Lecture
Focus on Residues

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Focus on residues relevant to milk and dairy products

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Teagasc
Agriculture and Food Development Authority

Focus on residues

- Introduction
- Iodine
- TCM - Trichloromethane
- Chlorates
- NBPT - Protected urea
- QACs - Quaternary ammonium compounds
- Phthalates
- POEAs - PolyOxyEthylene Amines
- PFAS - Perfluoroalkylated substances
- Future trends and conclusion

Introduction:

- Milk on its journey from cow to consumer is exposed to various sources of residues
- Some residues are potentially harmful if present at significant levels, others impact product quality - perceived by consumers/ regulators
- Significance of residues is evident now - complex legal, regulatory and guiding recommendations on acceptable levels in dairy products
- Supported by highly sensitive detection methods which can detect residues at low limits of quantification
- Research now focusing on sources of different residues, their entry into milk/dairy products and strategies for their mitigation
- Many have potential for accumulation within dairy products such as butter and milk powders (often used as ingredients in infant milk formula)

Why is milk iodine a quality issue

- Ireland is one of leading infant formula manufacturers worldwide
- Market has significant potential for growth
- Two mechanisms :
  - Milk powder as an ingredient can be sourced abroad
  - Milk powder can be produced and sourced at home
    - Preferable; Needs to have correct levels and balance of minerals including iodine
- Target for iodine in milk powder as an ingredient in IMF :
  - 100 μg iodine/ 100g powder
  - equates to <150μg iodine/kg milk
- Difficult to source at times of year
- Iodine is an essential trace element - humans and animals

International recommendations on iodine requirements for cows: 0.5mg/kg DM intake or approximately 10-12 mg/cow/day

Iodine sources that can lead to high milk iodine

1. Concentrate feed

- Early 1990s – 139 µg iodine/kg milk; 97% of pasture samples – subnormal iodine levels
- Recommended adequate supplementation of iodine
- 12-60 mg of iodine /cow per day
  - ~12 mg of iodine /cow per day advised for routine continuous use
  - ~60 mg of iodine /cow per day advised for national use in a 5-month mineral programme for deficient dairy cows

2. Teat disinfection

- Used routinely on-farm post-milking and sometimes pre-milking
- Pre-milking disinfection poses substantial risk of transfer to milk
- Dependent on the degree of removal from the teats prior to cluster attachment
- Some processors recommend not to use iodine for this purpose

High iodine intake risks

- Cows fed high concentrate levels with high levels of iodine added
- Iodine routinely added to feed rations at **5-30 mg/kg**
- Cows typically fed 3 kg/cow/day in early lactation or higher – can potentially receive **15-90 mg/cow/day**
- Level of iodine in feed set a/c to the volume fed – but when actual feed levels > planned feed levels – then excessive iodine intakes

Cows require 0.5mg/kg DM/day = 10 mg iodine/day	2 kg meal with 3 mg/kg iodine	= 6.0 mg iodine contributed by meal
	Cow intake at grass is 18kg DM/day	= 4.5 mg iodine contributed by grass

Conclusions

- Recommended supplementation level is 0.5 mg/kg DM/cow/day or ~10 – 12 mg/kg/cow/day
- Research has indicated high iodine levels in meal – now being addressed
- Excess iodine ingested through feed is excreted in milk and urine
- Seasonal problem in Ireland; early lactation spring and winter milk production
- Milk iodine level is most important in areas where milk is destined for IMF
- At a limit of 150 µg/kg – little flexibility
- Methodology now set up at Moorepark – ICPMS
- Supplement more precisely to meet requirement – H20

Chlorine residues - Context

Significant work in terms of maintaining the national milk supply suitable for product manufacture from a residue perspective

Two residue standards: TCM and chlorate

Critically important standards – health related:

TCM – butter - carcinogenic issues

Chlorate - chlorate associated with the inhibition of iodine uptake in humans with infants and young children identified as a high-risk demographic - particular concern where milk powder is an ingredient to IMF

TCM: by-product formed when chlorine containing disinfectant is used for cleaning MCS

TCM can form and accumulate in milk fat and butter

TCM development (Trichloromethane)

- TCM is a by-product of the disinfection process when chlorine is used
- Chlorine – very hygienically effective, cost effective and efficient disinfectant
- **BUT** if milk is not rinsed from milk contact surfaces before the detergent-sterilizer containing chlorine is used
- Chlorine binds to the milk - total organic chlorine
- Clear, colourless and volatile
- Accumulates in fat portion of milk and fat rich product
- **Target level in butter = 0.024 mg/kg or less**
- Fat association – concentration in fat from milk to butter
- **» Target level in milk = 0.00124 mg/kg**
- Need to meet this to maintain good markets, e.g. German market

TCM target levels

Initially: <0.002 mg/kg for milk and <0.03 mg/kg for butter (competitiors; 0.07 mg/kg in Ire)

