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Levels of Sulphite-Reducing Clostridia in New Zealand Nutritional Dairy Powders

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Scientific Interpretive Summary

This SIS is prepared by MPI risk assessors to provide context to the following report for MPI risk managers and external readers

Levels of sulphite-reducing clostridia in New Zealand nutritional dairy powders

As a consequence of the detection of high levels of sulphite-reducing clostridia (SRC) in whey protein concentrate and nutritional powders manufactured by Fonterra, and the subsequent actions taken by MPI, Fonterra and other stakeholders, MPI has described a series of Interim Measures to strengthen customer and consumer assurance around New Zealand's dairy powder production.

Interim Measure #6 required MPI to obtain a baseline dataset of levels of contamination of sulphite-reducing clostridia (SRC) in New Zealand nutritional dairy powders to facilitate comparison with unexpected results and to evaluate the implications of imposition of microbiological criteria on New Zealand dairy manufacturers.

The observed high prevalence but generally very low counts of SRCs in New Zealand nutritional dairy powders and inhomogeneity of SRC distribution in contaminated batches leads to the conclusion that it is unlikely that routine testing of dairy powders for SRCs in New Zealand, and setting of microbiological criteria, will provide additional assurances on the hygiene of processing or safety of such powders than current assurance programmes.

However, if monitoring programmes for SRCs are required, careful design of the sampling plan would be needed as the testing of single samples could be highly misleading with respect to both product quality and food safety assurances. Multiple samples would be required to provide an accurate assessment of the contamination level to properly inform decisions on the efficacy and performance of control measures under "risk-based" food control programmes.

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1 Executive summary

As a consequence of the detection of high levels of sulphite-reducing clostridia (SRC) in whey protein concentrate and nutritional powders manufactured by Fonterra, and the subsequent actions taken by MPI, Fonterra and other stakeholders, MPI has described a series of Interim Measures to strengthen customer and consumer assurance around New Zealand's dairy powder production.

Interim Measure #6 required MPI to obtain a baseline dataset of levels of contamination of sulphite-reducing clostridia (SRC) in New Zealand nutritional dairy powders to facilitate comparison with unexpected results and to evaluate the implications of imposition of microbiological criteria on New Zealand dairy manufacturers. MPI collated SRC test results for 2107 batches of nutritional dairy powders voluntary supplied by New Zealand dairy processors.

The limit of m=100 recommended by the ICMSF was exceeded in only six out of 826 batches of whey protein concentrate powder (WPC). This represents just 0.7% of the product batches tested.

Analysis of data for 821 batches of nutritional dairy products for infants and young children showed that levels are not substantially different between products with 10 CFU/g exceeded in 1% of dairy powders for 0 to 6 month old infants, 6% of dairy powders for 6 to 12 month old infants, and 6% of milk powdered products for young children (12 to 36 months of age).

Low levels of SRC in WPC are most likely to have originated from the raw material. Higher levels of contamination observed in a few batches suggesting contamination from within the process.

All methods of analysis for SRCs employed, with the possible exception of the FDA BAM method, appeared to perform similarly; the latter was used by just one premises and therefore differences in results compared to the national average cannot be attributed to the method itself.

In conclusion, it is unlikely that routine testing of nutritional dairy powders for SRCs in New Zealand, and setting of microbiological criteria, will provide additional assurances on the hygiene of processing or safety of such powders than current assurance programmes.

2 Introduction

As a consequence of the detection of high levels of sulphite-reducing clostridia (SRC) in whey protein concentrate and nutritional powders manufactured by Fonterra, and the subsequent actions taken by MPI, Fonterra and other stakeholders, MPI has described a series of Interim Measures to strengthen customer and consumer assurance around New Zealand's dairy production. In particular the response relates to the following nutritional dairy powders:

- Infant Formula (IF) products for infants (stage 1 IF for 0-6m and stage 2 or follow-on-formula for 6-12months) and Dairy Based Formulated Powdered Supplementary Foods (DBFPS) for young children (1-3y)
- Nutritional base powders (NBP) for the above products
- Whey Protein Concentrate (WPC) as an ingredient for the above products

SRC include a number of species widely found in the environment and in faeces. They grow anaerobically and produce spores that protect them from the rigors of the harsh environment. Their presence in a food or water may not be of immediate concern but may serve as a hygiene indicator for processing of milk and milk products. However, some of the SRC are potentially foodborne pathogens, meaning that their presence in elevated numbers has additional spoilage and food safety implications.

