine efficacy.

1.6.1.5 Limitations of the process

The significant contributors to the limitation of effectiveness of the use of chlorine compounds on food contact surfaces are inadequate cleaning and preparation of surfaces prior to disinfection, improper concentration of free chlorine or active species in the use solution, inadequate exposure time and lack of complete coverage or accessibility to target microorganisms. The latter may be due to improper design of processing equipment or equipment that has not been suitably maintained, allowing for the harborage of microbial niches. The effectiveness of chlorine compounds during processing operations is limited by the accumulation of organic matter, although some treatment systems strive to reduce the presence of soil prior to the disinfection step through the use of scrapers or brushes. Disinfectants more tolerant of organic matter are clearly better suited for most in-process applications.

Also critical is the quality of the water in a food processing facility used to dilute concentrated chlorine chemicals to working use solutions. Although not directly affected by hard water salts, upward drifts in pH may limit the efficacy of free chlorine. Alternatively, reductions in pH levels below 4 may result in the generation of chlorine gas and/or cause corrosion of stainless steel surfaces. Stainless steel corrosion is of particular concern to food manufacturers processing acidic foods, such as tomato products. Residual food soils, if left on food contact surfaces, may combine with chlorine solutions, resulting in pitting of stainless steel. Pitting can present harborage sites for accumulation of food soils and microorganisms.

Use of chlorine dioxide to disinfect food contact surfaces is limited by the innate instability of the chemistry, the need to generate the active chemical on site and the safety risks that chlorine dioxide gas poses to workers if ventilation systems are inadequately designed or maintained. The high initial capital cost of a chlorine dioxide generator is another factor limiting its use.

1.6.2 *Disinfection of food contact surfaces using non-chlorine-based alternative compounds*

Several non-chlorine-based alternative biocidal compounds are utilized to disinfect hard non-porous food contact surfaces. They have functions and target microorganisms similar to those of chlorine-based compounds.

1.6.2.1 Alternative compounds used

The most widely used inorganic peroxide on food contact surfaces is hydrogen peroxide. Organic peroxygen compounds used for the sanitization of food contact surfaces include peroxyacetic acid, peroxyoctanoic acid and mixtures of the two. Hydrogen peroxide is widely used for sterilization of equipment and containers in aseptic packaging for foods and drinks. In the USA, it is approved by the USFDA for this application (USFDA, 1990). Peroxyacetic and peroxyoctanoic acids are widely used to disinfect food contact surfaces. Peroxyacetic acid has application as well for use as a commercial sterilant in aseptic packaging operations.

Iodophors, which are mixtures of iodine and surface-active agents that act as carriers and solubilizers for the iodine, are commonly used on food contact surfaces in the beverage industry.

Also commonly used on food contact surfaces are QACs. QACs approved as no-rinse disinfectants for food contact surfaces include the “second generation” QAC, *n*-alkyldimethylbenzylammonium chloride; the “third generation” dual QACs, *n*-alkyldimethylbenzylammonium chloride and *n*-alkyldimethylethylbenzylammonium chloride; the “fourth generation” twin or dual chain QACs, didecyldimethylammonium chloride and dioctyldimethylammonium chloride; and “fifth generation” mixtures of fourth-generation and second-generation QACs.

Ozone is a powerful and naturally unstable oxidizing gas that, when dissolved in water, is used for the sanitization of food contact surfaces. Because of its instability, it must be produced on site at the food processing facility.

Peroxyacetic and/or peroxyoctanoic acids, QACs and ozonated water may be applied to conveyor belts and slicers during processing.

1.6.2.2 Treatment conditions

Treatment conditions for the application of non-chlorine-based alternatives to food contact surfaces are presented in Table 1.13. Generally, environmental, application and regulatory conditions are similar to those applicable to the use of chlorine-based compounds on food contact surfaces, described above.

1.6.2.3 Effectiveness of alternative compounds

As with the chlorine compounds, these alternative compounds are generally effective if food contact surfaces are sufficiently prepared (i.e. cleaned and rinsed) prior to the application of the biocide. Appropriate design and maintenance of processing equipment are also essential to ensure contact between the active chemical and the target microorganisms.

Table 1.13. Treatment conditions for alternative compounds applied to food contact surfaces prior to operation

Compound	Application	Exposure time	Concentration (mg/kg) ^a	pH	Temperature (°C)
Peroxyacetic acid	No-rinse disinfection ^b	1 min	Up to 315	3–4.5	12–21
	Disinfection	10 min	Up to 2320	3–4.5	12–21
	Commercial sterilization	Up to 20 s		3–4.5	40–60
Peroxyoctanoic acid	No-rinse disinfection	1 min	Up to 122	1.5–2	12–21
	Disinfection	10 min	Up to 547	1.5–2	12–21
Hydrogen peroxide	Commercial sterilization	3–7 s	35%	2–3.5	21
Iodophor	No-rinse disinfection	1 min	Up to 25	2–5	12–21
	Disinfection	10 min	Up to 75	2–5	12–21
QACs	No-rinse disinfection	1 min	Up to 200 (1st–4th generation); up to 400 (5th generation)	7–8	12–21
	Disinfection	10 min	800–1200	7–8	12–21
Ozonated water	No-rinse disinfection	1 min	1.5–4	6–8.5	12–21

^a Unless otherwise specified.

^b Limited to the application of USEPA-registered sanitizers for food contact surfaces.

The effectiveness of peroxyacetic and peroxyoctanoic acids has been reviewed (Block, 2001; Cords et al., 2005). Their efficacy is influenced by numerous factors, including concentration, contact time, temperature and pH of the use solution. Other factors include the presence of organic material and, to a lesser extent, the impact of hard water salts. Organic peroxygen compounds achieve a broad spectrum of activity over a broader pH range than hypochlorous-generating chlorine compounds. Antimicrobial activity has been observed to diminish above pH 7 (Cords et al., 2005). The effect of pH may be a result of the shifting of the equilibrium action of the peroxygenated compounds in a use solution. Peroxyacetic and peroxyoctanoic acids exhibit significant bactericidal activity at low temperatures, a characteristic that lends itself to wide use in food and beverage processing environments, including broad applications in clean-in-place systems. The presence of organic material has less impact on the efficacy of these organic peroxygen compounds compared with chlorine (Block, 2001). Holah et al. (1990) evaluated 12 commonly used surface disinfectants using bacterial biofilms developed on stainless steel. The authors concluded that peroxyacetic acid was the most effective of the compounds tested. Similar results were observed in studies reported by Stopforth et al. (2002), Krysinski, Brown & Marchisello (1991) and Carpentier & Cerf (1993), in which peroxyacetic acid was compared with other biocides. Fatemi & Frank (1999) presented similar results using organic challenges.

Iodine, unlike chlorine, is bactericidal over a fairly broad pH range against a wide spectrum of microorganisms, including yeasts and moulds. Iodophors may also provide a weak acid rinse for mineral buildup control and are less irritating to the skin than chlorine (Cords et al., 2005). In many cases, iodophors are effective at much lower concentrations than chlorine (Gershenfeld & Witlin, 1955; Trueman, 1971). Lindsay & von Holy (1999) investigated the effectiveness of an iodophoric preparation at 35 mg/l as iodine to reduce

populations of planktonic and sessile *Bacillus subtilis* and *Pseudomonas fluorescens*. The iodophor performed as well as the peroxyacetic acid-based and chlorhexidine-based sanitizers also analysed. Iodophors do not lose antimicrobial efficacy as rapidly as chlorine in the presence of organic material (Cords et al., 2005). This is especially true at low pH (Davis, 1962). At higher pH, an organic matter effect becomes apparent. Generally, iodophors are more adversely affected by hard water salts than chlorine, and the degree of influence depends on the specific type of iodophor being evaluated.

Because of the diversity of QACs commercially available, general statements regarding the effectiveness of QACs and the environmental conditions that influence them are difficult. The pH, temperature, organic matter and water hardness may all influence activity. Much of the early research that examined the effect of hydrogen ion concentrations on the antimicrobial activity of QACs suggests that maximum efficacy is exhibited in the alkaline pH range (Soike, Miller & Elliker, 1952). However, further work has indicated that the effect of pH may vary with bacterial species, with Gram negatives being more susceptible to QACs in the acid pH range and Gram positives in the alkaline pH range (Cords et al., 2005). QACs are generally not as effective as chlorine, iodophors or peroxyacids at cold temperatures. The activity of various QAC formulations against bacterial biofilms was studied by Krysinski, Brown & Marchisello (1991). The residual activity of QACs has been noted (Cords et al., 2005) and is an attribute often sought after by food processors.

Ozone is a powerful broad-spectrum biocide. Reviews of the applicability of ozonated water in food processing suggest the range of ozone concentrations needed to achieve effective sanitization of a food contact surface is 1.5–4 mg/kg (Kim, Yousef & Dave, 1999; Weavers & Wickramanayake, 2001). Ozone is quite unstable and has limited solubility in water at high temperature and pH.

1.6.2.4 Limitations of alternative compounds

The general limitations of the alternative compounds in terms of their ability to effectively sanitize or disinfect food contact surfaces are similar to those described above. Additionally, each alternative biocide may be associated with limitations specific to its chemical nature.

Peroxyacetic and peroxyoctanoic acids are sensitive to metal ions, so the quality of water used in the preparation of working solutions is critical. These biocides are also corrosive to soft metals, such as brass, copper, mild steel and galvanized steel. Corrosivity is accelerated by the presence of high concentrations of chloride in the water (>75 mg/kg). High temperatures will also exacerbate the corrosion rate. Concentrated peroxyacetic acid has a strong, pungent odour.

QACs, when used in mechanical operations, can foam and therefore are not recommended for use in clean-in-place systems. They are also not effective at low temperatures (Cords et al., 2005) and have little tolerance of hard water salts.

A large capital investment is required of food processors implementing the use of ozone for disinfection of their facility. Ozone must be generated and monitored on site. Additionally, many applications require adequate ventilation systems to operate within established exposure limits (e.g. <0.1 mg/l continuous 8 h exposure). Validation that the process is achieving required thresholds of disinfection effectiveness is required.

1.6.2.5 Summary

Active chlorine compounds are broadly used in food processing facilities to disinfect food contact surfaces prior to the beginning of operation. Of the active chlorine compounds,

sodium hypochlorite is the most commonly used. The process is generally effective if surfaces are properly cleaned and prepared before the application of the biocide. Several non-chlorine-based alternative compounds are utilized as well, including peroxyacids, iodophors, QACs and ozonated water.

Additionally, biocides are used to mitigate the accumulation of bacterial populations on food contact surfaces during production. Hypochlorite, ASC, peroxyacids, QACs and ozonated water may be used for this application.

Requirements related to completing the cleaning and disinfection cycle with a potable water rinse vary globally from region to region and from country to country. The final step of the cycle in food processing facilities within the USA is the application of a USEPA-registered no-rinse food contact disinfectant. The practice mandates that treated surfaces be adequately drained prior to production, but it is expected that chemical residues contact food. Potable water rinsing is generally not practised in those applications in which biocides are applied to food contact surfaces (e.g. conveyor belts and slicers) during production. Because this application is practised in close proximity to the contact of the treated surface with food, one can expect chemical residues to come into contact with the food as well. There is, however, little information available regarding the quantification of such residuals on foods.

1.7 References

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2. CHEMISTRY OF DISINFECTANTS AND FORMATION OF DISINFECTION BY-PRODUCTS IN FOOD AND WATER

2.1 Introduction

This chapter describes the most common disinfectants/sanitizers used in food processing and summarizes information on their chemistry and the by-products that may be produced during their interactions with foods during processing. These disinfectants include chlorine-based disinfectants, such as acidified sodium chlorite (ASC), *N*-chloramines (especially monochloramine), chloramine-T, chlorine dioxide, hypochlorite-related compounds and sodium dichloroisocyanurate, as well as non-chlorine-based alternative disinfectants, including 1,3-dibromo-5,5-dimethylhydantoin, hydrogen peroxide, ozone, peroxyacids, quaternary ammonium salts, such as cetylpyridinium chloride (CPC), iodophors, sodium metasilicate and trisodium phosphate (TSP).

The common disinfectants are oxidants and chemically reactive and differ in their disinfection efficacy. They also vary in their oxidation capability and other chemical activity (Table 2.1). The ideal disinfectant would have high broad-spectrum efficacy against microorganisms and low by-product formation potential.

Table 2.1. Relative characteristics of oxidants/disinfectants^a

Oxidant	Disinfecting efficiency	Oxidizing efficiency	Halogenation capability
Chlorine	High	High	High
Chlorine dioxide	High	High	Low
Monochloramine	Low	Low	Low
Ozone	High	High	None (no bromide)
Hydrogen peroxide	Low	Moderate	None
Bromine	High	Moderate	High
Iodine	High	Low	Low

^a From Rice & Gomez-Taylor (1986).

The chemistry of disinfection by-products (DBPs) that may be formed in water and on foods—bromate, chloral hydrate, chlorate, chlorite, dimethylhydantoin, haloacetic acids (HAAs), haloacetonitriles (HANs), halofuranones (MX and MX analogues), *N*-nitrosamines and trihalomethanes (THMs)—is also addressed in this chapter, in connection with the respective treatments that may generate the by-products.

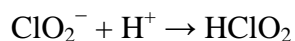
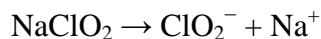
Other disinfectants may have some applications in food and/or water processing, but they were beyond the scope of this assessment. Among these are ionizing radiation (e.g. gamma), ultraviolet (UV) light, electron beam radiation and copper ionization.

2.2 Acidified sodium chlorite

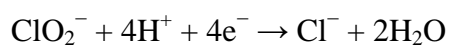
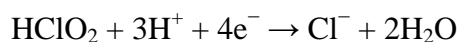
2.2.1 Chemistry

ASC (NaClO₂) is a combination of sodium chlorite (25%) and a food-grade acid (50%). It is clear and colourless. The chemical name is sodium chlorite (chlorous acid,

sodium salt; Chemical Abstracts Service [CAS] registry number 7758-19-2). Sodium chlorite is activated with any food-grade acid at levels sufficient to reach pH values in the range 2.3–2.9 for spray and dip solutions. The active components are chlorous acid, which is a strong oxidizing agent, and chlorine dioxide. The addition of acid to sodium chlorite generates chlorous acid:



The oxidation/reduction of chlorous acid and chlorite ion (ClO_2^-) may also generate chloride ion via the following reactions:



2.2.2 Application and fate in foods

ASC is used as a broad-spectrum disinfectant in poultry chiller water as well as in processing of meats, poultry, seafoods, fruits and vegetables. Its antimicrobial action is derived from chlorous acid and chlorine dioxide, the concentrations of which are dependent on the pH of the solution (USDA, 2002). ASC is approved under several national regulations for application onto the surface of different types of fresh and processed foods at a sodium chlorite concentration range of 50–1200 mg/l (e.g. FSANZ, 2006; USFDA, 2006). The sodium chlorite concentrations used are within the range 500–1200 mg/l for spray and dip solutions (pH 2.3–2.9) and 50–150 mg/l for chiller water (pH 2.8–3.2). Fresh and processed fruits and vegetables are subjected to a water rinse after ASC application followed by a 24 h withholding time (for cut produce only). Treatment of whole or parts of poultry carcasses, sausages or delicatessen meats (cold cuts) is carried out by spraying or dipping prior to or after chilling. ASC is also used to treat pre-chilling and chilling water at relatively low levels (i.e. 50–150 mg/l as sodium chlorite) into which poultry carcasses are submerged. Poultry and meat products are not rinsed subsequent to treatment.

Chlorine dioxide, chlorite ion and chlorate ion (ClO_3^-) are generated as reaction products; chloride is the final reduction product. The respective concentrations will vary depending on the pH of the mixture. The dissociation of chlorite to chlorous acid is about 31% at pH 2.3, 10% at pH 2.9 and 6% at pH 3.2, and the amount of chlorine dioxide does not exceed 1–3 mg/l (USDA, 2002). Thus, a 1200 mg/l solution of ASC is expected to convert to chlorous acid at 376 mg/l at pH 2.3 or 123 mg/l at pH 2.9; a 50 mg/l solution of ASC is expected to convert to chlorous acid at 16 mg/l at pH 2.3 or 3 mg/l at pH 3.2 (FAO, 2007).

The residual concentrations of chlorite and chlorate as reported in the data submitted to the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) (WHO, 2008a) for raw products of three food categories that had been treated with ASC solution were as follows: meat and meat products, including poultry, 0.1 mg/kg for both chlorite and chlorate; fish and fish products, 0.01 mg/kg for chlorite and 0.1 mg/kg for chlorate; and fruits and vegetables, 0.01 mg/kg for chlorite for all fruits and vegetables, except for leafy vegetables (0.23 mg/kg), and 0.01 mg/kg for chlorate. The treatment was at the proposed sodium chlorite use level of 1200 mg/l and under optimum conditions to fulfil the technological purpose (with sufficient time of spray or immersion and drip with water wash and holding time). The results showed

that residues of chlorite and chlorate in most foods treated with ASC declined to levels below the limits of detection with time (after treatment, rinsing and a holding period).

Residues of chlorite and chlorate were reported by SCVPH (2003) for poultry carcasses immersed in a 150 mg/l ASC solution at pH 2.8 and 5 °C for 1 h, then drained for 5 min and rinsed for 5 min in clean water. The residue levels were lower than the detection limit (chlorate <19 µg/kg) or became so after 2 h (chlorite <16 µg/kg). Furthermore, cooking of foods treated with chlorite solutions may either drive off chlorite and chlorate residues or reduce them to chloride (USFDA, 1995). Therefore, the concentrations of chlorite and chlorate in poultry and seafood after cooking would be negligible. In treatments with ASC at 500, 850 and 1200 mg/l for 5 s and dip in rinse water, both raw and cooked meat samples were below the estimated detection limit (0.03 µg/cm² of meat surface) for chlorite and chlorate (USFDA, 1997). However, residues would remain in seafood consumed raw. One industry sponsor estimated that 1 mg/kg as chlorite and 9 mg/kg as chlorate could remain in raw seafood (USFDA, 1998a).

A manufacturer provided analyses of different fish and seafood (salmon, snapper, catfish, scallops and shrimp) treated by immersion for 30 s in ASC at 1200 mg/l at pH 2.3 and allowed to drip for 30 s. The chlorate and chlorite residues were analysed without a potable water rinse post-treatment after 0, 24 and 48 h post-treatment. No chlorate was detected (limit of detection [LOD] 0.1 mg/kg) at any point, and chlorite was not detected (LOD 0.01 mg/kg) after 24 h. When a post-treatment potable water rinse was performed, no chlorite residues were detected in salmon, scallops and shrimp at any time; chlorite was detected in grouper and catfish samples at 0 h, but the concentration was below the LOD after 24 h (USFDA, 2004a). No total organic halide residues were detected (LOD 0.01 mg/kg) in any control or treated seafood samples (USFDA, 2004a).

Residue levels were measured in several fruits and vegetables after treatment with ASC at 1200 mg/l for 5 or 10 s and then either not rinsed and air-dried or rinsed with water. Primary results were given as residue weight per item, not in units of concentration. The concentrations were then calculated using average weights for each fruit or vegetable (USFDA, 1998b), as shown in Table 2.2 for the air-dried samples (not rinsed).

Table 2.2. Chlorite and chlorate residue levels after treatment with ASC solution

Fruit/vegetable	Chlorite concentration (mg/kg)	Chlorate concentration (mg/kg)
Apple (medium)	0.29	<0.07
Orange (Florida)	0.30	<0.06
Carrot (19.1 cm)	2.29	<0.14
Cantaloupe quarter (medium)	32.83	<0.07
Potato (medium)	0.34	<0.08
Lettuce (one leaf)	8.80	495

A potable water rinse following ASC treatment would probably reduce the levels of residual chlorite and chlorate. This was observed in a later study (USFDA, 2001) in which fruits (melons, apples, oranges, strawberries) and vegetables (carrots, lettuce, onions and french fries) were treated with ASC at 1200 mg/l at pH 2.5 and analysed for chlorite and chlorate after dwell times of 1, 2, 6, 24 and 48 h. The protocols were as follows:

- 1) 30 s ASC dip followed by 5 s post-treatment deionized water rinse;
- 2) 30 s ASC dip with no post-treatment water rinse;
- 3) 30 s ASC spray followed by 5 s post-treatment deionized water rinse;
- 4) 30 s ASC spray with no post-treatment water rinse.

The analysis revealed that the chlorate concentration was below the LOD (0.1 mg/kg) for any of the tested samples and conditions used. In the case of chlorite, the values were below 0.1 mg/kg for all the tested foods except for the following: carrots with 1.49 mg/kg in protocol 2 and 0.89 mg/kg in protocol 4; melons with 1.04 mg/kg in protocol 2 and 1.1 mg/kg in protocol 4; lettuce with 0.23 mg/kg in protocol 1, 15.3 mg/kg in protocol 2, 0.56 mg/kg in protocol 3 and 2.98 mg/kg in protocol 4; oranges with 0.23 mg/kg in protocol 2; and onions with 16.82 mg/kg in protocol 4. In the case of lettuce, the residues of chlorite were very high, and the manufacturer proposed a water rinse followed by a dip or spray treatment for 30 s with ASC at 1200 mg/l and a post-treatment rinse with deionized water with 0, 1, 2, 4, 6, 24 and 48 h dwell times. The chlorite concentration was reduced to <0.01 mg/kg after a 24 h dwell time for the dip and a 6 h or 48 h dwell time for the spray treatment, even though 0.99 mg/kg was still detected at 24 h.

2.2.3 Reactions of acidified sodium chlorite with food components

ASC may interact with either organic matter in solution or proteins, fats or other compounds in the foods with the potential for the formation of different reaction products. The potential reactions are described below.

A treatment of poultry carcasses under exaggerated conditions (immersion in ASC at 2525 mg/l, pH 2.78, for 5 min) was performed by a manufacturer to check the effect on amino acids in comparison with controls. In both cases, proteins were hydrolysed, and the distribution of amino acids in the disinfected carcasses was identical to that in the controls. This also includes amino acids such as cysteine, tyrosine, threonine and tryptophan, which may be prone to oxidation due to easily oxidizable functional groups. Other reaction products that could be potentially generated were not analysed (EFSA, 2005).

The potential formation of chlorinated organic compounds after ASC treatment of poultry carcasses under different conditions was tested as follows (EFSA, 2005):

- 1) Poultry carcasses were immersed in ASC at 2525 mg/l, pH 2.78, for 5 min, rinsed with distilled water, then blotted dry and soaked in hexane overnight for the extraction of lipid residues. The analysis of the samples by gas chromatography did not detect any chlorinated organic compounds. The LOD was about 0.05 mg/kg.
- 2) Poultry carcasses were sprayed with ASC at 1200 mg/l, pH 2.5, for 15 s, then air chilled for 2 h. The analysis did not reveal increases of organically bound chlorine (LOD 0.05 mg/kg).

The poultry carcasses were also screened to detect oxidation or changes in the fatty acid profiles under different treatment conditions:

- 1) immersion of the poultry carcasses in ASC at 1200 mg/l for 5 s, then 5 min dripping and 1 h immersion in water (pre-chill study);
- 2) immersion of the carcasses in ASC at 150 mg/l for 1 h and then 5 min dripping (chiller study);
- 3) dipping the carcasses in ASC at 1200 mg/l for 15 or 30 s with no rinsing and dwell times of 1, 2, 4 and 8 h (post-chill study);
- 4) dipping the carcasses in ASC at 1200 mg/l for 15 or 30 s, followed by 5 s water rinsing and 30 s dwell time (post-chill study);
- 5) dipping the carcasses in ASC at 1200 mg/l for 15 or 30 s with no rinsing and 30 s dwell time (post-chill study).

The analysis did not reveal any chlorinated organics (LOD 0.05 mg/kg). In all cases, samples and controls were cooked.

The results of the analysis of fatty acids in the lipid fractions of the carcasses after all ASC treatments were found to be similar to the controls. Similar results were reported for red meat treated with ASC at 500, 850 and 1200 mg/l for 5 s and dipped in rinse water (USFDA, 1997) and for seafoods treated with ASC at 1200 mg/l at pH 2.3 (USFDA, 2004a). The 2-thiobarbituric acid reactive substances (TBARS) analysis was also performed to detect any oxidation of fatty acids. TBARS values were higher in the skin after the treatments but not in the muscle, which remained unaffected regardless of the treatment. A study of the skin treated with ASC at 150 mg/l at pH 3.05 and 5 °C for 45 min (chiller treatment) gave TBARS increases equivalent to 2.8 times the control (USFDA, 1995). The chiller treatment gave higher TBARS values in the skin than did the use of ASC in spray. However, cooking gave much higher TBARS values, even in the controls. In the case of red meat treated as described above, the TBARS values were 0.29–0.36 mg/kg for treated samples in comparison with 0.26 mg/kg for the controls. The TBARS values for cooked samples were 5–6 times those of the raw samples, probably due to oxidation of fatty acids by heating (USFDA, 1997). In the case of seafood, no significant increase of TBARS values was reported after immersion for 30 s in ASC at 1200 mg/l at pH 2.3 (USFDA, 2004a).

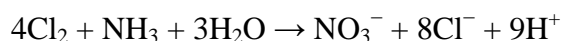
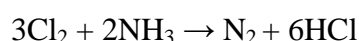
2.2.4 Summary

Based upon the available data, chlorate and chlorate are the main by-products that may remain as residues on food.

2.3 N-Chloramines

2.3.1 N-Chloramine chemistry

N-Chloramines are produced from the chemical reactions between ammonia or organic amines and chlorine. The most common form used as a disinfectant is monochloramine (NH₂Cl; CAS No. 10599-90-3). Chloramines may be deliberately produced by combining the ammonia or amines with chlorine prior to contact with the medium to be disinfected (food or water), or they may be spontaneously formed whenever chlorine is used if ammonia or amines are present in the medium:

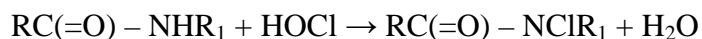


The two final common products of ammonia oxidation with excess chlorine are nitrogen and nitrate (Montgomery, 1985), and this is called breakpoint chlorination. The process is a function of chlorine to ammonia ratio, temperature, and pH and alkalinity of the solution. The goal for disinfection is to maximize monochloramine formation and minimize dichloramine and trichloramine formation. This is achieved by maintaining the pH between 6.5 and 8.5 with a chlorine to ammonia ratio of approximately 4:1 (Zentox, 2007). In addition

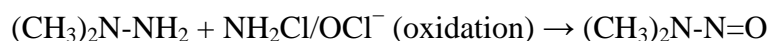
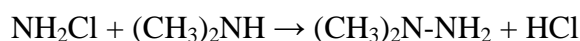
to inorganic *N*-chloramine, there are analogous organic *N*-chloramines. *N*-Chlorodimethylamine is an example of an organic amine formed from dimethylamine. *N*-Chloramines are labile, so they will exchange halogens as well as transfer halogens to other amine or amide compounds with which they are in proximity.

Because of its low oxidizing efficiency and low halogenation capability in water, monochloramine has a lesser tendency to produce halogenated DBPs and oxidation products, but it also has much lower disinfection potency than free chlorine under most conditions. However, some of its DBPs are of greater concern than many of the chlorination products.

Related chemicals are *N*-chloroamides that could be formed by reactions of chlorine with amides such as protein peptides:



Monochloramine can react in water with secondary amines such as dimethylamine to produce dimethylhydrazine, which can be oxidized in the presence of the monochloramine to *N*-nitrosodimethylamine (NDMA; CAS No. 62-75-9) (Choi & Valentine, 2002; Mitch & Sedlak, 2002a):



The ability of chloramines to form nitrosamines with diverse secondary amine precursors has been demonstrated in laboratory studies (Mitch & Sedlak, 2002b). The most efficient formation of a nitrosamine appears to result when chloramine forms a hydrazine intermediate, which reacts with a secondary amine to form the nitrosamine (Choi & Valentine, 2002; Mitch & Sedlak, 2002a).

NDMA formation has been extensively documented (Valentine et al., 2005). The typical ranges produced in drinking-water are shown in Table 2.3. Recent measurements have shown that NDMA is generally present at low concentrations (2–180 ng/l) in chloraminated/chlorinated drinking-water (WHO, 2006). However, these values apply predominantly to water supplies that use monochloramine and whose source waters are significantly impacted by upstream wastewater discharges. A survey of wastewater plants revealed nanogram per litre concentrations, and one plant produced NDMA at concentrations as high as 960 ng/l (Valentine et al., 2005). However, many drinking-water plants produce no NDMA, demonstrating the requirement for the presence of dimethylamine or a precursor of this secondary amine with respect to this formation mechanism. Certain ion exchange resins or polymers used as flocculants have been shown to be precursors of NDMA. The occurrence of *N*-nitrosomorpholine (CAS No. 59-89-2), *N*-nitrosodiethylamine (CAS No. 55-18-5) and *N*-nitrosopyrrolidine (CAS No. 930-55-2) has been observed in some drinking-waters resulting from disinfection when the corresponding secondary amines are also present (Charrois et al., 2004).

2.3.2 Application and fate in foods

Monochloramine is proposed for use as an antimicrobial agent in poultry process chiller water at levels up to 50 mg/l (Russell & Axtell, 2005; USFDA, 2008a).¹

¹ Note that the petition (USFDA, 2008a) was being held in abeyance by the United States Food and Drug Administration (USFDA) as of 1 June 2009 (<http://www.fda.gov/Food/FoodIngredientsPackaging/FoodAdditives/ucm082418.htm>). Note also that the Food Safety and Inspection Service (FSIS) of the United

Chloraminated drinking-water is regulated so as not to exceed 4 mg/l as chlorine (Cl₂) in the United States of America (USA) (USEPA, 2009); the WHO *Guidelines on Drinking-water Quality* guideline value is 3 mg/l as monochloramine (WHO, 2008b).

Table 2.3. NDMA occurrence in drinking-waters disinfected with chlorine or monochloramine^a

Site	Total number of samples	Number of samples with NDMA detected	Percentage of total	NDMA concentrations (ng/l)
Drinking-water plant influents	81	6	7.4	0.6–1.8
Drinking-water plant effluents	81	28	35	0.6–30
Distribution samples	79	49	62	0.6–24

^a From Valentine et al. (2005).

Organic *N*-chloramines have long been known to form in drinking-water treated with chlorine or monochloramine. They are largely regarded as a nuisance, as they reduce the disinfectant activity by decreasing the available free chlorine. There has been little systematic work to characterize the forms of organic *N*-chloramine that are present in water beyond the formation of the *N*-chloramines of α -amino acids. Organic *N*-chloramines produced from α -amino acids present in many foods are generally more readily formed and degrade more readily than compounds either that have no carboxyl group or whose carboxyl group is further removed from the amine group. While slower in formation, dichloramines are more readily formed with non-amino acid nitrogens at physiological pH and probably in drinking-water (Nightingale et al., 2000). At the macromolecular level, exocyclic nitrogens of purine and pyrimidine bases react more readily to form *N*-chloramines; over time, however, the chlorine is transferred to cyclic nitrogen-containing moieties.

In a comparison of total chlorine levels in poultry carcasses immersion-chilled with tap water (presumably chlorinated) or water containing monochloramine at 50 mg/l, the skin and fat levels ranged between 0.3 and 0.7 mg/kg, and the concentrations in the tap water-chilled products were higher than those in the monochloramine-chilled products (Axtell, Russell & Berman, 2006). Levels of lipid peroxidation products as measured by TBARS in roasted chicken tissues were in a small range: in breast meat, from 3.86 mg/kg (tap water) to 2.73 mg/kg (monochloramine roasted and stored); in thigh meat, from 3.62 mg/kg (monochloramine fresh roasted) to 3.39 mg/kg (monochloramine stored and roasted); and in skin and fat, from 2.96 mg/kg (monochloramine stored and roasted) to 2.96 mg/kg (monochloramine fresh roasted). Fatty acid profiles in the various tissues prepared with tap water (presumably chlorinated) versus water with monochloramine at 50 mg/l and roasted showed similar distributions of oleic (33.9–45.2 mg/kg), linoleic (16–18.9 mg/kg), linolenic (0.15–0.39 mg/kg) and arachidonic acids (0.10–1.00 mg/kg).

2.3.3 Nitrosamine residues in foods

There are extensive published data on the presence of nitrosamine residues in numerous types of foods. Their formation is attributable to several mechanisms, of which interaction with active chlorine compounds is a minor contributor. Nitrosamines may be formed by nitrosation of secondary amines by nitrite/nitrous acid, reactions of *N*-chloramines

States Department of Agriculture (USDA) lists the monochloramine poultry chiller antimicrobial treatment system on its February 2006 list of new technologies that it has reviewed and has no objection to their use in FSIS establishments (USDA, 2006).

with secondary amines, thermal/cooking processes and undoubtedly others, including biological processes. Detected nitrosamines have included NDMA, *N*-nitrosoproline (CAS No. 7519-39-0), *N*-nitrosopyrrolidine and *N*-nitrosopiperidine (CAS No. 100-75-4). Concentrations of nitrosamines in foods are summarized in Appendix A at the end of the chapter (Jakszyn et al., 2004a).

In a study of potential in situ nitrosamine formation in three sets of poultry carcasses that were chilled in 1) iced distilled water containing monochloramine at 50 mg/l, 2) iced distilled water containing sodium hypochlorite at 50 mg/l and 3) iced distilled water only, the carcasses were in contact for 6 h versus the usual ~1 h contact. The chickens were then roasted at 160 °C for 45 min (Zentox, 2007). *N*-Nitrosomorpholine, *N*-nitrosodiethylamine, NDMA, *N*-nitrosodibutylamine and *N*-nitrosopiperidine were not detected in any of the samples, with a detection limit of 1 µg/kg. However, *N*-nitrosopyrrolidine was detected in all three treatment conditions at 3.53 µg/kg (monochloramine), 2.92 µg/kg (sodium hypochlorite) and 2.74 µg/kg (distilled water). It initially appeared that the monochloramine-treated roasted chickens showed a slightly increased production of *N*-nitrosopyrrolidine. In a retest in which the cooking time was determined by reaching an internal temperature of 80 °C, the extent of *N*-nitrosopyrrolidine formation was proportional to the cooking time (Zentox, 2007) and independent of chiller treatment water disinfectant. Thus, its formation was not related to the disinfection system.

2.4 Chloramine-T

The chemical name of chloramine-T (CAS No. 127-65-1) is *N*-chloro-4-methylbenzenesulfonamide trihydrate, sodium salt. The molecular formula is $C_7H_7ClNOS^- \cdot Na^+ (3H_2O)$. The trihydrate form of chloramine-T ($C_7H_8ClNO_2SNa \cdot 3H_2O$) is CAS No. 7080-50-4. Other names for chloramine-T are sodium *p*-toluene sulfonamide, *N*-chloro-*p*-toluenesulfonylamide, sodium chloro[(4-methyl phenyl)sulfonyl]azanide and *N*-chlorotosylamide, sodium salt (Figure 2.1). Chloramine-T is a white crystalline powder that decomposes at 130 °C and is highly soluble in water (~15% at 25 °C; IPCS, 2004). It is used as a biocide and mild disinfectant. The commercial product chloramine-T is synthesized through the chlorination of benzene sulfonamide or *p*-toluene sulfonamide (Haneke, 2002).

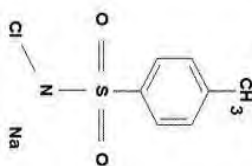


Figure 2.1. Chemical structure of chloramine-T

As an *N*-chloro-compound, chloramine-T contains electrophilic chlorine and can be compared with the *O*-chlorinated sodium hypochlorite or *N*-chloramines. Chloramine-T is nearly neutral (pH typically 8.5). In water, it hydrolyses to hypochlorite (OCl^-). After chlorine is released into solution, the stable residue would be *p*-toluenesulfonamide ($C_7H_9NO_2S$; CAS No. 70-55-3). Chloramine-T is used for disinfection and as an algicide, bactericide and germicide, for parasite control and for drinking-water and food application disinfection. The molecular structure of toluenesulfonylamide is similar to that of *p*-aminobenzoic acid, an intermediate in bacterial metabolism that can be disrupted by this sulfonamide. Therefore, chloramine-T is capable of inhibiting bacterial growth by two mechanisms, with both the phenylsulfonamide moiety and the electrophilic chlorine. It is a

method for delivering stabilized chlorine. Chloramine-T is used to disinfect food contact surfaces and equipment that are then specified to be rinsed with water prior to use. Solution concentrations are 0.3–0.5%. As the surfaces are specified to be rinsed with clean water prior to use, the amounts of either chloramine-T or *p*-toluenesulfonamide that could be transferred to food would be very small (Axcentive, 2008).

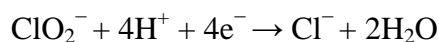
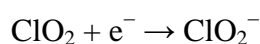
2.5 Chlorine dioxide

Chlorine dioxide (ClO₂; CAS No. 10049-04-4) is an antimicrobial agent recognized for its disinfectant properties since the early 1900s. The mechanism of action by which chlorine dioxide inactivates microorganisms is not entirely understood. However, it is known that chlorine dioxide kills microorganisms by either altering or disrupting transport of nutrients across the cell wall and also penetrating into the cell and disrupting protein synthesis (Young & Setlow, 2003; EFSA, 2005).

2.5.1 Chemistry

Chlorine dioxide is a greenish-yellow gas at room temperature that is very soluble in water (EFSA, 2005). It may be produced by 1) mixing a solution of chlorine with a solution of sodium chlorite, 2) acidification of chlorates with hydrochloric or sulfuric acid, 3) reduction of chlorates in acid medium, 4) reacting acids with chlorites and 5) electrolysis, using sodium chloride, sodium chlorite and water (Dychdala, 2001).

The chemistry of chlorine dioxide differs from that of other chlorine compounds, in that hypochlorous acid is not formed from reduction of chlorine dioxide. Chlorine dioxide is reduced in water, generating the chlorite ion, which is then reduced to chloride ion:



In the absence of oxidizable substances and in the presence of alkali in water, chlorine dioxide is reduced, generating chlorite and chlorate ions:

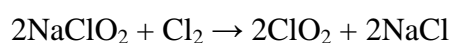


The chlorite ion is further reduced to the chloride ion, as shown above.

Chlorine dioxide has a relative molecular mass of 67.45, a melting point of –59 °C, a boiling point of 11 °C and a solubility of 3.01 g/l at 25 °C and 4.6 kPa.

2.5.2 On-site generation of chlorine dioxide

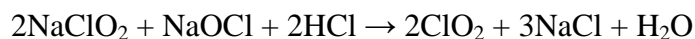
Because chlorine dioxide is unstable as a gas, it is almost always used as a dissolved gas in water at a concentration of 0.5–10 g/l, and it must be generated on site at the point of use:



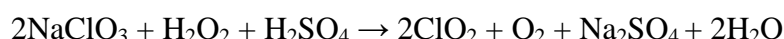
Whereas either the oxidation or acidification of sodium chlorite solution will generate chlorine dioxide, the oxidative method gives much better yields. Most commercial generators

use sodium chlorite as the common precursor chemical to generate chlorine dioxide for drinking-water application (USEPA, 1999). Chlorine dioxide is 10 times more soluble than chlorine gas in water, depending upon the pH, and does not hydrolyse in solution. It remains as a “true” dissolved gas that retains its biocidal properties throughout the entire pH 2–10 range (SCVPH, 2003).

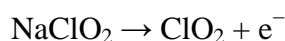
A combination of sodium hypochlorite (NaOCl) and hydrochloric acid (HCl) solutions is also used when chlorine gas, the most common oxidizing agent, is not desired:



Chlorine dioxide can also be generated from the reaction of aqueous solution of sodium chlorate with hydrogen peroxide and sulfuric acid:



or from electrolysis of an aqueous solution of sodium chlorite:



Conversion of sodium chlorite to chlorine dioxide by chemical oxidation can exceed 95%. The USFDA (2005) requires that the generator effluent contain at least a weight fraction of 90% of chlorine dioxide with respect to all chlorine species, as determined by Method 4500 ClO₂-E of *Standard Methods for the Examination of Water and Wastewater* (APHA, AWWA & WEF, 1998).

2.5.3 Application and fate in foods

Chlorine dioxide gas and liquid formulations have many commercial food uses: 1) washing fruit and vegetables, 2) disinfecting meat and poultry, 3) disinfecting fish and seafood, 4) disinfecting food processing equipment and 5) sanitizing water. In the USA, chlorine dioxide is currently regulated for use as an antimicrobial agent in water used for poultry processing and in water used to wash fruit and vegetables that are not raw agricultural commodities (when followed by a potable water rinse), in an amount not to exceed 3 mg/kg as residual chlorine dioxide (USFDA, 2005). Once applied, chlorine dioxide quickly breaks down into chlorite, chlorate and chloride ions.

2.5.3.1 Fresh produce

Chlorine dioxide is efficacious on a variety of vegetables and fruits largely because its efficacy is little affected by pH and organic matter and it does not react with ammonia to form chloramines, as is the case with sodium hypochlorite and hypochlorous acid. Traditionally, aqueous chlorine dioxide at concentrations of 50–200 mg/kg is widely used to wash fruits and vegetables; however, its effectiveness is limited to a reduction of 1–2 log colony-forming units (cfu) for pathogenic and spoilage bacteria (Brackett, 1992).

Washing produce with an aqueous chlorine dioxide solution has limited efficacy due to the hydrophobic nature of the produce and organic matter on its surface. However, as a gas has greater surface penetration than a liquid, chlorine dioxide gas may be more effective for surface sanitation than aqueous chlorine dioxide (Han et al., 2001). Treatment of cut and peeled fruits and vegetables with dilute chlorine dioxide solution (approximately 10 mg/kg) did not result in the detection of any halocarbons (USFDA, 1994).

2.5.3.2 Poultry and red meat

Poultry chiller water typically initially contains chlorine dioxide at 20–50 mg/l, which rapidly decomposes to chlorite and chlorate in a ratio of 7:3, leaving generally approximately 5% of the initial chlorine dioxide concentration. Thus, the resulting concentrations in poultry chiller water are approximately 2.5 mg/l as chlorine dioxide, 33 mg/l as chlorite and 14 mg/l as chlorate (SCVPH, 2003). Chlorite itself is an oxidant and can be ultimately reduced to chloride (reduction potential: +0.76 V). The poultry carcasses would absorb chlorite and chlorate, which may react with components of poultry tissues during processing and storage or be further reduced during the poultry chilling process. Hence, a decontamination process of 1 h applying chlorine dioxide would result in maximum residue levels of 0.13 mg/kg carcass for chlorite and 0.06 mg/kg carcass for chlorate (SCVPH, 2003). The poultry will be cooked prior to consumption, so chlorine-containing residues would be volatilized or react to form more innocuous species (e.g. chloride), which would reduce the level of any residues of chlorine dioxide and its by-products (chlorite and chlorate) on poultry as consumed.

Chlorine dioxide is expected to react with poultry components (i.e. biomolecules such as lipids, vitamins, proteins, etc.) as well as organic materials present in chiller water. Studies of TBARS values for malonaldehyde, a secondary lipid oxidation product, and fatty acid profiles have suggested that the potential lipid oxidation in poultry (USFDA, 1993) and ground beef (Jiménez-Villarreal et al., 2003) is not significant. The use of a chlorine dioxide solution (approximately 3 mg/kg) on poultry did not appreciably affect TBARS values of chilled poultry (USFDA, 1993). Furthermore, there is no consistent pattern in fatty acid levels that would suggest more pronounced oxidation and loss of unsaturated fatty acids from chlorine dioxide-treated poultry compared with untreated poultry. TBARS analyses have also indicated that chlorine dioxide-treated (200 mg/l) beef trimmings and untreated ground beef patties showed little differences in lipid oxidation (Jiménez-Villarreal et al., 2003).

2.5.3.3 Fish and other seafood

Chlorine dioxide is employed as a disinfectant in water and ice used to rinse, wash, thaw, transport or store seafood. No chlorine residuals were present following chlorine dioxide treatment (10–40 mg/l). Also, total organic halogen analysis of shrimp and crawfish indicated that no chlorine by-products were produced from sanitizing treatment with chlorine dioxide (Kim et al., 1999).

2.5.4 *Reactions with food components*

Chlorine dioxide in water and DBP chemistry have been described by Rice & Cotruvo (1978). Aqueous chlorine dioxide can react with carbohydrates, lipids, amino acids, peptides and proteins (Fukayama et al., 1986; Rice & Gomez-Taylor, 1986). Chlorine dioxide, which also contains a mixture of chlorite and chlorate in water, acts primarily as an oxidant rather than as a chlorinating agent, and its redox potential in aqueous solution ($\text{ClO}_2 + e^- = \text{ClO}_2^-$, 1.15 V) is less than that of hypochlorous acid ($\text{HClO} + \text{H}^+ + 2e^- = \text{Cl}^- + \text{H}_2\text{O}$, 1.49 V). Therefore, chlorine dioxide is likely to be less reactive and produce fewer by-products than chlorine in the reaction during food processing, such as in poultry chiller water (Tsai, Higby & Schade, 1995). Chlorine dioxide is a comparatively weak oxidizing agent and has a lower oxidation potential than ozone, chlorine or hypochlorous acid. Because chlorine dioxide has lower oxidation strength, it is more selective in its reactions. Typically, chlorine dioxide will react with compounds that have activated carbon bonds, such as phenols, or with other active

compounds, such as sulfides, cyanides and reduced iron and manganese compounds (Fukayama et al., 1986; SCVPH, 2003). Most importantly, chlorine dioxide is very specific in its reactivity and enters into only a few side reactions compared with chlorine. Further, if chlorine dioxide is pure, it does not chlorinate organic material and therefore does not form THMs and other chlorinated DBPs.

Chlorine dioxide can oxidize simple carbohydrates (e.g. glucose) to form carbonyl derivatives that are subsequently oxidized to carboxylic acids. Polysaccharides (e.g. cellulose) are also susceptible to oxidation and may produce gluconic acid. However, some of these reactions require elevated temperatures (>80 °C) and are not likely to occur in foods treated with aqueous chlorine dioxide unless processed under elevated temperatures. Further, meat, poultry and fish do not contain carbohydrates in appreciable amounts.

Proteins are subject to oxidation, substitution and addition reactions following treatment with aqueous chlorine dioxide. However, no significant effects on the protein content of salmon and red grouper fillets were reported after treatment with chlorine dioxide (20–200 mg/l in brine solution for 5 min). Also, there was no obvious change in the lipid content or fatty acid composition in both salmon and red grouper fillets after treatment (Kim et al., 1998). Therefore, there are no specific data available on chlorine dioxide by-product formation from fish proteins or lipids. Furthermore, no effects on the vitamin content or on proximate composition of fish have been reported, with the exception of significant reductions in thiamine (salmon and red grouper) and riboflavin (red grouper) levels after treatment (Kim et al., 1998).

Unsaturated fatty acids in lipids can react with chlorine dioxide and produce a variety of compounds, such as unsaturated ketones, chloroketones, chlorohydrins, dichloro-addition products and epoxides (Rice & Cotruvo, 1978; Rice & Gomez-Taylor, 1986). Saturated aliphatic hydrocarbons are neither oxidized nor chlorinated by chlorine dioxide or chlorine. In commercial poultry chiller water in the presence of chlorine, saturated and unsaturated aliphatic aldehydes (pentanal, hexanal, heptanal, octanal, *trans*-2-octenal, nonanal, *trans*-2-nonenal, decanal, 2,2-nonadienal, *trans*-2-decenal, 2,4-decadienal and *trans*-2-undecenal) were detected by gas chromatographic/mass spectrometric (GC/MS) analysis, and hexanal and nonanal were the two major aldehydes detected (Tsai, Mapes & Huxsoll, 1987). The presence of aldehydes is indicative of autoxidation in the poultry chiller water.

Chlorine dioxide is relatively inert towards individual amino acids, and reactions are pH dependent (Tan et al., 1987). Chlorine dioxide oxidizes tryptophan to form indoxyl, isatine and indigo red (Fukayama et al., 1986). Tyrosine formed dopaquinone upon oxidation by chlorine dioxide. Sulfur-containing amino acids (cystine and methionine) are oxidized to bisulfoxide and sulfonic acid derivatives (Rice & Gomez-Taylor, 1986). The reaction of aqueous chlorine dioxide with peptides and proteins is considered to be mainly due to interaction with individual amino acid moieties in the peptides.

Chlorine dioxide, unlike chlorine, does not react with ammonia or water (Rice & Gomez-Taylor, 1986). Additionally, the reaction of the bromide ion (Br^-) with chlorine dioxide is thermodynamically unfavourable. However, with intense sunlight and high concentrations of chlorine dioxide, chlorine dioxide does oxidize the bromide ion to hypobromite (BrO^-) and bromate (BrO_3^-) (Rice & Gomez-Taylor, 1986).

Phenols and hydroquinones can be oxidized in reactions with chlorine dioxide; *p*-benzoquinone and aromatic carboxylic acids are produced when chlorine dioxide is present in excess (Wajon, Rosenblatt & Burrows, 1982). Chlorine dioxide does not produce THMs from reactions with humic acid and other natural materials in raw water when pure, but it is reported to produce oxidation products (i.e. benzenepolycarboxylic acids, aliphatic dibasic acids, carboxyphenylglyoxylic acids and aliphatic monobasic acids). Several derivatives of

furan and dioxane were also identified in the reaction with humic acid and other natural materials (Rice & Gomez-Taylor, 1986).

A trace amount of chloroform (<2–30 µg/kg) was reported to be formed when poultry carcasses were exposed to water containing chlorine dioxide (Robinson, Mead & Barnes, 1981). However, this volatile compound was considered to be an artefact of the GC/MS analytical method, formed as a result of the reaction between chlorine dioxide and 2,6-diphenyl-*p*-phenylene oxide that was used in the sample concentrator, and was not formed by the reaction of chlorine dioxide (USFDA, 1993). Also, the above discussion on the lack of volatile halocarbons (i.e. chloroform) being formed in the treatment of fresh produce with chlorine dioxide also supports the absence of chlorination reactions.

More than 40 DBPs were detected in finished drinking-water from a water plant using chlorine dioxide (Richardson et al., 1994). Multispectral identification techniques were employed, but the products were not quantified. The predominant identified products were organic esters, acids and olefins, and only two aldehydes (benzaldehyde and ethylbenzaldehyde) were detected. A few halogenated compounds were detected, probably from some chlorine in the treatment process. Numerous aliphatic carboxylic acids were detected, including maleic acid/anhydride. It is possible that other aldehydes were formed and oxidized during treatment or processing, and also that some of the products were formed from precursors that were not ordinarily part of the natural organic matter (NOM) in the water.

2.5.5 Summary

Chlorine dioxide may induce chemical changes in food. The residues or transformation products that could possibly result from food processing with chlorine dioxide are inorganic oxychlorine anions (i.e. chlorite, chlorate), chloroorganics (i.e. chlorinated lipids, chlorinated proteins) and oxidized organics (i.e. oxidized lipids, oxidized amino acids).

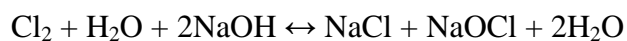
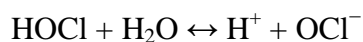
2.6 Hypochlorite-related compounds (chlorine gas, sodium hypochlorite, calcium hypochlorite, hypochlorous acid, hypochlorite ion)

2.6.1 Chemistry

Chlorine, whether in the form of chlorine gas (Cl₂; CAS No. 7782-50-5) or as the solids sodium hypochlorite (NaOCl; CAS No. 7681-52-9) or calcium hypochlorite (Ca(OCl)₂; CAS No. 7778-54-3), dissolves in water to form hypochlorous acid (HOCl; CAS No. 7790-92-3) and hypochlorite ion (OCl⁻). Chlorine is the most common form of active chlorine used in food sanitation; it is certainly the most common disinfectant used in drinking-water and wastewater treatment. It was introduced into drinking-water treatment in the first decade of the 20th century and resulted in immediate reductions in the risk of transmission of waterborne diseases. Drinking-water treatment and chemistry are of interest in this food context, because chlorinated drinking-water or more highly chlorinated water is frequently used as the vehicle for food contact sanitation, and some of the chemical products in the water may be accumulated to some degree in the food product, in addition to whatever products form from the contact of the food with the disinfectant.

Chlorine is produced from electrolysis of sodium chloride and is provided commercially as chlorine gas or in various concentrations in basic solution as the hypochlorite (e.g. common bleach), partly due to handling and storage difficulties associated with gaseous chlorine (Montgomery, 1985). Electrolysis of sodium chloride salt that contains some

bromide will also produce hypobromous acid (HOBr) and bromate as by-products, which will be carried forward in the chlorine product:



HOCl + organics → oxidation products and organochlorines



HOBr + organics → oxidation products and organobromines

A chlorine solution at about pH 7.4 is approximately 50% hypochlorite and 50% hypochlorous acid (Asano et al., 2007); at pH 10, it is approximately 100% hypochlorite. It should not be used below pH 5 due to the excessive presence of gaseous chlorine in the equilibrium mixture. The biocidal effectiveness is greatest in the acid form as hypochlorous acid, but hypochlorite is also an effective, but slower-acting, biocide.

Hypochlorites are available as powders or liquids, depending on the type of salt used. Calcium hypochlorite comprises the majority of the powdered offerings, whereas sodium hypochlorite and potassium hypochlorite are generally available as liquid solutions (Dychdala, 2001). Commercial solutions of sodium hypochlorite usually contain 12.5–17% available chlorine (household bleach may be approximately 5–10% sodium hypochlorite), but the composition will change upon storage, particularly under the influence of light and heat; chlorate (not a disinfectant) and chlorite are major products of this decomposition (disproportionation). For example, a 16.7% solution stored at 26.7 °C will lose 10% of its available chlorine in 10 days, 20% in 25 days and 30% in 43 days (Asano et al., 2007), so it should be stored in a cool place and used relatively quickly. For that reason, disinfectant solutions are made to approximate concentrations, and then concentrations are specifically determined by measurement of active chlorine residuals.

Chlorine as hypochlorous acid or hypochlorite is a very reactive chemical, and it can engage in numerous chemical processes under mild environmental conditions, including in iced water. It can function as both an oxidizing agent and a halogenating agent (Rice & Gomez-Taylor, 1986). Oxidation is probably the predominant chemical process occurring in chlorine's water and food contact applications, but the halogenated by-products have received the most attention. Chlorine will oxidize bromide to hypobromous acid, which is an active brominating agent. The chemistry and distribution of by-products produced differ somewhat with the pH of the solution as well as the composition of the precursor chemicals that are available for reaction. For example, in chlorination of bromide-containing fresh waters or seawater, which contains bromide at about 60–80 mg/l, organobromine DBPs will predominate over organochlorine DBPs (Huang, Chen & Peng, 2004; Westerhoff, Chao & Mash, 2004; Cotruvo et al., in press). Although chlorine produces DBPs, its high efficacy, ease of use and low cost make it the disinfectant of choice in many applications.

There are numerous detailed assessments and reviews of the chemistry and toxicology of chlorine and by-products in water (IPCS, 2000; Woo et al., 2002; Bull et al., 2006; USEPA, 2006; WHO, 2008b), and some in foods (FAO/WHO, 2000). Several of those DBPs formed between chlorine and organic substances have been regulated (Appendix B), either

for their own sake or as THMs and HAAs, principally as indirect indicators of the presence of other non-quantified or unidentified by-products (Cotruvo, 1981, 1982).

2.6.1.1 Chemistry of chlorine interactions with organic matter

The chemistry of and by-product formation from chlorine interactions with foods is much less studied than that of interactions with organic matter in drinking-water. This is partly because the chemistry of food contact is much more complex than drinking-water chemistry; thus, direct comparisons are difficult to make in the absence of adequate information on chlorine chemistry in food contact. Analyses are much more difficult because of the medium and contact conditions. In drinking-water, many of the halogenated DBPs are small molecules, hydrophobic and volatile; some have higher molecular weights. Contact times between disinfectant and precursor chemicals may be several days during water storage and distribution to consumers, and the formation chemistry will continue as long as disinfectant and precursors are present. Heating the water for beverage use will drive reactions to completion, consume the residual disinfectant and deplete volatile organics and DBPs, but less volatile substances will remain.

With food contact, precursor chemicals that can react with the disinfectant could include the complex natural organics in the water and fats/lipids, proteins, carbohydrates and numerous other chemical products in the food. For example, it has been demonstrated under *in vitro* conditions that hypochlorous acid is reactive with both free and peptide-bound tyrosine, *N*-acetyltyrosine and bovine serum albumin, and it can generate chlorotyrosine, 3,5-dichlorotyrosine and chlorinated aldehydes (Fu et al., 2000). However, food contact conditions are much different from *in vitro* conditions, and they differ among meats in cold water chillers and sprays, iced seafood and sprayed fresh fruits and vegetables. As foods are sprayed or immersed in water containing disinfectants, water may be absorbed into the food product and carry with it DBPs that were present in the water. This would add to the potential exposure from the food. On the other hand, storage and/or cooking of the foods probably result in losses of DBPs from volatilization and degradation.

2.6.2 Disinfection by-products in drinking-water

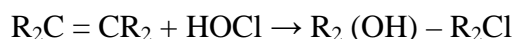
Chlorinated/brominated by-products from chlorination are the most extensively studied chemicals produced from disinfectants in contact with water and food. One reason is that, compared with non-halogenated compounds, they are more readily separated from water solutions for analysis because of their hydrophobicity. In the 1970s, when standard gas chromatographic analytical procedures were first used to analyse drinking-water, it was discovered that THMs were being formed in microgram per litre concentrations from reactions of chlorine/hypochlorite with the NOM commonly present in water sources, particularly in surface water sources.

The chemistry of chlorine interacting with organic precursors in drinking-water is highly complex, and most of the specific precursors and mechanisms are not known in detail. There may be a limited relationship to DBPs from chlorine and food contact. The complexity is probably best illustrated by the numerous categories of halogenated by-products that have been detected in one or more studies (see Tables 2.4 and 2.5 below). Naturally occurring polyphenolic compounds are some of the most likely precursors for many of the products. THMs are halogen-substituted single-carbon compounds with the general formula CHX_3 , where X may be fluorine, chlorine, bromine or iodine, or a combination thereof. The THMs of principal interest are chloroform (CHCl_3 ; CAS No. 67-66-3), bromodichloromethane (BDCM) (CHBrCl_2 ; CAS No. 75-27-4), dibromochloromethane (DBCM) (CHBr_2Cl ; CAS

No. 124-48-1) and bromoform (CHBr₃; CAS No. 75-25-2); several other THMs have been detected more rarely and at lower concentrations. Since the initial analyses from the 1970s, numerous families and hundreds of individual halogenated DBPs have been identified and quantified in chlorinated drinking-water. Among these are HAAs, HANs, haloketones, halopicrins, halophenols and halofuranones, in addition to non-halogenated oxidized products such as acids, aldehydes and ketones. The THMs and HAAs usually account for the largest portion of the identifiable DBPs in chlorinated drinking-water (up to 50%). Another chemical group of compounds that has been detected at nanogram per litre levels in chlorinated drinking-water is the MX-related chemicals. MX is the common name applied to one member (3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone) of a group of halomethylhydroxy-furanones formed from oxidation, halogenation and ring cleavage of phenolic-type natural organics in the water. They are cyclic lactones or open chain carboxyl compounds and would not be highly volatile. Levels of MX up to 310 ng/l have been detected in drinking-water (Weinberg et al., 2002).

In an attempt to identify substances of interest for further studies, structure–activity techniques and genotoxicity data were applied as a method for pre-screening of 209 DBPs in order to rank them with respect to carcinogenic potential from long-term exposure (Woo et al., 2002). In a study on structure–activity relationships of novel by-product formation from substructures of haloquinones identified in NOM, quantitative structure–activity relationships and analogies with related compounds were used to identify other by-products that could be of interest (Bull et al., 2006). Chemicals identified in this study included those identified by Woo et al. (2002), but the study also provided an additional list of probable by-products. Those considered to be of most concern were a number of halogenated quinones, halogenated cyclopentenoic acid derivatives, halonitriles and various *N*-chloramines. The formation of the major by-products goes through a series of intermediates with various phenolic (Figure 2.2) and other unidentified precursors that naturally occur in surface waters, but may not occur in many foods. Changing from processes that utilize free chlorine to the use of monochloramine has a high likelihood of preserving some of the intermediate species. Figure 2.2 illustrates the changes in products that would be expected by reactions with phenol treated with monochloramine in place of free chlorine. A variety of quinone structures have been shown to occur with monochloramine that will be destroyed by ring cleavage with free chlorine (Heasley et al., 2004). It has long been known that various phenolic precursors are intermediates in the formation of most of the THMs and HAAs. Excess chlorine results in cleavage of the phenolic ring to give rise to haloacids and THMs. If monochloramine is utilized in place of free chlorine to reduce THMs and HAAs, it is likely that higher concentrations of halogenated quinones will be encountered (Heasley et al., 2004; Bull et al., 2006).

Other basic chemical oxidation processes that can occur include oxidation of alcohols to aldehydes, ketones and carboxylic acids as well as formation of chlorohydrins:



Chlorine will oxidize bromide in water to HOBr, which is a more active halogenating agent than HOCl; thus, in the presence of bromide, the analogous brominated and mixed halogenated by-products will be formed.

The aggregated total concentration of halogenated organic products in drinking-water may range from a few micrograms per litre in very low organic carbon groundwater or membrane-treated water to perhaps a milligram per litre or more in some waters with high levels of NOM precursors, depending upon the chlorine dosage, quantity of NOM precursors, pH, temperature and contact time. In the presence of ammonia and organic amines,

chloramines will rapidly form. They are poor halogenating and oxidizing agents, so the presence of ammonia will suppress the formation of most of the halogenated and oxidized DBPs; however, chloramines are low-efficacy disinfectants.

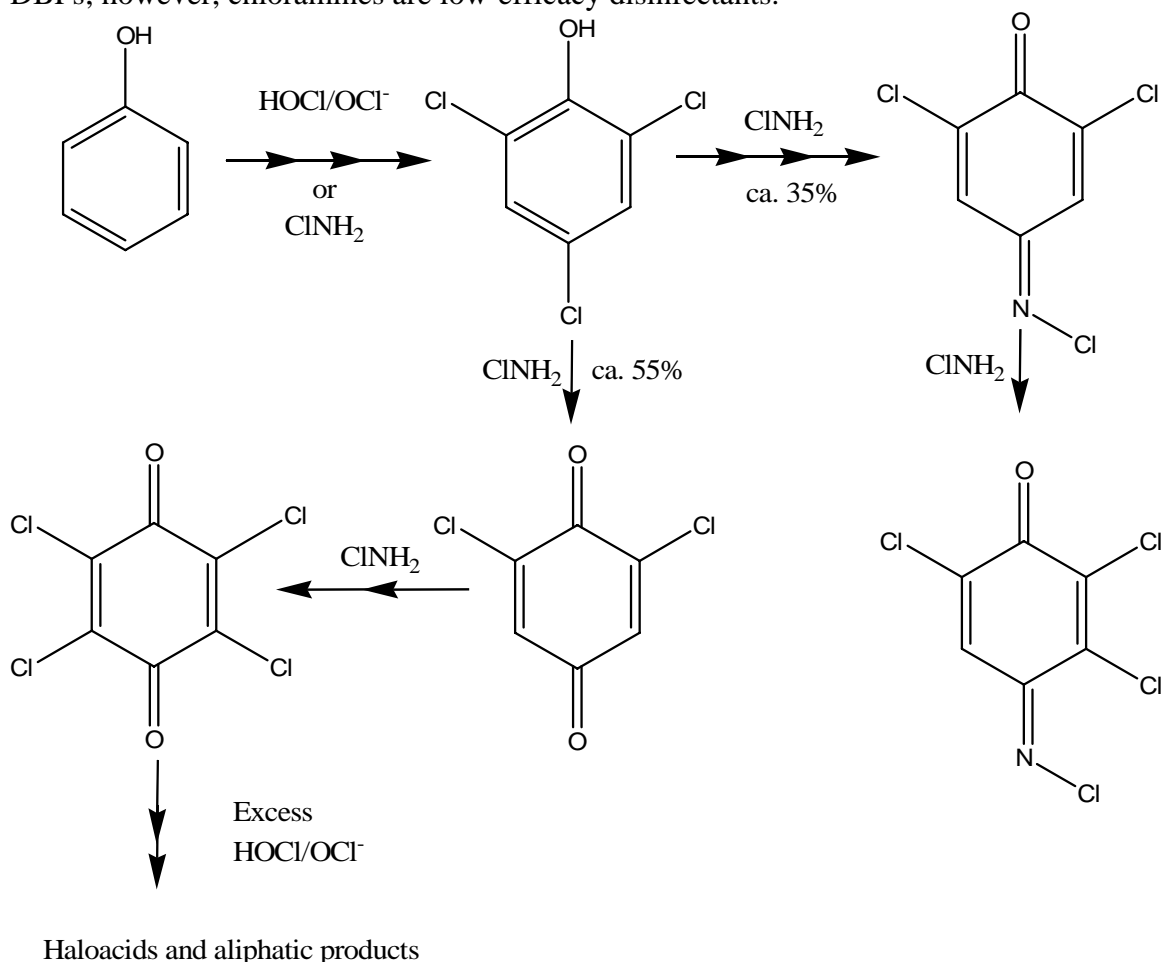


Figure 2.2. Formation of haloquinones

Although many DBPs have been identified, there are good comprehensive quantitative data available for only a few dozen in water supplies. Data from a recent study of 12 utilities in the USA and Canada by the United States Environmental Protection Agency (USEPA) (Weinberg et al., 2002) are provided in Tables 2.4 and 2.5, which are not comprehensive, but are probably indicative of many water supplies. The disinfectants used varied among these utilities, with ozone (one utility) and chlorine dioxide (four utilities) employed in some utilities, but all systems employed chlorine or chloramines at some stage in the treatment. These data may differ slightly from those reported, because the means represent the average of utility means, rather than an overall mean of all samples. There are earlier data sets that are available (e.g. USEPA/AMWA, 1989), but those surveys included fewer by-products. As the USEPA-funded survey included only 12 utilities and mixed disinfectants, it probably does not reflect extreme occurrences of DBPs. Nevertheless, the variation of DBP concentrations among the 12 utilities ranges up to 2 orders of magnitude. It should not be assumed that the concentrations co-vary with one another in dependable patterns among water utilities or even within the same system in different seasons of the year (Wright et al., 2002; Bull et al., 2009).

