Milk Quality Workshop

Cavan, 25 January 2024

TCM update and focus on residues of potential concern for the dairy industry

Dr. Bernadette O'Brien

Animal and Grassland Research and Innovation Centre,
Teagasc, Moorepark, Fermoy, Co.Cork, Ireland.

Teagasc
Agriculture and Food Development Authority

Introduction

- TCM - Trichloromethane - update
- NBPT - Protected urea
- QACs - Quaternary ammonium compounds
- Phthalates
- POEAs - PolyOxyEthylene Amines
- PFAS - Perfluoroalkylated substances
- Micro plastics
- Nitrates/ nitrites
- Current issues and trends

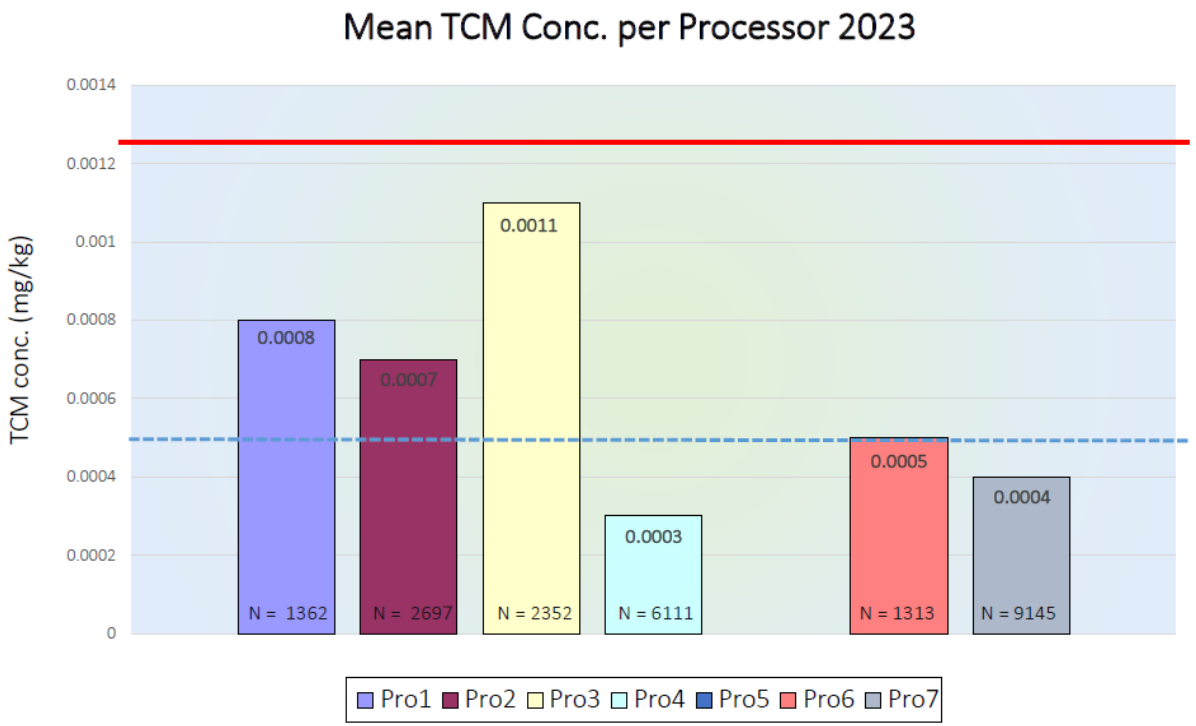
TCM residue

- Value of Irish Dairy exports in 2022 was €6.8bn, an increase of 48% from 2017.
- Butter accounted for 19% of the export value, in 2022
- TCM residue - chlorine related
- TCM can form and accumulate in milk fat and butter
- Critically important standards – health associations:

- **Target level in butter = 0.024 mg/kg or less**
- **» Target level in milk = 0.00124 mg/kg**

Raw milk results 2023 (Jan – Dec)