The ICMSF[1] considers that monitoring for SRC is appropriate as an indicator of the control of anaerobic spore formers and to determine adherence to good hygiene and manufacturing practices during the production of dehydrated dairy ingredients or products such as powdered IF. ICMSF suggests that SRC levels in WPC exceeding a limit of m = 100 cfu/g, would point to there being conditions conducive to the multiplication of anaerobic clostridia in the processing lines, or a source of external contamination that is amplified through concentration of the dairy product.

While manufacturers may test for the presence of SRC for their own control purposes, there has not previously been a collation of this data on a national data to assess the levels that occur. Interim Measure #6 of MPI's response to the recommendations of the WPC Inquiry is the requirement to undertake a targeted microbiological survey of nutritional dairy powders. The outcomes of the survey are reported here.

This dataset is then used to evaluate the relevance of microbiological criteria for New Zealand dairy manufacturers, and to enable the evaluation of potential designs for monitoring programmes to meet possible market access requirements.

This project complements a MPI survey of SRC levels in raw milk, and contributes to the agreed suite of MPI Interim Measures to improve regulatory oversight of the dairy industry for nutritional dairy powders following the WPC2013 response.

[1] ICMSF 2013. Usefulness of testing for sulphite-reducing clostridia in powdered infant formula and dairy-based ingredients for infant formula. International Commission on Microbiological Specifications for Foods.

3 Methods

3.1 DATA COLLECTION

All New Zealand manufacturers of nutritional dairy powders that are performing SRC product testing were asked to provide MPI with all results (with available metadata) for SRCs and specified indicator organisms (e.g. aerobic plate count, total coliforms, *Bacillus cereus)* from company monitoring programmes.

Companies were asked to specify which validated method was used for SRC detection. Validated methods included:

- ISO 15213,
- Modified ISO (Danone/CLF validated internal method),
- NZTM2.59.3, or
- US FDA BAM method (using PCR confirmation procedures).

All methods were specified as being able to detect one spore per gram of dairy powder.

The total number of results required in the dataset for each group of products to inform the development of quantitative microbiological criteria was calculated from the expected prevalence of SRCs as determined from a limited dataset previously acquired. Based on the prevalence of positive samples for which the concentration of SRCs exceeded 10 spores per gram, a minimum of 164 results for each of the three products, apportioned across the respective manufacturing premises, was estimated as required to ensure 95% confidence intervals for the prevalence of the range 1-7%.

3.2 DATA ANALYSIS

Data analysis was performed separately for the formula products, their base powders and WPC. The dairy nutritional powders were split into three risk groups:

- Infant formula (IF) and its base powder products for infants from 0 to 6 month
- Follow-On-Formula [FOF] and its base powder products for infants from 6 to 12 months.
- Growing-Up-Milk (GUM) and its base powder products for young children from 12 to 36 months.

The prevalence of SRC spores was calculated on a nationwide basis for the collated dataset.

3.3 INTERNATIONAL CRITERIA

MPI is not aware of any international regulatory criteria for SRCs in nutritional dairy products, and SRCs are not specified under Codex dairy standards. MPI notes that interest in SRCs in dairy products has previously related solely to functional/aesthetic defects in cheese.

However, notwithstanding the absence of regulatory limits, suppliers to Danone Corporation have historically been required to comply with Danone's Food Safety Risk Indicator Criteria[2] for SRC spores in nutritional milk powders (Table 1).