2.6.3 Disinfection by-products in foods

Chlorine and hypochlorite are commonly used in chillers and sprays for sanitization of food products. Poultry, meats, fish, fruits and vegetables, and other foods (e.g. milk, cheese) are exposed for various periods of time, ranging from seconds to hours (Fukayama et al., 1986). A summary of these treatments is provided in chapter 1. Chlorine and chlorine-containing compounds and by-products present in water used in food processing may penetrate into the surface biofilms to some degree. Quinone derivatives are less likely to be formed in the produce per se but may be formed in processing water and taken up because of their relatively non-polar character. Their stability upon heating is not known.

Table 2.4. Disinfection by-products in 12 drinking-water utilities in the USA and Canada^a

DBP	Number of utilities	Concentration ($\mu\text{g/l}$) ^b		
		Mean	Median	Range
Chloroform	12	16	12	0.5–47
BDCM	12	10	12	2.2–19
DBCM	12	6.5	4.7	0.1–20.5
Bromoform	12	2.1	0.7	nd–6.4
Dichloriodomethane	12	1.1	0.45	0.08–1.5
Bromochloriodomethane	12	0.4	0.3	nd–2.5
Dibromiodomethane	10	0.29	nd	nd–2.5
Chlorodiodomethane	12	0.11	nd	nd–1.1
Bromodiodomethane	12	0.03	nd	nd–0.4
Iodoform	12	0.04	nd	nd–0.4
Monochloroacetic acid	12	1.6	nd	nd–3.9
Monobromoacetic acid	12	0.3	0.27	nd–1.0
Dichloroacetic acid	12	14	15	1.4–22
Bromochloroacetic acid	12	5.9	4.4	1.7–11
Dibromoacetic acid	12	3.4	1.2	nd–12
Trichloroacetic acid	12	9.4	6.1	0.5–35
Bromodichloroacetic acid	12	4.6	5.5	nd–9.4
Dibromochloroacetic acid	12	2.2	1.5	nd–5.9
Tribromoacetic acid	12	0.12	nd	nd–0.9
Chloroacetonitrile	12	0.07	0.055	nd–0.26
Bromoacetonitrile	12	0.005	nd	nd–0.04
Dichloroacetonitrile	12	1.4	1.2	0.1–4.1
Bromochloroacetonitrile	11	0.8	0.6	nd–2.6
Dibromoacetonitrile	12	0.6	0.3	nd–2.3
Trichloroacetonitrile	12	0.02	nd	nd–0.15
Bromodichloroacetonitrile	12	nd	nd	nd–0.4
Dibromochloroacetonitrile	12	0.01	nd	nd–0.15
Tribromoacetonitrile	12	nd	nd	nd
Dichloroacetaldehyde	12	2.2	1.7	0.4–11.1
Bromochloroacetaldehyde	12	0.5	0.32	nd–1.3
Chloral hydrate	12	2.2	1.8	0.2–5.9
Tribromoacetaldehyde	12	0.19	0.04	nd–0.93
Chloropropanone	12	0.22	0.11	nd–1.1

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DBP	Number of utilities	Concentration ($\mu\text{g/l}$) ^b		
		Mean	Median	Range
1,1-Dichloropropanone	12	0.61	0.58	0.12–1.3
1,3-Dichloropropanone	12	nd	nd	nd
1,1-Dibromopropanone	12	0.032	nd	nd–0.12
1,1,1-Trichloropropanone	12	1.3	1.4	0.03–3.6
1,1,3-Trichloropropanone	12	0.02	0.02	nd–0.13
1-Bromo-1,1-dichloropropanone	12	0.24	0.2	nd–0.95
1,1,1-Tribromopropanone	12	nd	nd	nd
1,1,3-Tribromopropanone	12	0.005	nd	nd–0.033
1,1,3,3-Tetrachloropropanone	12	0.05	nd	nd–0.26
1,1,1,3-Tetrachloropropanone	12	0.08	0.07	nd–0.13
1,1,3,3-Tetrabromopropanone	12	0.05	nd	nd–0.025
Chloronitromethane	12	0.04	nd	nd–0.16
Bromonitromethane	12	0.02	nd	nd–0.08
Dichloronitromethane	12	0.12	0.24	nd–0.38
Bromodichloronitromethane	12	0.11	nd	nd–0.42
Dibromonitromethane	12	0.07	nd	nd–0.19
Chloropicrin	12	0.26	0.16	0.04–0.92
Bromodichloronitromethane	12	0.32	0.24	nd–1.0
Dibromochloronitromethane	12	0.30	0.18	nd–0.44
Bromopicrin	12	0.35	nd	nd–0.63

^a Adapted from Weinberg et al. (2002).

^b The concentrations reported as “nd” were not detected. The LODs for the various substances were between 0.02 and 3 $\mu\text{g/l}$, but these LODs varied slightly between sampling occasions and analytical methods used.

Table 2.5. Additional analyses of disinfection by-products and total organic halogen in a survey of 12 utilities in the USA and Canada^a

DBP	Number of utilities	Concentration ($\mu\text{g/l}$) ^b		
		Mean	Median	Range
Monochloroacetaldehyde	12	0.42	0.22	nd–1.3
Dichloroacetaldehyde	12	3.4	2.7	0.5–9.5
Bromochloroacetaldehyde	11	1.2	1.1	0.1–3.5
3,3-Dichloropropenoic acid	12	0.43	0.14	nd–2.7
Bromochloromethylacetate	12	0.036	nd	nd–0.4
Monochloroacetamide	8	0.14	nd	nd–0.5
Monobromoacetamide	8	0.24	nd	nd–1.1
2,2-Dichloroacetamide	12	1.5	1.7	nd–3.8
Dibromoacetamide	8	0.87	0.25	nd–2.8
Trichloroacetamide	8	0.51	0.30	nd–1.1
BMX-1	10	0.034	nd	nd–0.13
BEMX-1	10	0.10	nd	nd–0.72
BMX-2	10	0.028	nd	nd–0.15
BEMX-2	10	0.12	nd	nd–0.81

DBP	Number of utilities	Concentration ($\mu\text{g/l}$) ^b		
		Mean	Median	Range
BMX-3	10	0.004	nd	nd–0.04
BEMX-3	10	0.097	nd	nd–0.41
MX	12	0.11	0.020	nd–0.18
Red-MX	2	0.033	nd	nd–0.29
EMX	12	0.013	nd	nd–0.10
ZMX	10	0.011	nd	nd–0.12
Ox-MX	10	nd	nd	nd
Mucochloric acid (ring)	12	0.085	0.01	nd–0.71
Mucochloric acid (open)	12	0.081	0.09	nd–0.19
TOX	12	169	182	65–236

BEMX-1, BEMX-2, BEMX-3: corresponding brominated analogues of EMX; BMX-1: 3-chloro-4-(bromochloromethyl)-5-hydroxy-2(5H)-furanone; BMX-2: 3-chloro-4-(dibromomethyl)-5-hydroxy-2H(5H)-furanone; BMX-3: 3-bromo-4-(dibromomethyl)-5-hydroxy-2H(5H)-furanone; EMX: (*E*)-2-chloro-3-(dichloromethyl)-4-oxobutenoic acid; MX: 3-chloro-4-(dichloromethyl)-5-hydroxy-2H(5H)-furanone; Ox-MX: oxidized MX, (*Z*)-2-chloro-3-(dichloromethyl)butenedioic acid; Red-MX: reduced MX, 3-chloro-4-(dichloromethyl)-2(5H)-furanone; TOX: total organic halides; ZMX: (*Z*)-2-chloro-3-(dichloromethyl)-4-oxobutenoic acid

^a Adapted from Weinberg et al. (2002).

^b The concentrations reported as “nd” were not detected. The LODs for the various substances were between 0.1 and 3 $\mu\text{g/l}$, but these LODs varied slightly between sampling occasions and analytical methods used.

For example:

- *Carrots*: Cut carrots were washed with chlorinated water at 4 °C, then with warm tap water at 50 °C, and it was reported that by-product formation due to chlorination was negligible (Klaiber et al., 2005).
- *Cheese*: Chloroform was reported at concentrations ranging from 2.4 to 17 $\mu\text{g/kg}$ in cheese (Entz, Thomas & Diachenko, 1982).
- *Butter*: Chloroform was reported at 56 $\mu\text{g/kg}$ and BDCM at 7 $\mu\text{g/kg}$ (Entz, Thomas & Diachenko, 1982).
- *Shrimp*: Following immersion in a 150 mg/l solution of hypochlorous acid, 2% of the chlorine was incorporated into shrimps, with 75% in the edible portion; 73% of the 2% taken up was as chloride ion (Cunningham & Lawrence, 1977).
- *Poultry*: Chloroform (447 $\mu\text{g/kg}$) was found in fresh uncooked poultry after immersion in 50 mg/l aqueous chlorine. However, it was not determined whether the chloroform came from the water or from reactions with the tissues. The highest levels were in depot fat (Robinson, Mead & Barnes, 1981).
- *Poultry*: Chloroform levels were reported in tissues from chickens that had been immersed in chiller water, then stored or roasted immediately; a control used tap water (presumably chlorinated) instead of chiller water (Axtell, Russell & Berman, 2006). The concentrations of chloroform did not vary greatly in all of the tested products and were in the range of 0.27–0.3 mg/kg. The skin and fat chloroform concentrations were very similar for all three conditions and ranged between 0.18 and 0.22 mg/kg.
- *Poultry patties*: No significant differences in triglycerols, phospholipids or fatty acid compositions were found between stored hypochlorous acid and non-chlorinated treated chicken patties (Erickson, 1999).

Semicarbazide ($\text{NH}_2\text{NHCONH}_2$; CAS No. 79-17-4) was shown to be formed in foods under usually extreme conditions of contact and room temperature incubation with hypochlorite solutions at concentrations ranging from 0.015% to 12%. Concentrations of semicarbazide in the range of 1–20 mg/kg were detected only at chlorine concentrations of about 1% and higher. Hypochlorite reactions forming semicarbazide occurred *in vitro* with arginine, creatine, creatinine and urea, but not with histidine and citrulline, at a hypochlorite concentration of 0.015% (Hoenicke et al., 2004). Under usual food processing conditions, it is unlikely that semicarbazide is formed.

2.6.4 Other reactions with foods

Chlorinated poultry chiller water was analysed by GC/MS for the presence of saturated and unsaturated aliphatic aldehydes (Tsai, Mapes & Huxsoll, 1987). These aldehydes were pentanal, hexanal, heptanal, octanal, *trans*-2-octenal, nonanal, *trans*-2-nonenal, decanal, 2,2-nonadienal, *trans*-2-decenal, 2,4-decadienal and *trans*-2-undecenal. Their presence is indicative of autoxidation occurring in the chiller water, which may also involve the presence of oxidizer sanitizers. There is buildup of filterable and non-filterable solids in chiller water to a variable degree, depending on chiller design, production rate, cleanliness, fat content on the carcass surface and other factors. The solids content tends to reach a steady state from incoming carcasses and overflow as processing continues. The fatty acid and lipid content in the chicken skin and flesh are a function of feed type. The aldehydes identified are predictably formed by autoxidation of fatty acids. From the presence of those acids and esters in chlorinated chiller water, it would be expected that secondary organic chlorine-containing compounds may be formed in the presence of oxygen (Fukayama et al., 1986), but none were detected in the study (Tsai, Mapes & Huxsoll, 1987).

2.7 Sodium dichloroisocyanurate

2.7.1 Chemistry

Sodium dichloroisocyanurate (NaDCC; 1,3-dichloro-1,3,5-triazinane-2,4,6-trione; $\text{NaC}_3\text{N}_3\text{O}_3\text{Cl}_2$) is the sodium salt of a chlorinated hydroxytriazine. It is a form of stabilized chlorine, which provides a convenient way to handle chlorine. The product contains 55–62% available chlorine; it is very soluble in water. When dissolved in water, it undergoes equilibrium-controlled dissociation into chlorine and several isocyanurate chemicals and ultimately isocyanuric acid as the stable end product (FAO, 2003). It is marketed in an anhydrous, >97% pure form (CAS No. 28933-78-9) and as a dihydrate, >99% pure form ($\text{NaC}_3\text{N}_3\text{O}_3\text{Cl}_2 \cdot 2\text{H}_2\text{O}$; CAS No. 51580-86-0). The principal impurity in NaDCC is sodium chloride.

2.7.2 Application and fate in foods

NaDCC is used like chlorine, especially for outdoor swimming pool disinfection, because it reduces the solar decomposition of hypochlorite, as an emergency drinking-water disinfectant for short-term use and in food sanitation applications. In its applications, it can be treated as though it were chlorine, although because of its control of chlorine release and concentration in solution, it should produce smaller amounts of DBPs. In addition, a residue of stable cyanuric acid will remain in solution (e.g. 1 mg of anhydrous NaDCC corresponds to 0.59 mg of cyanuric acid).

An example of a food sanitizing application includes treatment of salad vegetables. NaDCC was used in a solution of 100 mg/l available chlorine, and the pH was adjusted to 5 with hydrochloric acid (Nicholl, McInerney & Prendergast, 2004). Salad greens and cabbage were soaked and drained. The maximum free available chlorine on the vegetables was 0.8 mg/l, and cyanuric acid residues were not detected.

2.8 1,3-Dibromo-5,5-dimethylhydantoin (active bromine)

DBDMH (CAS No. 77-48-5) is used as an alternative to active chlorine in the disinfection of water.

2.8.1 Chemistry

DBDMH is a stable, white crystalline solid. Dissolution of DBDMH in water quantitatively produces two molecules of hypobromous acid and one molecule of dimethylhydantoin (DMH) (Figure 2.3), which are in equilibrium until the hypobromous acid is consumed in other reactions. However, atomic bromine may be postulated as a transfer intermediate that could convert to hypobromous acid. Hypobromous acid, like hypochlorous acid, is an excellent oxidizing agent and has found use as a disinfectant to treat water for drinking, recreational waters (e.g. swimming pools, spas and hot tubs) (Seidel, 2004) and water used in food processing.

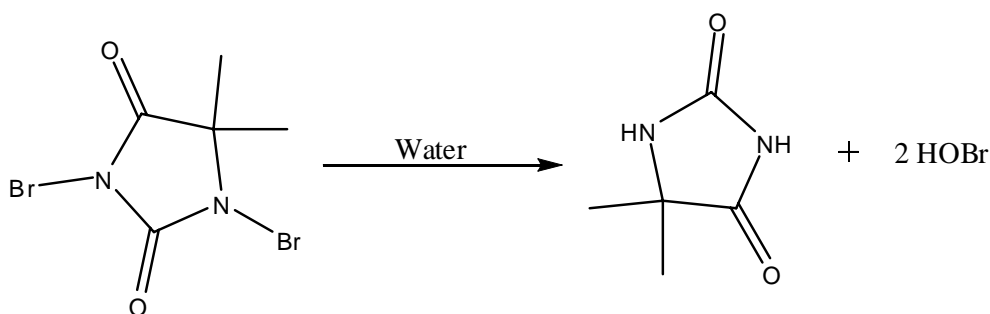


Figure 2.3. Hydrolysis of DBDMH

2.8.2 Application and fate in foods

DBDMH is authorized in the USA for use as a disinfectant in water and ice used in the processing of poultry and as a disinfectant in water used to process beef hides, carcasses, heads, trim, parts and organs. The use level of DBDMH in poultry process water and ice is limited to 100 mg/kg as available bromine, which is equivalent to 90 mg/kg as DBDMH. The use level of DBDMH in process water used to treat beef carcasses is limited to 300 mg/kg as available bromine, or 270 mg/kg as DBDMH. As DBDMH decomposes in water and with heat, it is not expected to be present on food at the time of consumption. However, its breakdown product, DMH, would be an expected residue on foods that are not washed or further processed before consumption. In addition, other DBPs, such as organobromine DBPs, bromide and bromate, would also be potential residues on food treated with aqueous solutions of DBDMH.

2.8.2.1 DMH

The amount of DMH that remains on poultry carcasses after processing was estimated using 1) the maximum use level of DBDMH in poultry chiller water (90 mg/kg), 2) the water uptake by poultry carcasses (8% by weight), 3) the assumption that DMH and other breakdown products will be absorbed by the carcass in an amount proportional to the amount of water taken up by the carcass while it is in the chiller tank and 4) the amount of chiller water allowed to be recirculated (50% in the USA). The concentration of DMH on raw poultry is estimated to be 0.005 mg/g. The concentration of DMH in the chiller tank at any given time would be no greater than 60 mg/kg (USFDA, 2003). Therefore, the concentration of DMH in poultry would not be greater than 0.005 mg/g chicken, or 5 mg/kg chicken.

The maximum use level of DBDMH in water used to process beef is limited to 300 mg/kg as active bromine, which is equivalent to 270 mg/kg as DBDMH. The amount of DMH that remains on beef carcasses after processing can be estimated using 1) the maximum use level of DBDMH in water applied to beef as a spray (270 mg/kg), 2) the assumption that the amount of DMH absorbed by the carcass is proportional to the amount of water taken up by the carcass while it is treated with the disinfectant spray (1%) (USFDA, 2008b) and 3) the molecular weights of DBDMH (285 g/mol) and DMH (128 g/mol). The concentration of DMH on raw beef would be approximately 0.001 mg/g.

2.8.2.2 Bromide

The quantity of residual bromide on a poultry carcass treated with a solution of DBDMH can be estimated using assumptions 2, 3 and 4 from section 2.8.2.1 above and the worst-case assumption that 100% of the bromine liberated from DBDMH is converted to bromide; however, organobromine products actually account for a portion of the initial bromine. Therefore, a worst-case estimate for residual bromide is 6 mg/kg in raw chicken (USFDA, 2003). Using a conservative estimate of residual bromide on beef, assuming 100% conversion of the active bromine to bromide, the concentration of bromide on beef would be approximately 0.002 mg/g.

2.8.2.3 Trihalomethanes

Chloroform is not expected to be present in the poultry or poultry processing water or ice beyond what is normally observed in potable water produced using accepted disinfection processes. However, the USFDA (2003) estimated a bromoform concentration of approximately 0.005 µg/g raw chicken and DBCM or BDCM concentrations of less than 0.0004 µg/g raw chicken. The residue values for DBCM and BDCM are data from the USFDA (2003) indicating that DBCM and BDCM were not detected in the poultry process water above the LOD of 5 µg/l.

Chloroform is not expected to be present in the beef or beef processing water beyond what is normally observed in potable water produced using accepted disinfection processes. However, the average concentration of bromoform found in the spray used to treat beef was 5.5 µg/kg. The above assumptions give a residual bromoform level of 0.000 06 µg/g beef (USFDA, 2008b). The presence of DBCM and BDCM on beef is related to the method used to generate the potable water used in the beef processing water and to the use of DBDMH. Data from the USFDA (2008b) indicate that these compounds were not detected in the process water above the LOD of 5 µg/kg. Using the assumptions above and the LOD, the concentration of either DBCM or BDCM would be less than 0.000 05 µg/g raw beef.

2.8.2.4 Bromate

Although bromate may potentially be generated in small amounts during the use of DBDMH and may migrate to poultry during processing, bromate is a strong oxidant (Seidel, 2004) and is expected to be reduced to bromide during cooking (USFDA, 2003). Therefore, bromate is not expected to be present on food at the time of consumption.

2.8.2.5 Brominated and iodinated compounds

The type of water used in food processing and the disinfectants added may have an influence on the formation of brominated and iodinated compounds. The use of seawater to process seafood will be associated with higher concentrations of bromide and some iodide. These salts will then be converted to hypobromous or hypoiodous acids in the presence of chlorine and some other disinfectants and result in the production of brominated and iodinated by-products in addition to chlorinated by-products. Organobromine by-products will also be produced when fresh waters containing bromide are chlorinated. Reactions with proteins or lipids in the foods may be possible; however, there is no reported evidence for the formation of brominated organic species in food under conditions approved in, for example, the USA (USFDA, 2003, 2008b).

2.8.3 Summary

Considering the available data on treatment of poultry and beef with DBDMH, it is unlikely that significant amounts of DBPs would be formed and would remain as residues. Chemical residues could include DMH, bromide, DBCM, BDCM and bromoform.

2.9 Ethyl lauroyl arginate

Ethyl lauroyl arginate (synonyms: lauramide arginine ethyl ester, LAE) is synthesized by esterifying L-arginine with ethanol to obtain ethyl arginate hydrochloride (HCl), which is then reacted with lauroyl chloride to form the active ingredient ethyl-*N*^α-lauroyl-L-arginate hydrochloride (C₂₀H₄₁N₄O₃Cl; CAS No. 60372-77-2). It is a cationic surfactant that has a wide spectrum of activity against bacteria, yeasts and moulds. *N*^α-Lauroyl-L-arginine is a principal by-product in the manufacture of ethyl-*N*^α-lauroyl-L-arginate HCl and is also formed by enzymatic action in fresh food. In the USA, ethyl lauroyl arginate is generally recognized as safe for use on meat and poultry products and other food products, including flavoured drinks, fish, dried legumes and prepared salads, at levels up to 200 mg/kg (FAO, 2008).

The extent of hydrolysis of ethyl lauroyl arginate under various conditions was determined by measurement of the percentage of ethyl-*N*^α-lauroyl-L-arginate HCl recovered in each sample. In 24 out of 33 samples, no hydrolysis process took place. Only 9 samples showed interaction with the components of the sample. In 4 of these 9 samples, ethyl lauroyl arginate was hydrolysed to *N*^α-lauroyl-L-arginine, which is the main metabolite. In the remainder of the samples, in which it was combined with nitrite, meat or soya proteins or ovo-albumin or lacto-albumin, more extensive hydrolysis occurred. In spite of this, no formation of nitrosamines was observed (FAO, 2008).

The stability of ethyl-*N*^α-lauroyl-L-arginate HCl was evaluated in eight different food matrices. Five of these matrices were examples of processed foods, and the rest were examples of fresh foods. It was found to be stable throughout the duration of the study in all processed food matrices; only in the fresh food matrices was a decrease in concentration observed (FAO, 2008).

2.10 Ozone (active oxygen)

Ozone (triatomic oxygen, O₃; CAS No. 10028-15-6), either in the gaseous phase or in an aqueous solution, is used as a disinfectant in the processing, treatment and storage of foods, including fresh produce, meat and poultry. Ozone treatment is approved for such uses in the USA (USFDA, 2003) and Australia (FSANZ, 2006). There are currently no restrictions on its use, save that Good Manufacturing Practice must be followed.

2.10.1 Chemistry and preparation

Ozone is unstable and must be generated at the point of application using one of three major methods: 1) irradiation of air using high-intensity UV lamps (185 nm), 2) corona discharge (used to produce large volumes of ozone) and 3) passage of dry air or oxygen across a high-voltage discharge gap (Kirk-Othmer, 2004). Additional methods of generation of ozone have been described (Kim, Yousef & Dave, 1999). In the gas phase, the decomposition of ozone is catalysed by light, trace organic matter, nitrogen oxides, peroxides, metals and metal oxides. The mechanisms of decomposition are outlined in Figures 2.4 and 2.5, where M represents any species present in the gas phase (Kirk-Othmer, 2004).

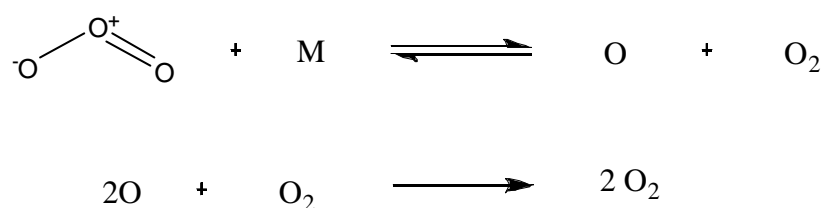


Figure 2.4. Decomposition of ozone

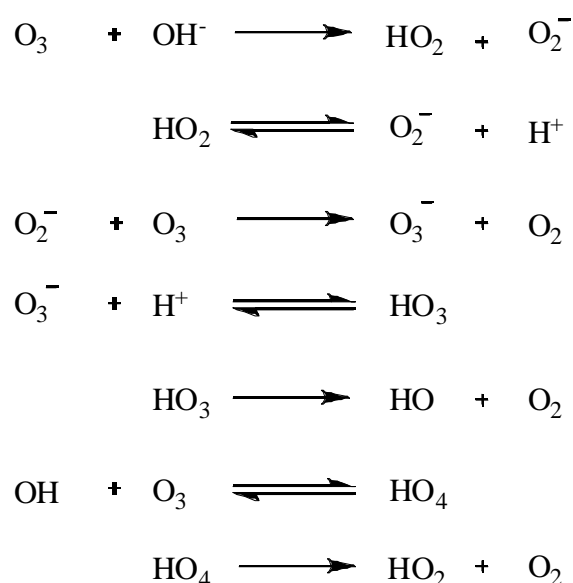


Figure 2.5. Decomposition of ozone in water

Although the decomposition of gaseous ozone is relatively simple and produces only oxygen as a by-product, the decomposition of ozone in the aqueous phase is far more

complex, generating a large number of reactive species that can participate further in numerous side reactions or hasten its decomposition. In pure water, ozone decomposes by a radical chain reaction initiated by hydroxide and propagated by superoxide and hydroxyl radicals (Kirk-Othmer, 2004).

Ozone in water and its reaction product and by-product chemistry have been described in an early review (Rice & Cotruvo, 1978). Owing to its high oxidation potential ($E^0 = 2.07$ V), ozone reacts with a large number of compounds. For example, halogens, with the exception of fluorine, form hypohalite ions that, in the presence of excess ozone, are oxidized to halites (Rice & Cotruvo, 1978; Rice & Gomez-Taylor, 1986; Kirk-Othmer, 2004). Metal ions such as Fe^{2+} and Mn^{2+} are converted to hydroxides ($\text{Fe}(\text{OH})_3$) or metal oxides (MnO_2) (Kirk-Othmer, 2004). In addition, ozone reacts with most organic substrates, including, but not limited to, olefins, acetylenes, aromatics, and C–H, C=N, N=N, Si–H and Si–C bonds (Kirk-Othmer, 2004). Under extended reaction times and high concentrations of ozone, hydrocarbons can be broken down into carbon dioxide and water (Rice & Gomez-Taylor, 1986). The most common transformation induced by ozone is the cleavage of olefin double bonds, forming, depending on the location and substitution of the double bond, ketones or aldehydes (Figure 2.6).

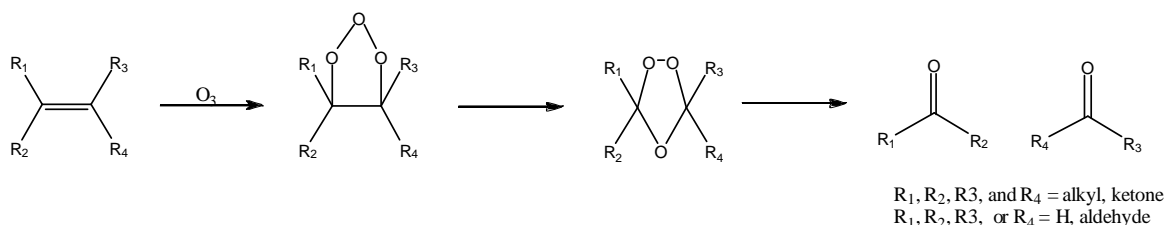


Figure 2.6. Ozonation of olefins

The reactivity of ozone in solution depends greatly on the conditions employed during ozonation. For example, at pHs below 6 and at or below room temperature, ozone reacts directly with organic molecules. Above pH 8, ozone decomposes to highly energetic hydroxyl radicals that react non-selectively with materials via electron transfer, hydrogen abstraction, addition reaction, etc. Between pH 6 and 8, ozone can react by both pathways (Rice & Gomez-Taylor, 1986). Therefore, the conditions under which ozone is used as a disinfectant must be closely monitored and controlled to give the desired result.

2.10.2 Application and fate in foods

Ozone is used to disinfect water and ice used in the processing of foods, including seafood and fish, and there is the potential for reaction of ozone with components of water, such as bromide and chloride. Reaction of ozone with halides can produce oxyhalides, such as hypochlorous acid or hypobromous acid. The hypohalous acids would react with organic matter in the water, and chlorate and bromate could be formed in reaction with additional ozone (IPCS, 2000). Bromate formed through reactions with molecular ozone may contribute in the range of 30–80% to the overall bromate ion formation in waters containing NOM. The presence of bromide ion in the aqueous solution treated with ozone may lead to formation of additional by-products, such as bromoform and other brominated THMs, dibromoacetonitrile and dibromoacetone. Also, aldehydes, ketones, ketoacids and carboxylic acids may be formed by ozonation, with aldehydes, such as formaldehyde, being dominant.

Ozone is extremely reactive and would be expected to react with most components of food (e.g. proteins, fatty acids, vitamins, etc.) that contained unsaturation or were oxidizable. There are reports that, under laboratory conditions, hypobromous acid reacts with proteins, peptides and amino acids, producing brominated tyrosine and short-lived *N*-brominated species, such as bromamines and bromamides. Hawkins & Davies (2005) reported that greater than 40% of hypobromous acid generated in the presence of bovine serum albumin is converted to short-lived bromamides and bromamines. Above 4 °C, these protein-derived *N*-bromo compounds decompose rapidly (either directly or through the formation of free radicals) by a number of pathways, including oxidation of tyrosine, formation of carbonyl moieties in proteins, and rearrangement and fragmentation of proteins. Although bovine serum albumin and fish muscle proteins are not identical, they contain tyrosine. There would, however, be variation in the quantities of the reaction products owing to the macromolecular configuration of the individual proteins. Given the reactive nature of hypobromous acid and the *N*-bromo compounds and the variation of the chemical composition of protein chains and their macromolecular configuration, small quantities of numerous compounds would be expected. However, specific compounds or classes of compounds have not been identified. Although brominated tyrosine is expected to be stable under these conditions, the data by Hawkins & Davies (2005) indicate that the concentration of these brominated compounds in fish and seafood would be insignificant.

2.10.3 Summary

Ozone and its rapid decomposition limit its reactivity to the surface of foods. The quantities of oxidation products resulting from the treatment of seafood and fish would be small compared with those resulting from oxidation due to the cooking of food; however, brominated DBPs could be formed with available bromide.

2.11 Peroxyacids and peroxides

A number of oxygen-based alternatives to chlorine-containing disinfectants are currently being used in the processing of fresh meat, poultry, fish and fresh and processed fruits and vegetables. They include hydrogen peroxide and peroxyacids, as well as ozone (see section 2.10). Peroxy compounds are a group of peroxide compounds containing at least one pair of oxygen atoms (-O-O-) bonded by a single covalent bond. Peroxides may be divided into two groups: inorganic and organic peroxy compounds.

2.11.1 Chemistry of peroxyacids and hydrogen peroxide

JECFA recently evaluated peroxyacid-based antimicrobials containing 1-hydroxyethylidene-1,1-diphosphonic acid (HEDP) (C₂H₈O₇P₂; CAS No. 2809-21-4) as a stabilizer (FAO, 2004; FAO/WHO, 2005). The following is a summary of the chemistry of the peroxyacid antimicrobial washes from these reports. Peroxyacid antimicrobial solutions are typically prepared by mixing aqueous hydrogen peroxide (4–12%) (CAS No. 7722-84-1) and aqueous acetic acid (40–50%) (CAS No. 64-19-7), which results in an equilibrium mixture of acetic acid, peroxyacetic acid (CAS No. 79-21-0), hydrogen peroxide and water (Figure 2.7).

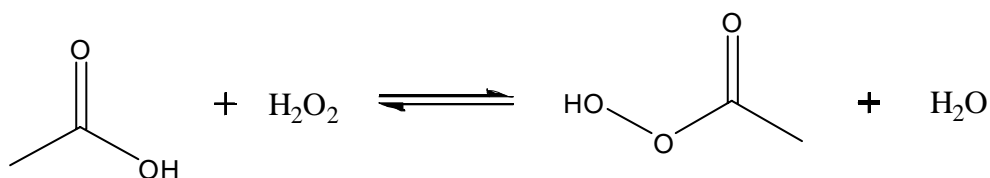


Figure 2.7. Peroxyacid formation from hydrogen peroxide

These antimicrobial washes may sometimes contain 3–10% octanoic acid (CAS No. 124-07-2), which, when treated with hydrogen peroxide, produces an equilibrium mixture of octanoic acid and peroxyoctanoic acid (CAS No. 33734-57-5). The peroxyacid solutions are typically sold as concentrates and are diluted with water to a total peroxyacid concentration of 80–200 mg/kg.

Peroxyacids are inherently unstable and decompose into non-toxic chemicals in the presence of heat, acids and certain transition metal ions (e.g. copper). Two mechanisms for the decomposition are 1) hydrolysis to their corresponding organic acid and hydrogen peroxide and 2) decomposition to their corresponding organic acid and oxygen (Figure 2.8) (FAO, 2004). The hydrogen peroxide in these solutions decomposes into water and oxygen. To counteract the deleterious effects of metal ions, manufacturers incorporate <1% HEDP as a chelating agent. Unlike hydrogen peroxide and the peroxyacids, HEDP is stable and is expected to remain in the antimicrobial wash and on food after treatment.

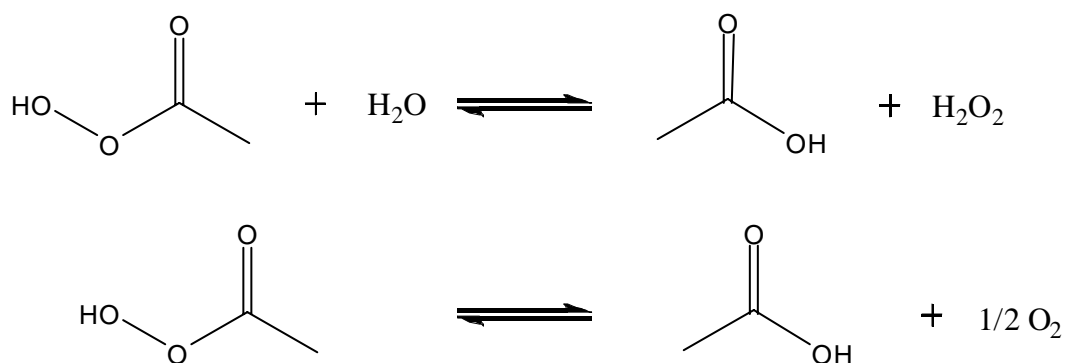


Figure 2.8. Decomposition equilibria of peroxy compounds

2.11.2 Application and fate in foods

Given the highly reactive nature of the peroxyacids and hydrogen peroxide, these compounds are not expected to be present on foods at the time of consumption. However, their breakdown products (e.g. acetic acid or octanoic acid) and residual HEDP would be expected residues on foods that are not washed, peeled or further processed before consumption. HEDP residues will remain on foods that are not washed or further processed. Being less reactive than hypochlorite, peroxyacids may survive longer in contact with organic matter and may penetrate biofilms more effectively; however, they are also lesser biocides than hypochlorite.

The peroxyacids would be expected to react with components of food (e.g. proteins, fatty acids, vitamins). However, the data available to JECFA on the TBARS values (as a measure of the oxidation of fatty acids) and fatty acid profiles of raw and cooked poultry and beef indicated that there were no significant differences between treated and control samples.

In the USA, the use of peroxyacid disinfectants on poultry carcasses and red meat is currently authorized; the maximum concentration of peroxyacids is 220 mg/kg as peroxyacetic acid, the maximum concentration of hydrogen peroxide is 85 mg/kg and the maximum concentration of HEDP is 11 mg/kg (USFDA, 2009). The use of peroxyacid disinfectants in wash water and chilling water for fruits and vegetables is authorized in the USA, with a limit of HEDP of 9.6 mg/kg. The worst-case scenario that was estimated for leafy greens was 0.53 mg/kg as HEDP (USFDA, 2007a). The use of peroxyacid disinfectants in water and ice used to commercially process fish and seafood is also authorized in the USA, with a limit of HEDP of 10 mg/kg in the wash water and ice. Given that 1 kg of fish retains approximately 9 g of water, the residue level of HEDP on fish would be around 90 µg/kg fish (USFDA, 2007b).

2.11.3 Summary

The only chemical residue in food resulting from the use of peroxyacid disinfectants in food processing is HEDP.

2.12 Quaternary ammonium compounds (including cetylpyridinium chloride)

Quaternary ammonium compounds, commonly referred to as QACs or Quats, are widely used as surface sanitizers in hospital settings, nurseries (Rutala, 2005) and food processing facilities. QACs are organically substituted ammonium compounds in which the nitrogen atom has a valency of five. They have the general structure $R_4N^+X^-$, where the Rs can be numerous alkyl or alkylbenzyl moieties, including several different groups in the same molecule, and the X is a halide ion, often chloride. They are ionic and water soluble. However, their solubility can be affected by water quality factors (e.g. hard water) and pH. They are commonly used on food contact surfaces, and several are registered as “no-rinse sanitizers” (Cords et al., 2005), which would be indicative of a regulator’s conclusions of their low toxicity under those conditions of use and residue transport. No-rinse sanitizers for food contact surfaces include the “second-generation” QAC, *n*-alkyldimethylbenzylammonium chloride; the “third-generation” dual QACs, *n*-alkyldimethylbenzylammonium chloride and *n*-alkyldimethylethylbenzylammonium chloride; the “fourth-generation” twin or dual-chain QACs, didecyldimethylammonium chloride and dioctyldimethylammonium chloride; and “fifth-generation” mixtures of fourth-generation and second-generation QACs. They are also common components of antiseptic hand soaps (Sattar, 2004).

2.12.1 Cetylpyridinium chloride

CPC is a QAC found in an anhydrous form ($C_{21}H_{38}NCl$; CAS No. 123-03-5) or as cetylpyridinium chloride monohydrate ($C_{21}H_{38}NCl \cdot H_2O$; CAS No. 6004-24-6). CPC has been approved for food contact use in the USA (USFDA, 2004b) as an antimicrobial agent to treat the surface of raw poultry carcasses only in systems that collect and recycle solution that is not carried out of the system with the treated poultry carcasses. CPC should be applied at a maximum level of 0.66 g/kg of raw poultry carcass as a fine mist spray of an ambient-temperature aqueous solution to raw poultry carcasses prior to immersion in a chiller. The aqueous solution should also contain propylene glycol at a concentration 1.5 times that of the CPC. The requirement for collection of the solution is due to the fact that water from poultry processing may be recycled into animal feed. Water retention in poultry carcasses may be

initially up to 12% by weight (Zentox, 2007), so the maximum would be a function of the concentration in the chiller water and the amount of retained chiller water.

The safety analysis connected with the promulgation of the regulation in the USA (USFDA, 2004b) contained information related to residual CPC on poultry carcasses following treatment using a number of different protocols. The carcasses were treated and cooked in a manner to simulate consumer practices. In five different studies involving more than 400 carcasses, it was noted that the residue of CPC on the carcass was directly proportional to the level in the wash and that use of a potable water wash following treatment did not result in significantly lower residues of CPC on the carcass than allowing the carcass to drip dry following treatment. The average residual level of CPC on carcasses ranged from 4.4 mg/kg for a 0.05% solution wash to 20 mg/kg for a 2.0% solution wash. The concentration of CPC in treatment solutions used in the USA is limited to no more than 0.8% CPC.

2.13 Iodophors

Iodophors are widely used as surface sanitizers in hospital settings, nurseries (Rutala, 2005) and food processing facilities. They are also common components of antiseptic hand soaps (Sattar, 2004). Iodophors are mixtures of iodine (I_2 ; CAS No. 7553-56-2) and surface-active agents such as alcohols and polyethoxyols that act as carriers and solubilizers for the iodine. Iodine has low solubility in water, so the solubilizers help to keep it in suspension as well as act as a dispensing medium to control the continuous release of iodine into the water and stabilize the concentration of iodine in the water (Gottardi, 2001). The result is a water-soluble material that releases free iodine (12.5–25 mg/l) in solution.

Iodophors are primarily produced from polyethoxylated nonylphenol or polyol, which is a block copolymer of propylene and ethylene oxide. Polyethoxyphenols, including nonylphenoethoxylates, which are commonly used surfactants, have been suspected of being weak endocrine-active agents in water. Various other surfactants, including anionics, cationics, amphoteric and other nonionics, have also been used (Batey, 1976). The nature of the interaction between the iodine and the surfactant has not been clearly defined. It is known, however, that the iodine is bound in micellar aggregates in the carrier and that, on dilution, the micelles are dispersed and the linkage of the iodine is progressively reduced (Twomey, 1968, 1969).

Iodine easily undergoes oxidation and reduction to iodide and iodate, and it can react with organic thiols, such as cysteine, as well as amines and peptides. After ingestion, it is assumed that iodide and/or iodate are available for bioconversion to forms that are part of the iodine pool.

2.14 Sodium metasilicate

Sodium metasilicate (waterglass) is commercially available in three forms: anhydrous (Na_2SiO_3 ; CAS No. 6834-92-0), pentahydrate ($Na_2SiO_3 \cdot 5H_2O$; CAS No. 10213-79-3) and nonahydrate ($Na_2SiO_3 \cdot 9H_2O$; CAS No. 13517-24-3) (IPCS, 1997). Sodium metasilicate is used in solution as a detergent-type cleaning and degreasing agent for surfaces of poultry, beef and pork in slaughtering process operations. It is used in concentrations of about 1.1–1.6% in both pre-chiller and post-chiller topical applications to sanitize the carcasses. Sodium metasilicate seems to function as a bactericide principally due to the high pH of the working solutions, which ranges from about 12.6 to 13.3, and it is used at higher temperatures (30–

40 °C) and lower temperatures (7–13 °C). Residues on treated poultry carcasses were reported to be a maximum 171 mg/kg.

2.15 Trisodium phosphate

TSP (Na_3PO_4 ; CAS No. 7601-54-9) can be obtained in anhydrous or hydrated form and is also referred to as trisodium monophosphate or trisodium orthophosphate. It has a variety of uses in manufacturing of detergents (as builders, i.e. substances added to soaps or detergents to increase their cleansing action) due to the ability to sequester cations and because of the fairly high pH of solutions with TSP. Its use has declined, as phosphate discharges in wastewaters can contribute to environmental effects. The pH of a 1% solution is 11.5–12.5. The distribution of phosphate forms in solution is a function of solution pH.

TSP is used in aqueous solution typically at 8–12%, in which it is ionized to sodium (Na^+) and phosphate ions (PO_4^{3-}). These ions can be absorbed into food, but further reactions are considered unlikely (EFSA, 2005). Poultry treated with a 12% TSP solution for 15 min at 3 °C and pH 13.03, drained and then stored at 3 °C had a longer shelf life and lower bacterial populations. The pH of the treated poultry decreased to approximately 8 at day 0, then declined to about 6.2 after 5 days of storage (Del Río et al., 2007). Phosphate residue levels were not reported.

2.16 Other considerations

2.16.1 Vaporization and loss of residue chemicals

Many of the halogenated DBPs are non-polar/non-ionic organic chemicals and therefore have sufficient vapour pressures to result in spontaneous losses from foods during storage, processing and cooking, thus reducing residue levels. The Henry's Law constant is an indication of the volatility of a chemical. It characterizes the equilibrium distribution of dilute concentrations of volatile soluble chemicals between gas and liquid (USEPA, 2007). The Henry's Law constant will be temperature dependent and also subject to numerous physical factors of the medium. It can be presented on a concentration basis (1-Pa/mol), but also as a dimensionless value for relative comparisons. Table 2.6 illustrates the dimensionless Henry's Law constants for the principal THMs and 2-chlorophenol at 25 °C and 50 °C as an indication of relative loss potential during processing.

Table 2.6 Dimensionless relative Henry's Law constants

Chemical	Henry's Law constants	
	25 °C	50 °C
Chloroform	0.147–0.150	0.351–0.412
BDCM	0.0654–0.0832	~0.179
DBCM	0.0320–0.0431	~0.0707
Bromoform	0.0198–0.0218	0.0728–0.0821
2-Chlorophenol	0.0159	0.0655

In Table 2.6, the series from chloroform to bromoform is a set of somewhat polarized neutral compounds with increasing molecular weights and concurrent reducing volatility, and the values of the constants decline concurrently. The values increase at the higher listed

temperature (50 °C), which is well below cooking temperatures. Although the molecular weight of 2-chlorophenol is similar to that of chloroform, its constant is considerably lower than that for bromoform, because it has more ionic character, and therefore its volatility is lower. Residue data and before and after cooking data were not available for most of the DBPs that are usually produced at much lower concentrations than THMs, but Henry's Law constants can give an approximate indication of their loss propensity relative to THMs.

2.16.2 Opportunities for further studies

Most of the data available on DBPs in the environment have been obtained from studies of drinking-water disinfection. Existing qualitative and quantitative residue studies of DBPs in food products have tended to focus on THM (including chloroform) measurements in processed and cooked foods, particularly poultry. This is probably due to the fact that THMs were the first DBPs regulated in drinking-water and because of the relative ease of analysis. It might not have been understood that the THMs were regulated primarily as indicators or surrogates for the unquantified mix of other DBPs that are generated during water treatment processes from the precursors present in natural source waters.

Very limited information is available on actual DBP residues in food products. Extrapolations from DBPs in drinking-water to DBPs in food are difficult to make because the conditions of the chemical interactions, dosages, temperatures, contact times and especially the precursors are very different. In addition, the consequences of cooking may reduce the presence of volatile compounds (e.g. chloroform), but also form additional compounds (e.g. in the case of nitrosamines). As a particular point, under some oxidation conditions, bromide can be converted to hypobromous acid, which would shift the composition of by-products to organobromine compounds.

Additional, more detailed studies of the formation and composition of DBPs in foods are needed to improve the ability to determine whether any significant risks may be associated with the use of disinfectant treatments in food production and food processing, and in particular how cooking and other types of food preparation may alter the composition of DBPs in foods.

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Appendix A: Data on nitrosamines in foods

Food type	Concentrations of nitrosamines (one or more, combined; µg/100 g)
Potato	0.015–1.44
Cabbage	0.014–0.19
Corn	0.002–0.83
Tomato	0.187–0.27
Fermented vegetables	nd–0.50
Cheese	0.02–9.75
Milk	0.03–3.70
Milk (sour)	0.08–11.9
Flour	0.02–1.44
Bacon	nd–6.50
Beef	Up to 788
Frankfurters	Up to 27
Ham	0.1–79
Salami	Up to 131
Sausage	nd–0.42
Fish	nd–140
Fish (processed)	nd–3.9
Seafood/shrimp	nd–13.1
Oil	nd–0.38
Beer	Up to 6.8
Tea	0.2–1.5
Coffee	Up to 0.5

nd, not detected

Appendix B: Drinking-water guidelines and regulations

The THMs were originally regulated in 1978 in the USA at 0.100 mg/l as indicator chemicals for the unidentified DBPs that are produced during the chlorination process. HAAs were regulated later as individual contaminants as well as general indicators. In the WHO *Guidelines for drinking-water quality* (GDWQ) (WHO, 2008b) and the USEPA (2009) regulations, guideline values (GVs) and maximum contaminant levels (MCLs), respectively, have been set for many of the THMs and several other DBPs.

Table 2B.1 provides WHO guidelines and USEPA and European Union (EU) regulations for selected disinfectants and DBPs.

Table 2B.1. WHO guidelines and USEPA and EU regulations^a

Disinfectant/DBP	GDWQ GV (mg/l)	USEPA MCL (mg/l)	EU standard (mg/l)
Chloroform	0.3	0.08	–
Bromoform	0.1	0.08	–
BDCM	0.06	0.08	–
DBCM	0.1	0.08	–
Total THMs	–	0.08	0.1 ^b
Trichloroacetaldehyde (chloral hydrate)	– ^c	–	–
Cyanogen chloride	– ^d	–	–
Chloroacetic acid	0.02	0.06	–
Bromoacetic acid	–	0.06	–
Dibromoacetic acid	–	0.06	–
Dichloroacetic acid	0.05 (P)	0.06	–
Trichloroacetic acid	0.2	0.06	–
Total of 5 HAAs	–	0.06	–
Dibromoacetonitrile	0.07	–	–
Dichloroacetonitrile	0.02 (P)	–	–
Bromate	0.01 (P)	0.010	0.01
Chlorate	0.7 (P)	–	–
Chlorite	0.7 (P)	1	–
Chlorine	5 ^e	4 ^b	–
Monochloramine	3 ^e	4 ^b (as chlorine)	–
NDMA	0.0001	–	–

P, provisional guideline value

^a After EU (1998); WHO (2008b); USEPA (2009).

^b Maximum allowed value. The other values are normally average values of multiple samples over a specified time period.

^c A health-based value of 0.1 mg/l can be calculated for chloral hydrate. However, because chloral hydrate usually occurs in drinking-water at concentrations well below those at which toxic effects are observed, it is not considered necessary to derive a formal guideline value.

^d Although a GV of 0.07 mg/l was included in the third edition of the GDWQ, it has been proposed that the GV be withdrawn in the fourth edition, because cyanogen chloride is unlikely to be present at concentrations of toxicological concern. As it is not considered necessary to derive a formal guideline value, a health-based value of 0.3 mg/l as cyanide is proposed (M. Sheffer, personal communication, 2009).

^e Partly for organoleptic aspects.

3. CHEMICAL RISK ASSESSMENT

3.1 Toxicology and exposure assessment

3.1.1 Introduction

3.1.1.1 Chemical risk assessment

The chemical risk assessments are mostly based on existing authoritative assessments that were available at the international or national level, rather than re-evaluating original publications and undertaking risk characterization de novo. However, original studies used for risk characterization are cited.

In reality, the direct exposure by ingestion to many of the chemically reactive disinfectants and some of their inorganic halogenated by-products will most likely be less than calculated here, as they will be partially or completely degraded in saliva or stomach juice after ingestion. However, at this time, these effects were not included because of the lack of quantitative data.

3.1.1.2 Dietary exposure assessment for foods (other than drinking-water)

Dietary exposure assessments were drafted for all chlorine-based disinfectants, alternative disinfectants and disinfection by-products (DBPs) that were relevant to the processes described in chapter 1 and the chemistry in chapter 2. These assessments drew primarily on existing authoritative assessments that were available at the national or international level, rather than re-evaluating occurrence data and undertaking an exposure assessment de novo.

The occurrence (i.e. concentration in food) data available for this assessment and supporting other authoritative assessments were relatively limited. There is therefore a relatively high level of uncertainty associated with the dietary exposure assessments. In some cases, very conservative assumptions were applied to compensate for this uncertainty. The degree of uncertainty and conservatism is articulated for each of the chemicals for which an exposure assessment is undertaken. The level of uncertainty and conservatism needs to be taken into consideration in the risk–benefit assessments (see chapter 6). For some of the by-products, no occurrence data were available for food, other than drinking-water.

3.1.1.3 Dietary exposure assessment for drinking-water

An exposure assessment for drinking-water was conducted for each of the DBPs for which occurrence data were available. The World Health Organization (WHO) uses a default consumption value of 2 litres for drinking-water and a typical body weight of 60 kg to estimate the WHO drinking-water guideline values (WHO, 2008d). This usually represents a conservative value for water consumption. However, the default assumption of 2 litres/day is not always appropriate or conservative for some populations and climates. Reference hydration value intakes could differ, for example, under average conditions: 2.2 litres for adult women, 2.9 litres for adult men and 1 litre for children. For physically active persons and increased temperatures, the reference values could be 4.5 litres for men, women and children; 4.8 litres for pregnant women; and 3.8 litres for lactating women (WHO, 2003a). In Australia, the mean consumption of water in food (all respondents), based on a 1995 national

nutrition survey, was reported as 969 g/day, equivalent to 0.969 litre/day. The average body weight associated with the survey was 68 kg, with respondents being 2 years of age and older (FSANZ, 2008).

In the United States of America (USA), analysis of data from the 1994–1996 and 1998 Continuing Survey of Food Intakes by Individuals (CSFII), which includes children, indicated that average estimated daily per capita ingestion of community water and all water sources was 0.926 litre/day and 1.233 litres/day, respectively. This represented 75% from community water, 13% from bottled water, 10% from other sources (well, spring, cistern, etc.) and 2% from non-identifiable sources. The consumption values did not include water found naturally in foods (biological water) and water added by commercial food and beverage manufacturers (commercial water). The average self-reported body weight associated with the same survey was 65 kg (USEPA, 2004). The community water consumption value is considered the most representative of water to which chlorine-containing disinfectants may have been applied.

For Europe, data for “tap water” from the Concise European Food Consumption Database were available for Belgium, the Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, the Netherlands, Norway, Slovakia, Sweden and the United Kingdom. These data were for adults and generally related to the age group 16–64 years (EFSA, 2008).

A summary of the food consumption and body weight values used in the dietary exposure assessments for drinking-water is presented in Table 3.1.

3.1.1.4 Other information

Concentrations of chemicals are given in SI units (Système international d’unités) (e.g. mg/kg, mg/l), in keeping with Food and Agriculture Organization of the United Nations (FAO)/WHO policy.

The expressions “acceptable daily intake” (ADI) and “tolerable daily intake” (TDI) are used as stated in the original publications and may therefore not be used consistently throughout the document (e.g. TDI is usually used for substances that are contaminants). This may be the case also for the expressions no-observed-(adverse-)effect level (NO(A)EL) and lowest-observed-(adverse-)effect level (LO(A)EL).

3.1.2 Chlorine-containing disinfectants

3.1.2.1 Acidified sodium chlorite

Introduction

Acidified sodium chlorite (ASC), which is produced by combining sodium chlorite with a food-grade acid, is used as a broad-spectrum disinfectant. The active ingredient is chlorous acid, and its reaction products are chlorine dioxide, chlorite and chlorate.

ASC was evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2007 (WHO, 2008a). JECFA noted that residual chlorine dioxide is lost by evaporation; hence, chlorite, chlorate and chloride are the principal residues expected. The chloride generated as a result of treatment with ASC is negligible compared with the chloride already present in food. As chlorine dioxide acts as an oxidizing agent, it does not form trihalomethanes (THMs) or by-products other than chlorite and chlorate ions. The residues of the food-grade acids (e.g. phosphate, citrate, malate, sulfate) are commonly present in food and have previously established ADIs. Therefore, JECFA focused its toxicological evaluation

on ASC, chlorite and chlorate. The review of the chemistry of ASC in section 2.2 confirms that this approach is justified.

Table 3.1. Summary of the drinking-water consumption and body weight data used for the drinking-water exposure assessments

Country	Approximate number of respondents	Mean body weight of all respondents (kg)	Mean consumption of drinking-water for all respondents ^a (litre/day)
Australia	13 800	68	0.969
Belgium	1 720	71	0.100
Czech Republic	1 750	75	0.288
Denmark	3 150	74	0.840
Finland	2 010	77	0.886
France	2 000	66	0.283
Germany	3 550	77	0.071
Hungary	930	73	0.001
Iceland	1 080	76	0.670
Ireland	1 370	75	0.284
Italy	1 540	66	0.206
Netherlands	4 290	75	0.209
Norway	2 310	73	0.312
Slovakia	2 210	75	0.224
Sweden	1 090	73	0.480
United Kingdom	1 720	76	0.205
USA	25 000	65	0.926
WHO	–	60	2 ^b

^a For the European countries, data for “tap water” were used (EFSA, 2008). The consumption of tap water in Hungary was reported as being only 1 ml/day (mean consumption for all respondents).

^b The WHO consumption value is for the model drinking-water diet used in the WHO drinking-water guidelines (WHO, 2008d).

In order to assess the safety of ASC, JECFA set ADIs for sodium chlorite (0.03 mg/kg body weight [bw] per day, expressed as chlorite [ClO_2^-]) and sodium chlorate (0.01 mg/kg bw per day, expressed as chlorate [ClO_3^-]) (WHO, 2008a).

The European Food Safety Authority (EFSA) has also reviewed ASC for treatment of poultry carcasses, confirming the JECFA evaluation, as no further data had been made available (EFSA, 2005). EFSA (2005) concluded that the exposure to chlorite residues arising from treated poultry carcasses would be of no safety concern.

Toxicological data

JECFA concluded that the available toxicological data were sufficient to assess the safety of ASC by setting ADIs for chlorite and chlorate (WHO, 2008a). The available studies on ASC related to a germicidal product, and some of these involved parenteral administration. These studies were not directly relevant to oral exposure but provided useful supplementary information that did not raise concern about the use of acidified chlorite as a processing aid.

The toxicological information relating to chlorate and chlorite is considered in sections 3.1.4.3 and 3.1.4.4, respectively.

Dietary exposure

There is no direct dietary exposure to ASC. The dietary exposure to residues resulting from use of ASC is considered in sections 3.1.4.3 and 3.1.4.4.

Risk characterization

As there is no direct dietary exposure to ASC, it is not a risk to consumers. The toxicologically relevant residues (i.e. chlorate and chlorite) are considered in sections 3.1.4.3 and 3.1.4.4.

3.1.2.2 Chloramine (monochloramine)

Introduction

The toxicology of monochloramine was evaluated and described in Environmental Health Criteria 216 (IPCS, 2000). The WHO *Guidelines for drinking-water quality* (WHO, 2006a) as well as original publications have also been used as sources of information on monochloramine. A TDI of 94 µg/kg bw per day for monochloramine was derived in the WHO *Guidelines for drinking-water quality* (WHO, 1993). This was confirmed in subsequent evaluations (IPCS, 2000; WHO, 2004a, 2006a).

Toxicological data

The NOAEL after chronic oral exposure, used for establishing the TDI, was identified from a study by the United States National Toxicology Program (NTP, 1992). Monochloramine was administered for 2 years to male and female F344/N rats and B6C3F1 mice at 0, 50, 100 or 200 mg/l in the drinking-water. These solutions were prepared from gaseous chlorine and neutralized to pH 9 by the addition of sodium hydroxide. At this pH, almost all chlorine will be available as hypochlorite. Monochloramine was generated by adding the buffered sodium hypochlorite solution to a dilute ammonium hydroxide solution. Stability studies indicated that 92% of the initial target concentration remained after 2 days of preparation. The buffered hypochlorite stock solutions were prepared once weekly, and solutions for drinking were prepared 4 times weekly.

The monochloramine concentrations corresponded to average doses of 0, 2.9, 5.2 and 9.4 mg/kg bw per day in male F344/N rats and 0, 3.1, 5.7 and 10.2 mg/kg bw per day in female rats. There were no clinical findings or alterations in haematological parameters considered to be attributable to the consumption of chloraminated water. There were no biologically significant differences in survival or in absolute or relative organ weights between dosed and control groups. Mean body weights of rats given the highest dose were 5–10% lower than those of their respective control groups throughout the study. Based on these considerations, the authors considered the NOAELs for this study to be 5.2 and 5.7 mg/kg bw per day for male and female rats, respectively. Feed consumption by dosed animals was similar to controls. However, it is probable that the observed weight decreases were a direct result of the unpalatability of the drinking-water, as a dose-related decrease in water consumption was seen in both sexes from the first week and throughout the study. The water consumption during the second year of the study by high-dose rats was 34% lower than controls for males and 31% lower for females. No treatment-related non-neoplastic lesions were observed in either male or female rats (NTP, 1992). There was no evidence of carcinogenic activity in the male rats. In the female rats, there was equivocal evidence of carcinogenic activity based on a significant increase in the incidence of mononuclear cell leukaemia above the concurrent and historical controls. The incidences were 8/50 for controls, 11/50 for the low dose, 15/50 for the intermediate dose and 16/40 for the high dose. The following factors did not support an association between the occurrence of mononuclear

cell leukaemia and the consumption of chloraminated drinking-water: the increases in leukaemia incidence in dosed female rats were small and not clearly dose related, there was no decrease in tumour latency in the dosed groups, the effect was not observed in male rats or in female and male mice (see below), and the incidence in concurrent controls was less than the mean incidence in historical controls.

B6C3F1 mice were exposed for 2 years to average doses of monochloramine in their drinking-water of 0, 5.4, 9.8 and 17.0 mg/kg bw per day for males and 0, 5.8, 10.6 and 19.0 mg/kg bw per day for females. The authors reported that there were no clinical findings or alterations in haematological parameters attributable to the consumption of chloraminated water. There were no biologically significant differences in survival or in absolute or relative organ weights between dosed and control groups. As was observed in rats, there were dose-related decreases in water consumption—in high-dose mice, 42% lower than controls in males and 40% lower in females. Feed consumption by dosed male mice was similar to that of controls throughout the study. In females, mean feed consumption was similar in all treatment groups except the high-dose group, in which it was slightly lower than in the other groups. There was a dose-related decrease in mean body weights of both sexes of dosed mice compared with controls throughout most of the study. No treatment-related non-neoplastic lesions were observed in either male or female mice. There was no evidence of carcinogenic activity in male or female B6C3F1 mice (NTP, 1992; WHO, 2004a).

Although monochloramine has been shown to be mutagenic in some *in vitro* studies, it did not induce micronucleus formation, chromosomal aberrations or aneuploidy in the bone marrow of CD-1 mice or sperm abnormalities in B6C3F1 mice (IARC, 2004). Monochloramine induced the formation of micronuclei in erythrocytes of newt larvae *in vivo* (IARC, 2004). The International Agency for Research on Cancer (IARC) evaluated monochloramine as not classifiable as to its carcinogenicity to humans (Group 3) (IARC, 2004), because there was inadequate evidence in humans and experimental animals.

WHO (1993) derived a TDI of 94 µg/kg bw per day by applying an uncertainty factor of 100 (for intraspecies and interspecies variation) to the dose of approximately 9.4 mg/kg bw per day, which was the highest dose administered in the 2-year NTP rat drinking-water study. This was considered to be a NOAEL rather than a LOAEL because of the probability that the small reduction in body weight at this dose was caused by the unpalatability of the drinking-water (NTP, 1992).

Dietary exposure

In the USA, monochloramine has been proposed for use for poultry chiller water disinfection at levels up to 50 mg/l (USFDA, 2008), whereas the maximum residual level for drinking-water in the USA is 4 mg/l as chlorine (USEPA, 2009). Dietary exposure to monochloramine (as chlorine) from the consumption of drinking-water could be 8 mg/day (0.13 mg/kg bw per day for a 60-kg person), assuming that one consumes 2 litres of water per day. Dietary exposure from the consumption of meat would be lower, as per the analyses below.

Zentox (2007) developed a conservative hypothetical estimation of dietary exposure to monochloramine following the chiller treatment of poultry in water. Assumptions included a 12% uptake (by weight) of chiller water containing monochloramine at 50 mg/l; therefore, a carcass that weighed 1 kg would contain 6 mg of monochloramine.

Table 3.2 shows the consumption of meat in three European countries, estimated from the Concise European Food Consumption Database by EFSA (2005). Combining these meat consumption figures with the potential residual levels in meat gives a dietary exposure of up to 2 mg/person per day, or 0.04 mg/kg bw per day for a 60-kg person consuming meat at the 99th percentile.

Table 3.2. Consumption of meat and meat products (including offal) in the adult population of France, Italy and Sweden

Country	Number of subjects	Number of consumers	Average daily consumption in consumers only (g/day)						
			Mean	SD	50th	90th	95th	97.5th	99th
France	1875	1861	120	66	110	206	243	274	321
Italy	1425	1419	137	67	127	224	264	292	351
Sweden	1214	1204	151	68	141	233	263	297	346

SD, standard deviation

At the international level, the use of the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) consumption cluster diets (WHO, 2007b) allows the preparation of another conservative estimate of dietary exposure to monochloramine. Cluster K shows the highest consumption of poultry products at 145.9 g/day, with cluster M having the highest total meat consumption at 279.3 g/day. Using these food consumption figures yields dietary exposure estimates up to 0.9 mg/person per day (poultry), again assuming that no monochloramine is lost upon treatment and that there is a 12% uptake of water into the meat product. The dietary exposure assessments for each of the GEMS/Food consumption cluster diets are presented, on a kilogram body weight basis, in Table 3.3.

Table 3.3. Estimates of per capita dietary exposure to monochloramine, using a hypothetical residue concentration, following the dipping of chicken in chlorine, based on the 13 GEMS/Food consumption cluster diets

	Per capita dietary exposure (µg/kg bw per day) ^{a,b,c}												
	A	B	C	D	E	F	G	H	I	J	K	L	M
Chicken meat	0.55	4.31	2.86	2.21	3.86	2.63	1.2	4.34	1.5	0.44	6.4	2.68	9.67
Poultry ^d	0.71	5.85	3.19	2.4	6.1	2.73	1.76	13.13	2.51	0.47	14.59	2.77	11.51

^a Assuming a 60-kg average body weight.

^b WHO consumption cluster diets based on food balance sheet data; August 2006 version used (<http://www.who.int/entity/foodsafety/chem/ClusterDietsAug06.xls>).

^c Hypothetical concentration of 6 mg/kg in chicken and other poultry was used for the exposure assessment.

^d The poultry exposure assessment has been presented on the assumption that the dipping use of chlorine is also applied to other poultry.

