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SURVEY OF MILK FOR MYCOTOXINS

This supplement should be read in conjunction with the original Food Survey Information Sheet, which contains the full details of the survey including the background, the sampling strategy, sample details and analytical quality assurance.

Key facts

- Mycotoxins are naturally occurring toxins produced by moulds, which may grow on cereals and other commodities used as animal feeds. In dairy cattle, some of these toxins can be metabolised and their metabolites may be transferred into milk.
- In the original Food Survey Information Sheet the results of the analyses of 100 milk samples (50 retail and 50 farm gate) for aflatoxin M_1 and ochratoxin A were reported. This Supplement reports the results for the analysis of the milk samples for the ochratoxin A metabolite, ochratoxin α, and zearalenone, α-zearalenol and β-zearalenol and their conjugates.
- Ochratoxin α, α- and β-zearalenol and α- and β-zearalenol conjugates were not detected in any of the samples.
- Out of the 100 samples surveyed in total, zearalenone was detected at low levels in 3 retail samples. Zearalenone conjugates were detected at low levels in 3 retail samples and 2 farm gate samples.
- Data from this survey of retail and farm gate milk samples show that exposure levels to zearalenone and its conjugates do not present safety implications for public health. Consumers do not need to change their diet as a result of the findings of this survey.

Summary

This survey analysed 50 retail and 50 farm-gate milk samples, produced by both conventional and organic methods, for the mycotoxins: ochratoxin α and zearalenone, α- and β-zearalenol and their conjugates. Milk samples that contain residues of mycotoxins indicate that they originated from a dairy herd, which had consumed contaminated feed.

Zearalenone was detected in 3 out of 50 retail milk samples at levels ranging from 1.2 to 5.5 micrograms/l. Zearalenone conjugates were found in 3 out of the 50 retail milks and 2 out of the 50 farm-gate milks at levels ranging from 3.4 to 12.5 micrograms/l. All the samples containing detectable levels of zearalenone or its conjugates were produced by conventional means. It is interesting to note that zearalenone conjugates were not detected in the same samples as zearalenone. However, the metabolic fate of zearalenone will depend on the capacity of rumen microflora to metabolise the toxin once ingested, which will be affected by an animal's diet, and this could account for such discrepancies in the transmission of residues to cows' milk.

There were considerable technical problems encountered in the initial analysis of the zearalenol conjugates. Studies using a different method of analysis and bulked milk samples to enhance sensitivity demonstrated that no α-zearalenol conjugates were present in the samples. Ochratoxin α, α- and β-zearalenol and α- and β-zearalenol conjugates were not detected in any of the samples.

Background

Zearalenone is an estrogenic compound produced by various *Fusarium* moulds which are frequently found as contaminants in most cereal crops, particularly maize and wheat, grown in temperate climates[1-5] and in cereal products[6,7] and animal feedingstuffs.[8,9] The mould species are important plant pathogens, which can cause various pre-harvest diseases such as ear blight in cereals.[10] The moulds are able to produce zearalenone and other possible toxins before harvest, although toxin production may also take place during storage of improperly dried grain.[11-12]

In 2000, the EC's Scientific Committee on Food (SCF) considered zearalenone and established a temporary tolerable daily intake (tTDI)[#] of 0.2 micrograms/kg bodyweight based on the oestrogenic effects of zearalenone.[13]

Zearalenone and its metabolites, α- and β-zearalenol are capable of being transmitted to tissues and milk of ruminants at elevated feed levels.[14,15] Until the current survey zearalenone and its metabolites α- and β-zearalenol had not previously been monitored in milk produced in the UK. However, zearalenone has been found in UK animal feeds.[16]

Background information on the production, occurrence and toxicity of ochratoxin A is given in the main Food Survey Information Sheet. Ochratoxin A residues are generally not found to any significant extent in the tissues of ruminants, or in the milk except at high levels of ingestion.[17] Ochratoxin A is metabolised to the non-toxic metabolite ochratoxin α.[18] Although ochratoxin α is non-toxic, and therefore not a potential concern to consumer health, its presence in milk serves as an indicator of the presence of significant levels of ochratoxin A contamination in animal feed. Ochratoxin α was not detected in any of the milk samples in this survey.

