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ORIGINAL RESEARCH

Evaluation of trichloromethane formation from chlorine-based cleaning and disinfection agents in cow's milk

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The development of trichloromethane (TCM) in milk can result from the use of chlorine-based detergent solutions during the milk production process. Different factors affecting the development of TCM in milk were investigated. Gas chromatography with electron capture detector was the method of analysis. Combining milks of different TCM levels resulted in milk TCM levels similar to that calculated mathematically. Increased chlorine concentration in the detergent and reduced rinse water volume resulted in increased TCM concentrations ($P < 0.05$) in milk, while the addition of chlorine to the rinse water also increased ($P < 0.001$) milk TCM.

Keywords Milk, Trichloromethane (TCM), Gas chromatography (GC), Milking machine wash routines.

INTRODUCTION

Cleaning and disinfection of equipment used for transfer and storage of milk and the manufacture of dairy products is important to ensure a high standard of food hygiene. Sodium hypochlorite is commonly used throughout the dairy processing chain from on-farm production to product manufacture because of its effectiveness in washing and decontamination of contact surfaces and also because of its relatively low cost (Cousins 1977; Schmidt 1997). Sixty-seven per cent of liquid detergent and detergent steriliser products surveyed for application in the cleaning of Irish milking equipment are chlorine based (Gleeson and O'Brien 2011). However, there are disadvantages associated with chlorine misuse, namely, the development of disinfection by-products in food. Disinfection by-products are contaminants, 'not intentionally added to foods but are present as a result of the production process' (Codex Alimentarius Commission 2002).

If chlorine comes into contact with organic material, it can form total organic chlorine (Tiefel and Guthy 1997). Total organic chlorine consists of volatile organic chlorine (VOX) and nonvolatile organic chlorine. The most important of the VOX group is the contaminant trichloromethane (TCM), also called chloroform (Tiefel and Guthy 1997). The formation of VOX can occur via a haloform reaction. The classic haloform reaction is that which occurs between a halogen (chlorine, bromine, fluorine and iodine) and a methylketone to form a haloform (Fuson and Bull 1934). Milk and milk products contain acetoin, diacetyl and other methyl-ketones (Mick *et al.* 1982) that can react with chlorine to yield VOX in the form of TCM. Chlorinated hydrocarbons, such as TCM, accumulate in fat-rich portions, in products like milk, butter and vegetable oil (Hubbert *et al.* 1991), often producing undesirable food taints. Fleming-Jones and Smith (2003) concluded that in the USA, dairy products contained the highest levels of TCM, compared with other food products.

A level of 0.1 mg/kg, well below the concentration that would be considered carcinogenic, has been established as the acceptable limit for all Trihalomethanes in drinking water supplies by the EU (1998). Trihalomethanes are a group of chemical compounds that contain a halogen such as chlorine, fluorine, bromine or iodine, or a combination of these (WHO 2005). The EU has not published regulations on the acceptable limits of TCM in foods. However, strict regulations have been enacted in Germany on the acceptable levels of TCM in foods. The German legal limit for TCM in food, set by the Verordnung über Höchstmengen an Schadstoffen in Lebensmitteln (2003), is the same as

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the EU drinking water limit for Trihalomethanes. Furthermore, TCM target levels of <0.03 and <0.002 mg/kg in butter and milk, respectively, have been recommended. Thus, it is economically important that individual countries competing for market share achieve these TCM targets as well as the legal targets.