The estimates of dietary exposure presented herein are highly conservative. Although monochloramine is known to be less reactive than chlorine and other alternative chlorine antimicrobials, it does decompose when in contact with organic materials. Studies with poultry have shown, however, that levels of haloforms and total chlorine-containing material are higher in cooked poultry that had been immersed in control (non-sanitized) water compared with monochloramine-treated water. Additionally, there is no measurable difference in fatty acid profiles of poultry treated with monochloramine compared with control water-treated poultry after cooking. Treatment of poultry with monochloramine followed by roasting resulted in no greater formation of *N*-nitrosopyrrolidine than in the controls.

The dietary exposure to monochloramine can be expected to be negligible in comparison with that from treated water. For consumers not exposed to monochloramine-treated waters, a conservative estimate of dietary exposure would be 2 mg/person per day (0.04 mg/kg bw per day).

Risk characterization

The estimated dietary exposure of 40 µg/kg bw per day is well below the TDI of 94 µg/kg bw per day. Exposure to monochloramine in drinking-water has the potential to exceed the TDI and to be up to about 4 times the exposure from monochloramine-treated food. Therefore, no health concern was identified with use of monochloramine in poultry chiller water.

3.1.2.3 Chloramine-T

Introduction

Chloramine-T has been evaluated by the European Medicines Agency (EMA) as a disinfectant used as treatment for bacterial gill disease in cultured fish (EMA, 1999), for teat and udder disinfection in lactating cows (EMA, 2001) and for treatment of skin disease in horses (EMA, 2005). In addition, information was found in a document submitted by industry (Axcentive SARL, 2008) and in a literature review prepared for the United States National Institute of Environmental Health Sciences (Haneke, 2002b).

p-Toluenesulfonamide (PTSA) is the primary reaction product and marker metabolite of chloramine-T (EMA, 2005). *o*-Toluenesulfonamide is not formed in aqueous solutions of chloramine-T and is therefore not relevant for the safety evaluation of chloramine-T (EMA, 1999). Chloramine-T is also converted very quickly in the stomach/gastrointestinal system into PTSA (Axcentive SARL, 2008).

No ADI or TDI has been identified for chloramine-T or for PTSA.

Toxicological data

EMA evaluated the available toxicity studies on chloramine-T and PTSA in 1999. No chronic studies were found in rats or mice, and a TDI could not be established. However, the highest dose of chloramine-T tested without any effect (NOAEL) was approximately 15 mg/kg bw per day, from 300 mg/kg in the feed, in a 90-day study in rats (EMA, 1999, 2001). In this study, Wistar rats (10 per sex per group) were exposed to diets containing chloramine-T at 0, 100, 300, 1000 or 3000 mg/kg feed, equivalent to approximately 0, 5, 15, 50 or 150 mg/kg bw per day. A slight reduction of weight gain and food efficiency was observed in females at 3000 mg/kg feed. Relative kidney weight was significantly increased in both sexes at doses equal to or higher than 1000 mg/kg feed. In females at 1000 and 3000 mg/kg feed, increased severity and frequency of calcareous deposits in kidneys were observed. The NOAEL was 300 mg/kg feed, equivalent to approximately 15 mg/kg bw per day. The rest of the toxicity studies evaluated by EMA in 1999 were for an exposure duration shorter than 90 days, were performed in dogs, gave no effects or were performed or reported in such a way that a NOAEL could not be identified.

Axcentive SARL (2008) reported a subchronic 90-day dietary study in rats (conducted according to Organisation for Economic Co-operation and Development [OECD] Test Guideline 408) with PTSA given at 1000, 3000 and 10 000 mg/kg in feed. A decreased body weight gain in animals at the highest dose, up to 21% in males and up to 11% in females, was observed. Also, a minimal degree of hyperplasia of the urothelium of the urinary bladder was observed in two males. These effects were observed only at the highest level of 10 000 mg/kg feed, which was equal to PTSA doses of 738 mg/kg bw per day in males and 795 mg/kg bw per day in females; converted to chloramine-T, the doses were 1210 mg/kg bw per day for males and 1303 mg/kg bw per day for females. At the other dose levels, no effects were observed. Based on these results, the NOEL was 3000 mg/kg feed—that is, 214 mg/kg bw per day as PTSA, corresponding to 351 mg/kg bw per day as chloramine-T. The LOAEL was the highest dose tested, equivalent to 1210 mg/kg bw per day

as chloramine-T, according to Axcentive SARL (2008). No raw data or details other than those reported above were provided; therefore, the validity of the NOEL and LOAEL values cannot be evaluated.

Decreased body weight in rats exposed to 351 mg/kg bw per day (3000 mg/kg feed) in the 90-day subchronic dietary study was regarded as the critical effect of chloramine-T (Axcentive SARL, 2008). As the pharmacokinetic studies indicated no potential for bioaccumulation, Axcentive SARL (2008) proposed that the default safety factor of 100 could be used to derive a TDI of 3.51 mg/kg bw per day for chloramine-T. However, this proposal has not been supported by an independent expert body.

In a two-generation study in rats (conducted according to OECD Test Guideline 416), with PTSA given at concentrations of 1000, 3000 and 10 000 mg/kg in feed, dose-related decreased body weight gain and changes in absolute and relative organ weights were observed at 3000 and 10 000 mg/kg feed in the parent and F₁ groups (Axcentive SARL, 2008). The NOAEL for parent and F₁ animals in this experiment was 1000 mg/kg feed—that is, 52–78 mg/kg bw per day as PTSA for males and 75–161 mg/kg bw per day as PTSA for females, corresponding to 85–128 mg/kg bw per day as chloramine-T for males and 123–264 mg/kg bw per day as chloramine-T for females.

The genotoxicity of chloramine-T has been assayed in the following tests: a non-Good Laboratory Practice (GLP) *Salmonella* microsomal test (*S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538, with and without metabolic activation); a non-GLP deoxyribonucleic acid (DNA) repair test on *Escherichia coli*, with and without activation; a GLP-compliant gene mutation assay in mouse lymphoma L5178Y cells, with and without activation; and a GLP-compliant micronucleus assay in mice treated by gavage with 300, 600 and 1200 mg/kg bw per day for 2 days. All these tests gave negative results (EMEA, 1999).

In a non-GLP *Salmonella* microsomal test, PTSA was evaluated in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538, with and without metabolic activation, with negative results. Based on these available data, neither chloramine-T nor PTSA is genotoxic (EMEA, 1999). One bacterial reverse mutation test and one gene mutation test in mouse lymphoma cells in vitro and one in vivo micronucleus test were also reported by industry as negative (no data provided) (Axcentive SARL, 2008). No carcinogenicity studies have been found for either chloramine-T or PTSA.

None of the available studies involved chronic exposure, nor were the available results reported in sufficient detail to be properly evaluated. The expert meeting did not establish a TDI for chloramine-T.

Dietary exposure

The United States Food and Drug Administration (USFDA) models the situation in which sanitizing solutions are not washed off prior to the use of the preparation surface, and all food consumed in a day would be in contact with this surface (USFDA, 1993). This approach leads to a very conservative estimate that the expert meeting considered not relevant in this context.

The expert meeting calculated a more refined dietary exposure estimate in which it was assumed that *one* meal per day was prepared on the treated, unwashed surface. Parameters are used from the USFDA model (USFDA, 1993), assuming that treatment results in chloramine-T residues of 4.6 µg/cm² surface. It is then assumed that the food would contact 4000 cm² of this treated surface, resulting in a dietary exposure to chloramine-T of 6 mg/day, equivalent to 0.1 mg/kg bw per day for a 60-kg individual. This model still represents a conservative estimate of dietary exposure, given the assumption relating to the amount of chloramine-T residues, lack of rinsing after disinfection and daily consumption of such prepared food.

There are no data on chloramine-T-treated water, either consumed as such or used on food, to allow an estimate of exposure from this route. However, chloramine-T decomposes rapidly in cooking, and human exposure would probably be intermittent and at much lower levels than estimated by this model.

Risk characterization

No TDI could be established for chloramine-T due to the lack of long-term toxicity data and limited detail on the available studies. The dietary intake of 0.1 mg/kg bw per day has been estimated, which is a margin of 150 times lower than the NOAEL of 15 mg/kg bw per day for effects on the kidney in a 90-day rat study. Taking into account that actual exposure is likely to be much lower and intermittent, the margin is expected to be much larger, and no health concern was identified.

3.1.2.4 Chlorine dioxide

Introduction

Chlorine dioxide is an unstable gas that has to be generated at the point of use as an antimicrobial agent. It is produced by oxidation or acidification of sodium chlorite, by combination of sodium hypochlorite and hydrochloric acid or by reaction of sodium chlorate with hydrogen peroxide and sulfuric acid.

In its review of ASC, JECFA noted that residual chlorine dioxide is lost by evaporation; hence, chlorite, chlorate and chloride are the principal residues expected. As chlorine dioxide acts as an oxidizing agent, it does not form THMs or by-products other than chlorite and chlorate ions. The review of the chemistry in chapter 2 confirms that chlorite and chlorate are the main residues in food expected to result from use of chlorine dioxide as a disinfectant.

Chlorine dioxide was most recently evaluated by EFSA (2005), which referred to the TDI of 0.03 mg/kg bw per day for chlorite set by WHO (IPCS, 2000) and confirmed by JECFA (WHO, 2008a).

Toxicological data

The toxicological information relating to chlorate and chlorite is considered in sections 3.1.4.3 and 3.1.4.4, respectively.

Dietary exposure

There is no direct dietary exposure to chlorine dioxide. The dietary exposure to residues resulting from use of chlorine dioxide is considered in sections 3.1.4.3 and 3.1.4.4.

Risk characterization

As there is no direct dietary exposure to chlorine dioxide, it is not a risk to consumers. The toxicologically relevant residues are considered in sections 3.1.4.3 and 3.1.4.4.

3.1.2.5 Hypochlorite-related compounds (chlorine gas, sodium hypochlorite, calcium hypochlorite, hypochlorous acid, hypochlorite ion)

Introduction

Chlorine, whether in the form of chlorine gas from a cylinder or as the solids sodium hypochlorite or calcium hypochlorite, dissolves in water to form hypochlorous acid and hypochlorite ion (WHO, 2006a). Therefore, based on considerations of the chemistry of either chlorine gas or hypochlorites used in aqueous solutions as disinfectants in the food

industry, the main components expected to be of toxicological relevance are hypochlorite ion and, possibly, hypochlorous acid. Chlorine gas, hypochlorous acid and hypochlorite ion are in equilibrium with each other, their concentrations depending on the pH of the solution. At pH 7, the chlorine solution is approximately 50% hypochlorite and 50% hypochlorous acid. Its biocidal effectiveness is greatest when it is in the acid form as hypochlorous acid and is a function of the concentration of the residual active chlorine, temperature and pH of the solution, and contact time.

The mechanisms of the toxicity of aqueous chlorine (i.e. chlorine gas, hypochlorous acid and hypochlorite) are basically similar (ATSDR, 2007).

The toxicology of hypochlorite-related substances has been described in Environmental Health Criteria 216 (IPCS, 2000). The Agency for Toxic Substances and Disease Registry's draft *Toxicological profile for chlorine* (ATSDR, 2007) and the WHO *Guidelines for drinking-water quality* (WHO, 2006a), as well as some original publications, have also been used as sources of information on hypochlorite-related compounds.

A TDI of 150 µg/kg bw per day for free chlorine was established in the WHO *Guidelines for drinking-water quality* (WHO, 1993). IPCS (2000) indicated that there were no new data to suggest that this TDI should be changed.

Toxicological data

The NOAEL used for establishing the TDI was obtained from a 2-year NTP bioassay (NTP, 1992). Chlorine was administered to F344/N rats and B6C3F1 mice (70 per sex per group) at 0, 70, 140 or 275 mg/l (expressed as elemental chlorine, Cl) in drinking-water. Groups of 10 rats or mice of each sex were predesignated for evaluation at 14 or 15 weeks and 66 weeks. The solutions were prepared from gaseous chlorine and neutralized to pH 9 by the addition of sodium hydroxide. At this pH, almost all chlorine will be available as hypochlorite. Stability studies indicated that 85% of the initial target concentration remained after 3 days of preparation. Stock solutions were prepared once weekly, and solutions for drinking were prepared 4 times weekly. Based on body weight and water consumption, the doses were approximately 0, 4, 7 and 14 mg/kg bw per day for male rats; 0, 4, 8 and 14 mg/kg bw per day for female rats; 0, 7, 14 and 24 mg/kg bw per day for male mice; and 0, 8, 14 and 24 mg/kg bw per day for female mice. A dose-related decrease in water consumption was observed throughout the study in the treated groups from both sexes in both rats and mice. Water consumption by high-dose rats during the second year of the study was 21% lower than controls for males and 23% lower than controls for females. Water consumption by high-dose mice was 31% lower than controls for males and 26% lower than controls for females. Mean body weights and food consumption were comparable between treated and control groups. There were no clinical findings attributable to treatment, no alterations in haematological parameters and no biologically significant differences in survival rates or absolute or relative organ weights between treated and control groups. No treatment-related non-neoplastic lesions were observed in either rats or mice. There was no evidence of carcinogenic activity in male F344/N rats receiving 70, 140 or 275 mg/l as atomic chlorine. There was equivocal evidence of carcinogenic activity of chlorinated water in female F344/N rats, based on a significant increase in the incidence of mononuclear cell leukaemia in mid-dose, but not high-dose, female rats receiving chlorinated water compared with controls ($P = 0.014$ by the life table test) (controls, 8/50; low dose, 7/50; intermediate dose, 19/51; high dose, 16/50). The factors not supporting this association include the following: the increase in leukaemia in dosed female rats was slight and not clearly dose related, there was no decrease in tumour latency, the incidence in concurrent controls was less than in historical controls and there was no supporting evidence of this effect in male

rats. There was no evidence of carcinogenic activity of chlorinated water in male or female B6C3F1 mice receiving 70, 140 or 275 mg/l as atomic chlorine.

The lowest NOAEL from this study was 14 mg/kg bw per day as chlorine for female rats, based on absence of findings in histopathology of tissues and organs and haematological parameters. The lowest NOAEL for B6C3F1 mice in the same study was 24 mg/kg bw per day as chlorine in females, based on absence of findings in histopathology of tissues and organs and haematological parameters.

Based on the lowest NOAEL value of 14 mg/kg bw per day as chlorine (rounded to 15 mg/kg bw per day as chlorine) and using an uncertainty factor of 100 (10 each for intraspecies and interspecies variation), WHO (1993) established a TDI of 150 µg/kg bw per day for free chlorine (WHO, 1993), which was confirmed by IPCS (2000).

Although sodium hypochlorite has been shown to be mutagenic in some in vitro studies, it did not induce micronucleus formation or chromosomal aberrations in the bone marrow of mice in vivo (ATSDR, 2007). Sodium hypochlorite induced the formation of micronuclei in erythrocytes of newt larvae in vivo (ATSDR, 2007). Hypochlorite salts were assigned to Group 3: the compounds are not classifiable as to their carcinogenicity to humans by IARC (1991), based on inadequate evidence for the carcinogenicity in experimental animals and no available data from studies in humans.

Dietary exposure

Chlorine gas is approved for use in red meat and poultry processing in the USA (USDA, 2007). No dietary exposure to chlorine gas following such use is expected. Chlorine in the form of hypochlorous acid and hypochlorite ion is highly reactive and is expected to result in the formation of DBPs when it comes into contact with food. Nitrosamines, chloroform and chloramines can be produced from the chemical reactions between ammonium or amines present in food and free active chlorine. The dietary exposure to hypochlorous acid and hypochlorite ion per se will therefore be minimal. The dietary exposures to the DBPs that are formed are considered elsewhere within this chapter.

Risk characterization

Chlorine in the form of hypochlorous acid and hypochlorite ion is expected to react on contact with food to form DBPs. There is no direct dietary exposure to chlorine, and therefore it is not a risk to consumers. The toxicologically relevant DBPs are considered under the respective headings in this chapter.

3.1.2.6 Dichloroisocyanurate

Introduction

Sodium dichloroisocyanurate (NaDCC) is used as a source of free available chlorine (in the form of hypochlorous acid) (WHO, 2004b, 2007a).

The description of the toxicology of dichloroisocyanurate is based mainly on WHO Food Additives Series, No. 52 (WHO, 2004b). However, a background document for the WHO *Guidelines for drinking-water quality* (WHO, 2007a) and some original publications have also been used as sources of information on NaDCC.

JECFA (WHO, 2004b) concluded that studies of the toxicity of sodium cyanurate were appropriate for assessing the safety of NaDCC, because any residues of intact NaDCC in drinking-water would be rapidly converted to cyanuric acid on contact with saliva. JECFA established a TDI for anhydrous NaDCC of 0–2.0 mg/kg bw per day for intake from drinking-water treated with NaDCC for the purpose of disinfection.

Toxicological data

In a 2-year study, groups of 80 male and 80 female Charles River CD-1 rats were given drinking-water containing sodium cyanurate at a concentration of 0, 400, 1200, 2400 or 5375 mg/l, corresponding to estimated doses of 0, 26, 77, 154 or 371 mg/kg bw per day, with control groups receiving drinking-water containing an equivalent amount of sodium hippurate or untreated drinking-water (IRDC, 1985). Survival was slightly lower in the group receiving the highest dose compared with the control group receiving untreated drinking-water, but not compared with the control group receiving sodium hippurate. There was no substance-related increase in tumour incidence. Multiple lesions of the urinary tract (calculi and hyperplasia, bleeding and inflammation of the bladder epithelium, dilated and inflamed ureters and renal tubular nephrosis) and cardiac lesions (acute myocarditis, necrosis and vascular mineralization) were reported in males that died during the first year of the study and that were receiving a dose of 371 mg/kg bw per day. No toxicologically significant treatment-related effects were observed at 154 mg/kg bw per day, which was considered to be the NOAEL in this study. In a similar 2-year study in which B6C3F1 mice received a dose of sodium cyanurate equivalent to 0, 30, 110, 340 or 1523 mg/kg bw per day in drinking-water (from concentrations of 0, 100, 400, 1200 and 5375 mg/l), survival was similar in all groups, and there were no treatment-related changes in the incidence of tumours or other histopathological lesions (Serota et al., 1986).

Sodium cyanurate was not mutagenic in *in vitro* *Salmonella typhimurium* mutagenicity tests, with or without activation, in mouse lymphoma cells or in a test of sister chromatid exchanges in Chinese hamster ovary (CHO) cells. No effects were observed for cytogenetic alterations in bone marrow of rats *in vivo* at a dose of 5000 mg/kg bw (WHO, 2004b).

The NOAEL for sodium cyanurate derived from the 2-year study in rats was 154 mg/kg bw per day, equivalent to 220 mg/kg bw per day as anhydrous NaDCC. With the application of an uncertainty factor of 100, a TDI for anhydrous NaDCC of 0–2.0 mg/kg bw per day was established for intake from drinking-water treated with NaDCC for the purpose of disinfection (WHO, 2004b).

Dietary exposure

NaDCC decomposes in water to release free chlorine, which is then available for the disinfection of drinking-water. Consequently, there is no direct human dietary exposure to NaDCC. Conventional chlorination of drinking-water with elemental chlorine gives rise to a number of by-products as a result of the reaction of free available chlorine with natural organic matter (NOM). The safety of these by-products has been addressed by WHO, with the development of guidelines for drinking-water quality. The use of NaDCC as a source of free available chlorine is not expected to lead to greater production of such by-products than does the use of elemental chlorine. The sixty-first meeting of JECFA (FAO/WHO, 2004) concluded that the continued reaction of NaDCC-released free chlorine with organics in water would eventually result in residues of cyanuric acid in water; hence, this was the only organic by-product for which human exposure was estimated.

Human exposure to cyanuric acid was evaluated by assuming that 1 mol of NaDCC results ultimately in 1 mol of cyanuric acid in treated water. The daily intake of cyanuric acid from the consumption of water by adults, assuming a maximum application of NaDCC of 3.2 mg/l (equivalent to 2 mg/l as free chlorine) and consumption of 2 litres of water per day, would be equivalent to 6.4 mg/person per day, expressed as NaDCC (equivalent to 0.03 mg/kg bw per day for a 60-kg person), or 4.2 mg/day as cyanuric acid.

Risk characterization

The estimated dietary exposure of 0.03 mg/kg bw per day for a 60-kg person is well below the upper end of the TDI range of 0–2.0 mg/kg bw per day, expressed as NaDCC. Therefore, no health concern was identified.

3.1.3 Alternative disinfectants

3.1.3.1 1,3-Dibromo-5,5-dimethylhydantoin

Introduction

No comprehensive toxicological evaluations of 1,3-dibromo-5,5-dimethylhydantoin (DBDMH) were found.

Toxicological data

No data were found for DBDMH on the end-points chronic toxicity, mutagenicity, carcinogenicity, or developmental or reproductive toxicology. No data were available with which to establish a TDI for DBDMH.

Dietary exposure

Currently in the USA, DBDMH is authorized for use as a disinfectant in water and ice used in the processing of poultry and as a disinfectant in water used to process beef hides, carcasses, heads, trim, parts and organs. Given that DBDMH rapidly decomposes in water to hypobromous acid and dimethylhydantoin (DMH), it is not expected to be present on food at the time of consumption. Therefore, there is no direct dietary exposure to DBDMH. Exposures to DMH (see section 3.1.4.5) and potential DBPs, such as bromate (see section 3.1.4.1), dibromochloromethane (DBCM) (see section 3.1.4.10), bromodichloromethane (BDCM) (see section 3.1.4.10) and bromoform (see section 3.1.4.10), are considered separately.

Risk characterization

As there is no direct dietary exposure to DBDMH, no health concern was identified.

3.1.3.2 Ethyl lauroyl arginate

Introduction

Ethyl lauroyl arginate is a cationic surfactant that has a wide spectrum of activity against bacteria, yeasts and moulds. *N*^α-Lauroyl-L-arginine is a principal by-product in the manufacture of the active ingredient ethyl-*N*^α-lauroyl-L-arginate hydrochloride and is also formed by enzymatic action in fresh food.

Ethyl lauroyl arginate was evaluated by JECFA in 2008 (WHO, 2009) and was previously evaluated by EFSA (2007). The toxicological data are not available in the public domain but are described in the JECFA monograph (WHO, 2009) and in the EFSA opinion (EFSA, 2007).

JECFA established an ADI of 0–4 mg/kg bw for ethyl lauroyl arginate, expressed as ethyl-*N*^α-lauroyl-L-arginate hydrochloride (WHO, 2009).

Toxicological data

Ethyl lauroyl arginate is well absorbed and rapidly metabolized by hydrolysis of the ethyl ester and lauroyl amide, via *N*^α-lauroyl-L-arginine and, to a lesser extent, L-arginine ethyl ester, to arginine, lauric acid and ethanol. Arginine subsequently undergoes normal

amino acid catabolism via the urea and citric acid cycles, with ultimate elimination as carbon dioxide in the expired air and urea in the urine. Lauric acid enters normal fatty acid metabolism, and ethanol is converted to acetate, which enters normal biochemical pathways. Both lauric acid and ethanol are also present naturally in foods. Given the rapid degradation of ethyl lauroyl arginate, exposure to this compound and *N*^α-lauroyl-L-arginine in vivo is likely to be short.

Ethyl lauroyl arginate is of low acute toxicity. In feeding studies in rats at high dietary concentrations, the major observations were forestomach changes. JECFA concluded that these changes represented local irritation in the forestomach caused by storage of ingested diet and thus were not indicative of systemic toxicity. A reduction in the concentration of leukocytes in the peripheral blood was also reported at some doses and time points. These differences were due to lower concentrations of neutrophils or lymphocytes with occasional effects on monocytes and large unstained cells, with no consistent pattern of changes in leukocytes. In addition, evidence of neurobehavioural effects (higher low- and high-beam motor activity) was seen in the male rats at 18 000 mg/kg feed. In the absence of other evidence for an effect on the nervous system, this higher level of exploratory behaviour was considered of doubtful association with treatment and not indicative of neurotoxicity. JECFA noted that the observed effects on leukocytes were inconsistent within and between studies and were not likely to be biologically significant. Furthermore, the changes were not accompanied by histopathological changes in the progenitor cell populations of the bone marrow or lymphoid tissue, which would be expected if the effect were due to systemic toxicity. Therefore, JECFA concluded that the highest dietary concentration tested, 18 000 mg/kg (equal to average doses of ethyl lauroyl arginate of approximately 900 mg/kg bw per day in male rats and 1100 mg/kg bw per day in female rats) was the NOAEL for systemic toxicity. Long-term studies of carcinogenicity were not available.

A range of studies in vitro (bacterial mutation, cytogenetics and gene mutation in mouse lymphoma cells) with ethyl lauroyl arginate and *N*^α-lauroyl-L-arginine did not provide evidence of genotoxicity. The absence of pre-neoplastic lesions in the 52-week study and the absence of genotoxic activity do not suggest that ethyl lauroyl arginate has carcinogenic potential.

In two studies of reproductive toxicity in rats, ethyl lauroyl arginate at a dietary concentration of 15 000 mg/kg delayed vaginal opening by 4 days in the female offspring. Although this effect was not accompanied by functional changes, JECFA considered this effect to be potentially adverse and concluded that the NOAEL for the dams was a dietary concentration of 6000 mg/kg, corresponding to 502 mg/kg bw per day expressed as ethyl lauroyl arginate or 442 mg/kg bw per day expressed as the active component, ethyl-*N*^α-lauroyl-L-arginate hydrochloride.

JECFA established an ADI of 0–4 mg/kg bw for ethyl lauroyl arginate, expressed as ethyl-*N*^α-lauroyl-L-arginate hydrochloride, based on the NOAEL of 442 mg/kg bw per day identified in studies of reproductive toxicity and a safety factor of 100.

Dietary exposure

The dietary exposure to ethyl lauroyl arginate was estimated by combining food consumption data for beef and poultry with the maximum use level of 200 mg/kg in the USA. This mean dietary exposure was 2.5 mg/kg bw per day, and the dietary exposure was 4.7 mg/kg bw per day for someone consuming these foods at the 90th percentile.

EFSA has also prepared an estimate of dietary exposure to ethyl lauroyl arginate as part of its overall safety evaluation of the preservative. The potential dietary exposure to ethyl lauroyl arginate was estimated based on United Kingdom food consumption data and on the assumption that it would be present in all food categories for which use levels are proposed.

The mean potential exposure to ethyl lauroyl arginate in consumers only ranged from 0.11 mg/kg bw per day in the elderly to 0.83 mg/kg bw per day in children aged 1.5–4.5, whereas high potential exposure (97.5th percentile in consumers only) ranged from 0.37 mg/kg bw per day in the elderly to 2.89 mg/kg bw per day in children aged 1.5–4.5. EFSA concluded that, based on the data available, the average dietary exposure to ethyl lauroyl arginate across Europe would be unlikely to exceed 1 mg/kg bw per day, and high-level exposure (at the 97.5th percentile) would be unlikely to exceed 3 mg/kg bw per day.

Risk characterization

JECFA concluded that some estimates of high-percentile dietary exposure to ethyl lauroyl arginate exceed the ADI of 0–4 mg/kg bw, but recognized that these estimates were highly conservative and that actual intakes were likely to be within the ADI. Therefore, no health concerns were identified.

3.1.3.3 Ozonated water

Introduction

Because of its reactivity, the toxicity of ozone is mostly related to its reaction products, especially after oral exposure. The presence of bromide ion in the aqueous solution treated with ozone may lead to the formation of, for example, hypobromite ion, bromate ion, bromoform and other brominated THMs, dibromoacetonitrile (DBAN) and dibromoacetone (IPCS, 2000). Aldehydes, ketones, ketoacids and carboxylic acids may also be formed by ozonation.

The use of ozone in disinfection of drinking-water is described in IPCS (2000) and WHO (2006a), but no toxicity or risk characterization of ozone itself is given in these documents. Therefore, no evaluations of the toxicity of ozone from oral exposure have been found.

A review of available chemical data supports the hypothesis that rapid decomposition of ozone and its breakdown products limits their reactivity to the surface of food, and residues often will be removed by washing or peeling before eating or volatilized and decomposed during cooking.

Toxicological data

No evaluations of the toxicity of ozone from oral exposure have been found (see section 3.1.4.1 for bromate).

Dietary exposure

No dietary exposure to ozone is expected (see section 3.1.4.1 for information on exposure to bromate).

Risk characterization

As there is no direct dietary exposure to ozone, no health concerns were identified.

3.1.3.4 Peroxyacids and peroxides

Introduction

Peroxyacid antimicrobial solutions are typically prepared by mixing hydrogen peroxide and acetic acid in aqueous solution, which results in an equilibrium mixture of acetic acid, peroxyacetic acid, hydrogen peroxide and water. Preparations may also contain octanoic acid, which, when treated with hydrogen peroxide, produces an equilibrium mixture

of octanoic acid and peroxyoctanoic acid. As described in chapter 2, peroxyacids decompose to their corresponding organic acid and hydrogen peroxide or oxygen. The hydrogen peroxide in these solutions decomposes into water and oxygen. Preparations may contain 1-hydroxyethylidene-1,1-diphosphonic acid (HEDP), which is stable and is expected to remain in the antimicrobial wash and on food after treatment.

Peroxyacid solutions were most recently evaluated by JECFA in 2005 (WHO, 2006c). JECFA considered that, owing to the high reactivity of peroxyacids and hydrogen peroxide towards organic matter, they would break down into acetic acid, octanoic acid and water, respectively, and therefore be of no safety concern (WHO, 2006c). This is the most recent international evaluation of peroxyacids.

EFSA has also reviewed peroxyacids for treatment of poultry carcasses and concluded that the estimated intakes of residues of peroxyacetic acid, hydrogen peroxide, acetic acid, octanoic acid and HEDP arising from the treatment of poultry carcasses would be of no safety concern (EFSA, 2005).

Toxicological data

In 2005, JECFA considered the safety of antimicrobial solutions using HEDP as a sequesterant or stabilizer and containing three or more of the following components: acetic acid, hydrogen peroxide, octanoic acid and peroxyacetic acid (WHO, 2006c). These solutions are intended to be diluted before use to achieve peroxyacid concentrations in the range 80–220 mg/kg. JECFA concluded that the peroxy compounds in these solutions would break down into acetic acid and octanoic acid and that small residual quantities of these acids on foods at the time of consumption would not pose a safety concern; JECFA therefore focused its evaluation on the residues of HEDP that are anticipated to remain on foods (WHO, 2006c).

JECFA noted that absorption of HEDP from the gastrointestinal tract is very limited and that its metabolism is negligible. HEDP did not show evidence of mutagenic activity. In 90-day toxicity studies in dogs and rats, the NOELs were 250 mg/kg bw per day and 500 mg/kg bw per day, respectively (WHO, 2006c). In reproductive toxicity studies, a NOEL of 50 mg/kg bw per day was identified for both rats and rabbits. HEDP has not shown any evidence of mutagenic activity. Based on the available toxicity data, together with a margin of exposure of >1000 when comparing the highest estimate of intake of HEDP with the starting oral dose of 5 mg/kg bw per day used in clinical treatment of patients with Paget disease, JECFA concluded that HEDP does not pose a safety concern at the concentrations of residue that are expected to remain on foods (WHO, 2006c).

JECFA evaluated acetic acid in 1974, allocating an ADI “not limited”¹ (FAO/WHO, 1974a). This ADI was retained at a subsequent evaluation in 1997 (FAO/WHO, 1999). In evaluating the acceptance of acetic acid, emphasis was placed on its established metabolic pathways (metabolized to carbon dioxide) and its consumption by humans as a normal constituent of the diet. Also in 1997, JECFA concluded that use of octanoic acid as a flavouring agent posed no safety concerns at intakes of up to 63 µg/kg bw per day (FAO/WHO, 1999). JECFA evaluated hydrogen peroxide in 1966 as a preservative and sterilizing agent for use in milk, concluding that it was not possible to set an ADI for humans because of the instability of hydrogen peroxide in contact with food (FAO/WHO, 1966). However, it was noted that hydrogen peroxide may be used only in circumstances where more acceptable methods of milk preservation are not available (FAO/WHO, 1966). This was confirmed in a subsequent evaluation in 1974 (FAO/WHO, 1974b).

¹ This is a term no longer used by JECFA that has the same meaning as ADI “not specified” (see Annex 4).

Dietary exposure

Human exposure to components of antimicrobial peroxyacid solutions was evaluated by the sixty-third meeting of JECFA (FAO/WHO, 2005). Additionally, an EFSA evaluation was published in 2005. Consistent with what is known about the chemistry of peroxy compounds, no residues of hydrogen peroxide, peroxyacetic acid or peroxyoctanoic acid are anticipated to be present on foods that have been washed in, sprayed with or otherwise treated using peroxyacid solutions derived from acetic or octanoic acid and subsequently cooked. Regardless, the EFSA evaluation included a highly conservative estimate of 1.46 µg/kg bw per day for possible residual peroxyacids and hydrogen peroxide (at the 99th percentile). This estimate was based on a detection limit of 1 mg/l, assuming that peroxide concentrations no higher than 0.25 mg/kg carcass would be present 2 min after treatment.

Acetic and octanoic acids present at equilibrium in the solutions and as by-products from the corresponding peroxyacids would be expected to remain on any treated foods that are not washed or further processed after treatment. JECFA reported that the mean intake of octanoic acid from foods consumed as part of the diet in the USA had been estimated to be approximately 200 mg/day. A highly conservative estimate of exposure to octanoic acid resulting from the use of the antimicrobial solutions of 1.9 mg/day was noted (WHO, 2006c). The intake of acetic acid was not explicitly analysed for JECFA, but its use in and on foods (as vinegar) would result in a greater exposure than that from the use of peroxyacid antimicrobial solutions. There would be no need to further consider exposure to these common food acids. The EFSA evaluation did not consider exposure to the fatty acid by-products.

HEDP is expected to remain on foods that are treated with antimicrobial solutions and that are not further washed, processed or cooked. JECFA reported that, on the international level, the highest estimate of intake of HEDP, prepared using GEMS/Food diets, was that for the European diet: 3.6 µg/kg bw per day, for the upper-bound estimate using a model for vegetables with a high surface area. JECFA also considered national estimates of intake from the Czech Republic, the USA and the United Kingdom. The upper-bound estimate of exposure was 2.2 µg/kg bw per day for the Czech Republic. The mean and 90th-percentile upper-bound estimates of intake for the USA were 2.2 and 4.7 µg/kg bw per day, respectively. The mean and 90th-percentile upper-bound estimates of intake for the United Kingdom were 1.8 and 3.3 µg/kg bw per day, respectively. The EFSA estimate of dietary exposure to HEDP was 1 µg/kg bw per day at the 99th percentile. EFSA noted that its estimates did not consider washing or food preparation and that actual dietary consumption is likely to be lower.

JECFA was aware of non-food uses of HEDP. HEDP is used as an anti-scalant for water treatment and in boilers worldwide (the regulatory limit for this use is 25 µg/l in the USA). HEDP is also used as a drug to treat Paget disease and in some over-the-counter cosmetic and pharmaceutical formulations. The USEPA (1998) estimated that exposure to HEDP from all these uses was not more than 6 µg/kg bw per day, including 0.04 µg/kg bw per day from its use on food. JECFA noted that this estimate of exposure resulting from food uses of HEDP was much less conservative than that used in the present evaluation.

Overall, a conservative estimate of the chronic dietary exposure to HEDP would be 5 µg/kg per day, based on the 90th-percentile national estimate from the USA.

Risk characterization

As JECFA concluded that HEDP does not pose a safety concern at the concentrations of residue that are expected to remain on foods, no health concerns were identified.

3.1.3.5 Quaternary ammonium compounds (cetylpyridinium chloride)

Introduction

Quaternary ammonium compounds (QACs) are organically substituted ammonium compounds that are commonly used as surface sanitizers in processing facilities. Cetylpyridinium chloride (CPC) is a QAC found in an anhydrous form or as the monohydrate.

The toxicity data used in this document are from reports provided to the USFDA in connection with a toxicological evaluation of the use of CPC as an antimicrobial agent on the surface of raw poultry carcasses (secondary direct food additive) (USFDA, 2007a,b). The USFDA established an ADI for CPC of 8 µg/kg bw per day (USFDA, 2007a).

Toxicological data

No chronic (2-year) or carcinogenicity studies of CPC were found. CPC was reported to be not mutagenic in *Salmonella typhimurium* strains TA97a, TA98, TA100 and TA1535 and *Escherichia coli* WP2 *uvrA* (pkM101), with or without activation, and it was not clastogenic in the in vitro chromosomal aberration assay with CHO cells, with and without activation (USFDA, 2007a).

Twenty male and 20 female Sprague-Dawley rats per treatment were fed CPC-containing diet at a dose level of 0, 125, 250, 500 or 1000 mg/kg for 90 days, equivalent to 0, 8.97, 17.94, 35.36 or 70.23 mg/kg bw per day in males and 0, 10.85, 22.30, 42.40 or 82.66 mg/kg bw per day in females (USFDA, 2007a). Feed consumption, clinical observations, body weights and absolute and relative organ weights were recorded, and haematology, serum chemistry, urinalysis, ophthalmic and neurological examinations, gross examination and histopathology were performed. The study conclusions were that 1000 mg/kg feed was the LOAEL in rats based on decreased body weights and body weight gain in both sexes and reduced heart weight in females. The NOEL was 500 mg/kg feed, equivalent to 35.36 and 42.40 mg/kg bw per day, respectively, for male and female rats.

Male and female purebred Beagle dogs, four animals per sex per dose, were fed diets containing CPC at 0, 250, 375, 500 or 1000/500 mg/kg feed for 90 days. The test compound was withheld from animals in the 1000 mg/kg group from study day 29 until day 42/41 (males/females) because of significant weight loss in this dose group. The CPC treatment was then resumed, but at 500 mg/kg feed for the duration of the study. Corresponding time-weighted average doses of CPC were 0, 7.82, 11.76, 14.15 and 16.65 mg/kg bw per day in males and 0, 8.01, 10.79, 17.29 and 17.14 mg/kg bw per day in females, respectively. Feed consumption, clinical observations, body weights, and absolute and relative organ weights were recorded, and haematology, serum chemistry, urinalysis, ophthalmic and neurological examinations, gross examination and histopathology were done. The study conclusions were that, based on the reduction in body weight gain seen in both sexes in the CPC-treated dogs and decreased red blood cell parameters (i.e. red blood cell count, haemoglobin level and haematocrit), the LOAEL was 375 mg/kg feed. A NOEL of 250 mg/kg feed, equivalent to 8 mg/kg bw per day (7.82 and 8.01 mg/kg bw per day for males and females, respectively), was established.

Because the NOEL in the 90-day dog study was lower than the NOEL in the 90-day rat study, to be conservative, the NOEL from the dog study was used to calculate the ADI for CPC. Using the NOEL of 8 mg/kg bw per day from the dog study and applying a safety factor of 1000, the USFDA established an ADI for CPC of 8 µg/kg bw per day, or 0.48 mg/day for a person with a body weight of 60 kg (USFDA, 2007a).

Dietary exposure

Consumption data for chicken taken from a survey in the USA were used to calculate exposure to CPC. As it was shown in the residue studies that the CPC exposure was almost exclusively due to consumption of skin, only data for skin-on poultry were used (with 8.8% of poultry weight being skin). Poultry consumption of 22 g/day was combined with the CPC residual data taken from the studies using 0.8% solutions (mimicking the United States regulation; see section 2.12.1) to give a CPC exposure of 26 µg/person per day.

Dietary exposure to CPC from consumption of treated poultry can be generalized using the GEMS/Food database for consumption data. The highest consumption of poultry meat is for cluster K, 146 g/person per day. Using this figure (assuming that the skin is consumed with the poultry at 8.8%, as above) with the maximum mean residual level of 20 mg/kg (from use of a 2.0% solution of CPC; see section 2.12.1) gives an exposure to CPC of 260 µg/person per day, equal to 4.3 µg/kg bw per day for a 60-kg person.

It is noted that CPC also has potential uses in dentifrices at 0.005–2% of product, from which some ingestion can occur during tooth brushing (USP, 1991).

Risk characterization

The estimated dietary exposure of 4.3 µg/kg bw per day is below the ADI for CPC of 8 µg/kg bw per day established by the USFDA. Therefore, no health concern was identified with use of CPC on food contact surfaces.

3.1.3.6 Iodophors

Introduction

Iodophors are mixtures of iodine and surface-active agents that act as carriers and solubilizers for the iodine. The result is a water-soluble material that releases free iodine (12.5–25 mg/l) in solution. The information used here is from the background document on iodine for development of the WHO *Guidelines for drinking-water quality* (WHO, 2003b).

Toxicological data

Iodine is an essential element in the synthesis of the thyroid hormones thyroxine (T₄) and triiodothyronine (T₃) through the precursor protein thyroglobulin and the action of the enzyme thyroid peroxidase. The estimated dietary iodine requirement for adults ranges from 80 to 150 µg/day (WHO, 2003b). Chronic consumption of iodinated drinking-water has not been shown to cause adverse health effects in humans, although some changes in thyroid status have been observed (WHO, 2003b).

In 1988, JECFA set a provisional maximum tolerable daily intake (PMTDI) for iodine of 1 mg/day (17 µg/kg bw per day) from all sources, based mainly on data on the effects of iodide (WHO, 1989). However, recent data from studies in rats indicate that the effects of iodine in drinking-water on thyroid hormone concentrations in the blood differ from those of iodide (WHO, 2003b). Available data therefore suggest that derivation of a guideline value for iodine on the basis of information on the effects of iodide is inappropriate, and there are few relevant data on the effects of iodine. Because iodine is not recommended for long-term disinfection, only for emergency disinfection of drinking-water in the field, lifetime exposure to iodine from water disinfection is unlikely. For these reasons, a guideline value for iodine has not been established (WHO, 2003b).

Dietary exposure

Currently in the USA, iodophores are regulated for use as sanitizers on hard surfaces that may contact food; they are not used directly on food. However, because sanitizers used

in the USA are not washed from food contact surfaces before the surfaces are used to process food, there may be some carryover from the surface to the food. The USFDA (1993) models this situation as follows. It is assumed that 1 mg of end user solution (maximum allowable iodine concentration in the USA is 25 mg/l) resides on each square centimetre of treated surface. For iodophores, this results in a residual iodine level of 0.03 µg/cm² surface. It is then assumed that all food consumed in a day would contact 4000 cm² of this treated surface. This highly conservative model derives a dietary exposure to iodophores of 0.1 mg/person per day (about 2 µg/kg bw per day for a 60-kg individual).

WHO (2003b) estimated that the main natural sources of dietary iodide are seafood (200–1000 µg/kg) and seaweed (1000–2000 mg/kg). Iodide is also found in cow's milk (20–70 µg/l) and may be added to table salt (100 µg of potassium iodide per gram of sodium chloride) to ensure an adequate intake of iodine. Exposure to iodine may occur through drinking-water, pharmaceuticals and food. At a concentration of 4 µg/l in drinking-water, adult human daily intake will be 8 µg iodine, on the assumption that 2 litres of drinking-water are consumed per day.

The WHO dietary exposure assessment did not take into account emergency disinfection of drinking-water in the field. However, lifetime exposure to iodine from water disinfection is unlikely.

Risk characterization

The highly conservative estimate of dietary exposure to iodine from use of iodophores (2 µg/kg bw per day) is well below the PMTDI of 17 µg/kg bw per day. There are other sources of iodine in the food. If these result in total dietary exposure approaching or exceeding the PMTDI, the contribution from iodophores would be minimal. Therefore, no health concerns were identified.

3.1.3.7 Sodium metasilicate

Introduction

Sodium metasilicate (waterglass) is commercially available in three forms: anhydrous (Na₂SiO₃; CAS No. 6834-92-0), pentahydrate (Na₂SiO₃·5H₂O; CAS No. 10213-79-3) and nonahydrate (Na₂SiO₃·9H₂O; CAS No. 13517-24-3) (IPCS, 1997).

Sodium metasilicate is not included in WHO's *Guidelines for drinking-water quality* (WHO, 2006a). It is described in an IPCS (1997) document and has been evaluated by the USEPA (2006), by the OECD (2004) in a Screening Information Dataset document on soluble silicates, including sodium metasilicate, and in a document prepared for the United States National Institute of Environmental Health Sciences (Haneke, 2002a). In addition, a document was submitted by industry on products containing either anhydrous or pentahydrate forms of sodium metasilicate (DANISCO, 2007). These reports have been used as sources of information in this section.

In 1973, JECFA allocated an ADI "not limited" for silicon dioxide and certain silicates except magnesium silicate and talc (FAO/WHO, 1974b). This was on the basis of the biological inertness of these compounds.

Toxicological data

No lifetime (2-year) studies were found on sodium metasilicate. In a 2-year study reported in USEPA (2006), rats and mice were fed silicon dioxide (SiO₂, a degradation product of sodium metasilicate pentahydrate) at dietary levels of up to 50 000 mg/kg (5% of the diet), giving doses of approximately 2500 and 7500 mg/kg bw per day for rats and mice, respectively. The only effect noted was a significant reduction in body weight at the highest

dose at the 10-week point in mice, which continued throughout the rest of the study. This was likely attributable to a nutritional imbalance rather than a toxic effect of the high percentage of silica in the daily diet of the mice. No adverse effects were observed in rats. (The lower doses were not stated, and the reference to the study was not given.)

In a 90-day study in rats, anhydrous sodium metasilicate was administered in drinking-water at concentrations of 200, 600 and 1800 mg/l, corresponding to approximately 26.4, 76.2 and 227.1 mg/kg bw per day for males and approximately 32.1, 97.6 and 237.2 mg/kg bw per day for females. No clearly treatment-related effects were found; therefore, the NOAEL was 227–237 mg/kg bw per day (the highest dose tested) in the rats (OECD, 2004).

In a 90-day study in mice, anhydrous sodium metasilicate was administered in drinking-water at concentrations of 300, 900 and 2700 mg/l to males and 333, 1000 and 3000 mg/l to females, corresponding to approximately 96–100, 264–280 and 776–832 mg/kg bw per day in males and approximately 88–104, 260–284 and 716–892 mg/kg bw per day in females (OECD, 2004). Body weight, urinalysis, clinical chemistry, haematology, organ weights and histopathology were examined. No fatalities occurred. In females, a significant decrease in pituitary gland weight was observed in the high-dose group. Other effects occasionally observed were single incidences and not dose related. The NOAELs were therefore 776–832 mg/kg bw per day in males (highest dose tested) and 260–284 mg/kg bw per day in females. The LOAEL was 716–892 mg/kg bw per day in female mice.

The chemical structure of sodium metasilicate does not contain elements that raise concern for genotoxicity (OECD, 2004). None of the substances sodium metasilicate, silicic acid and silicon dioxide showed point mutation activity in three bacterial test species (USEPA, 2006). Anhydrous sodium metasilicate at concentrations of 0.005–0.5 mol/l (<6.2%) was not genotoxic in DNA damage and repair assays conducted on *Bacillus subtilis* recombination repair-deficient and wild-type strains without metabolic activation (OECD, 2004; DANISCO, 2007). Anhydrous sodium metasilicate was negative in the Ames test with *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537, with and without metabolic activation, at concentrations of 0.1–10 mg/plate and in the mouse bone marrow chromosomal aberration test in vivo after single oral doses of 740–1340 mg/kg bw (OECD, 2004).

There are no Codex Alimentarius Commission maximum residue levels established for residues of sodium metasilicate (USEPA, 2006).

Dietary exposure

Sodium metasilicate is widely used in cosmetics, hair and skin products, detergents and a variety of cleaning products and as an active ingredient in insecticides, fungicides and antimicrobial pesticides at concentrations up to 4% (USEPA, 2006). There are therefore several potential sources of exposure to sodium metasilicate.

Silica and silicates are permitted for use as direct food additives, primarily as flow agents in powdered foods or to absorb water (Haneke, 2002a; EFSA, 2004; USEPA, 2006). The sodium metasilicate pentahydrate is also classified by the USFDA as “generally recognized as safe” (GRAS) as an indirect food additive for use in washing mixtures of fruits and vegetables, in sanitizing solutions on food contact surfaces, in boiler water and for other uses (Haneke, 2002a; USEPA, 2006). Residues of the pentahydrate, when used in fruit and vegetable washes, are expected to be orders of magnitude less than the estimated daily dietary consumption of 20–30 mg silica (silicon dioxide, silicon) from natural sources and drinking-water (USEPA, 2006). EFSA (2004) referenced earlier work where the daily intake from the British diet had been estimated to be 20–50 mg (Pennington, 1991; Bellia, Birchall & Roberts, 1994). The relative contributions were 55% from water, coffee and beer, 14% from grain products and 8% from vegetables. The dietary silicon exposure estimated from the

British diet was 20–50 mg/day, corresponding to 0.3–0.8 mg/kg bw per day for a 60-kg person, and EFSA considered that these intakes were unlikely to cause adverse effects.

The EFSA (2004) opinion also referred to silicon, in the form of silica, being found in supplements. According to the doses recommended by the manufacturers, the supplements (e.g. products on the Norwegian market, according to the Norwegian Institute of Public Health) provide 1–75 mg/day as silicon, corresponding to 0.017–1.25 mg/kg bw per day for a 60-kg adult. Silicon in the form of amorphous silica, silicates and dimethylpolysiloxane is added to food as an anti-caking and anti-foaming agent. Dimethylpolysiloxane is used for the treatment of infant colic.

The OECD (2004) also estimated the summed systemic exposure of consumers to soluble silicates through oral, dermal and inhalation contact with detergents and cleaners to be 12.3 µg/kg bw per day, which is about 1–2 orders of magnitude lower than the estimated daily silica intake through ubiquitous natural occurrence in the diet (soluble silicates include sodium metasilicate, sodium silicate [CAS No. 1344-09-8] and potassium silicate [CAS No. 1312-76-1]). This study also reported that another important route of exposure is through the addition of sodium silicate to drinking-water as a corrosion inhibitor and sequestering agent.

Risk characterization

Dietary exposure resulting from use of metasilicate as a disinfectant is insignificant in comparison with other dietary sources of silicates, and no health concerns were identified.

3.1.3.8 Trisodium phosphate

Introduction

Trisodium phosphate (TSP) was most recently evaluated by EFSA (2005), which cited the maximum tolerable daily intake (MTDI) of 70 mg/kg bw established by JECFA in 1982 for the group of phosphoric acid and phosphate salts (WHO, 1982). The European Scientific Committee for Food (SCF, 1991) also endorsed the MTDI.

Toxicological data

JECFA evaluated TSP in 1982 as part of the group of phosphoric acid and phosphate salts (WHO, 1982). JECFA noted that the toxicological end-point of most concern was ion imbalance in the diet, with high phosphate intakes leading to calcification of soft tissues, especially the kidneys, and loss of bone density. In a series of experiments, Sherman diets containing 1%, 2.5% and 5% sodium diphosphate were fed for 16 weeks to groups of 20 male and female rats weighing between 90 and 115 g; a similar group received a diet containing 5% sodium monophosphate. In the sodium phosphate groups, growth was normal up to the 2.5% level; kidney weight was increased at the 2.5% level (females) and above; and kidney function (concentration test) was decreased at the 2.5% level (males) and above. Kidney damage (calcification, degeneration and necrosis) was observed in a greater percentage of rats in the 1% group than in the controls. At the higher concentration of sodium diphosphate, more severe kidney damage occurred; in addition, some of the animals had hypertrophy and haemorrhages of the stomach. The latter abnormality was not found in the 5% sodium monophosphate group. Other studies found no effects on the kidney at higher doses. JECFA considered the rat to be exquisitely sensitive to calcification and hydronephrosis upon exposure to acids forming calcium chelates or complexes and identified 1% as the lowest level of dietary phosphorus that might conceivably lead to nephrocalcinosis in rats. This was extrapolated to humans using the equivalent daily caloric intake to derive a phosphorus dose of 6600 mg/day. JECFA noted that “the usual calculation for provision of a margin of safety is probably not suitable for food additives that are also nutrients” and established an MTDI of

70 mg/kg bw, expressed as phosphorus, for the sum of phosphates naturally present in food and derived from additives.

The SCF (1991) noted that phosphate salts are not mutagenic in a number of test systems.

Dietary exposure

TSP is approved for use in raw unchilled poultry carcasses and giblets in the USA (USDA, 2007). TSP is also used as a food additive, and in these uses all sodium phosphates may be referred to collectively as sodium phosphate or by International Numbering System No. 339. TSP was most recently evaluated by EFSA (2005). Another report, prepared for the government in the USA (USDA, 2002), considered the efficacy and safety of TSP use in poultry processing. The report considered oral exposure, although no dietary exposure assessment was undertaken.

The EFSA (2005) assessment includes an exposure assessment estimated using the Concise European Food Consumption Database. Exposure assessment using mean and high percentiles of consumption was conducted for three European countries. Mean and high consumptions of meat and meat products (including offal) by adults were extracted from the three national food consumption surveys—namely, France (Volatier, 2000), Italy (Turrini et al., 2001) and Sweden (Becker & Pearson, 2002)—which are based on 7-day records for individuals. Average mean daily consumption of meat (edible portion) is given in Table 3.2 in section 3.1.2.2. Potential dietary exposure to all substances was estimated based on the conservative hypothesis that the concentration in the edible part of the meat is identical to the concentration in the carcass.

Previous calculations by the Scientific Committee on Veterinary Measures relating to Public Health (SCVPH, 2003) indicated that the treatment of poultry carcasses with TSP would incorporate TSP at a concentration of 480 mg/kg carcass. Using these calculations and the meat consumption data for European adults, as reported above, EFSA (2005) estimated that the potential daily exposure to TSP for a 60-kg individual would be up to 1.21 mg/kg bw at the mean meat consumption and up to 2.08 and 2.80 mg/kg bw at the 95th and 99th percentiles of meat consumption, respectively.

Risk characterization

The dietary exposure estimated to result from the use of TSP in treatment of poultry carcasses is considerably lower than the MTDI of 70 mg/kg bw, expressed as phosphorus, for the total sum of phosphates. Therefore, no health concerns were identified.

3.1.4 Disinfection by-products

3.1.4.1 Bromate

Introduction

Bromate is not normally found in water, but may be formed in water during ozonation when the bromide ion is present (WHO, 2005a). Bromate may also be present in hypochlorite solutions used to disinfect drinking-water, as a result of the presence of bromide in the raw materials (chlorine and sodium hydroxide) used in the manufacture of sodium hypochlorite and the high pH of the concentrated solution (WHO, 2005a). The toxicology and mechanisms of in vivo bromate carcinogenicity have been examined in detail (Bull & Cotruvo, 2006).

The toxicology of bromate is evaluated and described in Environmental Health Criteria 216 (IPCS, 2000), in the WHO *Guidelines for drinking-water quality* (WHO, 2006a) and in the bromate background document for development of these guidelines (WHO,

2005a). In addition, IARC monographs (IARC, 1986, 1987, 1999b) and a USEPA toxicological review of bromate (USEPA, 2001), as well as some original publications of pivotal studies, have been used as sources of information in this section.

Both a carcinogenicity assessment based on the linearized multistage model and a TDI of 1 µg/kg bw based on a non-linear approach for the carcinogenicity of bromate have been developed (IPCS, 2000).

Toxicological data

The systemic toxicity of bromate (administered as the potassium salt) has been reported from long-term studies designed to evaluate the carcinogenicity of bromate in F344 rats and B6C3F1 mice (Kurokawa et al., 1983, 1986a,b; DeAngelo et al., 1998). The data show that the kidney is the major target organ of bromate-associated toxicity and that rats are more sensitive than mice to bromate exposure (WHO, 2005a). A NOAEL could not be determined from the studies of Kurokawa et al. (1983, 1986a,b). A NOAEL for bromate of 1.1 mg/kg bw per day was identified in male F344 rats based on kidney effects (i.e. renal pelvis urothelial hyperplasia), and a NOAEL of 59.6 mg/kg bw per day was identified in male B6C3F1 mice based on studies in which no effects on survival, body weight, organ weight, serum chemistry or incidence of non-neoplastic lesions were observed (DeAngelo et al., 1998).

A physiologically based toxicokinetic model for bromate metabolism and detoxification is in the later stages of development, based upon *in vivo* studies in the rat (J.A. Cotruvo, personal communication, 2008). The liver is not a target organ, and it has been shown that the liver is significantly less susceptible than the kidney for cytotoxicity or DNA damage. Even at fairly high doses, the half-life in the rat is in minutes. Indications are that environmentally relevant bromate doses are rapidly metabolized in the liver and blood, thus significantly reducing or virtually eliminating doses to target organs. Thus, previous risk models most likely significantly overestimated the low-dose risks from bromate ingestion (J.A. Cotruvo, personal communication, 2008).

The weight of evidence demonstrated that bromate is clearly mutagenic *in vitro* and *in vivo* (IPCS, 2000; WHO, 2005a). The clearest evidence of bromate-induced cancer comes from the studies of F344 rats. In summary (WHO, 2005a), bromate produced tumours at multiple sites in male rats, including the kidney (adenomas and carcinomas), the thyroid gland (follicular cell adenomas and carcinomas) and the peritoneum (mesotheliomas) (Kurokawa et al., 1983, 1986a,b, 1987; DeAngelo et al., 1998). In the female rat, only kidney tumours were observed (Kurokawa et al., 1983, 1986b). Further, a clear dose–response relationship exists with tumour incidence and the severity/progression of tumours. The weight of evidence from the rat bioassays clearly indicates that bromate has the potential to be a human carcinogen at high doses. Bromate also caused a treatment-related, but not dose-related, increase in the incidence of renal tumours in male B6C3F1 mice (DeAngelo et al., 1998).

WHO (IPCS, 2000; WHO, 2005a) noted that there were insufficient data to conclude on the mode of carcinogenic action of bromate, whether it is cytotoxicity and reparative hyperplasia, oxidative stress, such as lipid peroxidation and free radical production, and/or DNA reactivity (genotoxicity), and stated that the mechanisms may also differ for tumours at various sites. Thiol-dependent oxidative damage to the guanine base in DNA was considered a plausible mode of action for bromate-induced cancer (Bull & Cotruvo, 2006).

The kidney is the major target organ of bromate-associated carcinogenicity, and male rats are significantly more sensitive than female rats, mice or hamsters to bromate exposure (Gold, 2005).

IARC (1986, 1987, 1999b) evaluated the carcinogenicity of potassium bromate and concluded that it is possibly carcinogenic to humans (Group 2B). There was inadequate evidence in humans and sufficient evidence in experimental animals for the carcinogenicity of potassium bromate (IARC, 1999b). The USEPA (2001) has also classified bromate in Group B2 as a probable (likely) human carcinogen by the oral route of exposure on the basis of no evidence in humans and adequate evidence of carcinogenicity in male and female rats.

Because of insufficient information on the mode of carcinogenic action of bromate, both a carcinogenicity assessment based on the linearized multistage model as well as a TDI based on a non-linear approach for the carcinogenicity of bromate were developed (WHO, 2005a). A TDI of 1 µg/kg bw was calculated based on a no-effect level for the formation of renal cell tumours in rats at 1.3 mg/kg bw per day in the study of Kurokawa et al. (1986a) and the use of an uncertainty factor of 1000 (10 each for interspecies and intraspecies variation and 10 for possible carcinogenicity). The calculated upper 95% confidence limit of 0.1 µg/kg bw per day for a 10^{-5} excess lifetime cancer risk (WHO, 2005a) was based on an increased incidence of renal tumours in male rats given potassium bromate in drinking-water for 2 years using the same study (Kurokawa et al., 1986a).

The more recent study by DeAngelo et al. (1998) was selected for the derivation of a guideline value for drinking-water (WHO, 2005a, 2006a), because this study used lower doses and more animals per group and the tumour findings were similar to those observed in the earlier study. To estimate cancer risks based on low-dose linear extrapolation, a one-stage Weibull time-to-tumour model was applied to the incidence of each tumour type (mesotheliomas, renal tubule tumours and thyroid follicular tumours) in male rats given potassium bromate in drinking-water, using the 12-, 26-, 52- and 77-week interim kill data (DeAngelo et al., 1998). Individual cancer potency estimates were summed using Monte Carlo analysis (USEPA, 2001). The upper-bound estimate of the cancer potency for bromate was $0.19 \text{ (mg/kg bw per day)}^{-1}$. The concentrations in drinking-water associated with upper-bound excess lifetime cancer risks of 10^{-4} , 10^{-5} and 10^{-6} were 20, 2 and 0.2 µg/l, respectively.

Dietary exposure

As discussed above, bromate may be formed in water during ozonation when the bromide ion is present. Bromate may also be generated during the use of DBDMH as an antimicrobial on beef and poultry. However, bromate is a strong oxidant (Seidel, 2004) and is expected to be reduced to bromide during cooking (USFDA, 2003). Therefore, bromate is not expected to be present on beef or poultry at the time of consumption.

WHO has reported that for most people, exposure to bromate is unlikely to be significant (IPCS, 2000).

Risk characterization

Because bromate is not expected to be present on meat at the time of consumption, no health concerns were identified.

3.1.4.2 Chloral hydrate (2,2,2-trichloroethane-1,1-diol)

Introduction

Chloral hydrate ($\text{Cl}_3\text{CCH}(\text{OH})_2$; CAS No. 302-17-0) may be formed in reactions between NOM and hypochlorous acid or hypobromous acid (IPCS, 2000).

The information in this section is based mostly on IPCS (2000). In addition, IARC (1995) and some original publications have been used. A TDI of 16 µg/kg bw per day has been derived for chloral hydrate (IPCS, 2000).

Toxicological data

In a 2-year study, chloral hydrate at 1 g/l of drinking-water (166 mg/kg bw per day) induced liver tumours in male B6C3F1 mice (Daniel et al., 1992a). Lower doses were not evaluated. It is probable that the liver tumours induced by chloral hydrate involve its metabolism to trichloroacetic acid (TCA) and/or dichloroacetic acid (DCA), which are considered to act as tumour promoters (IPCS, 2000). Chloral hydrate has been shown to induce chromosomal anomalies in several in vitro tests, but it has been largely negative when evaluated in vivo (IARC, 1995). IARC (1995) has classified chloral hydrate in Group 3 (not classifiable as to its carcinogenicity to humans).

Chloral hydrate administered to Sprague-Dawley rats for 90 days in drinking-water induced hepatocellular necrosis at concentrations of 1200 mg/l and above, with no effect being observed at 600 mg/l (approximately 60 mg/kg bw per day) (Daniel et al., 1992b). Hepatomegaly was observed in male CD-1 mice at doses of 144 mg/kg bw per day administered by gavage for 14 days, whereas no effects were seen at 14.4 mg/kg bw per day for 14 days (Sanders et al., 1982). Mild hepatomegaly was observed in male CD-1 mice when chloral hydrate was administered in drinking-water at 70 mg/l (16 mg/kg bw per day) in a 90-day follow-up study (Sanders et al., 1982).

Based on the mild hepatomegaly observed when chloral hydrate was administered in drinking-water at 16 mg/kg bw per day to male CD-1 mice in the 90-day follow-up study (Sanders et al., 1982) and applying an uncertainty factor of 1000 (10 each for interspecies and intraspecies variation and 10 for the use of a LOAEL instead of a NOAEL), a TDI of 16 µg/kg bw per day was derived (IPCS, 2000).

Dietary exposure

No occurrence data on the levels of chloral hydrate in food, other than drinking-water, were identified. Occurrence data relating to the concentration of chloral hydrate in drinking-water in North America are summarized in Table 2.4 in chapter 2.

An estimate of mean dietary exposure arising from the consumption of drinking-water has been calculated and is presented in Table 3.4. Dietary exposure resulting from the consumption of drinking-water was within the range 0.000–0.073 µg/kg bw per day.

Table 3.4. Mean dietary exposure to chloral hydrate from the consumption of drinking-water^a

Country	Exposure (µg/kg bw per day)	Country	Exposure (µg/kg bw per day)
Australia	0.031	Ireland	0.008
Belgium	0.003	Italy	0.007
Czech Republic	0.008	Netherlands	0.006
Denmark	0.025	Norway	0.009
Finland	0.025	Slovakia	0.007
France	0.009	Sweden	0.014
Germany	0.002	United Kingdom	0.006
Hungary	0.000	USA	0.031
Iceland	0.019	WHO	0.073

^a The mean concentration of chloral hydrate from 12 drinking-water utilities in the USA and Canada was used in the estimate of dietary exposure.

Risk characterization

No data have been identified in relation to residues of chloral hydrate in food resulting from use of chlorine-based disinfectants. Therefore, no health concern was identified, but residue data are needed.

3.1.4.3 Chlorate

Introduction

Chlorate is generated as a reaction by-product from the use of ASC or chlorine dioxide. It is also a decomposition product of stored sodium hypochlorite and can be present in treated food.

JECFA evaluated chlorate as part of its evaluation of ASC in 2007 and set an ADI for chlorate of 0.01 mg/kg bw per day (WHO, 2008a). This is the most recent evaluation of chlorate.

In reviewing ASC, EFSA (2005) noted the dietary exposure to chlorate residues, but did not specifically comment on the health implications.

Toxicological data

Chlorate is rapidly absorbed and distributed throughout the body. It is excreted primarily in the urine in the form of chloride, with lesser amounts of chlorite and chlorate.