Table 1 Danone Corporation Food Safety Risk Indicator Criteria for SRC spores in nutritional milk powders

Products		Aliquot (g)	n	c	m	M
For infants	0-6 months	1	5	1	5	10
For infants	6-12 months	1	5	1	10	25
For children	12-36 months	1	5	1	10	50

As an example of performance against an internationally recognised microbiological criterion, the Danone criteria was applied to the data from the microbiological survey.

[2] Danone internal standard, 2013

4 Results

Ten of the larger dairy processors provided data from SRC batch testing during the period from September 2012 to May 2014. Most of the tests were carried out as a component of quality assurance and product grading procedures, although some testing occurred during trace-back activities following incidents.

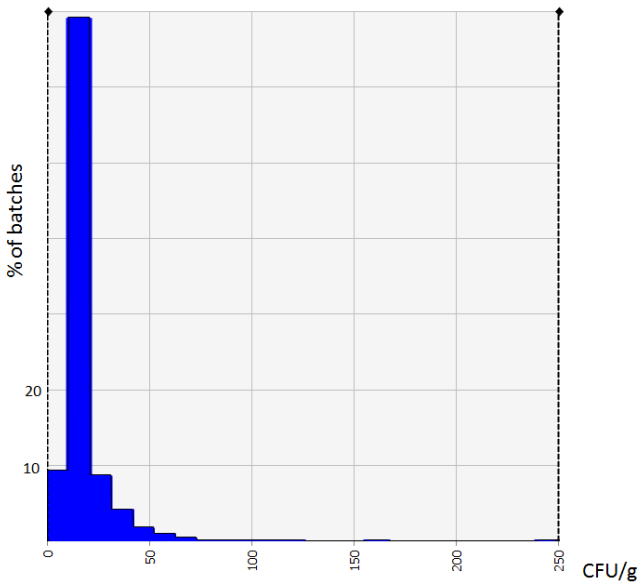
Most of the batches of product in the dataset were tested, in addition to SRC, for aerobic plate count, total coliforms and *Bacillus cereus*.

The dataset contains test results for 821 batches of IF and DBFPS, 460 batches of NBP for the above products and 826 batches of WPC. This exceeded the requirement for a minimum dataset of 164 samples per product group. Where batches had multiple test results, only the highest SRC count was entered into the dataset.

4.1 WHEY PROTEIN CONCENTRATE

Results for 826 WPC batches that were tested for the presence of SRC spores are presented in Table 2 and also summarised in the Figure 1. Additional microbiological data, if available, is presented for batches where the SRC count exceeded or was equal 100 cfu/g (Table 2).

Figure 1 SPC test results from WPC.



Only six apparently independent batches of WPC had SRC spores at counts ≥ 100 cfu/g (0.7%; 95% CI 0.3%, 1.6%). The majority of the test results were substantially below 100 cfu/g; 98.3% (95% CI 97.2%, 99.1%) <50 cfu/g, 90.9% (95% CI 88.6%, 92.6%) <20 cfu/g.

Table 2. SPC, *B. cereus* and indicator (APC; aerobic plate count) test results for WPC.

SRC count range (cfu/g)	Batches tested (n)	%	Additional microbiological results (cfu/g)
Not detected	81	9.8%	-
1-<10	594	72%	-
10-<20	75	9.1%	-
20-<30	37	4.39%	-
30-<40	16	1.9%	-
40-<50	9	1.1%	-
50-<60	4	0.5%	-
60-<70	2	0.22%	-
70-<80	2	0.22%	-
90-<100	1	0.11%	-
100-<110	2	0.22%	1.APC=1800; *B. cereus* =40 2. APC 19,000; *B. cereus* < 10 coliforms <1 for both batches
110-<120	1	0.11%	APC=40,000; *B. cereus* =10 coliforms <1
150-<160	1	0.11%	APC= 4,500,000*; B. cereus* =40; coliforms <1
160-<170	1	0.11%	APC=3,400,000; *B. cereus*= 100 CFU/g; coliforms <1
240-<250	1	0.11%	APC= 260,000*; B. cereus* no data; coliforms <1

Aerobic plate counts (APC) ranged from 1,800 to 4,500,000 cfu/g and total coliforms were not detected in any of the six batches with SRC spores counts ≥ 100 cfu/g. *B. cereus* was detected in four of the six batches but with counts ≤100 cfu/g.