The presence of TCM in food has previously been associated with chlorinated drinking water (Rook 1974). However, it is now known that cleaning and disinfection solutions containing chlorine can also be considered a source of contamination (Resch and Guthy 2000). The authors showed the formation of TCM in recycled cleaning and disinfection solutions when rinsing (pre- and post-washing) with water was omitted from the milking machine wash procedure. Lack of rinsing permitted direct contact between the detergent solution and milk. Consequently, the TCM concentration increased in the detergent solution and in the subsequent milk passing through the milking machine. Recycling of the detergent solution for a further wash occasion allowed TCM to further increase in milk. In Ireland, the two most common procedures for cleaning and disinfection of milking equipment are: hot circulation cleaning using a formulated detergent steriliser (sodium hydroxide and sodium hypochlorite) and cold circulation cleaning using a formulated powder-based alkaline detergent (O'Brien 2009). Chlorine is used in both routines because it represents the steriliser portion of the detergent steriliser product used for the hot circulation cleaning and is used in conjunction with a weekly hot wash in the cold circulation cleaning system. In a scenario where acid washing is used daily and the solution is discarded after use, the currently recommended chlorine concentration in the detergent steriliser solution ranges between 100 and 200 mg/L chlorine (Clegg and Bacic 1968; Reinemann *et al.* 2003). Both hot and cold circulation cleaning involve three stages, a pre-washing rinse (directly after milking), washing, and a post-washing rinse (Palmer 1991). Chlorine may also be added to the water of the post-rinse of the cold circulation cleaning on some Irish farms, as an extra hygiene step. The recommended chlorine concentration in the post-rinse water is 29 mg/L chlorine. The recommended volume of rinse water required for both the pre- and post-rinses of a milking plant is 14 L/milking unit (L/MU) (O'Brien 2009). However, water is a resource which has to be purchased and paid for on many farms and this may influence the volume of water used in the rinse cycle. The effect of reduced rinse water usage on the development of TCM in milk with Irish wash procedures has not been investigated.

As the milk purchasing cooperative/company collects the milk from each farm, the individual milk is mixed with the larger milk pool. These individual farm supplies may have varying TCM concentrations. Thus, the TCM content of milk for processing into dairy products is a function of the TCM levels of all of the contributing milks. The objectives of this study were to investigate: (i) the effect of combining milks of different TCM levels on the overall TCM concentration of the resulting milk pool, (ii) the effect of two concentrations of chlorine (263 and 473 mg/L) and four levels of rinse water (7, 10, 14 and 16.3 L/MU) used in milking machine washing routines, on TCM levels in milk, and (iii) the effect of varying chlorine concentrations (0, 29, 58 and 116 mg/L) added to the final rinse water of the milking machine washing routine on TCM residues in milk.

MATERIALS AND METHODS

Measurement method

Static head-space gas chromatography (HS-GC) with electron capture detector (ECD) was chosen as the analytical procedure and was used as described by Resch and Guthy (1999). The GC settings used for the analysis of TCM in milk are shown in Table 1. Resch and Guthy (1999) showed that the use of an internal standard method was beneficial when comparing similar samples. The authors also showed that the external standard method gave improved recovery for TCM in milk when compared with the internal standard method alone. Therefore, both an internal and external standard were used for analysis throughout.

Effect of combining milks of varying TCM concentrations

Milk was obtained directly from a cow by hand milking (as opposed to machine milking) and placed in two glass containers that had been thoroughly washed, rinsed with ethanol and distilled water, oven dried and cooled. Milk obtained in this

Table 1 Gas chromatography conditions for the analysis of trichloromethane in milk

Auto-Sampler	CTC analytics Combi-pal[1]
Carrier gas	Helium
Incubation time and temperature	1 h, 80 °C
Agitator	500 rpm, 5 s on 2 s off
Injection time	30 s
Temperature of injection needle	85 °C
Transfer liner	90 °C
Cycle time	38.5 min
Gas chromatography	Agilent 7890A
Detector	Electron Capture Detector
Split	10:1
Make-up gas	Nitrogen 10 mL/min
Helium gas pressure	80 bar
Column	
Injector temperature	90 °C
Detector	280 °C
Program	50 °C 5 min
	Ramped to 130 °C at 5 °C/min
	Immediately ramped to 200 °C at 20 °C/min
	Hold for 10 min
Total time of analysis	34.5 min

[1]CTC Analytics AG Industriestrasse 20 CH-4222 Zwingen Switzerland.

manner had no contact with cleaning and disinfection agents and had nondetectable levels of TCM. This milk volume was sub-divided into Milk A and Milk B. Milk B was spiked with a known concentration (0.027 mg/kg) of TCM (high). Six combinations of milks were prepared in the following order: 100:0, 80:20, 60:40, 40:60 and 20:80, 0:100 Milk A and Milk B (high), respectively. The samples were stored at 4 °C for 24 h before analysis. This procedure was repeated five times and each sample was analysed in duplicate.