In common with sodium chlorite, sodium chlorate has been reported to have effects on erythrocytes, but JECFA concluded that the most sensitive effects were changes to the thyroid gland of male rats in a 2-year carcinogenicity study (NTP, 2005). Groups of 50 male and 50 female F344/N rats were exposed to sodium chlorate in the drinking-water for 2 years at doses equivalent to approximately 5, 35 and 75 mg/kg bw per day for males and 5, 45 and 95 mg/kg bw per day for females. There were positive trends in the incidence of thyroid gland follicular cell carcinoma in male rats and thyroid gland follicular cell adenoma and carcinoma (combined) in male and female rats. The incidence of thyroid gland follicular cell hypertrophy was significantly increased in all exposed male groups and in the mid- and high-dose groups of females. Thyroid gland focal follicle mineralization occurred in most females in the mid- and high-dose groups. The incidences of haematopoietic cell proliferation in the spleen of high-dose males and bone marrow hyperplasia in the mid- and high-dose male groups were significantly greater than controls. Because a NOAEL was not identified in this study, JECFA decided to apply a benchmark dose (BMD) approach to derive a point of departure on the dose–response curve. Rats are considered to be highly sensitive to the effects of agents that disrupt thyroid hormone homeostasis. JECFA considered that humans are likely to be less sensitive than rats to these effects and that a safety factor for interspecies variation was not required. The rat thyroid gland follicular cell hypertrophy data were modelled in order to derive the BMD for a 10% increase in follicular cell hypertrophy (BMD₁₀) and the corresponding 95% lower confidence limit (BMDL₁₀). The BMDL₁₀ values for chlorate ranged from 1.1 to 4.4 mg/kg bw per day, with the lowest value representing the best fit.

Some positive results have been found in bacterial mutation assays *in vitro* using chlorate, but no positive results have been observed in *in vivo* genotoxicity assays. Based on the negative *in vivo* genotoxicity data and the nature of the histopathological observations, JECFA concluded that a non-genotoxic mode of action was likely for the induction of thyroid tumours by sodium chlorate. This mode of action is likely to be mediated via decreased serum thyroid hormones, leading to increased release of thyroid stimulating hormone (TSH) and consequent stimulation of thyroid cell proliferation and thyroid gland growth, which can lead to thyroid tumours in rodents (WHO, 2008a).

JECFA established an ADI of 0–0.01 mg/kg bw for chlorate on the basis of the BMDL₁₀ of 1.1 mg/kg bw per day, applying a safety factor of 10 to allow for intraspecies variability and an additional factor of 10 to allow for deficiencies in the database, particularly with respect to investigation of possible neurodevelopmental effects.

Dietary exposure

Dietary exposure to chlorate was considered by JECFA (WHO, 2008a) in the context of the use of ASC as a spray or dipping solution for poultry, meats, vegetables, fruits and seafoods and in poultry chilling water. JECFA stated that residual chlorine dioxide is lost by evaporation, and chloride is considered to be negligible compared with the chloride already present in food; hence, chlorite and chlorate are the principal by-product residues expected.

Potential dietary exposures were estimated by JECFA on the basis of the residual concentrations of chlorate as reported in the submitted data for raw products of three food categories (see section 2.2.2) using the 13 GEMS/Food consumption cluster diets (WHO, 2007b) and individual food consumption data from European countries for the general population using the Concise European Food Consumption Database (EFSA, 2005). International mean dietary exposures were estimated to be 0.1–0.6 µg/kg bw per day for chlorate for the 13 GEMS/Food consumption cluster diets, assuming a body weight of 60 kg. National estimates for European countries of mean to 95th-percentile daily dietary exposures in the general population were 0.3–0.6 µg/kg bw for chlorate.

The expert meeting noted that these estimates were highly conservative, as it was assumed that all the treated foods would be consumed daily over a lifetime and that all treated foods consumed contained the maximum residual level of chlorate reported in experimentation on raw products.

Risk characterization

The estimated high-end dietary exposure to chlorate of 0.6 µg/kg bw per day is well below the ADI of 0–10 µg/kg bw. Therefore, no health concerns were identified.

3.1.4.4 Chlorite

Introduction

Chlorite is generated as a reaction by-product from the use of ASC or chlorine dioxide. It is also a decomposition product of stored sodium hypochlorite and can be present in food with any of these agents.

JECFA evaluated chlorite as part of its evaluation of ASC in 2007 and set an ADI of 0–0.03 mg/kg bw per day (WHO, 2008a). This is the most recent evaluation of chlorite. EFSA (2005) also reviewed ASC for treatment of poultry carcasses and concluded that the exposure to chlorite residues arising from treated poultry carcasses would be of no safety concern.

The ADI set by JECFA, and the basis of its derivation, is the same as the TDI set by IPCS (2000).

Toxicological data

Chlorite is rapidly absorbed and distributed throughout the body. It is excreted primarily in the urine in the form of chloride, with lesser amounts of chlorite and chlorate. Toxicological studies conducted with sodium chlorite in a number of species demonstrated that the most consistent finding is oxidative stress associated with changes in erythrocytes.

Sodium chlorite has given positive results in some, but not all, in vitro genotoxicity assays and in one of the two available in vivo mouse micronucleus assays involving

intraperitoneal administration. Negative results were obtained in several *in vivo* assays involving oral administration of sodium chlorite to mice. Sodium chlorite was not carcinogenic following a number of long-term studies, although these were not conducted to current standards (WHO, 2008a).

In a two-generation reproductive study (Gill et al., 2000), Sprague-Dawley rats (30 per sex per dose) received drinking-water containing sodium chlorite at 0, 35, 70 or 300 mg/l for 10 weeks and were then paired for mating. Males were exposed through mating, then sacrificed. Exposure for the females continued through mating, pregnancy, lactation and until necropsy following weaning of their litters. Dosing continued through two generations with chlorite doses for the F₀ animals of 0, 3.0, 5.6 or 20.0 mg/kg bw per day for males and 0, 3.8, 7.5 or 28.6 mg/kg bw per day for females. For the F₁ animals, chlorite doses were 0, 2.9, 5.9 or 22.7 mg/kg bw per day for males and 0, 3.8, 7.9 or 28.6 mg/kg bw per day for females. There were reductions in water consumption, food consumption and body weight gain in both sexes in all generations at various times throughout the experiment, primarily in the 70 and 300 mg/l groups; these were attributed to a lack of palatability of the water. At 300 mg/l, reduced pup survival, reduced body weight at birth and throughout lactation in the F₁ and F₂ generations, lower thymus and spleen weights in both generations, lowered incidence of pups exhibiting a normal righting reflex, delays in sexual development in males and females in the F₁ and F₂ generations and lower red blood cell parameters in the F₁ generation were noted. Significant reductions in absolute and relative liver weights in F₀ females and F₁ males and females, reduced absolute brain weights in F₁ and F₂ animals and a decrease in the maximum response to an auditory startle stimulus on postnatal day 24 but not at postnatal day 60 were noted in the 300 and 70 mg/l groups. Minor changes in red blood cell parameters in the F₁ generation were seen at 35 and 70 mg/l, but these appear to be within normal ranges based on historical data. The NOEL in this study was 35 mg/l (2.9 mg/kg bw per day), based on lower auditory startle amplitude, decreased absolute brain weight in the F₁ and F₂ generations and altered liver weights in the two generations.

JECFA applied an uncertainty factor of 100 to the NOEL to allow for interspecies and intraspecies variability, resulting in an ADI of 0–0.03 mg/kg bw per day, expressed as the chlorite ion. This ADI was supported by the results of studies in human volunteers showing no adverse effects at this intake.

Dietary exposure

Dietary exposure to chlorite was considered by JECFA (WHO, 2008a) in the context of the use of ASC as a spray or dipping solution for poultry, meats, vegetables, fruits and seafoods and in poultry chilling water. JECFA stated that residual chlorine dioxide is lost by evaporation, and chloride is considered to be negligible compared with the chloride already present in food; hence, chlorite and chlorate are the principal by-product residues expected.

Potential dietary exposures were estimated by JECFA on the basis of the residual concentrations of chlorite as reported in the submitted data for raw products of three food categories (see section 2.2.2) using the 13 GEMS/Food consumption cluster diets (WHO, 2007b) and individual food consumption data from European countries for the general population using the Concise European Food Consumption Database (EFSA, 2005). International mean dietary exposures were estimated to be 0.2–0.7 µg/kg bw per day for chlorite for the 13 GEMS/Food consumption cluster diets, assuming a body weight of 60 kg. National estimates for European countries of mean to 95th-percentile daily dietary exposures in the general population were 0.9–3 µg/kg bw for chlorite.

The expert meeting noted that these estimates were highly conservative, as it was assumed that all the treated foods would be consumed daily over a lifetime and that all treated

foods consumed contained the maximum residual level of chlorite reported in experimentation on raw products.

Risk characterization

The estimated high-end dietary exposure to chlorite of 3 µg/kg bw per day is well below the ADI of 0–30 µg/kg bw. Therefore, no health concerns were identified.

3.1.4.5 Dimethylhydantoin

Introduction

DMH (CAS No. 77-71-4) is generated from the decomposition of DBDMH upon dissolution in water. DMH is stable and, unless washed from raw poultry or beef prior to cooking, is expected to be present on cooked poultry and beef during consumption.

No comprehensive toxicological evaluations of DMH were found. The toxicity experiments described for DMH were found at TOXNET (2008). No ADI or TDI values have been identified for DMH.

Toxicological data

DMH is listed as a suspected central nervous system depressant in humans (TOXNET, 2008).

When DMH was tested in two studies in rats (at doses of 0, 100, 320 or 1000 mg/kg bw per day and 0, 100, 300 or 1000 mg/kg bw per day, respectively) and in two studies in mice (doses as for rats) for 1.5–2 years, the chronic NOELs were 100 and 300 mg/kg bw per day in rats and 300 and 320 mg/kg bw per day in mice (TOXNET, 2008). In the first study in rats, the NOEL of 100 mg/kg bw per day was set on the basis of increased incidence of wet and dried yellow matting in the urogenital area, primarily in males at or above 320 mg/kg bw per day. There were no treatment-related effects on haematology, clinical chemistry, urinalysis, ophthalmology or histopathology. In the second study in rats, the NOEL of 300 mg/kg bw per day was based on a decreased survival time relative to controls in both sexes at 1000 mg/kg bw per day; however, it was statistically significantly decreased only in males. Body weights were statistically significantly lower (9–14%) in females at 1000 mg/kg bw per day later in the study. Body weight gains were significantly decreased in males compared with controls at 1000 mg/kg bw per day. No effects were observed in relation to haematology, clinical chemistry, urinalysis, ophthalmology, organ weights or pathology.

In the first study in mice, the NOEL of 300 mg/kg bw per day was based on decreased body weights in males and increased amyloidosis in females at 1000 mg/kg bw per day. In the second study, the NOEL of 320 mg/kg bw per day was based on slightly decreased body weights (5%) in males and increased food consumption, primarily during weeks 58–69 in both sexes, at 1000 mg/kg bw per day.

DMH was negative for carcinogenicity when tested in these studies in mice and rats for 1.5–2 years. The NOELs for carcinogenicity were >1000 mg/kg bw per day in all four studies; in other words, no treatment-related effects were observed at any of the tested doses (TOXNET, 2008).

DMH was negative when tested in various in vitro tests for mutagenicity/genotoxicity (bacterial reverse mutation assay, unscheduled DNA synthesis in rat primary hepatocytes, forward mutations in TK locus in L5178Y mouse lymphoma cells, mammalian cell transformation assay and mammalian chromosomal aberrations) (TOXNET, 2008). It was also negative when tested in vivo in a bone marrow chromosomal aberration study in rats given single doses up to 2000 mg/kg bw (TOXNET, 2008).

A number of one-generation reproductive/developmental toxicity studies of DMH, three in rabbits and four in rats, were reported in TOXNET (2008). The reported NOELs/NOAELs for maternal toxicity were 500, 1000 and 1050 mg/kg bw per day for rabbits and 500, 1000, 1000 and 1000 mg/kg bw per day for rats in the various studies. The reported NOELs/NOAELs for developmental toxicity were 100, 1000 and 1050 mg/kg bw per day for rabbits and 1000, 1000, 1000 and 2000 mg/kg bw per day for rats. In one rabbit study, no developmental effects were observed at 100 mg/kg bw per day, whereas the percentages of fetuses with 27 presacral vertebrae was numerically increased at the higher dose levels of 500 and 1000 mg/kg bw per day, and adactyly and brachydactyly of the number 1 digit on both forepaws were noted in four fetuses in the same litter at the highest dose. In addition, a two-generation reproductive/developmental toxicity study in rats using doses of 0, 250, 500 and 1000 mg/kg bw per day found NOELs for systemic toxicity in both male parents and pups of 250 mg/kg bw per day, for systemic toxicity in female parents of 1000 mg/kg bw per day and for reproductive toxicity of 1000 mg/kg bw per day. No developmental toxicity was reported in this study. Slightly (<10%) decreased mean body weights in F₁ weanling males at or above 500 mg/kg bw per day and increased absolute and relative kidney (F₀) and pituitary (F₁) weights in males at 1000 mg/kg bw per day were observed. Significantly decreased body weights were observed in F₁ pups at or above 500 mg/kg bw per day and in F₂ pups at 1000 mg/kg bw per day. F₂ mean live litter size was significantly decreased at 1000 mg/kg bw per day prior to culling.

Dietary exposure

Dietary exposure to DMH from the consumption of beef and poultry treated with DBDMH can be estimated using the United States CSFII 1994–1996, 1998. The consumption of beef and poultry at the 90% percentile (94% eaters) is 150 g/person per day. Using this value and assuming that the DMH residue level of 0.005 mg/g meat (see section 2.8.2.1) represents a worst-case value for DMH residue on beef and poultry gives an exposure to DMH of 0.8 mg/person per day, or 0.013 mg/kg bw per day for a 60-kg adult.

Risk characterization

No ADI or TDI values have been identified for DMH. The margin of exposure between the lowest NOAEL of 100 mg/kg bw per day in a number of toxicity studies and the estimated dietary exposure to DMH of 0.013 mg/kg bw per day is approximately 8000. As the available information suggests that DMH is not genotoxic or carcinogenic and the database includes studies of carcinogenicity and reproductive effects, this large margin of exposure does not raise concerns for the health of consumers.

3.1.4.6 Haloacetic acids (HAAs)

Introduction

Haloacetic acids (HAAs) produced in the chlorination of drinking-water consist of a series of chlorinated and brominated forms. The chlorinated HAAs have been more thoroughly characterized toxicologically than their brominated analogues (IPCS, 2000). Dihaloacetates and trihaloacetates occur in significantly higher concentrations than the monohaloacetates (IPCS, 2000). The HAAs described in this section are the dominant forms found in drinking-water and the ones for which extensive toxicological data have been developed.

The description of the toxicology of HAAs in this section is based mainly on Environmental Health Criteria 216 (IPCS, 2000) and references therein.

Trichloroacetic acid/trichloroacetate (TCA)

Toxicological data

TCA (Cl₃CCOOH; CAS No. 76-03-9) is one of the weakest activators of the peroxisome proliferator-activated receptor known (Issemann & Green, 1990). It appears to be only marginally active as a peroxisome proliferator, even in rats (DeAngelo et al., 1989). Furthermore, treatment of rats with high levels of TCA in drinking-water does not induce liver tumours (DeAngelo et al., 1997). These data strongly suggest that TCA presents little, if any, carcinogenic hazard to humans at the low concentrations found in drinking-water.

From a long-term study of TCA given in drinking-water for 576 days to female B6C3F1 mice 7–8 weeks of age, a NOAEL of 40 mg/kg bw per day was estimated for absence of hepatic toxicity (Pereira, 1996). Application of an uncertainty factor of 1000 (10 each for interspecies and intraspecies variation and 10 for possible carcinogenicity) gave a TDI of 40 µg/kg bw per day (IPCS, 2000). IARC (1995) has classified TCA in Group 3 (not classifiable as to its carcinogenicity to humans).

Dietary exposure

No occurrence data on the levels of HAAs in foods, other than drinking-water, were identified by the expert meeting. Occurrence data relating to the concentration of TCA, DCA and dibromoacetic acid (DBA) in drinking-water in North America are summarized in Table 2.4 in chapter 2.

An estimate of mean dietary exposure to TCA, DCA and DBA arising from the consumption of drinking-water is presented in Table 3.5.

Table 3.5. Mean dietary exposure to HAAs from the consumption of drinking-water^a

Country	Exposure (µg/kg bw per day)		
	TCA	DCA	DBA
Australia	0.134	0.200	0.048
Belgium	0.013	0.020	0.005
Czech Republic	0.036	0.054	0.013
Denmark	0.107	0.159	0.039
Finland	0.108	0.161	0.039
France	0.040	0.060	0.015
Germany	0.009	0.013	0.003
Hungary	0.000	0.000	0.000
Iceland	0.083	0.123	0.030
Ireland	0.036	0.053	0.013
Italy	0.029	0.044	0.011
Netherlands	0.026	0.039	0.009
Norway	0.040	0.060	0.015
Slovakia	0.028	0.042	0.010
Sweden	0.062	0.092	0.022
United Kingdom	0.025	0.038	0.009
USA	0.134	0.199	0.048
WHO	0.313	0.467	0.113

^a The mean concentrations of the DBPs from the 12 drinking-water utilities in the USA and Canada were used in the estimate of dietary exposure.

Risk characterization

No data have been identified in relation to residues of TCA in food resulting from use of chlorine-based disinfectants. Therefore, no health concern was identified, but residue data are needed.

Dichloroacetic acid/dichloroacetate (DCA)

Toxicological data

The induction of mutations by DCA (Cl_2CHCOOH ; CAS No. 79-43-6) is very improbable at the low doses that would be encountered in chlorinated drinking-water (IPCS, 2000). The available data indicate that DCA differentially affects the replication rates of normal hepatocytes and hepatocytes that have been initiated (Pereira & Phelps, 1996). Based upon the above considerations, it was suggested that cancer risk estimates for DCA should be modified by incorporation of newly developing information on its comparative metabolism and modes of action to formulate a biologically based dose–response model, when such data become available (IPCS, 2000).

The effects of DCA appear to be closely associated with doses that induce hepatomegaly and glycogen accumulation in mice (IPCS, 2000). The NOAEL for these effects was approximately 40 mg/kg bw per day in an 8-week study in male B6C3F1 mice treated with DCA doses of approximately 20–600 mg/kg bw per day in drinking-water (Kato-Weinstein et al., 1998). By applying an uncertainty factor of 1000 (10 each for interspecies and intraspecies variation and 10 for the short duration of the study and possible carcinogenicity), a TDI of 40 $\mu\text{g}/\text{kg}$ bw per day was calculated (IPCS, 2000).

IARC (1995) has classified DCA in Group 3 (not classifiable as to its carcinogenicity to humans).

Dietary exposure

For details of dietary exposure to DCA, see the dietary exposure section for TCA above.

Risk characterization

No data have been identified in relation to residues of DCA in food resulting from use of chlorine-based disinfectants. Therefore, no health concern was identified, but residue data are needed.

Dibromoacetic acid/dibromoacetate (DBA)

Brominated acetic acids are formed in water that contains bromide, which strong oxidizers such as chlorine and ozone are capable of oxidizing to hypobromous acid. There are very few data available on the toxicity of these chemicals.

Toxicological data

Data on the carcinogenicity of brominated acetic acids are too preliminary to be useful in risk characterization (IPCS, 2000). However, there are data on the effects of DBA (Br_2CHCOOH ; CAS No. 631-64-1) on male reproduction.

No effects were observed on male reproduction in rats at daily doses of 2 mg/kg bw per day by gavage for 79 days, whereas higher doses, from 10 mg/kg bw per day, led to progressively more severe effects (increased retention of step 19 spermatids, marked atrophy of the seminiferous tubules) (Linder et al., 1997). From this NOAEL of 2 mg/kg bw per day, using an uncertainty factor of 100 (10 each for interspecies and intraspecies variation), a TDI of 20 $\mu\text{g}/\text{kg}$ bw per day was derived (IPCS, 2000).

Dietary exposure

For details of dietary exposure to DBA, see the dietary exposure section for TCA above.

Risk characterization

No data have been identified in relation to residues of DBA in food resulting from use of chlorine-based and ozone disinfectants. Therefore, no health concern was identified, but residue data are needed.

3.1.4.7 Haloacetonitriles (HANs)

Introduction

Toxicological data are quite limited on haloacetonitriles (HANs). Dichloroacetonitrile (DCAN) (CHCl_2CN ; CAS No. 3018-12-0), bromochloroacetonitrile (BCAN) (CHBrClCN ; CAS No. 83463-62-1) and dibromoacetonitrile (DBAN) (CHBr_2CN ; CAS No. 3252-43-5) are the most important in terms of concentrations found in drinking-water (IPCS, 2000). Without appropriate human data or an animal study that involves a substantial portion of an experimental animal's lifetime, there is no generally accepted basis for estimating carcinogenic risk from the HANs (IPCS, 2000).

The description of the toxicology of HANs in this section is based mainly on Environmental Health Criteria 216 (IPCS, 2000) and references therein.

Dichloroacetonitrile (DCAN)

Toxicological data

There are some data on the reproductive toxicity of DCAN. A NOAEL of 15 mg/kg bw per day was determined for DCAN in a reproductive toxicity study in Long-Evans rats in which DCAN was given at doses of 0, 5, 15, 25 or 45 mg/kg bw per day from days 6 to 18 of gestation (Smith et al., 1989). By applying an uncertainty factor of 1000 (10 each for intraspecies and interspecies variation and 10 for severity of effects), a TDI of 15 $\mu\text{g}/\text{kg}$ bw per day was derived (WHO, 1993).

Dietary exposure

No occurrence data relating to HANs in food, other than drinking-water, were identified by the expert meeting. Occurrence data relating to the concentration of the HANs in drinking-water in North America are summarized in Table 2.4 in chapter 2.

An estimate of mean dietary exposure arising from the consumption of drinking-water containing those HANs for which toxicological information was available has been calculated and is presented in Table 3.6.

Risk characterization

No data have been identified in relation to residues of DCAN in food resulting from use of chlorine-based disinfectants. Therefore, no health concern was identified, but residue data are needed.

Dibromoacetonitrile (DBAN)

Toxicological data

Reproductive and developmental effects were observed for DBAN only at doses that exceeded those established for general toxicity (about 45 mg/kg bw per day) (Smith et al., 1987).

Table 3.6. Mean dietary exposure to HANs from the consumption of drinking-water^a

Country	Exposure (µg/kg bw per day)		
	DCAN	DBAN	TCAN
Australia	0.020	0.009	0.000
Belgium	0.002	0.001	0.000
Czech Republic	0.005	0.002	0.000
Denmark	0.016	0.007	0.000
Finland	0.016	0.007	0.000
France	0.006	0.003	0.000
Germany	0.001	0.001	0.000
Hungary	0.000	0.000	0.000
Iceland	0.012	0.005	0.000
Ireland	0.005	0.002	0.000
Italy	0.004	0.002	0.000
Netherlands	0.004	0.002	0.000
Norway	0.006	0.003	0.000
Slovakia	0.004	0.002	0.000
Sweden	0.009	0.004	0.000
United Kingdom	0.004	0.002	0.000
USA	0.020	0.009	0.000
WHO	0.047	0.020	0.001

^a The mean concentrations of the DBPs from the 12 drinking-water utilities in the USA and Canada were used in the estimate of dietary exposure.

A NOAEL of 23 mg/kg bw per day was determined for DBAN given at doses of 6, 23 or 45 mg/kg bw per day dissolved in corn oil in a 90-day toxicity study in CD rats (Hayes, Condie & Borzelleca, 1986). By applying an uncertainty factor of 1000 (10 each for intraspecies and interspecies variation and 10 for the short duration of the study), a TDI of 23 µg/kg bw per day was derived (WHO, 1993; IPCS, 2000).

Dietary exposure

For dietary exposure to DBAN, see the dietary exposure section for DCAN above.

Risk characterization

No data have been identified in relation to residues of DBAN in food resulting from use of chlorine-based and ozone disinfectants. Therefore, no health concern was identified, but residue data are needed.

Trichloroacetonitrile (TCAN)

Toxicological data

LOAELs for TCAN (CCl₃CN; CAS No. 545-06-2) were identified as 7.5 mg/kg bw per day for embryotoxicity and 15 mg/kg bw per day for developmental effects in rats (Smith et al., 1988). However, later studies suggest that these responses were dependent upon the vehicle used (Christ et al., 1996).

No TDI could be established for TCAN (IPCS, 2000).

Dietary exposure

For dietary exposure to TCAN, see the dietary exposure section for DCAN above.

Risk characterization

No data have been identified in relation to residues of TCAN in food resulting from use of chlorine-based and ozone disinfectants. Therefore, no health concern was identified, but residue data are needed.

3.1.4.8 Halofuranones (MX and MX analogues)

Introduction

3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) (C₅H₃Cl₃O₃; CAS No. 77439-76-0) is formed by the reaction of chlorine with complex organic matter in drinking-water or aqueous solutions after chlorination or chloramination. Brominated analogues are formed when bromide is present in addition to organic material. MX is the member of the hydroxyfuranone class that has been most extensively studied; much less is known about the other chlorinated and brominated halofuranones.

The MX-related halofuranones were ranked by expert structure–activity relationship judgement with emphasis on genotoxic cancer potential (Woo et al., 2002). Of 10 MX-related halofuranones, 3 analogues—3-chloro-4-(bromochloromethyl)-5-hydroxy-2(5H)-furanone (BMX-1), 3-chloro-4-(dibromomethyl)-5-hydroxy-2(5H)-furanone (BMX-2; CAS No. 132059-52-0) and 3-bromo-4-(dibromomethyl)-5-hydroxy-2(5H)-furanone (BMX-3)—were considered to be of moderate to high concern because of their structural analogy to MX, which has been shown to be a multitarget carcinogen in the rat (see below), and their positive mutagenicity data in the Ames test with potencies comparable to those of MX. One analogue, 2,3-dichloro-4-oxobutenoic acid (3,4-dichloro-5-hydroxy-2(5H)-furanone, mucochloric acid; CAS No. 87-56-9), was of moderate concern, because of structural analogy to MX and positive genotoxicity data (Ames test, *Escherichia coli*, sister chromatid exchange in CHO cells), but less active than MX. Four MX analogues were considered to be of low to moderate concern: (*E*)-2-chloro-3-(dichloromethyl)-4-oxobutenoic acid (EMX), 3-chloro-4-(dichloromethyl)-2(5H)-furanone (red-MX; CAS No. 122551-89-7), dihydro-4,5-dichloro-2(3H)-furanone and 5-hydroxy-5-trichloromethyl-2-furanone, with more or less structural analogy to MX, but less potency. Two analogues were of marginal concern: 2-chloro-3-(dichloromethyl)-butenedioic acid (ox-MX) and (*E*)-2-chloro-3-(dichloromethyl)-butenedioic acid (ox-EMX, in later papers called ox-MX; Krasner et al., 2006). As the data available so far indicate that none of these analogues has higher carcinogenic potential than MX itself, the toxicity of MX is used to represent the “worst-case” toxicity to halofuranones. Non-cancer effects, as well as CAS numbers, are not known for most of these substances. Other brominated EMX analogues are reported more recently: (*E*)-2-chloro-3-(bromochloromethyl)-4-oxobutenoic acid (BEMX-1), (*E*)-2-chloro-3-(dibromomethyl)-4-oxobutenoic acid (BEMX-2) and (*E*)-2-bromo-3-(dibromomethyl)-4-oxobutenoic acid (BEMX-3), and an isomer of EMX, (*Z*)-2-chloro-3-(dichloromethyl)-4-oxobutenoic acid (ZMX) (Krasner et al., 2006; Richardson et al., 2007).

The toxicology of MX is evaluated and described in Environmental Health Criteria 216 (IPCS, 2000). In addition, IARC (2004), the WHO *Guidelines for drinking-water quality* (WHO, 2006a) and some original publications have been used as sources of information in this section.

Toxicological data

The critical effects of MX appear to be its mutagenicity and carcinogenicity, and this section therefore has focused on the carcinogenicity.

MX was administered to Wistar rats (50 per sex per group) in drinking-water for 104 weeks at 0, 0.4, 1.3 or 5.0 mg/kg bw per day for males and 0, 0.6, 1.9 or 6.6 mg/kg bw per day for females (Komulainen et al., 1997). Dose-dependent increases in the incidence of several tumours were observed in the rats, whereas the same MX doses had no obvious toxic effects on the animals. Increases in tumours of the lung, mammary gland, haematopoietic system, liver, pancreas, adrenal gland and thyroid were observed, but few showed a clear dose-response (IPCS, 2000). In IPCS (2000), it was noted that the data from this experiment indicate that MX induces thyroid and bile duct tumours. An increased incidence of thyroid tumours was seen at the lowest dose of MX administered (0.4 mg/kg bw per day in males and 0.6 mg/kg bw per day in females). The induction of thyroid tumours with high-dose chemicals has long been associated with halogenated compounds. The induction of thyroid follicular tumours could involve modifications in thyroid function or a mutagenic mode of action. Mean plasma levels of thyroid hormones (T_4 , T_3 and TSH) at the end of the study were not significantly different between MX-treated rats and controls, suggesting that the thyroid tumours were not caused indirectly by excess hormonal stimulation. A dose-related increase in the incidence of cholangiomas and cholangiocarcinomas was also observed, beginning at the low dose in female rats, with a more modest response in males. The increase in cholangiomas and cholangiocarcinomas in female rats was used to derive a slope factor for cancer. The 95% upper confidence limit for a 10^{-5} lifetime cancer risk based on the linearized multistage model was calculated to be 0.06 $\mu\text{g}/\text{kg}$ bw per day (IPCS, 2000).

McDonald & Komulainen (2005) calculated cancer potency for MX from the carcinogenicity experiment of Komulainen et al. (1997), using either a linearized multistage model or a BMD model and Monte Carlo analysis. They obtained similar results by both methods: a mean cancer potency of $2.3 (\text{mg}/\text{kg per day})^{-1}$ and an upper 95th-percentile estimate of $4.5 (\text{mg}/\text{kg per day})^{-1}$. Using the upper 95th-percentile estimate of cancer potency of $4.5 (\text{mg}/\text{kg per day})^{-1}$, an intake of 2 litres/day and a 70-kg body weight resulted in an estimated concentration of 7.8 ng/l corresponding to a 10^{-6} lifetime cancer risk for MX.

There were no studies of toxicity or metabolism of MX or related compounds reported in humans (IPCS, 2000). There are data to suggest that MX or a mutagenically active metabolite reaches the systemic circulation in experimental animals (IPCS, 2000). Mutagenic activity has been detected in various organs and tissues using doses as low as 4.3 mg/kg bw. The available data are too limited to provide much more than very general guidance as to whether MX or a metabolite reaches critical target organs in humans also (IPCS, 2000).

MX is a potent, direct-acting mutagen that induces primarily GC \rightarrow TA transversions in both bacterial and mammalian cells (IARC, 2004). It induces DNA damage in bacterial and mammalian cells, as well as in rodents in vivo. MX is a chromosomal mutagen in mammalian cells and in rats, and it induces mammalian cell transformation in vitro. The MX-associated thyroid gland tumors in rats described above are caused by mechanisms other than TSH-mediated hormonal promotion. An overall evaluation of all the mutagenicity and genotoxicity data shows that MX is mutagenic and genotoxic both in vitro and in vivo. There is inadequate evidence in humans and limited evidence in experimental animals for the carcinogenicity of MX. IARC (2004) has classified MX in Group 2B: possibly carcinogenic to humans on the basis of rat tumorigenicity and its strong mutagenicity.

Dietary exposure

No occurrence data for halofuranones in food, other than drinking-water, were identified. Occurrence data relating to the concentration of the halofuranones in drinking-water in North America are summarized in Table 2.5 in chapter 2.

An estimate of mean dietary exposure arising from the consumption of drinking-water has been calculated and is presented in Table 3.7. Occurrence data were also available for ox-MX, with the concentrations being reported as “0” (i.e. not detected), although the limit of detection (LOD) was not available to the reviewer.

Table 3.7. Mean dietary exposure to halofuranones (MX and MX analogues) from the consumption of drinking-water^a

Country	Exposure (µg/kg bw per day)									
	BMX-1	BEMX-1	BMX-2	BEMX-2	BMX-3	BEMX-3	MX	Red-MX	EMX	ZMX
Australia	0.0005	0.0014	0.0004	0.0017	0.0001	0.0014	0.0016	0.0005	0.0002	0.0002
Belgium	0.0000	0.0001	0.0000	0.0002	0.0000	0.0001	0.0002	0.0000	0.0000	0.0000
Czech Republic	0.0001	0.0004	0.0001	0.0005	0.0000	0.0004	0.0004	0.0001	0.0000	0.0000
Denmark	0.0004	0.0011	0.0003	0.0014	0.0000	0.0011	0.0012	0.0004	0.0001	0.0001
Finland	0.0004	0.0012	0.0003	0.0014	0.0000	0.0011	0.0013	0.0004	0.0001	0.0001
France	0.0001	0.0004	0.0001	0.0005	0.0000	0.0004	0.0005	0.0001	0.0001	0.0000
Germany	0.0000	0.0001	0.0000	0.0001	0.0000	0.0001	0.0001	0.0000	0.0000	0.0000
Hungary	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Iceland	0.0003	0.0009	0.0002	0.0011	0.0000	0.0009	0.0010	0.0003	0.0001	0.0001
Ireland	0.0001	0.0004	0.0001	0.0005	0.0000	0.0004	0.0004	0.0001	0.0000	0.0000
Italy	0.0001	0.0003	0.0001	0.0004	0.0000	0.0003	0.0003	0.0001	0.0000	0.0000
Netherlands	0.0001	0.0003	0.0001	0.0003	0.0000	0.0003	0.0003	0.0001	0.0000	0.0000
Norway	0.0001	0.0004	0.0001	0.0005	0.0000	0.0004	0.0005	0.0001	0.0001	0.0000
Slovakia	0.0001	0.0003	0.0001	0.0004	0.0000	0.0003	0.0003	0.0001	0.0000	0.0000
Sweden	0.0002	0.0007	0.0002	0.0008	0.0000	0.0006	0.0007	0.0002	0.0001	0.0001
United Kingdom	0.0001	0.0003	0.0001	0.0003	0.0000	0.0003	0.0003	0.0001	0.0000	0.0000
USA	0.0005	0.0014	0.0004	0.0017	0.0001	0.0014	0.0016	0.0005	0.0002	0.0002
WHO	0.0011	0.0033	0.0009	0.0040	0.0001	0.0032	0.0037	0.0011	0.0004	0.0004

^a The mean concentration of the DBPs from the 12 drinking-water utilities in the USA and Canada were used in the estimate of dietary exposure.

Risk characterization

No data have been identified in relation to residues of halofuranones in food resulting from use of chlorine-based and ozone disinfectants. Therefore, no health concern was identified, but residue data are needed.

3.1.4.9 N-Nitrosamines

Introduction

N-Nitrosamines are well-known environmental chemicals that can be metabolized into potent genotoxic agents. N-Nitrosodimethylamine (NDMA) is a model compound for this class of substances. Currently, five N-nitrosamines have been defined as DBPs in

drinking-water, and they are found to increase in concentration in the distribution system: NDMA, *N*-nitrosopyrrolidine (NPYR), *N*-nitrosomorpholine (NMOR), *N*-nitrosodiphenylamine (NDPA; CAS No. 86-30-6) and *N*-nitrosopiperidine (NPIP) (Richardson et al., 2007). Nitrosamines detected in food are NDMA, *N*-nitrosoproline (NPRO), NPYR and NPIP (Jakszyn et al., 2004a).

N-Nitrosamines were not included in Environmental Health Criteria 216. The information in this document has been taken from the WHO *Guidelines for drinking-water quality* (WHO, 2008b,c), IARC (1978, 1982, 1987), a Concise International Chemical Assessment Document (IPCS, 2002), the toxicology database Integrated Risk Information System, or IRIS (USEPA, 2008), as well as some original publications.

Toxicological data

As NDMA and the *N*-nitrosamines as a group are potent genotoxic and carcinogenic substances, cancer is the critical end-point for risk characterization. In addition to being the best-characterized end-point, in general, tumours occur at the lowest concentration compared with those typically reported to induce non-cancer effects (IPCS, 2002). In this section, the emphasis has therefore been put on cancer as the end-point of chronic toxicity. However, effects of NDMA on the liver and kidney in repeated-dose toxicity studies (>0.2 mg/kg bw per day), embryo toxicity and embryo lethality in single-dose developmental studies (20–30 mg/kg bw) and a range of immunological effects, such as suppression of humoral and cell-mediated immunity, reversible at lowest concentrations (5 mg/l), have been reported (IPCS, 2002). In case reports, liver failure, brain haemorrhage and death have been attributed to the ingestion of NDMA by humans (doses not stated) (IPCS, 2002).

N-Nitrosamines can be metabolized into potent genotoxic agents. The genotoxicity of NDMA, the model compound for this class, is well studied. The results show that NDMA is mutagenic and clastogenic in a wide array of test systems *in vitro* in the presence or absence of metabolic activation in bacterial and mammalian cells (human and rodents) (IARC, 1978; Liteplo & Meek, 2001). Clear evidence of genotoxicity in many organs is also observed in various test systems *in vivo*. NDMA is activated to a mutagen mainly by cytochrome P450 2E1, whereas other *N*-nitrosamines were activated by various other P450 enzymes in strains of *Salmonella* containing human P450 genes (Fujita & Kamataki, 2001). The mutagenic and genotoxic potency varies between the *N*-nitrosamines. NDPA, unlike most of the other nitrosamines, is not clearly mutagenic and genotoxic in bacterial or mammalian cells *in vitro* or *in vivo*, as most studies were negative or gave conflicting results (McGregor, 1994). There were also fewer studies available showing carcinogenicity of NDPA in experimental animals.

Many nitrosamines have been tested extensively for carcinogenicity, and nearly all have shown carcinogenic effects in a variety of species exposed through various routes (IARC, 1978, 1982, 1987). The primary sites of tumour formation for the nitrosamines are the oesophagus and liver. However, other organs, including the urinary bladder, brain and lungs, are also target organs. A mixture of three *N*-nitrosamines in low doses—NPYR (0.4 mg/kg bw per day), *N*-nitrosodiethylamine (NDEA) (0.1 mg/kg bw per day) and *N*-nitrosodiethanolamine (NDELA) (CAS No. 1116-54-7; 2.0 mg/kg bw per day)—given in drinking-water to rats for their lifetime showed additivity for liver tumours (Berger, Schmähl & Zerban, 1987). A study evaluated liver and oesophageal tumours induced by NDEA or NDMA in 4080 rats at 15 doses given in drinking-water during the lifetime of the rats (Peto et al., 1991a,b). The results from this study showed that exposures to concentrations of NDEA or NDMA as low as 1 mg/l in the drinking-water resulted in 25% of the animals developing liver tumours, a dose of 0.1 mg/l caused about 2.5% and a dose of 0.01 mg/l caused about 0.25%. etc., with no indication of a threshold effect (Peto et al., 1991a). The liver tumour risk from 2 years of chronic exposure of rats to very low doses of NDMA would

be in the order of 0.03% (males) and 0.04% (females) per microgram per kilogram body weight per day, and for NDEA, in the order of 0.06% (males) and 0.1% (females) (Peto et al., 1991b).

Although no epidemiological data were available at the time, IARC (1978) found sufficient evidence in animals for the carcinogenicity of several *N*-nitrosamines and noted that these compounds should be regarded as if they were carcinogenic to humans. IARC (1987) has classified two *N*-nitrosamines in Group 2A: probably carcinogenic to humans: NDMA and NDEA, based on no adequate data in humans and sufficient evidence of carcinogenicity in experimental animals. Several other *N*-nitrosamines are classified by IARC (1987) in Group 2B: possibly carcinogenic to humans, including NPYR, NMOR, NPIP and NDELA, based on no adequate data in humans and sufficient evidence of carcinogenicity in experimental animals. Other *N*-nitrosamines are classified by IARC (1987) in Group 3: not classifiable as to their carcinogenicity to humans, including NDPA and NPRO, based on no adequate data in humans and limited or inadequate evidence of carcinogenicity in experimental animals. IRIS (USEPA, 2008) identifies the following nitrosamines as B2, probable human carcinogens: NDMA, NDEA, NDELA, NPYR and NDPA.

The WHO *Guidelines for drinking-water quality* (WHO, 2008c) indicate that for NDMA, a concentration of 100 ng/l in drinking-water is associated with an upper-bound excess lifetime cancer risk of 1 in 100 000. The USEPA (2008) provided values of 2, 100, 200 and 70 000 ng/l for NDEA, NDELA, NPYR and NDPA, respectively, as representing the 95% lower bound on the estimated concentration of the chemical in drinking-water associated with a cancer risk of 1 in 100 000.

Dietary exposure

Water treatment plants incorporating a chlorination process (e.g. sodium hypochlorite and/or chloramine) produce nitrosamines, including NDMA, as DBPs (Richardson, 2003). In assessing the dietary exposure to nitrosamines formed as a result of the use of chlorine-containing disinfectants, it is important to consider their other environmental sources. WHO (2006b) reported a number of different routes by which NDMA enters the environment and drinking-water, including through 1) being a by-product of industrial processes for industries such as rubber manufacturing, leather tanning, pesticide manufacturing, food processing, foundries and dye manufacturing, 2) sewage treatment plant effluent, 3) runoff from agricultural production and 4) being a contaminant in pesticide formulations. Furthermore, the addition of nitrites and nitrates to foodstuffs to reinforce the preserving effect of smoking, salting or cooking can lead to the formation of nitrosamines (EFSA, 2003).

The ingestion of drinking-water that contains NDMA appears to contribute only a small fraction to the overall NDMA exposure (Environment Canada & Health Canada, 2001). Rough estimates of the exposure to various sources of NDMA in Canada indicate that water contributes less than 10% to the overall exposure (IPCS, 2002). A report from the USEPA (Fristachi & Rice, 2005) indicates that the trace levels of NDMA in drinking-water contribute from 0.001% to 0.55% (or less than 1%) to overall human exposure to NDMA.

Based on a worst-case estimation of exposure to NDMA-contaminated air, water and food, the daily NDMA intake of a 20- to 59-year-old would be 0.005–0.016 µg/kg bw per day (IPCS, 2002). Daily intake of NDMA from ingestion of drinking-water was estimated at 0.0003–0.001 µg/kg bw per day, based on a mean NDMA concentration of 0.012 µg/l and a maximum concentration of 0.04 µg/l obtained from 20 samples from four water treatment plants using a pre-blended polyamine/alum product during the treatment process (IPCS, 2002). The low-end value is similar to those observed in some chloramine-treated drinking-water, which shows that human exposure to NDMA via drinking-water is likely to provide a relative contribution below 10% of total exposure.

In a home not containing environmental tobacco smoke, the major source of exposure to NDMA is food, at 0.0043–0.011 µg/kg bw per day (WHO, 2006b). If there is regular indoor exposure to environmental tobacco smoke, then this source would exceed all the other sources combined by almost an order of magnitude, at 0.05 µg/kg bw per day (WHO, 2006b).

Risk characterization

It can be concluded that the formation of nitrosamines is attributable to several mechanisms, interaction with active chlorine compounds being only a minor one. Although there are no data available on nitrosamine residues in food resulting from disinfection processes, they are likely to be minimal compared with other sources of exposure. Therefore, no health concerns are identified.

3.1.4.10 Trihalomethanes (THMs)

Introduction

THMs are generally the most prevalent by-products of drinking-water disinfection by chlorine (IPCS, 2000). A variety of non-neoplastic toxic effects have been associated with short-term and long-term exposure of experimental animals to high doses of THMs. The four most common THMs—chloroform, BDCM, DBCM and bromoform—have been shown to be carcinogenic to rodents in high-dose chronic studies, and therefore cancer following chronic exposure is the primary hazard of concern for this class of DBPs (IPCS, 2000).

The description of the toxicology of THMs in this section is based mainly on Environmental Health Criteria 216 (IPCS, 2000) and references therein.

Chloroform

Chloroform is generally the predominant THM in chlorinated water and is also the most extensively studied chemical of this class (IPCS, 2000).

Toxicological data

Owing to the weight of evidence indicating that chloroform can induce cancer in animals only after chronic exposure to cytotoxic doses, it is clear that exposures to low concentrations of chloroform in drinking-water do not pose carcinogenic risks (IPCS, 2000). Direct DNA reactivity and mutagenicity cannot be considered to be key factors in chloroform-induced carcinogenesis in experimental animals. A substantial body of data demonstrates a lack of direct *in vivo* or *in vitro* genotoxicity of chloroform. If THMs produce their genotoxic effects primarily via the glutathione conjugation mechanism, the results of Pegram et al. (1997) indicate that chloroform would be mutagenic in mammals only at lethal doses. There is, however, compelling evidence to support a mode of action for tumour induction based on metabolism of chloroform by the target cell population, followed by cytotoxicity of oxidative metabolites and regenerative cell proliferation. A number of recent studies support the hypothesis that chloroform acts to produce cancer in rodents through a non-genotoxic/cytotoxic mode of action, with carcinogenesis resulting from events secondary to chloroform-induced cytolethality and regenerative cell proliferation (Larson, Wolf & Butterworth, 1994a,b; Pereira, 1994; Larson et al., 1996; Templin et al., 1996a,b,c, 1998). These studies have shown that organ toxicity and regenerative hyperplasia are associated with the tumorigenicity of chloroform and are apparently the key steps in its carcinogenic mode of action. Thus, sustained toxicity would result in tumour development. Chloroform induces liver and kidney tumours in long-term rodent cancer bioassays only at doses that induce frank cytotoxicity in these target organs. Furthermore, there are no instances of chloroform-induced

tumours that are not preceded by this pattern of dose-dependent toxic responses (Golden et al., 1997).

The NOAEL for cytolethality and regenerative hyperplasia in female mice was 10 mg/kg bw per day after administration of chloroform in doses of 0, 3, 10, 34, 238 or 477 mg/kg bw per day in corn oil (5 days/week) for 3 weeks (Larson, Wolf & Butterworth, 1994b). Based on the mode of action evidence for chloroform carcinogenicity and applying an uncertainty factor of 1000 (10 each for intraspecies and interspecies variation and 10 for the short duration of the study) to this NOAEL for cytotoxicity in mice, a TDI of 10 µg/kg bw per day was derived for chloroform (IPCS, 2000). Subsequently, IPCS (2004) proposed a TDI of 15 µg/kg bw per day, based upon a study in which fatty cysts developed in the liver of dogs given chloroform orally at 15 mg/kg bw per day for 7.5 years. This slightly higher TDI was adopted in the more recent WHO drinking-water guidelines (WHO, 2005b).

Dietary exposure

For the purpose of the exposure assessment, presented in Table 3.8, a chloroform concentration of 0.3 mg/kg was used, representing the highest level found in cooked chicken (see section 2.6.3).

Table 3.8. Estimates of per capita dietary exposure to chloroform, following the dipping of chicken in chlorine, based on 13 GEMS/Food consumption cluster diets

	Exposure (µg/kg bw per day) ^{a,b,c}												
	A	B	C	D	E	F	G	H	I	J	K	L	M
Chicken meat	0.03	0.22	0.14	0.11	0.19	0.13	0.06	0.22	0.08	0.02	0.32	0.13	0.48
Poultry ^d	0.04	0.29	0.16	0.12	0.31	0.14	0.09	0.66	0.13	0.02	0.73	0.14	0.58

^a Assuming a 60-kg average body weight.

^b WHO consumption cluster diets based on food balance sheet data; August 2006 version used (<http://www.who.int/entity/foodsafety/chem/ClusterDietsAug06.xls>).

^c Concentration of 0.3 mg/kg in chicken and other poultry was used for the exposure assessment.

^d The poultry exposure assessment has been presented on the assumption that the dipping use of chlorine is also applied to other poultry.

Occurrence data relating to the concentration of the four most important THMs in drinking-water in North America are summarized in Table 2.4 in chapter 2. An estimate of mean dietary exposure arising from the consumption of drinking-water has been calculated and is presented in Table 3.9.

Risk characterization

The estimated range of dietary exposure to chloroform from active chlorine-treated poultry, based on the highest detected concentration in cooked chicken, is up to 0.73 µg/kg bw per day. Adding this to the highest estimated intake from drinking-water (0.53 µg/kg bw per day) results in a total dietary exposure that is well below the TDI of 10 µg/kg bw per day (or the higher TDI of 15 µg/kg bw per day, based on a study in dogs; IPCS, 2004).

Bromodichloromethane (BDCM)

Toxicological data

Of the brominated THMs, BDCM is of particular interest because it has produced tumours in both rats and mice and at several sites (liver, kidney, large intestine) after gavage in corn oil (NTP, 1987). The induction of colon tumours in rats by BDCM is also interesting because of the epidemiological associations of THM with colorectal cancer (IPCS, 2000).

BDCM and other brominated THMs are also weak mutagens (Pegram et al., 1997; IARC, 1999a). In the NTP (1987) study, BDCM caused tumours at lower doses and at more target sites compared with any of the other THMs (IPCS, 2000).

Table 3.9. Mean dietary exposure to THMs from the consumption of drinking-water^a

Country	Exposure (µg/kg bw per day)			
	Chloroform	BDCM	DBCM	Bromoform
Australia	0.228	0.143	0.093	0.030
Belgium	0.023	0.014	0.009	0.003
Czech Republic	0.061	0.038	0.025	0.008
Denmark	0.182	0.114	0.074	0.024
Finland	0.184	0.115	0.075	0.024
France	0.069	0.043	0.028	0.009
Germany	0.015	0.009	0.006	0.002
Hungary	0.000	0.000	0.000	0.000
Iceland	0.141	0.088	0.057	0.019
Ireland	0.061	0.038	0.025	0.008
Italy	0.050	0.031	0.020	0.007
Netherlands	0.045	0.028	0.018	0.006
Norway	0.068	0.043	0.028	0.009
Slovakia	0.048	0.030	0.019	0.006
Sweden	0.105	0.066	0.043	0.014
United Kingdom	0.043	0.027	0.018	0.006
USA	0.228	0.142	0.093	0.030
WHO	0.533	0.333	0.217	0.070

^a The mean concentrations of the DBPs from the 12 drinking-water utilities in the USA and Canada were used in the estimate of dietary exposure.

In a 2-year bioassay, BDCM was given by corn oil gavage 5 days/week to F344 rats and B6C3F1 mice (50 animals per sex per group) at doses of 0, 50 or 100 mg/kg bw per day (male and female rats), 0, 25 or 50 mg/kg bw per day (male mice) or 0, 75 or 150 mg/kg bw per day (female mice) (NTP, 1987). BDCM induced tumours, in conjunction with cytotoxicity and increased proliferation, in the kidneys of mice and rats at doses of 50 and 100 mg/kg bw per day, respectively (NTP, 1987). Large intestinal tumours in rats occurred after exposure to both 50 and 100 mg/kg bw per day.

However, a more recent study by NTP (2006) of BDCM given in drinking-water to male F344/N rats and female B6C3F1 mice gave no indication of carcinogenicity. In this 2-year drinking-water study, there was no evidence of carcinogenic activity of BDCM in male F344/N rats exposed to target concentrations of 0, 175, 350 or 700 mg/l (equivalent to average daily BDCM doses of approximately 0, 6, 12 or 25 mg/kg bw). There was no evidence of carcinogenic activity of BDCM in female B6C3F1 mice exposed to target concentrations of 0, 175, 350 or 700 mg/l (equivalent to average daily BDCM doses of approximately 0, 9, 18 or 36 mg/kg bw). In this study, no effects on survival rates and no non-neoplastic effects were found in either rats or mice (NTP, 2006). In the rats, the body weights were similar in the exposed groups and the control animals. In the mice, all exposed groups showed lower final body weights than controls, but that was attributed to decreased water consumption because of poor palatability of the dosed water (NTP, 2006).

Factors such as the stability of BDCM in water, the influence of the corn oil vehicle, different rates of absorption and delivery of parent compound to target organs, and different rates of metabolism after gavage and drinking-water exposure may have contributed to the contrasting results in the two studies (NTP, 2006). The results of in vitro mutagenicity studies with BDCM were mixed, with negative effects in *Salmonella* and for chromosomal aberrations in CHO cells, but positive results for mutations in mouse lymphoma cells and sister chromatid exchange in CHO cells, in the presence but not the absence of metabolic activation. In vivo studies of chromosome damage were negative (NTP, 2006).

Dietary exposure

Dietary exposure to BDCM may occur as a result of the use of DBDMH in the processing of poultry or from the treatment of beef with aqueous solutions of DBDMH. Dietary exposure to BDCM from the consumption of beef and poultry treated with DBDMH can be estimated using the CSFII 1994–1996, 1998. The consumption of beef and poultry at the 90% percentile (94% eaters) is 150 g/person per day. Using this value and assuming that the residue level of 0.0004 µg/g (see section 2.8.2.3) represents a worst-case value for BDCM residue on beef and poultry gives an exposure to BDCM of 0.06 µg/person per day, or 0.001 µg/kg bw per day for a 60-kg person.

Risk characterization

The margin between the lowest dose of BDCM found to cause tumours in rats and mice when administered by gavage in corn oil (50 mg/kg bw per day) and the estimated human dietary exposure resulting from residues in treated meat is in the region of 50 million. No effects were observed in the more recent carcinogenicity study with BDCM administered in drinking-water to male rats and female mice at doses up to approximately 25 and 36 mg/kg bw per day, respectively (NTP, 2006).

In view of the lack of mutagenicity in vivo and the lack of carcinogenicity in the recent NTP study with administration of BDCM in drinking-water, it is considered highly unlikely that BDCM residues present a concern for health.

Dibromochloromethane (DBCM)

Toxicological data

In a 2-year corn oil gavage study, DBCM was given for 5 days/week to F344/N rats at doses of 0, 40 or 80 mg/kg bw per day and to B6C3F1 mice at doses of 0, 50 or 100 mg/kg bw per day (NTP, 1985). DBCM induced hepatic tumours in female mice, but not in rats, at a dose of 100 mg/kg bw per day (NTP, 1985).

The brominated THMs are considered to be weakly mutagenic, with activation involving glutathione conjugation. DBCM and bromoform have been reported to be more potent than other brominated THMs (DeMarini et al., 1997; Pegram et al., 1997). However, WHO (2005b) considered the evidence of genotoxicity to be inconclusive. IARC (1991) has classified DBCM in Group 3 (not classifiable as to its carcinogenicity to humans).

In previous evaluations, it has been suggested that the corn oil vehicle may play a role in the induction of tumours in female mice by affecting the bioavailability of DBCM in the long-term study (WHO, 1996). A NOAEL for DBCM of 30 mg/kg bw per day was established in a 13-week corn oil gavage study, based on the absence of histopathological effects in the liver of rats (NTP, 1985). Based on this NOAEL and using an uncertainty factor of 1000 (10 each for interspecies and intraspecies variation and 10 for the short duration of the study and possible carcinogenicity), a TDI for DBCM of 30 µg/kg bw per day was derived (IPCS, 2000). In a subsequent evaluation, the NOAEL was corrected to allow for

gavage exposure on 5 days/week, resulting in establishment of a TDI of 21.4 µg/kg bw per day (WHO, 2005b).

Dietary exposure

DBCM is a potential DBP resulting from the use of DBDMH in the processing of poultry and beef. Dietary exposure to DBCM from the consumption of beef and poultry treated with DBDMH can be estimated using the CSFII 1994–1996, 1998. The consumption of beef and poultry at the 90% percentile (94% eaters) is 150 g/person per day. Using this value and assuming that the residue level of 0.0004 µg/g meat (see section 2.8.2.3) represents a worst-case value for DBCM residue on beef and poultry gives an exposure to DBCM of 0.06 µg/person per day, or 0.001 µg/kg bw per day for a 60-kg person.

Risk characterization

The estimated dietary exposure of 0.001 µg/kg bw per day (upper bound) is considerably below the DBCM TDI of 21.4 µg/kg bw per day, and therefore no health concerns were identified.

Bromoform

Toxicological data

In a 2-year corn oil gavage study, bromoform was given to F344/N rats (50 per sex per dose) and female B6C3F1 mice (50 per dose) at doses of 0, 100 or 200 mg/kg bw per day, 5 days/week (NTP, 1989). Male mice (50 per dose) received doses of 0, 50 or 100 mg/kg bw per day. Bromoform induced a small increase in tumours of the large intestine in rats at a dose of 200 mg/kg bw per day (NTP, 1989).

Bromoform was weakly mutagenic in a number of assays, with activation mediated via glutathione conjugation (DeMarini et al., 1997; Pegram et al., 1997). However, WHO (2005b) considered the evidence of genotoxicity to be inconclusive. IARC (1999b) has classified bromoform in Group 3 (not classifiable as to its carcinogenicity to humans).

A NOAEL for bromoform is 25 mg/kg bw per day based on the absence of liver lesions in rats after 13 weeks of dosing by corn oil (NTP, 1989). Based on this NOAEL and using an uncertainty factor of 1000 (10 each for interspecies and intraspecies variation and 10 for the short duration of the study and possible carcinogenicity), a TDI for bromoform of 25 µg/kg bw per day was derived (IPCS, 2000). In a subsequent evaluation, the NOAEL was corrected to allow for gavage exposure on 5 days/week, resulting in establishment of a TDI of 17.9 µg/kg bw per day (WHO, 2005b).

Dietary exposure

Bromoform is a potential DBP resulting from the use of DBDMH in the processing of poultry and beef. Dietary exposure to bromoform from the consumption of beef and poultry treated with DBDMH can be estimated using the CSFII 1994–1996, 1998. The consumption of beef and poultry at the 90% percentile (94% eaters) is 150 g/person per day. Using this value and assuming that the bromoform residue level of 0.005 µg/g meat (see section 2.8.2.3) represents a worst-case value for bromoform residue on beef and poultry gives an exposure to bromoform of 0.8 µg/person per day, or 0.013 µg/kg bw per day for a 60-kg person.

Risk characterization

The estimated dietary exposure of 0.013 µg/kg bw per day is considerably below the bromoform TDI of 17.9 µg/kg bw per day, and therefore no health concerns were identified.

3.2 Epidemiological review

3.2.1 Introduction

Several disease outbreaks associated with microbially contaminated foods have occurred in a number of countries in recent years, including outbreaks of foodborne illness associated with verotoxigenic *Escherichia coli* and *Listeria monocytogenes* in processed meat products and *E. coli* O157:H7 in spinach (see chapter 4). However, it is unknown whether any of these outbreaks could be attributed to a lack of proper disinfection procedures during food processing rather than a lack of good hygienic practices. Therefore, the expert meeting did not further consider epidemiological studies of pathogen outbreaks associated with food.

In addition, no epidemiological studies on the health effects associated with exposure to disinfectants and DBPs in food products have been identified. Instead, all epidemiological studies to date have focused on DBPs in drinking-water. Therefore, this section focuses on epidemiological studies of DBPs, mainly chlorination by-products, in drinking-water; one study deals with ozonation.

Epidemiological studies on disinfectants and DBPs in drinking-water and swimming pools have been conducted since the 1970s, when it became clear that DBPs could be formed as part of the disinfection process. The focus of epidemiological studies has generally been on the DBPs rather than the disinfectants as a putative agent. As the DBPs occur as a mixture, the epidemiological studies have compared health risks for water type (e.g. groundwater versus surface water), the absence versus presence of some disinfection process (e.g. chlorination versus chloramination) or the level of DBPs, often indicated by THMs, the most common group of DBPs. However, little information is generally provided by the studies on how these indicator variables (i.e. THMs) relate to the underlying mixture of the more than 600 known by-products (Richardson, 1998). Some studies have examined the effects of individual by-products (e.g. individual THMs or HAAs), but if there is little information on the correlation with other DBPs, then it is unclear whether these specific compounds relate to the observed risks or still act as a marker. Furthermore, many epidemiological studies have not specifically taken into account the amount of water ingested, whereas few have specifically taken into account exposure routes other than ingestion (e.g. inhalation, dermal absorption) that may contribute significantly to the uptake of substances such as THMs during, for example, showering, bathing and swimming (e.g. Backer et al., 2000; Nieuwenhuijsen, Toledano & Elliott, 2000; Lynberg et al., 2001; Miles et al., 2002; Nuckols et al., 2005; Gordon et al., 2006; Leavens et al., 2007). The review below should be read in the light of these comments.

As most epidemiological studies of DBPs and cancer were conducted before 2004, they have been extensively described and evaluated in the IPCS (2000) and IARC (2004) documents on disinfectants and DBPs. These studies will not be further discussed beyond what the two documents concluded. New studies since 2004 will be described. As many of the reproductive epidemiological studies on DBPs have taken place after 2000, a more in-depth description of these studies will be given.

3.2.2 IPCS (2000) conclusions

IPCS (2000) performed a detailed evaluation of the epidemiological studies on disinfectants and DBPs and summarized the findings as described below.

Epidemiological studies have not identified an increased risk of cardiovascular disease associated with chlorinated or chloraminated drinking-water.

Based on the entire cancer–chlorinated drinking-water epidemiological database, there is better evidence for an association between exposure to chlorinated surface water and bladder cancer than for other types of cancer. However, the latest published study by Cantor et al. (1998) noted several inconsistencies in results among the studies for smokers/non-smokers and males/females, and the evidence is still considered insufficient to allow a judgement as to whether the association is causal and which water contaminants may be important. Evidence for a role of THMs is weak. Poole (1997) also noted that “The basic conclusion of the present report is that the hypothesis of a causal relationship between consumption of chlorination by-products and the risk of any cancer, including bladder cancer and rectal cancer, is still an open question”.

The overall findings of Cantor et al. (1998) support the hypothesis of an association between bladder cancer and duration of use of chlorinated surface water or groundwater and estimated THM exposures, but aspects of these results caution against a simple interpretation and raise additional questions about the nature of the association. An increase in bladder cancer risk was found with duration of chlorinated groundwater use, as well as with total duration of chlorinated drinking-water (surface water plus groundwater) use, with relative risks similar to those observed with chlorinated surface water. This finding is unexpected, because the levels of by-products from most chlorinated groundwaters are much lower than those in treated surface water. In addition, risk was found to increase with duration of chlorinated surface water use among ever-smokers, but not women. This raises questions of internal consistency, as well as consistency with other findings. In contrast, Cantor et al. (1998) found associations for both sexes, primarily among never-smokers. Cantor, Hoover & Hartge (1985) noted:

In Ontario, King and Marrett [1996] noted somewhat higher risk estimates for never-smokers associated with duration of chlorinated surface water. In Colorado, McGeehin et al. [1993] reported similar patterns of risk among smokers and never-smokers, and among men and women. Finally, in a case–control study from Washington County, Maryland, Freedman et al. [1997] reported results that parallel the current findings, namely that the risk associated with chlorinated surface water was primarily observed among men and among smokers. Reasons for differences among these observations and differences with results from our study are unclear. A possible explanation for the apparent discrepancies in findings for smokers and never-smokers among studies may reside in water quality and water treatment differences in the respective study areas, with resulting variations in the chemical composition of byproduct mixtures. Nevertheless, results should not differ by sex.

IPCS (2000) concluded that the existing epidemiological data were insufficient to allow a conclusion that the observed associations between bladder or any other cancer and chlorinated drinking-water or THMs are causal or provide an accurate estimate of the magnitude of risk. Any association between exposure to chlorinated surface water, THMs or the mutagenicity of drinking-water and cancer of the colon, rectum, pancreas, brain and other sites cannot be evaluated at this time because of inadequate epidemiological evidence. However, the findings from well-conducted studies associating bladder cancer with chlorinated water and THMs cannot be completely dismissed, even though inconsistencies have been noted for risks among men and women and among smokers and non-smokers. Because of the large number of people exposed to chlorinated drinking-water, it is important to resolve this issue using studies designed with sound epidemiological principles. Additional studies to resolve the questions about the associations that have been reported for chlorinated surface water, THMs, fluid and tap water consumption, and bladder cancer and reproductive and developmental effects must focus on the resolution of various problems noted in previous studies, especially consideration of exposures to other DBPs.

IPCS (2000) noted that the existing epidemiological data are insufficient to allow the importance of the observed associations of chlorinated drinking-water or THMs with adverse

pregnancy outcomes to be assessed. Although several studies have suggested that increased risks of neural tube defects and miscarriage may be associated with THMs or selected THM species, additional studies are needed to determine whether the observed associations are spurious.

A recently convened scientific panel (USEPA, 1997) concluded that the results of published epidemiological studies do not provide convincing evidence that DBPs cause adverse pregnancy outcomes. The panel recommended that additional studies be conducted, specifically that the Waller et al. (1998) study be expanded to include additional exposure information about by-products other than THMs and that a similar study be conducted in another geographic area.

3.2.3 IARC (2004) conclusions

IARC (2004) evaluated the carcinogenicity of some disinfectants and DBPs that are found in most chlorinated and chloraminated drinking-water (chloral hydrate, DCA, TCA, MX and monochloramine) and concluded that several studies were identified that analysed risk with respect to one or more measures of exposure to complex mixtures of these DBPs. No data specifically on these substances were available to the IARC working group.

3.2.4 Evaluation of studies published since IPCS (2000) and IARC (2004)

3.2.4.1 Cancer

Villanueva et al. (2003) conducted a meta-analysis to evaluate whether consumption of chlorinated drinking-water was associated with bladder cancer. They selected studies evaluating individual consumption of chlorinated drinking-water and bladder cancer, extracted from each study risk estimates for intermediate and long-term (>40 years) consumption of chlorinated water, stratified by sex when possible, and performed meta-analysis for the two exposure levels. They included six case-control studies (6084 incident bladder cancer cases, 10 816 controls) and two cohort studies (124 incident bladder cancer cases). Ever consumption of chlorinated drinking-water was associated with an increased risk of bladder cancer in men (combined odds ratio [OR] = 1.4, 95% confidence interval [CI] = 1.1–1.9) and women (combined OR = 1.2, 95% CI = 0.7–1.8). The combined OR for mid-term exposure in both sexes was 1.1 (95% CI = 1.0–1.2) and for long-term exposure was 1.4 (95% CI = 1.2–1.7). The combined estimate of the slope for a linear increase in risk was 1.13 (95% CI = 1.08–1.20) for 20 years and 1.27 (95% CI = 1.15–1.43) for 40 years of exposure in both sexes.