Industry Average = 0.0006**mg/kg**
Weighted Average = 0.0005**mg/kg**

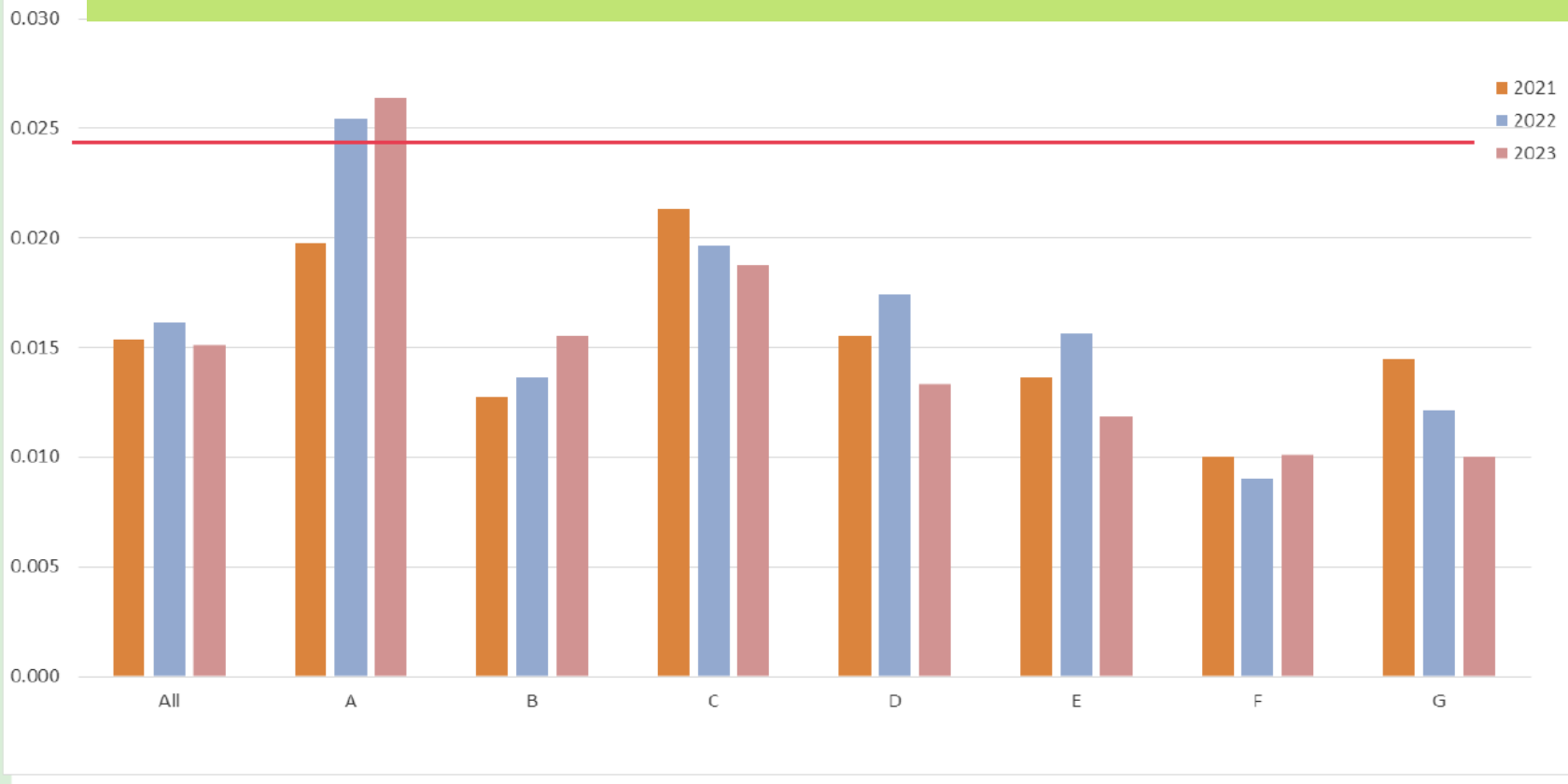


Target limit
0.00124mg/kg

Weighted
Average

eagasc
Agriculture and Food Development Authority

TCM levels in bulk butter 2021 - 2023



Ornua
THE HOME OF IRISH DAIRY

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

- NBPT is a urease inhibitor utilised in urea-based fertilizers
- Can delay urea hydrolysis and reduce ammonia losses
- But concerns arise re: potential residues in milk products
- Toxicity assessment of NBPT – considered as a Category 3 substance