Effect of different chlorine concentrations and rinse water volumes on TCM levels in milk

Where daily acid washing is not used, the recommended chlorine concentration of detergent steriliser solution is between 200 and 300 mg/L (Gleeson and O'Brien 2011). Therefore, the effect of two chlorine concentrations (263 and 473 mg/L) representing the recommended concentration and an abuse concentration of detergent steriliser solution used in the main hot circulation wash cycle for a 30 unit milking plant was tested. The hot circulation cleaning regime included:

1. an initial pre-wash rinse with cold water at a volume of 14 L/MU,
2. the main wash with detergent steriliser at chlorine concentrations of 263 and 473 mg/L at a volume of 12 L/MU and
3. a post-wash rinse with varying levels of cold water (7, 10, 14 and 16.5 L/MU) tested at each of the two chlorine concentrations.

All milking machine detergent/steriliser washes were completed using hot water at 70 °C. After each 5-day period, the milking machine received an acid wash with hot water (70 °C) to descale equipment surfaces, followed by an alkaline detergent/steriliser wash using hot water (70 °C).

Each detergent steriliser chlorine concentration was evaluated in association with each rinse water volume over a 5-day period (10 milking events). Milk samples were taken from the milk line prior to the plate cooler. Milk samples were collected at the start of milking as the initial milk was pumped from the receiver jar to the bulk tank. These samples were referred to as 'first milk' samples (in the first 4.5 L). Subsequent milk samples were taken mid-way through milking and were referred to as 'mid milk' samples. Approximately, 100 mL of milk was taken per sample. All milk samples were cooled to 4 °C and stored at −22 °C until analysis. It had previously been shown that storage at −22 °C for 1 week did not impact on TCM levels (data not shown). Sampling for each treatment took place during morning (am) and evening (pm) milkings. As part of the experimental dairy farm procedure, a milking interval of 17/7 was in place, that is, 17 h between pm and am milking and 7 h between am and pm milking.

Effect of the addition of sodium hypochlorite to the final rinse water on TCM levels in milk

The hot circulation cleaning routine included: (i) a post-milking/pre-wash rinse using 16.5 L/MU of water, (ii) a main wash including detergent steriliser containing 263 mg/L chlorine with 12 L/MU wash solution, (iii) a post-wash rinse using 16.5 L/MU, and (iv) an additional post-wash rinse conducted immediately after the first post-wash rinse. The additional post-wash rinse cycle contained 0, 29, 58 and 116 mg/L free available chlorine and treatments were evaluated over a 5-day period (10 milking events). The recommended chlorine concentration is 29 mg/L, and two other elevated chlorine concentrations (58 and 116 mg/L) used in the post-rinse water on levels of TCM in milk were tested. Milk sampling was conducted as described earlier (at the commencement and mid-way through milking).

Statistical analysis

Statistical analysis was completed using ProcMixedSAS (SAS Institute Inc 2009). Linear auto-regression was used to establish the relationship between bulk milk volume and TCM concentration in milk.

Results of varying rinse water volume and chlorine concentration on TCM formation in milk were analysed using a repeated measures model. A diagonal covariance structure was assumed. The model included terms for chlorine concentration (mg/L), rinse water volume (L), stage of sampling (first, mid), time (am, pm) and their interactions. Pairwise comparisons were performed and a Tukey–Kramer adjustment was used.

Results from the addition of hypochlorite to the final rinse water of milking machine wash routines were analysed using a repeated measures model. A diagonal covariance structure was assumed. The model included terms for Time (am/pm), Stage (Sampling stage), and Treatment (chlorine concentration). Time was treated as the main effect. Pairwise comparisons were performed and a Tukey–Kramer adjustment was used. Statistically significant differences were determined at 5% probability level.