Ranmuthugala et al. (2003) conducted a cohort study in 1997 in three Australian communities with varying levels of DBPs in the water supply. Exposure was assessed using both available dose (total THM concentration in the water supply) and intake dose (calculated by adjusting for individual variations in ingestion, inhalation and dermal absorption). Micronuclei in urinary bladder epithelial cells were used as a preclinical biomarker of genotoxicity. Cells were scored for micronuclei for 228 participants, of whom 63% were exposed to DBPs and 37% were unexposed. Available dose of total THMs for the exposed group ranged from 38 to 157 µg/l, whereas intake dose ranged from 3 to 469 µg/kg bw per day. Relative risk for DNA damage to bladder cells, per 10 µg/l of available dose of total THMs, was 1.01 (95% CI = 0.97–1.06) for smokers and 0.996 (95% CI = 0.961–1.032) for non-smokers. Relative risk, per 10 µg/kg bw per day of intake dose of total THMs, was 0.99 (95% CI = 0.96–1.03) for smokers and 1.003 (95% CI = 0.984–1.023) for non-smokers.

Villanueva et al. (2004) pooled the primary data from six case–control studies of bladder cancer that used THMs as a marker for DBPs. Two studies were included from the USA and one each from Canada, Finland, France and Italy. Inclusion criteria were the availability of detailed data on THM exposure and individual water consumption. The analysis included 2806 cases and 5254 controls, all of whom had measures of known exposure for at least 70% of the exposure window of 40 years before the interview. Cumulative exposure to THMs was estimated by combining individual year-by-year average THM level and daily tap water consumption. There was an adjusted OR of 1.24 (95% CI = 1.09–1.41) in men exposed to an average THM concentration of more than 1 µg/l compared with those who had lower or no exposure. Estimated relative risks increased with increasing exposure, with an OR of 1.44 (95% CI = 1.20–1.73) for exposure higher than 50 µg/l. Similar results were found with other indices of THM exposure. Among women, THM exposure was not associated with bladder cancer risk (OR = 0.95, 95% CI = 0.76–1.20). ORs for cumulative THM exposure are given in Table 3.10. Cumulative exposure was estimated by combining individual year-by-year average THM level and daily tap water consumption.

Table 3.10. Pooled analysis of bladder cancer and cumulative exposure to THMs^a

THM exposure level (mg)	Male ORs	Female ORs
0–15	1.00	1.00
>15–50	1.22	0.92
>50–400	1.28	0.94
>400–1000	1.31	1.02
>1000	1.50	0.92

^a After Villanueva et al. (2004).

Chevrier, Junod & Cordier (2004) used data from a case–control study of bladder cancer conducted between 1985 and 1987 in seven French hospitals. They compared 281 cases and 272 controls for whom they could reconstruct at least 70% of the residential exposure to drinking-water contaminants over a 30-year period. They found that the risk of bladder cancer decreased as duration of exposure to ozonated water increased (OR = 0.60, 95% CI = 0.3–1.3, for 1–9 years; OR = 0.31, 95% CI = 0.1–0.7, for 10 years or more). Simultaneously, the risk of bladder cancer increased with duration of exposure to chlorinated surface water (OR = 2.02, 95% CI = 1.0–4.3, for 0 versus ≥29 years), with the estimated THM content of the water (OR = 2.99, 95% CI = 1.1–8.5, for <1 versus >50 µg/l) and cumulative exposure to THMs (OR = 3.39, 95% CI = 1.2–9.6, for 0 versus >1500 (µg/l)·year).

Do et al. (2005) reported results from a population-based case–control study of 486 incident cases of pancreatic cancer and 3596 age- and sex-matched controls. Exposure to chlorination by-products was estimated by linking lifetime residential histories to two different databases containing information on chlorination by-product levels in municipal water supplies. Logistic regression analysis found no evidence of increased pancreatic cancer risk at higher chlorination by-product concentrations (all ORs <1.3). Null findings were also obtained assuming a latency period for pancreatic cancer induction of 3, 8 or 13 years.

Villanueva et al. (2007) examined whether bladder cancer risk was associated with exposure to THMs through ingestion of water and through inhalation and dermal absorption during showering, bathing and swimming in pools. Lifetime personal information on water consumption and water-related habits was collected for 1219 cases and 1271 controls in a 1998–2001 case–control study in Spain and was linked with THM levels in geographic study areas. Long-term THM exposure was associated with a 2-fold increase in bladder cancer risk,

with an OR of 2.10 (95% CI = 1.09–4.02) for average household THM levels of >49 µg/l versus ≤8 µg/l. Compared with subjects not drinking chlorinated water, subjects with THM exposure of >35 µg/day through ingestion had an OR of 1.35 (95% CI = 0.92–1.99). The OR for duration of shower or bath weighted by residential THM level was 1.83 (95% CI = 1.17–2.87) for the highest compared with the lowest quartile. Swimming in pools was associated with an OR of 1.57 (95% CI = 1.18–2.09). Furthermore, they identified genetically susceptible groups, such as those with glutathione *S*-transferase enzymes GSTT1 and GSTZ1 (Cantor et al., 2006).

Bove, Rogerson & Vena (2007b) examined the relationship between the estimated concentrations of THMs in drinking-water and the risk for urinary bladder cancer in a case–control study of 567 white men aged 35–90 years in western New York State, USA. They used logistic regression to estimate ORs and to assess the effects of THM consumption on cancer risk. Higher levels of consumption of THMs led to increased risk for cancer of the urinary bladder (OR = 2.34, 95% CI = 1.01–3.66). Results were most significant for bromoform (OR = 3.05, 95% CI = 1.51–5.69), and risk was highest (OR = 5.85, 95% CI = 1.93–17.46) for those who consumed the greatest amount of water at points within the distribution system with the oldest post-disinfected tap water.

Bove, Rogerson & Vena (2007a) assessed the effects of estimated exposure to some of the components of the THM group on the ORs and probabilities for rectal cancer in white males in a case–control study of 128 cases and 253 controls, conducted in Monroe County, western New York State, USA. The spatial patterns of THMs and individual measures of tap water consumption provided exposure estimates. The risk for rectal cancer did not increase with the total level of THMs, but increasing levels of bromoform (measured in µg/day) did correspond with an increase in the risk (OR = 1.85, 95% CI = 1.25–2.74) for rectal cancer. The highest quartiles of estimated consumption of bromoform (1.69–15.43 µg/day) led to increased risk for rectal cancer (OR = 2.32, 95% CI = 1.22–4.39). Two other THMs were marginally associated with an increase in risk—DBCM (OR = 1.78, 95% CI = 1.00–3.19) and BDCM (OR = 1.15, 95% CI = 1.00–1.32).

Karagas et al. (2008) conducted an exploratory analysis of the hypothesis that exposure to DBPs may enhance risk of cancers of skin. They used data accrued in a completed population-based case–control study of keratinocyte-derived malignancies—basal cell carcinomas (BCC) and squamous cell carcinomas (SCC)—from New Hampshire, USA, originally designed to examine the effects of drinking-water arsenic. Newly diagnosed cases of BCC and SCC were identified through a state-wide network of dermatologists, dermatopathologists and pathologists, and age- and sex-matched controls were selected from population lists. The study comprised 293 SCC cases, 603 BCC cases and 540 controls. Residents of towns or cities with multiple water systems were assigned the average THM value weighted by the proportion of the population served by these systems. Among individuals who reported using public water systems, the ORs for those with levels above 40 µg/l were 2.4 (95% CI = 0.9–6.7) for BCC and 2.1 (95% CI = 0.7–7.0) for SCC compared with those below 1 µg/l.

3.2.4.2 Reproductive outcomes

A summary of the results of reproductive epidemiological studies is given in Table 3.11.

Table 3.11. Summary of epidemiological studies on chlorinated DBPs and adverse reproductive outcomes

Reference	Study details (location, time, sample size)	Cases	Exposure assessment	Other risk factors included	Main positive findings OR (95% CI)
Aschengrau, Zierler & Cohen (1989)	Massachusetts, USA Sample population: 1677	286 spontaneous abortions	Surface water versus groundwater Chlorination versus chloramination	Smoking habits Contraceptive use Medical and obstetrical history Metals	Surface water versus groundwater 2.2 (1.3–3.6)
Kramer et al. (1992)	Iowa, USA 151 towns with a single water source 1989–1990 Sample population: 4028	588 (total) 159 LBW 342 pre-term delivery 187 IUGR/SGA	Based on maternal residential address and one municipal water survey to estimate individual THM levels (2 or 3 exposure categories)	Maternal age Parity Marital status Education Smoking Prenatal care	No versus medium (1–9 µg/l) versus high (≥10 µg/l): Chloroform LBW: 1 versus 1.1 (0.7–1.6) versus 1.3 (0.8–2.2) IUGR: 1 versus 1.3 (0.9–1.8) versus 1.8 (1.1–2.9) DBCM IUGR: 1 versus 1.2 (0.8–1.7) versus 1.7 (0.9–2.9)
Aschengrau, Zierler & Cohen (1993)	Massachusetts, USA 2 hospitals 1977–1980 Sample population: 2348	1171 (total) 1039 major congenital malformations Urinary tract defects Respiratory tract defects 77 stillbirths 55 neonatal deaths	Based on maternal residential address to ascertain type of water supply, chlorination versus chloramination and groundwater/mixed water versus surface water	Maternal age Pregnancy history Alcohol Ethnicity Hospital payment Other water contaminants	Chlorinated versus chloraminated: Stillbirth: 2.6 (0.9–7.5) Neonatal deaths: 1.1 (95% CI not provided) Congenital malformations: - major malformations: 1.5 (0.7–2.1) - respiratory defects: 3.2 (1.1–9.5) - urinary tract defects: 4.1 (1.2–14.1)

Reference	Study details (location, time, sample size)	Cases	Exposure assessment	Other risk factors included	Main positive findings OR (95% CI)
Bove et al. (1995)	New Jersey, USA 75 towns with a public water supply 1985–1988 Sample population: 81 602	29 268 (total) <i>Live births:</i> 1853 LBW 905 VLBW 4082 SGA 7167 pre-term 594 fetal deaths <i>All births: defects:</i> 669 surveillance 118 CNS defects 83 oral cleft 56 NTD 108 major cardiac	Based on maternal residential address and municipal water surveys to estimate monthly TTHM levels (5 or 6 exposure categories)	Maternal age Ethnicity Sex of baby Primipara Prenatal care Education Previous stillbirth or miscarriage Other contaminants	TTHM levels >100 µg/l versus ≤20 µg/l: LBW: 1.4 (50% CI 1.2–1.7) IUGR/SGA: 1.5 (90% CI 1.2–1.9) TTHM levels >80 µg/l versus ≤20 µg/l: Surveillance register defects: 1.6 (90% CI 1.2–2.0) CNS defects: 2.6 (90% CI 1.5–4.3) NTD: 3.0 (90% CI 1.3–6.6) Major cardiac defects: 1.8 (90% CI 1.0– 3.3) TTHM levels >100 µg/l versus ≤20 µg/l: Oral cleft defects: 3.2 (90% CI 1.2–7.3)
Savitz, Andrews & Pastore (1995)	North Carolina, USA 6 hospitals 1988–1991 Sample population: 1003	548 (total) 126 spontaneous abortion 244 pre-term 178 LBW	Based on maternal residential address and quarterly municipal water surveys to estimate average TTHM levels Analysis of: a) surface water versus groundwater source b) TTHM levels (3 exposure categories) c) consumption during pregnancy d) water source x amount e) TTHM dose (level x amount)	Maternal age Ethnicity Hospital Education Marital status Poverty level Smoking Alcohol consumption Employment Nausea	TTHM concentration 40.8–59.9 versus 81.1–168.8 µg/l: Spontaneous abortion: 1.2 (0.6–2.4) TTHM concentration 40.8–63.3 versus 82.8–168.8 µg/l: LBW: 1.3 (0.8–2.1) Per 50 µg/l incremental change in TTHM concentration: Spontaneous abortion: 1.7 (1.1–2.7)

Reference	Study details (location, time, sample size)	Cases	Exposure assessment	Other risk factors included	Main positive findings OR (95% CI)
Kanitz et al. (1996)	Liguria, Italy 2 hospitals 1988–1989 Sample population: 676	548 live births in "exposed" area 50 pre-term 141 caesarean section 133 neonatal jaundice 20 LBW 288 small body length 370 small cranial circumference	Based on maternal residential address to ascertain type of water source (chlorine dioxide and/or hypochlorite versus not treated)	Maternal age Education Smoking Alcohol Sex of child	Sodium hypochlorite-treated (TTHM concentration 8–16 µg/l) versus non- treated water: Neonatal jaundice: 1.1 (0.7–2.8) LBW: 6.0 (0.6–12.6) Small body length: 2.3 (1.3–4.2) Small cranial circumference: 3.5 (2.1– 8.5)
Gallagher et al. (1998)	Colorado, USA 28 census blocks in 2 water districts 1990–1993 Sample population: 1244 live births	72 LBW 29 term LBW 68 pre-term delivery	Based on maternal residential address and municipal water surveys Estimate of household TTHM level during last trimester based on hydraulic modelling (4 exposure categories)	Maternal age Smoking Marital status Parity Education Employment Prenatal care	High TTHM level (≥ 61 µg/l) versus lowest (≤ 20 µg/l): LBW: 2.1 (1.0–4.8) Term LBW: 5.9 (2.0–17.0)
Waller et al. (1998)	California, USA 3 regions of surface water, groundwater and mixed drinking- water 1989–1991 Sample population: 5144 pregnancies	499 spontaneous abortions	Based on maternal residential address and quarterly municipal water surveys to estimate average TTHM and individual THM levels Analysis based on: a) THM levels (3 or 10 exposure categories) b) consumption during first trimester from interview (2 exposure categories)	Maternal age Gestational age Smoking History of pregnancy loss Ethnicity Employment	High TTHM dose (≥ 5 glasses/day + ≥ 75 µg/l) versus low dose (<5 glasses/day + <75 µg/l): Spontaneous abortion: 1.8 (1.1–3.0) High BDCM dose (≥ 5 glasses/day + ≥ 18 µg/l) versus low dose (<5 glasses/day + <18 µg/l): Spontaneous abortion: 3.0 (1.4–6.6)

Reference	Study details (location, time, sample size)	Cases	Exposure assessment	Other risk factors included	Main positive findings OR (95% CI)
Dodds et al. (1999)	Nova Scotia, Canada 1988–1995 Sample population: 49 842 births	4673 SGA 2393 LBW 342 VLBW 2689 pre-term delivery 77 NTD 82 cleft defect 430 major cardiac defects 197 stillbirth 96 chromosomal abnormalities	Based on maternal residential address and TTHM levels for public water facilities (3 sampling locations) modelled using linear regression on the basis of observations by year, month and facility (4 exposure categories)	Maternal age Parity Maternal smoking Attendance at prenatal classes Neighbourhood family income Sex Pregnancy and pre-delivery weight	TTHM concentration 0–49 µg/l versus >100 µg/l Stillbirth: 1.66 (1.09–2.52) Chromosomal abnormalities: 1.38 (0.73–2.59) Small for gestation age: 1.08 (0.99–1.18) NTDs: 1.18 (0.67–2.10)
Klotz & Pynch (1999)	New Jersey, USA 1993–1994 Sample population: all births, of which 112 cases and 248 controls selected	112 NTD	Based on residential address and public water facility TTHM data and tap water sampling for TTHMs, HANs and HAAs (3–5 exposure categories)	Sociodemographics Pregnancy and medical history Parental occupational Use of vitamins	TTHM public monitoring data, known residence and isolated cases <5 µg/l versus 40+ µg/l NTDs: 2.1 (1.1–4.0)
Magnus et al. (1999)	Norway Sample population: 141 077	2608 all birth defects 62 NTD 250 major cardiac defects 91 respiratory defects 122 urinary defects 143 oral cleft	Chlorination yes versus no Colour high versus low (in chlorinated water, average TTHMs = 9.4 µg/l, average HAAs = 14.6 µg/l)	Maternal age Parity Geographical placement Population density Industry profile	No chlorination low colour versus chlorination high colour All birth defects: 1.14 (0.99–1.31) Urinary tract defects: 1.99 (1.10–3.57) NTDs: 1.26 (0.61–2.62) Major cardiac defects: 1.05 (0.76–1.46) Respiratory tract defects: 1.07 (0.52–2.19)

Reference	Study details (location, time, sample size)	Cases	Exposure assessment	Other risk factors included	Main positive findings OR (95% CI)
Källén & Robert (2000)	Sweden 1985–1994 Sample population: No chlorination: 74 324 singletons Sodium hypochlorite: 24 731 singletons Chlorine dioxide: 15 429 singletons	Multiple births Gestational duration Birth weight Intrauterine growth Body length Head circumference Body mass index Infant survival up to 1 year Perinatal death Apgar score Neonatal jaundice Congenital malformations, including NTDs Childhood cancer Hypothyroidism	No versus sodium hypochlorite (no versus chlorine dioxide)	Year of birth Maternal age Parity Maternal education Maternal smoking Congenital malformations and childhood cancer Maternal age Year of birth	No versus sodium hypochlorite LBW: 1.15 (1.05–1.26) <32 weeks' gestation: 1.22 (1.00–1.48) <37 weeks' gestation: 1.09 (1.01–1.17) <43 cm length: 1.97 (1.30–2.97) <47 cm length: 1.25 (1.10–1.43) Body mass index >16 kg/m ² : 1.27 (1.19–1.37) <31 cm head circumference: 1.46 (1.07–1.98) Spine malformation: 3.2 (1.0–10.0)
King, Dodds & Allen (2000)	Nova Scotia, Canada 1988–1995 Sample population: 49 756	214 stillbirths (72 asphyxia-related stillbirths)	Based on maternal residential address and TTHM, chloroform and BDCM levels for public water facilities (3 sampling locations) modelled using linear regression on the basis of observations by year, month and facility (4 exposure categories) ($r =$ 0.44 for TTHM and BDCM)	Maternal age Parity Maternal smoking Attendance at prenatal classes Neighbourhood family income Sex Pregnancy and pre- delivery weight	Chloroform concentration 0–49 µg/l versus >100 µg/l Stillbirth: 1.56 (1.04–2.34) Asphyxia-related stillbirth: 3.15 (1.64–6.03) BDCM concentration <5 µg/l versus >20 µg/l Stillbirth: 1.98 (1.23–3.49) Asphyxia-related stillbirth: 1.75 (0.72–4.22)

Reference	Study details (location, time, sample size)	Cases	Exposure assessment	Other risk factors included	Main positive findings OR (95% CI)
Yang et al. (2000a)	China, Province of Taiwan Sample population: 18 025 first-parity births Chlorinated: 10 007 Non-chlorinated: 8018	LBW Pre-term delivery (<37 weeks)	Chlorinated (>95% population served chlorinated water) versus non-chlorinated (<5% population served chlorinated water)	Maternal age Marital status Maternal education Sex	Chlorinated versus non-chlorinated Pre-term delivery: 1.34 (1.15–1.56)
Yang et al. (2000b)	China, Province of Taiwan Sample population: Chlorinated: 24 882 Non-chlorinated: 20 460	Sex ratio	Chlorinated (>95% population served chlorinated water) versus non-chlorinated (<5% population served chlorinated water)		No association
Dodds & King (2001)	Nova Scotia, Canada 1988–1995 Sample population: 49 842 births	77 NTDs 430 cardiovascular anomalies 82 cleft defects 96 chromosomal abnormalities	Based on maternal residential address and TTHM, chloroform and BDCM levels for public water facilities (3 sampling locations) modelled using linear regression on the basis of observations by year, month and facility (4 exposure categories) ($r =$ 0.44 for TTHM and BDCM)	Maternal age Parity Maternal smoking Attendance at prenatal classes Neighbourhood family income Sex Pregnancy and pre- delivery weight	BDCM concentration ≥ 20 $\mu\text{g/l}$ versus < 5 NTDs: 2.5 (1.2–5.1)

Reference	Study details (location, time, sample size)	Cases	Exposure assessment	Other risk factors included	Main positive findings OR (95% CI)
Jaakkola et al. (2001)	Norway Sample population: 137 145	6249 LBW ? SGA 7886 pre-term delivery	Chlorination yes versus no Colour high versus low (in chlorinated water, average TTHMs = 9.4 µg/l, average HAA5 = 14.6 µg/l)	Maternal age Parity Geographical placement Population density Industry profile	No chlorination low colour versus chlorination high colour Pre-term delivery: 0.91 (0.84–0.99)
Waller et al. (2001)	See Waller et al. (1998)	See Waller et al. (1998)	See Waller et al. (1998)	See Waller et al. (1998)	Reanalysis of Waller et al. (1998) Utility-wide subset sample highest OR High TTHM dose (≥5 glasses/day + ≥75 µg/l) versus low dose (≥5 glasses/day + <75 µg/l): Spontaneous abortion: 5.1 (1.8–14.7) Little relationship with showering
Cedergren et al. (2002)	Sweden Sample population: 58 669	Cardiac defects	TTHM concentration >10 µg/l versus ≤10 µg/l in surface water Hypochlorite and chlorine dioxide versus hypochlorite in surface water Groundwater versus surface water	Maternal age Parity Smoking Education	TTHM concentration >10 µg/l versus ≤10 µg/l Cardiac defects: 1.30 (1.08–1.56) Groundwater versus surface water Cardiac defects: 1.32 (1.10–1.58) Hypochlorite and chlorine dioxide versus hypochlorite Cardiac defects: 1.85 (1.42–2.39)
Hwang, Magnus & Jaakkola (2002)	Norway Sample population: 285 631	Any birth defect NTD - anencephalus - spina bifida - hydrocephalus Cardiac defects	Chlorination (yes/no) and level of water colour (mg Pt/l): <10, 10–19.9, ≥20	Maternal age Parity Socioeconomic status: - centrality - population density	Chlorination (yes) and level of water colour: <10 versus ≥20 mg Pt/l All birth defects: 1.18 (1.02–1.36) Ventricular septal defect: 1.81 (1.05–3.09) Chlorination (yes) and level of water colour: <10 versus ≥10 mg Pt/l

Reference	Study details (location, time, sample size)	Cases	Exposure assessment	Other risk factors included	Main positive findings OR (95% CI)
		- ventricular septal defects - atrial septal defects Respiratory defects Oral cleft defects - Cleft palate - Cleft lip Urinary tract defect - Obstructive urinary tract defect			All birth defects: 1.13 (1.01–1.25) Cardiac defects: 1.37 (1.00–1.89) Respiratory defects: 1.89 (1.00–3.58) Urinary tract defects: 1.46 (1.00–2.13)
Nieuwen-huijsen, Northstone & Golding (2002)	England Sample population: 11 462	Birth weight	Amount of swimming (h)	Maternal age Maternal education Smoking Alcohol use Drug use Gestational age Ethnicity Infant sex	No association
Wright, Schwartz & Dockery (2003)	Massachusetts, USA Sample population: 56 513	Birth weight LBW SGA Gestational age Pre-term delivery	TTHM concentration 0–60, >60–80, >80 µg/l or per 20 µg/l increase in TTHM concentration	Maternal age Maternal education Ethnicity Smoking Parental care Parity Infant sex	TTHM concentration 0–60 or >80 µg/l Birth weight: –32 g (–47 to –18) SGA: 1.14 (1.02–1.26) Gestational age (weeks): 0.08 (0.01–0.14) per 20 µg/l increase in TTHM concentration Birth weight: –2.8 g (–5.5 to –0.2) Gestational age: 0.02 (0.01–0.03)

Reference	Study details (location, time, sample size)	Cases	Exposure assessment	Other risk factors included	Main positive findings OR (95% CI)
Shaw et al. (2003)	California, USA Sample population: Study 1: 538 NTD cases and 539 controls Study 2: 265 NTD cases, 207 conotruncal heart defect cases, 409 orofacial cleft cases and 481 controls	Study 1: NTDs (anencephaly and spina bifida) Study 2: NTDs (anencephaly and spina bifida), conotruncal heart defects, orofacial clefts	Study 1 and study 2: Continuous TTHM Categorical: 0, 1–24, 25–49, 50–74 and ≥ 75 $\mu\text{g/l}$ Also study 1: ≥ 50 versus < 50 $\mu\text{g/l}$ and < 5 glasses ≥ 50 versus < 50 $\mu\text{g/l}$ and > 5 glasses Study 1: Chloroform ≥ 12.2 versus < 12.2 $\mu\text{g/l}$ BDCM ≥ 4.2 versus < 4.2 $\mu\text{g/l}$ DBCМ ≥ 1.7 versus < 1.7 $\mu\text{g/l}$ Study 2: Chloroform ≥ 15.0 versus < 15.0 $\mu\text{g/l}$ BDCM ≥ 9.6 versus < 9.6 $\mu\text{g/l}$ DBCМ ≥ 3.6 versus < 3.6 $\mu\text{g/l}$	Ethnicity Education Body mass index Use of vitamins Methylenetetra- hydrofolate reductase genotype	Study 1: NTDs NTD risk inversely related to TTHM exposure but only occasionally significant for one category Chloroform concentration ≥ 12.2 versus < 12.2 $\mu\text{g/l}$: 0.50 (0.34–0.75) BDCM concentration ≥ 4.2 versus < 4.2 $\mu\text{g/l}$: 0.66 (0.45–0.97) DBCМ concentration ≥ 1.7 versus < 1.7 $\mu\text{g/l}$: 0.69 (0.47–1.0) Study 2: Multiple cleft palate/lip Chloroform concentration ≥ 15.0 versus < 15.0 $\mu\text{g/l}$: 0.21 (0.05–0.90)
Aggazzotti et al. (2004)	Italy 9 towns Sample population: 1194 subjects	343 pre-term delivery 239 SGA at term	Water sampling directly at mothers' homes to determine THM levels and chlorite/chlorate levels Questionnaire on personal habits to determine: - type of water consumption - frequency of bath/shower - swimming pool attendance	Maternal age Education Sex Smoking Alcohol Coffee	Pre-term delivery No significant associations Term SGA > 200 $\mu\text{g/l}$ low inhalation exposure versus < 200 $\mu\text{g/l}$: 1.52 (0.91–2.52) > 200 $\mu\text{g/l}$ high inhalation exposure versus < 200 $\mu\text{g/l}$: 1.70 (0.97–3.00)

Reference	Study details (location, time, sample size)	Cases	Exposure assessment	Other risk factors included	Main positive findings OR (95% CI)
Dodds et al. (2004)	Nova Scotia and eastern Ontario, Canada Sample population: 112 stillbirths and 398 live birth controls	Stillbirth	Various indices: 0, 1–49, 50–79 and >80 µg/l for TTHM and chloroform 0, 1–4, 5–9 and >9 µg/l for BDCM Quintiles for total exposure (ingestion/showering/bathing) for TTHM, chloroform and BDCM Concentration and duration	Age Province Household income	Stillbirth: TTHM concentration >80 µg/l versus 0: 2.2 (1.1–4.4) TTHM highest versus lowest quintile: 2.4 (1.2–4.6) Drinking 5+ drinks per day and THM 50+ µg/l versus <1 drink and THM = 0: 4.0 (1.4–11) Chloroform and BDCM generally follow TTHM trend
Infante- Rivard (2004)	Montreal, Quebec, Canada Sample population: 493 cases, 472 controls	IUGR (10th percentile)	Regulatory data on THMs, >90th percentile versus ≤90th percentile	Gestational age Sex Race Mother's weight gain Body mass index Smoking Primiparity Pre-eclampsia Previous IUGR	IUGR No association with THMs only, but with CYP2E1*5 (G1259C): 13.2 (1.19–146.7) in newborns
Wright, Schwartz & Dockery (2004)	Massachusetts, USA Sample population: 196 000 registry based	Birth weight Gestational age SGA Pre-term delivery	TTHM Individual THMs HAAs MX Mutagenicity	SGA TTHM concentration >74 versus ≤33 µg/l: 1.13 (1.07–1.20) Chloroform concentration >63 versus ≤26 µg/l: 1.11 (1.04–1.17) BDCM concentration >13 versus ≤5 µg/l: 1.15 (1.08–1.22) >2250 versus ≤1250 revertants/l (elevated mutagenic activity): 1.25 (1.04–1.51)	

Reference	Study details (location, time, sample size)	Cases	Exposure assessment	Other risk factors included	Main positive findings OR (95% CI)
Yang (2004)	China, Province of Taiwan Sample population: 182 796	LBW Pre-term delivery	15 non-chlorinating municipalities and 128 chlorinating municipalities	Maternal age Education Gestational age Birth weight Sex	Similar results for birth weight Pre-term delivery Non-chlorinating municipalities versus chlorinating municipalities: 1.37 (1.20– 1.56)
Hinckley, Bachand & Reif (2005)	Sample population: 48 119	LBW, IUGR, pre-term delivery	THMs and HAAs	Maternal age Ethnicity Education Parity Smoking Kessner index	IUGR TTHM concentration ≥ 53 versus < 40 $\mu\text{g/l}$: 1.09 (1.00–1.18) Term LBW DBA concentration ≥ 5 versus < 4 $\mu\text{g/l}$: 1.49 (1.09–2.04) IUGR DCA concentration ≥ 8 versus < 6 $\mu\text{g/l}$: 1.28 (1.08–1.51) TCA concentration ≥ 6 versus < 4 $\mu\text{g/l}$: 1.19 (1.01–1.41) Weeks 37–40 IUGR DCA concentration ≥ 8 versus < 6 $\mu\text{g/l}$: 1.27 (1.02–1.59) Weeks 33–36 Term LBW DBA concentration ≥ 5 versus < 4 $\mu\text{g/l}$: 1.49 (1.10–2.02)
King et al. (2005)	Nova Scotia and eastern Ontario, Canada	Stillbirth	HAAs	Maternal age Province Income	Stillbirth No significant results after adjustments for THMs

Reference	Study details (location, time, sample size)	Cases	Exposure assessment	Other risk factors included	Main positive findings OR (95% CI)
	Sample population: 112 cases, 398 controls			Occupation Smoking	
Porter et al. (2005)	Maryland, USA 4 regions	IUGR	THMs and HAAs	Smoking Ethnicity Prenatal care Alcohol Marital status	No association
Toledano et al. (2005)	3 water regions in United Kingdom Sample population: 920 571 stillbirths and live births (1993–1998) and 969 304 live births (1992–1998)	Stillbirth LBW VLBW	THMs	Maternal age Deprivation	Stillbirths TTHM concentration ≥ 60 versus < 30 $\mu\text{g/l}$: 1.11 (1.00–1.23)
Lewis, Suffet & Ritz (2006)	Sample population: 36 259 births	Term LBW	THMs (weekly)	Trimester Age Sex Marital status Ethnicity Education Parity Smoking Conception/birth	Term LBW Second trimester: All: TTHM concentration ≥ 70 versus < 40 $\mu\text{g/l}$: 1.50 (1.07–2.10) Per 10 $\mu\text{g/l}$ TTHM concentration: 1.08 (1.00–1.20) Non-Caucasians: TTHM concentration ≥ 70 versus < 40

Reference	Study details (location, time, sample size)	Cases	Exposure assessment	Other risk factors included	Main positive findings OR (95% CI)
				season	$\mu\text{g/l}$: 1.60 (1.03–2.47)
				Maternal disease	Per 10 $\mu\text{g/l}$ TTHM concentration: 1.10 (1.00–1.22)
Savitz et al. (2006)	Sample population: 2409	Spontaneous abortion	THMs, HAAs, total organic halides	Maternal age, race, ethnicity, education, marital status, income, smoking, alcohol intake, caffeine consumption, body mass index, age at menarche, employment status, diabetes, pregnancy loss history, induced abortion history, vitamin use	No association
Lewis et al. (2007)	Sample population: 37 498	Pre-term birth	THMs	Maternal age Ethnicity Education Previous birth Marital status Maternal disease Income Kessner index Sex	No association (with some exception for some groups on government pay)
Yang et al. (2007)	Sample population: 90 848	LBW, IUGR, pre-term delivery	THMs	Maternal age Maternal education Marital status Only first birth	No association

Reference	Study details (location, time, sample size)	Cases	Exposure assessment	Other risk factors included	Main positive findings OR (95% CI)
Nieuwen- huijzen et al. (2008)	Sample population: 2 605 226	Congenital anomalies	THMs	Maternal age Deprivation Sex	Isolated ventricular septal defects TTHM concentration ≥ 60 versus < 30 $\mu\text{g/l}$: 1.43 (1.00–2.04) Subset isolated major cardiovascular defects Bromoform concentration 2–<4 versus <2 $\mu\text{g/l}$: 1.13 (0.99–1.29) Bromoform concentration ≥ 4 versus <2 $\mu\text{g/l}$: 1.18 (1.00–1.39) Isolated gastroschisis Bromoform concentration 2–<4 versus <2 $\mu\text{g/l}$: 1.11 (0.85–1.45) Bromoform concentration ≥ 4 versus <2 $\mu\text{g/l}$: 1.38 (1.00–1.92)

CNS, central nervous system; IUGR, intrauterine growth retardation (restriction); LBW, low birth weight; NTD, neural tube defect; SGA, small for gestational age; TTHM, total trihalomethanes; VLBW, very low birth weight

A number of studies found statistically significant positive associations between THMs and neural tube defects, one of the most studied groups of congenital anomalies (Bove et al., 1995; Klotz & Pyrch, 1999; Dodds & King, 2001), whereas others did not (Dodds et al., 1999; Magnus et al., 1999; Källén & Robert, 2000; Hwang, Magnus & Jaakkola, 2002; Shaw et al., 2003; Nieuwenhuijsen et al., 2008). Klotz & Pyrch (1999) found a statistically significant association between total THM levels in the water and neural tube defects, but not with HAN and HAA levels. Also, the effects were most pronounced in offspring from women who did not take supplementary vitamins, but these findings were not confirmed by the Shaw et al. (2003) study. Inclusion of information on ingestion, showering, bathing and swimming made little difference to the risk estimates.

Hwang, Magnus & Jaakkola (2002) and Cedergren et al. (2002) found significant associations between chlorinated water and levels of total THMs above 10 µg/l, respectively, and respiratory congenital anomalies, but other studies did not find such an association (Bove et al., 1995; Dodds et al., 1999; Magnus et al., 1999; Källén & Robert, 2000; Dodds & King, 2001; Shaw et al., 2003; Nieuwenhuijsen et al., 2008). Studies on chlorinated water and respiratory congenital anomalies have been rare, but two studies found a significant positive association (Aschengrau, Zierler & Cohen, 1993; Hwang, Magnus & Jaakkola, 2002), whereas one did not (Nieuwenhuijsen et al., 2008). Similarly, for urinary tract defects, three studies reported statistically significant associations (Aschengrau, Zierler & Cohen, 1993; Magnus et al., 1999; Hwang, Magnus & Jaakkola, 2002), and one did not (Nieuwenhuijsen et al., 2008). Studies on oral cleft or cleft palate have largely been negative, except for the study by Bove et al. (1995). In a meta-analysis, Hwang & Jaakkola (2003) reported evidence for an effect of exposure to chlorination by-products on the risk of neural tube and urinary system defects, but results for respiratory system, major cardiac and oral cleft defects were heterogeneous and inconclusive. The exposure index they used, though, was fairly crude, without actual levels of DBPs. The meta-analyses also did not include the largest study to date, and larger than all the previous studies combined, by Nieuwenhuijsen et al. (2008), which reported no association between THM levels and cleft palate/lip, abdominal wall, major cardiac, neural tube, urinary and respiratory defects; except for a restricted set of anomalies with isolated defects, there were excess risks in the highest exposure categories of total THMs for ventricular septal defects and of bromoform for major cardiovascular defects and gastroschisis.

Only a few studies have assessed the relationship between DBPs and spontaneous abortion. A California, USA, study has attracted the most attention, as it found a statistically significant association between total THMs and BDCM and spontaneous abortion (Waller et al., 1998). The ORs were even larger after reanalysis when restricting it to subjects with more confidence in the exposure data (Waller et al., 2001). In a study trying to replicate these results, Savitz et al. (2006) found no evidence for an association between a number of DBPs and spontaneous abortion, even though the exposure assessment was more refined.

A number of Canadian studies and one English study found statistically positive associations between DBPs and stillbirths (Dodds et al., 1999, 2004; King, Dodds & Allen, 2000; Toledano et al., 2005). However, a small case-control study by Dodds et al. (2004) did not show a monotonic relationship between THM levels and stillbirth, and they did not find an association between HAAs and stillbirth (King et al., 2005).

Studies on pre-term delivery have generally shown no association with DBPs (Bove et al., 1995; Savitz et al., 1995; Gallagher et al., 1998; Wright, Schwartz & Dockery, 2003, 2004; Aggazzotti et al., 2004; Hinckley, Bachand & Reif, 2005; Lewis et al., 2007; Yang et al., 2007), with the exception of the study by Yang et al. (2000a) and Yang (2004). Study results on low birth weight have been mixed, with some studies reporting statistically significant associations (Bove et al., 1995; Gallagher et al., 1998; Källén & Robert, 2000;

Lewis, Suffet & Ritz, 2006) and others not (Kramer et al., 1992; Savitz et al., 1995; Kanitz et al., 1996; Dodds et al., 1999; Jaakkola et al., 2001; Wright, Schwartz & Dockery, 2003; Toledano et al., 2005; Yang et al., 2007). Studies on small for gestational age and/or intrauterine growth retardation or restriction showed some more consistent results, and a good proportion of them have found statistically significant associations (Kramer et al., 1992; Bove et al., 1995; Gallagher et al., 1998; Wright, Schwartz & Dockery, 2003, 2004; Aggazzotti et al., 2004; Hinckley, Bachand & Reif, 2005), whereas others did not (Porter et al., 2005; Yang et al., 2007). Wright, Schwartz & Dockery (2004) found statistically significant associations with THMs and a measure of mutagenicity, but not with HAAs or MX. Infante-Rivard (2004) found that the association between THMs and intrauterine growth retardation or restriction was modified by a metabolic polymorphism, with newborns without the CYP2E1 (G1259C) variant at high risk.

Two small epidemiological studies have investigated the relationship between DBPs and semen quality. Fenster et al. (2003) found that total THM levels were not associated with decrements in semen quality. The per cent normal morphology decreased and the per cent head defects increased at higher levels of an ingestion metric; at the highest level of the ingestion metric, the investigators observed a small difference in per cent morphologically normal sperm compared with the lowest level. BDCM exposure was inversely related to linearity (a motility parameter). Luben et al. (2007) studied the relationship between exposure to classes of DBPs and sperm concentration and morphology, as well as DNA integrity and chromatin maturity, but found no association or consistent pattern of increased abnormal semen quality with elevated exposure to THMs or HAAs.

The above studies generally have occurred in areas where they used chlorination or chloramination as the main water treatment. When chlorine dioxide is used as the disinfecting agent, chlorite and chlorate are the main DBPs. Aggazzotti et al. (2004) conducted a case-control study in nine Italian provinces and found a small increase in the risk of small for gestational age at term and high levels of chlorite in drinking-water. Tuthill et al. (1982) conducted a study that was difficult to interpret, but only pre-term delivery appeared to be higher in water treated with chlorine dioxide compared with chlorinated water, and there were no statistical differences in jaundice, birth weight and defects or stillbirths. Kanitz et al. (1996) found an increase in jaundice and pre-term delivery and an increase in low birth weight, small body length and cranial circumference in chlorine dioxide-treated water compared with non-treated water, but the effects were similar to those observed with chlorinated water, and the study was small. Källén & Robert (2000) found no increase in jaundice, pre-term delivery, birth weight and other characteristics, death or malformations in chlorine dioxide-treated water. Cedergren et al. (2002) found an increased risk for cardiac defects in hypochlorite- and chlorine dioxide-treated water compared with only hypochlorite-treated water.

3.2.5 Summary

The overall evidence from a number of recent studies suggests an association between exposure to DBPs and the risk of bladder cancer. There have been a number of studies on colon cancer and other cancers, but the results have been mixed and are inconclusive.

In a pooled analysis of six large epidemiological studies on bladder cancer in relation to drinking-water DBPs, it is suggested that the risk of bladder cancer among men may be increased by 30% above a lifetime intake of 15 mg total THMs (used as a marker of the total DBPs) from drinking-water (Villanueva et al., 2004). This is equivalent to a daily intake of 1 µg/day of THMs from drinking-water, which is about 3 µg/day (or in the order of 1 µg/day for chloroform) if we assume that drinking-water represents only one third of all sources of

THMs (other sources being showering, bathing or swimming in pools). This estimate of chloroform exposure is close to the estimate of intake of chloroform from food that could be derived from data on poultry processing (see section 3.1).

The expert meeting noted, however, that information in the study was related to the profile of DBPs found in drinking-water and that the relationship between these DBPs and those found in food is not known.

The studies on small for gestational age have generally shown a significant excess risk, but the results for other reproductive outcomes have generally been inconsistent and inconclusive.

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4. THE EFFECT OF DISINFECTANTS IN FOOD PROCESSING ON MICROBIOLOGICAL SAFETY AND HEALTH

4.1 Introduction

Chlorinated compounds are used extensively in the food industry as disinfectants to control both spoilage bacteria and pathogenic bacteria on food. Their use is designed either to prevent an increase in the microbiological load on foods or to reduce the microbiological load on foods. In the former capacity, chlorinated compounds are introduced into food processing water or used to disinfect food contact surfaces to control the buildup of bacteria and prevent cross-contamination of foods. In the latter capacity, they are directly applied to the surface of foods to inactivate contaminating microorganisms. Details on the specific use of chlorine in the food industry are provided in chapter 1.

The focus in this chapter is on evaluating the effect of chlorinated compounds and certain other disinfectants on the reduction in the prevalence and numbers of pathogenic microorganisms on food. Considered are specific uses (as described in chapter 1) and those pathogenic bacteria that are known hazards associated with the food commodities reviewed. Although disinfectant chemicals will also control spoilage bacteria and, hence, increase the shelf life and stability of foods, this aspect is not considered here, as it has no direct impact on health risks.

Although there is now a considerable body of scientific literature on disinfectants, not all studies have indicated a beneficial effect (i.e. reduction in pathogen load), and the evidence obtained must be examined critically in relation to the relevance of the study to practical processing conditions. To differentiate between evidence from different studies, it is necessary to develop criteria to distinguish their relative contribution to the general body of evidence.

It is generally accepted that studies whereby pathogenic bacteria are inoculated onto food prior to assessing disinfectants generate data that overestimate the activity of the disinfectant compared with data from studies where the pathogen contamination is natural. This tends to be a result of inefficient attachment of pathogens to food using artificial inoculation methods. Therefore, for the purposes of assessing data in this chapter, studies using inoculation of food with pathogens were considered to contribute less to the body of evidence on disinfectant effectiveness than those studies using natural contamination. Similarly, studies that generate data on the effect of disinfectants using industrial-scale equipment are more likely to accurately describe disinfectant effects in practice compared with studies conducted in laboratories using experimental equipment. Thus, studies in industrial settings generally contribute more to the body of evidence.

The data on pathogen reduction achieved by food disinfectants that have been identified in this chapter were assessed using the matrix shown in Table 4.1. In each case, adjustments were made to this general categorization to accommodate the specific details of the study, such as suitable controls or clear articulation of the disinfection conditions.

Table 4.1. Relative strength of the contribution of study data to the general body of evidence based on study type

	Natural contamination	Inoculated studies
Industrial data	High ^a	–
Pilot-scale data ^b	High ^c	Medium ^d
Laboratory data	Medium ^d	Low ^e

^a Ideal data also quantify counts and prevalence of pathogens with statistical analysis.

^b Experiments using industrial equipment in non-industrial settings.

^c If the pilot process is representative of the industrial process; otherwise, evidence makes a “medium” contribution to the body of evidence.

^d Data would not be sufficient to inform a quantitative microbial risk assessment or to allow definitive conclusions on risk reduction.

^e Data are indicative of a disinfectant effect that may be reproducible in practice, but on their own do not allow definitive conclusions on risk reduction.

4.2 Poultry

4.2.1 Pathogens

Several pathogenic bacteria have been associated with raw poultry. These are *Salmonella enterica* subsp. *enterica*, *Campylobacter* spp., *Clostridium perfringens*, *Listeria monocytogenes*, *Staphylococcus aureus*, pathogenic *Escherichia coli* and *Yersinia enterocolitica* (Cox et al., 2005). However, the main pathogenic bacteria associated with human illness resulting from the consumption of poultry and poultry products are species of the genera *Salmonella* and *Campylobacter*.

Campylobacter is the leading cause of zoonotic enteric infections in most developed and developing countries (Aarestrup & Engberg, 2001). The reported incidence rates of *Campylobacter* infections vary widely among countries; in 2004, rates ranged from 12.8 cases per 100 000 inhabitants in the United States of America (USA) to 299.1 cases per 100 000 inhabitants in New Zealand. Some of the variation may in part be explained by differences in surveillance systems, diagnostic methods and means of reporting, so caution should be used when drawing inferences from these data. Estimates of campylobacteriosis in developing countries, developed from laboratory-based surveillance studies in the general population, range from 5% to 20%, with significantly higher incidence rates in children (Coker et al., 2002).

Over 2500 *Salmonella enterica* serotypes are recognized, and all are regarded as capable of producing disease in humans. Worldwide, salmonellosis is a leading cause of enteric infectious disease attributable to foods. Illnesses caused by the majority of *Salmonella* serotypes range from mild to severe gastroenteritis and, in some patients, bacteraemia and a variety of associated longer-term conditions (FAO/WHO, 2002a).

Modern poultry processing is rapid, intensive and highly mechanized. As it is a wet process, there are considerable opportunities for the spread of *Salmonella* and *Campylobacter* spp. This section focuses on evaluating the evidence associated with the main disinfection processes in common use today in some countries and their effectiveness at reducing the contamination risks associated with *Salmonella* and *Campylobacter* spp. on poultry.

4.2.2 Common disinfection practices

Chlorine gas and hypochlorite are historically the common forms of chlorine that have been used in the poultry industry. However, other forms of chlorine have emerged, including

acidified sodium chlorite (ASC), chlorine dioxide and electrolysed water containing chloride ions. In addition, there are several non-chlorine-based alternative disinfectants that are available, such as trisodium phosphate (TSP), cetylpyridinium chloride and peroxyacetic acid. These disinfectants are primarily used for the purpose of post-processing sanitization of plant and equipment as well as reducing contamination of the raw product with pathogenic and spoilage bacteria and control of microbial cross-contamination. A review of commercial disinfectants used in poultry processing was conducted by Oyarzabal (2005). This review cites the approved disinfectants in the USA and their approved conditions of use. Similar conditions of use are employed in some other countries.

Whereas there are many potential chemicals and points of application during poultry processing, there are a few in common use that have been identified (chapter 1) for which the data have been summarized. These include:

- hypochlorite for carcass washing pre-chill or post-chill;
- hypochlorite in carcass chillers;
- ASC as a carcass wash pre-chill and post-chill;
- chlorine dioxide as a carcass wash or in chiller water;
- peroxyacetic acid for carcass spraying.

The following section summarizes the available information related to the effectiveness of these practices at reducing the contamination risks associated with *Salmonella* and *Campylobacter* in poultry.

4.2.3 Effectiveness of common disinfection practices

A keyword search (focusing on *Salmonella* and *Campylobacter* in poultry) of the current published scientific literature, including e-journals, was conducted. The *Journal of Food Protection*, *Poultry Science* and *Journal of Food Science* publishers' databases were also searched. The reference sections of identified papers were also used as a source of relevant papers. The results from a call for data put out by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) were considered when relevant. In total, 39 suitable scientific papers from 1965 to 2007 were obtained and reviewed for this exercise.

4.2.3.1 Hypochlorite for carcass washing pre-chill and post-chill

In a laboratory experiment using artificially inoculated poultry, Olson et al. (1981) demonstrated that dipping inoculated chicken wings in chlorinated water (20 mg/l) reduced the numbers of *Salmonella* Typhimurium from 0.91 log colony-forming units (cfu)/g to 0.54 log cfu/g and that this result was statistically significant compared with *Salmonella* numbers on wings that were not dipped.

The effectiveness of an inside–outside bird washer (IOBW) followed by chilling in tap water for 45 min at 4 °C was evaluated on a pilot scale using chicken carcasses artificially inoculated with *Salmonella* Typhimurium (Yang, Li & Slavik, 1999). Washing with a 50 mg/l chlorine solution at 20 °C (17 s spray time with 60 s contact time before wash-off) compared with washing in tap water provided a statistically significant reduction in *Salmonella* of 0.63 log cfu/carcass in only one trial of the three performed. In the second trial, the result was not statistically significant, and in the final trial, the water wash removed more *Salmonella* than the chlorine wash.

Northcutt et al. (2005) conducted similar studies on the effectiveness of chlorine washes. Carcasses were inoculated with caecal contents containing nalidixic acid-resistant *Salmonella*. Water at 21.1, 43.3 and 54.4 °C with and without available chlorine at 50 mg/l was sprayed onto carcasses for 5 s with an IOBW. Neither water temperature nor chlorine level was found to have a statistically significant effect on the counts of *Salmonella*. However, the physical action of washing alone resulted in a reduction of 0.7–1.1 log cfu/ml in *Salmonella* on the inoculated carcasses.

Stopforth et al. (2007), in an industrial study using natural contamination, showed that spraying poultry carcasses with 20–50 mg/l chlorinated water after defeathering reduced *Salmonella* prevalence by 8 percentage points, from 34% to 26%. They also showed that spraying carcasses with 20–50 mg/l chlorinated water after evisceration reduced *Salmonella* prevalence by 9 percentage points, from 45% to 36% (statistically significant). Spray application of 20–50 mg/l chlorinated water after neck removal using an IOBW reduced *Salmonella* prevalence by 5 percentage points, from 25% to 20% (not statistically significant). A second IOBW after the first IOBW using 20–50 mg/l chlorinated water reduced *Salmonella* prevalence by 4 percentage points, from 16% to 12% (not statistically significant). Spray-washing carcasses after chilling with 20–50 mg/l chlorinated water did not affect the prevalence of *Salmonella*. However, a post-chiller wash of carcasses with 20–50 mg/l chlorinated water after sizing reduced *Salmonella* prevalence by 6 percentage points, from 10% to 4% (not statistically significant). None of these treatments were compared with similar washing treatments using unchlorinated water alone, and therefore any additional effect of hypochlorite over the physical washing effect of water alone cannot be established. Evidence from other studies (e.g. Northcutt et al., 2005) suggests that the physical action of washing alone can remove pathogenic bacteria inoculated onto poultry samples, although the washing effect on natural contamination is not clear.

Villarreal, Baker & Regenstein (1990) studied the effect of a commercial carcass washer (chlorine concentration 20 mg/l) on natural *Salmonella* contamination of turkey carcasses. *Salmonella*-positive carcass prevalence rates dropped from 75% and 65% to 10% and 20%, respectively, using a spray carcass rinse with chlorine at 20 mg/l. This reduction was statistically significant compared with unwashed carcasses, but there was no control using washing in unchlorinated water.

The effectiveness of a chlorine carcass wash was evaluated in a study in which poultry carcasses were inoculated with caecal material containing *Campylobacter* (Northcutt et al., 2005). Water at 21.1, 43.3 and 54.4 °C with and without available chlorine at 50 mg/l was sprayed onto carcasses for 5 s with an IOBW. Neither water temperature nor chlorine level was found to have a statistically significant effect on the counts of *Campylobacter*. The physical action of washing alone resulted in a reduction of 2.1–2.8 log cfu of *Campylobacter* per carcass, although numbers were not reduced below those of the natural *Campylobacter* load on carcasses prior to inoculation. In another study on naturally contaminated poultry in a commercial plant, an IOBW with hypochlorinated water resulted in a *Campylobacter* reduction of 0.7 log cfu/carcass (statistically significant) and 0.34 log cfu/carcass (not statistically significant) in two experiments, but the prevalence of *Campylobacter*-contaminated carcasses was not affected (Oyarzabal et al., 2004). However, no unchlorinated washing controls were evaluated.

Chlorine was found to be effective against *Campylobacter* in a laboratory study of extended washing conducted on inoculated chicken breast skin (Park, Hung & Brackett, 2002). Chicken wing sections were inoculated with *Campylobacter* and immersed in the test solutions of chlorine (~50 mg/l) with a deionized water control at 4 °C and 23 °C for 10 and 30 min with agitation before analysis. *Campylobacter* was reduced by 1.14 and 1.21 log cfu/g at 23 °C for 10 and 30 min, respectively, in deionized water alone. Hypochlorite resulted in

further reductions of 1.64 and 1.76 log cfu/g at 23 °C for 10 and 30 min, respectively, and 1.47 and 1.6 log cfu/g at 4 °C for 10 and 30 min, respectively, in comparison with washing in deionized water alone. However, the contact times in this experiment were substantially longer than those employed in commercial premises.

Bashor et al. (2004) made a comprehensive study of carcass washing in four poultry processing plants. A single IOBW with 25 mg/l chlorinated water reduced *Campylobacter* numbers by 0.31 log cfu/carcass, and a series of three IOBW units reduced *Campylobacter* numbers by 0.45 log cfu/carcass. The prevalence of *Campylobacter*-positive carcasses was reduced from 86.6% pre-wash to 80% post-triple wash. Similar results were achieved in a second plant using a similar setup of three IOBW units in series, but all with a higher level of chlorinated water, at 35 mg/l. A reduction in *Campylobacter* of 0.63 log cfu/carcass was observed after the three washing units, and the prevalence of *Campylobacter*-positive carcasses was reduced from 83% pre-wash to 80% post-triple wash. Unfortunately, statistical analysis of the between-plant effects of the different chlorine concentrations was not reported by the authors. The effect of washing alone in water without any chemical addition was not reported, but from other studies cited above, it is possible that the physical washing action alone contributed substantially to the reductions achieved.

Summary

Table 4.2 summarizes the effects of hypochlorite on *Salmonella* and *Campylobacter* during carcass washing before and after chilling.

Industrial studies by Stopforth et al. (2007) and Villareal, Baker & Regenstein (1990) demonstrated an effect of washing carcasses in hypochlorite solution on the prevalence of *Salmonella*. However, these did not include an evaluation of the effect on *Salmonella* numbers of washing in water alone in the absence of chlorine. Other studies (Yang, Li & Slavik, 1999; Northcutt et al., 2005) showed that washing in water alone resulted in most of the reductions in *Salmonella* inoculated onto poultry. Therefore, it is not possible to make a definitive statement on the effectiveness of hypochlorite against *Salmonella* during carcass washing on an industrial scale based on these studies. It is likely that washing in water alone is a moderately effective intervention and that hypochlorite does not provide a significant additional effect.

Laboratory-based experiments have shown reductions in *Campylobacter* on carcasses of less than 2 log units, but only over extended washing times (up to 30 min). Other experiments using more practical conditions show reductions of less than 1 log unit on *Campylobacter* in comparison with no washing. However, when compared with washing in water alone, there was no effect on *Campylobacter* inoculated onto carcasses washed in water with hypochlorite (Northcutt et al., 2005). The industrial studies by Bashor et al. (2004) showed log reductions in *Campylobacter* in the order of 0.5 log units and prevalence reductions of between 3 and 7 percentage points after extensive washing in a series of three IOBW units. However, the action of washing in water alone was not evaluated. Therefore, as with *Salmonella*, it is likely that washing in water alone is a moderately effective intervention and that hypochlorite does not provide a significant additional effect.

The removal of pathogenic bacteria from poultry carcasses during physical washing procedures on an industrial scale is predominantly a feature of the physical action of the water rather than the use of hypochlorite in the water.

Table 4.2. Studies of hypochlorite for poultry carcass washing pre-chill and post-chill

Conditions of use	Pathogen	Setting	Contamination type	Contribution to body of evidence	Effect on numbers and prevalence	Reference
20 mg/l (manual spray)	<i>Salmonella</i>	Industrial	Natural	High	75% reduced to 10% ^a 65% reduced to 20% ^a	Villarreal, Baker & Regenstein (1990)
20–50 mg/l (various spray-washing methods pre-chill and post-chill)	<i>Salmonella</i>	Industrial	Natural	High	Between 4 and 8 percentage point reduction, depending on method ^a	Stopforth et al. (2007)
50 mg/l	<i>Salmonella</i>	Pilot	Inoculated	Medium	No consistent log reduction over water washing alone	Yang, Li & Slavik (1999)
50 mg/l	<i>Salmonella</i>	Pilot	Inoculated	Medium	No log reduction over water washing alone	Northcutt et al. (2005)
20 mg/l	<i>Salmonella</i>	Experimental	Inoculated	Low	0.37 log reduction ^a	Olson et al. (1981)
Not stated	<i>Campylobacter</i>	Industrial	Natural	Medium ^b	0.7 log reduction ^a	Oyarzabal et al. (2004)
50 mg/l	<i>Campylobacter</i>	Pilot	Inoculated	Medium	0.34 log reduction ^a	Northcutt et al. (2005)
50 mg/l (4 °C and 23 °C), 10 min and 30 min wash	<i>Campylobacter</i>	Experimental	Inoculated	Low	No log reduction over water washing alone	Park, Hung & Brackett (2002)
25 mg/l (three washers in sequence)	<i>Campylobacter</i>	Industrial	Natural	High	1.47–1.76 log reductions over water washing alone	Bashor et al. (2004)
35 mg/l (three washers in sequence)	<i>Campylobacter</i>	Industrial	Natural	High	0.31 log reduction (one washer), 0.45 log reduction (three washers), 6.6 percentage point reduction (after three washers) ^a	Bashor et al. (2004)
					0.63 log reduction (three washers), 3 percentage point reduction (after three washers) ^a	

^a Reductions were not compared with a control using water washing alone, and therefore it is not possible to separate the reduction resulting from the physical action of spraying carcasses with water and any additional effect of using hypochlorite in the wash water.

^b Contribution rating reduced because no conditions of use or concentration of chlorine provided.

4.2.3.2 Hypochlorite in carcass chillers

Hypochlorite is routinely used in poultry process lines in countries where chilling by water immersion is allowed. It is added to the chiller water to prevent the buildup of bacteria in the water during processing. Several studies have produced quantitative data on the effect of chlorinated chiller water both on the reduction of *Salmonella* and *Campylobacter* numbers on contaminated poultry carcasses and also in prevention of cross-contamination of uncontaminated carcasses from bacteria released into the chiller water from contaminated carcasses.

The effect of sodium hypochlorite (50 mg/l) in chiller water was evaluated in a study conducted on a pilot and commercial scale (Russell & Axtell, 2005). In a pilot-scale poultry chiller (5 °C, 1 h), mean log counts of nalidixic acid-resistant strains of *Salmonella* inoculated onto chicken carcasses were not reduced by hypochlorite. Immersion chilling in tap water alone reduced the count from 2.9 to 1.6 log cfu/ml. The addition of chlorine to the tap water had no additional effect over tap water alone. The statistical significance of these differences was not reported.

Thomson, Cox & Bailey (1976) conducted a laboratory study into the effects of water treated with sodium hypochlorite on a nalidixic acid-resistant marker strain of *Salmonella* Typhimurium. Inoculated carcasses were pre-chilled in a stirred water tank at 18 °C for 3 min before being transferred to a chilling regime consisting of a stirred chill tank at 18 °C for 10 min and then a second chill tank containing slush ice for 20 min. When the pre-chill and chill treatments with chlorine at 50 mg/l were compared with chilling in water alone with no pre-chill treatment, the carcass prevalence of the marker strain *Salmonella* dropped from 85% to 45% for inoculated carcasses and from 15% to 2% for uninoculated carcasses. This demonstrated an effect of chlorine and pre-chill agitation on *Salmonella* prevalence for infected carcasses as well as prevention of cross-contamination of uninfected carcasses. However, it is not possible to separate the individual effects of chlorine and pre-chill agitation because of a lack of a suitable control.

In a later study, Thomson et al. (1979) again used carcasses inoculated with the nalidixic acid-resistant marker strain of *Salmonella* Typhimurium. Here, inoculated carcasses were pre-chilled in a stirred water tank at 18 °C for 10 min before being transferred to a stirred chill tank containing slush ice for 20 min. The water in the tanks was chlorinated to an available chlorine level of 20 or 50 mg/l at pH 6.0. They noted that there was no statistically significant effect on the prevalence of *Salmonella* recovered from inoculated carcasses at either 20 or 50 mg/l. Uninoculated carcasses processed alongside inoculated carcasses were contaminated with the marker strain of *Salmonella* at a prevalence rate of 80% in the absence of chlorine. However, there was a statistically significant reduction in the prevalence of *Salmonella*-positive uninoculated carcasses at both chlorine concentrations: at 20 mg/l (to 33% at a flow rate of 1.9 litres per carcass and 58% at a flow rate of 0.95 litres per carcass) and at 50 mg/l (to 10% at a flow rate of 1.9 litres per carcass and 8% at a flow rate of 0.95 litre per carcass).

In a further study, the mean prevalence of *Salmonella*-positive carcasses was unaffected by chilling in water containing chlorine at 20–50 mg/l at pH 6.5–7.0 (Stopforth et al., 2007). Similarly, no statistically significant changes in the mean prevalence of *Salmonella*-positive carcasses were observed after immersion chilling poultry carcasses in water containing chlorine at 25 mg/l at the inflow and 9 mg/l at the outflow (James et al., 1992). However, this study showed that chilling in water without chlorination resulted in an increase in *Salmonella* prevalence on carcasses from 48% to 72%.

Lillard (1980) studied the effects of hypochlorite in chiller water on the prevalence of *Salmonella*-positive poultry carcasses. Chilling carcasses in water with chlorine at 20 and 34

mg/l resulted in an average carcass *Salmonella* prevalence rate reduction from 14.3% in untreated water to between 4.5% and 1.9% in chlorinated water. The effect of chlorine concentration was statistically insignificant.

Yang, Li & Johnson (2001) studied the effect of chlorine in chiller water on the death kinetics of inoculated nalidixic acid-resistant *Salmonella* Typhimurium on chicken skin. They reported that a 50 mg/l addition of chlorine resulted in a residual free chlorine level of 34 mg/l after 1 min, decreasing to 20 mg/l after 50 min, and this had little effect on the death kinetics of *Salmonella* (D-value 78.7 min). With older chiller water, where organic material had built up, the residual concentration of free chlorine was approximately zero after 1 min, and here the D-value for *Salmonella* on chicken skin increased to 167.7 min. This clearly illustrates the inactivation of chlorine by organic matter, its effect on *Salmonella* and the need to maintain chlorine addition to chiller water during processing to achieve the necessary free chlorine concentration.

In a study in an industrial plant by Bashor et al. (2004), a reduction in *Campylobacter* of 0.13 log cfu/carcass was achieved after chilling in water with chlorine at 25 mg/l, and the prevalence of *Campylobacter*-positive carcasses was reduced from 80% post-wash to 73.3% post-chill. In a second plant using a chill tank with a higher level of chlorinated water, at 35 mg/l, a reduction in *Campylobacter* of 0.25 log cfu/carcass was observed after chilling. The prevalence of *Campylobacter*-positive carcasses was reduced from 80% post-wash to 70% post-chill. Unfortunately, statistical analysis of the between-plant effects of the different chlorine concentrations was not reported by the authors.

The effect of chlorine in chiller water on the death kinetics of inoculated *Campylobacter jejuni* was studied on chicken skin (Yang, Li & Johnson, 2001). Chilling in chlorinated water with 50 mg/l added chlorine (free chlorine residual level of 34 mg/l after 1 min, decreasing to 20 mg/l after 50 min) resulted in a D-value for *Campylobacter* on chicken skin of 73 min. However, using older chiller water initially with chlorine at 50 mg/l, where organic material had built up, the residual concentration of free chlorine was approximately zero after 1 min. Chilling in this water resulted in a D-value for *Campylobacter* on chicken skin of 344.8 min. A similar result was seen with *Salmonella*, confirming the need to maintain free residual chlorine levels in chiller water during processing. However, Yang, Li & Johnson (2001) demonstrated that chlorine was effective at killing free *Campylobacter* in chiller water but did not examine the effect that this might have had on carcass prevalence.

In another study on naturally contaminated poultry in a commercial plant, a chiller with chlorinated water resulted in a *Campylobacter* reduction of 1.09 log cfu/carcass (statistically significant) and 1.3 log cfu/carcass (statistically significant) in two experiments. The prevalence of *Campylobacter*-contaminated carcasses was not affected in the first experiment but was reduced from 95% to 77.5% in the second experiment (Oyarzabal et al., 2004). However, no unchlorinated chiller water controls were evaluated.

Mead, Hudson & Hinton (1995) examined the effect of the chlorination of process water at several stages in the poultry slaughter process using hypochlorite in the chiller water and chlorine gas to chlorinate in-plant water (the forms of chlorine were not stated in the paper but were confirmed by personal communication). Water was chlorinated at the killing machine, the three defeathering machines, the head puller, conveyor belt to evisceration line, evisceration machines and other machinery in contact with birds, as well as in the chiller, to between 28 and 38 mg/l as available chlorine. Carcass neck skin samples were tested for *Campylobacter*. Individual process steps were not tested for their effect on *Campylobacter* reduction; instead, this was done for the process as a whole. Therefore, the effect of chlorine alone cannot be evaluated. However, a comparison of flocks before and after process changes involved only those flocks with similar levels of caecal carriage of *Campylobacter*. Before changes, 100% of samples were positive for *Campylobacter* after exsanguinations, with a log

geometric average count of 3.7 log cfu/g, and 91% of samples were positive after packing, with a log geometric average count of 1.8 log cfu/g. Following changes, 100% of samples were still positive after exsanguinations, with a log geometric average count of 3.9 log cfu/g, but 85% of samples were positive after packing, with a log geometric average count of 1.2 log cfu/g.

