CHLORINATED COMPOUNDS FORMED DURING CHLORINE WASH OF CHICKEN MEAT

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by

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CONTENTS

LIST OF TABLES

SUMMARY

Chlorine has a long history of use for the microbial disinfection of potable waters and use in water for food processing. However, in addition to its biocidal activity, chlorine is known to form disinfection by-products (DBPs) of public health concern during the chlorination process.

The current report aims to determine:

- Chlorinated compounds that may be formed during the chlorine washing of chicken meat;
- If any of these are likely to present any public health risk; and
- Appropriate risk management options that can be implemented to protect public health.

Considering the importance of chlorinated disinfectants to the food industry and the relatively long history of use, there is relatively little information available on the formation of potentially toxic chlorinated compounds due to the reaction of disinfectants with food components.

While there is some evidence for formation of chlorinated compounds in chicken treated with aqueous chlorine, oxidation reactions appear to predominate for chlorine dioxide and acidified sodium chlorite. Studies on ASC treatment of poultry carcasses indicated no significant treatment-related changes in fatty acid and amino acid composition of carcasses. While no equivalent information is available for chlorine dioxide, it is a less potent oxidising agent than ASC and the lack of significant changes in carcass composition due to ASC treatment suggest that chlorine dioxide treatment is similarly unlikely to affect carcass composition.

There is little information on the identity of chlorinated compounds formed from aqueous chlorine treatment of chicken. Formation of semicarbazide, a chemical belonging to a family of chemicals (hydrazines) known to cause cancer in animals, has been demonstrated following exposure of chicken flesh to aqueous chlorine. However, semicarbazide formation only occurred at chlorine concentrations well in excess of those used in the poultry industry.

Chloroform has been detected in chicken treated with aqueous chlorine. It is probable that this was due to absorption of chloroform from chiller water, rather than direct formation in the chicken flesh. Exposure modelling suggests that the levels of chloroform observed would result in exposure to chloroform at levels well below the tolerable daily intake (TDI) for this compound. While other halogenated disinfectant byproducts (bromodichloromethane, dibromochloromethane, bromoform) could potentially be absorbed by chicken carcasses from chiller or dip water, studies of absorption of these compounds have not been reported in the literature. Based on monitoring results, the composition of New Zealand potable water generally does not favour production of these brominated compounds. Water used for poultry processing in New Zealand must be of a potable standard.

Exposure modelling of potential chlorite and chlorate residues in chicken, due to use of chlorine dioxide or acidified sodium chlorite, indicates that exposure would be well below TDIs, even at the 95% level of exposure. This is consistent with conclusions from a European assessment.

In conclusion, no safety issues were identified due to the use of chlorine dioxide and ASC for poultry carcass disinfection. Chlorination reactions appear to be insignificant for these compounds and oxidation reactions do not appear to result in significant alteration of the fatty acid and amino acid composition of poultry carcasses.

Aqueous chlorine-based disinfection is widely used throughout the food processing industry, and direct disinfection of food with hypochlorite is particularly common in the produce sector. Chlorine has been reported to exert its microbial disinfection activity by both chemical (oxidation, enzyme inhibition) and physical (disruption of cell walls) mechanisms and it is to be expected that there will be some reactions between components of the disinfection system and food components. However, the information collected by this review indicates that, at least for carcinogens and mutagens, the concentrations of chlorine required to induce formation are well in excess of those used in practice.

During 2007-2008, FAO and WHO have considered the issue of chlorine usage in food processing, including an expert meeting on the associated risks and benefits. The meeting was held during May 2008 and the report from the meeting is not yet available. Unless the consultation identifies new areas of concern, we consider that there is insufficient evidence to justify further investigation of the risks to human health from the use of these disinfection chemicals in poultry processing.

1 INTRODUCTION

Internationally, poultry has been consistently associated with foodborne disease due to *Salmonella* and *Campylobacter*. Surveillance carried out in New Zealand has detected both of these pathogens on retail poultry (Wong *et al.*, 2007a; Wong *et al.*, 2007b) and poultry meat is now recognised as a primary source of *Campylobacter* in New Zealand (http://www.nzfsa.govt.nz/publications/news-current-issues/campy-main-web-page.htm).

The New Zealand Food Safety Authority (NZFSA) has developed a risk management strategy for *Campylobacter* in poultry, which includes consideration of potential controls at a number of points along the farm to fork continuum (http://www.nzfsa.govt.nz/consumers/food-safety-topics/foodborne-illnesses/campylobacter/strategy/index.htm). One potential control is the application of sprays or washes containing antimicrobial agents, during poultry processing.

A number of antimicrobial agents have been considered in this context, including (Scientific Committee on Veterinary Measures Relating to Public Health, 2003):

- Chlorine dioxide
- Acidified sodium chlorite
- Chlorine
- Trisodium phosphate
- Peroxyacids
- Organic acids
- Ozone
- Cetylpyridinium chloride

The first three of these (chlorine dioxide, acidified sodium chlorite and chlorine) are classed as chlorinated antimicrobials or chlorinated disinfectants.

Chlorine has a long history of use for the microbial disinfection of potable waters (International Programme on Chemical Safety, 2000). However, in addition to its biocidal activity, chlorine is known to form disinfection by-products (DBPs) of public health concern during the chlorination process.