5-6 years ago: <0.00155 mg/kg in milk and 0.027 mg/kg for butter

More recently: 0.00124 mg/kg in milk and 0.024mg/kg in butter

Teagasc approaches to addressing the problem

1. Chemical analysis of detergent-sterilizer products
    1. Independent testing of chlorine containing cleaning products –strengths of the working solutions of chlorine – found many of them too strong – too much chlorine
    2. Web page set up to inform industry on different detergent products based on chemical analysis
2. TCM analysis
    1. Set up of Methodology
    2. Developed test – GC – link with MULA in Germany
    3. Static HS-GC with electron capture detector was used
    4. Current annual sample throughput = up to 42,000 samples annually
    5. Identified source of the problem at farm level
    6. Funded by Ornua/ 7 milk processors
    7. Three GC machines; two technicians
    8. Meeting chaired by Teagasc and Ornua, 4 occasions/year

3. Knowledge transfer and liaising with processor and suppliers to correct TCM problems on-farm

- Farm visits together with report and advice on solving problem
- Continuous communication of results and information with milk quality personnel and engagement and presenting results and advice to stakeholders :
- Dairy farmers; Milk quality advisors of processing plants; Service providers to farmers, e.g. bulk tank technicians; Detergent companies; Teagasc advisors;

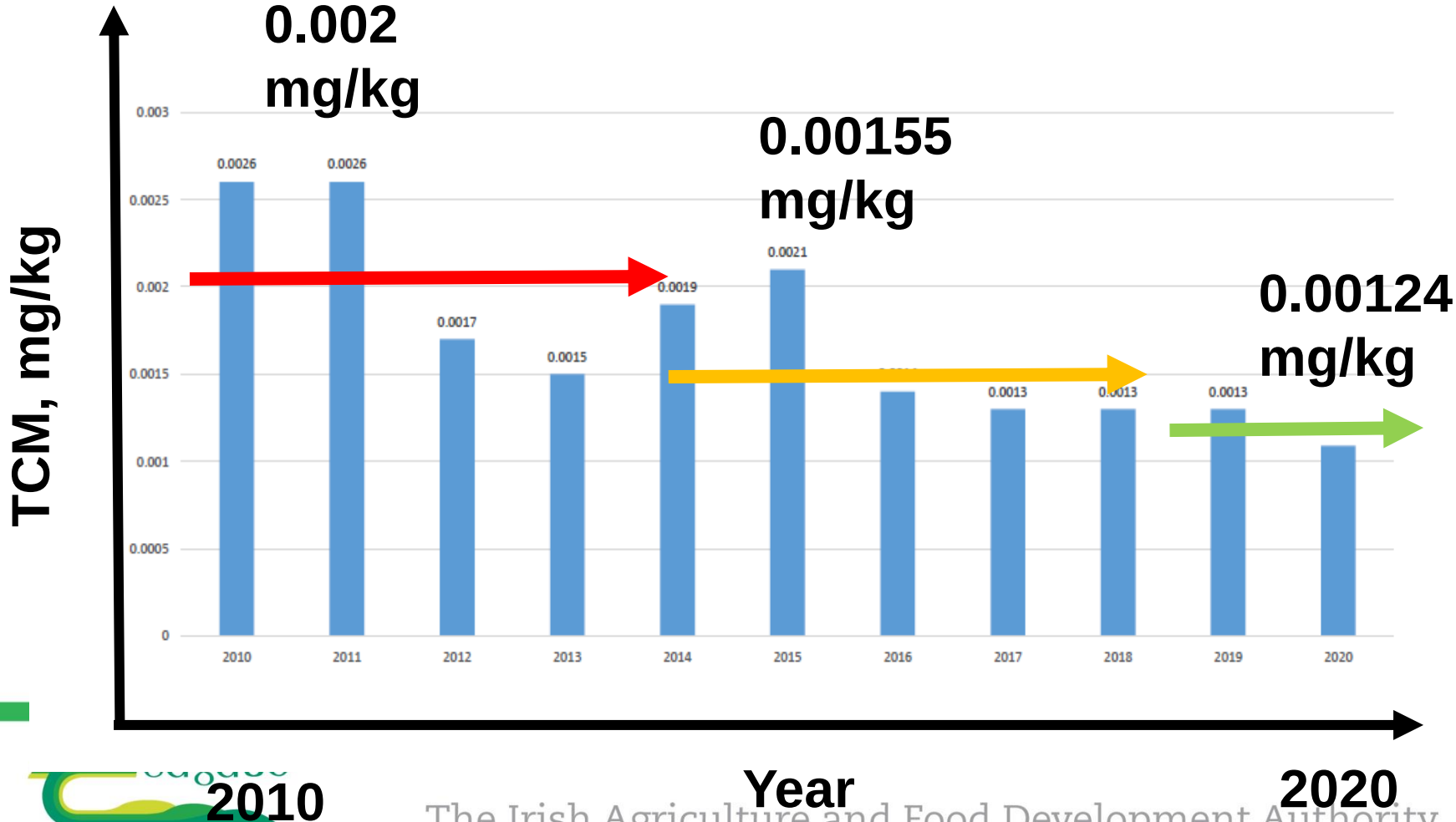
Strategy for control

1. All suppliers sampled approximately twice per year
2. Samples analysed
3. Results back to processors within 6-day timeline
4. Processor personnel work with farmer to solve an issue
5. Communication through milk quality meetings with processing industry, technical personnel; written articles in popular press.