Summary

Table 4.3 summarizes the studies on the effect of hypochlorite in chiller tanks of *Salmonella* and *Campylobacter* on poultry. Studies evaluating the numbers of *Salmonella* on carcasses before and after chilling are few. However, Russell & Axtell (2005) noted a reduction in *Salmonella* numbers inoculated onto carcasses caused by the physical movement of carcasses in the chiller water rather than the presence of hypochlorite in the chiller water. Experiments by Thomson et al. (1979) showed that greater reductions in the prevalence of *Salmonella* (inoculated) on carcasses were achieved with chlorinated water than with non-chlorinated water, by a combination of pre-chill and chill treatments.

Overall, the studies show that if chlorine is not present in chiller water, then the prevalence of *Salmonella* on carcasses increases because of cross-contamination. This is supported by Lillard (1980), who showed that the prevalence of *Salmonella* in chiller water treated with chlorine at 34 and 20 mg/l was reduced from 41.7% (untreated water) to “not detected” and 17.3%, respectively. Other data not elaborated here also demonstrate the effectiveness of chlorine in killing free *Salmonella* and *Campylobacter* in chiller water (Yang, Li & Johnson, 2001).

The effects of chlorinated chiller water on the prevalence of *Campylobacter*-contaminated carcasses and also mean contamination concentrations per carcass seem to be slightly greater than the effects on *Salmonella*, but reports are inconsistent. Small reductions in both numbers and prevalence of *Campylobacter* on carcasses were observed when chiller water was chlorinated.

Rapid inactivation of chlorine by organic matter greatly reduced its ability to kill *Campylobacter* and *Salmonella* in the chiller water itself. Hence, chlorine must be continually dosed into chiller water to maintain residual activity.

4.2.3.3 ASC as a carcass wash pre-chill and post-chill

The effectiveness of ASC was evaluated by Stopforth et al. (2007) as part of a study on multiple sequential interventions conducted in three poultry processing plants in the USA. Spray application of ASC (500–1200 mg/l as sodium chlorite acidified with citric acid to pH 2.5–2.9) reduced the prevalence of *Salmonella* on carcasses from 17% to 9% (statistically significant). Dipping carcass parts in ASC had an even bigger effect, reducing the prevalence from 29% to 1%. Controls to evaluate the physical effect of dipping and spraying carcasses in water alone were not included.

Spray treatment of poultry carcasses with ASC followed by chilling was studied in five poultry plants in the USA (Kere-Kemp et al., 2001). Carcasses that were visibly contaminated with faecal matter were tested after evisceration, after the IOBW, after spray treatment with ASC (1100 mg/l as sodium chlorite acidified with citric acid at 9000 mg/l, pH 2.5, for 15 s at 14–18 °C) and after chilling. The IOBW reduced the prevalence of *Salmonella*-positive carcasses from 37.3% to 31.4%. Treatment with the IOBW followed by ASC spray resulted in a reduction in the prevalence of *Salmonella*-positive carcasses from 37.3% to 10%. Controls to evaluate the physical effect of spraying carcasses in water alone were not included.

Table 4.3. Studies of hypochlorite in poultry carcass chillers

Conditions of use	Pathogen	Setting	Contamination type	Contribution to body of evidence	Effect on numbers and prevalence	Reference
50 mg/l	<i>Salmonella</i>	Pilot	Inoculated	Medium	1.3 log reduction in water alone; no additional effect of hypochlorite	Russell & Axtell (2005)
50 mg/l (18 °C pre-chill in tank with stirring and two-stage chill in water, then ice slush)	<i>Salmonella</i>	Pilot	Inoculated	Medium	85% reduced to 45% (inoculated carcasses) 15% reduced to 2% (uninoculated carcasses)	Thomson, Cox & Bailey (1976)
20 and 50 mg/l (18 °C pre-chill in tank with stirring chill in ice slush)	<i>Salmonella</i>	Pilot	Inoculated	Medium	No change in prevalence (inoculated carcasses) 80% reduced to 33% or 10% (uninoculated carcasses)	Thomson et al. (1979)
20–50 mg/l (chiller tank pH 6.5–7.0)	<i>Salmonella</i>	Industrial	Natural	High	No reduction in prevalence	Stopforth et al. (2007)
25 mg/l (drag-through chiller)	<i>Salmonella</i>	Industrial	Natural	High	48% increased to 72% (unchlorinated water)	James et al. (1992)
20–34 mg/l (chiller tank)	<i>Salmonella</i>	Industrial	Natural	High	No change in prevalence (chlorinated water) 14.4% (untreated water) reduced to between 4.5% and 1.9% (concentration of chlorine insignificant)	Lillard (1980)
25 mg/l (chiller tank)	<i>Campylobacter</i>	Industrial	Natural	High	0.13 log reduction, 80% reduced to 73.3% ^a	Bashor et al. (2004)
35 mg/l (chiller tank)	<i>Campylobacter</i>	Industrial	Natural	High	0.25 log reduction, 80% reduced to 70% ^a	Bashor et al. (2004)
Not stated	<i>Campylobacter</i>	Industrial	Natural	Medium ^b	1.09 log reduction, no change in prevalence ^a 1.3 log reduction, 95% reduced to 77.5% ^a	Oyarzabal et al. (2004)

^a Reductions were not compared with a control using water chilling alone, and therefore it is not possible to separate the reduction resulting from the physical action of carcass agitation in water and any additional effect of using hypochlorite in the chiller water.

^b Contribution rating reduced because no conditions of use or concentration of chlorine provided.

The effect of ASC on *Salmonella* Enteritidis was also studied on inoculated chicken legs followed by chill storage at 3 °C over 5 days (Del Río et al., 2007). Sodium chlorite (1200 mg/l) was acidified with citric acid to pH 2.7 and applied to the inoculated legs as a dip for 15 min. Treatment resulted in mean log reductions over untreated controls of 2.05, 2.42, 2.25 and 1.65 log cfu/g skin on sampling days 0, 1, 3 and 5, respectively. However, the mean log reductions were not significantly different from each other on any one sampling day. A water dip control achieved a 0.33 log cfu/g reduction in *S. Enteritidis*, but in that case, the pathogen grew on the samples during storage over the 5-day period. Sexton et al. (2007) studied ASC treatment of chicken carcasses in a plant after the screw chiller using birds naturally contaminated with *Salmonella*. Sodium chlorite (900 mg/l) was acidified with citric acid to pH 2.5–2.6. Carcasses were dipped in the treatment solution after chilling for 20 s before testing after a maximum of 4 h. The prevalence of *Salmonella*-positive carcasses dropped from 90% to 10% after treatment. However, the log mean count on positive carcasses remained similar between untreated carcasses (–1.8 log cfu/cm²; standard deviation [SD] 0.56 log cfu/cm²) and treated carcasses (–1.85 log cfu/cm²; SD 0.55 log cfu/cm²). Controls to evaluate the physical effect of the dipping carcasses in water alone were not included.

ASC carcass treatment followed by chilling was studied in an industrial setting for activity against *Campylobacter* (Kere-Kemp et al., 2001). Carcasses that were visibly contaminated with faecal matter were sampled after evisceration, after IOBW, after ASC spray treatment and after chilling. Sodium chlorite (1100 mg/l) acidified with citric acid (9000 mg/l, pH 2.5) was sprayed (15 s) onto carcasses at 14–18 °C. The IOBW reduced the *Campylobacter* numbers on contaminated carcasses by an average of 1.08 log cfu/carcass but did not affect the prevalence of *Campylobacter*-positive carcasses (73.2% post-evisceration versus 74.8% post-IOBW). The IOBW followed by ASC spray treatment resulted in a reduction in *Campylobacter* of 2.56 log cfu/carcass, and the prevalence of *Campylobacter*-positive carcasses was reduced from 73.2% to 49.1%. Controls to evaluate the physical effect of the spraying of carcasses with water alone were not included.

ASC was studied for its effects on *Campylobacter* inoculated onto chicken breast skin in a laboratory study (Arritt et al., 2002). ASC (0.1% volume by volume [v/v]) was sprayed as a fine mist onto skin samples for 3 s with 0.5, 3 and 10 min contact time. Treatment with water alone resulted in a reduction in *Campylobacter* of 0.15 log cfu/skin sample, whereas treatment with ASC resulted in a reduction of 1.52 log cfu/skin sample. These reductions were mean reductions across all contact times, as contact time was found to have no significant effect on the ability of the antimicrobial agent to kill *Campylobacter*. Arritt et al. (2002) also demonstrated that the antimicrobial agents were even more effective at killing *Campylobacter* when the bacteria were applied to skin samples after application of the antimicrobial agent.

The activity of ASC (900 mg/l as sodium chlorite acidified with citric acid to pH 2.5–2.6) was also tested as a carcass dip on carcasses naturally contaminated with *Campylobacter* after a screw chiller in a commercial plant (Sexton et al., 2007). The prevalence of naturally contaminated *Campylobacter*-positive carcasses was reduced from 100% to 23% by ASC treatment of carcasses, and the mean count on positive carcasses dropped from 1.59 log cfu/cm² (SD 0.51 log cfu/cm²) to –2.21 log cfu/cm² (SD 0.17 log cfu/cm²) compared with untreated control carcasses. The effect of a control dip in water alone was not reported. Oyarzabal et al. (2004) studied the use of an ASC dip for controlling *Campylobacter* on broiler carcasses after chilling in a commercial plant. ASC (600–800 mg/l as sodium chlorite acidified to pH 2.5–2.7) was used as a carcass dip with 15 s contact time. Mean log reduction of *Campylobacter* was 0.92 log cfu/carcass, and prevalence rates dropped from 100% of carcasses to 12.5%, compared with untreated carcasses. In a second experiment, mean log

reduction of *Campylobacter* was 1.2 log cfu/carcass, and prevalence rates dropped from 77.5% of carcasses to 2.5%, compared with untreated carcasses. The effect of a control dip in water alone was not reported. Bashor et al. (2004) studied the effectiveness of an ASC spray treatment against *Campylobacter* in a commercial plant. They found that ASC reduced *Campylobacter* populations on average by 1.26 log units. The effect of a carcass spray with water alone was not studied. Overall, in these three studies, the absence of controls for carcass washing in water alone makes it difficult to draw definitive conclusions regarding the effect of including ASC in the wash water independent of the physical effects of spraying or dipping.

The effect of chlorine and Alcide (a product containing an activator of 16.7% lactic acid and a base containing 3.03% sodium chlorite) on *Salmonella* on turkey carcasses was evaluated in a process plant (Villarreal, Baker & Regenstein, 1990).¹ *Salmonella* prevalence was reduced to zero from 75% and 65% following chlorine rinse (20 mg/l) and chilling of the rinsed carcasses in iced water containing the Alcide solution (1 part Alcide base : 200 parts water : 1 part Alcide activator). However, dip-rinsing carcasses for 20 s in Alcide (1 part Alcide base : 20 parts water : 1 part Alcide activator), with or without chilling in water with the Alcide solution, also reduced the contaminated carcass prevalence rate from 75% and 65% to zero. No controls were used to study the effect of rinsing and chilling carcasses in untreated water alone.

In a study of post-chill carcass treatment, chicken skin samples inoculated with *Campylobacter jejuni* were exposed to ASC (0.1% sodium chlorite, 0.9% citric acid, pH 2.43) for up to 5 days (Ozdemir, Gugukoglu & Koluman, 2006). Reductions in *Campylobacter* compared with immersion in tap water alone were 1.9, 2.5, >3.3 and >3.0 log cfu/g skin after 0, 1, 3 and 5 days of chill storage at 4 °C, respectively. Similar results were also found using a second inoculated strain of *C. jejuni*.

Summary

Table 4.4 summarizes the effects of ASC on *Salmonella* and *Campylobacter* on poultry. ASC is an effective means of reducing the prevalence of *Salmonella*-contaminated carcasses during spray or dip treatments both pre-chill and post-chill. However, reliable data on the effect of ASC on numbers of *Salmonella* on carcasses were not found.

ASC was shown to be more effective against *Campylobacter*. As a spray or dip either pre-chill or post-chill, it resulted in log reductions of around 1.5 log cfu/g in industrial settings. The prevalence of *Campylobacter* was also reduced significantly. ASC activity appeared to extend into chill storage, but quantitative results were available only from laboratory-based experiments rather than commercial situations.

Most studies, particularly those conducted in the industrial setting, suffered from the lack of a control for the physical action of water alone as a spray or dip. However, evidence from a laboratory study that contained this control suggested that there was only a small effect on inoculated *Salmonella* of 0.15 log cfu/skin sample (Arritt et al., 2002). Also, studies tended not to use IOBW or high-volume sprays, and therefore they would be less likely to exert a physical reduction effect on bacteria on carcasses.

¹ The expert meeting recognizes that Villarreal, Baker & Regenstein (1990) considered Alcide to be a slow-release chlorine dioxide product. However, more recent understanding of the chemistry involved indicates that the appropriate active chemical should more correctly be referred to as either chlorous acid or ASC (S. Burnett, personal communication, 2009).

Table 4.4. ASC for poultry carcass washing pre-chill and post-chill

Conditions of use	Pathogen	Setting	Contamination type	Contribution to body of evidence	Effect on numbers and prevalence	Reference
500–1200 mg/l as sodium chlorite, pH 2.5–2.9 with citric acid (pre-chill spray)	<i>Salmonella</i>	Industrial	Natural	High	17% reduced to 9% ^a	Stopforth et al. (2007)
500–1200 mg/l as sodium chlorite, pH 2.5–2.9 with citric acid (pre-chill dip)	<i>Salmonella</i>	Industrial	Natural	High	29% reduced to 1% ^a	Stopforth et al. (2007)
1100 mg/l as sodium chlorite, pH 2.5 (15 s pre-chill spray)	<i>Salmonella</i>	Industrial	Natural	High	31.4% reduced to 10% ^a	Kere-Kemp et al. (2001)
1200 mg/l as sodium chlorite, pH 2.7 with citric acid (dip)	<i>S. Enteritidis</i>	Laboratory	Inoculated	Low	Additional 1.65–2.42 log reduction over 0.33 log cfu/g reduction (water dip alone)	Del Río et al. (2007)
900 mg/l as sodium chlorite, pH 2.5–2.6 with citric acid (post-chill dip)	<i>Salmonella</i>	Pilot	Natural	High	90% reduced to 10%, no change in log mean counts ^a	Sexton et al. (2007)
1100 mg/l as sodium chlorite, pH 2.5 (15 s pre-chill spray)	<i>Campylobacter</i>	Industrial	Natural	High	1.48 log reduction above the effect of an IOBW alone, 73.2% reduced to 49.1% ^a	Kere-Kemp et al. (2001)
0.1% v/v (spray as fine mist)	<i>Campylobacter</i>	Laboratory	Inoculated	Low	0.15 log reduction (water control) 1.52 log reduction (ASC)	Arritt et al. (2002)
1200 mg/l as sodium chlorite, pH 2.5	<i>Campylobacter</i>	Industrial	Natural	High	1.26 log reduction, 87% reduced to 63% ^a	Bashor et al. (2004)
900 mg/l as sodium chlorite, pH 2.5–2.6 with citric acid (post-chill dip)	<i>Campylobacter</i>	Pilot	Natural	High	100% reduced to 23%, 3.8 log reduction ^a	Sexton et al. (2007)

Conditions of use	Pathogen	Setting	Contamination type	Contribution to body of evidence	Effect on numbers and prevalence	Reference
Chlorine rinse (20 mg/l), then chilled in water with 1 part Alcide : 200 parts water : 1 part activator	<i>Salmonella</i>	Pilot	Natural	High	75% and 65% reduced to "not detected" (chlorine rinse 20 mg/l and chill in chlorinated water, 75% and 65% reduced to 25%) ^a	Villarreal, Baker & Regenstein (1990)
1 part Alcide : 20 parts water : 1 part activator (pre-chill dip)	<i>Salmonella</i>	Pilot	Natural	High	65% and 75% reduced to "not detected" ^a	Villarreal, Baker & Regenstein (1990)
600–800 mg/l as sodium chlorite, pH 2.5–2.7 (post-chill dip)	<i>Campylobacter</i>	Industrial	Natural	High	0.92 log reduction, 100% reduced to 12.5% ^a 1.2 log reduction, 77.5% reduced to 2.5% ^a	Oyarzabal et al. (2004)

^a Reductions were not compared with a control using water spray/dip alone, and therefore it is not possible to separate the reduction resulting from the physical action of water spray/dip and any additional effect of using ASC (see summary text for discussion).

4.2.3.4 Chlorine dioxide as a carcass wash or in chiller water

There are few studies examining the effect of chlorine dioxide on bacteria in poultry, and even fewer on pathogenic bacteria. These are summarized in Table 4.5.

Lillard (1980) studied the effects of chlorine dioxide in chiller water on the prevalence of *Salmonella*-positive poultry carcasses. Chilling carcasses in water with chlorine dioxide at 3 and 5 mg/l resulted in a reduction of the average carcass *Salmonella* prevalence rate from 14.3% with untreated water to 2.1% and 1%, respectively. The effect of chlorine dioxide concentration was statistically insignificant. Lillard (1980) also showed that the prevalence of *Salmonella* in chiller water treated with chlorine dioxide at 3 and 5 mg/l was reduced from 41.7% in the untreated water control to not detected and 25%, respectively. In another study, Thiessen, Osborne & Ogg (1984) reported that the prevalence rates of *Salmonella* on carcasses were reduced from 97.3% in untreated water to not detected, with residual chlorine dioxide at 1.33 mg/l or higher in the chiller water. Significant reductions of *Salmonella* were also reported in the chiller water itself with chlorine dioxide present.

Overall, the limited data set available suggests that chlorine dioxide is effective against *Salmonella* and *Campylobacter* on poultry. It is also active against *Salmonella* in chiller water and would therefore help to reduce cross-contamination.

4.2.3.5 Peroxyacetic acid for carcass spraying

The only data available on the effectiveness of peroxyacetic acid at reducing pathogens on poultry are laboratory-based data with artificial inoculation (see Table 4.6). Del Río et al. (2007) studied the effect of peroxyacetic acid (220 mg/l, pH 3.75) on *Salmonella* inoculated on poultry legs during an experimental dipping process. *Salmonella* was reduced by 0.36 ± 0.7 log cfu/g from 6.93 ± 0.47 log cfu/g by a 15 min dip. Subsequent storage over 5 days showed a statistically significant increase in the reduction achieved, up to 1.1 ± 0.59 log cfu/g. However, a control dipped in water alone resulted in a reduction of 0.33 ± 0.35 log cfu/g. Therefore, there was virtually no effect of peroxyacetic acid. Over 5-day storage, *Salmonella* on the water-dipped legs grew, whereas *Salmonella* on the peroxyacetic acid-dipped legs continued to die off.

In a study conducted by Ecolab (unpublished data, 2001), *S. Typhimurium* artificially inoculated on chicken skin was reduced by 0.75 log cfu/g after spray treatment with peroxyacetic acid at a concentration of 200 mg/l. The same study found that dipping chicken parts in peroxyacetic acid also had an effect, with wing and liver contamination reduced by 0.32 cfu/g and 0.45 log cfu/g, respectively. The statistical significance of these results was not reported, however, and there was no water spray control.

It appears from these limited data that peroxyacetic acid is not as effective as other antimicrobial agents against *Salmonella*. However, prevalence was not tested, and studies in industrial settings were not found. Peroxyacetic acid may have use as a means of preventing *Salmonella* growth on processed poultry, but more studies would be required. The lack of spray-wash controls with water alone to evaluate the effect of the physical action of water on pathogens on carcasses means that definitive conclusions on the effectiveness of peroxyacetic acid cannot be drawn. No data on the effect of peroxyacetic acid on *Campylobacter* were found in the search conducted.

Table 4.5. Chlorine dioxide in chiller water for poultry

Conditions of use	Pathogen	Setting	Contamination type	Contribution to body of evidence	Effect on numbers and prevalence	Reference
3 mg/l as chlorine dioxide (0.3–0.6 mg/l as free residual chlorine) (chiller water)	<i>Salmonella</i>	Industrial	Natural	High	14.3% (untreated water) reduced to 2.1%, numbers on positive carcasses 0.4–48 cells/g reduced to <0.4 cells/g	Lillard (1980)
5 mg/l as chlorine dioxide (0.5–1.0 mg/l as free residual chlorine) (chiller water)	<i>Salmonella</i>	Industrial	Natural	High	14.3% (untreated water) reduced to 1%, numbers on positive carcasses 0.4–48 cells/g reduced to <0.4 cells/g	Lillard (1980)
1.33 mg/l	<i>Salmonella</i>	Industrial	Natural	High	97.7% reduced to not detected	Thiessen, Osborne & Ogg (1984)

^a Reductions were not compared with a control using water spray/dip alone, and therefore it is not possible to separate the reduction resulting from the physical action of water spray/dip and any additional effect of using chlorine dioxide.

Table 4.6. Peroxyacetic acid as a wash or dip for poultry

Conditions of use	Pathogen	Setting	Contamination type	Contribution to body of evidence	Effect on numbers and prevalence	Reference
200 mg/l (skin spray)	<i>S. Typhimurium</i>	Laboratory	Inoculated	Low	0.75 log reduction ^a	Ecolab, unpublished data, 2001
200 mg/l (wing immersion)	<i>S. Typhimurium</i>	Laboratory	Inoculated	Low	0.32 log reduction ^a	Ecolab, unpublished data, 2001
200 mg/l (liver immersion)	<i>S. Typhimurium</i>	Laboratory	Inoculated	Low	0.48 log reduction ^a	Ecolab, unpublished data, 2001
220 mg/l (leg dipping, 15 min)	<i>S. Enteritidis</i>	Laboratory	Inoculated	Low	0.36 log reduction (peroxyacetic acid) 0.33 log reduction (water alone)	Del Río et al. (2007)

^a Reductions were not compared with a control using water spray/dip alone, and therefore it is not possible to separate the reduction resulting from the physical action of water spray/dip and any additional effect of using peroxyacetic acid.

4.2.4 Quantitative microbial risk assessment to evaluate the public health impact of the use of disinfectants in poultry processing

To evaluate the effect of chlorinated disinfectants on microbiological risk, it is necessary to establish the risks to health that certain food commodities pose in the absence of these chemicals. In the case of poultry, two quantitative risk assessment models have been previously developed for FAO/WHO, one on *Salmonella* and one on *Campylobacter* (FAO/WHO, 2002a,b). Only the *Campylobacter* model is suitable for illustrating the possible impact of food disinfectant use on public health outcomes. It has been possible to adapt this model to incorporate quantitative data on the effect of chlorine-based disinfectants on these *Campylobacter* in poultry production systems. As a result, a quantitative estimate of the risk reduction brought about by the use of chlorine-based disinfectants has been possible. However, in other food commodities reviewed—namely, red meat, fish and fishery products and fresh produce—no suitable quantitative risk assessment models were available.

The detailed model use and risk reduction outcome is shown in Appendix 1.

4.3 Red meat

4.3.1 Pathogens

Red meat is an important vehicle of foodborne human illness in many parts of the world and may be contaminated with a range of pathogenic bacteria (Skovgaard, 1999). When present, the organisms are usually carried asymptotically in the alimentary tract and on the skin or hide of animals. Meat can become contaminated at any of the stages involved in slaughter and carcass dressing or subsequently during handling or further processing in different parts of the supply chain. The principal pathogens of concern in primary processed meats are *Salmonella*, *Campylobacter* and verotoxigenic *Escherichia coli* (VTEC). VTEC are mainly associated with ruminants, especially cattle and sheep. VTEC are also a risk in some fermented products, and outbreaks of disease associated with this pathogen in salami-type products have been reported in a number of countries due to uncontrolled fermentations. Strains of *Listeria monocytogenes* are also commonly found in the primary processed product, but their public health significance in this context remains unclear. In further processed products, *L. monocytogenes* is of substantial concern, and a number of outbreaks of disease associated with this pathogen in these products have been reported.

4.3.2 Common disinfection practices

With respect to primary meat processing, a spray-chilling system is used in some abattoirs to reduce water loss and increase the chilling rate of carcasses by evaporative cooling, thus ensuring that the deep muscle reaches 10.0 °C within 24 h and 7.2 °C within 36 h (National Advisory Committee on Microbiological Criteria in Foods, 1993). During the first 12 h of chilling at about -3 °C, carcasses may be exposed intermittently (e.g. for 2 min every 30 min) to a fine mist of chilled water containing free chlorine concentrations up to 50 mg/l. Although this is not strictly an antimicrobial treatment, it was thought to contribute to the control of pathogenic and spoilage bacteria on the meat (Swift & Company, 1973; Dickson & Anderson, 1992). Other chemical-based antimicrobial treatment of carcasses is likely to be applied before the chilling process, with the aim of maximizing the effect on microbial contamination. These treatments vary, but generally include spraying of whole carcasses, primal or subprimal cuts, organs and trim with various antimicrobial chemicals

(including chlorine-based ones) in water. In the case of primal cuts, subprimal cuts and organs, immersion in water with antimicrobial compounds may also occur. Carcasses may also be sprayed with antimicrobial agents, which can be chlorine based, prior to hide removal in an attempt to reduce transfer of microorganisms from the hide to the surface of the meat. In the case of further processed products, contamination with *L. monocytogenes* often occurs post-cooking. Attempts to control this pathogen generally entail spraying the meat with, or immersing it in, a solution of antimicrobial chemical. Although chlorine-based products have been experimentally used in this context, their application in commercial processing facilities is very infrequent. In contrast, chlorine-based products may be used to control microorganisms on food contact surfaces during processing in both primary and secondary red meat processing in many parts of the world. This use, however, is often sporadic, and the degree of transfer of antimicrobial compounds to the meat remains undetermined. The most common antimicrobials used are hypochlorite, ASC and lactic acid (see chapter 1).

4.3.3 Effectiveness of common disinfection practices

Several studies have been carried out on pre-chill carcasses of beef, lamb and pork to determine the effects of spray-washing with superchlorinated water on either aerobic plate counts or counts of specific indicator bacteria. For example, Kotula et al. (1974) used chlorine at 200 mg/l at either 12.8 °C or 51.7 °C over a pH range of 4–7. When carcasses were sampled 45 min after treatment, aerobic plate counts were reduced by 1–2 log units, extending to more than 2 log units after 24 h. By increasing the washing pressure from 85 to 498.5 kPa, counts were reduced by more than 2 and 3 log units after 45 min and 24 h, respectively. Similar results were obtained by other workers (reviewed by Dickson & Anderson, 1992), and reductions in count ranged from 1 to 3 log units, depending on the experimental conditions. In other studies, however, there was no significant effect of chlorine on carcass contamination, and this may have been due to the initial presence of unusually low numbers of organisms or to the treatment conditions used.

There have been few studies on the effectiveness of chlorine-containing compounds against specific pathogens of concern, and even fewer in processing plants. Emswiler-Rose & Kotula (1984) used a model system rather than carcass meat to determine the chlorine sensitivity of pure cultures of various organisms. In each case, an agar plate was spread-inoculated with the test organism, and a disc of filter paper soaked in a chlorine solution at a specific concentration was placed on the surface. After incubation of the plate, the diameter of any zone of inhibition was measured. The lowest chlorine concentration at which inhibition occurred under these conditions was 78 mg/l for *Campylobacter jejuni*, 177 mg/l for *Yersinia enterocolitica* and 362 mg/l for *Salmonella* Typhimurium.

Cutter & Siragusa (1995) reported that an 800 mg/l chlorine spray-wash reduced counts of *E. coli* O157:H7 on inoculated beef carcass tissue by only 1.04 log cfu/cm², and spray treatments with 50, 100, 250 or 500 mg/l resulted in reductions of <1 log cfu/cm². Inoculated beef carcass tissue was also used by Stopforth et al. (2004) to determine the effect of chlorine sprays on acid-habituated and non-habituated *E. coli* O157:H7 under simulated chilling conditions. The meat samples were held at –3 °C for 10 h and sprayed for 30 s every 30 min with a 500 mg/l sodium hypochlorite solution at 4 °C. The samples were then transferred to 1 °C for a further 38 h. With acid-habituated cells, chlorine had no significant effect on the counts obtained immediately after spraying, but there was a 1.2 log cfu/cm² reduction after the full 48 h, a result comparable to that obtained by spraying plain water. Similarly, chlorine reduced counts of non-habituated cells by 0.6 log cfu/cm² and by a further 1.2 log cfu/cm² after 48 h. Again, the effects resembled those observed with water alone.

The efficacy of chlorine dioxide as a carcass decontaminant for beef was studied by Cutter & Dorsa (1995). Fresh beef carcass tissue was inoculated with bovine faeces and spray-treated in a pilot-scale washer for 10 s at 16 °C and 520 kPa, using chlorine dioxide at concentrations ranging from 0 to 20 mg/l. Regardless of chlorine dioxide concentration, bacterial populations were reduced by no more than 0.93 log cfu/cm², and the results were not statistically different from those obtained with plain water. Even with a chlorine dioxide concentration of 20 mg/l and an increase in water pressure to 690 kPa for up to 60 s, count reductions were no greater than those achieved with water. It was concluded that spray treatment with chlorine dioxide was no more effective than water for reducing bacterial contamination of beef.

Two forms of ASC were studied by Castillo et al. (1999), one activated by phosphoric acid, the other by citric acid. Trials involved inoculation of various sites on hot-boned beef carcasses, using either *E. coli* O157:H7 or *S. Typhimurium*. For both pathogens, counts were reduced by 3.8–3.9 log units when a water wash was followed by spraying with phosphoric acid-activated ASC and by 4.5–4.6 log units after spray-washing with citric acid-activated ASC. The corresponding reduction with water alone was 2.3 log units. All sites on the carcass were treated effectively, apart from the inside round, which showed lower reductions. With both forms of ASC, there was a clear reduction in count for organisms that spread beyond the initial inoculation site. In a study entailing the dipping of meat inoculated with *E. coli* O157:H7 or *S. Typhimurium* into ASC, a similar reduction in number (1.4–2.1 log units) as for the spray treatment for both of the pathogens was obtained (Harris et al., 2006).

The study of Stopforth et al. (2004) utilized samples of beef carcass tissue that were inoculated with either acid-habituated or non-habituated strains of *E. coli* O157:H7. Exposure to simulated conditions of carcass chilling involved –3 °C for 10 h, followed by 1 °C for a further 38 h. During the initial 10 h period, carcass samples were sprayed for 30 s every 30 min with either water or 0.12% ASC. The effect of ASC treatment was similar for both acid-habituated and non-habituated cells. Immediately after treatment, there was a 1.7–2.2 log reduction in count and a further decline of 0.9–1.1 log after the full 48 h of chilling, which was about 2.0 log units greater than that achieved with water alone.

Lactic acid is a non-chlorine-containing compound commonly used in sprays and washes for the control of pathogens during primary processing of red meat. Harris et al. (2006) demonstrated that the dipping of meat inoculated with *E. coli* O157:H7 or *S. Typhimurium* into 2% lactic acid gave a reduction in numbers (1.5–2.0 log units) similar to that achieved with ASC at 1200 mg/l. In another study, Sawyer et al. (2008) showed a 1.3–1.6 log unit reduction in numbers of the same two pathogens on meat dipped in a 2.5% lactic acid solution.

Although there is little information in the literature on the effect of chlorine usage in abattoirs on specific pathogens on meat, experiments with the model system of Emswiler-Rose & Kotula (1984) showed that *C. jejuni* was among the more chlorine sensitive of the organisms tested and notably more so than some strains of *Salmonella*. However, the studies on poultry described previously suggest that this difference in chlorine sensitivity is of little consequence in relation to spray treatment of carcasses. Under commercial conditions, spray-washing pre-chill red meat carcasses with chlorine has had a variable effect on aerobic plate counts or counts of indicator bacteria, and some studies have found no effect. Whether used in spray-cooling of carcasses or in a separate spray-washing process, there was little or no effect of chlorine on *E. coli* O157:H7, even at a concentration of 800 mg/l, and spray-washing with chlorine dioxide was similarly ineffective. Hence, both chlorine and chlorine dioxide, when used in these ways, have only a minimal effect on pathogens associated with beef carcasses, and therefore risk reduction is likely to be negligible.

Of the chlorine-containing products tested, only ASC, especially when activated by citric acid, was an effective antimicrobial in both spray-washing and spray-cooling systems, and counts of *E. coli* O157:H7 were reduced by approximately 2 log units on inoculated beef carcass tissue. These findings parallel those on poultry and the effects of ASC on *Salmonella* and *Campylobacter* described previously. As the incidence of enteric pathogens on raw red meat is usually low, use of ASC would be expected to have a significant effect in reducing risk, although only to a small extent, because the treatment tends to be less effective on naturally occurring contaminants than it is on inoculated organisms.

In some countries, lactic acid is commonly used during processing as an antimicrobial agent for red meat. However, studies on this compound suffer from the same limitations as for those on chlorine-containing compounds—namely, a lack of data on effectiveness under commercial conditions. In practice, lactic acid is likely to be as effective as ASC. This has been confirmed, for example, by Harris et al. (2006), who compared the efficacy of the two against the same pathogens, tested under identical conditions. From the available data, lactic acid appears to be a suitable alternative to chlorine-based compounds for reducing pathogen contamination of red meat.

Summary

Although no in-plant studies have been reported, Table 4.7 summarizes some laboratory-based work that has examined the effects of commonly used antimicrobial agents on pathogens present on meat.

Overall, spray treatment of the meat with hypochlorite at 50–800 mg/l reduced counts of *E. coli* O157 by only 0.1–1.0 log units and therefore was largely ineffective. By contrast, ASC applied as a spray or dip treatment at 1200 mg/l yielded a 1.4–1.5 log reduction in *E. coli* O157 and a 1.6–2.1 log reduction in *Salmonella*, suggesting that the treatment would be beneficial under practical conditions. Similar results were obtained with lactic acid, which could be used as an alternative to ASC.

4.4 Fishery products

4.4.1 Product

Fishery products are highly diverse, ranging from raw whole fish to ready-to-eat products. Fish and fishery products are generally considered safe, and surveillance data from a few developed countries show that these products account for only a small percentage of foodborne illnesses. During 1992–2003 in England and Wales, fish and shellfish accounted for 14% of foodborne illnesses, whereas desserts accounted for 15%, poultry 24% and red meat 20% (Hughes, Gillespie & O'Brien, 2007). In the USA, seafood accounts for only 10–19% of foodborne illnesses (Butt, Aldridge & Sanders, 2004). Most of these illnesses are associated with consumption of live bivalve molluscs or are due to histamine in some marine fish, and chlorine has no specific use to overcome these hazards. However, ready-to-eat fishery products such as cold-smoked fish have been occasionally implicated in illnesses due to *Listeria monocytogenes* (Rocourt, Jacquet & Reilly, 2000).

Table 4.7. Relevant antimicrobial chemical effectiveness studies against important pathogens on red meat

Conditions of use	Pathogen	Study setting	Contamination type	Contribution to body of evidence	Effect on numbers and prevalence ^a	Control used ^a	Reference
Hypochlorite							
50 mg/l (spray)	<i>E. coli</i> O157	Laboratory	Inoculated	Low	0.74 log	Water (0.57 log)	Cutter & Siragusa (1995)
100 mg/l (spray)	<i>E. coli</i> O157	Laboratory	Inoculated	Low	0.49 log	Water (0.57 log)	Cutter & Siragusa (1995)
250 mg/l (spray)	<i>E. coli</i> O157	Laboratory	Inoculated	Low	0.79 log	Water (0.57 log)	Cutter & Siragusa (1995)
500 mg/l (spray)	<i>E. coli</i> O157	Laboratory	Inoculated	Low	0.51 log	Water (0.57 log)	Cutter & Siragusa (1995)
800 mg/l (spray)	<i>E. coli</i> O157	Laboratory	Inoculated	Low	1.04 log	Water (0.57 log)	Cutter & Siragusa (1995)
50 mg/l (spray)	<i>E. coli</i> O157 (acid habituated)	Laboratory	Inoculated	Low	0.1 log	Water (0.3 log)	Stopforth et al. (2004)
50 mg/l (spray)	<i>E. coli</i> O157 (non-acid habituated)	Laboratory	Inoculated	Low	0.6 log	Water (0.5 log)	Stopforth et al. (2004)
ASC							
1200 mg/l (dip)	<i>E. coli</i> O157	Laboratory	Inoculated	Low	1.4 log	Water	Harris et al. (2006)
1200 mg/l (dip)	<i>Salmonella</i>	Laboratory	Inoculated	Low	2.1 log	Water	Harris et al. (2006)
1200 mg/l (spray)	<i>E. coli</i> O157	Laboratory	Inoculated	Low	3.8 log	Water (2.3 log)	Castillo et al. (1999)
1200 mg/l (spray)	<i>Salmonella</i>	Laboratory	Inoculated	Low	3.9 log	Water (2.3 log)	Castillo et al. (1999)
Lactic acid							
2% (dip)	<i>E. coli</i> O157	Laboratory	Inoculated	Low	1.5 log	Water	Harris et al. (2006)
2% (dip)	<i>Salmonella</i>	Laboratory	Inoculated	Low	2.0 log	Water	Harris et al. (2006)
2.5% (dip)	<i>E. coli</i> O157	Laboratory	Inoculated	Low	1.3 log	Water	Sawyer et al. (2008)
2.5% (dip)	<i>Salmonella</i>	Laboratory	Inoculated	Low	1.6 log	Water	Sawyer et al. (2008)

^a Effects are given as log cfu/ml or log cfu/g reduction of organisms in all cases.

Use of hazard analysis and critical control point (HACCP)-based approaches has led to marked improvements in the safety of fish and fishery products, and a sanitation plan is a prerequisite for implementation of HACCP. The sanitation plan includes safety of processing water, hygiene of food contact surfaces, prevention of cross-contamination, hand washing, employee health and exclusion of pests as important components. Chlorine usage is important to ensure water safety, hygiene of food contact surfaces and prevention of cross-contamination. The FAO/WHO risk assessment of choleraenic *Vibrio cholerae* O1 in warm-water shrimp in international trade (FAO/WHO, 2005) considered data on detection of this pathogen in warm-water shrimp imported by the USA, Japan and Denmark during 1995–2000. Of over 20 000 samples analysed, only 2 samples in 1995 (early period of HACCP implementation) were positive for this pathogen. On the other hand, *V. cholerae* O1 has been reported at a much higher frequency from domestically marketed shrimp and fish in southern Asia (Chen et al., 2004; Saravanan et al., 2007) and occasionally in Latin America (De Paola et al., 1993), when hygienic practices have been inadequate.

4.4.2 Pathogens

There are very few human pathogenic microorganisms (e.g. *Vibrio parahaemolyticus*) that are naturally associated with fish and fishery products. In fish that are cultured in coastal environments or inland in fresh water, pathogens such as *Listeria monocytogenes* and *Salmonella* could be of concern because of their presence in the environment. *Vibrio parahaemolyticus* is generally present at low levels—for example, 10^2 /g or lower in shrimp (Karunasagar, Venugopal & Karunasagar, 1984) and ~ 88 /g in finfish (Chan et al., 1989). The infective dose for *V. parahaemolyticus* is $\sim 10^6$ cells (FAO/WHO, in press); therefore, multiplication in seafood is necessary before an infective dose is reached. *Listeria monocytogenes* is widespread in the aquatic environment and has been frequently isolated from several fish species (Huss, Jorgensen & Vogel, 2000). It may colonize the fish processing environment and may be difficult to eliminate (Huss, Jorgensen & Vogel, 2000). Its prevalence in fish smoking plants typically ranges from 10% to 40%, but may sometimes reach 100% (Jorgensen & Huss, 1998; Autio et al., 1999).

4.4.3 Common disinfection practices

Usage of chlorine in most types of fish processing industry is mainly as a hygienic processing aid rather than as a decontamination treatment. Mostly calcium or sodium hypochlorite is used to treat water used for washing fish and for making ice. For these purposes, water containing chlorine at concentrations below 10 mg/l is generally used. However, for cleaning boxes, cleaning fish processing tables and washing floors, water containing chlorine concentrations of 50–200 mg/l is used. Use of chlorine to reduce pathogen levels is common in the fish processing industry to produce ready-to-eat products such as cold-smoked fish fillets or shrimp for the sushi and sashimi market. In cold-smoked fish, *L. monocytogenes* is the target organism; in sashimi-grade shrimp, *V. parahaemolyticus* is the target pathogen. In these industries, use of chlorine dips at levels ranging from 50 to 200 mg/l has been reported. *Listeria monocytogenes* is particularly difficult to eliminate from the processing environment, and a decontamination step using chlorine at levels of 100–200 mg/l has been recommended to control this pathogen (El-Kest & Marth, 1988). This is a common practice in the industry producing ready-to-eat smoked fish.

4.4.4 Effectiveness of common disinfection practices

Washing fish using chlorinated water is important to clean the fish surface. Use of non-potable water at this stage could result in contamination of fish with pathogens such as *Salmonella* or choleraogenic *Vibrio cholerae* O1. Chlorination of processing water would eliminate these waterborne pathogens and prevent contamination of fish. Chlorination of drinking-water played an important role in the elimination of typhoid fever in Europe and the USA. Washing of fish would also reduce the microbial load on the surface of the fish. Reduction in surface microflora of fish by washing could contribute to improved shelf-life of fish (Shewan, 1971). In the case of pathogens such as *V. parahaemolyticus* and *V. vulnificus*, which are indigenous to coastal and estuarine environments, about 90% reduction in levels can be achieved by washing shrimp with water containing chlorine at 10 mg/l (Table 4.8). Washing of contaminated surfaces with potable water brought about 2 log reductions in levels of *Salmonella*, *V. parahaemolyticus*, *V. vulnificus*, *V. cholerae*, *Escherichia coli* and *Staphylococcus aureus*, and washing these surfaces with water containing residual chlorine levels of 100 mg/l completely eliminated the pathogens (Dinesh, 1991).

Listeria monocytogenes is a pathogen that is widely distributed in the environment and may be present in fish. This organism is of concern in ready-to-eat products such as smoked fish, because it is known to persist in the fish processing environment and may contaminate cold-smoked fish after processing (FAO/WHO, 2004). Use of water containing chlorine at 20–30 mg/l for thawing frozen salmon has been found to reduce the level of *L. monocytogenes* (Eklund et al., 1997). Under laboratory conditions, chlorine at levels of 20–25 mg/l has been shown to be effective in killing both *E. coli* and *L. monocytogenes* in a fish model system. Shin, Chang & Kang (2004) reported a 2–3 log reduction in levels of *L. monocytogenes* in fish stored in ice made with water containing chlorine dioxide at 20–100 mg/l. Bremer & Osborne (1998) evaluated an industrial-scale finfish washing system using gilled and gutted king salmon (*Oncorhynchus tshawytscha*). Exposure of salmon to free chlorine at 200 mg/l at a turnover rate for the total wash solution of 2.25 cycles/h for 120 min resulted in a 96–99% decrease in total plate count. Further, washing could eliminate 99.79% of *L. monocytogenes* cells that had been artificially inoculated on the surface of gilled and gutted fish. A study in Iceland (Cormier et al., 2007) showed that implementation of HACCP in plants producing ready-to-eat shrimp and lobster minimized the probability of finding *L. monocytogenes* in ready-to-eat products. Although use of chlorine in the fish smoking industry will not result in a product that is free from this pathogen, the prevalence and numbers of pathogens are significantly reduced. Cases of human illness are due to foods containing more than 10^2 cells of *L. monocytogenes* per gram, and measures that reduce the frequency of contamination would imply a proportional reduction in the rates of illness, provided the proportion of high contamination is reduced similarly (FAO/WHO, 2004). Available data suggest that use of chlorine can reduce prevalence and also reduce the number of organisms, hence contributing to risk reductions.

In the case of *V. parahaemolyticus*, the infective dose is $\sim 10^6$ cells (FAO/WHO, in press); therefore, multiplication in seafood is necessary before an infective dose is reached. Washing fish in chlorinated water would bring about over a 90% reduction in levels of *V. parahaemolyticus* (Table 4.8), thus greatly reducing the human health risk due to this organism.

Table 4.8. Studies showing pathogen reduction following use of chlorine in fish processing

Conditions of use	Pathogen	Study setting	Contamination type	Contribution to body of evidence	Effect on numbers and/or prevalence	Reference
Washing of fish in water containing chlorine at 200 mg/l	<i>L. monocytogenes</i>	Pilot	Inoculation	Medium	99.79% reduction	Bremer & Osborne (1998)
Thawing frozen fish in water containing chlorine at 20–25 mg/l	<i>L. monocytogenes</i>	Laboratory	Inoculation	Low	Elimination	Eklund et al. (1997)
Fish storage in ice containing chlorine dioxide at 20–100 mg/l	<i>L. monocytogenes</i> , <i>S. Typhimurium</i> , <i>E. coli</i> O157:H7	Laboratory	Inoculation	Low	2–3 log reduction	Shin, Chang & Kang (2004)
Immersion of shrimp in water containing chlorine at 50 mg/l for 30 min	<i>V. parahaemolyticus</i>	Laboratory	Natural	Medium	85–97% reduction	Chaiyakosa et al. (2007)
Washing shrimp in water containing chlorine at 10 mg/l	<i>V. parahaemolyticus</i> , <i>V. cholerae</i> , <i>V. vulnificus</i> , <i>Salmonella</i>	Laboratory	Inoculation	Low	>90% reduction	Dinesh (1991)
Washing of processing surface with water containing chlorine at 100 mg/l	<i>V. parahaemolyticus</i> , <i>V. cholerae</i> , <i>V. vulnificus</i> , <i>Salmonella</i>	Laboratory	Inoculation	Low	Elimination	Dinesh (1991)

4.5 Fresh produce

4.5.1 Product

Fresh produce includes fruits and vegetables that are consumed with little or no further processing or preparation by the consumer. Produce can be distributed and sold loose or pre-packed in either unprocessed or minimally processed form. Many products are ready to eat, and no further antimicrobial process is applied before consumption.

4.5.2 Pathogens

The Centre for Science in the Public Interest in the USA compiles a database of outbreaks associated with foods. Between 1990 and 2005, this database captured information on 639 outbreaks of foodborne illness due to produce, involving 31 496 illnesses (CSPI, 2008). The most publicized outbreak in recent years occurred in the USA in 2006: spinach from the Salinas Valley in California was contaminated with *Escherichia coli* O157:H7. In 26 states, 204 persons were infected with *E. coli* O157:H7, 102 were hospitalized, 31 developed haemolytic-uraemic syndrome and 3 died.

Fresh produce becomes contaminated primarily in the field during production via contaminated water (irrigation, pesticide application, flooding), by contact with soil and soil improvers contaminated with animal or human faeces, as a result of the presence of livestock or wildlife in the production areas or from the equipment or workers during harvesting. Contamination is also possible during post-harvest operations by cross-contamination from contaminated wash water, from contaminated food contact surfaces or from workers and equipment. Microorganisms associated with fresh produce are controlled by a combination of good agricultural practices during production, good hygienic practices during harvesting and packing, processing and distribution, as well as the use of antimicrobial chemicals during processing and a cold chain during distribution.

A non-exhaustive list of the main pathogenic microorganisms that have been associated with human illness as a result of the consumption of fresh produce includes VTEC, *Salmonella* spp., *Shigella* spp., *Cryptosporidium* spp., *Yersinia enterocolitica*, *Listeria monocytogenes*, *Giardia lamblia* and *Cyclospora cayetanensis*, as well as various enteric viruses.

4.5.3 Common disinfection practices

Chlorinated compounds are perhaps the most universal disinfectants used in the fresh produce industry. Chlorine is used to decontaminate processing equipment, to control the microbial load in wash waters as well as in the disinfection of food contact surfaces and the fresh produce itself. Chapter 1 identified that the most commonly used chlorinated compounds in the fresh produce industry are sodium/calcium hypochlorite and aqueous chlorine dioxide. Chlorine delivered by use of hypochlorous acid and hypochlorite is used by the industry at levels between 25 and 200 mg/l (contact time <2 min) as post-harvest spray or dip and then at concentrations of between 10 and 50 mg/l in flume water (contact time 0.5–15 min). The aqueous form of chlorine dioxide is also used by the industry at up to 3 mg/l in flume water. The fresh produce industry also uses peroxyacetic acid as an alternative to chlorine at about 40 mg/l in flume water.

The use of other disinfectants, such as ASC, gaseous chlorine dioxide, ozone and hydrogen peroxide, is less common in the industry, or the disinfectants have been examined only at the experimental phase.

4.5.4 Effectiveness of common disinfection practices

In this section, data have been identified that concern the effect of those disinfectants considered in common industrial use in chapter 1 and summarized in the previous section. These data were identified during literature searches conducted by FAO/WHO and also from information provided to these organizations in the call for data accompanying this expert meeting. They may not constitute all of the available studies on pathogens on fresh produce, but they do provide a representative cross-section of data.

4.5.4.1 Hypochlorite in flume water and as a dip/spray

Table 4.9 summarizes these studies and the strength of their individual contribution to the body of evidence concerning the effect of chlorine. Treatments (up to 200 mg/l) with chlorine solutions can reduce populations of pathogens by up to 2 log units compared with water washing generally. In contrast, Wu et al. (2000) reported that treatment of whole parsley leaves with free chlorine at 150 mg/l reduced the populations of *Shigella sonnei* by more than 6 log cfu/g. It is clear that each bacterial species exhibits different sensitivity to chlorine. The physical structure of the vegetable also has an impact on the efficacy of chlorine. In addition, there are a variety of methods (e.g. time for inoculation of pathogens and treatment with chlorine, temperature, concentration of chlorine) used to study the effect of chlorine on fresh produce. Different experimental methods will affect the results. Akbas & Olmez (2007) reported that increasing the treatment time from 2 to 5 min did not result in any further significant decrease in *Escherichia coli* population on lettuce pieces. Li et al. (2001) reported that survival of *E. coli* O157:H7 on lettuce pieces after agitation in a chlorine solution of 20 mg/l at 20 °C and 50 °C was not significantly different. Although the effect of the different conditions individually might be small, the combination might have a greater impact on the result.

Although there are many studies providing data on pathogen reduction on produce due to chlorine, they are confined to experimental methods; as such, they would make a smaller contribution to the body of evidence on the likely effect of chlorine used in practice during spray-washing of produce or in flume water. No identified studies have examined the effect of chlorine in flume water on the prevalence of pathogens on produce; hence, definitive conclusions cannot be drawn on its effect on preventing cross-contamination due to pathogens in the process water or on contact surfaces.

The primary health concern with fresh produce is foodborne illness from the consumption of ready-to-eat leafy green vegetables such as lettuce and spinach. The data shown in Table 4.9 for leafy greens suggest that chlorine use at levels between 20 and 200 mg/l for contact times between 1 and 10 min results in reductions of between 0.2 and 1.7 log units of *L. monocytogenes*, 0.3 and 2 log units of *Salmonella*, 0.3 and 1.7 log units of *E. coli* O157 and 0.2 and 6.0 log units of *Shigella* over washing in water alone. In general, larger reductions are achieved at higher concentrations of chlorine, but data seem too inconsistent to be definitive. Data are also inconsistent between studies on the effect of contact time. Those studies that included a water wash control showed log reductions in pathogens between 0.5 and 1.0 log units, depending on the type of leafy green tested and the pathogen species. Given that these experiments use pathogens artificially inoculated onto produce, it is likely that these effects are an overestimate of the effects of chlorine in washes or flume water in the industrial setting.

Table 4.9. Studies showing pathogen reduction after produce treatment with chlorine via hypochlorite

Conditions of use	Fresh produce	Pathogen	Study setting	Study type	Contribution to body of evidence	Effect on numbers and/or prevalence ^a	Control used ^a	Reference
100 mg/l, dipping (2 min)	Lettuce pieces	<i>L. monocytogenes</i>	Laboratory	Inoculation	Low	1.5 log	Water (0.6 log)	Akbas & Olmez (2007)
100 mg/l, dipping (5 min)	Lettuce pieces	<i>L. monocytogenes</i>	Laboratory	Inoculation	Low	1.7 log	Water (0.7 log)	Akbas & Olmez (2007)
20 mg/l, dipping (30 s)	Lettuce pieces	<i>L. monocytogenes</i>	Laboratory	Inoculation	Low	1–1.2 log	No treatment	Li et al. (2001)
300 mg/l (3 min)	Lettuce pieces	<i>E. coli</i> O157:H7	Laboratory	Inoculation (internalized in leaves by vacuum perfusion)	Low	0.5 log	No treatment	Niemira (2007)
600 mg/l (3 min)	Lettuce pieces	<i>E. coli</i> O157:H7	Laboratory	Inoculation (internalized in leaves by vacuum perfusion)	Low	0.5 log	No treatment	Niemira (2007)
300 mg/l (3 min)	Spinach pieces	<i>E. coli</i> O157:H7	Laboratory	Inoculation (internalized in leaves by vacuum perfusion)	Low	0.5 log	No treatment	Niemira (2007)
600 mg/l (3 min)	Spinach pieces	<i>E. coli</i> O157:H7	Laboratory	Inoculation (internalized in leaves by vacuum perfusion)	Low	0.5 log	No treatment	Niemira (2007)
25 mg/l, stirring (10 min, 4 °C)	Lettuce pieces	<i>L. monocytogenes</i>	Laboratory	Inoculation	Low	0.2 log	Tap water	Zhang & Farber (1996)
50 mg/l, stirring (10 min, 4 °C)	Lettuce pieces	<i>L. monocytogenes</i>	Laboratory	Inoculation	Low	0.8 log	Tap water	Zhang & Farber (1996)

Conditions of use	Fresh produce	Pathogen	Study setting	Study type	Contribution to body of evidence	Effect on numbers and/or prevalence ^a	Control used ^a	Reference
100 mg/l, stirring (10 min, 4 °C)	Lettuce pieces	<i>L. monocytogenes</i>	Laboratory	Inoculation	Low	1.0 log	Tap water	Zhang & Farber (1996)
200 mg/l, stirring (10 min, 4 °C)	Lettuce pieces	<i>L. monocytogenes</i>	Laboratory	Inoculation	Low	1.3 log	Tap water	Zhang & Farber (1996)
25 mg/l, stirring (10 min, 22 °C)	Lettuce pieces	<i>L. monocytogenes</i>	Laboratory	Inoculation	Low	0.6 log	Tap water	Zhang & Farber (1996)
50 mg/l, stirring (10 min, 22 °C)	Lettuce pieces	<i>L. monocytogenes</i>	Laboratory	Inoculation	Low	1.0 log	Tap water	Zhang & Farber (1996)
100 mg/l, stirring (10 min, 22 °C)	Lettuce pieces	<i>L. monocytogenes</i>	Laboratory	Inoculation	Low	1.2 log	Tap water	Zhang & Farber (1996)
200 mg/l, stirring (10 min, 22 °C)	Lettuce pieces	<i>L. monocytogenes</i>	Laboratory	Inoculation	Low	1.7 log	Tap water	Zhang & Farber (1996)
200 mg/l, immersion (10 min)	Lettuce pieces	<i>Salmonella</i>	Laboratory	Inoculation	Low	1.0 log	Distilled water (0.7 log)	Kondo, Murata & Isshiki (2006)
200 mg/l, immersion (10 min)	Lettuce pieces	<i>E. coli</i> O157:H7	Laboratory	Inoculation	Low	1.2 log	Distilled water (0.7 log)	Kondo, Murata & Isshiki (2006)
200 mg/l, immersion (10 min)	Lettuce pieces	<i>S. aureus</i>	Laboratory	Inoculation	Low	1.4 log	Distilled water (1.1 log)	Kondo, Murata & Isshiki (2006)
100 mg/l, stirring (10 min)	Lettuce pieces	<i>Y. enterocolitica</i>	Laboratory	Inoculation	Low	2.36–2.68 log	Distilled water	Escudero et al. (1999)
100 mg/l, stirring (10 min)	Lettuce pieces	<i>Y. enterocolitica</i>	Laboratory	Inoculation	Low	2.55–3.15 log	Distilled water	Escudero et al. (1999)
200 mg/l, agitation (1 min)	Lettuce pieces	<i>E. coli</i> O157:H7	Laboratory	Inoculation	Low	0.86–0.88 log	No treatment and water (0.58–0.59 log)	Koseki et al. (2003)

Conditions of use	Fresh produce	Pathogen	Study setting	Study type	Contribution to body of evidence	Effect on numbers and/or prevalence ^a	Control used ^a	Reference
200 mg/l, agitation (1 min)	Lettuce pieces	<i>Salmonella</i>	Laboratory	Inoculation	Low	0.96–1.04 log	No treatment and water (0.53–0.67 log)	Koseki et al. (2003)
20 mg/l, agitation (20 °C, 1 min)	Lettuce pieces	<i>E. coli</i> O157:H7	Laboratory	Inoculation	Low	1.0 log	No treatment and immersion in water (0.7 log)	Li et al. (2001)
120 mg/l, shaking (40 s)	Shredded lettuce	<i>Salmonella</i>	Laboratory	Inoculation	Low	0.8 log	Deionized water	Weissinger, Chantaranont & Beuchat (2000)
200 mg/l, shaking (40 s)	Shredded lettuce	<i>Salmonella</i>	Laboratory	Inoculation	Low	0.8 log	Deionized water	Weissinger, Chantaranont & Beuchat (2000)
100 mg/l, wash (1 min)	Shredded lettuce	<i>L. monocytogenes</i>	Laboratory	Inoculation	Low	0.7 log	Water (0.5 log)	Hellstrom et al. (2006)
100 mg/l, mixing (5 min)	Chinese cabbage pieces	<i>E. coli</i> O157:H7	Laboratory	Inoculation	Low	2.0–2.7 log	Water (0.7–1.0 log)	Inatsu et al. (2005)
100 mg/l, immersion (5 min)	Parsley bunches	<i>Salmonella</i>	Laboratory	Inoculation	Low	1.7–2.0 log	Deionized water	Lapidot, Romling & Yaron (2006)
200 mg/l, immersion (5 min)	Parsley bunches	<i>Salmonella</i>	Laboratory	Inoculation	Low	1.7–2.0 log	Deionized water	Lapidot, Romling & Yaron (2006)
800 mg/l, immersion (5 min)	Parsley bunches	<i>Salmonella</i>	Laboratory	Inoculation	Low	2.2–2.6 log	Deionized water	Lapidot, Romling & Yaron (2006)

Conditions of use	Fresh produce	Pathogen	Study setting	Study type	Contribution to body of evidence	Effect on numbers and/or prevalence ^a	Control used ^a	Reference
1600 mg/l, immersion (5 min)	Parsley bunches	<i>Salmonella</i>	Laboratory	Inoculation	Low	2.6–3.0 log	Deionized water	Lapidot, Romling & Yaron (2006)
5 mg/l, agitation (5 min)	Parsley bunches	<i>Shigella</i>	Laboratory	Inoculation	Low	1.2 log	Deionized water	Wu et al. (2000)
10 mg/l, agitation (5 min)	Parsley bunches	<i>Shigella</i>	Laboratory	Inoculation	Low	2.3 log	Deionized water	Wu et al. (2000)
100 mg/l, agitation (5 min)	Parsley bunches	<i>Shigella</i>	Laboratory	Inoculation	Low	4.3 log	Deionized water	Wu et al. (2000)
150 mg/l, agitation (5 min)	Parsley bunches	<i>Shigella</i>	Laboratory	Inoculation	Low	>6 log	Deionized water	Wu et al. (2000)
Sodium hypochlorite (50 mg/l) + citric acid to pH 6.5, agitation (60 s)	Leafy greens (many varieties)	<i>E. coli</i> O157:H7	Laboratory	Inoculation	Low	2.4 log	Water (1.0 log)	Stopforth et al. (2008)
Sodium hypochlorite (50 mg/l) + citric acid to pH 6.5, agitation (60 s)	Leafy greens (many varieties)	<i>L. monocytogenes</i>	Laboratory	Inoculation	Low	2.2 log	Water (0.6 log)	Stopforth et al. (2008)
Sodium hypochlorite (50 mg/l) + citric acid to pH 6.5, agitation (60 s)	Leafy greens (many varieties)	<i>Salmonella</i>	Laboratory	Inoculation	Low	2.1 log	Water (0.6 log)	Stopforth et al. (2008)
Sodium hypochlorite (50 mg/l) + citric acid to pH 6.5, agitation (90 s)	Leafy greens (many varieties)	<i>E. coli</i> O157:H7	Laboratory	Inoculation	Low	2.3 log	Water (1.0 log)	Stopforth et al. (2008)
Sodium hypochlorite (50 mg/l) + citric acid to pH 6.5, agitation (90 s)	Leafy greens (many varieties)	<i>L. monocytogenes</i>	Laboratory	Inoculation	Low	2.1 log	Water (1.4 log)	Stopforth et al. (2008)

Conditions of use	Fresh produce	Pathogen	Study setting	Study type	Contribution to body of evidence	Effect on numbers and/or prevalence ^a	Control used ^a	Reference
Sodium hypochlorite (50 mg/l) + citric acid to pH 6.5, agitation (90 s)	Leafy greens (many varieties)	<i>Salmonella</i>	Laboratory	Inoculation	Low	2.8 log	Water (1.1 log)	Stopforth et al. (2008)
Sodium hypochlorite (50 mg/l) + sodium acid sulfite to pH 6.5, agitation (60 s)	Leafy greens (many varieties)	<i>E. coli</i> O157:H7	Laboratory	Inoculation	Low	2.3 log	Water (1.0 log)	Stopforth et al. (2008)
Sodium hypochlorite (50 mg/l) + sodium acid sulfite to pH 6.5, agitation (60 s)	Leafy greens (many varieties)	<i>L. monocytogenes</i>	Laboratory	Inoculation	Low	2.0 log	Water (0.6 log)	Stopforth et al. (2008)
Sodium hypochlorite (50 mg/l) + sodium acid sulfite to pH 6.5, agitation (60 s)	Leafy greens (many varieties)	<i>Salmonella</i>	Laboratory	Inoculation	Low	2.2 log	Water (0.6 log)	Stopforth et al. (2008)
Sodium hypochlorite (50 mg/l) + sodium acid sulfite to pH 6.5, agitation (90 s)	Leafy greens (many varieties)	<i>E. coli</i> O157:H7	Laboratory	Inoculation	Low	2.5 log	Water (1.0 log)	Stopforth et al. (2008)
Sodium hypochlorite (50 mg/l) + sodium acid sulfite to pH 6.5, agitation (90 s)	Leafy greens (many varieties)	<i>L. monocytogenes</i>	Laboratory	Inoculation	Low	1.7 log	Water (1.4 log)	Stopforth et al. (2008)
Sodium hypochlorite (50 mg/l) + sodium acid sulfite to pH 6.5, agitation (90 s)	Leafy greens (many varieties)	<i>Salmonella</i>	Laboratory	Inoculation	Low	2.1 log	Water (1.1 log)	Stopforth et al. (2008)
Sodium hypochlorite at 25 mg/l (2 min)	Spinach	<i>Salmonella</i> Hadar	Laboratory	Inoculation	Low	0.56 log	No treatment	Pirovani et al. (2000)

Conditions of use	Fresh produce	Pathogen	Study setting	Study type	Contribution to body of evidence	Effect on numbers and/or prevalence ^a	Control used ^a	Reference
Sodium hypochlorite at 75 mg/l (2 min)	Spinach	<i>Salmonella</i> Hadar	Laboratory	Inoculation	Low	0.82–0.95 log	No treatment	Pirovani et al. (2000)
Sodium hypochlorite at 125 mg/l (2 min)	Spinach	<i>Salmonella</i> Hadar	Laboratory	Inoculation	Low	0.62 log	No treatment	Pirovani et al. (2000)
Sodium hypochlorite at 25 mg/l (5 min)	Spinach	<i>Salmonella</i> Hadar	Laboratory	Inoculation	Low	0.62–0.80 log	No treatment	Pirovani et al. (2000)
Sodium hypochlorite at 75 mg/l (5 min)	Spinach	<i>Salmonella</i> Hadar	Laboratory	Inoculation	Low	0.75–0.84 log	No treatment	Pirovani et al. (2000)
Sodium hypochlorite at 125 mg/l (5 min)	Spinach	<i>Salmonella</i> Hadar	Laboratory	Inoculation	Low	0.96–1.00 log	No treatment	Pirovani et al. (2000)
Sodium hypochlorite at 25 mg/l (8 min)	Spinach	<i>Salmonella</i> Hadar	Laboratory	Inoculation	Low	0.60 log	No treatment	Pirovani et al. (2000)
Sodium hypochlorite at 75 mg/l (8 min)	Spinach	<i>Salmonella</i> Hadar	Laboratory	Inoculation	Low	0.98–1.01 log	No treatment	Pirovani et al. (2000)
Sodium hypochlorite at 125 mg/l (8 min)	Spinach	<i>Salmonella</i> Hadar	Laboratory	Inoculation	Low	1.30 log	No treatment	Pirovani et al. (2000)

^a Effects are given as log cfu/ml or log cfu/g reduction of organisms in all cases.

