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

3

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

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29   **Summary**

30   The experiments reported in this research paper aimed to investigate differences in the levels

31   of chlorate (CHLO), perchlorate (PCHLO), trichloromethane (TCM) and iodine residues in

32   bulk tank (BT) milk produced at different milk production periods, and to monitor those

33   levels throughout a skim milk powder (SMP) production chain (BTs, collection tankers

34   [CTs], whole milk silo [WMS] and skim milk silo [SMS]). Chlorate, PCHLO and iodine

35   were measured in SMP, while TCM was measured in the milk cream. The CHLO, TCM and

36   iodine levels in the mid-lactation milk stored in the WMS were lower than legislative and

37   industrial specifications (0.0100 mg/ kg, 0.0015 mg/ kg and 150 µg/ L, respectively);

38   however, in late-lactation, those levels were numerically higher than the mid-lactation levels

39   and specifications. Consequently, CHLO and iodine levels in SMP were numerically higher

40   in late-lactation than in mid-lactation. Trichloromethane accumulated in the cream portion

41   after separation. Perchlorate was not detected in any of the samples. Regarding iodine, the

42   levels in mid-lactation reconstituted SMP were higher than that required by manufacturers

43   (100 µg/ L), indicating that the levels in milk should be lower than 142 µg/ L. The higher

44   residue levels observed in late-lactation could be related to the low milk volume produced

45   during that period and changes in sanitation practices, while changes in feed management

46   could have affected iodine levels. This study could assist in controlling and setting limits for

47   CHLO, TCM and iodine levels in milk, ensuring premium quality dairy products.

48

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

50

51   International markets are setting high specifications for milk and dairy product quality,

52   including stringent guidelines on concentrations of residues that could occur in milk.

53   Potential milk contaminants of most concern include chlorate (CHLO, ClO_3^-), perchlorate

54   (PCHLO, ClO_4^-) and trichloromethane (TCM, $CHCl_3$), which arise as a consequence of

55   sanitation with chlorine products.

56   Chlorate and PCHLO were reported to result in thyroid dysfunctions (EFSA, 2015), while

57   TCM could possibly be carcinogenic to humans (ICAR, 1999). There are a few studies

58   available that have discussed contributing factors on-farm (Gleeson *et al.*, 2013; Ryan *et al.*,

59   2013); however, the dynamic of residue concentrations when subjected to different milk

60   processing conditions is not fully understood. Sodium hypochlorite, chlorine gas or dioxide

61   may be used for the sanitation of water, while chlorine-based detergents are used for the

62   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

97 different milk production periods (mid- and late-lactation). Chlorate, PCHLO and iodine

98 were measured throughout the production stages of skim milk powder (SMP), while TCM

99 was measured throughout the production stages of milk and cream, which were destined for

100 butter manufacture. The second objective was to investigate differences in residue levels in

101 bulk tank (BT) milk produced during mid- and late-lactation. The milk used in this study was

102 produced on commercial dairy farms and processed in a commercial SMP processing plant.

103

104 **Materials & Methods**

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

106 In Ireland a seasonal spring-calving production system is practiced, with all cows calving

107 within a 10-week period approximately (February to April). This experiment was performed

108 on one occasion during each of mid- (May; 80 DIM) and late-lactation (December; 290

109 DIM). The farms that supplied milk to the factory (mid-lactation: 67 farms; late-lactation:

110 150 farms), milk storage conditions on-farm, amount of milk produced, milk collection and

111 the skim milk powder manufacturing process was the same as described by Paludetti *et al.* (in

112 press). A schematic drawing of the SMP manufacturing process is shown in supplementary

113 Figure S1.

114 In mid-lactation, samples were collected at various points of the manufacturing process

115 between the farm BTs and the SMP [BTs, collection tankers (CTs), whole milk silo (WMS),

116 skim milk silo (SMS) and final SMP] and were tested for CHLO, PCHLO and iodine. In late-

117 lactation, samples were collected at various points between the CTs and the SMP [CTs,

118 WMS, cream silo (CS), SMS and final SMP] and were tested for CHLO, PCHLO and iodine.

119 In both lactation periods, TCM was quantified in all samples, with exception of the SMP

120 samples (supplementary Figure S1). Due to the high number of farms (150) necessary to

121 supply sufficient milk volume to undertake the manufacturing process in late-lactation

122 (December), it was not possible to undertake collection and analysis of all individual BT

123 samples. The collection of samples and preparation of SMP samples for analysis were

124 performed as described by Paludetti *et al.* (in press).