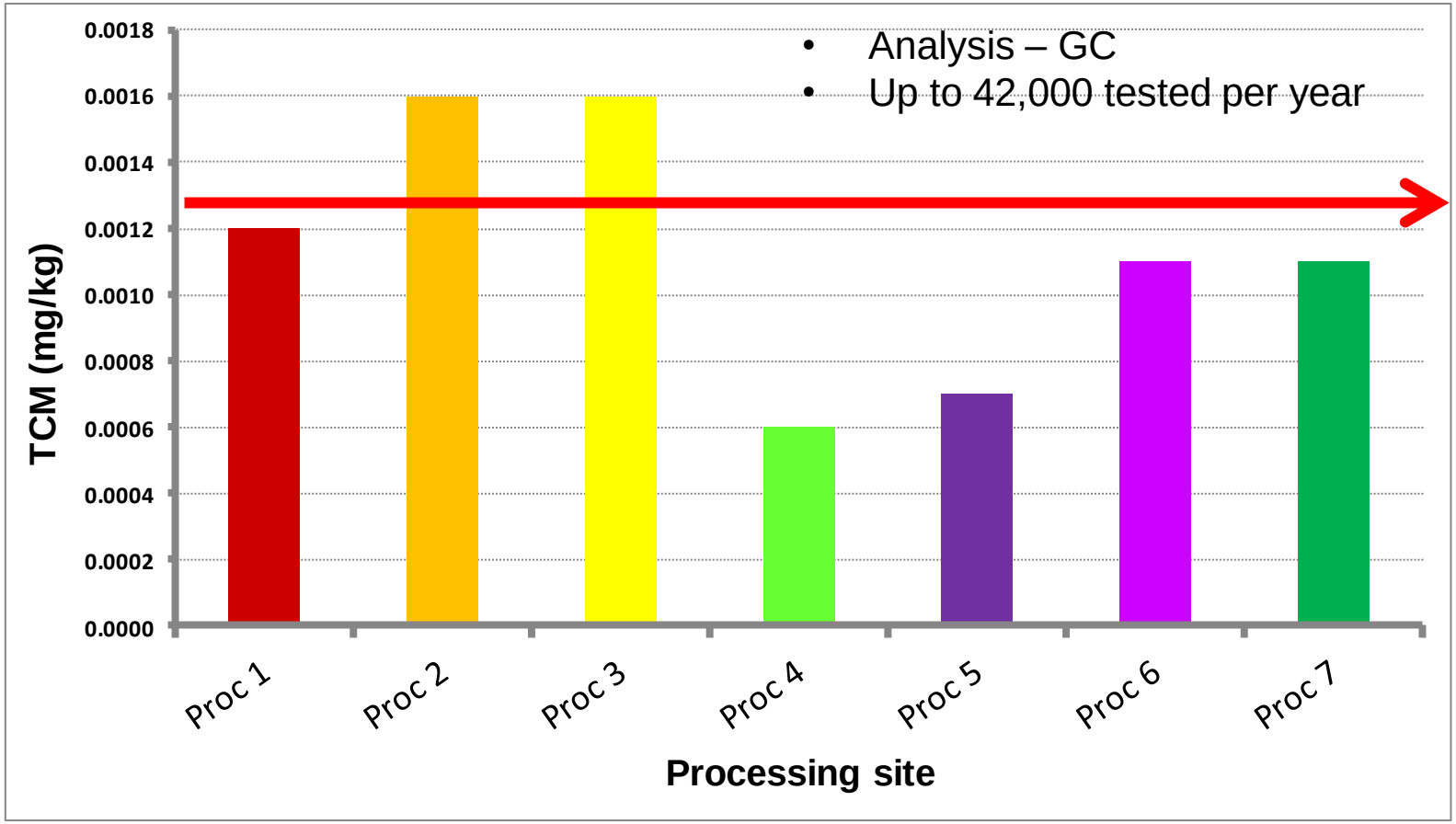
4. Research - Controlled experiments on reducing TCM/ Cl in milk while maintaining good micro quality

- Laboratory, milking parlour and commercial farm based studies, e.g. rinse water volumes; washing protocols

TCM Industry Combined Mean 2010-2020 (milk)

