AOAC

Topic Advisor Report 2005

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Aflatoxin M₁

Late 2004, the English edition of FAO Food and Nutrition Paper 81 “Worldwide regulations for mycotoxins in food and feed in 2003” appeared in print (1), early 2005 followed by Chinese, French and Spanish editions. The document provides insight in limits and regulations on mycotoxins in food and feed in more than 120 countries. Some 60 countries have set specific limits for aflatoxin M₁ in milk and milk products. Some details about aflatoxin M₁ regulations were already published in 2004 (2), when the draft publication approached the stage of finalization. These details have essentially not changed.

New batches of whole milk reference materials, certified for their aflatoxin M₁ content are now available through the European Commission’s Directorate General Joint Research Centre (JRC) (3). The certified mass fractions of aflatoxin M₁ are <0.02 μg/kg (ERM-BD282), 0.111 +/- 0.018 μg/kg (ERM-BD283) and 0.44 +/- 0.06 μg/kg (ERM-BD284). Details of the preparation, homogeneity and stability testing of the materials have been published (4).

Whereas HPLC has been the most common technique to determine aflatoxin M₁ in milk and milk products now for many years, a tendency is visible to use alternative techniques. In the reporting year five papers were published specifically about analytical methodology for aflatoxin M₁, none of these dealt with conventional HPLC methodology. Instead, these papers described enzyme-linked immunosorbent assay (ELISA) (5), chemiluminescent enzyme immunoassay (6), flow-injection immunoassay (7), tandem mass spectrometry (8) and head space sensor assay (9). For most of these techniques some performance details were published, but interlaboratory validation data were not provided, which makes it difficult to judge about the value of the techniques, in comparison to HPLC, for which AOAC final action methods exist. The developments are interesting though, and should be closely followed.

In a duplicate 24-hour diet study carried out in the Netherlands, in which 123 participants collected duplicates of their 24-hour diets in 1994, samples were investigated for aflatoxin M₁, in addition to many other contaminants (10). For this purpose a method of analysis was developed, that could simultaneously determine aflatoxin M₁, aflatoxin B₁, and ochratoxin A at very low levels. The method involved chloroform extraction, liquid-liquid extraction, immunoaffinity cleanup and liquid chromatography. The method was in-house validated. The toxin was detectable in 48 % of the samples, but the toxin contents remained below the limit of quantitation in all samples. The conclusion was that human exposure to aflatoxin M₁ in the Netherlands was low. The study has been repeated with samples collected in 2004, results will be published in 2006.
In particular ELISA is more and more used in survey work. Out of nine papers published in the past year about occurrence of aflatoxin M₁ in milk and milk products, five made use of ELISA, and several of these studies were done in Turkey. Raw milk (11), cheese (12), and cheese and butter (13) were investigated in Turkey with this technique. Other Turkish studies on raw milk (14) and cheese (15) made use of HPLC. From the Turkish studies, it can be concluded that significant percentages of the raw milk samples (33-60%) and of the cheese samples (0-80 %) did not fulfill Turkish legal limits for aflatoxin M₁ in milk and cheese of 0.05 μg/kg. In Brazil, 24 % of samples of raw milk analysed for aflatoxin M₁ with ELISA, contained detectable amounts of the toxin, whereas 7 % exceeded the MERCOSUR legal limit of 0.5 μg/kg milk (16). In Greece, where ELISA was used to analyse ewe’s milk, and the produced curd and Feta cheeses, all aflatoxin M₁ levels were below the EU limit (0.05 μg/kg milk) (17). Samples of fresh milk, milk powder and drinking yoghurt collected in Taiwan in 2002, were all found to meet Taiwanese regulatory requirements of 0.5 μg/kg milk (18). In Iran, where a limit for aflatoxin M₁ in milk is in force of 0.05 μg/kg, 40 % of the investigated samples of raw milk obtained from dairy plants did not fulfil legal requirements (19).

A Brazilian study on aflatoxin M₁ in human milk showed that, out of 50 samples collected from a human milk bank in Sao Paulo and analyzed with HPLC, only one was contaminated with aflatoxin M₁, at 0.024 μg/l (20).

A study on carry-over of aflatoxin B₁ from feed to milk of dairy ewes was published (21). The mean rates of transfer ranged from 0.032-0.112 %, much lower than values reported for dairy cattle and goats. The authors concluded that sheep have a better ability to degrade aflatoxin B₁. In another study the carry-over of aflatoxin M₁ from milk to kefir and kefir grain was investigated (22) at laboratory conditions, with aflatoxin M₁ levels of 0.1, 0.2 and 0.5 μg/kg. Carry-over percentages to kefir ranged from 60-80 %, whereas the ratios in kefir grains ranged from 1.6 -2.6 %. The results demonstrated that the carry-over of aflatoxin M₁ from kefir and kefir grain is higher at higher levels of the toxin. Storage of kefir at 4 +/- 2 °C did not influence the aflatoxin M₁ content.

The topic advisor was informed that the molar absorptivity of aflatoxin M₁ in benzene/acetonitrile (9+1) was changed from a value of 18815 in the 15th edition of AOAC Official Methods of Analysis in 1995 to a value of 18000 in the 16th edition of AOAC Official Methods of Analysis in 2000. No rationale for the change is known and the value of 18000 in the 16th edition does not seem correct. In the absence of convincing arguments for this change, steps will be taken to publish the value of 18815 again in the coming 17th edition of AOAC Official Methods of Analysis in 2005.

References

(3) IRMM Reference materials catalogue. www.irmm.jrc.be/
Recommendations:

- The Topic Advisor recommends that the molar absorptivity for aflatoxin M₁ in benzene/acetonitrile (9+1) should be 18815, and that this value be published in the 17th edition of AOAC Official Methods of Analysis.