The current report aims to determine:

- Chlorinated compounds that may be formed during the washing of chicken meat with chlorinated disinfectants;
- If any of these are likely to present any public health risk; and
- Appropriate risk management options that can be implemented to protect public health.

The current report only considers chlorinated compounds formed or potentially formed in chicken carcasses and not compounds formed in chlorinated chiller or wash water. Compounds formed in chlorinated water will be discussed where there is specific evidence of absorption into chicken carcasses.

2 CHLORINATED ANTIMICROBIALS USED FOR DISINFECTION OF CHICKEN CARCASSES

Three main types of chlorinated antimicrobials have been used internationally for the disinfection of poultry carcasses; aqueous chlorine, chlorine dioxide and acidified sodium chlorite. While other chlorinated disinfectants exist (sodium dichloroisocyanurate, monochloramine), these three are currently permitted as bleaching agents, washing and peeling agents, under the Australia New Zealand Food Standards Code and have a history of use in the poultry industry worldwide.

While all three species have some potential to initiate oxidation and chlorination reactions, their antimicrobial activity is mainly related to their oxidative potential.

2.1 Aqueous Chlorine

Chlorine has been used for many years by the food industry as the main sanitising and disinfection agent. In the US, chlorine and hypochlorites have GRAS (generally recognised as safe) status for specified food processing uses and for use in the preparation of bottled water (Fukayama *et al.*, 1986).

Chlorine is produced as a diatomic gas (Cl_2). Chlorine gas hydrolyses rapidly and almost completely in water to form hypochlorous acid (HOCl) which dissociates to form the hypochlorite ion (OCl^-) (Fukayama *et al.*, 1986):

$$Cl_2 + H_2O \rightleftharpoons HOCl + HCl$$
$$HOCL \rightleftharpoons OCl^- + H^+$$

Chlorine, hypochlorous acid and the hypochlorite ion are all present in equilibrium in an aqueous solution, with their relative proportions dependent on the pH of the solution. Hypochlorous acid is the dominant species at neutral pH, while the hypochlorite ion dominates at alkaline pH (above approximately pH 7.7 at temperatures above about 10ºC). While all three species will readily oxidise organic matter, hypochlorous acid has the highest redox potential (+1.49 V), followed by chlorine (+1.36 V) and the hypochlorite ion (+0.90 V) (Fukayama *et al.*, 1986). Hypochlorous acid is the main antimicrobial agent in aqueous chlorine.

Aqueous chlorine has been reported to have three major disadvantages for use as a decontaminant in the poultry industry (Kemp *et al.*, 2000):

- Its relative ineffectiveness against *Salmonella*;
- Its ability to act as a corrosive on plant machinery; and
- Its ability to combine with organic matter to generate mutagenic substances (Haddon *et al.*, 1996; Masri, 1986; Tsai *et al.*, 1997).

2.2 Chlorine Dioxide

Chlorine dioxide is a polar gas that is highly soluble in water, but does not react with water (Fukayama *et al.*, 1986). While the redox potential of chlorine dioxide (+0.95 V) is lower than hypochlorous acid or chlorine, its oxidation capacity is greater as its primary reduction product, the chlorite ion, is also an oxidising agent with a redox potential of +0.78 V:

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

The antimicrobial activity of chlorine dioxide is virtually independent of pH. Chlorine dioxide is primarily an oxidising agent. Chlorine dioxide may chlorinate a wide range of organic compounds, however, the rates of these reactions are much lower than for reactions with hypochlorous acid (Fukayama *et al.*, 1986). Chlorination due to chlorine dioxide should be considered to be a minor reaction pathway.

2.3 Acidified Sodium Chlorite (ASC)

Acidified sodium chlorite (ASC) is produced by the addition of food-grade acid (citric, phosphoric, hydrochloric, malic or sodium hydrogen sulphate) to an aqueous solution of sodium chlorite. Under these circumstances chlorite is converted to chlorous acid, which can subsequently form a mixture of chlorite, chlorate, chlorine dioxide and chloride (Scientific Committee on Veterinary Measures Relating to Public Health, 2003):

$$NaClO_2 \rightarrow ClO_2^- + Na^+ \quad \text{(Chlorite ion)}$$
$$ClO_2^- + H^+ \square HClO_2 \quad \text{(Chlorous acid)}$$
$$ClO_2^- + H_2O \rightarrow ClO_3^- + 2H^+ + 2e^- \quad \text{(Chlorate ion)}$$
$$2ClO_3^- + 8H^+ + 7e^- \rightarrow ClO_2 + Cl^- + 4H_2O \quad \text{(Chlorine dioxide, chloride)}$$

Solutions of ASC consist mainly of chlorite ion (65% at pH 2.3 to 95% at pH 3.2), hydrogen ions and chlorous acid (25% at pH 2.3 to 5% at pH 3.2) (Food Standards Australia New Zealand, 2003).

3 CONCENTRATION OF CHLORINATED DISINFECTANTS USED IN THE POULTRY INDUSTRY

3.1 Aqueous Chlorine

Chlorinated water is used in New Zealand poultry processing for spray washes of the carcasses during primary processing and for immersion chilling at the end of primary processing. Information gathered during a visit by ESR staff to a poultry processing plant and from discussions with an industry representative (Roy Biggs, Tegel Foods, personal communication), indicated use patterns were:

- Carcass sprays at chlorine concentration of 45-80 mg/L; and
- Immersion chiller at chlorine concentration of 45-80 mg/L, with residence time of 70-75 minutes. Chlorine concentration of water exiting the immersion chiller should be 0.5-5 mg/L (the residual chlorine concentration equals the initial dose less the chlorine demand).