125

126 *Comparison between the residue levels in the same 67 farm bulk tanks in mid- and late-*

127 *lactation (May and November)*

128 The concentrations of CHLO, PCHLO, TCM and iodine residues were measured in raw milk

129 produced on the same 67 dairy farms sampled in mid-lactation (May, 80 DIM) and in late-

130   lactation period (November; 260 DIM), to investigate the effect of milk production period on

131   residue levels. Milk samples were collected as described by Paludetti *et al.* (in press).

132

133   *Quantification of chlorate and perchlorate*

134   The quantification of CHLO and PCHLO was performed by high-performance liquid

135   chromatography coupled to tandem mass spectrometry (LC/ MS-MS) with ESI electrospray

136   ionisation in negative mode (-ESI). The mid-lactation milk and SMP samples, as well as the

137   67 late-lactation farm BT samples, were analysed in the laboratory of Labor Friedle GmbH

138   group (Labor Friedle GmbH, Von-Heyden-Straβe 11, D-93105, Tegernheim, Germany),

139   while the late-lactation samples from the factory (CT, WMS, SMS and SMP samples) were

140   analysed in Teagasc Ashtown (Dublin, Ireland). The methodologies used are based on the

141   procedures described in the European Quick Polar Pesticides method (QuPPe) (EURL-SRM,

142   2015). In the present study, some of the milk samples were analysed by both laboratories and

143   the results were statistically similar ($P > 0.05$). The detection limit of CHLO and PCHLO in

144   milk was 0.0010 mg/ kg and in SMP was 0.010 mg/ kg.

145

146   *Quantification of trichoromethane*

147   Trichloromethane was quantified in the milk using static head-space gas chromatography

148   (HS-GC) with electron capture detector (ECD), fitted with a low thermal mass system (LTM)

149   (Agilent 7890A, Agilent Technologies, Santa Clara, California, USA). The trichloromethane

150   detection limit in this analysis was 0.0001 mg/ kg. The methodology applied was an adaption

151   of the procedure of Resch & Guthy (1999). This analysis was performed in the Milk Quality

152   laboratory in Teagasc Moorepark (Fermoy, Co. Cork, Ireland).

153

154   *Quantification of iodine*

155   Iodine was quantified in milk and reconstituted SMP samples using inductively coupled

156   plasma mass spectrometry (ICP-MS), using an Agilent ICP-MS 7700x (Agilent

157   Technologies, Santa Clara, California, USA). The methodology used was based on the

158   procedures described in the standard method for the determination of iodine compounds in

159   foodstuffs (BS EN 15111:2007, 2007). Standard solutions of Tellurium and 1% TMAH were

160   used to obtain a calibration curve. The limit of detection was 1.31 µg/ L. The mid-lactation

161   milk and SMP samples were analysed in Teagasc Moorepark (Fermoy, Co. Cork, Ireland),

162   while the late-lactation milk and SMP samples were analysed in FBA laboratories

163 (Capoquinn, Co. Waterford, Ireland). Those laboratories used the same methodology and

164 samples analysed by both laboratories had statistically similar results (P>0.05).

165

166 *Statistical analysis*

167 *Influence of individual farm milk volumes on the residue concentration in each CT and*

168 *influence of CT milk on the residue concentration in WMS*

169 The statistical analyses were performed using the software SAS 9.3 (SAS Institute, 2016). In

170 mid-lactation, the iodine and TCM concentrations of each CT were predicted using the

171 volume and iodine or TCM concentrations measured in the milk of all farms that supplied

172 each respective CT. In mid- and late-lactation, the iodine and TCM concentrations in the

173 WMS were also predicted using the volume and iodine or TCM concentrations in the milk of

174 all CTs that supplied that silo. Those predictions were calculated as volume-weighted means

175 with estimated confidence intervals. The actual iodine or TCM concentrations measured in

176 each CT and WMS samples were compared to the respective confidence interval for those

177 predicted means. Agreement plots were also used to check for bias in the relationship

178 between actual and predicted means. It was not possible to perform the same analyses with

179 the CHLO and PCHLO results, due to the low number of samples in which those residues

180 were detected.

