Monitoring residue concentrations in milk from farm and throughout a milk powder manufacturing process

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Short title: **Milk quality from farm to milk powder**

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Summary

The experiments reported in this research paper aimed to investigate differences in the levels of chlorate (CHLO), perchlorate (PCHLO), trichloromethane (TCM) and iodine residues in bulk tank (BT) milk produced at different milk production periods, and to monitor those levels throughout a skim milk powder (SMP) production chain (BTs, collection tankers [CTs], whole milk silo [WMS] and skim milk silo [SMS]). Chlorate, PCHLO and iodine were measured in SMP, while TCM was measured in the milk cream. The CHLO, TCM and iodine levels in the mid-lactation milk stored in the WMS were lower than legislative and industrial specifications (0.0100 mg/ kg, 0.0015 mg/ kg and 150 µg/ L, respectively); however, in late-lactation, those levels were numerically higher than the mid-lactation levels and specifications. Consequently, CHLO and iodine levels in SMP were numerically higher in late-lactation than in mid-lactation. Trichloromethane accumulated in the cream portion after separation. Perchlorate was not detected in any of the samples. Regarding iodine, the levels in mid-lactation reconstituted SMP were higher than that required by manufacturers (100 µg/ L), indicating that the levels in milk should be lower than 142 µg/ L. The higher residue levels observed in late-lactation could be related to the low milk volume produced during that period and changes in sanitation practices, while changes in feed management could have affected iodine levels. This study could assist in controlling and setting limits for CHLO, TCM and iodine levels in milk, ensuring premium quality dairy products.

Keywords: chlorate, perchlorate, trichloromethane, iodine, milk quality

International markets are setting high specifications for milk and dairy product quality, including stringent guidelines on concentrations of residues that could occur in milk. Potential milk contaminants of most concern include chlorate (CHLO, ClO_3^-), perchlorate (PCHLO, ClO_4^-) and trichloromethane (TCM, $CHCl_3$), which arise as a consequence of sanitation with chlorine products.

Chlorate and PCHLO were reported to result in thyroid dysfunctions (EFSA, 2015), while TCM could possibly be carcinogenic to humans (ICAR, 1999). There are a few studies available that have discussed contributing factors on-farm (Gleeson *et al.*, 2013; Ryan *et al.*, 2013); however, the dynamic of residue concentrations when subjected to different milk processing conditions is not fully understood. Sodium hypochlorite, chlorine gas or dioxide may be used for the sanitation of water, while chlorine-based detergents are used for the sanitation of milking or processing equipment. Chlorine products generally have good bactericidal properties and are widely used because of their effectiveness and low cost (Garcia-Villanova *et al.*, 2010). The decomposition of chlorine compounds results in the production of oxyhalide species (ClO^- and ClO_2^-), which react and form CHLO. Further reactions of CHLO with those oxyhalides result in the formation of PCHLO (Gordon & Tachiyashiki, 1991). Residual chlorine, CHLO or PCHLO on the surfaces of processing equipment can contaminate milk (Asami *et al.*, 2013). The contamination of infant formula with CHLO is a major concern due to the risk of intoxication in infants, which have lower tolerance than adults. The contact of chlorine with milk could also result in the formation of TCM (Tiefel & Guthy, 1997). Chlorinated hydrocarbons accumulate in fat-rich fractions; therefore, products such as butter and cream could contain high concentrations of TCM if milk is contaminated with high levels (Hubbert *et al*., 1996).

Excessive levels of residual iodine in raw milk are another concern in the Irish dairy industry, especially in the manufacture of infant formula. Iodine is an essential micronutrient for the synthesis of hormones by the thyroid gland (Leung & Braverman, 2014). Even though iodine is a nutrient of extreme importance to the human organism, the daily consumption of iodine at higher levels than recommended could result in dysfunctions of the thyroid gland. Bovine milk is one of the main sources of iodine for humans and its content depends on the daily iodine intake by dairy cows (Flachowsky *et al*. 2014). The US National Research Council (2001) recommends that the daily iodine intake per cow should be 10 mg, which is the reference value applied in Ireland. The utilisation of rations with higher levels of iodine than required or overfeeding cows can result in excessive iodine concentrations secreted into milk. Over supplementation of Irish herds is of most concern during early and late-lactation and during winter milk production (O'Brien *et al*., 2013). O'Brien *et al.* (1999) recorded an average of 227 ug/ L iodine in Irish milk, while concentrations of 510 and 180 ug/ L were recorded for December and June, respectively. Those levels were not a food safety concern at the time; however, processors are currently requiring lower levels of iodine in raw milk destined for the production of infant formula, in order to meet requirements of the international market. Some Irish dairy processors require that raw milk should contain less than 150 µg/ L of iodine. Other iodine sources in milk include mineral-added water, boluses, mineral licks and grass (Magowan *et al.*, 2010). The use of iodine-based teat disinfectants could also contribute to iodine content in milk, as those products are absorbed through the teat skin if not completely removed prior to milking (Flachowsky *et al*. 2014).