- **Question:** does NBPT bio-transfer from pasture, through the grazing animal and into milk

- No legislative requirements established for NBPT in cow's milk
- Field trials necessary to develop a database

- **Experiment** conducted at the Teagasc, Johnstown Castle Research Centre

Teagasc investigation of NBPT residue in milk

- **Research herd**
  - The average N rate for the overall herd was 181 kg N/ha/year
  - All N was applied as urea+NBPT
  - Bulk milk collected from Feb 2020 to 2021
- **Control herd on a commercial farm**
  - NO urea and NBPT used
  - Bulk milk collected from Mar to Oct 2020
- **Research herd - individual cow milk**
  - Pasture received highest N rate of 234 kg N ha^{-1} and highest NBPT
  - Milk collected from individual cows (*n* = 20) twice/month (June to August 2020)
- Analysed at Teagasc Ashtown using UHPLC-MS/MS method
- 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 and thus, highly unlikely to pose any toxic threat to humans

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

Quaternary Ammonium Compounds (QACs)

- QACs are human-made chemicals used as disinfectants for controlling bacteria, viruses, etc on stainless steel surfaces, in laboratories'/manufacturing plants
- But antimicrobial resistance
- Over 2,000 products containing QACs; found in ready-to-use sprays, aerosols, wipes, e.g. sanitizing wipes and some teat disinfection products or food processing equipment
- German Institute of Risk Analysis reported that 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
- But insufficient data to draw any definite conclusion
- Test method developed at Teagasc for measurement of QACs

Necessary to monitor QACs

- US management recommendations (Arnold et al, 2023) https://doi.org/10.1021/acs.est.2c08244

  suggest growing evidence of adverse environmental and human health impacts of QACs - suggests reduced exposure through the 'essential-use approach'

- Zheng et al. (2022) https://doi.org/10.1038/s41370-022-00439-4

  reported the first study on detection of QACs in breast milk and identified breastfeeding as an exposure pathway to QACs in infants and higher QAC levels found in breast milk from mothers who used QAC-containing disinfecting products and disinfected more often

- Considered - the significant increase in the use of disinfectants containing QACs during COVID-19 warrant research on QAC toxicity and human health effects

Phthalates

- Group of chemicals used in common household products: carpets, cables, tubes, packaging materials and historically in children's plastic toys
- Added to PVC to enhance flexibility – can leach out over time
- ~ 30 different phthalates – e.g. diethylhexyl phthalate (DEHP)
- Often replaced now with non-phthalate alternatives

What are the health effects from phthalates in foods?