4.5.4.2 Aqueous chlorine dioxide in flume water and as a spray/dip

There is less information about the effectiveness of chlorine dioxide compared with hypochlorite as a disinfectant for fresh produce. The effect of chlorine dioxide on pathogenic bacteria on fresh produce is shown in Table 4.10. Zhang & Farber (1996) showed that concentrations of chlorine dioxide in water up to 5 mg/l could inactivate up to 90% of *L. monocytogenes*. Inactivation of *Salmonella* and *E. coli* O157 was similar with chlorine dioxide at 20 mg/l, around 1 log unit over water alone, with a slightly greater effect on apples than on lettuce (Huang et al., 2006). Han et al. (2001) showed that there was little effect of chlorine dioxide at 0.3 mg/l on *L. monocytogenes* on green peppers. Treatment of uninjured green pepper surfaces with chlorine dioxide at 3 mg/l resulted in a 2.3 log reduction of *L. monocytogenes*, whereas no effect was seen on injured green pepper surfaces.

From the limited data available, at the chlorine dioxide concentrations below 3 mg/l that are commonly used in the fresh produce industry, the effect on pathogens is limited to no more than 1 log unit over and above water treatment alone. Data on *Salmonella* and *E. coli* O157 are available only at high experimental concentrations, but even then, inactivation was low. It appears that aqueous chlorine dioxide is no more effective than chlorine at reducing the numbers of pathogens on leafy greens.

4.5.4.3 Peroxyacetic acid in flume water and as a spray/dip

Peroxyacetic acid is used in the fresh produce industry in flume water as an alternative to chlorine. However, data on its effect on pathogen reduction on fresh produce are limited. Table 4.11 shows data quantifying the effects on pathogens. Oh, Dancer & Kang (2005) demonstrated that peroxyacetic acid at 40 mg/l reduced *E. coli* O157 and *L. monocytogenes* by 0.8 and 0.3 log, respectively, with 10 min contact time, but *Salmonella* was more susceptible (2.5 log reduction). To achieve reductions in the other pathogens similar to those in *Salmonella*, it was necessary to increase contact time to 30 min, whereupon similar log reductions of between 2 and 3 log units were achieved for all pathogens studied. Higher reductions of up to 4.5 log units were detected with contact times of 60 min, but this is unrealistic in the industrial setting when peroxyacetic acid is used in flume water. Other studies show similar results. Generally, peroxyacetic acid seems more effective at killing pathogens than chlorine with similar contact times. However, the effect of water alone in these studies was not reported, although other studies on other disinfectants suggest that water may result in up to a 1 log reduction in pathogens alone without disinfectant.

Under commercial conditions, as described in chapter 1, the extent of pathogen reduction by peroxyacetic acid in flume water would depend on the pathogen and would range from 0.3 to 2.5 log units.

Table 4.10. Studies showing pathogen reduction after produce treatment with aqueous chlorine dioxide

Conditions of use	Fresh produce	Pathogen	Study setting	Study type	Contribution to body of evidence	Effect on numbers and/or prevalence ^a	Control used ^a	Reference
500 mg/l, mixing (15 min)	Chinese cabbage	<i>E. coli</i> O157:H7	Laboratory	Inoculated	Low	0.9–1.2 log	Water (0.4–0.6 log)	Inatsu et al. (2005)
20 mg/l, stirring (10 min)	Lettuce leaves	<i>E. coli</i> O157:H7	Laboratory	Inoculated	Low	2.3 log	Water (1.3 log)	Huang et al. (2006)
20 mg/l, stirring (10 min)	Lettuce leaves	<i>Salmonella</i>	Laboratory	Inoculated	Low	2.2 log	Water (1.5 log)	Huang et al. (2006)
20 mg/l + sonication 170 Hz, stirring	Lettuce leaves	<i>E. coli</i> O157:H7	Laboratory	Inoculated	Low	2 log	Water (1.3 log)	Huang et al. (2006)
20 mg/l + sonication 170 Hz, stirring	Lettuce leaves	<i>Salmonella</i>	Laboratory	Inoculated	Low	3.2 log	Water (1.5 log)	Huang et al. (2006)
20 mg/l, stirring (10 min)	Apples	<i>E. coli</i> O157:H7	Laboratory	Inoculated	Low	2 log	Water (0.5 log)	Huang et al. (2006)
20 mg/l, stirring (10 min)	Apples	<i>Salmonella</i>	Laboratory	Inoculated	Low	2.5 log	Water (0.5 log)	Huang et al. (2006)
20 mg/l + sonication 170 Hz, stirring	Apples	<i>E. coli</i> O157:H7	Laboratory	Inoculated	Low	4 log	Water (0.5 log)	Huang et al. (2006)
20 mg/l + sonication 170 Hz, stirring	Apples	<i>Salmonella</i>	Laboratory	Inoculated	Low	4 log	Water (0.5 log)	Huang et al. (2006)
10 mg/l, agitation (10 min)	Lettuce pieces	<i>E. coli</i> O157:H7	Laboratory	Inoculated	Low	1.55 log	Water (0.88 log)	Singh et al. (2002)
1 mg/l, stirring (10 min, 4 °C)	Lettuce pieces	<i>L. monocytogenes</i>	Laboratory	Inoculated	Low	<0 log	Tap water	Zhang & Farber (1996)
2 mg/l, stirring (10 min, 4 °C)	Lettuce pieces	<i>L. monocytogenes</i>	Laboratory	Inoculated	Low	0.6 log	Tap water	Zhang & Farber (1996)
3 mg/l, stirring (10 min, 4 °C)	Lettuce pieces	<i>L. monocytogenes</i>	Laboratory	Inoculated	Low	0.4 log	Tap water	Zhang & Farber (1996)
5 mg/l, stirring (10 min, 4 °C)	Lettuce pieces	<i>L. monocytogenes</i>	Laboratory	Inoculated	Low	1.1 log	Tap water	Zhang & Farber (1996)

Conditions of use	Fresh produce	Pathogen	Study setting	Study type	Contribution to body of evidence	Effect on numbers and/or prevalence ^a	Control used ^a	Reference
1 mg/l, stirring (10 min, 22 °C)	Lettuce pieces	<i>L. monocytogenes</i>	Laboratory	Inoculated	Low	0 log	Tap water	Zhang & Farber (1996)
2 mg/l, stirring (10 min, 22 °C)	Lettuce pieces	<i>L. monocytogenes</i>	Laboratory	Inoculated	Low	0.5 log	Tap water	Zhang & Farber (1996)
3 mg/l, stirring (10 min, 22 °C)	Lettuce pieces	<i>L. monocytogenes</i>	Laboratory	Inoculated	Low	0.4 log	Tap water	Zhang & Farber (1996)
5 mg/l, stirring (10 min, 22 °C)	Lettuce pieces	<i>L. monocytogenes</i>	Laboratory	Inoculated	Low	0.8 log	Tap water	Zhang & Farber (1996)
0.3 mg/l, agitation (10 min)	Green pepper pieces (injured)	<i>L. monocytogenes</i>	Laboratory	Inoculated	Low	0.54 log/5 g	Water (0.51 log/5 g)	Han et al. (2001)
0.3 mg/l, agitation (10 min)	Green pepper pieces (uninjured)	<i>L. monocytogenes</i>	Laboratory	Inoculated	Low	1.87 log/5 g	Water (1.53 log/5 g)	Han et al. (2001)
3.0 mg/l, agitation (10 min)	Green pepper pieces (injured)	<i>L. monocytogenes</i>	Laboratory	Inoculated	Low	0.44 log/5 g	Water (0.39 log/5 g)	Han et al. (2001)
3.0 mg/l, agitation (10 min)	Green pepper pieces (uninjured)	<i>L. monocytogenes</i>	Laboratory	Inoculated	Low	3.67 log/5 g	Water (1.35 log/5 g)	Han et al. (2001)

^a Effects are given as log cfu/ml or log cfu/g reduction of organisms in all cases.

Table 4.11. Studies showing pathogen reduction after produce treatment with peroxyacetic acid

Conditions of use	Fresh produce	Pathogen	Study setting	Study type	Contribution to the body of evidence	Effect on numbers and/or prevalence ^a	Control used ^a	Reference
40 mg/l, 10 min	Lettuce leaves	<i>E. coli</i> O157:H7	Laboratory	Inoculated	Low	0.8 log	No	Oh, Dancer & Kang (2005)
40 mg/l, 10 min	Lettuce leaves	<i>L. monocytogenes</i>	Laboratory	Inoculated	Low	0.3 log	No	Oh, Dancer & Kang (2005)
40 mg/l, 10 min	Lettuce leaves	<i>Salmonella</i>	Laboratory	Inoculated	Low	2.5 log	No	Oh, Dancer & Kang (2005)
40 mg/l, 30 min	Lettuce leaves	<i>E. coli</i> O157:H7	Laboratory	Inoculated	Low	2.2 log	No	Oh, Dancer & Kang (2005)
40 mg/l, 30 min	Lettuce leaves	<i>L. monocytogenes</i>	Laboratory	Inoculated	Low	3.3 log	No	Oh, Dancer & Kang (2005)
40 mg/l, 30 min	Lettuce leaves	<i>Salmonella</i>	Laboratory	Inoculated	Low	2.7 log	No	Oh, Dancer & Kang (2005)
40 mg/l, 60 min	Lettuce leaves	<i>E. coli</i> O157:H7	Laboratory	Inoculated	Low	3.4 log	No	Oh, Dancer & Kang (2005)
40 mg/l, 60 min	Lettuce leaves	<i>L. monocytogenes</i>	Laboratory	Inoculated	Low	4.5 log	No	Oh, Dancer & Kang (2005)
40 mg/l, 60 min	Lettuce leaves	<i>Salmonella</i>	Laboratory	Inoculated	Low	3.8 log	No	Oh, Dancer & Kang (2005)
50 mg/l, 60 s	Precut iceberg lettuce	<i>L. monocytogenes</i>	Laboratory	Inoculated	Low	1.7 log	Potable water	Helstrom et al. (2006)
80 mg/l, 2–5 min	Lettuce leaves (whole and shredded)	<i>E. coli</i> O157:H7	Laboratory	Inoculated	Low	~4.4 log	Tap water	Rodgers et al. (2004)
80 mg/l, 2–5 min	Lettuce leaves (whole and shredded)	<i>L. monocytogenes</i>	Laboratory	Inoculated	Low	~4.4 log	Tap water	Rodgers et al. (2004)

^a Effects are given as log cfu/ml or log cfu/g reduction of organisms in all cases.

4.6 Food contact surfaces

The purpose of the disinfectant on food contact surfaces is to reduce cross-contamination where pathogens attached to equipment become dislodged and attach to the surfaces of food in contact with them. The standard method of assessing the effect of disinfectants is by suspension tests with the bacteria of concern. Here, different concentrations of the disinfectant are used to establish the minimum inhibitory concentration. However, in practice, spoilage and pathogenic bacteria attach to surfaces and can form a biofilm. Biofilms respond differently to disinfectants compared with bacteria in suspension. This section considers the effects of disinfectants only on biofilms either in industrial situations or under laboratory conditions using bacteria grown on model contact surfaces such as stainless steel coupons. This assessment is not a comprehensive review of the subject, but aims to quantify the general effects of key disinfectants.

4.6.1 Studies on test surfaces

Sodium hypochlorite is the most common surface disinfectant used in the food industry. Joseph et al. (2001) studied hypochlorite effects against biofilms of *Salmonella* on plastic, cement and stainless steel surfaces. Biofilms on plastic challenged with chlorine solutions at concentrations up to 100 mg/l for up to 25 min resulted in reductions in *Salmonella* from less than 2 log units (chlorine at 10 mg/l for 25 min) up to 7.53 log units (chlorine at 100 mg/l for 20 min). On cement, *Salmonella* biofilm numbers were reduced by 3.53 log units (chlorine at 100 mg/l for 20 min), reflecting the difficulty in sanitizing porous surfaces. On steel, *Salmonella* biofilm numbers were reduced by 5.47 log units (chlorine at 100 mg/l for 15 min). Ramesh et al. (2002) studied the effect of several disinfectants on *Salmonella* numbers in 4-day-old biofilms grown on galvanized steel surfaces. Sodium hypochlorite at 250 mg/l for 2 min resulted in a reduction of 7.18 log cfu/cm².

Listeria monocytogenes has been shown to adhere to various surfaces after a short contact time at 4 °C and 20 °C (Mafu et al., 1990a). Biofilms of *L. monocytogenes* grown on stainless steel and plastic surfaces were challenged with sodium hypochlorite in a study by Jeyasekaran, Karunasagar & Karunasagar (2000). A 100 mg/l chlorine solution resulted in an additional 3.27 log cfu/cm² reduction from 5.72 log cfu/cm² over the effect of the water control on stainless steel. However, on plastic, the same concentration of chlorine resulted in only a 0.75 log cfu/cm² reduction from 5.16 log cfu/cm² over the effect of the water control. Clearly, plastic surfaces were more difficult to disinfect. Higher concentrations of chlorine (200 mg/l) resulted in an additional 5.72 and 2.3 log cfu/cm² reduction on stainless steel and plastic, respectively, over the effect of a water control. Another study on *L. monocytogenes* was conducted by Mustapha & Liewen (1989). Concentrations of chlorine up to 800 mg/l resulted in a reduction in *L. monocytogenes* biofilm numbers on stainless steel of between 1 and >4 log cfu/ml. Smooth stainless steel was found to be easier to disinfect than pitted stainless steel. Meylheuc, Renault & Bellon-Fontaine (2006) also studied the effect of sodium hypochlorite on *L. monocytogenes*. They reported a 3.9 and 4.0 log reduction in *L. monocytogenes* on stainless steel for cells grown at 20 °C and 37 °C, respectively, using a solution with active chlorine at 1.23 mg/l and a 5 min contact time. On polytetrafluoroethylene with the same solution, log reductions were 3.4 and 3.5 log cfu for cells grown at 20 °C and 37 °C, respectively. Mafu et al. (1990b) found that hypochlorite at 100 mg/l as chlorine was effective as a sanitizer against *L. monocytogenes* on food contact surfaces.

Other disinfectants, such as peroxyacetic acid, hydrogen peroxide, iodophores and quaternary ammonium compounds (QACs), have also been tested against pathogenic bacteria in biofilms on hard surfaces. QACs (50–800 mg/l) were tested against *L. monocytogenes* in

biofilms (Mustapha & Liewen, 1989). QACs at 50 mg/l were effective at reducing *L. monocytogenes* biofilm numbers by >4 log cfu/ml on smooth and pitted stainless steel. Peroxyacetic acid was found to be effective against *L. monocytogenes* as 4 h adherent mixed culture biofilm with *Pseudomonas* on stainless steel. The mixed culture attachment was 10^8 cfu/cm², and this was reduced to 4 cfu/cm² after 1 min contact with peroxyacetic acid at 40 mg/l (Fatemi & Frank, 1999). A combination of peroxyacetic acid and hydrogen peroxide was tested against *L. monocytogenes* cells adhered to stainless steel or polytetrafluoroethylene (Meylheuc, Renault & Bellon-Fontaine, 2006). Peroxyacetic acid/hydrogen peroxide containing peroxyacetic acid at 5.13 mg/l resulted in a 3.6 and 3.0 log reduction for cells grown at 20 °C and 37 °C, respectively, on stainless steel. On polytetrafluoroethylene, log reductions of 3.7 and 3 log cfu were reported for cells grown at 20 °C and 37 °C, respectively. Iodophors were studied against *Salmonella* biofilms (Joseph et al., 2001). Available iodine concentrations between 1 and 50 mg/l were used with contact times between 5 and 25 min. A maximum 3.5 log cfu/cm² reduction was achieved with iodine (I₂) at 50 mg/l for 5 min on plastic. A 6 log cfu/cm² reduction was achieved with iodine at 50 mg/l for 25 min on cement. A 5.5 log cfu/cm² reduction was achieved with iodine at 50 mg/l for 20 min on stainless steel. Jeyasekaran, Karunasagar & Karunasagar (2000) studied the effect of iodophors on biofilms of *L. monocytogenes*. An iodophor solution of 10 mg/l resulted in an additional 1.78 log cfu/cm² reduction from 5.72 log cfu/cm² over the effect of the water control on stainless steel. However, on plastic, the same concentration of iodophor resulted in only a 0.18 log cfu/cm² reduction from 5.16 log cfu/cm² over the effect of the water control. Higher concentrations of iodophor (20 mg/l) resulted in an additional 3.21 and 1.77 log cfu/cm² reduction on stainless steel and plastic, respectively, over the effect of a water control.

Frank, Ehlers & Wicker (2003) tested a number of disinfectants against *L. monocytogenes* biofilms grown on stainless steel and coated in chicken serum albumin and rendered chicken fat. Static cleaning with sodium hypochlorite at 200 mg/l resulted in log reductions in the coated biofilm of 4.27, 4.56 and 5.41 log units at 1, 10 and 30 min exposure, respectively. QACs (2 ml/l) resulted in log reductions in the coated biofilm of 4.78, 5.56 and 6.06 log units at 1, 10 and 30 min exposure, respectively. ASC (7.5% with 6% phosphoric acid) resulted in log reductions in the coated biofilm of 5.76, 6.32 and 6.16 log units at 1, 10 and 30 min exposure, respectively. Peroxyacetic acid (2 ml/l) resulted in log reductions in the coated biofilm of 4.48, 4.59 and 5.26 log units at 1, 10 and 30 min exposure, respectively.

4.6.2 Studies on industrial equipment surfaces

Mead, Hudson & Hinton (1994) demonstrated that an antimicrobial-resistant *E. coli*-inoculated knife in an automatic poultry killer spread contamination to at least 500 poultry carcasses; chlorinated water spray (10 mg/l) resulted in contamination of 250–400 carcasses at levels 0.4–1.3 log units lower than with the unwashed knife. Similar results were detected with the head puller, which spread contamination to 500 carcasses, but a water spray with chlorine at 25 mg/l stopped the spread after only 25–100 carcasses. Superchlorinated water may prevent biofilm formation on working surfaces and equipment, reducing the likelihood of cross-contamination and facilitating post-processing cleaning (Arnold, 2005). Bailey et al. (1986) found that using chlorine at 40 mg/l in wash water to combat bacteria in a chicken fat matrix on stainless steel reduced numbers of *Salmonella* by 96% compared with a 50% reduction by using an unchlorinated water spray.

Disinfectants are also used in the meat industry to decontaminate equipment surfaces, especially knives (Taormina & Dorsa, 2007). Knives were inoculated with raw pork residues and the pathogens *Escherichia coli* O157, *Salmonella* Typhimurium or *Clostridium*

perfringens. Blades were dipped for 1–15 s in hot water (82.2 °C), warm water (48.9 °C) or warm disinfectant (neutral or acid QAC at 400 mg/l or peroxyacetic acid in combination with hydrogen peroxide [peroxyacetic acid at 165 mg/l and hydrogen peroxide at 700 mg/l]). Reductions on knives dipped for 1 s were less than 1 log unit, with no significant difference between treatments. Reductions in *E. coli* O157 after 15 s in hot water, neutral QAC, acid QAC or peroxyacetic acid were 3.02, 2.38, 3.04 and 1.52 log units, respectively. Reductions in *S. Typhimurium* after 15 s in hot water, neutral QAC, acid QAC or peroxyacetic acid were 2.39, 1.49, 1.66 and 1.34 log units, respectively. Reductions in *C. perfringens* after 15 s in hot water, neutral QAC, acid QAC or peroxyacetic acid were 2.03, 1.50, 1.18 and 1.41 log units, respectively.

In the fish processing industry, hypochlorite is mostly used in Thailand, India, Bangladesh and Indonesia at concentrations of 20–100 mg/l for decontamination of container and table surfaces. A study of tote box cleaning (Powney & Dunsmore, 1986) demonstrated that fish fillets with low counts stored in clean boxes took 10 days to reach 10^7 cfu/g, whereas in dirty boxes they took only 7 days to reach the same numbers. Several cleaning regimes were assessed, including chlorinated alkaline detergent, a phosphoric acid detergent and an acidic QAC compound detergent/sanitizer. In a study on the general microbial ecology of fish processing plants, Bagge-Ravn et al. (2003) observed that in four different fish industries (two of cold-smoked salmon, semipreserved herring and caviar), disinfection was carried out with hypochlorite in three of them (alone or in association with other products); only in one industry was the disinfecting agent peroxyacetic acid.

Summary

Cross-contamination is a complex process that is difficult to quantify in experimental and industrial settings. The experiment by Mead, Hudson & Hinton (1994) in poultry plants provides one of the best examples of how surface decontamination can prevent cross-contamination of food. It is difficult to quantify the effects of cross-contamination on pathogen numbers on food, but it is widely recognized that the use of disinfectants in food processing is important to prevent cross-contamination and therefore reduce consumer exposure to pathogens.

Data on the quantitative effects of disinfectants on food pathogens are available based on studies in industrial, pilot and laboratory settings. These data are not always equivalent. Assessment of the effectiveness of disinfectants based on studies in industrial settings is difficult. This is because the microflora, including pathogens, in the process environment is already being controlled by the ongoing use of disinfectant. Hence, attempting to measure the effectiveness at individual steps does not accurately reflect what would happen if no disinfectants had ever been used in the process prior to the study. The end result of this is that the incremental effectiveness of the individual control steps is underestimated.

Laboratory studies demonstrate that biofilms of *Salmonella* and *L. monocytogenes* can be inactivated by a range of disinfectants at suitable concentrations with appropriate contact times. Taormina & Dorsa (2007) demonstrated the effectiveness of disinfectants against *E. coli* O157, *S. Typhimurium* and *C. perfringens* on knives. Hypochlorite is effective at concentrations between 100 and 200 mg/l, depending on the porosity and smoothness of the surface being treated. Peroxyacetic acid is also an effective disinfectant alone and in combination with hydrogen peroxide. QACs are effective at concentrations up to 50 mg/l. Iodophors are active against *Salmonella* and *L. monocytogenes* but seem less effective than chlorine when used at concentrations up to 20 mg/l. Limited data on ASC show that this chemical also has surface disinfectant potential.

4.7 References

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Appendix 1: Risk modelling of the effect of chlorinated compounds on *Campylobacter* in poultry

This appendix illustrates how risk assessment (which consists of four steps: hazard identification, hazard characterization, exposure assessment and risk characterization), risk modelling and its outputs can be incorporated into the risk–benefit decision-making process. The overall objectives of a risk model are to translate the level or frequency of contamination of a product into a human health risk outcome. In the current illustration, the impact of the use of chlorine during poultry processing on *Campylobacter* contamination can be translated into an estimate of infections avoided. Translating the impact of an intervention on pathogens on a product to the human health outcome is helpful, because it allows us to compare different interventions acting in different ways and at different points in the process into a common metric for comparison across strategies or when conducting a cost–benefit assessment.

Campylobacter risk model description

FAO/WHO (2002b) developed a risk model for *Campylobacter* in poultry, which can be adapted and applied in the current project, as the basis for estimating the risk from *Campylobacter* in poultry and to quantify the potential implications of the use of chlorine in the processing of poultry in terms of risk reductions.

The risk modelling part of any microbial risk assessment can be divided into two primary components: the exposure assessment (which estimates the prevalence and level of a pathogen by considering processing effects as well as human consumption and behaviour); and the hazard characterization (which translates the outputs from the exposure assessment into a human health outcome, typically done using a dose–response relationship).

An overview of the risk assessment model for *Campylobacter* in broilers developed by FAO/WHO (2002b) is outlined in Figure 4A.1. The model considered the occurrence and number of *Campylobacter* present in chicken products throughout the process and up to the point of consumption. The stages from rearing of broilers to the consumption of chicken products are grouped into four main modules: 1) Farm & Transport, 2) Processing, 3) Storage and 4) Preparation. The exposure assessment initially evaluates the frequency and levels of *Campylobacter* on the farm, estimating the probability that a random flock is *Campylobacter* positive, the within-flock prevalence and the levels of colonization and contamination of the broilers (internally and externally). Subsequently, the stages of transport, processing, storage and preparation by the consumer are explored and combined to predict the overall impact that these stages will have upon the contaminating *Campylobacter* load on a random chicken carcass or product to determine the final exposure level.

The risk model relies on a human feeding trial study that was conducted (Black et al., 1988) using just over 100 healthy young adult volunteers (in the USA) in order to derive the dose–response relationship. Data for *C. jejuni* A3249 and 81-176 were pooled and fit to the beta-Poisson dose–response model. The response being measured in the model is infection; however, in order to estimate the probability of illness, the conditional probability of illness following infection was estimated using a dose-independent probability derived from the same study. The dose–response relationship is shown in Figure 4A.2.

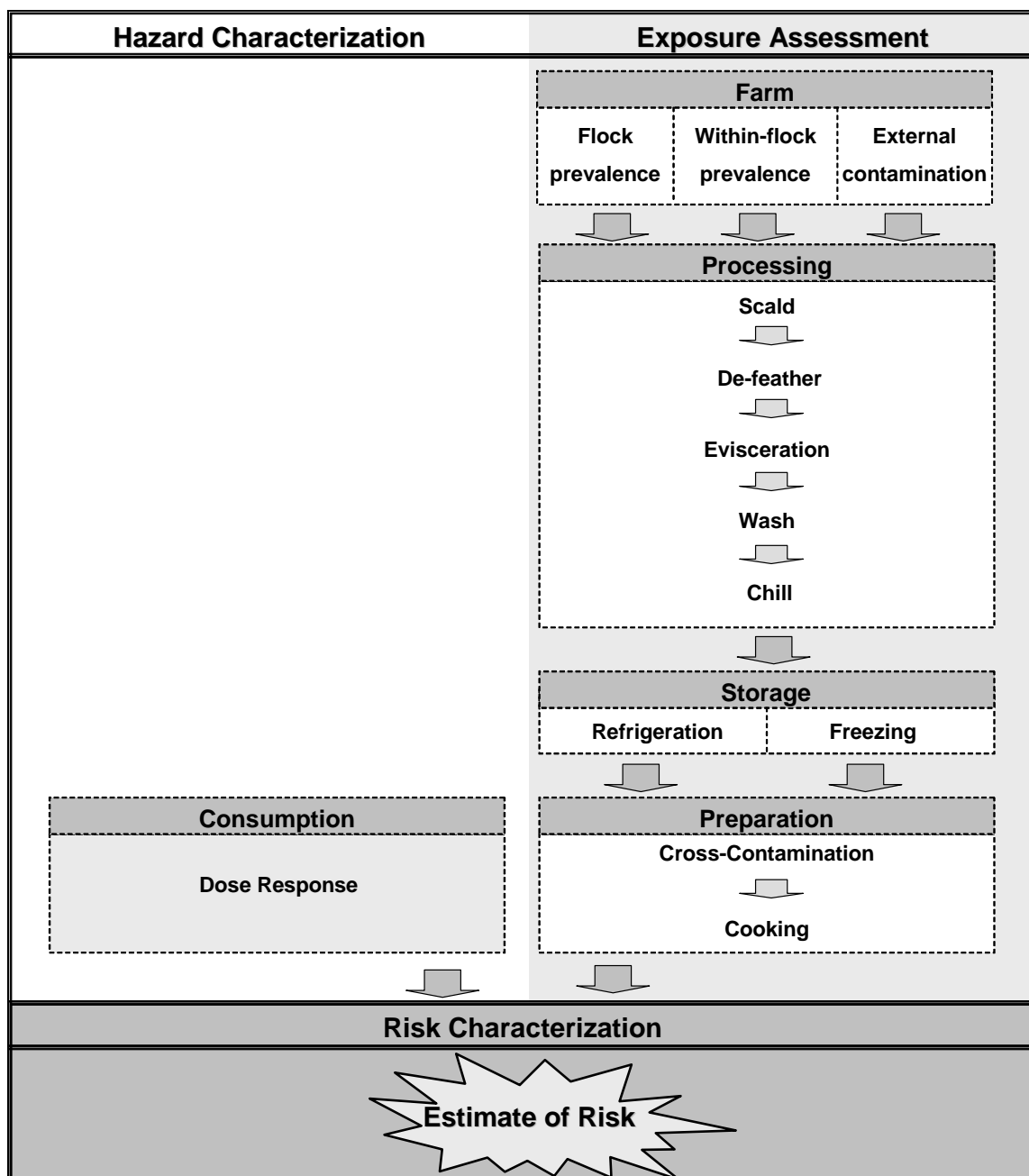


Figure 4A.1. Graphical representation of a *Campylobacter* in poultry exposure assessment. The model developed by FAO/WHO (2002b) begins at the end of the hazard characterization, the second step of risk assessment.

Model application

The FAO/WHO (2002b) risk model focuses on both fresh and frozen whole broilers prepared and consumed in the home and can be analysed using Monte Carlo simulation implemented with @RISK software.

Every iteration of the model tracks a randomly selected chicken from the farm, through processing, storage, preparation and cooking, to consumption, and the exposures that arise as a result of preparing that serving. In the model, chickens are probabilistically assigned to be either contaminated or not contaminated given the on-farm prevalence of

Campylobacter. Chickens originating from negative and positive flocks are then simultaneously simulated, the number of *Campylobacter* organisms present on the resulting product is estimated from statistical distributions based on reported data, and the changes in the level of contamination from farm to fork are modelled. The variability in these processes is described by probability distributions derived from published and unpublished data. In addition, the model also estimates the conversion of previously negative chickens into positive chickens as a result of cross-contamination, or vice versa.

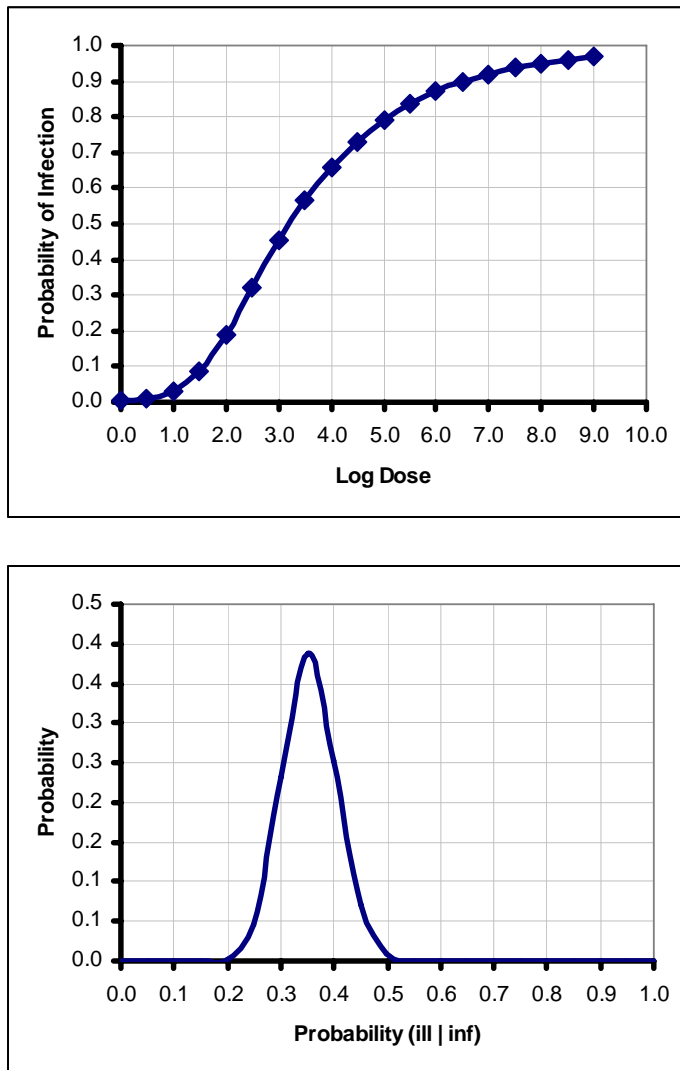


Figure 4A.2. FAO/WHO (2002b) dose–response model used to estimate the probability of infection upon exposure to *Campylobacter* and conditional probability of illness upon infection

Ultimately, the objective of the risk model is to translate pathogen contamination rates and levels, and their subsequent reductions as a result of an intervention, into a human health outcome. In order to do this using the existing model, some modifications were made. These modifications were primarily a simplification of the model to create a more efficient model that would still be appropriate for current purposes. Specifically, the FAO/WHO (2002b) model included detailed bird-by-bird contamination transfer at various stages of the processing plant with a very detailed and mechanistic model of the defeathering process, which tended to be very computationally expensive.

As the current project was primarily interested in the impact of chlorine on the contamination levels exiting the plant, and as the most frequent use of chlorine in the processing plant occurs during washing or chilling, both of which happen near the end of the process, the earlier processes were collapsed. The existing model was simulated for 10 000 iterations using an input value of 80% for on-farm prevalence with all other inputs at their default settings, and the resulting pre-washing prevalence and contamination distribution were estimated (Figure 4A.3). The concentration on carcasses originating from positive flocks was described using a normal distribution with a mean of 3.8 log cfu/carcass and standard deviation of 1.3 log cfu/carcass, whereas the concentration on carcasses originating from negative flocks was described with a normal distribution with a mean of 1.62 log cfu/carcass and a standard deviation of 1.3 log cfu/carcass. These distributions were then used as the starting point for all subsequent simulations used to estimate the effect of chlorine use on pathogen risk. In essence, the baseline model against which all results are compared is one for which the prevalence of *Campylobacter*-contaminated flocks on farms is 80%.

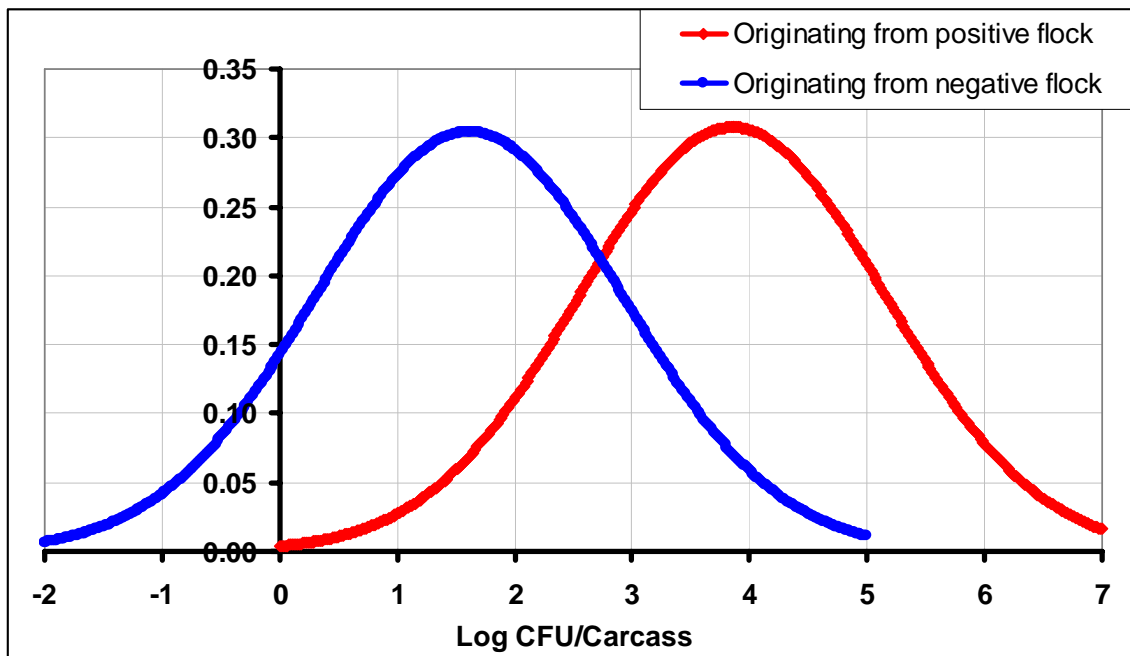


Figure 4A.3. Resulting distributions for contamination levels on chickens prior to washing used as inputs to the modified model

The impact of chlorine use during chicken processing and its subsequent estimated public health impact through the reduction of pathogen risk are presented in the following scenarios. The detailed quantitative data on the effect of chlorine on *Campylobacter* on poultry carcasses have been summarized previously in this chapter. The following scenarios are constructed based on a subset of the information in order to illustrate how pathogen reduction estimates, reported at various points in the process, can be translated into a common human health risk outcome.

Model scenarios

The following scenarios were constructed based on the data presented and applied to the modified risk model, in order to estimate the potential risk reduction as a result of the use of chlorine or other disinfectants in poultry processing.

Baseline scenario

The baseline scenario represents the risk estimates generated based on the current model without any additional steps to the described process. The baseline model, summarized graphically in Figure 4A.1, includes a washing step with plain water and a chilling step in water with no free chlorine.

1) Use of chlorine in an IOBW

As summarized previously, the primary effect from washing is the physical removal of contamination rather than a chemical decontamination effect. Northcutt et al. (2005) evaluated the effectiveness of a chlorine carcass wash in a study where poultry carcasses were inoculated with caecal material containing *Campylobacter*. Water at various temperatures with and without available chlorine at 50 mg/l was sprayed onto carcasses for 5 s with an IOBW. Neither water temperature nor chlorine level was found to have a statistically significant effect on the counts of *Campylobacter*. Although the effect was not statistically significant, the use of chlorine produced on average approximately 0.1 log greater reduction compared with just water alone. As this appendix is an illustrative exercise, we can assume that the effect of adding chlorine to the wash water could produce anywhere from no effect to a generous 0.1 log reduction.

2) Use of an ASC spray decontamination wash (based on Kere-Kemp et al., 2001; Bashor et al., 2004; Oyarzabal et al., 2004; Sexton et al., 2007)

This scenario estimates the effect of an additional decontamination step during processing that consists of the use of ASC at concentrations ranging from 600 to 1200 mg/l, resulting in log reductions from 0.9 to 3.8 log.

Most studies, particularly those conducted in the industrial setting, suffered from the lack of a control for the physical action of water alone as a spray or dip. However, evidence from a laboratory study that contained this control suggested that there was no significant effect on *Salmonella* (Arritt et al., 2002). Also, studies tended not to use IOBW or high-volume sprays, and therefore they would be less likely to exert a physical reduction effect.

3) Use of an alternative to chlorine-based disinfectant spray (based on Bashor et al., 2004)

This scenario estimates the impact on pathogen risk of using an alternative to chlorine-based disinfectant spray. The use of TSP (12% solution) was studied by Bashor et al. (2004) and was found to reduce *Campylobacter* by approximately 1 log when sprayed on carcasses for 15 s.

This study suffered from the lack of a control for the physical action of water alone as a spray or dip. However, evidence from a laboratory study that contained this control suggested that there was no significant effect on *Salmonella* (Arritt et al., 2002). Also, studies tended not to use IOBW or high-volume sprays, and therefore they would be less likely to exert a physical reduction effect

4) The use of chlorine in the chill tank

This scenario is based on one used in the FAO/WHO (2002b) risk assessment model, which assumes that when there is sufficient free chlorine in the chill tank, the frequency with which cross-contamination occurs is reduced (50–75% of the time); when it does occur, the amount of cross-contamination is less (0–4 log without chlorine to 0–3 log with chlorine). This is supported by the results of Yang, Li & Johnson (2001) presented below, in which the use of chlorine in the chill tank had a very short D-value.

The data available from the literature search that are relevant to the use of chlorine in the chill tank and its impact on the load of *Campylobacter* on carcasses do not provide any directly usable information for incorporation into the risk model. Although various authors have shown that there is a reduction in the contamination levels on carcasses exiting the chill tank, there is no clear way to determine how much of an effect the use of water alone might have had. One study done by Yang, Li & Johnson (2001) using inoculated *Campylobacter* on chicken does provide some indication of the effect that chlorine has on carcass contamination levels. These authors found that the chilling of chicken in water containing chlorine at 50 mg/l had a D-value of 73 min for *Campylobacter* contamination on the chicken. In other words, it would take 73 min to produce a 1 log reduction on chicken carcasses immersed in chiller water containing chlorine at 50 mg/l. When these authors looked at chiller water with a higher amount of organic content (as might be expected as the processing operation continues), the D-value was increased to 344 min. Based on this study, the chilling of carcasses using chlorinated chiller water is unlikely to be a significant decontamination step.

The biggest potential impact from the use of chlorine in chill tanks is not necessarily from the reduction in contamination on already contaminated chickens, but the prevention of or reduction in cross-contamination from *Campylobacter* being deposited on either uncontaminated or previously very low level contaminated chicken.

Yang, Li & Johnson (2001) also conducted a study to look at reduction of *Campylobacter* in chiller water as a function of chlorine concentration. These results are presented in Table 4A.1.

Table 4A.1. Effect of chlorine concentration in chiller water on the survival of *Campylobacter* as a function of chlorine concentration and water age (organic material buildup)

Chemical	Concentration (mg/l)	Water age (h)	D-value (min)
Chlorine	10	0	17.2
Chlorine	30	0	1.3
Chlorine	50	0	0.5
Chlorine	10	8	113.6
Chlorine	30	8	15.2
Chlorine	50	8	6.0

These results indicate that *Campylobacter* can be rapidly deactivated in chlorinated chill tank water, provided the amount of free chlorine is sufficient to overcome the organic material that builds up during processing. At a concentration of 50 mg/l, we would expect 90% reductions in the water within 30 s, whereas this would get extended to about 6 min when the organic load increases. Continuous dosing to ensure a sufficient free chlorine concentration in the water would be required in order for the cross-contamination to be prevented, as evidenced by the fact that a chlorine concentration of 10 mg/l has a D-value of 17.2 min in 0-h-old water, whereas the D-value gets extended to 113.6 min in 8-h-old water.

The results from incorporating these scenarios into the model are presented in Table 4A.2.

Conclusions

It is difficult to determine the risk reduction achievable by the use of disinfectants and therefore the impact of these chemicals on public health. Models that estimate these effects, like the one used in this work, carry a high degree of uncertainty as a result of the lack of appropriate data.

Table 4A.2. Summary of model estimates of relative risk reduction

Scenario description	Mean risk estimate	Estimated reduction in risk (%)
Baseline: Fresh chicken produced without chlorine in either the chill tank or during washing	1.63E-03	–
Scenario 1: Use of an IOBW with chlorine at 50 mg/l	1.57E-03	4 ^a
Scenario 2: Use of ASC decontamination spray	4.82E-04	71 ^b
Scenario 3: Use of a chlorine alternative TSP decontamination spray	7.49E-04	54 ^b
Scenario 4: Use of chlorine in chill tank at concentration to ensure sufficient free chlorine	5.10E-04	69
Scenario 5: Combination of Scenarios 1 and 4 (chlorine in wash water and chill tank)	4.76E-04	71 ^{a,b}
Scenario 6: Combination of Scenarios 2 and 4 (ASC decontamination spray and chlorine in chill tank)	4.58E-05	97 ^b
Scenario 7: Combination of Scenarios 3 and 4 (TSP decontamination spray and chlorine in chill tank)	1.26E-04	92 ^b

^a Chlorine had no statistically significant additional effect compared with unchlorinated water alone. The effect was due to the physical action of washing.

^b It is important to recognize that these studies did not compare the effect of a carcass spray or dip with water alone against the effect when the chemical agent was used. As a result, the true additional effect of the disinfectant in the spray/dip water cannot be assessed.

The use of an IOBW can result in significant reductions in *Campylobacter* numbers; however, the addition of chlorine to the water has no real significant additional effect (Northcutt et al., 2005). The model estimates that if an allowance is given to assume up to 0.1 log additional reduction due to chlorine addition in the wash water, then this translates to a *Campylobacter* risk reduction of 4% compared with the baseline scenario. This upper range in risk reduction (benefit) would need to be carefully tempered with the potential additional risk from adding chlorine to the wash water. Specifically, is the questionable and minimal benefit greater than the corresponding risks that would be calculated?

When ASC is used as a disinfectant spray, this results in an estimated 71% reduction in the risk of campylobacteriosis. However, data are not available that allow the effect of the ASC to be disaggregated from the physical effect of spraying/dipping carcasses in water alone. However, data from other studies suggest that the removal of bacteria from carcasses by the physical action of water is minimal (e.g. Arritt et al., 2002) unless high-pressure, high-volume water is used, as in an IOBW (Northcutt et al., 2005). The use of a TSP spray-wash was estimated to result in a 54% reduction in mean risk of campylobacteriosis, although this estimate is also subject to caveats similar to those in the estimate with ASC discussed previously.

The use of chlorine as a disinfectant to remove *Campylobacter* from chill tank water and hence prevent cross-contamination resulted in an estimated mean risk reduction in campylobacteriosis of 69%. When combined with an ASC or TSP carcass wash, use of chlorine in the chill tank resulted in an estimated mean risk reduction in campylobacteriosis of 97% and 92%, respectively, subject to the caveat discussed previously for the ASC and TSP carcass wash scenario. The combination of IOBW carcass wash with carcass chilling in chlorinated water resulted in an estimated mean risk reduction in campylobacteriosis of 71%. The model therefore demonstrates the enhanced risk reduction that can be achieved by the use of multiple interventions in series during poultry processing.

5. UNINTENDED CONSEQUENCES

The primary intended benefits of disinfection processes are the reduction of microbial foodborne disease risk and the control of contamination of food by pathogenic and non-pathogenic microorganisms during food production and food processing. However, use of antimicrobial compounds in the food processing industry can have consequences other than those intended. These include the development of antimicrobial resistance, the disruption of normal microflora, and nutritional and organoleptic changes in treated foods. Studies on the nature of such unintended consequences are described in this chapter.

5.1 Development of antimicrobial resistance

Microorganisms exposed to sublethal concentrations of antimicrobial compounds may develop the ability to survive in the presence of normally lethal concentrations. As acquired resistance to one type of antimicrobial agent may confer protection against other types, the widespread use of biocides by the food industry has led to concern about its impact on the development of resistance to therapeutic drugs. Sanitizers used by the food industry inactivate microorganisms by reacting at multiple sites within the cell. Therefore, microorganisms cannot develop resistance to these agents through modification of a specific target site, as is the case for therapeutic antimicrobial compounds. However, there are reports of microorganisms developing tolerance to chemical sanitizers after sublethal exposure in the laboratory, and sanitizer-tolerant microorganisms have been isolated from processing plant environments (Meyer, 2006).

Active chlorine compounds and peroxides kill through oxidation brought about by the generation of free radicals. As multiple free radicals may be produced, their specific interactions with cell components are complex. The specific mechanism by which hypochlorous acid kills bacterial cells is still unknown (Mokgatia, Gouws & Brozel, 2002). As multiple components of the cell are susceptible to oxidative damage, tolerance to oxidative sanitizers is based on the ability of the cell to neutralize free radicals, counter the effects of oxidative damage and excrete polymers that inactivate the biocide before it reaches the cell. Mokgatia, Gouws & Brozel (2002) isolated a hypochlorous acid-tolerant strain of *Salmonella* from a poultry processing plant. The tolerance was related to increased catalase and membrane-bound dehydrogenase production and increased ability to repair deoxyribonucleic acid (DNA). Hypochlorous acid tolerance in *Listeria monocytogenes* induced by exposure to sublethal levels in the laboratory is associated with increased biofilm formation (Folsom & Frank, 2007). Cells within a biofilm are protected from inactivation by the production of exocellular polymers. Published research has not associated the development of tolerance to hypochlorous acid with the acquisition of resistance to therapeutic antimicrobial compounds.

More information is available on acquired tolerance to quaternary ammonium compounds (QACs) compared with chlorine tolerance, perhaps because microorganisms exhibiting this characteristic are more frequently isolated from processing plant environments than are microorganisms that tolerate active chlorine biocides. QACs inactivate bacteria by modifying the cell membrane, causing loss of control over permeability (Block, 2001). Mullapudi, Siletzky & Kathariou (2008) found a high prevalence (51–60% of isolates) of benzalkonium chloride-tolerant *L. monocytogenes* in turkey processing plants, whereas Aase et al. (2000) observed 10% prevalence in strains isolated from poultry processing

environments. Some strains of *L. monocytogenes* adapt to sublethal exposure to QACs through stimulation of proton motive force-dependent efflux (Aase et al., 2000). Mereghetti et al. (2000) found evidence that the efflux pump-associated QAC resistance gene is not plasmid-borne, but Romanova, Favrin & Griffiths (2002) concluded that the gene (*mdrL*) can be both plasmid and chromosomal. Mereghetti et al. (2000) also found evidence that QAC tolerance in *L. monocytogenes* is associated with modification to the cell wall, as did To et al. (2002). These modifications involve changes to surface antigens and cell membrane fatty acids. Lunden et al. (2003) observed that the adaptive response of *L. monocytogenes* to various processing plant biocides resulted in cross-protection towards related and unrelated biocides. There is little information on the public health implications of pathogens acquiring QAC tolerance. Mullapudi, Siletzky & Kathariou (2008) reported that an outbreak strain of *L. monocytogenes* exhibited tolerance to benzalkonium chloride. However, there is no evidence that QAC tolerance in pathogens is associated with resistance to therapeutic agents or otherwise increased public health risk. Meyer (2006) concluded that there is no need for rotational use of biocides in food processing facilities, as biocide-tolerant microorganisms isolated from these environments remain susceptible to recommended usage levels.

A recent report from the European Food Safety Authority (EFSA, 2008) assessed the possible effect of chlorine dioxide, acidified sodium chlorite (ASC), trisodium phosphate (TSP) and peroxyacids on the emergence of antimicrobial resistance. These biocides are widely used in the food industry as alternatives to hypochlorous acid-based biocides. This report concluded that there is no published information to indicate that the use of these substances to treat poultry carcasses would lead to the development of resistance to therapeutic antimicrobial compounds.

5.2 Disruption of normal microflora

The use of active chlorine in food processing water is targeted at preventing the spread of pathogenic microorganisms and reducing levels of pathogens on food and equipment. However, active chlorine exhibits nonspecific activity and therefore also reduces levels of normal microflora. Possible negative consequences of disruption of native microflora include a reduction of microbial competition, which might allow increased growth of pathogen, and an increase in shelf life, which would provide more time for pathogen growth before loss of sensory quality.

One example where an application of technology that increases shelf life has a demonstrated potential to increase public health risk is the use of modified atmosphere packaging (MAP) for fresh produce. Berrang, Brackett & Beuchat (1989) observed that the application of MAP for some vegetables does not slow the growth of *Listeria monocytogenes*; because of this, the increase in shelf life may result in greater public health risk. No information is available to indicate that increases in shelf life resulting from use of active chlorine in food processing provide the opportunity for additional pathogen growth. Unlike MAP, there is no evidence that the use of active chlorine alters the growth environment of the food, and, unlike MAP, the initial pathogen load in the produce may be reduced.

The possibility that reduced microbial competition to chlorine treatment could allow increased growth of pathogens should also be considered. Many fruits and vegetables are sufficiently acidic to provide yeasts and moulds a competitive growth advantage over pathogens. Growth of yeasts and moulds can increase the pH of the fruit or vegetable or degrade the cellular structure so that growth of pathogens is increased (Beuchat, 2002). Wells & Butterfield (1997) found that *Salmonella* was potentially present in 18–20% of 401 fresh fruit and vegetable samples affected by soft rot that were obtained at market, whereas only 9–

10% of 402 healthy samples were potentially positive for the pathogen. When they induced soft rot in carrot, pepper and potato, *Salmonella* growth increased 10-fold. Brandl (2008) observed that soft rot due to growth of *Erwinia chrysanthemi* enhanced growth of *Escherichia coli* O157:H7 on lettuce. However, others have isolated native microflora from fresh produce that inhibits growth of pathogens. For example, *Salmonella syringae* prevented growth of *E. coli* O157:H7 in apple wounds (Janisiewicz, Conway & Leverentz, 1999), and Liao & Fett (2001) found that 6 of 120 isolates from fresh produce were able to inhibit growth of at least one human pathogen. Current evidence indicates that native microflora that inhibits growth of pathogens on fresh produce is less common than native microflora that has either no effect or a growth-promoting effect on pathogens. There are no data indicating that the disruption of native microflora on fresh fruits and vegetables by washing in chlorinated water as practised in the food industry would enhance the growth or survival of pathogenic microflora in the commercial product.

Use of chlorinated water in poultry processing will reduce the population of both normal and pathogenic microflora on the carcass. Patterson (1968) investigated the consequences of this microflora disruption and found that the spoilage microflora of chicken carcasses washed with chlorine at 200 and 400 mg/l was similar to that of water-washed carcasses. He concluded that chlorine-treated carcasses posed no greater risk to public health as a result of microflora disruption. There are no data indicating that the disruption of native microflora on poultry carcasses by chlorine treatment as practised in the food industry would enhance the growth or survival of pathogenic bacteria.

5.3 Nutritional and organoleptic changes in treated foods

This section covers the unintended effects of chlorine-based disinfectants and other alternatives, such as peroxyacids or ozone, in food production and food processing, focusing on nutritional and organoleptic changes in treated foods.

5.3.1 Effects on nutritional quality of treated foods

Little information is available at present in the scientific literature on the effect of the use of disinfectants on the nutritional quality of muscle foods. Most of the published studies have been performed with vegetables, probably because of their high surface to volume ratio, which can potentially facilitate more intense effects on nutritional components.

5.3.1.1 Meat, poultry, fish and fishery products

Poultry carcasses treated with ASC under exaggerated conditions showed amino acid and fatty acid profiles similar to those of controls. Lipid peroxidation, measured as an increase in thiobarbituric acid reactive substances (TBARS), was observed in the skin but not in the muscle (EFSA, 2005). Poultry carcasses treated with peroxyacids showed no significant alteration in either TBARS or fatty acid profiles in raw or cooked samples (EFSA, 2005). Beef trimmings for production of ground beef treated with chlorine dioxide (200 mg/l solution) showed oxidation profiles, measured as TBARS, similar to those of controls (Jiménez-Villarreal et al., 2003a).

The effect of chlorine dioxide treatment (20, 40, 100 and 200 mg/l in 3.5% brine for 5 min) on nutrients was evaluated in salmon and red grouper (Kim et al., 1998). Treatment did not result in variation in composition of major nutrients (protein and lipid) or moisture content, but decreased the concentrations of some vitamins. Red grouper had a higher initial

content of thiamine and riboflavin compared with salmon, and the relative effects were also more pronounced. The reduction in thiamine content for both fishes appeared to be dose related and reached almost 60% at 200 mg/l. Red grouper and salmon showed a reduction in riboflavin content (more than 30% and 15%, respectively). Niacin content did not correlate to the concentration of chlorine dioxide, and the mineral content was unaffected by the chlorine dioxide treatment (Kim et al., 1998).

5.3.1.2 Fresh fruits and vegetables

The content of L-ascorbic acid in shredded cabbage treated with hypochlorite (200 µg/l) was reduced by 30% (Sawai et al., 2001). Reduced concentrations of vitamin C (36%) and β-carotene (56%) were noted for fresh-cut iceberg lettuce treated with chlorine (dipped in 100 mg/ml chlorine solution at 20 °C for 2 min, pH 8.6) after 12 days of storage (Akbas & Ölmez, 2007). However, there were similar reductions in controls. Chlorine treatment (100 mg/l, pH 6.5) of rocket (arugula) leaves reduced vitamin C content by around 15% and 20% after 12 days of storage under air or MAP, respectively, in comparison with water-treated controls. The content of total polyphenols was not affected, but the total glucosinolate content was halved in treated produce after a 12-day storage under MAP (Martínez-Sánchez et al., 2006). Shredded carrots washed with chlorinated water (free chlorine at 100 mg/l) showed a 20% decrease in sugars, especially sucrose, probably due to leaching (Klaiber et al., 2004).

Shredded carrots were washed with ASC (100 mg/l at pH 2.71, 250 mg/l at pH 2.55 and 500 mg/l at pH 2.47) and stored up to 21 days at 5 °C. Other sanitizers included in the study were sodium hypochlorite (200 mg/l at pH 6.5) and peroxyacetic acid (40 mg/l at pH 3.72) (Ruiz-Cruz et al., 2007). In general, all sanitizers tended to retain antioxidant capacity. The shredded carrots washed with ASC at 250 mg/l and pH 2.55 showed a higher retention of antioxidant capacity than controls during the storage at 5 °C, which may be due to the retention of phenolic and flavonoid compounds and also carotenes. In fact, the reduction of carotenes was lower in treated produce compared with controls washed with water. The treatment also reduced the activity of peroxidase, and this may explain the observed control of whitening and maintenance of firmness in treated carrots (Ruiz-Cruz et al., 2007). In rocket (arugula) leaves washed with ASC at 250 mg/l and pH 2.63 (stored 8 and 12 days at 4 °C), no difference in vitamin C content compared with controls washed with water was reported after 8 days of storage under air or MAP. After 12 days of MAP storage, a 45% decrease in vitamin C content was noted in treated produce in comparison with controls (Martínez-Sánchez et al., 2006). Storage under MAP reduced the content of total polyphenols (more markedly than controls), mainly due to acylated flavonoid glycoside degradation. The total glucosinolate content was significantly reduced in ASC-treated leaves after 5 days of storage under MAP in comparison with the controls (Martínez-Sánchez et al., 2006).

Treatments with ozone or peroxyacids generate very reactive oxygen species that are potentially able to react with food components, such as amino acids (histidine, tryptophan, cysteine, cystine and methionine), vitamins (β-carotene, riboflavin, ascorbic acid, vitamin D and tocopherols), lipids (unsaturated fatty acids), sugars (glucose, fructose, sucrose and maltose) (Choe & Min, 2006) and even cell wall polysaccharides. However, it must be taken into account that while these treatments are strongly oxidative, they are limited to the external surface of the food, so that any expected effect on such nutrients would be restricted mainly to those located on the surface. Significant losses of vitamin C and β-carotene (30% and 55%, respectively) have been reported after 18 days of storage of fresh iceberg lettuce initially treated with ozone (4 mg/l for 2 min), but similar effects were seen in controls (Akbas & Ölmez, 2007). The vitamin C content of ozone-treated (up to 0.18 mg/l for 5 min) fresh-cut

celery was higher after 3, 6 and 9 days of refrigerated storage than those in controls (Zhang et al., 2005). In the same study, a decrease of total sugars was reported with time of storage, but there was no difference in relation to the control. Rocket (arugula) leaves washed with ozonated water (10 mg/l) or peroxyacetic acid solution (300 mg/l) showed reduced vitamin C content with storage—about 28% and 12%, respectively, when stored for 12 days under air, and about 40% and 30%, respectively, when stored for 12 days under MAP (Martínez-Sánchez et al., 2006). Peroxyacid treatment reduced the total glucosinolate content by 30% and 60% after 8 and 12 days, respectively, under MAP, but did not affect the total polyphenols content (Martínez-Sánchez et al., 2006). Ozone treatment reduced the total glucosinolate content by 55% and total polyphenolic content by 25% after 8 days' storage under MAP (Martínez-Sánchez et al., 2006). Treatment of fresh-cut tomatoes with hydrogen peroxide (dipping in up to 0.4 mol/l hydrogen peroxide solutions for 1 min) resulted in reduced phenolic and antioxidant levels (11% and 31%, respectively, in comparison with controls) after 7 days of storage at 4–6 °C. Reductions in vitamin C and lycopene contents were also reported, about 20% and 10%, respectively, at 1 day of storage, but the differences compared with controls were almost negligible after 7 days of refrigerated storage (Kim, Luo & Tao, 2007).

The use of disinfectants under typical conditions—hydrogen peroxide (5% for 30 min), hypochlorite (500 mg/l at pH 7.6 for 30 min), aqueous-phase ozone (8 mg/l for 30 min) and gaseous ozone (40 mg/l for 60 min)—resulted in significant losses of biothiols in vegetables (Qiang et al., 2005). These thiols are antioxidants and may act as such once consumed. This finding is important, as biothiols are present inside the vegetables. A hypothesis is that antioxidants near the surface have been previously oxidized, and therefore further oxidation can take place. The assayed biothiols were reduced glutathione, *N*-acetyl-L-cysteine, captopril, cysteine, homocysteine, γ -L-glutamyl-L-cysteine and oxidized glutathione. The effect and extent of the losses were dependent on the disinfectant and type of vegetable (Qiang et al., 2005). Higher losses were noted for all analysed biothiols in spinach, especially after peroxide treatment, with 70% biothiol reduction. Around 50% losses were reported after ozone and free chlorine treatments. Around 60–70% of the reduced glutathione was oxidized in red pepper. Reduction of *N*-acetyl-L-cysteine in cucumber was around 30% for all treatments. Smaller effects were reported in green beans and asparagus (Qiang et al., 2005).

5.3.2 Effects on organoleptic quality of treated foods

5.3.2.1 Meat and poultry

Meat treated with chlorinated water has been reported to increase more in weight than meat treated with non-chlorinated water (Cunningham & Lawrence, 1977). Also, chicken skin absorbed more water (130% in weight after 2 h in chlorinated water) than lean meat or fat.

Poultry carcasses were exposed to a chiller bath with chlorinated water (hypochlorous acid at 18 mg/l). Light (breast) and dark (leg/thigh) meats were removed and minced. Minces of patties were baked at 177 °C for 25 min. After cooling, patties were stored for 0, 1, 2 and 3 days under refrigeration and reheated at 177 °C for 20 min. Dark patties (from leg/thigh) did not show any difference for any of the sensory attributes in relation to the controls. Warmed-over flavour notes were observed in cooked chlorinated and non-chlorinated light patties (from breast); these off-flavours were higher for non-chlorinated samples till day 2, but after 2 days, off-flavours increased rapidly in chlorinated samples during storage and were significantly higher than in non-chlorinated samples (Erickson, 1999). The reason may

be the slowing down of initiation reactions for warmed-over flavour. In summary, chlorination did not affect the flavour of cooked, reheated dark chicken patties but had effects on light chicken patties consisting of a delay of warmed-over flavour up to 2 days of storage but an opposite effect after 3 days (Erickson, 1999). Concentrations of chlorine up to 200 mg/l have not been reported to cause an adverse effect on the appearance, taste or odour of the meat (SCVPH, 1998).

The use of chlorine dioxide (USDA, 2002a), ASC (USDA, 2002b) or peroxyacids (USDA, 2002c) as respective antimicrobial agents in poultry process water, under the prescribed and controlled conditions of use, have been reported to not alter the sensory properties of poultry. Some slight effects have been reported, such as a change in the colour of chicken breast skin from pinkish-white to greyish-white, but with no effect (no off-flavours) upon oven cooking (Thiessen, Osborne & Orr, 1984). Slight bleaching was also reported on the surface of turkey carcasses after chlorine dioxide treatment (Villarreal, Baker & Regenstein, 1990).

ASC treatments (1200 mg/l for 5 s) in the form of dips or sprays on the surface of dressed broilers were reported not to affect water holding capacity, appearance, smell, tenderness or overall acceptability (Sinhamahapatra et al., 2004). However, in another study in which chicken legs were treated with ASC (dipping into 1200 mg/l ASC solution, pH 2.7, for 15 min at 18 °C), legs turned slightly whiter initially, but no differences in smell or overall acceptability were found (Del Río et al., 2007). In the same study, it was reported that sensory quality (colour, smell and general acceptability) was improved in relation to the controls when the legs were stored at 3 °C for up to 5 days. A mild transitory whitening of the poultry skin after ASC treatment (1200 mg/l) has been also reported (Kemp, Aldrich & Waldroup, 2000). ASC treatment (300 mg/l) also maintained the organoleptic quality (colour, odour and taste) of raw ground beef, even in the cooked product. In both cases, the analysis was performed at 5, 8 and 12 days after the initial treatment; however, a more intense ASC treatment (600 mg/l) had a significant effect ($P < 0.05$) on raw and cooked ground beef, giving worse colour and odour in relation to the control (Bosilevac et al., 2004).

Beef trimmings were treated with chlorine dioxide (200 mg/l). The prepared ground beef had colour parameters (L , a and b), pH, TBARS, beef odour and off-odours similar to those of controls and followed the same trend up to 7 days' display (Jiménez-Villarreal et al., 2003a). When preparing ground beef patties, similar results were observed, except a little worse off-odour and better juiciness in the chlorine dioxide-treated beef trimmings (Jiménez-Villarreal et al., 2003b).

Peroxyacids can exert some slight whitening on poultry carcass surface that can be reverted after 24 h. Acids were reported to accumulate in the skin, affecting odour and flavour, such as a vinegar-like odour when peroxyacetic acid was used (SCVPH, 2003). However, chicken legs treated with peroxyacids (dipping into 220 mg/l peroxyacid solution, pH 3.75, for 15 min at 18 °C) did not show significant sensory differences compared with the untreated legs in terms of colour, smell or overall acceptability (Del Río et al., 2007).

Chicken legs treated with TSP at concentrations below 10% did not produce noticeable off-flavours or discoloration. So, chicken legs treated with TSP (dipping into 12% weight by volume TSP solution, pH 13.0, for 15 min at 18 °C) did not show significant sensory differences, or were even better after 5 days of storage at 3 °C, compared with the untreated legs in terms of colour, smell or overall acceptability (Del Río et al., 2007). However, chicken legs treated with higher concentrations had a detectable chemical odour and showed darker, less red and less yellow legs compared with untreated legs (Kim et al., 1999a).

5.3.2.2 Fish and fishery products

ASC treatment of salmon fillets (dipping in ASC at >100 mg/l, pH 3.24, for 1 min) resulted in a visible loss of colour. A similar change happened with another ASC treatment (50 mg/l, pH 3.29, for 2 min) that produced a very apparent change of colour, which would result in rejection by consumers. However, a reduction of the treatment to just 1 min did not result in a visible change of colour, even though the treated ASC solution had a light pink colour combined with a small degree of turbidity (Su & Morrissey, 2003).

Chlorine dioxide treatment of sea scallops did not show discernible effects until the scallops were exposed to concentrations above 3.8 mg/l for more than 10 min. Development of slime and loss of surface sheen were then noticeable, giving the product a drier appearance. Also, seepage about the product was evident (Kim et al., 1999b). Fillets of mahi-mahi experienced changes in colour from the preferred ruby-red to darker reddish brown. A bleaching effect was noticed at chlorine dioxide concentrations of 7.6 mg/l or higher, but was judged still acceptable. Chlorine dioxide treatment of shrimps did not cause discernible effects for the first 2 days of storage, and appearance was even better than control between 2 and 5 days. The exposure of shell-on shrimp to chlorine dioxide did not influence the sensory attributes. For all these treatments, the solutions experienced noticeable changes of colour, which were attributed to the formation of chlorinated reaction products (Kim et al., 1999b).