The first objective of this study was to investigate changes in the CHLO, PCHLO, TCM and iodine levels throughout the milk production chain, from farm to dairy product, in two different milk production periods (mid- and late-lactation). Chlorate, PCHLO and iodine were measured throughout the production stages of skim milk powder (SMP), while TCM was measured throughout the production stages of milk and cream, which were destined for butter manufacture. The second objective was to investigate differences in residue levels in bulk tank (BT) milk produced during mid- and late-lactation. The milk used in this study was produced on commercial dairy farms and processed in a commercial SMP processing plant.

Materials & Methods

Sampling procedure at the farms and throughout a skim milk powder manufacturing process

In Ireland a seasonal spring-calving production system is practiced, with all cows calving within a 10-week period approximately (February to April). This experiment was performed on one occasion during each of mid- (May; 80 DIM) and late-lactation (December; 290 DIM). The farms that supplied milk to the factory (mid-lactation: 67 farms; late-lactation: 150 farms), milk storage conditions on-farm, amount of milk produced, milk collection and the skim milk powder manufacturing process was the same as described by Paludetti *et al.* (in press). A schematic drawing of the SMP manufacturing process is shown in supplementary Figure S1.

In mid-lactation, samples were collected at various points of the manufacturing process between the farm BTs and the SMP [BTs, collection tankers (CTs), whole milk silo (WMS), skim milk silo (SMS) and final SMP] and were tested for CHLO, PCHLO and iodine. In late-lactation, samples were collected at various points between the CTs and the SMP [CTs, WMS, cream silo (CS), SMS and final SMP] and were tested for CHLO, PCHLO and iodine. In both lactation periods, TCM was quantified in all samples, with exception of the SMP samples (supplementary Figure S1). Due to the high number of farms (150) necessary to supply sufficient milk volume to undertake the manufacturing process in late-lactation (December), it was not possible to undertake collection and analysis of all individual BT samples. The collection of samples and preparation of SMP samples for analysis were performed as described by Paludetti *et al.* (in press).

Comparison between the residue levels in the same 67 farm bulk tanks in mid- and late-lactation (May and November)

The concentrations of CHLO, PCHLO, TCM and iodine residues were measured in raw milk produced on the same 67 dairy farms sampled in mid-lactation (May, 80 DIM) and in late-lactation period (November; 260 DIM), to investigate the effect of milk production period on residue levels. Milk samples were collected as described by Paludetti *et al.* (in press).

Quantification of chlorate and perchlorate

The quantification of CHLO and PCHLO was performed by high-performance liquid chromatography coupled to tandem mass spectrometry (LC/ MS-MS) with ESI electrospray ionisation in negative mode (-ESI). The mid-lactation milk and SMP samples, as well as the 67 late-lactation farm BT samples, were analysed in the laboratory of Labor Friedle GmbH group (Labor Friedle GmbH, Von-Heyden-Straβe 11, D-93105, Tegernheim, Germany), while the late-lactation samples from the factory (CT, WMS, SMS and SMP samples) were analysed in Teagasc Ashtown (Dublin, Ireland). The methodologies used are based on the procedures described in the European Quick Polar Pesticides method (QuPPe) (EURL-SRM, 2015). In the present study, some of the milk samples were analysed by both laboratories and the results were statistically similar ($P>0.05$). The detection limit of CHLO and PCHLO in milk was 0.0010 mg/ kg and in SMP was 0.010 mg/ kg.

Quantification of trichoromethane

Trichloromethane was quantified in the milk using static head-space gas chromatography (HS-GC) with electron capture detector (ECD), fitted with a low thermal mass system (LTM) (Agilent 7890A, Agilent Technologies, Santa Clara, California, USA). The trichloromethane detection limit in this analysis was 0.0001 mg/ kg. The methodology applied was an adaption of the procedure of Resch & Guthy (1999). This analysis was performed in the Milk Quality laboratory in Teagasc Moorepark (Fermoy, Co. Cork, Ireland).

Quantification of iodine

Iodine was quantified in milk and reconstituted SMP samples using inductively coupled plasma mass spectrometry (ICP-MS), using an Agilent ICP-MS 7700x (Agilent Technologies, Santa Clara, California, USA). The methodology used was based on the procedures described in the standard method for the determination of iodine compounds in foodstuffs (BS EN 15111:2007, 2007). Standard solutions of Tellurium and 1% TMAH were used to obtain a calibration curve. The limit of detection was 1.31 µg/ L. The mid-lactation milk and SMP samples were analysed in Teagasc Moorepark (Fermoy, Co. Cork, Ireland), while the late-lactation milk and SMP samples were analysed in FBA laboratories

(Capoquinn, Co. Waterford, Ireland). Those laboratories used the same methodology and samples analysed by both laboratories had statistically similar results (P>0.05).

Statistical analysis

Influence of individual farm milk volumes on the residue concentration in each CT and influence of CT milk on the residue concentration in WMS

The statistical analyses were performed using the software SAS 9.3 (SAS Institute, 2016). In mid-lactation, the iodine and TCM concentrations of each CT were predicted using the volume and iodine or TCM concentrations measured in the milk of all farms that supplied each respective CT. In mid- and late-lactation, the iodine and TCM concentrations in the WMS were also predicted using the volume and iodine or TCM concentrations in the milk of all CTs that supplied that silo. Those predictions were calculated as volume-weighted means with estimated confidence intervals. The actual iodine or TCM concentrations measured in each CT and WMS samples were compared to the respective confidence interval for those predicted means. Agreement plots were also used to check for bias in the relationship between actual and predicted means. It was not possible to perform the same analyses with the CHLO and PCHLO results, due to the low number of samples in which those residues were detected.