181

182 *Comparison between the residue levels in the same 67 farm bulk tanks in mid- and late-*

183 *lactation (May and November)*

184 Differences between the adjusted least square means of the 67 mid- and late-lactation milk

185 samples, collected in May and November, were calculated using the MIXED procedure in

186 SAS 9.3 (SAS Institute, 2016). The fixed effects included in each model were lactation

187 period (mid- and late-lactation) and farms (numbered from 1 to 67). Farms were considered

188 the experimental unit and the response variable was iodine or TCM. Residual checks were

189 made to ensure that the assumptions of the analysis were met.

190 It was not possible to statistically determine the differences between CHLO and PCHLO

191 levels measured in mid- and late-lactation milk samples, due to insufficient number of

192 samples in which those residues were detected. McNemar's test was applied to compare the

193 number of BT milk samples in mid- and late-lactation that had CHLO and TCM

194 concentrations $\geq$ 0.0010 and 0.0015 mg/kg, respectively. The GLM procedure was used to

195 determine the regression relationship between CHLO and TCM concentrations.

196

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

230 numerically similar. The mean TCM concentrations of the milk samples from the CTs and
231 WMS were also numerically similar in both mid- and late-lactation.

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

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

241

242 *Iodine*

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

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

257

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

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

264   The volume-weighted mean TCM concentration was significantly higher in late-lactation

265   (0.0015 ± 0.0014 mg/ kg; range: 0.0003 to 0.0074 mg/ kg) than in mid-lactation ($0.0009 \pm$

266   0.0008 mg/ kg; range: 0.0002 to 0.0043 mg/ kg) ($P < 0.0001$).

267   The volume-weighted mean iodine concentrations of the BT samples in mid- and late-

268   lactation (142.2 ± 129.2 and 119.7 ± 151.6 µg/ L, respectively) were not statistically different

269   ($P = 0.63$).

270

271   **Discussion**

272   *Residues related to the use of chlorine*

273   Concentrations of CHLO and PCHLO were monitored throughout the production chain of

274   SMP in mid- and late-lactation (May and December, respectively). In Europe, a default

275   threshold limit of 0.0100 mg/ kg of CHLO and PCHLO is applied for milk (EC no

276   396/2005). In mid-lactation (May), the volume-weighted mean CHLO concentration in the 14

277   BTs and 6 CTs (in which CHLO was detected) were lower than that limit; however, in late-

278   lactation (December), the mean CHLO concentration of the 6 CTs (in which CHLO was

279   detected) was higher than the EC limit and higher than the volume-weighted mean

280   concentration in mid-lactation.

281   In mid-lactation, the CHLO concentrations in each of the CTs could have been diluted as

282   CHLO was not detected in 53 of the BTs. For example, CHLO was not detected in 4 CT milk

283   samples, as only one of the BT milk volumes contributing to each of those CTs contained

284   CHLO. Additionally, CHLO was not detected in most of the BT milk supplied to the 6 CTs

285   in which CHLO was detected, indicating that the sanitation of those CTs could possibly have

286   influenced the CHLO levels. In both mid- and late-lactation, as CHLO was not detected in

287   most of the CT milk volumes, the CHLO concentrations could have also been diluted in the

288   WMS; therefore, it is likely that the sanitation practices of the silos did not influence the

289   CHLO levels. Consequently, the mean CHLO concentrations in the WMSs were lower than

290   the EC limit of 0.0100 mg/ kg. However, as the milk supplied to the factory during late-

291   lactation contained higher levels of CHLO than the mid-lactation milk, the CHLO levels in

292   the WMS in late-lactation were higher compared to mid-lactation; consequently, the CHLO

293   levels in the SMP were higher in late-lactation than in mid-lactation.