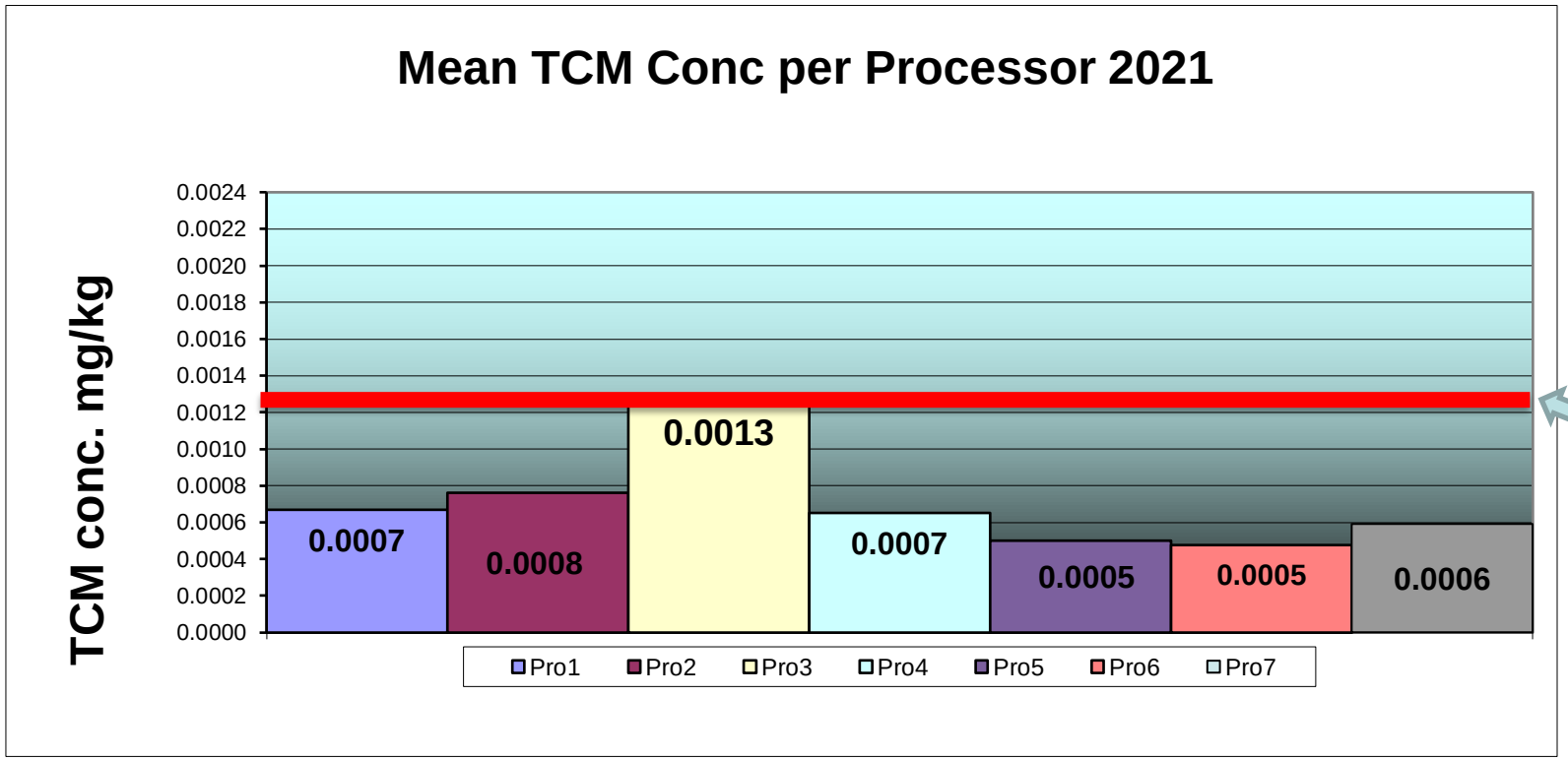


Developed a test for TCM
Milk TCM levels across different processors in 2020



Raw Milk Results 2021 (Jan –Nov)