Until the current survey ochratoxin A and its metabolite ochratoxin α had not previously been monitored in UK milk. However, ochratoxin A has been found in UK animal feed ingredients.[19,20]

Analytical methodology

Quality assurance

Details of the analytical procedures used and the limits of detection (LODs) are summarised in Table 1 and further detailed in the main Food Survey Information Sheet.

[#] An estimate of the amount of contaminant, expressed on a body weight basis, that can be ingested daily over a lifetime without risk to health.

Analytical quality assurance data are as described previously. Spiked samples were used to assess recovery, and recoveries between 70 and 110 per cent were classed as valid. Spiked samples were also used for quantification and all results were corrected for recovery (see Annex 1). The assessment of measurement uncertainty was targeted at samples containing mycotoxins at a range of levels. The measurement uncertainty determined for the analyses was ± 2 micrograms/l at a level of 10 micrograms/l of zearalenone. Measurement uncertainty data for ochratoxin α and α- and β-zearalenol are not required since they were not detected.

Confirmation of the presence of α-zearalenol conjugates

In the initial analysis for α-zearalenol, a sample of retail milk was pre-treated with the enzymes β-glucoronidase/sulphatase and analysed by HPLC with fluorescence detection. A peak that co-eluted with α-zearalenol prevented its detection. Ten further samples were taken from the same source and the enzyme treatment was repeated. The samples were then bulked and analysed by HPLC and the co-eluting peak was again present. The remainder of the bulked sample extract, was derivatised to form the trimethylsilyl derivatives and subjected to gas chromatography/mass spectrometry (GC/MS) using selective ion monitoring, alongside derivatised zearalenone and α- and β-zearalenol standards. No peak corresponding to α-zearalenol was found in the bulk derivatised milk sample. The GC/MS analysis of the milk samples was then carried out and demonstrated that α-zearalenol was not present in the milk post enzyme treatment (LOD < 1 microgram/l) and, therefore, α-zearanol conjugates were not present in the milk samples.

Results

A summary of the results is given in Tables 2 and 3. Zearalenone was detected in 3 conventional retail samples of milk, with the levels ranging from 1.2 – 5.5 micrograms/kg. Zearalenone conjugates were detected in 3 conventional retail and 2 conventional farm-gate milk samples at levels ranging from 5.9-12.5 and 3.4-8.3 micrograms/kg respectively. The conjugates were not detected in the same samples as zearalenone (see Table 2). Alpha-zearalenol and β-zearalenol and their conjugates were not detected in any of the samples.

Ochratoxin-α was not detected in any of the milk samples.

Dietary exposure estimates

Dietary exposure to mycotoxins has been estimated for adults and toddlers who consume average volumes of milk (mean consumers) and for those who consume significantly more than average (high level consumers). These estimates can then be compared with the tTDI set by the SCF. A tTDI of 0.2 micrograms/kg body weight was set for zearalenone, however a safety limit for zearalenone conjugates was not set due to insufficient data describing zearalenone metabolism in humans. These limits are used as a guideline to assess whether mycotoxin exposure through milk consumption is a risk to consumer health.

The Agency utilises information obtained from the National Diet and Nutrition Survey of British Adults[21] and children aged 1.5 – 4.5 years [22] to assess dietary exposure.

Adults

The results for UK adult consumers of all milk types (whole milk, semi-skimmed milk and skimmed milk) are shown in Table 4. The mean exposure to zearalenone for an adult consumer from all milk types was 2.32 nanograms/kg body weight/day. For a high level consumer the exposure was 5.10 nanograms/kg body weight/day for zearalenone. These compare to the SCF tTDI for zearalenone of 200 nanograms/kg body weight/day.

Toddlers

The results for UK toddler consumers of all milk types are shown in Table 4. The mean exposure for a toddler consumer from all milk types was 13.12 nanograms/kg body weight/day for zearalenone. For a high level consumer, the value was 29.92 nanograms/kg body weight/day. These compare to the SCF tTDI for zearalenone of 200 nanograms/kg body weight/day.

Interpretation

Zearalenone, α-zearalenol and β-zearalenol and their conjugates and ochratoxin-α have not previously been monitored in UK milk. Data from this survey of retail and farm gate milk samples show that exposure to zearalenone and its conjugates do not present safety implications for public health.