Comparison between the residue levels in the same 67 farm bulk tanks in mid- and late-lactation (May and November)

Differences between the adjusted least square means of the 67 mid- and late-lactation milk samples, collected in May and November, were calculated using the MIXED procedure in SAS 9.3 (SAS Institute, 2016). The fixed effects included in each model were lactation period (mid- and late-lactation) and farms (numbered from 1 to 67). Farms were considered the experimental unit and the response variable was iodine or TCM. Residual checks were made to ensure that the assumptions of the analysis were met.

It was not possible to statistically determine the differences between CHLO and PCHLO levels measured in mid- and late-lactation milk samples, due to insufficient number of samples in which those residues were detected. McNemar's test was applied to compare the number of BT milk samples in mid- and late-lactation that had CHLO and TCM concentrations $\geq$ 0.0010 and 0.0015 mg/kg, respectively. The GLM procedure was used to determine the regression relationship between CHLO and TCM concentrations.

Results

The mean CHLO, TCM and iodine concentrations of samples collected during mid- and late-lactation (May and December, respectively) throughout the milk powder production chain are shown in Table 1.

Chlorate and perchlorate

In mid-lactation (May), CHLO was detected in 14 of the 67 BT and 6 of the 11 CT samples. The weighted mean CHLO concentration was calculated at the basis of the milk volume supplied by those farms and CTs (Table 1). The volume-weighted mean CHLO concentrations of these farms and CTs were numerically similar.

In late-lactation (December), CHLO was detected in 6 of the 11 CT samples also, but the volume-weighted mean of those samples was higher compared to mid-lactation (Table 1).

In both mid- and late-lactation, the mean CHLO concentration in the WMS and SMS were numerically similar (Table 1).

The mean CHLO concentration of the SMP samples was higher in late-lactation (December) compared to mid-lactation (May) (Table 1). In both lactation periods, the CHLO concentration in powder increased approximately 50 times compared to the concentrations in SMS samples. In mid-lactation, the CHLO levels in the SMP samples decreased throughout the spray-dryer run. At the start, middle and end of the spray-drying process, the CHLO levels were: 0.0630 ± 0.0020, 0.0610 ± 0.0060 and 0.0470 ± 0.0020 mg/ kg, respectively. In contrast, the CHLO concentration of the late-lactation SMP samples did not vary throughout the spray-dryer run (start: 0.124 ± 0.003 mg/ kg; middle: 0.129 ± 0.011 mg/ kg; end: 0.126 ± 0.006 mg/ kg).

Perchlorate was not detected in any of the mid- and late-lactation samples collected throughout the manufacturing process.

Trichloromethane

Trichloromethane was detected in all BT and CT samples collected in mid-lactation (May) and in all CT samples collected in late-lactation (December). The volume-weighted mean TCM concentration of those samples was calculated considering the milk volume supplied by each BT or CT (Table 1). The volume-weighted mean TCM concentration of the CT milk samples was higher in late-lactation compared to mid-lactation. In mid-lactation, the volume-weighted mean TCM concentrations of the milk samples from the BTs and CTs were numerically similar. The mean TCM concentrations of the milk samples from the CTs and WMS were also numerically similar in both mid- and late-lactation.

The comparisons between the actual TCM concentration and the respective confidence interval for the predicted means for each mid-lactation CT sample, are shown in the supplementary Table S1. The TCM concentrations in all of the mid-lactation CT samples were within their respective confidence intervals. A similar comparison for the mid- and late-lactation WMS samples is shown in the supplementary Table S2. The TCM concentration in the WMS samples were also within their respective confidence interval in mid- and late-lactation.

In both lactation periods, the mean TCM concentration decreased in the SMS samples compared to the WMS samples, as expected (Table 1).

Iodine

In mid-lactation, the volume-weighted mean iodine concentration was numerically higher in the BT samples than in the CT samples. The volume-weighted mean iodine concentration of all of the CTs was numerically higher in late-lactation than in mid-lactation. In mid-lactation, the mean iodine concentrations in the CTs and WMS were similar, while in late-lactation, the mean concentration was numerically higher in the CTs compared to the WMS (Table 1). In both lactation periods, the iodine concentrations increased in SMS samples; and consequently, as levels were higher in late-lactation BT milk, the iodine concentration in SMP was higher in late-lactation than in mid-lactation.

The comparisons between the actual iodine concentrations of each mid-lactation CT sample with the respective confidence interval for the predicted means are shown in the supplementary Table S3, while such comparison for the mid- and late-lactation WMS samples are shown in the supplementary Table S4. All the iodine concentrations measured in each mid-lactation CT sample were within the respective confidence intervals, as well as the WMS samples collected in mid- and late-lactation.

Comparison between the residue levels in the same 67 farm bulk tanks in mid- and late-lactation (May and November)

The number of BT samples in which CHLO was detected was significantly higher in late-lactation (32 out of the 67 samples) than in mid-lactation (14 out of the 67 samples) ($P<0.0001$). Also, in contrast to mid-lactation, 8 out of the 67 late-lactation BT samples contained 0.0010 mg/ kg of PCHLO.