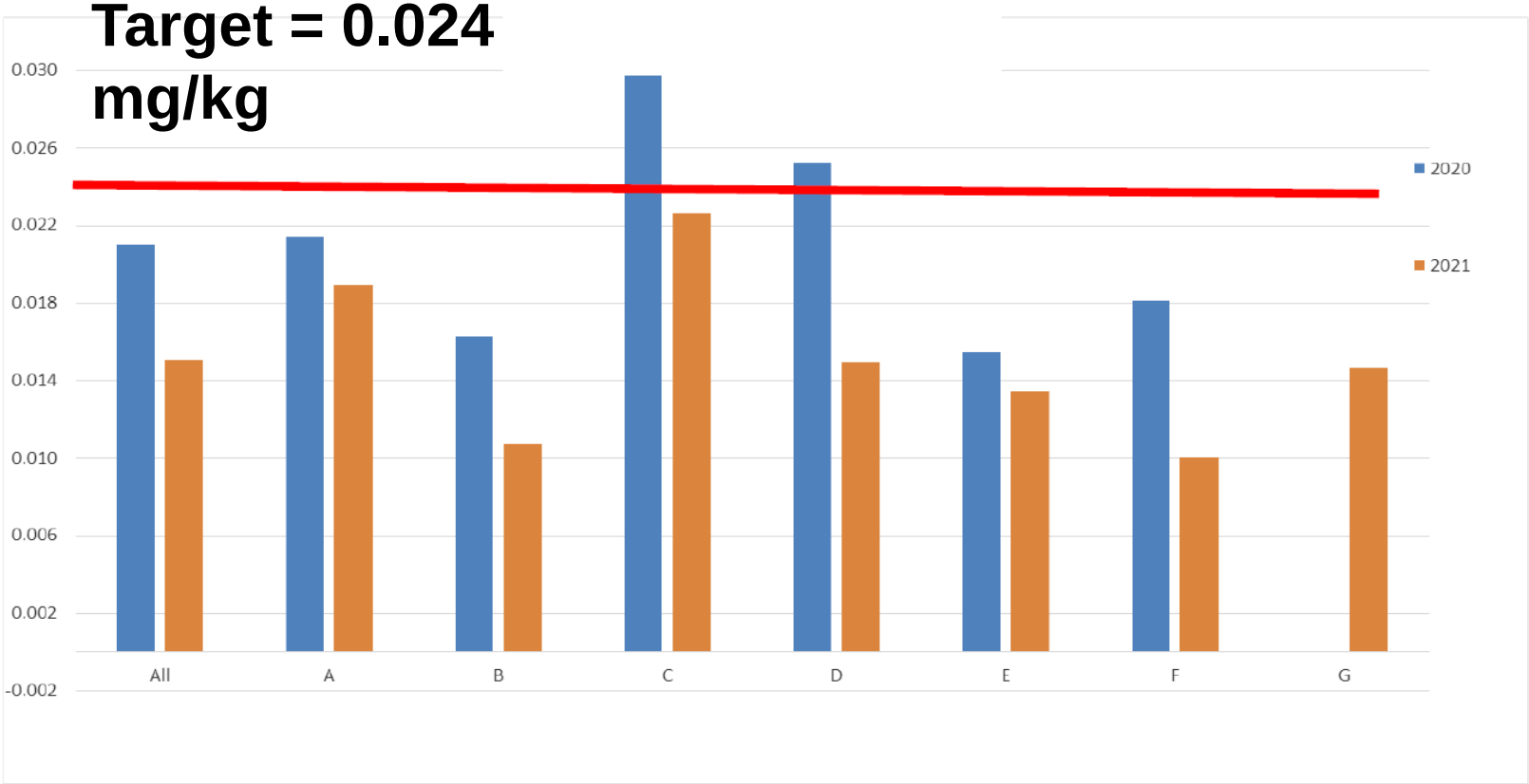
Industry Average = 0.0007mg/kg
Weighted Average = 0.0007mg/kg