Chlorination of process water can be achieved by addition of sodium hypochlorite or chlorine gas. Both of these processes have been reported to be used in New Zealand (Roy Biggs, Tegel Foods, personal communication).

Literature sources suggest similar concentrations of chlorine are used for immersion chilling in the USA (50 mg/L) (Robinson *et al.*, 1981).

3.2 Chlorine Dioxide

An EFSA assessment describes use of chlorine dioxide in poultry processing as (Scientific Panel on Food Additives Flavourings Processing Aids and Materials in Contact with Food, 2005a):

- Used in on-line reprocessing (sprays or washes) or in chiller baths at concentrations up to 50 mg/L, to maintain a residual concentration of 2.5 mg/L.

3.3 Acidified Sodium Chlorite

An application to use acidified sodium chlorite in poultry processing has been assessed by FSANZ (Food Standards Australia New Zealand, 2003). Intended use levels were:

- 50-150 mg/L ASC for whole carcass of poultry; and
- 500-1200 mg/L ASC for carcass parts of poultry; meats and formed meats (such as sausages, luncheon meats, and pressed hams); fruit and vegetables (intact and cut-up); and freshwater fish and seafood.

Equivalent permissions are in place in USA and Canada (Food Standards Australia New Zealand, 2003).

An EFSA assessment described use of ASC for poultry processing as (Scientific Panel on Food Additives Flavourings Processing Aids and Materials in Contact with Food, 2005a):

- 500-1200 mg/L, with pH in the range 2.3-2.9, for either a 15 second spraying or a 5-8 second dipping; or
- Immersion in chilling water at concentrations up to 150 mg/L, at pH 2.8-3.2. Residence time is typically one hour, but may be as long as three hours.

4 CHEMICAL SPECIES FORMED FROM CHLORINATED DISINFECTANTS DURING POULTRY PROCESSING

The literature discusses three main components of foods that chlorinated disinfectants may react with; lipids (fat), amino acids, peptides and proteins, and carbohydrates (Fukayama *et al.*, 1986; Scientific Panel on Food Additives Flavourings Processing Aids and Materials in Contact with Food, 2005a). Poultry contains negligible amounts of carbohydrate (<0.01 g/100 g) and reactions between carbohydrates and chlorinated disinfectants will not be discussed further in this report.

4.1 Reactions with Lipids

The fat content of poultry varies considerably. Skin typically containing approximately 30% w/w of fat, mainly as triacylglycerols, while breast contains 1% w/w of triacylglycerols and phospholipids and thigh contains 2-4% w/w of triacylglycerols (Scientific Panel on Food Additives Flavourings Processing Aids and Materials in Contact with Food, 2005a). Chicken fat typically contains approximately 60% mono- and poly-unsaturated fatty acids, most of which are oleic (C18:1) and linoleic (C18:2) acids (American Oil Chemists Society, 1997). Being an animal fat, the unsaturated component of chicken fat is lower than most plant oils, for example olive oil.

Reactions of chlorinated disinfectants with lipids are principally addition reactions to the unsaturated double bonds. Additions may be of chlorine or hydroxyl groups (Fukayama *et al.*, 1986).

4.1.1 Aqueous chlorine (hypochlorous acid)

Hypochlorous acid labelled with ^{36}Cl was used to measure the degree of incorporation of chlorine into free fatty acids (Ghanbari *et al.*, 1982). The amount of chlorine incorporated was proportional to the degree of unsaturation, with three times as much chlorine incorporated into arachidonic acid with four double bonds, as was incorporated into oleic acid with one double bond. However, in animal fats the fatty acids are present as triglycerides, not free fatty acids. Ghanbari *et al.* (1982) found much less incorporation of chlorine into triglycerides from olive oil or wheat germ when treated with hypochlorous acid. The chlorine concentration used was 180 mg/L, with reaction times up to 60 minutes.

Gas-chromatography-mass spectrometry (GC-MS) analysis of methylated solvent extracts from the reaction of aqueous chlorine and oleic acid identified six reaction products, of which only one was chlorinated (9-chloro-10-hydroxymethyl stearate), while the remainder were hydroxylated derivatives of oleic acid (Fukayama *et al.*, 1986). The chlorinated reaction product accounted for approximately 95% of the modified lipid products.

A series of studies comparing the metabolism of chlorinated and non-chlorinated lipids in rats concluded that chlorinated oleic acid was slightly less well absorbed than non-chlorinated oleic acid (Cunningham and Lawrence, 1976). Even greater discrimination against absorption and deposition of chlorinated linoleic and linolenic acids was observed (Cunningham and Lawrence, 1977a). Similarly, when radiolabelled oleic acid (^{3}H) and chlorinated oleic acid (^{36}Cl) were fed to pregnant and lactating rats, proportionally less ^{36}Cl

than ^{3}H was observed in foetal lipid or suckling rat lipid on the day following administration (Cunningham and Lawrence, 1977b).