5.3.2.3 Fresh fruits and vegetables

Processing of vegetables, especially physical stress during cutting procedures, creates wound signals. These may elicit physiological and biochemical reactions in tissues (adjacent and distant). These changes may be varied and can contribute to the accumulation of phenolic compounds that may serve as substrates to polyphenol oxidase and peroxidase, resulting in ortho-quinones that in turn can polymerize and form brown pigments (Baur et al., 2004a). Browning is one of the major causes of loss of quality in cut vegetables. Another important quality factor is the decrease in firmness and loss of integrity (Rico et al., 2006).

Phenolic metabolism may be affected by washings with sanitizers. Washing of shredded lettuce with chlorinated water (free chlorine at 100–200 mg/l) significantly reduced the activity of phenylalanine ammonia-lyase. The visual quality, the cut edge vascular tissue browning and favourable aroma preservation during 7 days of storage of shredded iceberg lettuces washed with chlorinated water were reported as better than when using tap water or ozone for washings. In this study, no off-odours or off-flavours caused by chlorine were perceived by the test panel (Baur et al., 2004b). Fresh-cut iceberg lettuce samples treated with chlorine (dipping in 100 mg/ml chlorine solution at 20 °C for 2 min, pH 8.6) or ozone (4 mg/l) did not reveal initial changes in colour, texture or moisture. Colour reduction followed a similar trend for all treatments as for the untreated samples during the 12 days of storage at 4 °C. Reported changes consisted of increases in *a* value (loss of green pigment), decreases in *b* value (loss of yellowness) and decreases in *L* value (lightness), which might be caused by phenolic oxidation or bacterial spoilage. No changes in texture and moisture were reported (Akbas & Ölmez, 2007). Sequential washes of sliced green bell peppers with chlorinated water (100 µg/l) produced a significant reduction in acetaldehyde, soluble solids (mostly sugars) and total phenols in relation to the non-washed controls (Toivonen & Stan, 2004). However, firmness retention was improved in washed slices, this being attributed to the removal of stress-related compounds produced during the cutting operation.

Fresh cilantro bunches were washed with 1-methylcyclopropene at 1.5 mg/l and then cut and washed for 1 min in either sodium hypochlorite (100 mg/l) or ASC (100 mg/l), dried, packaged and stored for up to 14 days. The control samples washed with water showed the

lowest quality score and high levels of yellowing. In contrast, samples washed with sanitizers had no off-odour and had higher colour score, near the initial green, and fresh appearance, with no yellowing or dehydration (Kim et al., 2007).

Apple slices treated with ASC (1.5–6 g/l dipped for 1 min) showed a smaller decrease in lightness (*L*) when stored for up to 24 h at 20 °C, indicating that treated slices showed significantly less browning than the water-treated control (Lu et al., 2007). However, this effect was not observed when the storage was prolonged to 14 days. Rocket (arugula) leaves washed with ASC (250 mg/l) and stored under air or under low oxygen and high carbon dioxide (MAP) were reported to keep a sensory quality (colour and visual quality) similar to that of controls washed with water (Martínez-Sánchez et al., 2006). ASC treatments (250 mg/l, pH 2.55, and 500 mg/l, pH 2.47) of shredded carrots and storage for up to 21 days at 5 °C showed a control of whitening and firmness maintenance (Ruiz-Cruz et al., 2007). ASC treatment of fermented Chinese cabbage (500 mg/l ASC pre-wash for 15 min) did not significantly influence the sensory (colour, odour, taste and texture) parameters analysed (Inatsu et al., 2005).

Chlorine dioxide treatment has been reported to cause browning of lettuce and cabbage attributed to oxidation of phenols by polyphenol oxidase (Sy et al., 2005), even though this enzyme appears to be inactivated by chlorine dioxide in apples (Fu et al., 2007). Gaseous chlorine dioxide was evaluated for its effectiveness to extend the shelf life of minimally processed lettuce and cabbage previously immersed in a cysteine solution to inhibit browning from occurring during chlorine dioxide treatment (Gómez-López et al., 2008). Chlorine dioxide treatment did not affect the respiration rate of iceberg lettuce but enhanced the respiration rate of cabbage. This change could be due to modifications of the metabolism of the tissue, probably due to oxidation of plant constituents. The previous addition of cysteine was effective in avoiding the development of brown pigments. Treated lettuce stored for 4 days at 7 °C under MAP showed higher off-odour and bad flavour above the acceptability limit as well as surface browning. Treated cabbage stored under similar conditions did not show variations in relation to controls and remained sensorily acceptable until 9 days of storage. However, practical application of cysteine before chlorine dioxide treatment is impaired due to its effect on the decontamination efficacy of chlorine dioxide (Gómez-López et al., 2008). Other authors have also observed significant discoloration of lettuce leaves after treatment with chlorine dioxide gas (0.5 mg/l for 2 min) in comparison with control samples at 0 days. The yellow-green colour changed to white-brown, and the *a* value (green to redness) increased for most treated samples. This effect was enhanced at higher concentrations of chlorine dioxide (0.5 mg/l for 10 min or 5 mg/l for 2 or 10 min) (Mahmoud & Linton, 2008). Significant discoloration of lettuce leaves was also reported at concentrations of chlorine dioxide higher than 0.2 mg/l for 60 min (D’Lima & Linton, 2002).

Several types of berries treated with chlorine dioxide gas (4.1 mg/l for 30 min and stored for up to 10 days at 8 °C) did not show significant changes in sensory quality (Sy, McWatters & Beuchat, 2005). Appearance, colour, aroma and overall quality of control and treated blueberries were not significantly different at day 0, and reductions in values were also similar during storage. The sensory attributes of treated strawberries and raspberries were significantly lower than controls at day 0. Sensory quality decreased during storage, but no differences were observed between treated and untreated samples. Initial bleached spots observed in treated samples of strawberries at day 0 were not evident after storage (Sy, McWatters & Beuchat, 2005).

Fresh-cut vegetables (cabbage, carrot and lettuce) treated with chlorine dioxide (1.4 mg/l for 10.5 min and then stored at 10 °C for 10 days) showed significant adverse changes in sensory quality (appearance, colour, aroma and overall quality) after 3 days of storage, particularly for lettuce leaves (Sy et al., 2005). Sensory quality also decreased during

further storage, but no differences were observed between treated and untreated samples, with some exceptions: treated fresh-cut carrots showed slight whitening in colour and significant adverse effects for all tested parameters, whereas fresh-cut lettuce showed slight brown discoloration and fresh-cut cabbage showed increased brown discoloration. In contrast, carrots treated with gaseous chlorine dioxide (1.3 mg/l at 28 °C for 6 min) and stored under MAP did not show significant sensory effects compared with the untreated samples (Gómez-López et al., 2007). Lettuce leaves treated with chlorine dioxide gas (for 30 min, 1 h and 3 h) did not show any visible difference in visual quality compared with the untreated control lettuce after 18 days of storage at 4 °C (Lee, Costello & Kang, 2004).

Various fruits (apple, tomato, onion and peach) were treated with chlorine dioxide gas (1.4 mg/l for 6 min) and stored at 21 °C for 10 days (tomatoes and peaches), 31 days (onions) and 41 days (apples) (Sy et al., 2005). Sensory quality (appearance, colour, aroma and overall quality) of peaches was significantly adversely affected by the treatment, which was evident even at 0 days; the quality of treated peach deteriorated very rapidly and markedly, so that the scores were unacceptable at 3 days. No significant differences were observed for tomatoes (even a trend towards better scores) or onions. Apples showed significant adverse effects of treatment for appearance, colour and overall quality after 9 days (Sy et al., 2005). Carrots treated with chlorine dioxide gas (1.3 mg/l at 1 min) and then stored under MAP at 7 °C for 8 days did not show significant differences compared with untreated carrots (Gómez-López et al., 2007). After 7 days, treated samples were unacceptable due to odour. In this case, no whitening was reported, in contrast to the results reported by Sy et al. (2005).

Potato strips were washed with several sanitizers and then either vacuum packaged or kept under adequate MAP, with the exception of samples treated with hypochlorite, and stored for 14 days at 4 °C. The treatments consisted of total chlorine (80 mg/l, adjusted to pH 6.5, from 10% sodium hypochlorite), sodium sulfite (2 g/l), peroxyacetic acid (300 mg/l), total ozone dose (20 mg/l, pH 7.5), and total ozone dose + peroxyacetic acid (20 mg/l + 300 mg/l, respectively) (Beltrán et al., 2005a). The respiratory activity was similar for all the treatments. Neither of the washing treatments resulted in browning promotion at 0 days. After 5 days, a moderate degree of browning was observed in peroxyacetic acid-treated and MAP-packaged samples. Only sodium sulfite-treated samples kept the initial visual appearance; the treatment controlled the browning at 5 days but produced off-odours. Fresh-cut potatoes treated with ozone, sodium sulfite and ozone-peroxyacetic acid and kept under vacuum had good results, with no browning and with full typical aroma and turgid texture. On the contrary, hypochlorite-treated potatoes gave some browning at 5 days. Vacuum packaging preserved the appearance better than MAP. Tomato slices treated with hydrogen peroxide (up to 0.4 mol/l for 1 min and stored up to 7 days at 4 °C) exhibited reduced red colour (Kim, Luo & Tao, 2007). Rocket (arugula) leaves were washed with various sanitizers (chlorine at 100 mg/l at pH 6.5, ASC at 250 mg/l at pH 2.6, lactic acid at 20 ml/l, ozone at 10 mg/l and peroxyacetic acid at 300 mg/l) and then stored at 4 °C under air or MAP (Martínez-Sánchez et al., 2006). Lactic acid treatment was detrimental to sensory quality. The visual quality, texture and freshness decreased during storage in a similar pattern as for the other assayed sanitizers, even though MAP storage generally gave significantly worse results. No off-odours were detected in any treatment.

Gaseous ozone treatment (21 400 mg/m³ for 30 min) did not affect the global sensory quality (visual quality, colour, translucency and soluble solids content) of fresh-cut cantaloupe melon after 8 days of storage under MAP at 5 °C; only aroma and firmness were slightly affected (Selma et al., 2008). Ozone-treated carrots showed a lighter (higher *L* values) and less intense (lower chromatic values) colour than control carrots. These effects increased with the ozone concentration (Liew & Prange, 1994). Other authors who have studied the effect of pre-washing of carrots with either chlorinated water (free chlorine at 200

mg/l for uncut and 100 mg/l for shredded carrot) or ozonated water (1.3 mg/l for uncut carrots) did not report significant sensory effects (colour, odour, texture or sweetness) (Klaiber et al., 2004). The authors reported a significant reduction in sweetness only for the shredded carrots treated with chlorinated water (free chlorine at 100 mg/l) as a consequence of about 20% loss of sugars due to sugar leaching caused by washing of the shredded carrots. The flavour of the carrots was also reported to be reduced. Fresh-cut lettuce treated with ozone showed excellent visual quality during storage, with no browning (Beltrán et al., 2005b).

In asparagus, some enzymes, such as phenylalanine ammonia-lyase and peroxidases, control lignification, which in turn is related to the toughening that occurs a few days after harvest and is a major factor for the determination of the spear quality. Ozone treatment (1 mg/l for 30 min) of fresh-cut green asparagus partially inhibited enzyme activity, and the levels of lignin, cellulose and hemicellulose, which play important roles in the texture attributes of the asparagus cell walls, increased at a slower rate than controls (An, Zhang & Lu, 2007). Polyphenol oxidase was partially inhibited by ozone treatment (up to 0.18 mg/l for 5 min), showing a concentration dependence in fresh-cut celery. The sensory quality (colour, visible structural integrity and general appearance) was reported to be better in ozone-treated celeries than in non-treated controls (Zhang et al., 2005). Polyphenol oxidase and pectin methylesterase activities in fresh-cut lettuce were also partially inhibited by ozone treatment (1 mg/l for 1 min and subsequent refrigerated storage for 10 days). The reduction of the methylesterase activity gave some negative effect on texture, as it was correlated with a lower crispness, whereas the fresh appearance was rated similar to the initial values until 7 days of storage (Rico et al., 2006). Fresh-cut salads consisting of chopped lettuce, shredded carrots and red cabbage were treated with ozone (2.5 mg/l for 10 min) or chlorine (100 mg/l as free chlorine for 10 min), and packages were kept under refrigeration for up to 25 days. Visual evaluation by a test panel reported browning, loss of integrity and overall poor appearance after 16 days in chlorine-treated salads. Ozone-treated salads showed slower degradation, with acceptable values at 21 days (García, Mount & Davidson, 2003).

5.4 Summary of findings

The nutrient contents and sensory quality of foods may be affected by treatments with disinfectants, even though the consequences show a large variability and to some extent contradictory results. The effects depend mainly on the type of food and mode of preparation, the type of sanitizer and conditions of the treatment (concentration, pH, time, temperature, full procedure), washing procedures and storage conditions (type of package, film permeability, time, temperature). In view of so many variables involved, recommendations should be given on a case-by-case basis.

Nutritional effects appear to be mainly focused on some vitamins (β -carotene, riboflavin, thiamine, ascorbic acid and tocopherols) and thiols (reduced and oxidized glutathione, captopril, *N*-acetyl-L-cysteine, γ -L-glutamyl-L-cysteine) that are particularly sensitive, especially in fruits and vegetables. Chlorine, ozone and peroxyacetic acid appear to be the most damaging for such vitamins and important antioxidant thiols. Some losses of sugars have also been reported after treatment with chlorine. Reductions of total polyphenols and glucosinolates have been reported during storage after the sanitizer treatment of vegetables.

Sensory quality, particularly colour, may be affected, depending on the intensity of the treatment. Some whitening in muscle foods and discoloration (browning) in vegetables and fruits have been reported. Even though there are some contradictory results in the

literature, the general trend shows that ASC and ozone treatments appear to keep and even improve the sensory quality during storage of fruits and vegetables, whereas chlorine dioxide and peroxyacids appear to be ineffective in preventing brown discoloration caused by phenolic oxidation, or even promote it. Off-odours may be detected during storage after specific treatment conditions for some sanitizers.

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6. RISK–BENEFIT ASSESSMENT

6.1 Introduction

Risk–benefit assessment can be defined as an activity that weighs the probability and severity of harm in a particular exposure scenario against the probability and magnitude of benefit as a basis for risk management decisions and communication to the public. Risk–benefit assessment can be performed to inform policy-makers, regulatory authorities and risk managers or consumers. The request for risk–benefit assessment must be unequivocally formulated, preferably in a dialogue between manager and assessor.

Risk–benefit assessment integrates the results of two separate activities: risk assessment and benefit assessment. Definitions and procedures for risk assessment have been well established in the scientific literature and in procedures adopted by international bodies, such as the Codex Alimentarius Commission. Similar definitions are not available for benefit assessment, but it is recommended that benefit assessment follows the same steps as risk assessment (e.g. EFSA, 2006). A general approach to risk–benefit assessment can be proposed (Table 6.1).

Table 6.1. General approach to risk–benefit assessment

Risk	Benefit
Hazard identification	Positive health effect identification ^a
Hazard characterization	Positive health effect characterization ^a
Exposure assessment	Exposure assessment
Risk characterization	Benefit characterization
Risk–benefit assessment	

^a A positive health effect (benefit) may also result from an intervention that leads to the reduction of the level of a hazard in food (i.e. a reduction in risk).

Risks and benefits should be assessed similarly and separately and for different population groups if necessary. The presentation of the results (risk characterization, benefit characterization, risk–benefit assessment) can be descriptive, semiquantitative or—if sufficient data are available—quantitative. Weighing of benefits against risks needs to take into account the time frame in which the effects become apparent and the severity and/or magnitude of these effects.

6.2 Current activities relating to risk–benefit analysis

Risk–benefit assessment is an actively developing field. Published studies have considered the risks and benefits associated with fish consumption (Ponce et al., 2000; FSA, 2004; Tuomisto et al., 2004; Cohen et al., 2005; Foran et al., 2005; Gochfeld & Burger, 2005; Hansen & Gilman, 2005; Verbeke et al., 2005; Norwegian Scientific Committee for Food Safety, 2006; Maycock & Benford, 2007), the risks and benefits of increased dietary exposure to folic acid (Lawrence, 2005; FSANZ, 2006; Hoekstra et al., 2007) and micronutrients (Renwick et al., 2004; Keijer et al., 2005; Shenkin, 2006).

The European Food Safety Authority (EFSA) Scientific Committee is preparing guidelines, and several European Union projects are ongoing: HiWATE (Health Impacts of Long-Term Exposure to Disinfection By-Products in Drinking Water; <http://www.hiwate.eu/>), INTARESE (Integrated Assessment of Health Risk of Environmental Stressors in Europe; <http://www.intarese.org/>), BENERIS (Benefit–Risk Assessment for Food: an Iterative Value-of-Information Approach; <http://www.beneris.eu/>), QALIBRA (Quality of life – integrated benefit and risk analysis; <http://www.qalibra.eu/>) and BRAFO (Benefit–Risk Analysis for Foods; <http://www.brafo.org/brafo/>). These projects study different aspects of risk–benefit analysis.

Only one published study on risk–benefit assessment of disinfectants was available. Havelaar et al. (2000) compared the risks of bromate formation due to ozonation of drinking-water with the benefits of reducing the concentration of viable *Cryptosporidium parvum*. Disability-adjusted life years (DALYs)—a metric that combines years of life lost due to premature mortality and years of life lost due to time lived in states of less than full health—were used to quantify the risks and benefits, and it was concluded that the health benefits of preventing gastroenteritis in the general population and premature death in patients with acquired immunodeficiency syndrome outweighed health losses by premature death from renal cell cancer. The application of DALYs in principle allowed a more explicit comparison of the public health risks and benefits of different management options. In practice, the application of DALYs was hampered by a substantial degree of uncertainty. The methodology used by Havelaar et al. (2000) was applied to optimize the ozone dosage in a drinking-water plant near Paris, France (Dilé-Mary et al., 2002).

6.3 Evaluation of the risks and benefits of disinfectants used in food production and processing

In the case of chlorine-based disinfectants used in food production and processing, there may be several benefits. From a public health perspective, the reduced exposure to pathogens is the key benefit. Other benefits, such as longer shelf life, are not considered here. The key potential risks are related to the increased exposure to chemical residues. Other potential risks, such as reduced consumer acceptance, are not considered in this assessment. Similar potential risks and benefits apply to other non-chlorine-containing chemical disinfectants, such as peroxyacetic acid.

Foodborne diseases are an important cause of morbidity and mortality worldwide, but the full extent and cost of unsafe food, and especially the burden arising from chemical and parasitic contaminants in food, are currently still unknown. Recently, the World Health Organization (WHO) has established the Foodborne Disease Burden Epidemiology Reference Group, which engages in estimating the global burden of foodborne illness using summary health metrics that combine morbidity, mortality and disability in the form of the DALY (Stein et al., 2007).

Several countries have published estimates of the incidence of illness related to the occurrence of pathogens in food. For example, it is estimated that in Australia, contaminated food caused approximately 5.4 million cases of gastroenteritis per year, along with 6000 non-gastrointestinal illnesses, 42 000 episodes of long-term effects (chronic sequelae) and 125 cases of premature mortality (Abelson, Potter Forbes & Hall, 2006). Such estimates can be based on reported cases, corrected for an estimate of the under-reporting ratio (e.g. USA—Mead et al., 1999; Australia—Hall et al., 2005), or can be based on population-based studies on the incidence of infectious intestinal illness (e.g. United Kingdom—Wheeler et al., 1999; Adak, Long & O'Brien, 2002; Adak et al., 2005; the Netherlands—De Wit et al., 2001).

These studies include attribution of a proportion of identified cases to food, as most pathogens can also be transmitted by other pathways, such as water, direct animal contact or between humans. Attribution studies may also include evaluation of the proportion of cases that is attributable to different food groups (beef, pork, poultry meat, fish, produce, etc.; Adak, Long & O'Brien, 2002; Hoffmann et al., 2007; Havelaar et al., 2008). Estimates for the disease burden (in DALYs) are available for the Netherlands (Kemmeren et al., 2006; Vijgen et al., 2007), whereas several countries (e.g. USA—USDA, 2009; Australia—Hall et al., 2005; the Netherlands—Kemmeren et al., 2006; Vijgen et al., 2007) have presented estimates of the costs associated with foodborne illness. In general, epidemiological information on foodborne illness is available at an aggregated level (“foodborne” or broad food categories, such as red meats, poultry, produce, etc.). At the level of specific food product–pathogen combinations, such information cannot be based on epidemiological studies, but would require the development of specific risk assessment models, with epidemiological information being used to calibrate or validate the risk assessment models. The public health impact of applying disinfectants in the food-chain can then be assessed using risk assessment models, as illustrated in Appendix 1 to chapter 4.

In general, it is difficult to attribute low levels of contaminant residues in food to the incidence of adverse health outcomes in the population, primarily because of the chronic nature of the potential health end-point. A conservative approach is usually taken whereby a chemical risk assessment is undertaken based on toxicological and other data. A limited number of countries have tried to characterize the adverse health outcomes associated with chemical residues across the population. In particular, the Netherlands has made an estimate of the number of DALYS that may result from the presence of naturally occurring contaminants (e.g. allergens and mycotoxins) and chemicals (e.g. nitrate and acrylamide) that arise in the production and processing of food (Baars, van Leeuwen & Kramers, 2006). No national assessment of potential adverse health outcomes across populations for disinfectants or their by-products was available to the expert meeting.

6.4 Approach taken by the expert meeting

The expert meeting developed a stepwise approach to risk–benefit analysis of chlorine-based compounds and alternative disinfectants used in food production and processing. This consisted of the following steps:

- listing the most predominant application practices used in food production and processing (i.e. use scenarios; see chapter 1 for details on the various uses of the disinfectants);
- performing risk assessments for the residues arising from each of the use scenarios; these residues may include both the parent disinfectant and its by-products (see chapter 3 for details);
- performing benefit assessments from pathogen reduction in the food (see chapter 4 for details).

The expert meeting identified some important gaps in the available data. These data gaps constrained the scope of the risk–benefit assessments. Consequently, the expert meeting agreed on a number of recommendations for further scientific studies and the development of standardized practices (see chapter 7). Where scientific data were available, an assessment of risk and/or benefit was undertaken, and the expert meeting categorized these situations in one of the following four categories:

- 1) No health concern identified; no benefits identified.
- 2) No health concern identified; benefits identified.
- 3) Health concern identified; no benefits identified.
- 4) Health concern identified; benefits identified.

Only use scenarios for which it was concluded that there are both health concerns and benefits were considered to need further evaluation. However, the expert meeting did not identify any use scenarios that were of this type (i.e. both health concerns and benefits identified).

6.5 Uncertainties

6.5.1 Chemical risk assessment

In the toxicological assessment, sufficient data or existing authoritative toxicological reviews were available to the expert meeting to allow the identification of a health reference value for most of the disinfectants identified in the scenarios as well as some by-products. However, the occurrence data (i.e. concentration in food) available for disinfectants and their by-products in food were relatively limited. These data are necessary to estimate the dietary exposure arising from the consumption of treated food. There is therefore a relatively high level of uncertainty associated with the dietary exposure assessments, although conservative assumptions were generally applied to compensate for this. In some cases, particularly for the disinfection by-products (DBPs) in food, there were very limited occurrence data available. For these DBPs, no dietary exposure assessment could be performed, and hence no complete risk assessment could be prepared. The data available on occurrence of DBPs on food were used to conclude on the likelihood of any health concerns, and the degree of uncertainty and conservatism is documented in chapter 3 where appropriate. The level of uncertainty and conservatism needs to be taken into consideration in the risk–benefit assessments.

There are only limited occurrence data available for trihalomethanes (THMs), some of which are genotoxic and carcinogenic. As THMs can be formed with hypochlorite but not with chlorine dioxide or acidified sodium chlorite (ASC), there is more uncertainty associated with the safety of the hypochlorite treatments than with that of the other processes, although definite data are not available.

6.5.2 Microbial risk assessment

The microbiological risk assessment contained a number of sources of uncertainty. The key sources were:

- data gaps where no experimental data were identified;
- lack of data on industrial-scale processes and the associated uncertainty of using only experimental data;
- use of data from studies in which the food was inoculated with the pathogen, rather than being naturally contaminated;
- inconsistencies between individual studies and the variability of these data;
- lack of appropriate controls used in studies.

These uncertainties were taken into consideration when evaluating the evidence and arriving at a risk–benefit conclusion, as shown in Table 6.2.

6.6 Results

From Table 6.2, it can be concluded that, where data were available, no health concerns were identified in relation to residues of disinfectants or the occurrence of DBPs. There were few scenarios in which some benefits were identified. These were the use of ASC to reduce counts of *Campylobacter* and *Salmonella* on poultry carcasses prior to chilling and the use of sodium hypochlorite or chlorine dioxide in chiller water for poultry to prevent cross-contamination. For other scenarios, the only documented benefits are based on laboratory studies using seeded cultures, and this evidence was considered insufficient to allow a conclusion to be reached about their effectiveness in practice.

Table 6.2. Risk–benefit assessment

Commodity	Chemical	Process parameters	Chemical risk assessment		Microbial risk assessment		Risk–benefit assessment
			Residue(s)	Conclusion	Pathogen(s)	Conclusion	
Poultry (Sequential scenario) ^a	Hypochlorite	Pre-chill carcass spray, 20–50 mg/l (including sequential treatment using three washers)	THMs and other organohalogens expected; data on chloroform only	No health concern identified (limited data)	<i>Salmonella</i> <i>Campylobacter</i>	No effect over washing in water alone discernible	No health concern identified; no benefit identified
		Chiller water, 50 mg/l as chlorine, aimed at 5 mg/l residual			<i>Salmonella</i> <i>Campylobacter</i>	Little to no reduction in numbers, but effective method for preventing cross-contamination of carcasses from chiller water	

Commodity	Chemical	Process parameters	Chemical risk assessment		Microbial risk assessment		Risk–benefit assessment
			Residue(s)	Conclusion	Pathogen(s)	Conclusion	
Poultry (Sequential scenario) ^a	ASC	Pre-chill spray or dip, 500–1200 mg/l, pH 2.5–2.9	Chlorite Chlorate	No health concern identified	<i>Salmonella</i> <i>Campylobacter</i>	Some evidence for prevalence reduction and up to 1.2 log reduction	No health concern identified; benefits identified
	Hypochlorite	Chiller, 20–50 mg/l as chlorine	THMs and other organohalogens expected; data on chloroform only	No health concern identified (limited data)	<i>Salmonella</i> <i>Campylobacter</i>	Little to no reduction in numbers, but effective method for preventing cross-contamination	
	ASC	Post-chill dip, 500–1200 mg/l, pH 2.5–2.9	Chlorite Chlorate	No health concern identified	<i>Salmonella</i> <i>Campylobacter</i>	Some evidence for prevalence reduction and up to 1.2 log reduction	
Poultry (Sequential scenario) ^b	Hypochlorite	Pre-chill rinse, 20 mg/l	THMs and other organohalogens expected; data on chloroform only	No health concern identified (limited data)	<i>Salmonella</i>	No effect over washing in water alone discernible	No health concern identified; no benefit identified
	ASC (Alcide)	Chiller, 1 part Alcide base : 200 parts water : 1 part Alcide activator	Chlorite Chlorate	No health concern identified	<i>Salmonella</i>	Some evidence for prevalence reduction	No health concern identified; benefits identified

Commodity	Chemical	Process parameters	Chemical risk assessment		Microbial risk assessment		Risk-benefit assessment
			Residue(s)	Conclusion	Pathogen(s)	Conclusion	
Poultry	ASC (Alcide)	Pre-chill dip, 1 part Alcide base : 20 parts water : 1 part Alcide activator	Chlorite Chlorate	No health concern identified	<i>Salmonella</i>	Some evidence for prevalence reduction	No health concern identified; benefits identified
Poultry	Chlorine dioxide	Chiller water, 3–5 mg/l as chlorine dioxide	Chlorite Chlorate	No health concern identified	<i>Salmonella</i>	No data on reduction in numbers, but an effective method for preventing cross-contamination of carcasses from chiller water	No health concern identified; benefits identified
Poultry	Peroxyacetic acid	Spray, 200 mg/l	HEDP	No health concern identified	<i>Salmonella</i>	Little to no effect on contamination levels over water washing alone (laboratory inoculation studies only)	No health concern identified; no benefits identified
Red meat	Hypochlorite	Carcass spray, 50–500 mg/l	THMs and other organohalogens expected; data on chloroform only	No health concern identified (limited data)	<i>E. coli</i> O157:H7	Little to no effect compared with water alone	No health concern identified; no benefits identified

Commodity	Chemical	Process parameters	Chemical risk assessment		Microbial risk assessment		Risk-benefit assessment
			Residue(s)	Conclusion	Pathogen(s)	Conclusion	
Red meat	ASC	Dip or spray, 1200 mg/l	Chlorite	No health concern identified	<i>E. coli</i> O157:H7	1.4–1.5 log reduction (laboratory inoculation studies only)	No health concern identified; potential benefits identified (only laboratory-based studies; more data needed)
			Chlorate			1.6–2.1 log reduction (laboratory inoculation studies only)	
Red meat	Lactic acid (as food-grade acid)	Dip, 2–2.5%	Lactate ^c	No health concern identified	<i>E. coli</i> O157:H7	1.3–1.5 log reduction (laboratory inoculation studies only)	No health concern identified; potential benefits identified (only laboratory-based studies; more data needed)
						1.6–2.0 log reduction (laboratory inoculation studies only)	
Shrimp	Hypochlorite	Immersion of shrimp in water containing 50 mg/l (for 30 min in laboratory studies)	THMs and other organohalogens expected; data on chloroform only	No health concern identified (limited data)	<i>V. parahaemolyticus</i>	Up to 1.5 log reduction (laboratory-based natural contamination studies)	No health concern identified; potential benefits identified (only laboratory-based studies; more data needed)
Fish and fishery products	Hypochlorite	Thawing fish in water containing 20–25 mg/l, or washing in water containing 200 mg/l	THMs and other organohalogens expected; data on chloroform only	No health concern identified (limited data)	<i>L. monocytogenes</i>	Up to approximately 2.5 log reduction (laboratory inoculation studies only)	No health concern identified (limited data); potential benefits identified (only laboratory-based studies; more data needed)

Commodity	Chemical	Process parameters	Chemical risk assessment		Microbial risk assessment		Risk-benefit assessment
			Residue(s)	Conclusion	Pathogen(s)	Conclusion	
Fish and fishery products	Chlorine dioxide	Fish storage in ice made with water containing chlorine dioxide at 20–100 mg/l	Chlorite Chlorate	No health concern identified	<i>Salmonella</i> <i>Typhimurium</i> <i>L. monocytogenes</i> <i>E. coli</i> O157:H7	2–3 log reduction (laboratory inoculation study only)	No health concern identified; potential benefits identified (only laboratory-based studies; more data needed)
Leafy greens	Hypochlorite	20–200 mg/l dips and sprays with contact times between 1 and 10 min	THMs and other organohalogen expected	No health concern identified (limited data)	<i>L. monocytogenes</i> <i>Salmonella</i> <i>E. coli</i> O157:H7	0.2–1.7 log reduction (laboratory inoculation study only)	No health concern identified; potential benefits identified (only laboratory-based studies; more data needed)
						0.3–2.0 log reduction (laboratory inoculation study only)	
						0.3–1.7 log reduction (laboratory inoculation study only)	
						0.2–6.0 log reduction (laboratory inoculation study only)	
Leafy greens	Chlorine dioxide	20 mg/l with 10–15 min contact time	Chlorite Chlorate	No health concern identified	<i>E. coli</i> O157:H7	Up to 1 log reduction (laboratory inoculation study only)	No health concern identified; potential benefits identified (only laboratory-based studies; more data needed)
						Up to 0.7 log reduction (laboratory inoculation study only)	

Commodity	Chemical	Process parameters	Chemical risk assessment		Microbial risk assessment		Risk-benefit assessment
			Residue(s)	Conclusion	Pathogen(s)	Conclusion	
Leafy greens	Chlorine dioxide	1–5 mg/l with 10 min contact time	Chlorite	No health concern identified	<i>L. monocytogenes</i>	Up to 1.1 log reduction (laboratory inoculation study only)	No health concern identified; potential benefits identified (only laboratory-based studies; more data needed)
			Chlorate				
Apples	Chlorine dioxide	20 mg/l with 10 min contact time	Chlorite	No health concern identified	<i>E. coli</i> O157:H7	Up to 1.5 log reduction (laboratory inoculation study only)	No health concern identified; potential benefits identified (only laboratory-based studies; more data needed)
			Chlorate				
Green peppers	Chlorine dioxide	0.3–3 mg/l with 10 min contact time	Chlorite	No health concern identified	<i>L. monocytogenes</i>	Up to 2.3 log reduction (laboratory inoculation study only)	No health concern identified; potential benefits identified (only laboratory-based studies; more data needed)
			Chlorate				

Commodity	Chemical	Process parameters	Chemical risk assessment		Microbial risk assessment		Risk-benefit assessment
			Residue(s)	Conclusion	Pathogen(s)	Conclusion	
Fresh produce	Peroxyacetic acid	40–80 mg/l with 10–60 min contact time	HEDP	No health concern identified	<i>E. coli</i> O157:H7	Up to 4.4 log reduction (laboratory inoculation studies, no control for effect of water alone)	No health concern identified; potential benefits identified (only laboratory-based studies; more data needed)
					<i>Salmonella</i>	Up to 3.8 log reduction (laboratory inoculation studies, no control for effect of water alone)	
					<i>L. monocytogenes</i>	Up to 4.5 log reduction (laboratory inoculation studies, no control for effect of water alone)	
Seeds for sprouting	Hypochlorite	20 000 mg/l	THMs and other organohalogens expected	No health concern identified (limited data)		No data identified	No health concern identified; no benefits identified (no data)
						No data identified	
Hydroponics	Hypochlorite	2 mg/l in irrigation water	THMs and other organohalogens expected	No health concern identified (limited data)		No data identified	No health concern identified; no benefits identified (no data)
						No data identified	
Food contact surfaces	Chloramine-T	0.5%	None expected	No health concern identified		No data identified	No health concern identified; no benefits identified (no data)
						No data identified	

Commodity	Chemical	Process parameters	Chemical risk assessment		Microbial risk assessment		Risk-benefit assessment
			Residue(s)	Conclusion	Pathogen(s)	Conclusion	
Food contact surfaces	Dichloroisocyanurate	0.005%	Cyanuric acid	No health concern identified		No data identified	No health concern identified; no benefits identified (no data)
Food contact surfaces	Iodophors	10–20 mg/l on plastic and metal surfaces	Iodine	No health concern identified	<i>L. monocytogenes</i>	0.18–3.21 log reduction (laboratory-grown biofilm studies only)	No health concern identified; potential benefits identified (only laboratory-based studies; more data needed)
Food contact surfaces	Hypochlorite	10–200 mg/l on porous and non-porous hard surfaces	THMs possible	No health concern identified (limited data)	<i>Salmonella</i>	0.75–7.5 log reduction (laboratory-grown biofilm studies only); 1 log reduction on biofilm in industrial setting	No health concern identified; potential benefits identified
					<i>L. monocytogenes</i>	0.75–5.7 log reduction (laboratory-grown biofilm studies only)	

ASC, acidified sodium chlorite; HEDP, 1-hydroxyethylidene-1,1-diphosphonic acid; THMs, trihalomethanes

^a Indicates that this is part of a series of processing steps used when chicken is water chilled. These steps are designed primarily to chill the carcass and not as a decontamination step; however, the use of chlorine during this step can be used as a risk mitigation method.

^b Indicates that this is a sequence of steps tested in studies that included a pre-rinse and the addition of Alcide (ASC) in the chill tanks.

^c Lactate is a natural constituent of food and the human body, so the expert meeting did not consider a separate risk assessment to be necessary.

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7. CONCLUSIONS AND RECOMMENDATIONS

The expert meeting's conclusions, key sources of uncertainty and recommendations for further scientific studies to fill gaps in knowledge and for the development of standardized practice are provided below, by chapter.

7.1 Description of current processes

- Poultry and fresh produce are the food products that have the most direct exposure to chlorine-containing disinfectants. The use of chlorine-based compounds in the fish and fishery product industry is mainly focused on the end-point disinfection of contact surfaces, and direct application to the edible portions of fish and shellfish is limited. The use of chlorine-containing disinfectants in red meat processing is uncommon.
- Sodium hypochlorite is the most widely used disinfectant, in particular in the production and processing of poultry meat, fresh produce (such as leafy greens), fish and fishery products, sprouts and hydroponics.
- Acidified sodium chlorite (ASC) solutions are commonly used as an alternative to sodium hypochlorite in specific poultry processing steps.
- Non-chlorine-based alternatives include (in addition to physical treatments, which were not considered) peroxyacids in poultry production and organic acids in meat production. These alternatives are effective disinfectants in some use scenarios.
- Active chlorine compounds are broadly used in food processing facilities to disinfect food contact surfaces prior to and during food processing operations in order to control cross-contamination and to obtain pathogen reduction. Requirements related to completing the cleaning and sanitization cycle with a potable water rinse vary globally from region to region and from country to country.
- The application of chlorine directly to food products to reduce virus levels has not been reported to date.

Recommendations

- Disinfectant treatment of water used in food processing must not be used to mask poor hygienic practices. It is recommended that disinfectants be used within the framework of good hygienic practices and a hazard analysis and critical control point (HACCP) system where applicable and subject to adequate process controls.

7.2 Chemistry of compounds used

- Chlorine (hypochlorite and hypochlorous acid) and chloramines, to a lesser degree, produce small quantities of oxidized and chlorinated disinfection by-products (DBPs); other disinfectants produce more oxidized products and lesser quantities of chlorinated by-products.
- There are limited data on the types and quantities of DBPs present as food residues after disinfection. Although most of the reported data on organic DBP formation on foods

involved chloroform measurements only, it can be assumed that if chloroform was detected, other trihalomethanes (THMs) and other DBPs were also formed.

- Substantial data are available on DBPs in drinking-water, but such data have limited applicability to scenarios of disinfection processes in food production and processing. Extrapolations from chloroform, other THMs and other DBPs in drinking-water to foods are difficult to make because the conditions of the chemical interactions, dosages, contact times, temperature and precursors are different.
- In addition, the chemical composition of food is more complex than that of water, and the contact/exposure conditions for disinfectants used in food processing are different. This may lead to the formation of different types and quantities of DBPs in treated foods compared with water.
- Cooking is likely to reduce the quantities of volatile compounds, such as chloroform, in foods.
- Nitrosamines are not likely to be present in most disinfected water used for food processing. If present, the quantities would be very small, especially in relation to the amount of nitrosamines commonly found in foods and produced by cooking.
- Under some oxidation conditions, bromide can be converted to hypobromous acid, which would shift the composition of DBPs to organobromine compounds. Chlorination of seawater or any water that contains bromide may lead to the formation of organobromine compounds; ozonation would also produce bromate.

Recommendations

- More research is needed on the formation, identity and amounts of DBPs in foods at consumption, reflecting the effects of processing, cooking, storage and other factors. Such studies should be interpreted in conjunction with the microbiological risk and shelf life benefits of the use of disinfectants.
- The formation of organobromines and bromate as a result of water chlorination should be studied further (e.g. in saltwater fish and shrimp processing).

7.3 Chemical risk assessment

- There is a lack of data on the by-products present in foods or in processing water following the use of chlorine-containing disinfectants. There is therefore a high degree of uncertainty in the dietary exposure assessments, although conservative assumptions were generally applied to compensate for this. Data on by-products were available for drinking-water, although these data have limited applicability to food.
- No epidemiological studies on the health effects of exposure to disinfectants and DBPs in food have been identified. The evidence from studies of drinking-water suggests an association between DBPs and increased risk of bladder cancer; however, the relationship between DBPs in drinking-water and those in food is not known.
- The toxicology of chlorine-containing compounds and alternatives has been extensively reviewed based on currently available risk assessments. For the identified residues of disinfectants and by-products, the estimated exposures did not raise toxicological concerns. The evidence with respect to hypochlorite use in poultry, fish and shellfish was weak, owing to a lack of qualitative and quantitative information on THMs.

Key sources of uncertainties

- Very limited data were available on the use of some of the substances (i.e. on which food commodities they were used, at which doses, etc.).
- Very few data were available for a number of DBPs in food, other than drinking-water.
- The authoritative international assessments used for chemical risk assessment and dietary exposure assessment may be some years old and therefore not always up to date.
- THMs, some of which are genotoxic and carcinogenic, are expected to result from hypochlorite use. However, data are available only for chloroform, which indicates the presence of other THMs, or are completely lacking.
- The concentrations of some DBPs could be decreased by volatilization during cooking or by degradation in the saliva or stomach, but quantitative data on such effects are lacking.

Recommendations

- Further research is needed on the toxicological effects of DBPs formed in water and in food.
- Studies of disinfectant residues and DBPs are needed, particularly for foods that might have substantial residues present when consumed.

7.4 Microbiological risk assessment

- Cross-contamination is a complex process that is difficult to quantify in experimental and industrial settings. It is difficult to quantify the effects of cross-contamination on pathogen numbers on food, but the use of disinfectants in food processing is important to prevent cross-contamination and thereby reduce consumer exposure to pathogens.
- Data on the quantitative effects of disinfectants on food pathogens are available based on studies in industrial, pilot and laboratory settings. These data are not always equivalent. It is considered that experimental studies using inoculated pathogens on food products may overestimate the effect of the disinfectant chemical on pathogens. However, this may not be the case when studying disinfectant use in wash or flume waters.
- ASC as a pre-chill and post-chill dip/wash is an effective disinfectant for reducing pathogens in poultry processing.
- In order to translate the impact of pathogen reductions into public health benefits, a risk assessment model is required. The Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) have a model available for *Campylobacter* in chicken. This model was used to test various scenarios, resulting in estimates of up to 70% reduction in campylobacteriosis risk from the use of ASC as a decontamination spray and up to approximately 97% reduction in campylobacteriosis risk from the use of a combination of chlorine in the immersion chill tank and an ASC decontamination spray.
- Laboratory studies have demonstrated that biofilms containing *Salmonella* spp. and *L. monocytogenes* can be inactivated by a range of disinfectants at suitable concentrations with appropriate contact times. The effectiveness of disinfectants against *Escherichia coli* O157, *Salmonella* Typhimurium and *Clostridium perfringens* on cutting tools has also been demonstrated.
- Assessment of the effectiveness of disinfectants based on studies in industrial settings is difficult. This is because the microflora, including pathogens, in the process environment

is already being controlled by the ongoing use of disinfectants. Hence, attempting to measure the effectiveness at individual steps does not accurately reflect what would happen if no disinfectants had been used in the process prior to the study. The end result is that assessments of the effectiveness of disinfectants based on studies in industrial settings are likely to underestimate the incremental effectiveness of the individual control steps.

Key sources of uncertainties

- There is a lack of data on industrial-scale processes and uncertainty associated with using only experimental data.
- Data from studies in which food is inoculated with a pathogen rather than being naturally contaminated tend to overestimate the efficacy of a disinfectant.
- There are inconsistencies between individual studies, and data are often variable within studies.
- It is difficult to quantify the effects of cross-contamination on pathogen numbers on food.
- Translation of pathogen reduction information into public health outcomes requires the use of quantitative microbial risk assessment models, which ideally should be done on a national level. These models are not always available or suitable. However, the public health impact on a relative basis can be achieved using international models (e.g. the FAO/WHO *Campylobacter* in poultry model).
- Data are available on individual disinfection steps in a process, but data are often lacking on the combined disinfection effects of serial or sequential control strategies.

Recommendations

- There is a need to develop more standardized protocols for studies of microbial reduction in the main food processing scenarios outlined in the report to address the problems of comparability of the results.

7.5 Unintended consequences

- There are no published reports indicating that the use of active chlorine or currently used alternatives to active chlorine are associated with acquired antimicrobial resistance to therapeutic agents. Chlorine and non-chlorine alternatives, including peroxyacids, ozone and other oxidants, as well as surfactants, including trisodium phosphate (TSP) and quaternary ammonium compounds (QACs), have nonspecific modes of action for which microorganisms may develop tolerance. However, this potential tolerance has not been associated with acquired antimicrobial resistance or the failure of biocides to be effective when used as recommended.
- Treatment of fresh fruits and vegetables and poultry carcasses with active chlorine can reduce the normal microflora of the produce. Currently available data indicate that such reduction in normal microflora does not result in increased survival or growth of pathogenic microorganisms.
- The nutrient contents (some vitamins and antioxidants) of foods may be affected by treatments with disinfectants, even though the effects are variable and sometimes contradictory. The effects depend on the type of food and mode of preparation, the type of disinfectant and conditions of use, and further processing (washing, type of packaging

and conditions of storage). The reported changes in nutrient content due to disinfectant use are low in relation to the normal dietary intake of these nutrients.

- The effect of disinfectant use on the sensory quality of foods is expected to be low when the disinfectant is used as recommended. Some studies show that ASC and ozone treatments appear to keep and even improve the sensory quality during storage of fruits and vegetables, whereas chlorine dioxide and peroxyacids appear to be ineffective in preventing, or even promote, the brown discoloration caused by phenolic oxidation.

Recommendations

- In view of the many variables involved in determining the effects of disinfection treatments on nutrient content and sensory quality of food, recommendations for best practices can be given only on a case-by-case basis.

7.6 Risk–benefit assessment

- Risk–benefit assessment is an activity that weighs the probability and severity of harm in a particular exposure scenario against the probability and magnitude of benefit. The expert meeting assessed the risks associated with exposure to the residues arising from the predominant application practices used in food production and processing and the benefits from pathogen reduction in the food.
- In principle, the results of risk–benefit assessments could be in four possible categories:
 - 1) No health concern identified; no benefits identified
 - 2) No health concern identified; benefits identified
 - 3) Health concern identified; no benefits identified
 - 4) Health concern identified; benefits identified

Only use scenarios resulting in category 4 (i.e. there are both health concerns and benefits) would need further evaluation and weighing of the risks and benefits.

- Based on the available data, no health concerns were identified from an evaluation of the toxicity and dietary exposure. This applies to both the disinfectant residues and, where data were available, the by-product residues. However, as discussed in chapters 2 and 3, there is greater uncertainty with respect to the use of hypochlorite than to the use of other chlorine and alternative disinfectants, owing to the potential formation of DBPs that are genotoxic and carcinogenic.
- There is evidence for reduction of pathogens on poultry carcasses and red meats by application of ASC and chlorine dioxide and by application of sodium hypochlorite in smoked fish production. There is some evidence for reduction of cross-contamination by the application of disinfectants (in particular sodium hypochlorite) in wash and flume waters.

ANNEX 1: LIST OF PARTICIPANTS

Joint FAO/WHO expert meeting on the benefits and risks of the use of chlorine-containing disinfectants in food production and food processing

Ann Arbor, Michigan, USA, 27–30 May 2008

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ANNEX 3: LIST OF ACRONYMS AND ABBREVIATIONS

ADI	acceptable daily intake
ASC	acidified sodium chlorite
BCAN	bromochloroacetonitrile
BCC	basal cell carcinoma
BDCM	bromodichloromethane
BEMX-1	(<i>E</i>)-2-chloro-3-(bromochloromethyl)-4-oxobutenoic acid
BEMX-2	(<i>E</i>)-2-chloro-3-(dibromomethyl)-4-oxobutenoic acid
BEMX-3	(<i>E</i>)-2-bromo-3-(dibromomethyl)-4-oxobutenoic acid
BMD	benchmark dose
BMD ₁₀	benchmark dose for a 10% increase in effect
BMDL ₁₀	95% lower confidence limit on the benchmark dose for a 10% increase in effect
BMX-1	3-chloro-4-(bromochloromethyl)-5-hydroxy-2(5H)-furanone
BMX-2	3-chloro-4-(dibromomethyl)-5-hydroxy-2(5H)-furanone
BMX-3	3-bromo-4-(dibromomethyl)-5-hydroxy-2(5H)-furanone
bw	body weight
CAS	Chemical Abstracts Service
cfu	colony-forming unit
CHO	Chinese hamster ovary
CI	confidence interval
CNS	central nervous system
CPC	cetylpyridinium chloride
CSFII	Continuing Survey of Food Intakes by Individuals (USA)
CYP	cytochrome P450
DALY	disability-adjusted life year
DBA	dibromoacetic acid
DBAN	dibromoacetonitrile
DBCM	dibromochloromethane
DBDMH	1,3-dibromo-5,5-dimethylhydantoin
DBP	disinfection by-product
DCA	dichloroacetic acid
DCAN	dichloroacetonitrile
DMH	dimethylhydantoin
DNA	deoxyribonucleic acid
EFSA	European Food Safety Authority
EMA	European Medicines Agency
EMX	(<i>E</i>)-2-chloro-3-(dichloromethyl)-4-oxobutenoic acid
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
GC/MS	gas chromatography/mass spectrometry
GDWQ	Guidelines for Drinking-water Quality (WHO)
GEMS/Food	Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme
GLP	Good Laboratory Practice
GRAS	generally recognized as safe
GST	glutathione <i>S</i> -transferase

GV	guideline value
HAA	haloacetic acid
HACCP	hazard analysis and critical control point
HAN	haloacetonitrile
HEDP	1-hydroxyethylidene-1,1-diphosphonic acid
HP	hydrogen peroxide
IARC	International Agency for Research on Cancer
IOBW	inside–outside bird washer
IPCS	International Programme on Chemical Safety (WHO)
IRIS	Integrated Risk Information System
IUGR	intrauterine growth retardation (restriction)
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
LAE	ethyl lauroyl arginate
LBW	low birth weight
LO(A)EL	lowest-observed-(adverse-)effect level
LOD	limit of detection
MAP	modified atmosphere packaging
MCL	maximum contaminant level (USA)
MTDI	maximum tolerable daily intake
MX	3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone
NA	not available
NaDCC	sodium dichloroisocyanurate
nd	not detected
NDEA	<i>N</i> -nitrosodiethylamine
NDELA	<i>N</i> -nitrosodiethanolamine
NDMA	<i>N</i> -nitrodimethylamine
NDPA	<i>N</i> -nitrosodiphenylamine
NMOR	<i>N</i> -nitrosomorpholine
NO(A)EL	no-observed-(adverse-)effect level
NOM	natural organic matter
NPIP	<i>N</i> -nitrosopiperidine
NPRO	<i>N</i> -nitrosoproline
NPYR	<i>N</i> -nitrosopyrrolidine
NTD	neural tube defect
NTP	National Toxicology Program (USA)
OECD	Organisation for Economic Co-operation and Development
OR	odds ratio
ox-EMX	(<i>E</i>)-2-chloro-3-(dichloromethyl)-butenedioic acid
ox-MX	2-chloro-3-(dichloromethyl)-butenedioic acid
PMTDI	provisional maximum tolerable daily intake
POA	peroxyacetic acid/hydrogen peroxide
PTSA	<i>p</i> -toluenesulfonamide
QAC	quaternary ammonium compound
red-MX	3-chloro-4-(dichloromethyl)-2(5H)-furanone
SCC	squamous cell carcinoma
SCF	the former Scientific Committee for Food in the European Union
SD	standard deviation
SGA	small for gestational age
SI	Système international d'unités
spp.	species

T ₃	triiodothyronine
T ₄	thyroxine
TBARS	2-thiobarbituric acid reactive substances
TCA	trichloroacetic acid
TCAN	trichloroacetonitrile
TDI	tolerable daily intake
THM	trihalomethane
TSH	thyroid stimulating hormone
TSP	trisodium phosphate
TTHM	total trihalomethanes
USA	United States of America
USDA	United States Department of Agriculture
USEPA	United States Environmental Protection Agency
USFDA	United States Food and Drug Administration
UV	ultraviolet
VLBW	very low birth weight
VTEC	verotoxigenic <i>Escherichia coli</i>
v/v	volume by volume
WHO	World Health Organization
ZMX	(Z)-2-chloro-3-(dichloromethyl)-4-oxobutenoic acid

ANNEX 4: GLOSSARY

Acceptable daily intake (ADI): An estimate of the amount of a substance in food or drinking-water, expressed on a body weight basis (usually milligrams per kilogram body weight), that can be ingested daily over a lifetime by humans without appreciable health risks.

Acceptable daily intake “not limited”: A term no longer used by the Joint FAO/WHO Expert Committee on Food Additives, which has the same meaning as acceptable daily intake “not specified”.

Acceptable daily intake “not specified”: A term applicable to a food substance of very low toxicity that, on the basis of the available chemical, biochemical and toxicological data as well as the total dietary intake of the substance, does not, in the opinion of the Joint FAO/WHO Expert Committee on Food Additives, represent a hazard to health. For that reason, the establishment of an acceptable daily intake expressed in numerical form is not deemed necessary.

Acquired resistance: Resistance to an antimicrobial treatment that is passed on to progeny.

Active chlorine: Chlorine in a form that is readily available for chemical reaction with microorganisms.

Antimicrobial: A disinfectant; an agent that kills or inactivates microorganisms.

Aquaculture: The farming during part or the whole of their life cycle of all aquatic animals, except mammalian species, aquatic reptiles and amphibians, intended for human consumption.

Bacteriocin: A peptide or small protein produced by bacteria that inhibits the growth of closely related strains or species.

Benefit assessment: An activity that estimates the probability and magnitude of benefit in a particular exposure scenario as a basis for risk management decisions and communication to the public.

Biocide: An active substance that inactivates microorganisms on animate or inanimate surfaces or in foods.

Biofilm: Microbial growth as a thin layer on a surface, including associated extracellular products.

By-product: A secondary or incidental product deriving from a manufacturing process, a chemical reaction or a biochemical pathway, not the primary product or service being produced. *See also* Disinfection by-products.

Chiller: A tank or vat containing cooled water or slush ice, used for cooling (e.g. poultry carcasses) in the food industry; sometimes used in series, with the first tank used for prechilling with tap water.

Chlorine alternative: A treatment or substance that replaces the use of chlorine-based compounds in a specified process by accomplishing the same functions without generating active chlorine compounds.

Colony-forming unit: A measure of viable cells in which a colony represents an aggregate of cells derived from a single progenitor cell.

Colour parameters (L, a and b): Descriptors of a globally recognized colour system, in which L represents lightness and a and b are colour space coordinates. They provide a standard, approximately uniform colour scale (known as the CIELAB colour scale) so that colours can be easily compared.

Cross-contamination: The transfer of microorganisms from an individual food item (animal carcass, single fish, whole fruit or vegetable, or single cut piece of these items) to another individual food item through air, water, handlers, contact with equipment surfaces or direct contact between individual items. This may occur between units within a batch or between batches.

D-value: A measure of the amount of time needed to provide a 1 log reduction in the number of microorganisms. A D-value of 73 min means that it would take 73 min to produce a 1 log reduction.

D₁₀ value: The radiation dose needed to inactivate 1 log of a target microorganism (measured in kilograys).

DALY: A time-based measure (disability-adjusted life year) that combines years of life lost due to premature mortality and years of life lost due to time lived in states of less than full health.

Depuration: A short-term process commonly used to reduce low levels of bacterial contamination in filter-feeding shellfish. Long-term relaying is required if there is the risk of high levels of contamination.

Disability-adjusted life year: *See* DALY.

Disinfectant: A substance used in aqueous solutions in food production and processing to eliminate or reduce the number of microorganisms on the food in washing, chilling and other processes. In some countries, a distinction is made between disinfection and sanitization, but for the purpose of this document, no such distinction is made.

Disinfection: The reduction by means of chemical agents and/or physical methods of the number of microorganisms in the environment to a level that does not compromise food safety or suitability.

Disinfection by-products (DBPs): Chemical compounds formed during disinfection processes, other than the original substances introduced in the aqueous solution used for disinfection.

End-point disinfection: The final treatment of a food product with disinfectant solution before retail distribution or the disinfection of a food contact surface immediately before use.

Flume: An elevated trough or pipe filled with wash water that keeps the product immersed for a certain minimum time as required by the treatment.

Further processed: A meat or poultry product that has undergone further processing, such as smoking, cooking or curing.

GEMS/Food consumption cluster diets: Per capita consumption of raw and semiprocessed agricultural commodities expressed in grams per person per day for distinct groups of the world's population that share similar dietary patterns. Based on food balance sheet data from the Food and Agriculture Organization of the United Nations, the diets were generated using a cluster analysis, which assigned countries to one of the 13 cluster diets.

Generally recognized as safe (GRAS): A designation used by the United States Food and Drug Administration, stating that a chemical or substance added to food is considered safe by experts and so is exempted from the usual Federal Food, Drug, and Cosmetic Act (i.e. the law in the USA that authorizes the United States Environmental Protection Agency to oversee the safety of foods, drugs and cosmetics) food additive tolerance requirements.

Hazard characterization: The qualitative and, wherever possible, quantitative description of the inherent properties of an agent or situation having the potential to cause adverse effects. This should, where possible, include a dose-response assessment and its attendant uncertainties.

Hazard identification: The identification of the type and nature of adverse effects that an agent has an inherent capacity to cause in an organism, system or (sub)population.

Infective dose: That amount of pathogenic organisms that will cause infection in susceptible subjects.

Iodophor: A mixture of iodine and surface-active agents that act as carriers and solubilizers for the iodine.

Log unit: "Log" stands for logarithm, which is the exponent of 10. For example, log 2 represents 10^2 or 10×10 or 100.

Log reduction: Log reduction stands for a 10-fold or one decimal or 90% reduction in numbers of recoverable bacteria in a test food vehicle. For example, a 1 log reduction would reduce the number of bacteria by 90%. This means, for example, that 100 bacteria would be reduced to 10 or 10 reduced to 1.

Lowest-observed-(adverse)-effect level (LO(A)EL): Lowest concentration or amount of a substance, found by experiment or observation, that causes an (adverse) alteration of morphology, functional capacity, growth, development or lifespan of the target organism distinguishable from normal (control) organisms of the same species and strain under the same defined conditions of exposure.

Margin of exposure: The ratio of the no-observed-adverse-effect level (NOAEL) or benchmark dose lower confidence limit for the critical effect to the theoretical, predicted or estimated exposure dose or concentration.

Margin of safety: The margin between the health-based guidance value (e.g. acceptable daily intake, tolerable daily intake) and the actual or estimated exposure dose or concentration. For some experts, the margin of safety has the same meaning as the margin of exposure.

Maximum tolerable daily intake (MTDI): *See* Provisional maximum tolerable daily intake (PMTDI).

Maximum tolerated dose (MTD): A high dose used in chronic toxicity testing that is expected, on the basis of an adequate subchronic study, to produce limited toxicity when administered for the duration of the test period.

Modified atmosphere packaging: A packaging technology for increasing shelf life in which the internal atmosphere is modified by reducing oxygen and replacing it with either carbon dioxide or nitrogen gas.

No-observed-(adverse)-effect level (NO(A)EL): Greatest concentration or amount of a substance, found by experiment or observation, that causes no detectable (adverse) alteration of morphology, functional capacity, growth, development or lifespan of the target organism under defined conditions of exposure.

Potable water: Drinking-water of sufficiently high quality that it can be consumed or used without risk of immediate or long-term harm.

Provisional maximum tolerable daily intake (PMTDI): The reference value, established by the Joint FAO/WHO Expert Committee on Food Additives, used to indicate the safe level of intake of a contaminant with no cumulative properties. Its value represents permissible human exposure as a result of the natural occurrence of the substance in food and drinking-water. In the case of trace elements that are both essential nutrients and unavoidable constituents of food, a range is expressed, the lower value representing the level of essentiality and the upper value the PMTDI. The tolerable intake is generally referred to as “provisional”, as there is often a paucity of data on the consequences of human exposure at low levels, and new data may result in a change to the tolerable level.

Residue: Chemicals that remain in or on food after, for example, disinfection, pesticide application, etc.

Resistance: An increased, genetic-based ability of a microorganism to survive a recommended usage level of an antimicrobial compound, resulting in a high

likelihood of treatment failure. This is similar to the definition of “clinical resistance” used by the European Food Safety Authority.

Risk assessment: A process intended to calculate or estimate the risk to a given target organism, system or (sub)population, including the identification of attendant uncertainties, following exposure to a particular agent, taking into account the inherent characteristics of the agent of concern as well as the characteristics of the specific target system. The risk assessment process includes four steps: hazard identification, hazard characterization, exposure assessment and risk characterization.

Risk–benefit assessment: An activity that weighs the probability and severity of harm in a particular exposure scenario against the probability and magnitude of benefit as a basis for risk management decisions and communication to the public.

Risk characterization: The qualitative and, wherever possible, quantitative determination, including attendant uncertainties, of the probability of occurrence of known and potential adverse effects of an agent in a given organism, system or (sub)population, under defined exposure conditions.

Spoilage microorganism: Microorganisms that cause undesirable changes to the colour, odour, taste and texture of food.

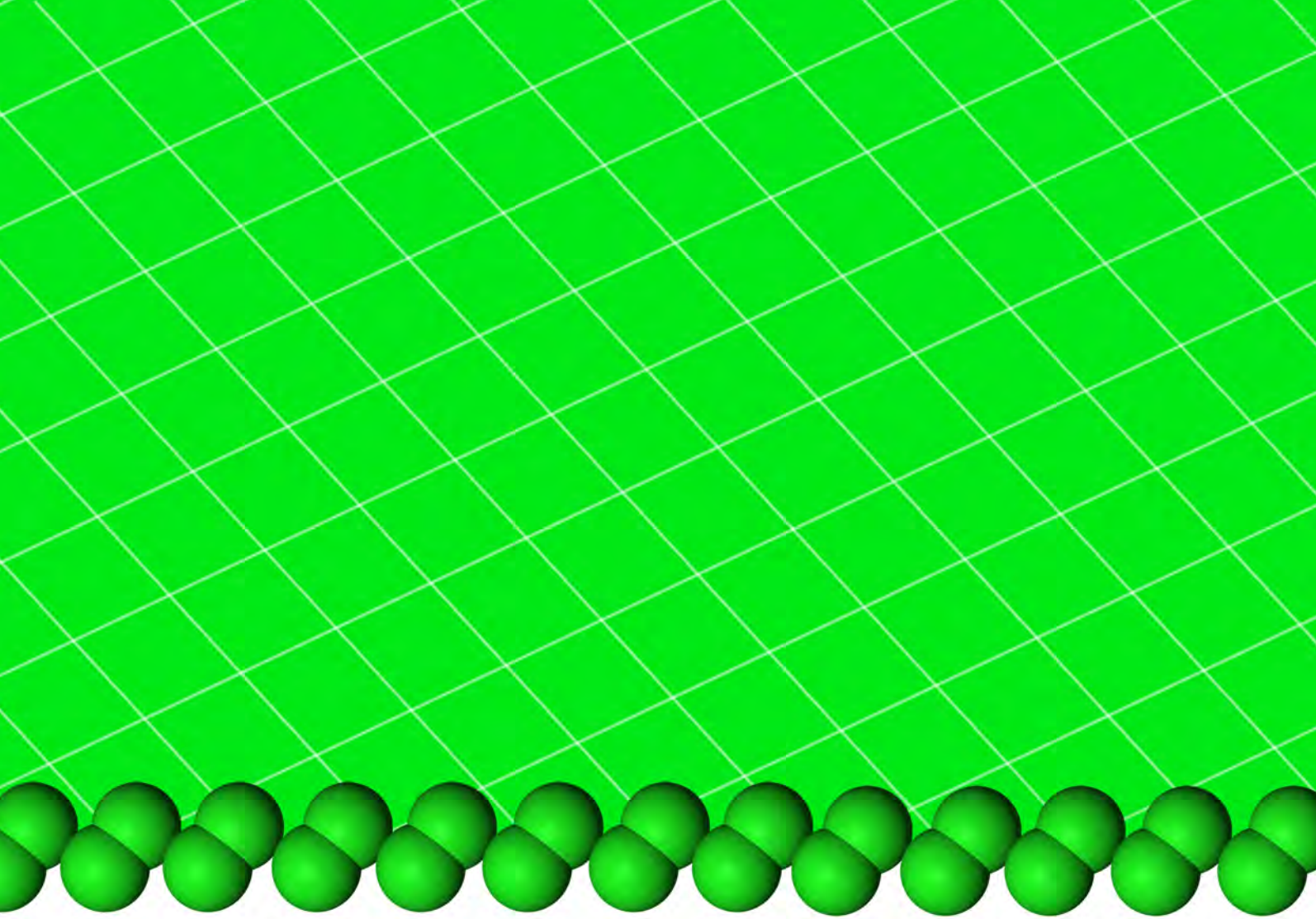
Superchlorination: Use of high chlorine dosage to ensure sufficient free chlorine residual to inactivate harmful microorganisms.

Target microorganism: A microbial species, genus or group for which lack of control during a specified process could result in adverse public health consequences.

2-Thiobarbituric acid reactive substances (TBARS): Biological specimens contain a mixture of TBARS, including lipid hydroperoxides and aldehydes, which increase as a result of oxidative stress. Plasma concentrations of TBARS are an index of lipid peroxidation and oxidative stress.

Tolerable daily intake (TDI): Analogous to acceptable daily intake (an estimate of the amount of a contaminant in food or drinking-water, expressed on a body weight basis, which can be ingested daily over a lifetime by humans without appreciable health risks). The term tolerable is used for agents that are not deliberately added, such as contaminants in food.

Tolerance: Reduced susceptibility of a microorganism to an antimicrobial treatment, usually determined as an increase in the minimum inhibitory concentration or minimum bactericidal concentration, that does not result in treatment failure, if the treatment is applied as recommended.



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