N =1907 N =3132 N =1551 N =9393 N =819 N =6072 N =11217

TCM levels in Butter 2020 - 2021

2020
2021



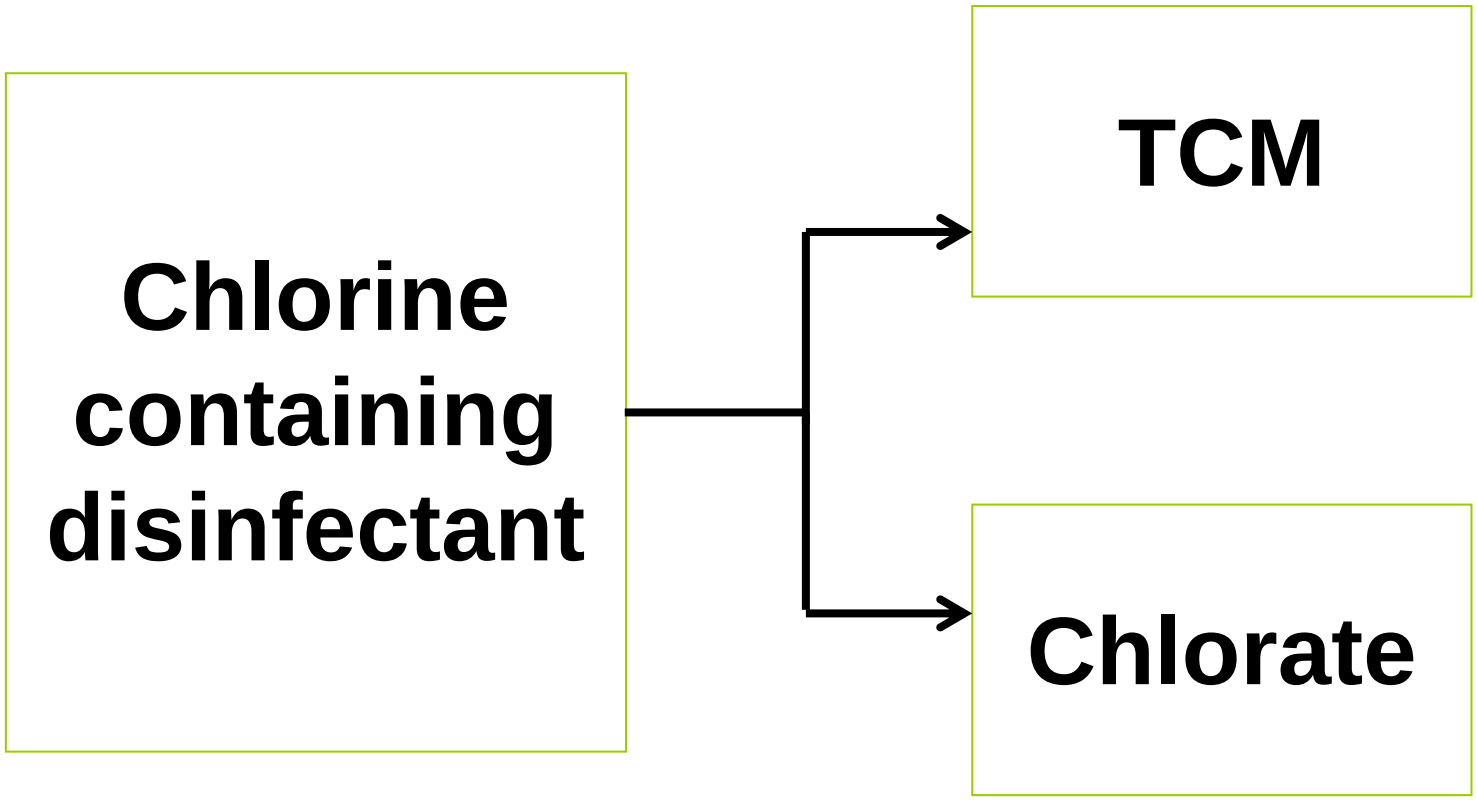
Main causes of high TCM

- Cleaning products had too high chlorine levels
- Improper usage of the chlorine containing cleaning product
- Insufficient rinsing of milking equipment after the wash cycle

Critical control points to maintain low TCM

- Rinse volume of water after milking and after detergent-sterilizer wash cycle
- Chlorine content of detergent-sterilizer and volume used
- Recycling of detergent-sterilizer solution
- Use of peracetic acid in place of chlorine for addition to rinse water or for cluster cleaning
- TCM residue has now largely been corrected as a consequence of the moratorium on the use of chlorine-based cleaning agents
- But it is absolutely critical that microbial hygiene and quality is not compromised

Chlorates



Chlorate formation

Oxidation reactions – chlorate formed during degradation of hypochlorite solutions

Chlorate formed is impacted by % chlorine of the solution; storage time; storage temperature and pH

A maximum residue level (MRL) of 0.01 mg/kg for chlorate applied to foods

Chlorate in milk and dairy products arises from the use of (i) detergents containing chlorate for cleaning of equipment and (ii) chlorinated water during processing

Processors have now converted to treatment of water by chlorine gas

Chlorine removal from washing protocols

Main precautions:

- A quick turnover of sodium hypochlorite storage
- Storage of hypochlorite solutions at a lower temperature
- Dilution of stored hypochlorite solutions on delivery
- Choose products with the recommended level of chlorine
- Use adequate volumes of water for rinsing
- Avoid teat disinfectants that contain chlorine dioxide/chloride

But lower levels of chlorate are now required in milk destined for IMF (0.001mg/kg) and lower levels of TCM required – new target of 0.00124mg/kg

So REMOVAL of chlorine is now the strategy

- But this requires strict adherence to new cleaning protocols, greater use of hot water, use of acids, etc
- **Constant vigilance of microbiological quality is critical**

Chlorate standards

Chlorate in milk detected at 0.002 mg/kg

But can be unsuitable for processing at this level e.g. high nutrition powders - chlorate levels of <0.01 mg/kg reqd in the final product e.g. infant and follow-on formulae

e.g. milk containing 0.002 mg/kg of chlorate, spray dried into powder (10 fold concentration), powder would contain 0.02 mg/kg of chlorate – thereby, outside of specification

Chlorate analysis

Teagasc Ashtown

Chlorates quantified using ultra performance liquid chromatography coupled with tandem mass spectrometry (UPLC –MS/MS)