4.1.2 Chlorine dioxide

Chlorine from chlorine dioxide (180 mg/L, reaction time up to one hour) radiolabelled with ^{36}Cl was incorporated into fatty acids and their methyl esters to a much lower extent than chlorine from radiolabelled aqueous chlorine (Ghanbari *et al.*, 1982). It has been concluded that the major reaction of chlorine dioxide with lipid is oxidation, rather than chlorination (Scientific Panel on Food Additives Flavourings Processing Aids and Materials in Contact with Food, 2005a).

4.1.3 Acidified sodium chlorite

The fatty acid profile of the lipid fraction from chicken carcasses was examined after immersion of the lipid in dips containing 150 or 1200 mg ASC/L (Scientific Panel on Food Additives Flavourings Processing Aids and Materials in Contact with Food, 2005a). Samples and controls were cooked before analysis, with no chlorinated organics being detected at a limit of detection of 0.05 mg/kg. No detectable changes were noted in the fatty acid profile, including the more sensitive polyunsaturated fatty acids. An increase in thiobarbituric acid reactive substances (TBARS) was observed in skin following ASC treatment, but not in muscle. TBARS is a measure of lipid oxidation. The skin has the highest fat content of the chicken carcass components.

4.2 Reactions with Amino Acids, Peptides and Proteins

Amino acids and their oligomers (peptides) and polymers (proteins) make up approximately 23% w/w of chicken breast, 13% w/w of chicken skin and 20% of chicken thigh. Proteins make up most of this group of compounds in chicken meat (Scientific Panel on Food Additives Flavourings Processing Aids and Materials in Contact with Food, 2005a). Table 1 summarises information on the major peptides found in poultry meat.

Table 1: Peptides found in poultry meat

Common name	Chemical name	Typical concentration (mg/kg)	Reference
Carnosine	β-alanyl-L-histidine	600-1800	(Aristoy and Toldrá, 2004)
Anserine	β-alanyl-L-1-methylhistidine	2000-7800	(Aristoy and Toldrá, 2004)
Balenine	β-alanyl-L-3-methylhistidine	20-100	(Aristoy and Toldrá, 2004)
Glutathione	L-γ-glutamyl-L-cysteinglycine	44-282	(Jahan *et al.*, 2004)
Carnitine	B-hydroxy γ-N-trimethyllysine	111-214	(Shimada *et al.*, 2004)

The free amino acid content of meat, before aging, is low with typical levels of 300 mg/kg (Scientific Panel on Food Additives Flavourings Processing Aids and Materials in Contact with Food, 2005a).

4.2.1 Aqueous chlorine (hypochlorous acid)

Hypochlorous acid has been shown to react rapidly with a range of amino acids in aqueous solution, with formation of N-chloro compounds (Fukayama *et al.*, 1986). Hypochlorous acid has also been shown to react with aromatic amino acids, resulting in ring cleavage and inclusion of chlorine (Kirk and Mitchell, 1980). However, the exact reaction products were not identified.

Peptides and proteins can undergo oxidation, substitution and addition reactions with aqueous chlorine (Fukayama *et al.*, 1986). Cunningham and Lawrence (1977c) exposed beef, pork and chicken meat to radiolabelled aqueous chlorine at 200 mg/L. Increases in aqueous and lipid soluble radio-chlorine in lean muscle meat were observed, with the degree of incorporation increasing with time. However, reaction products were not characterised (Cunningham and Lawrence, 1977c).

Considerably more research has been carried out on the interaction of chlorine and wheat proteins. Gaseous chlorine was shown to oxidise cysteine and methionine, destroy a proportion of the tyrosine and histidine and cause some deamidation, possibly due to the action of hydrochloric acid formed *in situ* (Ewart, 1968). Chlorine bleaching of wheat flour was shown to result in a progressive increase in extractability of the proteins in water and acetic acid (Tsen *et al.*, 1971). The changes were attributed to the dispersing, hydrolytic and oxidative actions of chlorine. Oxidation also resulted in changes in the sulphydryl (-S-H) content of the wheat proteins, degradation of the aromatic amino acids, and chlorination of tyrosine. These reactions have also been reported for reaction of hypochlorous acid with human proteins (Peskin and Winterbourn, 2001; Winterbourn, 2002).

Semicarbazide (SEM) has long been considered to be a side chain residue of the antibiotic nitrofurazone (Hoenicke *et al.*, 2004). Tests indicate that SEM is weakly mutagenic and carcinogenic (Scientific Panel on Food Additives Flavourings Processing Aids and Materials in Contact with Food, 2003). Hoenicke *et al*. (2004) demonstrated formation of SEM in chicken following treatment with active chlorine (sodium hypochlorite). Incubation of chicken overnight in solutions containing 1% and 12% active chlorine (10,000 and 120,000 mg/L) resulted in formation of SEM at concentrations of 1.2 and 2.5 μg/kg respectively. SEM was not detected after incubation of chicken in solutions of active chlorine at lower concentrations (0.015 and 0.05%; 150 and 500 mg/L). The authors speculated that SEM was formed through degradation of substances such as arginine, histidine, citrulline, creatine or creatinine. Treatment of these compounds and urea with active chlorine demonstrated significant SEM formation from arginine, creatine, creatinine and urea.