The volume-weighted mean TCM concentration was significantly higher in late-lactation (0.0015 ± 0.0014 mg/ kg; range: 0.0003 to 0.0074 mg/ kg) than in mid-lactation (0.0009 ± 0.0008 mg/ kg; range: 0.0002 to 0.0043 mg/ kg) ($P<0.0001$).

The volume-weighted mean iodine concentrations of the BT samples in mid- and late-lactation (142.2 ± 129.2 and 119.7 ± 151.6 µg/ L, respectively) were not statistically different ($P = 0.63$).

Discussion

Residues related to the use of chlorine

Concentrations of CHLO and PCHLO were monitored throughout the production chain of SMP in mid- and late-lactation (May and December, respectively). In Europe, a default threshold limit of 0.0100 mg/ kg of CHLO and PCHLO is applied for milk (EC no 396/2005). In mid-lactation (May), the volume-weighted mean CHLO concentration in the 14 BTs and 6 CTs (in which CHLO was detected) were lower than that limit; however, in late-lactation (December), the mean CHLO concentration of the 6 CTs (in which CHLO was detected) was higher than the EC limit and higher than the volume-weighted mean concentration in mid-lactation.

In mid-lactation, the CHLO concentrations in each of the CTs could have been diluted as CHLO was not detected in 53 of the BTs. For example, CHLO was not detected in 4 CT milk samples, as only one of the BT milk volumes contributing to each of those CTs contained CHLO. Additionally, CHLO was not detected in most of the BT milk supplied to the 6 CTs in which CHLO was detected, indicating that the sanitation of those CTs could possibly have influenced the CHLO levels. In both mid- and late-lactation, as CHLO was not detected in most of the CT milk volumes, the CHLO concentrations could have also been diluted in the WMS; therefore, it is likely that the sanitation practices of the silos did not influence the CHLO levels. Consequently, the mean CHLO concentrations in the WMSs were lower than the EC limit of 0.0100 mg/ kg. However, as the milk supplied to the factory during late-lactation contained higher levels of CHLO than the mid-lactation milk, the CHLO levels in the WMS in late-lactation were higher compared to mid-lactation; consequently, the CHLO levels in the SMP were higher in late-lactation than in mid-lactation.

In mid-lactation, the mean CHLO concentration of the SMP samples was lower than the limit applied by some Irish infant formula manufacturers (0.100 mg/ kg). The difference of 0.0016 mg/ kg between the mean CHLO concentration of the SMP samples collected at the end and start of the spray-drying run, indicated that the sanitation of the spray-dryer could have contributed to the CHLO levels in SMP. The interior surface of the spray-dryer could have contained residual CHLO, and the majority of that residue was transferred to the first batch of evaporated skim milk that entered the equipment. In late-lactation, the mean CHLO concentration of the SMP samples was higher than 0.100 mg/ kg, indicating that the CHLO level in the bulk milk stored in the WMS should had been lower than 0.0025 mg/ kg. Even though no variations in the CHLO concentration were observed in SMP samples collected throughout the spray-drying run in late-lactation, sanitation practices of that equipment could have also contributed to the increased CHLO levels in SMP. Additionally, the variations in CHLO concentrations throughout the spray-drying run that were observed in mid-lactation and not observed in late-lactation could be due to differences in the sanitation practices between production periods.

The concentrations of TCM were also monitored throughout the production chain of SMP in mid- and late-lactation (May and December, respectively). There are no European regulations that have defined a standard TCM limit for milk or dairy products; however, Irish dairy processors apply a limit of 0.0015 mg/ kg to milk destined for the production of lactic butter which should have less than 0.0300 mg/ kg of TCM, as required by the export market (Ryan et al., 2013). In mid-lactation, the mean TCM concentrations of the BTs, CTs and WMS were all lower than that limit; while, in late-lactation, the mean TCM concentrations of the CTs and WMS were higher than that limit and higher than the concentrations in mid-lactation. The agreement between the TCM concentrations of each mid-lactation CT sample and the contributions of each BT milk volume supplied, as well as the agreement between the TCM concentrations of the WMS samples and the contributions of each CT in both lactation periods, indicated that the cleaning protocol of the CTs or WMS did not contribute to any increases in the TCM levels in milk (Tables S1 and S2).

In both lactation periods, the decrease in the TCM concentrations in the SMS in relation to the WMS was expected, due to the accumulation of TCM in the cream during separation (Hubbert *et al.*, 1996; Table 1). As the levels of TCM were higher in late-lactation milk, the TCM concentration in late-lactation cream was possibly higher than the levels expected in cream produced with mid-lactation milk.