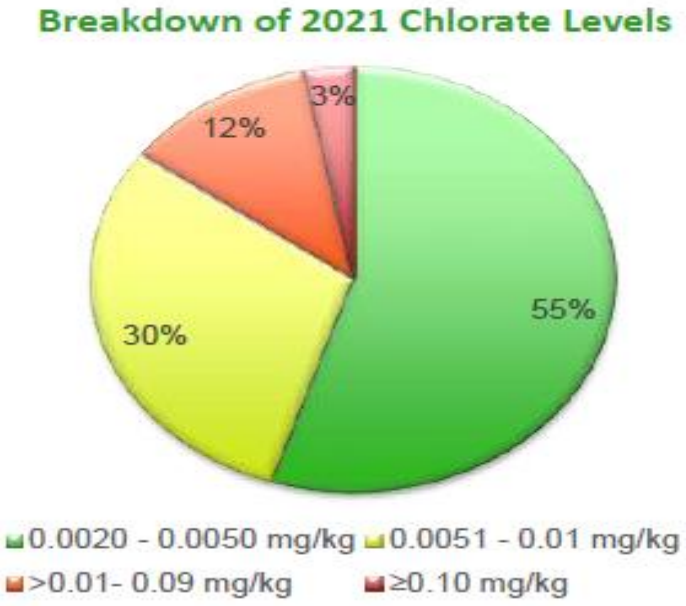
Analysed approximately 3,500 bulk milk samples for chlorate across 2020 & 2021.

7% reduction in the number of bulk milk samples with detected chlorate was observed in 2021 relative to 2020

Percentage of samples with detected chlorate in 2021 - 7% less than in 2020

2021

In 2021 **8%** of samples analysed displayed detectable levels of chlorate (≥0.0020 mg/kg).



Key point : 92% of samples had non - detectable levels, i.e. <0.002 mg/kg)

Chlorine free strategy

Lower chlorate levels now required in milk destined for IMF (0.001mg/kg) and lower TCM required – new target of 0.00124mg/kg

So REMOVAL of chlorine is now the strategy –since January 2021

BUT this carries a real risk of a deterioration in the microbiological quality of milk

Constant vigilance of microbiological quality is critical

Whole washing area needed to be re-designed.

New cleaning protocols developed with guidelines on how to use them

Examined on research and commercial farms

Chlorine-free cleaning has been found to be effective when carried out correctly

Chlorine- free washing protocols

5 chlorine-free cleaning protocols developed and tested

Correct use is crucial - ensure low TBC and Thermodurics

FIRM funded project

Teagasc and MTU; PhD student

Main focus:

Baseline levels of chlorate in Irish milk and dairy products on sale

Effectiveness of alternative non-chlorine based cleaning protocols and the potential risk of being a source of any alternative residues

The impact of water chlorination on chlorate levels

- What levels of chlorate in water cause a chlorate or TCM problem in milk
- Are those levels of chlorate prevalent in farm water supplies

N-(n-butyl) thiophosphoric triamide (NBPT)

- A urease inhibitor utilised in urea-based fertilizers
- NBPT enables efficient utilisation of urea by delaying urea hydrolysis and thereby reducing ammonia losses
- Fertilizer treated with NBPT is applied to pasture to mitigate both ammonia and nitrous oxide emissions, but concerns arise as to the potential for residues in milk products
- NBPT might transfer from pasture, through the cow and into milk
- Suggested as Category 3 substance capable of causing fertility issues
- A field study investigated potential transfer of NBPT residues into milk

Dairy farm experiment using protected urea as the fertiliser N source (NBPT farm) and a commercial farm that was not using a protected urea (Negative Control farm) Source: Nkwonta et al., 2021. Molecules, 26, 2890.

Teagasc investigation of NBPT residue in milk:

Bulk milk

- Bulk milk from herd at Johnstown Castle Feb 2020 to Feb 2021
- The average N rate for the overall herd was 181 kg N/ha/year
- All N was applied as urea+NBPT
- Bulk milk from control herd on a commercial farm

Individual cow milk

- A 20 cow herd established grazing paddocks fertilised at 234 kg N/ha/year - All N was applied as urea+NBPT
- Samples collected: twice/month in June; July; August

- All Samples analysed at Teagasc Ashtown
- **QUANTITATION USING LC-MS/MS METHOD DEVELOPED**
- LOQ:0.002mg/kg (2ppb)

Table 2. Sampling periods and summary result of NBPT and NBPTo residues in milk sampled on a farm using NBPT treated urea (NBPT Farm) and a farm not using NBPT treated urea (control farm).

Sampling Year	Month	Sample Source		
		Bulk Tank		Individual Cows
		Experiment Farm Samples (Johnstown Castle Farm)	Negative Control Farm (No urea + NBPT)	Grazing Pastures Fertilised at 234 kg N/ha as Urea + NBPT
2020	February	12	-	-
	March	24	3	-
	April	24	9	-
	May	24	12	-
	June	24	9	80
	July	24	9	80
	August	21	9	80
	September	26	8	-
	October	10		
	November	8		
	December	8		
2021	January	8		
	February	4		
Total sample		217	59	240
Sum of residues (NBPT and NBPTo) concentrations for all samples (mg kg^{-1})		<0.0020	<0.0020	<0.0020

Analysis of 516 milk samples from the field study found that NBPT concentrations were below the LOQ of 0.0020 mg/kg, suggesting very low risk of residues occurring in the milk