4.2 POWDERED DAIRY PRODUCTS FOR INFANTS AND YOUNG CHILDREN

Results of testing for the presence of SRC spores in powdered dairy products intended for consumption by infants and young children are presented in Tables 3 and 4. Additional microbiological data, if available, is presented for batches where the SRC count exceeded or was equal to 50cfu/g (Table 3).

Table 3. SPC, *B. cereus* and indicator test results for dairy powders for infants and children less than 36 months (IF base [IFB], FOF base [FOFB], GUM base [GUMB])

Product	Batches tested (n)	Test result range (number of batches in the range)						
		Not detected	1-<10 cfu/g	10-<20 cfu/g	20-<30 cfu/g	30-<40 cfu/g	40-<50 cfu/g	≥50 cfu/g
IFB[1]	122	53	62	5	0	1	0	1
IF[2]	221	108	110	1	1	0	0	1
IF+IFB	343	161	172	6	1	1	0	2
FOFB	115	50	62	1	1	1	0	0
FOF	259	80	163	14	2	0	0	0
FOF + FOFB	374	130	225	15	3	1	0	0
GUMB[3]	223	101	98	15	7	1	0	1
GUM	341	152	166	6	13	4	0	0
GUM + GUMB	564	253	264	21	20	5	0	1
Total	1281	544	661	42	24	7	0	3

[1] SRC=50, APC<100, coliforms=0, *B. cereus* <10
[2] SRC=480, APC=100, coliforms=0, *B. cereus* 10
[3] SPC≥50, APC=10, no other information

Table 4. SPC test results for dairy powders for infants and children less than 36 months (IF base [IFB], FOF base [FOFB], GUM base [GUMB])

Product	SRC count proportion (%)						
	Not detected	<10 cfu/g	<20 cfu/g	<30 cfu/g	<40 cfu/g	<50 cfu/g	≥50 cfu/g
IF+IFB	47	97	98.8	99	99.4	99.4	0.6
FOF + FOFB	34.8	95	98.9	99.7	100	100	0
GUM + GUMB	45	91.7	95.3	98.9	99.8	99.8	0.2
Total	42.5	94	97.3	99.2	99.8	99.8	0.2

SRC were detected at counts >10cfu/g in just 1.4 % (95% CI 0.2%, 3.9%) of IF (0 to 6 months) samples and 3% (95% CI 1.4%, 5.3%) of IF + IFB.

Similarly, SRC were detected at counts >10cfu/g in 6% (95% CI 3.6%, 9.8%) of FOF (6 to 12 months) samples and >20cfu/g in 0.8% (95% CI 0.09%, 2.8%). When combined with its base powder (FOF + FOFB), the corresponding prevalence was 5% (95% 3.1%, 7.8%) and 1.1% (95% 0.3%, 2.7%) respectively.

SRC were detected at counts >10cfu/g in 6.7% (95% CI 4.3%, 9.9%) of GUM (12 to 36 months) samples and >50cfu/g in 0% (95% CI 0%, 0.9%). When combined with its base powder (GUM + GUMB), the corresponding prevalence was 8% (95% 6.0%, 10.9%) and 0.2% (95% 0.005%, 0.98%) respectively.

Substantial variability in SRC counts was observed in batches from which multiple samples had been collected (but only the highest count entered into the datasets described above. For example, in the batch of IF with the highest SRC test result of 480 cfu/g, SRC were detected at <10 cfu/g in 3/7 samples but not detected in 1/7. Similarly, in the batch of GUM base with the highest SRC test result of 110 cfu/g, SRC were detected at <50 cfu/g in 4 /13 samples but not detected in 3/13.

High levels of *B. cereus,* total coliforms or the aerobic plate count did not correlate with contamination by SRCs and therefore have little utility as a tool to predict the presence or level of SRCs in nutritional dairy powders.