The concentrations of CHLO and TCM were also monitored in the same 67 farm BTs in mid- and late-lactation (May and November, respectively) to investigate if those concentrations could differ in milk produced by the same farm during different production periods. None of the mid-lactation BT samples contained CHLO levels higher than 0.0100 mg/ kg (EC limit), while 5 late-lactation BT samples contained levels higher than that limit. In relation to TCM, the number of BT samples that contained levels greater than 0.0015 mg/ kg was significantly higher in late-lactation (21 BT samples; range: 0.0016 to 0.0074 mg/ kg) than in mid-lactation (7 BT samples, range: 0.0017 to 0.0043 mg/ kg) (P = 0.002). Those increases in the levels of those residues in late-lactation could be related to changes in the sanitation practices on each farm. Chlorine detergent sterilisers should contain a maximum of 3.5% of chlorine and should be prepared and applied according to the manufacturer's instructions (Gleeson, 2016). According to Ryan et al. (2012), 14 L of rinse water per milking unit are recommended in order to totally remove the detergent solution, and the solutions should be rinsed immediately after the wash cycle. Additionally, the lower volume of milk produced per farm during late-lactation (1,683 ± 1,031 L) could have also contributed to the increase in CHLO or TCM levels during that period, as those residues could have been more concentrated. The presence of CHLO and TCM in milk was not correlated; therefore, if milk contains CHLO it will not necessarily contain TCM and *vice versa*. The contamination of milk with CHLO or TCM might be related to a combination of specific sanitation practices and further studies are necessary to determine them. In addition, the higher number of farms in late-lactation that supplied milk containing higher levels of CHLO or TCM indicated that extra care is required during that period for the production of milk powder or butter.

Iodine

Variations in the iodine concentrations were investigated throughout the production chain of SMP in mid- and late-lactation (May and December, respectively). The EFSA (2005) reported that the average iodine concentration in BT milk samples from several European studies was predominately between 100 and 200 µg/ L, which were suitable to meet the required iodine daily intake for children and adults. Some Irish dairy processors specify that the iodine levels in raw milk should be lower than 150 µg/ L to produce infant formula. In mid-lactation, the mean iodine concentration of the BT, CT and WMS samples were all lower than that limit; while in late-lactation, the mean concentrations of the CT and WMS were higher than that limit.

Flachowsky *et al*. (2014) suggested that iodine could undergo sublimation throughout processing, as more than 90% of iodine in milk is in the inorganic form. Small decreases in the mean iodine concentration observed from the BTs to CTs (mid-lactation) and from the CTs to WMS (late-lactation) could be associated with the sublimation of iodine (Table 1). The actual iodine concentrations measured in each CT (Table S3) and WMS (Table S4) were in agreement with the contributions of each BT and CT, respectively. However, the actual concentrations of each CT and WMS were slightly lower than the predicted concentrations (Tables S3 and S4), indicating that possibly a small amount of iodine underwent sublimation during transport and storage, but not sufficient to be significant. Those small losses could have resulted in those decreases in the mean iodine concentrations shown in Table 1.

In mid-lactation, two CT samples had levels higher than 150 µg/ L (390.8 and 202.9 µg/ L). One of those CTs collected milk from two farms that supplied milk containing 289.1 and 516.0 µg/ L of iodine. The other CT collected milk from 5 farms; however, most of the volume collected was from one farm that supplied milk containing 561.2 µg/ L of iodine. Therefore, it is important that individual milk suppliers control the iodine intake of their herds and correctly apply iodine-based teat disinfectants (US National Research Council, 2001; O'Brien *et al.*, 2013). In late-lactation, all of the CT samples had levels higher than 150 µg/ L, indicating that the iodine levels in BT milk were possibly higher in late-lactation than in mid-lactation. Those higher levels could be due to the contribution of the increased number of farms (150) and also due to high levels of iodine in ration supplied to the cows when indoors.

In both lactation periods, the mean iodine concentrations increased in the SMS when compared to the WMS. Prior to pasteurisation and cream separation, milk permeate (details were not disclosure by the manufacture) is added to standardise the protein and lactose content in milk; therefore, that permeate could have contributed to an increase in the iodine content in the SMS.

The International Council for Control of Iodine Deficiency Disorders (ICCIDD; Delange *et al.*, 1993) specified that the iodine content in reconstituted SMP should be lower than 100 µg/ L. The mean iodine concentrations of the SMP produced in mid- and late-lactation were higher than that limit (Table 1). Therefore, in the case of the conditions of this study, the iodine levels in the bulk milk supplied to the factory should be lower than 142 µg/ L to produce SMP containing iodine levels within the specification. Also, as the iodine levels were higher in late-lactation compared to mid-lactation milk, the iodine content in reconstituted SMP was also higher in late-lactation than in mid-lactation.

In order to investigate variations in the levels iodine in BT milk during different production periods, the concentrations of such residue were also measured in the same 67 farm BTs in mid- and late-lactation (May and November, respectively). In mid- and late-lactation, 13 and 12 BT samples had iodine concentrations higher than 150 µg/ L, respectively. Questionnaires were completed on some of those farms, capturing information regarding animal feed. It was established that the majority of those farms were using concentrates from one manufacturer, which contained at least 10 and a maximum of 43 mg of iodine/ kg of ration. Therefore, the iodine intake from ration per cow on those farms was likely to be higher than that recommended (10 mg per cow per day), as the average ration intake on those farms was 2.5 kg per cow per day. Other factors that were not included in the questionnaires could have contributed to iodine levels in milk such as grass, boluses, mineral-supplemented water and mineral licks. Furthermore, according to the questionnaires, five and two farms that were using iodine-based teat disinfectants supplied milk with iodine levels higher than 150 µg/ L, in mid- and late-lactation, respectively. O'Brien *et al*. (2013) also observed increases in the iodine levels in milk when applying those teat disinfectants post-milking. Those increases are associated with the absorption of iodine through the teat skin, particularly if pre-milking teat preparation is not being conducted.