294   In mid-lactation, the mean CHLO concentration of the SMP samples was lower than the limit

295   applied by some Irish infant formula manufacturers (0.100 mg/ kg). The difference of 0.0016

296   mg/ kg between the mean CHLO concentration of the SMP samples collected at the end and

297   start of the spray-drying run, indicated that the sanitation of the spray-dryer could have

298 contributed to the CHLO levels in SMP. The interior surface of the spray-dryer could have

299 contained residual CHLO, and the majority of that residue was transferred to the first batch of

300 evaporated skim milk that entered the equipment. In late-lactation, the mean CHLO

301 concentration of the SMP samples was higher than 0.100 mg/ kg, indicating that the CHLO

302 level in the bulk milk stored in the WMS should had been lower than 0.0025 mg/ kg. Even

303 though no variations in the CHLO concentration were observed in SMP samples collected

304 throughout the spray-drying run in late-lactation, sanitation practices of that equipment could

305 have also contributed to the increased CHLO levels in SMP. Additionally, the variations in

306 CHLO concentrations throughout the spray-drying run that were observed in mid-lactation

307 and not observed in late-lactation could be due to differences in the sanitation practices

308 between production periods.

309 The concentrations of TCM were also monitored throughout the production chain of SMP in

310 mid- and late-lactation (May and December, respectively). There are no European regulations

311 that have defined a standard TCM limit for milk or dairy products; however, Irish dairy

312 processors apply a limit of 0.0015 mg/ kg to milk destined for the production of lactic butter

313 which should have less than 0.0300 mg/ kg of TCM, as required by the export market (Ryan

314 et al., 2013). In mid-lactation, the mean TCM concentrations of the BTs, CTs and WMS were

315 all lower than that limit; while, in late-lactation, the mean TCM concentrations of the CTs

316 and WMS were higher than that limit and higher than the concentrations in mid-lactation.

317 The agreement between the TCM concentrations of each mid-lactation CT sample and the

318 contributions of each BT milk volume supplied, as well as the agreement between the TCM

319 concentrations of the WMS samples and the contributions of each CT in both lactation

320 periods, indicated that the cleaning protocol of the CTs or WMS did not contribute to any

321 increases in the TCM levels in milk (Tables S1 and S2).

322 In both lactation periods, the decrease in the TCM concentrations in the SMS in relation to

323 the WMS was expected, due to the accumulation of TCM in the cream during separation

324 (Hubbert *et al.*, 1996; Table 1). As the levels of TCM were higher in late-lactation milk, the

325 TCM concentration in late-lactation cream was possibly higher than the levels expected in

326 cream produced with mid-lactation milk.

327 The concentrations of CHLO and TCM were also monitored in the same 67 farm BTs in mid-

328 and late-lactation (May and November, respectively) to investigate if those concentrations

329 could differ in milk produced by the same farm during different production periods. None of

330 the mid-lactation BT samples contained CHLO levels higher than 0.0100 mg/ kg (EC limit),

331 while 5 late-lactation BT samples contained levels higher than that limit. In relation to TCM,

10

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 ($1,683 \pm 1,031$ 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

11

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,

400 which contained at least 10 and a maximum of 43 mg of iodine/ kg of ration. Therefore, the
401 iodine intake from ration per cow on those farms was likely to be higher than that
402 recommended (10 mg per cow per day), as the average ration intake on those farms was 2.5
403 kg per cow per day. Other factors that were not included in the questionnaires could have
404 contributed to iodine levels in milk such as grass, boluses, mineral-supplemented water and
405 mineral licks. Furthermore, according to the questionnaires, five and two farms that were
406 using iodine-based teat disinfectants supplied milk with iodine levels higher than 150 µg/ L,
407 in mid- and late-lactation, respectively. O'Brien *et al*. (2013) also observed increases in the
408 iodine levels in milk when applying those teat disinfectants post-milking. Those increases are
409 associated with the absorption of iodine through the teat skin, particularly if pre-milking teat
410 preparation is not being conducted.