4.2.2 Chlorine dioxide

Reaction of high levels of chlorine dioxide with proteins and amino acids does not appear to result in chlorinated derivatives (Meredith *et al.*, 1956; Moran *et al.*, 1953). Oxidation of cysteine and methionine, and reductions in levels of tryptophan and cystine were observed in wheat flour treated with chlorine dioxide (Moran *et al.*, 1953). A study of the reaction of chlorine dioxide with 21 amino acids found similar results, with only seven of the amino acids reacting (Tan *et al.*, 1987). Cysteine, tryptophan and tyrosine reacted rapidly at pH 3, 6 and 9. Methionine reacted only at pH 9, while histidine, hydroxyproline and proline reacted

at pH 6 and 9, with reactions at the higher pH being too rapid to monitor. The authors noted that chlorine dioxide only reacted with sulphur-containing or aromatic amino acids.

Chlorine dioxide was shown to react rapidly with two dipeptides (L-glycyl-L-tryptophan and L-tryptophylglycine), but not with a third (aspartame; L-aspartyl-L-phenylalanine methyl ester) (Tan *et al.*, 1987). This is consistent with the observed reactivity of the individual amino acids and the authors suggested that the reactions of the dipeptides were actually reactions of the tryptophan moiety. Two proteins (bovine serum albumin and casein) were also shown to react rapidly with chlorine dioxide (Tan *et al.*, 1987). The reaction products were not identified for reactions of chlorine dioxide with dipeptides or proteins.

4.2.3 Acidified sodium chlorite

No information was located on reactions of ASC with amino acids, peptides or proteins. However, available evidence suggests that treatment of poultry carcasses with ASC will results in oxidation reactions, rather than chlorination reactions. No chlorinated amino acids were detected in chicken wings soaked in 2,500 mg/L ASC for 5 minutes (Scientific Committee on Veterinary Measures Relating to Public Health, 2003). Comparison of amino acid profiles for untreated and ASC treated poultry carcasses were identical, with concentrations of the most reaction amino acids (cysteine, tyrosine, threonine, tryptophan) unchanged after ASC treatment (Scientific Panel on Food Additives Flavourings Processing Aids and Materials in Contact with Food, 2005a).

4.3 Absorption of Disinfection Byproducts From Water

Extensive research has been carried out on the formation of disinfection byproducts through reaction of disinfectants with organic material in water. However, little information is available on the occurrence of these byproducts in chicken.

4.3.1 Chloroform

Table 2 summarises information from a study that looked at the formation of chloroform in immersion chiller water and accumulation of chloroform in chicken flesh (Robinson *et al.*, 1981).

Table 2: Chloroform in chicken flesh following different immersion treatments

Treatment	Chloroform concentration (µg/kg) Mean (Range)		
	Skin	Fat[1]	Muscle
Control	5 (3-7)	NT	NT
Chlorine			
5 mg/L, 20 minutes, 1.5°C	3 (<3-6)	14 (<10-26)	3 (<2-4)
20 mg/L, spray, 13°C	68 (47-82)	159 (90-249)	12 (10-14)
50 mg/L, 5 minutes, 15-16°C	144 (102-192)	447 (53-1241)	177 (90-374)
50 mg/L, 20 minutes, 1.5-2.5°C	30 (17-22)	146 (56-295)	17 (13-25)
Chlorine Dioxide			
5 mg/L, 5 minutes, 15-16°C	19 (12-25)	20 (10-30)	2 (<2-6)
5 mg/L, 20 minutes, 1.5-2.0°C	6 (5-8)	14 (<8-20)	<2
Combined chlorine treatments			
Plus cooking	134 (113-164)	46 (26-82)	64 (61-71)

From Robinson *et al.* (1981)
NT = not tested
[1] Depot fat collected from the abdominal cavity and neck skin

Chloroform residues in chicken were generally greatest in fat, followed by skin, followed by muscle. Temperature appeared to have a greater impact on incorporation of chloroform into the carcass than contact time. The authors hypothesised that the chloroform observed after chlorine dioxide treatments may be have been due to hypochlorite used to generate the chlorine dioxide, rather than being due to the chlorine dioxide itself (Robinson *et al.*, 1981).

4.3.2 Brominated trihalomethanes

The oxidation potential of aqueous chlorine is sufficient to oxidise bromide ion to hypobromous acid (analogous to hypochlorous acid). As a result, waters containing bromide ion may also contain brominated members of the trihalomethane family ($CHBrCl_2$,$CHBr_2Cl$, $CHBr_3$). Brominated trihalomethanes can be the dominant members of the trihalomethane family even when bromide concentrations in the water prior to chlorination are less than 1 mg/L.

Increased bromine substitution in trihalomethanes increases their lipid solubility and the rate at which they permeate through human skin (Batterman *et al.*, 2002; Xu *et al.*, 2002). This suggests that if present in water, bromine-containing trihalomethanes are likely to appear in chicken fat at higher concentrations (on both a weight and molar basis) than chloroform.

The possible formation of brominated trihalomethanes is noted here, but in most New Zealand water supplies chloroform is the dominant member of the family. Of 881 samples analysed through the Ministry of Health's Priority 2 Determinand Identification Programme, chloroform was undetectable in 32% of samples and bromoform in 88% of samples (Nokes, 1999).

Haloacetic acids are the other major disinfection by-product formed in water. At the pH typical of potable waters these acids exist in their dissociated ionic form. The corresponding

haloacetate ions will have a low tendency to dissolve in fat and to migrate through skin (Xu *et al.*, 2002).