Conclusion

Incorrect sanitation practices on-farm can result in increases in the CHLO or TCM levels in milk throughout the year, while the production of lower volumes of milk is an additional contributing factor in late-lactation; therefore, extra care is necessary during that period. Consequently, increases in the CHLO or TCM levels in milk result in increased residue levels in SMP or cream, respectively. Therefore, it is important to control the initial residue levels in milk destined for processing, especially considering that those could concentrate greatly after evaporation and spray-drying processes or cream separation. Appropriate sanitation practices should also be carried out within the processing plant to avoid increases in the residue levels throughout the processing stages. In relation to iodine, this study indicated that some Irish dairy herds are over supplemented with iodine, while the use of iodine-based teat disinfectants also contributed to high levels in some BT samples. Also, the iodine content of the SMP produced in mid-lactation was not within the required specification, even though the WMS milk had lower iodine levels than specified, indicating that the levels in BT milk should be even lower. Finally, it is possible to calculate the expected residue levels in milk stored in the CTs or WMS based on the volumes and residue levels of milk supplied by each dairy farm, which could aid dairy processors to identify the stages that may have contributed to increases in those levels. This study highlights the importance of controlling the contributing factors on-farm and in the processing plant in order to maintain residues at safe and market-acceptable levels.

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Table 1. Mean (± SD) chlorate (CHLO), trichloromethane (TCM) and iodine concentrations in samples collected from the farm bulk tanks (BTs), collection tankers (CTs), whole milk silo (WMS), skim milk silo (SMS), cream silo (CS) and samples of skim milk powder (SMP) from the mid- and late-lactation periods.

	Mid-Lactation		
	CHLO (mg/ kg)	TCM (mg/ kg)	Iodine (µg/ L)
Farm BTs (n=67) †	0.0021 ± 0.0019 (0.0010 to 0.0070) ‡	0.0009 ± 0.0008 (0.0002 to 0.0043)	142.2 ± 129.2 (10.4 to 561.2)
CT (n=11) †	0.0020 ± 0.0010 (0.0010 to 0.0030) §	0.0009 ± 0.0003 (0.0006 to 0.0015)	134.2 ± 89.6 (58.3 to 390.8)
WMS (n=2)	0.0010 ± 0.0000	0.0009 ± 0.0000	135.5 ± 7.6
SMS (n=2)	0.0010 ± 0.0000	0.0002 ± 0.0000	142.1 ± 9.1
SMP (n=9)	0.0570 ± 0.0090 ¶		142.2 ± 10.0 (120.2 to 153.5)
	Late-Lactation		
	CHLO (mg/ kg)	TCM (mg/ kg)	Iodine (µg/ L)
CT (n=11) †	0.0410 ± 0.0554 (0.0020 to 0.1550) §	0.0020 ± 0.0007 (0.0010 to 0.0033)	437.6 ± 155.2 (225 to 709)
WMS (n=2)	0.0025 ± 0.0000	0.0018 ± 0.0000	419.0 ± 2.8
SMS (n=2)	0.0025 ± 0.0000	0.0005 ± 0.0000	450.0 ± 7.1
CS (n=2)		0.0190 ± 0.0000	
SMP (n=9)	0.1263 ± 0.0071 ¶		398.2 ± 22.8 (257 to 425)

n = number of samples; ranges are given between parentheses.

† Weighted means and standard deviations calculated considering the volumes of milk and residues concentrations of each farm or CT sample.

‡ Weighted mean CHLO of the 14 bulk tank milk samples in which chlorate was detected.

§ Weighted mean CHLO of the CT milk samples in which chlorate was detected (mid-lactation: 6 samples; late-lactation: 6 samples).

¶ Results for non-reconstituted skim milk powder

Monitoring residue concentrations in milk from farm and throughout a milk powder manufacturing process

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Short title: **Milk quality from farm to milk powder**

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SUPPLEMENTARY FILE

Materials & Methods

Comparison between the residue levels in farm bulk tanks in mid- and late-lactation

In late-lactation (November 2016), the average (± SD) milk volume that was stored in each BT of the 67 farms during sampling was 1,683 ± 1,031 L (range: 125 to 4,519 L), which were stored for an average (± SD) of 34 ± 15 h, at 3.3 ± 1.2 ˚C.

Quantification of trichoromethane

In each sample vial, 2 mL of milk or reconstituted SMP were added with 5 µL of internal standard and 5 µL of of ethanol. The internal standard consisted of a solution prepared using 2-bromo-1-chloropropane and ethanol (0.2 mg/ mL). Samples were placed on an autosampler tray (CTC analytics Combi-pal; CTC Analytics AG Industriestrasse 20 CH-4222, Zwingen, Switzerland) and were incubated for 15 min at 80 ˚C and agitated at 750 rpm. Samples were injected (500 µL) into an Agilent 19095J-121LTM column (10 m x 0.53 mm x 2.65 µm; Agilent Technologies, Santa Clara, California, USA) with a heated gas-tight syringe (90 ˚C). Helium was used as the carrier gas, and the column temperature was kept at a constant temperature of 200 ˚C, which decreased to 70 ˚C in the end of analysis.