RESULTS AND DISCUSSION

Effect of combining milk of varying TCM concentrations

A direct correlation between the proportion of 'high' TCM milk and the TCM concentration of the combined milks was observed ($P < 0.001$) (Fig. 1). Greater than 99% of the

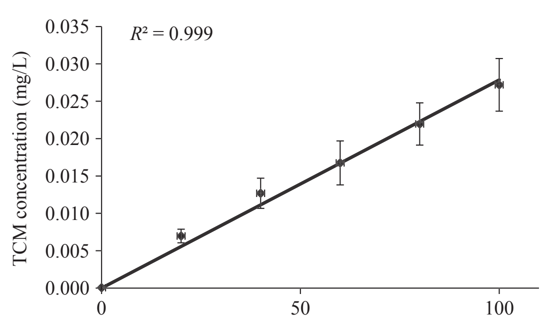

Figure 1 Effect of combining milks of different trichloromethane (TCM) concentrations on the average TCM concentration of the combined milk ('high' = 0.027 mg/kg).

variability in TCM concentration could be explained by varying the volume of 'high' TCM milk added. The TCM concentration of a milk pool is dependent on the TCM levels of the individual component milks and the volume of such milks. Therefore, bulk milk of 0.008 mg/kg TCM concentration has the potential to contaminate three similar quantities of milk of nondetectable TCM content resulting in a final TCM concentration of approximately 0.002 mg/kg in the milk mixture. This is of particular significance to milk processors as it shows that the number of milk suppliers with high TCM concentrations in their milk can influence the final TCM concentration of the combined milk load.

Effect of varying chlorine concentrations and rinse water volumes on TCM levels in milk

When the detergent sanitiser solution containing 263 mg/L chlorine was in place, average TCM residues in milk were increased as rinse water volumes were reduced from 16.5 to 7.0 L/MU ($P < 0.001$). Reducing the rinse water volume from 16.5 to 14.0 L/MU resulted in an increase in TCM concentration of 0.002 mg/kg ($P < 0.01$) (Table 2) in the 'first milk' sample. No such increase was observed in the 'mid milk' sample ($P > 0.05$). TCM residue in the 'first milk' sample was increased also when rinse water volumes were reduced from 14.0 to 7.0 L/MU ($P < 0.001$) but no significant effect was observed in the 'mid milk' samples. There was no significant difference in milk TCM concentrations observed when 263 and 473 mg/L chlorine in the detergent solution were compared ($P > 0.05$) (data not shown).

Average TCM residues in milk were increased as rinse water volumes were reduced from 16.5 to 7.0 L/MU when 473 mg/L chlorine was contained in the detergent steriliser solution ($P < 0.001$). Reducing the rinse water volume from 16.5 to 14.0 L/MU resulted in a numerical increase in TCM concentration of 0.001 mg/kg ($P > 0.05$) (Table 2) in the 'first milk' sample but not in the 'mid milk' sample. Reducing the rinse water volume from 14.0 to 7.0 L/MU resulted in an increase in TCM residue of 0.004 mg/kg ($P < 0.01$) in the 'first milk' sample and an increase of 0.002 mg/kg ($P < 0.001$) in the 'mid milk' sample.

The stage at which sampling occurred, that is, 'first' and 'mid', affected the resulting TCM concentration in milk. 'First milk' samples contained increased levels of TCM compared with 'mid milk' samples ($P < 0.001$). This effect was observed because of the 'first milk' sample being the first milk to come in contact with the cleaned equipment surfaces. The concentration of TCM in the 'mid milk' sample was diluted by the large milk volume processed during the milking period. Although high TCM levels were obtained for many of the 'first milk' samples, it is important to note that these figures would not be representative of the overall bulk milk TCM concentration. The TCM concentration of 'mid milk' samples would be more representative of bulk milk TCM concentrations.