Ninety-five percent of samples did not contain zearalenone conjugates above the detection limit of 1 microgram/kg. Of the 5 samples that did contain zearalenone conjugates the maximum concentration was 12.5 micrograms/kg. Assuming that, in humans, all the conjugate is metabolised to the parent toxin this equates, for a high level adult consumer, to an exposure of 0.12 micrograms/kg body weight/day. This exposure is below the SCF temporary TDI of 0.2 micrograms/kg body weight/day. Alpha- and β-zearalenol and their conjugates were not found in any of the samples above the LOD of 1.0 microgram/kg.

Conclusion

Zearalenone and zearalenone conjugates concentrations were detected in only 3 and 5 out of the 100 samples respectively. The results of this survey of retail and farm gate milk show that exposure to zearalenone and its conjugates does not present safety implications for public health. Research has suggested that cows' milk could be considered as a potential source of zearalenone and its metabolites in the human diet. This is of particular concern for young consumers, who have a relatively high dose to body weight ratio, and who may consume a large amount of cows' milk.[22] However, the findings of this survey do not raise any food safety concerns and consumers do not need to change their dietary habits.

The Food Standards Agency will continue to monitor milk for mycotoxins.

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Units of measurement

Milligram: 1 thousandth of a gram

Microgram: 1 millionth of a gram

Nanogram: 1 thousandth of a microgram

Kilogrammes (kg): 1 thousand grams

Litres (l): 1 thousand millilitres

µg/kg: micrograms per kilogram

ng/kg: nanograms per kilogram

Table 1: Summary of analytical methodology

Mycotoxin	Clean-up	Quantitation	LOD (mg/l)
Ochratoxin-α	OASIS (Waters oasis™) HLB (3cc)	HPLC with fluorescence detection	0.1
Zearalenone and α- and β-zearalenol	Immunoaffinity column	HPLC with fluorescence detection	1.0
Zearalenone, α- and β-zearalenol conjugates	Incubation with β-glucuronidase/sulphatase, and immunoaffinity column	HPLC with fluorescence detection	1.0

Table 2: Summary of the results for retail milk

Sample no.	Sample	Brand	Mycotoxin level (µg/l)	
			Zearalenone	Zearalenone conjugates
T21049	Fresh Pasteurised Milk Homogenised	Dairy Crest, Iceland	5.5	<1
T21052	Whole Fresh Pasteurised Milk	Homelands Farm, Londis	1.2	<1
T21058	Whole Fresh Pasteurised Milk	Spar	<1	5.9
T21066	Milk Pasteurised	Grahams, Lidl	<1	12.5
T21075	Whole Fresh Pasteurised Milk	Safeway	1.2	<1
T21092	Fresh Pasteurised Standardised Milk	Golden Vale	<1	11.3

Table 3: Summary of the results for farm milk

Sample no.	Sample	Location of farm	Mycotoxin level (µg/l)	
			Zearalenone	Zearalenone Conjugates
T21100	Conventional	Somerset	<1	8.3
T21106	Conventional	Somerset	<1	3.4

Table 4: UK exposure assessment for adults and toddlers from zearalenone in milk

Product	Intake of zearalenone (ng/kg b.w./day)			
	Adults[a]		Toddlers[b]	
	Mean consumer	High level consumer	Mean consumer	High level consumer
Whole Milk	1.73	4.55	10.58	28.78
Semi-skimmed milk	1.37	3.82	6.52	20.65
Skimmed milk	0.82	3.24	1.04	5.52
All above products[c]	2.32	5.10	13.12	29.92

[a] Average body weight for an adult = 70.1 kg

[b] Average body weight for a toddler = 14.5 kg

[c] Based on the consumption of a consumer who consumes a combination of whole milk and/or semi-skimmed milk and/or skimmed milk

Annex 1: Summary of recovery data

All analyses are conducted with spiked samples, i.e. to each sample matrix on each day a known amount of toxin was added prior to extraction. These samples were used to assess recovery and recoveries between 70% and 110% were classed as valid. Spiked samples were also used for quantification, thus making all the results recovery corrected. Recovery data throughout the study is shown below.

Mycotoxin	Total number of samples	Mean recovery	RSD (%)
Zearalenone	100	86	2.9
α-zearalenol	100	88	2.3
β-zearalenol	100	86	1.8
Ochratoxin α	100	86	5.8