4.3.3 Mutagenicity

Studies of poultry chiller water, and simulated poultry chiller water have examined the effect of chlorine and chlorine dioxide on the production of mutagenic compounds in the water (Tsai *et al.*, 1997). Mutagenic compounds were isolated and identified from simulated chiller water containing homogenised poultry meat and about 4% available chlorine (approximately 40,000 mg/L) (Haddon *et al.*, 1996). Water from actual poultry chillers was shown to be mutagenic in the Ames test when treated with 400 mg/L chlorine, but not 100mg/L chlorine dioxide (Tsai *et al.*, 1997). These authors commented that it cannot be assumed that chlorine use at lower "real-life" levels (20-50 mg/L) would have the same effect.

No reports have been found that examine whether these chemicals are taken up into poultry meat.

5 TOXICITY OF COMPOUNDS FORMED DURING TREATMENT OF CHICKEN CARCASSES WITH CHLORINATED DISINFECTANTS

Two classes of compounds will be considered in this section:

- The chlorinated disinfection compounds and their direct breakdown products; and
- Compounds formed by reactions between the chlorinated disinfection compounds and components of the chicken carcass.

5.1 Chlorinated Disinfection Products and their Breakdown Products

5.1.1 Hypochlorous acid and the hypochlorite ion

Hypochlorous acid and the hypochlorite ion together constitute aqueous chlorine. A WHO Working Party has established a tolerable daily intake (TDI) for chlorine of 150 μg/kg body weight/day (International Programme on Chemical Safety, 2000).

The TDI was based on a study carried out under the US National Toxicology Program (NTP). In a 2-year study, chlorine was fed via water to F344 rats and $B6C3F_1$ mice. The only treatment-related non-tumour effect was found to be a dilation of renal tubules in male mice receiving 24 mg/kg body weight/day for more than 66 weeks. No non-neoplastic lesions were observed in either male or female rats. A positive trend in stromal polyps of the uterus in female mice and an increased in mononuclear cell leukaemia for high-dose female rats were not considered to be treatment related.

Based on this study a No Observed Adverse Effect Level (NOAEL) of 15 mg/kg body weight/day was approximated. A safety factor of 100 was applied in deriving the TDI.

5.1.2 Chlorine dioxide

The toxicology of chlorine dioxide is considered to relate to its hydrolysis product, the chlorite ion (International Programme on Chemical Safety, 2000).

An oral TDI of 30 μg/kg body weight/day was assigned to chlorine dioxide on the basis of an NOAEL of 2.9 mg/kg body weight/day for neurodevelopmental effects of chlorite in rats, with inclusion of a safety factor of 100. The study was a two generation study in Sprague Dawley rats. The NOAEL related to lower auditory startle amplitude, decreased absolute brain weight in the F1 and F2 generations, and altered liver weights in two generations (International Programme on Chemical Safety, 2000).

5.1.3 Acidified sodium chlorite

The toxicology of ASC is considered in terms of the toxicity of the chlorite and chlorate ions (Benford *et al.*, 2008). The toxicity of the chlorite ion was discussed in the previous section.

For chlorate, the most sensitive endpoint was considered to be changes to the thyroid gland in male rats, specifically follicular cell hypertrophy in a two-year carcinogenicity and toxicity study (Benford *et al.*, 2008). No NOAEL could be derived from this study, but a Benchmark Dose (BMD) approach was used to derive a lowest BMD of 1.1 mg/kg body weight/day. Rats are highly sensitive to agents that disrupt thyroid hormone homeostasis and it was considered

likely that humans would be less sensitive than rats to thyroid effects. An interspecies safety factor was considered unnecessary. However, due to noted deficiencies in the toxicological database for this chemical an extra safety factor of 10 was applied. Along with the inter-individual safety factor of 10, this resulted in establishment of an ADI of 10 µg/kg body weight/day for the chlorate ion.

5.2 Disinfection Byproducts – Trihalomethanes

Cancer following chronic exposure is the primary hazard of concern for this class of compounds (International Programme on Chemical Safety, 2000).

5.2.1 Chloroform

Chloroform has been classified as a possible human carcinogen (group 2B) (IARC, 1999b). However, there is inadequate evidence to establish its carcinogenicity in humans and safety assessments have been based on its toxicity in laboratory animals.

Chloroform most commonly affects the liver and the kidneys. Several different approaches have been used to derive a tolerable daily intake (TDI) for chloroform, but have produced reasonably consistent TDIs in the range 10-15 µg/kg body weight/day.
http://www.inchem.org/documents/cicads/cicads/cicad58.htm

5.2.2 Bromodichloromethane

Bromodichloromethane has been classified as a Group 2B, possible human carcinogen, based in animal studies (IARC, 1999a).

In rats and mice, it induces tumor formation in the kidneys, and in rats there is tumour formation in the large intestine. As with all the brominated THMs, it is weakly mutagenic. Based on kidney tumor formation a dose of 2.1 µg/kg body weight/day is estimated to produce a risk level of 10^{-5} or an excess cancer risk of 1 in 100,000 (International Programme on Chemical Safety, 2000).

5.2.3 Dibromochloromethane

The IARC has classified dibromochloromethane in Group 3 (not classifiable as to its carcinogenicity to humans) (IARC, 1999a).