When data for am and pm milkings were compared, an increase in TCM concentration was observed in mean pm milk samples ($P < 0.001$). This may be due to the shorter milking interval between am and pm milking (7 h) and the consequent milk yield at the pm milking. The reduced milk volume at pm milkings may lead to less dilution and increased concentrations of TCM in milk. Current advice to farmers states that the first 4.5 L of milk, at the start of milking, should be run to waste.

Table 2 Effect of chlorine concentration and rinse water volume of milking machine wash routines (n = 20) on trichloromethane concentrations in milk (mg/kg)

Chlorine concentration	*Volume of rinse water L* 16.5	14	10	7	*SE*[1]	*P-value*[2]
263 mg/L						
'First Milk'	0.002^{a}	0.004^{b}	0.004^{ab}	0.010^{c}	0.125	*
'Mid Milk'	0.001^{a}	0.001^{a}	0.001^{a}	0.002^{a}	0.163	
473 mg/L						
'First Milk'	0.003^{a}	0.004^{ab}	0.006^{bc}	0.008^{c}	0.124	*
'Mid Milk'	0.000^{a}	0.000^{ac}	0.000^{ad}	0.002^{b}	0.174	*

Means with common superscript are not significantly different within rows.
[1]Standard Error of the mean.
[2]*P*-value *<0.01.

Effect of the addition of sodium hypochlorite to the final rinse water on TCM levels in milk

Increased concentrations of hypochlorite in the final rinse water of milking machine wash routines resulted in an increase in the formation of TCM in 'first milk' samples ($P < 0.001$). Increasing the concentration of chlorine from 0 to 29, 58 and 116 mg/L resulted in an increase of <0.001, <0.001 and 0.002 mg/kg TCM in 'first milk' samples, respectively ($P < 0.001$) (Table 3). Levels of TCM in average milk samples ('First' and 'Mid' milk samples) did not exceed the recommended level of 0.002 mg/kg upon the addition of the 29 and 58 mg/L chlorine to the final rinse water. However, the

Table 3 Effect of different hypochlorite concentrations in final rinse water of milking machine wash routines (n = 20) on trichloromethane concentrations in milk (mg/kg)

	Concentration of chlorine mg/L 0	29	58	116	*SE*[1]	*P-value*[2]
'First milk'	0.000^{a}	0.001^{b}	0.001^{cb}	0.003^{d}	0.065	*
'Mid milk'	0.000^{a}	0.000^{a}	0.000^{a}	0.000^{a}	0.065	

Means with common superscript are not significantly different within rows.
[1]Standard Error of the mean.
[2]*P*-value *<0.001.

addition of 116 mg/L chlorine to the final rinse water resulted in TCM levels exceeding the recommended level. However, this effect was not observed in 'mid milk' samples and TCM levels would be diluted in the bulk milk.

The concentrations of TCM were higher ($P < 0.001$) in the pm compared with am milk samples. This may be due to the lower volume of milk at the pm milking attributed to the 17/7 h milking interval in place on-farm.

CONCLUSION

The concentration of TCM in a milk pool obtained by mixing known volumes of milk of varying TCM levels may be calculated mathematically, and the number of milk loads with high TCM concentrations can influence the final TCM concentration of the overall combined milk load trichloromethane levels in 'first milk' samples (but not 'mid milk' samples) were increased as the volume of rinse water used in milking machine wash cycles was reduced. This was seen irrespective of the chlorine concentration of the detergent steriliser solution (263 or 473 mg/L). Thus, the currently recommended rinse water volume (14.0 L/MU) is effective in minimising milk TCM residues as 'mid milk' represents the combined milk pool. The risk of 'first milk' being included in the milk pool is reduced by adhering to the recommendation of allowing the first 4.5 L of milk to go to waste. Reducing rinse water volumes from 14.0 L/MU to 10.0 or 7.0 L/MU increased TCM residues irrespective of chlorine concentration. This study indicates that the minimum volume of rinse water must be 14 L/MU. The practice of adding sodium hypochlorite to the final rinse water did not have a negative effect on TCM levels in milk when used at the recommended level. When the addition of hypochlorite to the final rinse water exceeded the recommended level, an increase in TCM residues in milk was observed.

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