4.3 EFFECT OF TEST METHOD

There was little difference in prevalence of SRC spores at similar concentrations detected by the ISO 15213, NZTM2.59.3 or modified ISO methods. MPI believes that performance of these methods is similar given the natural slight variation between premises.

One laboratory representing one premises tested powders using the FDA BAM method with PCR for confirmation. While the detection level of SRC from that premises was lower than the national average, it remains unknown as to whether this was due to the method itself, sampling, premises hygiene or other factors.

5 Discussion and Conclusions

MPI collated SRC test results from 2107 batches of nutritional dairy powders voluntarily provided by New Zealand dairy processors.

SRC were detected in 90.2% of WPC and 57.5% of nutritional dairy powders [3] for infants and young children (Table 5).

Table 5. SPC prevalence in New Zealand whey protein concentrate (WPC) and nutritional dairy powders for infants up to 6 months of age (IF), infants from 6-12 months of age (FOF) and children from 12-36 months of age (GUM)

	Prevalence (rounded)			
	Not detected (<1)	Detected	Detected (<10)	Detected (>10)
WPC	10%	90%	72%	18%
IF	49%	51%	50%	1%
FOF	31%	69%	63%	6%
GUM	45%	55%	49%	6%

There was little difference in the levels of SRC between nutritional dairy powders.

New Zealand does not have regulatory criteria for the level of SRC in nutritional dairy powders. ICMSF suggested that SRC levels exceeding a limit of m = 100 cfu/g in WPC would point to conditions potentially conducive to the multiplication of anaerobic clostridia in the processing lines, or some source of external contamination. That limit was exceeded in six out of 826 batches of WPC (0.7% of the product tested).

In the recent MPI survey, prevalence of SRC spores in New Zealand raw milk was estimated at 3.3% (1.1%-7.6%) with the concentration in all positive samples being one spore/ml. Given that as little as one spore in one millilitre of raw milk would equate to about 10 spores in one gram of milk powder and more than 100 spores in a highly concentrated product such as WPC, the results of the current survey are likely to reflect the incoming milk as the source.

While not endorsing the application of commercial criteria, the Danone Food Safety Risk Indicators for SRC spores specify limits of M=10 and M=25 for dairy nutritional powders for infants from 0-6 months and 6-12 months, respectively, were used as an internationally recognised microbiological benchmark.

Using these criteria, 3% of New Zealand dairy nutritional powders for infants from 0-6 months and about 1% of dairy powders for infants from 6-12 months will fail. This failure rate would much higher if the marginal acceptance limit of m=5 for infants under six months and m=10 for all other nutritional dairy powders for infants and young children was applied.

[3] Includes final products and their bases.

The results of the survey indicate a high prevalence but generally low count of SRCs in New Zealand nutritional dairy powders (Table 5). Occasional detection of high numbers of SRC spores in nutritional powders for infants and young children and inhomogeneity of SRC distribution in the contaminated batches suggests that contamination during processing may occur, albeit infrequently.

High counts were observed in the WPC2013 event when routine cleaning procedures failed.

In conclusion, it is unlikely that routine testing of dairy powders for SRCs in New Zealand, and setting of microbiological criteria, will provide additional assurances on the hygiene of processing or safety of such powders than current assurance programmes.

However, testing for SRCs themselves may be more informative should future risk assessments indicate that the presence of anaerobic spore formers is a concern under usual New Zealand processing conditions.
Under the Animal Products Act (1999), manufacturers are required to understand their processes and the processing conditions that affect the safety and quality of their products. They need to understand which processes may be prone to growth of spore forming anaerobes, and design their monitoring programmes accordingly.

Care would be required, however, when designing such monitoring programmes for SRCs in nutritional dairy powders as the inhomogeneity of contamination and low counts means that testing single samples could be highly misleading with respect to the assurances, for both product quality and food safety, provided through compliance with any criteria applied. Multiple samples would be required to provide an accurate assessment of the contamination level to properly inform decisions on the efficacy and performance of control measures under “risk-based” food control programmes.