411

Conclusion

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

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564 **Table 1.** Mean (± SD) chlorate (CHLO), trichloromethane (TCM) and iodine concentrations in samples collected from the farm bulk tanks

565 (BTs), collection tankers (CTs), whole milk silo (WMS), skim milk silo (SMS), cream silo (CS) and samples of skim milk powder (SMP) from

566 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)

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

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

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

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

571 ¶ Results for non-reconstituted skim milk powder

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

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

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603 **SUPPLEMENTARY FILE**
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Materials & Methods

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

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

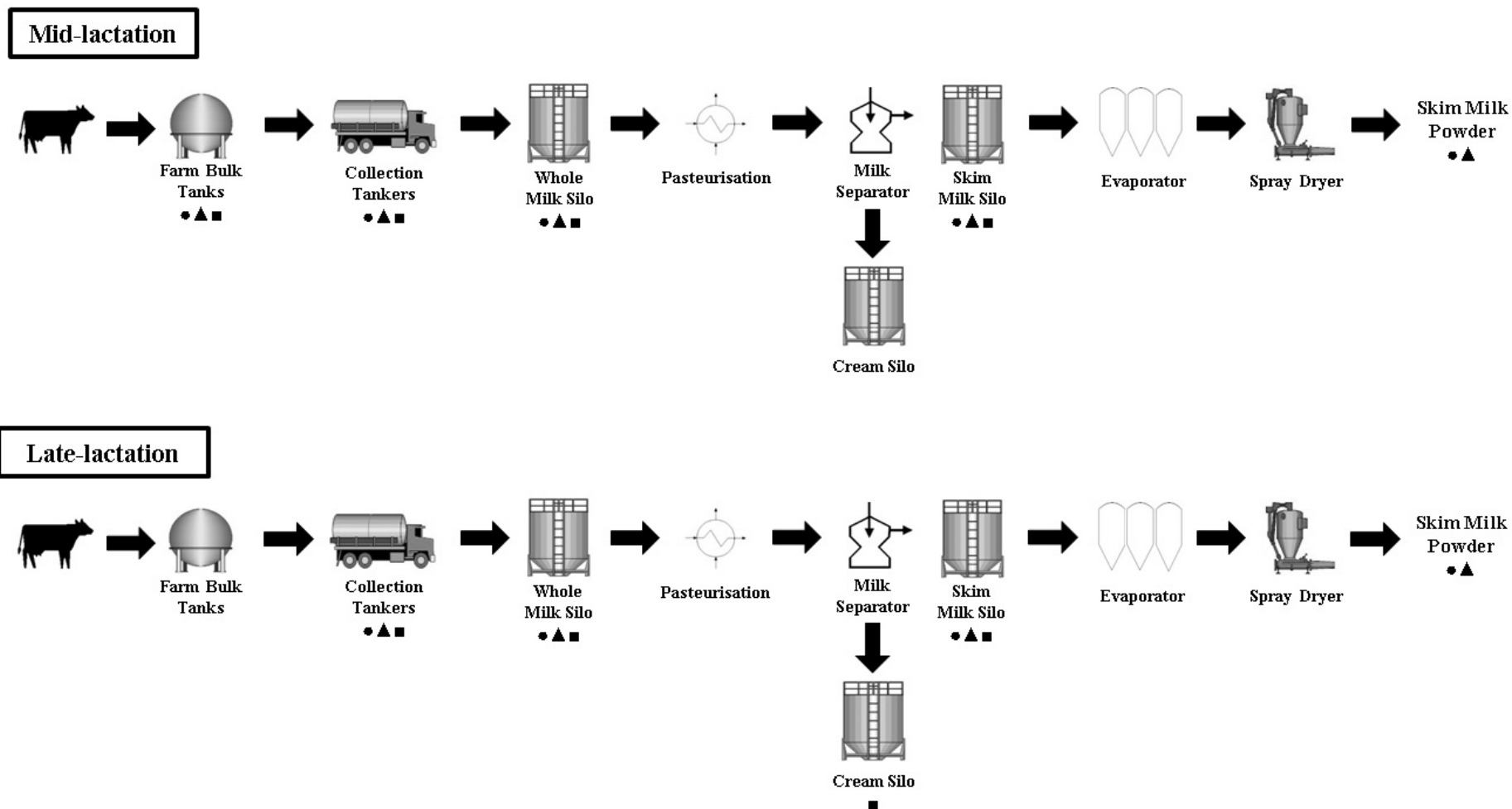‡ Confidence interval (CI), lower (LCL) and upper (UCL) confidence limits.

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.