Quantification of total iodine

In each vial, 1:1 (w/v) solutions of sample and 5% tetramethyl-ammonium hydroxide (TMAH) extraction solution were added and gently swirled. Vials were placed in an oven at 90 ˚C for 3 h and afterwards they were allowed to cool. The standards used for the calibration consisted of solutions of iodine with 0.5 mL of a Tellurium solution (1,000 µg/ mL), which contained 1% TMAH.

Table S1. Comparison of mean trichloromethane (TCM) concentrations measured in each collection tanker (CT: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11) during mid-lactation and those predicted (± standard error; S.E.) from the combined farm samples in each CT.

CT number	Number of farms	Total volume per tanker (L)	Mean (± SD) volume measured per farm (L)	Mean TCM concentration of each CT (mg/ kg)	Predicted TCM concentration (weighted means ± S.E.)† (mg/ kg)	95% CI‡		Mean TCM concentration of each CT covered by predicted C.I.
						LCL	UCL	
1	4	23771	5,943 ± 1,271	0.0015	0.0014 ± 0.0009	0.0000	0.0043	Yes
2	5	26503	5,301 ± 2,385	0.0008	0.0005 ± 0.0002	0.0000	0.0011	Yes
3	6	29122	4,854 ± 1,763	0.0012	0.0009 ± 0.0003	0.0001	0.0016	Yes
4	6	23780	3,963 ± 2,683	0.0012	0.0009 ± 0.0003	0.0002	0.0016	Yes
5	8	27585	3,448 ± 2,214	0.0008	0.0004 ± 0.0001	0.0002	0.0005	Yes
6	7	28628	4,090 ± 1,208	0.0011	0.0008 ± 0.0004	0.0000	0.0018	Yes
7	7	27188	3,884 ± 2,064	0.0006	0.0004 ± 0.0001	0.0002	0.0006	Yes
8	7	28470	4,067 ± 2,437	0.0007	0.0004 ± 0.0002	0.0001	0.0008	Yes
9	2	27147	13,574 ± 11,312	0.0010	0.0007 ± 0.00004	0.0002	0.0012	Yes
10	5	25248	5,050 ± 3,877	0.0007	0.0003 ± 0.0001	0.0000	0.0006	Yes
11	10	28561	2,856 ± 1,764	0.0008	0.0005 ± 0.0001	0.0003	0.0008	Yes

† Weighted means were calculated considering the volume of milk supplied by each farm or by each CT.

‡ Confidence interval (CI), lower (LCL) and upper (UCL) confidence limits.

Table S2. Comparison of mean trichloromethane (TCM) concentrations measured in the whole milk silo (WMS) during mid- and late-lactation and those predicted (± standard error; S.E.) from the combined collection tankers (CTs) samples.

	Mean TCM concentration of the WMS (mg/ kg)	**Mean (± SD) volume measured per CT (L)**	**Predicted TCM concentration (weighted means ± S.E.)† (mg/ kg)**	**95% CI‡**		**Mean TCM concentration of WMS covered by predicted C.I.**
				LCL	**UCL**	
Mid-lactation	0.0009	26,909 ± 1,902	0.0007 ± 0.00009	0.0005	0.0009	Yes
Late-lactation	0.0018	24,357 ± 3,768	0.0019 ± 0.0002	0.0014	0.0024	Yes

† Weighted means were calculated considering the volume of milk supplied by each CT.

‡ Confidence interval (CI), lower (LCL) and upper (UCL) confidence limits.

Table S3. Comparison of mean iodine concentrations measured in each collection tanker (CT: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11) during mid-lactation and those predicted (± standard error; S.E.) from the combined farm samples in each CT.

CT	Number of farms	Mean (± SD) volume measured per farm (L)	Total volume per CT (L)	Iodine concentration measured in each CT sample (μg/ L)	Predicted iodine concentrations (weighted means ± SE) (μg/ L) †	95% CI ‡		Mean iodine concentration of each CT covered by predicted CI
						LCL	UCL	
1	4	5,943 ± 1,271	23,771	83.9	89.2 ± 21.8	19.8	158.6	Yes
2	5	5,301 ± 2,385	26,503	81.8	90.0 ± 23.8	23.9	156.2	Yes
3	6	4,854 ± 1,763	29,122	120.0	117.9 ± 45.6	0.6	235.3	Yes
4	6	3,963 ± 2,683	23,780	58.3	61.2 ± 8.5	39.3	83.7	Yes
5	8	3,448 ± 2,214	27,585	125.9	141.0 ± 27.8	75.4	206.7	Yes
6	7	4,090 ± 1,208	28628	138.4	144.1 ± 55.7	7.9	280.3	Yes
7	7	3,884 ± 2,064	27188	112.0	116.7 ± 15.7	78.4	155.1	Yes
8	7	4,067 ± 2,437	28470	76.3	82.9 ± 20.9	31.6	134.1	Yes
9	2	13,574 ± 11,312	27147	390.8	335.7 ± 91.6	0	1,500	Yes
10	5	5,050 ± 3,877	25248	202.9	282.7 ± 121.2	0	619.7	Yes
11	10	2,856 ± 1,764	28561	80.0	101.7 ± 12.1	74.3	129.1	Yes

† Weighted means were calculated considering the volume of milk supplied by each farm.