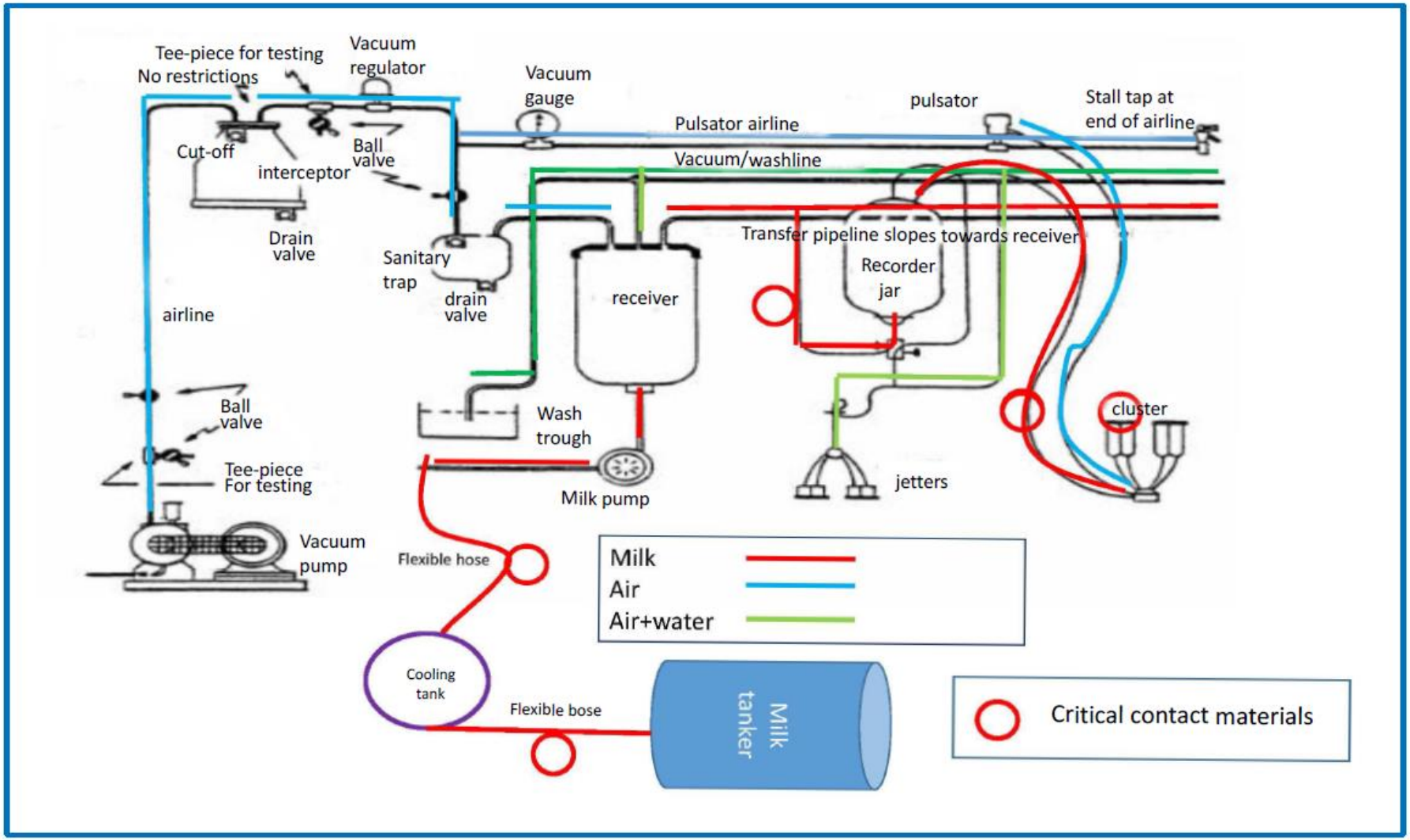
- Phthalates are regarded as endocrine disruptors i.e. can interfere with hormone systems
- Eales et al. (2022) found **robust evidence** of association between phthalates and neurodevelopment and risk of childhood asthma and **moderate evidence** of association with low birthweight, ADHD, Type 2 diabetes

What are the regulatory issues?

- Regulated in EU – but may not be as regulated elsewhere
- Some regulatory bodies have set migration limits or actual limits for phthalates (e.g. China). But no harmonised testing method - different laboratories - different methods - different limits of detection.

Phthalates – if present, are soluble in fat and can readily leach out of e.g. teat cup liners and dissolve into the fat portion of the milk

Likely transfer points of phthalates if present in rubberware/ liners



Phthalates in dairy products (μg kg^{-1}) (Giuliani et al, 2020)

Matrix	Phthalate	Average Concentration	Reference
Milk	DEHP	20-480	Samples from Scandinavian countries
Milk in silo and tanker	DEHP	60-140	
Cream 35% fat	DEHP	1060-1670	
Milk <1% fat	DEHP	20-40	
Milk	DEHP	10-40	Samples from Spain
Cream	DEHP	480-550	
Milk	DEHP	10-90	Samples from UK
Cheese	DEHP	600-3000	
Cream	DEHP	200-2700	