Dibromochloromethane has been found to induce tumours in livers of female mice, but not rats. Based on slope of the dose-response curve for the formation of these tumours, 1.5 µg/kg body weight/day is estimated to produce a risk level of 10^{-5} or an excess cancer risk of 1 in 100,000. A TDI of 30 µg/kg of body weight has been calculated based on the absence of histopathological effects in the liver of rats after 13 weeks exposure (International Programme on Chemical Safety, 2000).

5.2.4 Bromomethane (Bromoform)

The IARC has classified bromoform in Group 3 (not classifiable as to its carcinogenicity to humans) (IARC, 1999a).

Bromoform has been found to produce a small increase in tumours in the large intestine of rats. Based on this endpoint 7.7 μg/kg body weight/day equates to a risk level of based on these data of 10^{-5} or an excess cancer risk of 1 in 100,000. The absence of liver lesions in rats after 13 weeks exposure to bromoform has led to a TDI of 25 μg/kg body weight (International Programme on Chemical Safety, 2000).

6 EXPOSURE ESTIMATES FOR DISINFECTION BY-PRODUCTS

Exposure estimates for disinfection by-products by the New Zealand population were determined stochastically, using distributions for chicken consumption for adults and children determined previously (Cressey *et al.*, 2006). Body weights were also represented as distributions fitted to data from the 1997 National Nutrition Survey (Russell *et al.*, 1999) and the 2002 National Children's Nutrition Survey (Ministry of Health, 2003).

Distributions used were:

- Adult chicken consumption — Lognormal(124.32,230.33)
- Child chicken consumption — Lognormal(95.84,138.02)
- Adult body weight — InverseGauss(74.81,1550.35)
- Child body weight — InverseGauss(41.90,225.66)

Simulations were run for 10 000 iterations, with mean and 95th percentile confidence intervals for disinfectant by-product exposure being reported. The results of exposure assessments are summarised in Table 3-6 in the following sections.

6.1 Aqueous Chlorine

No information was found on chlorine residues in chicken flesh following treatment with aqueous chlorine.

6.1.1 Chloroform

The data of Robinson *et al.* (1981) was used to provide values for the concentration of chloroform in chicken following chlorinated disinfectant treatment. It was considered that fat, as defined in the study of Robinson *et al.* was unlikely to be commonly consumed. Excluding fat, the highest average concentration of chloroform (177 µg/kg) was observed in muscle meat following dipping in a solution of 50 mg/L chlorine for 5 minutes at 15-16ºC. While this concentration is consistent with our knowledge of chlorine use in the New Zealand poultry industry, the contact time and temperature are not. However, a chloroform concentration of 177 µg/kg was used to model the exposure of New Zealander to chloroform from consumption of chicken. Results are shown in Table 3.

Table 3: Estimated dietary exposure to chloroform from consumption of chicken

Population group	Estimated exposure to chloroform from treated chicken (µg/kg body weight/day)
New Zealand Adults (15+ years)	0.3 (0.01-1.6)
New Zealand Children (5-15 years)	0.5 (0.03-2.4)
TDI	10-15

95% CI 95th percentile confidence interval
TDI Tolerable Daily Intake

The results of Robinson *et al*. (1981) suggest that cooking of chicken may reduce the chloroform concentration significantly.

6.2 Chlorine Dioxide

No analytical data were found on chlorine dioxide residues in chicken. A European assessment hypothesised that treatment of chicken with chlorine dioxide in the chiller bath for 1 hour at a concentration of 20-50 mg chlorine dioxide/L would result in maximum residue levels in the chicken of 0.13 mg chlorite/kg and 0.06 mg chlorate/kg in the finished chicken carcass (Scientific Committee on Veterinary Measures Relating to Public Health, 2003). The chlorine dioxide present would add a further 0.01 mg/kg of residue in the form of chlorite.

Results of exposure simulation for the New Zealand population, using these concentration values, is presented in Table 4.

Table 4: Estimated dietary exposure to chlorine dioxide residues from consumption of chicken

Population group	Estimated exposure from treated chicken (µg/kg body weight/day)		
	Chlorite Mean (95% CI)	Chlorate Mean (95% CI)	Chlorine dioxide Mean (95% CI)
New Zealand Adults (15+ years)	0.2 (0.009-1.2)	0.1 (0.004-0.5)	0.02 (0.0007-0.09)
New Zealand Children (5-15 years)	0.3 (0.02-1.7)	0.2 (0.009-0.8)	0.03 (0.002-0.14)
European mean adult	0.33	0.15	0.02
TDI	30	10	30

95% CI 95th percentile confidence interval
TDI Tolerable Daily Intake

6.3 Acidified Sodium Chlorite

Table 5 summarises ASC manufacturer's (Alcide Corporation) data on residual levels of chlorite and chlorate on chicken carcasses following various treatment regimes (Scientific Panel on Food Additives Flavourings Processing Aids and Materials in Contact with Food, 2003). An application to FSANZ for use of ASC on a range of foods reported the results of residue analyses for chlorite and chlorate (Food Standards Australia New Zealand, 2003). While the results are presented in a different format (summarised for a range of foods), they appear to include the data in Table 5 for poultry.