Source: Nkwonta et al., 2021. Molecules, 26, 2890. https://doi.org/10.3390/molecules26102890

Quaternary Ammonium Compounds

- Quaternary ammonium compounds are disinfectant compounds
- Used in the manufacture of many detergent and disinfectant products either as the main ingredient or as an added compound to improve the antimicrobial effectiveness of the cleaning agent
- German Institute of Risk Analysis reported that QAC compound, DDAC is frequently detected in dairy products
- Levels are frequently detected in excess of the proposed EU Maximum Residue Limits (MRLs) of 0.5 mg/kg for food and 0.01 mg/kg for infant formula
- Proposed that DDAC residues may be due to disinfection of milking equipment or processing equipment
- But insufficient data to draw any definite conclusion

Quaternary Ammonium Compounds

RISK: chemical contamination of the final product

- Could give rise to problems of microbial resistance

QACs are not generally used for cleaning milking machines or bulk tanks in Ireland - excessive foaming

QACs are more likely to be used on stainless steel surfaces in laboratories'/manufacturing plants

QACs are present in sanitizing wipes and some teat disinfection products

Possibility of residues transferring to food from some teat cleaning products?-no evidence

Test method developed for measurement of QACs at Teagasc

Phthalates

- Phthalates - mostly used as plasticisers - added to PVC to enhance flexibility – can leach out over time
- Human exposure to phthalates - through food ingestion
- Some phthalates (e.g. DEHP) cause human health issues, e.g. disrupt the endocrine system
- Likely source - in FCMs such as flexible teat cup liners on milking machines, flexible hosing at the dairy farm/ factory, or dairy product packaging materials
- Soluble in fat: phthalates could dissolve into the fat portion of milk
- Consumer concern about effects on infants - addition of ingredients (milk powder) to infant formula contribute
- Cao (2010, *Comprehensive Reviews in Food Science and Food Safety* **9**: 21–43)
- Review on phthalates: the following phthalates found in milk and dairy products – DEHA, DEHP, DBP, DiDP, DiNP, BBzP

PolyOxyEthylene Amines (POEAs)

- **Food safety concerns exist in relation to POEAs**
- Can potentially occur in dairy ingredients
- POEAs are a surfactant often used in detergents and sanitizing agents
- They are synthetic wetting agents and improve wettability of hydrophobic surfaces e.g. in cleaning agents or as co-formulants in pesticide preparations
- Toxic effects related to POEAs have been reported in humans and the environment. Some manufacturing companies have banned the cleaning agents and sanitizers containing these substances
- In some instances, manufacturing companies have asked their suppliers of ingredients, e.g. dairy processors to investigate their use of these cleaning/sanitizing agents containing POEAs at their sites and to identify potential replacement options and a plan for phasing out those materials

Perfluoroalkylated substances (PFAS)

Chemicals used in household appliances, commercial foodstuff packaging materials and as flame-retardants

Key challenges: resistance to biodegradation, accumulation in environment and in humans, potential health effects - cancer, infertility and allergies

Contaminated water and food are the main exposure pathways of PFAS

The ability of PFAS to bind to β-lactoglobulin proteins in cow milk has made dairy milk a priority and ranked high among foods containing various PFAS

Also possibility of contamination of milk and infant formula through processing or packaging material

Suggested migration of PFAS from food contact paper used as repellent for water, oil and grease, leading to eventual ingestion of PFAS in packaged food

Perfluoroalkylated substances (PFAS)

- EU Commission recommendation that **PFAS** be monitored
- Current discussion on the need to set MRLs for PFAS
- MRL of 0.020 µg/kg is being proposed for milk
- Given the low levels of PFAS in milk, the industry emphasises that milk and dairy products contribute only slightly and that a MRL should not be set
- Also, PFAs are widespread, therefore, it is not easy for the industry to reduce background contamination; The influence of a farmer's production on PFAS levels in his products is very limited.
- More data needs to be collected and the sensitivity of analysis needs to be improved - currently a limit of detection of 0.5 µg/kg product, and this is greater than the MRL being proposed – so ???
- So the argument is to wait to gather sufficient analytical results before an MRL would be applied.

Future trends and conclusion

While risk of chlorine residues has largely been eliminated due to removal of chlorine-based cleaning agents, it is absolutely critical that microbial hygiene and quality is not compromised

Ongoing need for vigilance re: the emergence of other potential residues from the use of replacement (for chlorine) products

Need for vigilance Re: other new products, e.g. QACs, phthalates, PFAS

The following technological and knowledge gaps exist:

i) Analytical capabilities need to be improved - need for validated and robust methodologies to measure residues in milk and dairy products;

ii) knowledge on sources of contamination at milk production level; and

iii) Knowledge on causes of contamination at processing level

Focus must be maintained on contamination issues that may affect Irish exports and disadvantage the competitiveness of the agri-food industry.


Thank you