- Fierens *et al*. 2012 studied Belgian milk - showed DEHP migrated into raw milk - contaminated feed
- Fierens *et al*. 2013 study - at the processing facility, food contact materials, e.g. packaging materials increased the levels of DEHP in milk and powder
- **How can the dairy industry minimize phthalate contamination?**
- Ingredients for rubberware, liners, feeds, packaging must all be certified by suppliers as phthalate-free

Perfluoroalkylated substances (PFAS)

- PFAS are human-made chemicals
- Their entry pathways to the dairy chain may be
  - taken up directly by cows drinking water or eating grass or other plants
  - from silage
  - via ingredients used in dairy processing and food contact materials e.g. food packaging and consumer items such as pans, dishes, baking aids and paper wraps
- The ability of PFAS to bind to ß-lactoglobulin proteins in cow milk has made dairy milk a priority
- Increased levels of PFAS have been detected in butter and cheese samples
- Use of PFAS in food contact materials is increasingly prohibited - likelihood of PFAS arising from packaging is decreasing

PFAS

- Paper by Macheka et al. (Feb 2021) - first study on the occurrence and human exposure to PFAS in milk in S. Africa
- Low-level daily intake of PFAS found through dairy milk and infant formula consumption
- EDI of PFAS through the consumption of dairy milk and infant formula were lower than the daily tolerable limits
- Concluded: important due to long-term exposure and the cumulative effects of multiple exposure pathways
- The tests for PFAS are difficult and cost-intensive
- Ultra-high performance liquid chromatography method
- Sig challenge in analytical determination – Ogunbiyi et al. (2023) outlined different methods in 'Review of advances on analytical approaches'

PFAS

- EU Commission has recommended that **PFAS** be monitored
- Current discussion on the need to set MRLs for PFAS
- MRL of 0.020 µg/kg 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
- PFAS are widespread, therefore, the influence a farmer has on PFAS levels in the milk would be 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

PolyOxyEthylene Amines (POEAs)

- **Food safety concerns exist in relation to POEAs**
- Can potentially occur in dairy ingredients
- POEAs are surfactants derived from animal fat and often used in detergents and sanitizing agents
- They are synthetic wetting agents and improve wettability of hydrophobic surfaces e.g. in cleaning agents
- Toxic effects related to POEAs have been reported in humans and the environment
- Manufacturing companies have asked suppliers of ingredients, e.g. dairy processors to investigate their use of these cleaning/ sanitizing agents containing POEAs and to identify potential replacement options and a plan for phasing out those materials

Microplastics/ nanoplastics

- Microplastic pollution is a growing concern - risk of harm to human and ecosystem health - soil and water
- Contamination reports re:milk and dairy products in literature, but no data pertaining to the Irish dairy industry
- Microplastics - very resistant to (bio)degradation
- E.g. polyethylene, polypropylene, polystyrene, polyvinyl chloride
- Use of plastics in agriculture increasing – benefits: increasing production efficiency and reduced chemicals
- Several research studies aiming to assess health effects and their capability to cross the gut barrier
- FROM FAO 2021 Report: 12.5 million tonnes of plastic used annually in agriculture - degrades into micro plastics

37.3 m tonnes in food packaging

Entry to the dairy chain and measurement

- Entry into milk - through feed or water uptake by cow, absorption by their gastrointestinal tract, and excretion into the milk
- Can MP pass from the gastrointestinal tract to the udder ? Generally assumed that MP > 150 nm are excreted through faeces, but the fate of smaller particles is unknown
- MP may enter milk
  - on-farm from tubing, rubber, seals of milking equipment, etc
  - during milk collection, transport and processing
  - from packaging materials at the plant, during transport and distribution, and by the consumer opening the packaging
- Methodology focused on analysis of water samples at present
- Liquid milk and dairy products require additional sample preparation steps; methods specific for foods are still scarce

Occurrence in milk and dairy products

- Majority of data relates to MP in seafood and salt; no robust analytical methods for complex foods
- Limited information published for whole, semi-skimmed or skimmed milk
- Da Costa Filho *et al*. (2021) reported small sized MP in raw milk collected at farms immediately after the milking machine and in processed liquid milk and powdered dairy products
- Cushworth *et al*. (2023) reported significant difference in MP levels between sites where plastic crop covers were and were not used ($p \leq 0.001$) https://doi.org/10.1002/ppp3.10430 **UK**