Table 5: Residual chlorite and chlorate levels on chicken carcasses following differing treatments with acidified sodium chlorite

Treatment	Residual chlorite (µg/kg)	Residual chlorate (µg/kg)
Immersion in 1200 mg ASC/L (pH 2.5) for 5 seconds, post chiller tank and after 5 minutes drip	<9	<11
Immersion in 150 mg ASC/L (pH 2.8) for 1 hour in immersion chiller, immediately post treatment	540	<19
Immersion in 150 mg ASC/L (pH 2.8) for 1 hour in immersion chiller, 20 hours post treatment	<16	<19

ASC acidified sodium chlorite

It was considered that chlorite and chlorate concentration data from the 20 hour post treatment analyses were most applicable for human exposure assessment. Manufacturer's data indicated that both compounds were undetectable at this time. An upper bound exposure assessment was carried out by using the limit of detection values as surrogates for the concentration of chlorite and chlorate. These values (16 µg/kg chlorite and 19 µg/kg chlorate) and New Zealand food consumption and body weight data from the 1997 National Nutrition Survey (adults) and 2002 National Children's Nutrition Survey (children) were used to model potential chlorite and chlorate exposure due to use of ASC as a poultry disinfectant. Results are summarised in Table 6.

Table 6: Estimated dietary exposure to acidified sodium chlorite residues from consumption of chicken

Population group	Estimated exposure from treated chicken (µg/kg body weight/day)	
	Chlorite Mean (95th % CI)	Chlorate Mean (95th % CI)
New Zealand Adults (15+ years)	0.03 (0.001-0.15)	0.03 (0.001-0.17)
New Zealand Children (5-15 years)	0.04 (0.002-0.22)	0.05 (0.003-0.26)
European mean adult	0.04	0.05
TDI	30	10

95% CI 95th percentile confidence interval
TDI Tolerable Daily Intake

7 CONCLUSIONS

Considering the importance of chlorinated disinfectants to the food industry and the long history of use, there is relatively little information available on the identity or formation of chlorinated or oxidised compounds due to the reaction of disinfectants with food components.

While there is some evidence for formation of chlorinated compounds in chicken treated with aqueous chlorine, oxidation reactions appear to predominate for chlorine dioxide and acidified sodium chlorite. Studies on ASC treatment of poultry carcasses indicated no significant treatment-related changes in fatty acid and amino acid composition of carcasses. While no equivalent information is available for chlorine dioxide, it is a less potent oxidising agent than ASC and the lack of significant changes in carcass composition due to ASC treatment suggest that chlorine dioxide treatment is similarly unlikely to affect carcass composition.

There is little information on the identity of chlorinated compounds formed from aqueous chlorine treatment of chicken. Formation of semicarbazide, a chemical belonging to a family of chemicals (hydrazines) known to cause cancer in animals, has been demonstrated following exposure of chicken flesh to aqueous chlorine (Hoenicke *et al.*, 2004). However, semicarbazide formation only occurred at chlorine concentrations well in excess of those used in the poultry industry. A recent assessment concluded that semicarbazide is a weak non-genotoxic carcinogen for which a threshold mechanism can be assumed (Scientific Panel on Food Additives Flavourings Processing Aids and Materials in Contact with Food, 2005b). Likely human exposure is several orders of magnitude less than doses causing tumours in laboratory animals.

Chloroform has been detected in chicken treated with aqueous chlorine (Robinson *et al.*, 1981). It is probable that this was due to absorption of chloroform from chiller water, rather than direct formation in the chicken flesh. Exposure modelling suggests that the levels of chloroform observed would result in exposure to chloroform at levels well below the tolerable daily intake (TDI) for this compound. While other halogenated disinfectant byproducts (bromodichloromethane, dibromochloromethane, bromoform) could potentially be absorbed by chicken carcasses from chiller or dip water, studies of absorption of these compounds have not been reported in the literature. Based on monitoring results, the composition of New Zealand potable water generally does not favour production of these brominated compounds. Water used for poultry processing in New Zealand must be of a potable standard.

Exposure modelling of potential chlorite and chlorate residues in chicken, due to use of chlorine dioxide or acidified sodium chlorite, indicates that exposure would be well below TDIs, even at the 95% level of exposure. This is consistent with conclusions from a European assessment (Scientific Committee on Veterinary Measures Relating to Public Health, 2003).

In conclusion, no safety issues were identified due to the use of chlorine dioxide and ASC for poultry carcass disinfection. Chlorination reactions appear to be insignificant for these compounds and oxidation reactions do not appear to result in significant alteration of the fatty acid and amino acid composition of poultry carcasses.

Aqueous chlorine-based disinfection is widely used throughout the food processing industry, and direct disinfection of food with hypochlorite is particularly common in the produce sector (McIntyre *et al.*, 2008). Chlorine has been reported to exert its microbial disinfection activity by both chemical (oxidation, enzyme inhibition) and physical (disruption of cell walls) mechanisms (Morato *et al.*, 2003) and it is to be expected that there will be some reactions between components of the disinfection system and food components. However, the information collected by this review indicates that, at least for carcinogens and mutagens, the concentrations of chlorine required to induce formation are well in excess of those used in practice.

During 2007-2008, FAO and WHO have considered the issue of chlorine usage in food processing, including an expert meeting on the associated risks and benefits. The meeting was held during May 2008 and the report from the meeting is not yet available. Unless the consultation identifies new areas of concern, we consider that there is insufficient evidence to justify further investigation of the risks to human health from the use of these disinfection chemicals in poultry processing. For further information on the FAO/WHO project see:
http://www.who.int/ipcs/food/active_chlorine/en/
http://www.fao.org/ag/agn/agns/chemicals_chlorine_en.asp

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