

# A guide to testing for aflatoxin M1 in infant formula

Aflatoxins are mycotoxins mainly produced by two species of *Aspergillus*, *Aspergillus flavus* and *Aspergillus parasiticus*. Of the four major forms (B1, B2, G1 and G2), aflatoxin B1 is the most common as well as the most toxic.

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Ingested by cattle with their feed, aflatoxin B1 is metabolised and converted into a hydrolysed form called aflatoxin M1, which is then secreted into milk. The processing of milk and dairy products does not significantly degrade aflatoxin M1; as a result, the toxin can be found in milk powder, cheese and infant formula.

Research today has proven that nutrient intake in the early months of a child's life is of vital importance, so much so that it can impact health during adulthood. Babies and infants are much more susceptible to aflatoxins than adults. Both parents and the industry are becoming increasingly aware of this, and as a result, the infant diet is subject to a higher level of scrutiny.

Since milk, in many cultures, is an integral part of child nutrition, there is a high demand for infant formula all over the world. Though this represents a huge opportunity for the dairy industry, it also comes with challenges. Aflatoxin M1 has become a major concern in baby and infant food products, so one of the most important challenges is the avoidance of aflatoxin M1 contamination from infant formula.

## Very low maximum levels

Generally speaking, regulations set very low maximum or action levels for aflatoxin M1 contamination in milk products. These regulations, which also define levels for infant formula, are intended to protect humans, especially babies and infants, from ill effects arising from aflatoxin M1.

Depending on the country or region of the world in question, regulations governing

this mycotoxin in food differ. But they all have one thing in common: its maximum allowed level in infant formula is extremely low.

As far as European Union regulations are concerned, the limit for aflatoxin M1 is 50ppt in milk and dairy products, and only 25ppt for infant formula. Regulations in China limit the maximum concentration in milk powder for infant food (calculated in dry powder) to 500ppt. For the purpose of analysis, milk powder must be dissolved into 10ml of water.

Consequently, the maximum concentration of aflatoxin M1 in dissolved milk powder for infant food is set at 50ppt in China. In the United States, the action level of aflatoxin M1 in milk is 500ppt. By contrast, Singapore and Malaysia have at 25ppt far lower action levels for aflatoxin M1 in infant formula and follow-up formula.

In order to meet these strict regulations, a testing program that is robust, reliable, accurate and appropriate to testing for infant and baby food and formula must be in place. But which one should you choose?

## Accurate and precise testing: different methods

There are different ways of testing for aflatoxin M1. Along with the more traditional method of sending samples away to an analytical service provider, on-site rapid tests have become more prevalent in recent years.

Deciding whether to conduct the test yourself on-site or to send the samples to an analytical service laboratory depends on a variety of factors including the frequency of testing and the time period within which the results must be received. Additionally, the resources that are available are an important consideration.

In cases of frequent or high-volume testing, it is worth considering on-site analysis as the costs are generally low. If, however, you test only occasionally, it may be more convenient to send your samples away to an analytical service laboratory.

The two most common methods of on-site testing are ELISA (enzyme-linked immunosorbent assay) tests and lateral flow devices (LFD), also known as strip tests.

Deciding which one to use again depends on a variety of factors as both have advantages and disadvantages.

Among analytical service testing methods, HPLC (high performance liquid chromatography) and LC-MS/MS (liquid chromatography-tandem mass spectrometry) as well as ELISA are good candidates, depending on the offering of the analytical service laboratory.

Commercially available ELISA kits have the high degree of sensitivity necessary to detect and quantify very low levels of aflatoxin M1. This makes them appropriate for testing infant and baby food. HPLC-FLD (high-performance liquid chromatography with fluorescence detection) is another method that is able to detect and quantify the presence of aflatoxin M1 in a variety of matrices, including infant formula, at extremely low levels (ppt).

We recommend cleaning up samples of milk and dissolved milk powder before they are analysed to assist the detection of aflatoxin M1 by HPLC-FLD. This method delivers accurate and reliable results.

Another method is the LC-MS/MS method, which uses fully <sup>13</sup>C labelled internal standards to correct for matrix effects. This method allows for the detection and quantification of aflatoxin M1 in milk and various milk products with a limit of detection as low as 3.5ppt.

## Conclusion

The need for appropriate regulation and testing solutions to resolve or ameliorate health problems resulting from mycotoxin contamination in the general population is clear.

However, owing to the increased vulnerability of infants and babies to mycotoxins such as aflatoxin M1, much lower thresholds for infant food and formula are in effect. It is imperative that companies producing these ingredients and foods, and the laboratories that may support them in mycotoxin testing be aware of the maximum or action levels of aflatoxin M1 to select the testing method most appropriate to the matrix to be tested and the jurisdiction and market in which they operate. ■