Focus for work in this area

- Development /progression of methodology for testing – current method ~€1K/sample
- Establish any geographical or seasonal patterns in MP contamination of raw farm bulk milks
- Identify the key steps contributing to MP contamination along the dairy product manufacturing process
- Measure MP contamination in dairy products at point-of-sale

Nitrates and nitrites - potential issues

- Mechanisms of transfer to the dairy chain
  - Nitrate taken up by plants for protein synthesis - grazing dairy cows - excess nitrite and nitrate can transfer into milk as part of the excretion process
  - Drinking water - can contain nitrate levels up to ~ 100 mg/kg
  - Water used in the processing of dairy products
  - Descaling equipment with nitric acid
- Health issue - particularly when used for manufacture of baby and infant food
- Nitrate is not of dietary concern for humans, but can be reduced to nitrite in the mouth by nitrate reducing bacteria. These bacteria are low in adults because of low stomach pH, but infants have reduced stomach acidity and are more likely to have these bacteria

Nitrates and Nitrites

- Nitrite can further oxidize the ferrous ion in haemoglobin resulting in methaemoglobinemia - where haemoglobin becomes unable to properly release oxygen to the tissues, decreased oxygen capacity (blue baby syndrome)
- Also linked to: (a) increased risk of type 1 diabetes and (b) formation of nitrosamines in dairy products - carcinogenic
- Nitrates and nitrites in milk powders are critical when used in infant formula
- China became one of the 1st countries to implement strict regulations on nitrite in milk - linked to at least 3 deaths of infants in 2011; China has a limit of 2 mg/kg nitrite
- New Zealand indicates that nitrite concentrations less than 5 mg/kg are safe for human consumption

Studies on Nitrates and Nitrites

- Previous studies - suggested that a high nitrate diet does not mean high transfer of nitrates and nitrites to milk
- Study by Kammerer et al. (1992) showed no significant effect on nitrate in milk when cows had drinking water containing different nitrate levels (0-180 mg/l)
- Study by Croitoru et al. (2015) found concentrations of 2.0–3.5 mg/kg of nitrate and 0.035–0.80 mg/kg of nitrite in powdered milk (Romania)
- Study by Genualdi et al. (2020) measured the nitrate and nitrite concentrations in >150 US milks and milk powders; Av Nitrate = 17 ± 12 mg/kg; Av Nitrite > 2 mg/kg in 3 different brands of retail milk.

Measurement of Nitrates and nitrites

- Method developed at Teagasc capable of analysing nitrate and nitrite at trace levels below regulatory limits
- Ion chromatography allows simultaneous detection of nitrate and nitrite
- Analysis allows less rejection of exported dairy products in countries with very strict limits such as China

Milk Powder Sample Analysis Summary (LOR=limit of rejection)

Analyte	Total Samples	LOR (mg/kg)	<LOR	>LOR	Min (mg/kg)	Max (mg/kg)
Nitrate	91	0.4	6	85	<0.4	43.31
Nitrite		0.1	52	39	<0.10	3.36

Overview from IDF Meeting of SC on Residues and Chemical contaminants: 12 October 2023

What are the issues for the different countries

- Sweden = PFAs, Microplastics, Chlorate, mineral oils
- Australia = Bush fires creating PFAS problems
- France = Mineral oils, Mycotoxins
- Germany = PFAS
- Denmark = Issues with mineral oils, PFAS and hypochloric acid
- New Zealand = NZ currently focused on recycling issue; USDA passed a lot of recycling systems, but China have now found recycled plastics being used in packaging of pharmaceutical products
- USA = PFAS; some farms impacted by ground water contaminated with flame retardants
- Japan = PFAS are a risk management priority
- Switzerland = Mineral oil

Companies need to be able to sign off on clearance for exports. Residues are important due to health and safety but also due to economics, exports and legislation