‡ Confidence interval (CI), lower (LCL) and upper (UCL) confidence limits.

Table S4. Comparison of mean iodine concentrations measured in the whole milk silo (WMS) during the mid- and late-lactation periods and those predicted (± standard error; S.E.) from the combined collection tankers (CTs) samples.

	Mean (± SD) iodine concentration of the WMS (μg/ L)	Mean (± SD) volume measured per CT (L)	Predicted iodine concentration (weighted means ± SE) (μg/ L) †	95% CI ‡		Mean iodine concentration of the WMS covered by predicted CI
				LCL	UCL	
Mid-lactation	135.5 ± 7.6	26,909 ± 1,902	134.2 ± 28.3	71.0	197.3	Yes
Late-lactation	419.0 ± 2.8	24,357 ± 3,768	421.4 ± 50.5	308.8	534.0	Yes

† Weighted means were calculated considering the volume of milk supplied by each CT.

‡ Confidence interval (CI), lower (LCL) and upper (UCL) confidence limits.

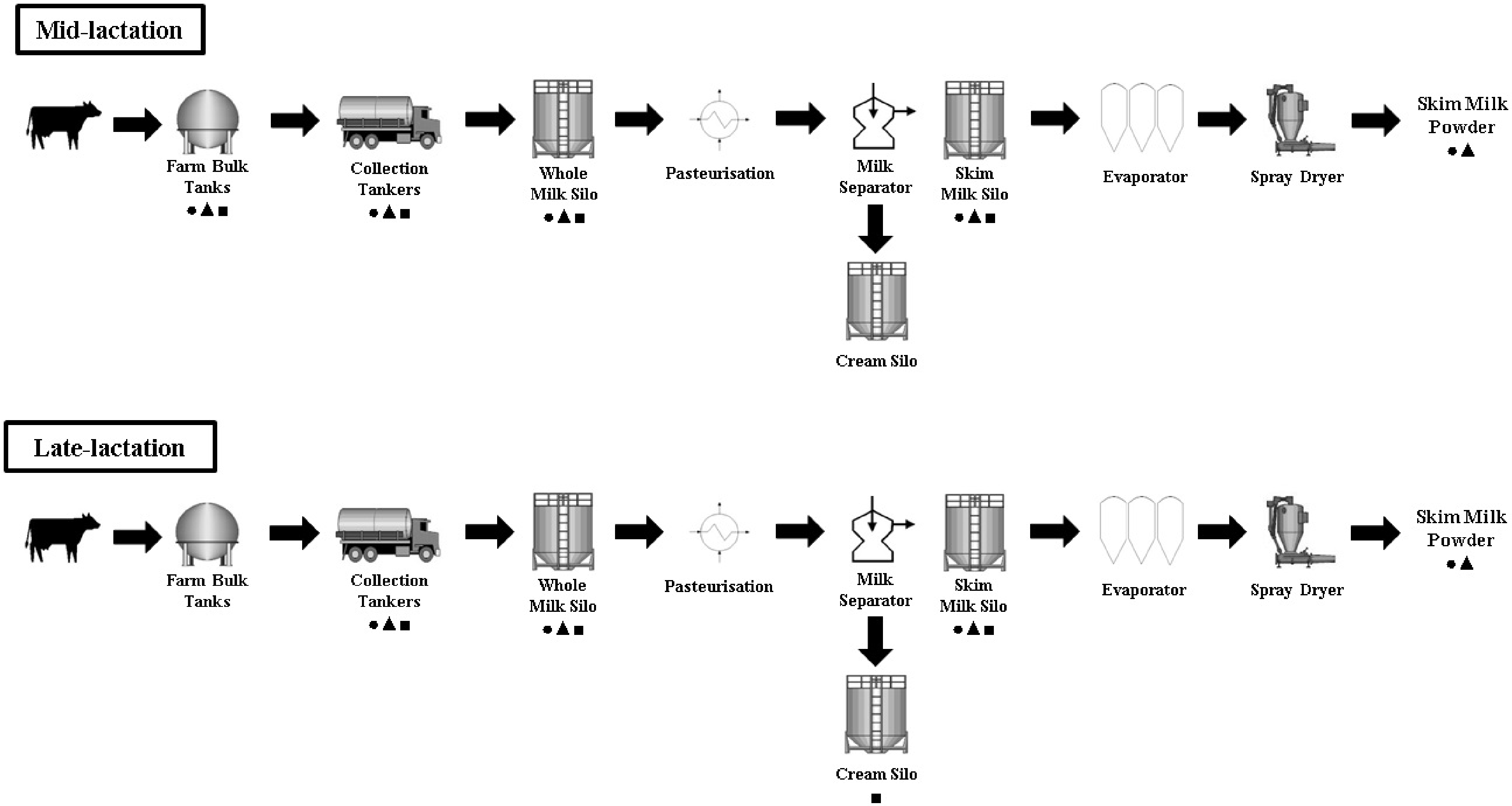

Figure S1. Milk supply chain and manufacturing process for conversion to low-heat skim milk powder, conducted in the mid- and late-lactation periods. The sampling points for chlorate (CHLO) and perchlorate (PCHLO), iodine and trichloromethane (TCM) are indicated with a ●, ▲and ■, respectively.