Mycotoxin Risk in Milk:
Why and How to Test for Aflatoxin M1

What is Aflatoxin?

Many genera of molds growing on agricultural products produce toxic substances, generally called mycotoxins. Mycotoxins are secondary metabolites of various, different fungal species and they differ in chemical structure, biosynthetic origins, and biological effects. One highly studied group of mycotoxins, aflatoxins, are produced by some species of *Aspergillus*. These toxic metabolites can be found in agricultural food and commodities, including nuts, grain, soybeans and feeds that are improperly stored under conditions where *Aspergillus* can thrive. Typically, contamination occurs under high temperature and humidity conditions, favorable for mold growth on soil, seeds, grains, and decaying vegetation. There are 4 major types of aflatoxins: B1, B2, G1 and G2, each named according to the ability to be detected under UV light as fluorescent blue (B) or green (G) colors. Aflatoxin B1 is the most prevalent and is highly toxic and carcinogenic to animals.

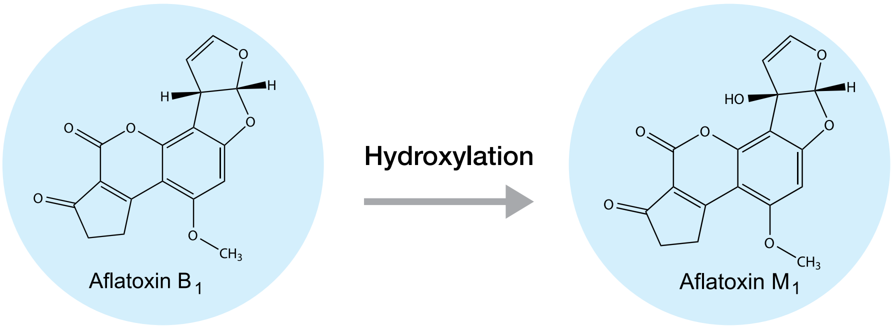


Figure 1. Aflatoxin M1 is a hydroxylated form of Aflatoxin B1. Aflatoxins are produced by *Aspergillus flavus* and *Aspergillus parasiticus*. (Fallah *et al*., 2009)

What Risks Do Aflatoxins Pose?

Humans can consume aflatoxins directly by consuming affected grain products. One of the main risks to human health comes indirectly from animals that ingest aflatoxins when they consume corn grain, silage and by-products as part of their daily feed. Taking this a step further - in dairy animals, aflatoxin B1 not only causes harm to the animal, but also is metabolized into aflatoxin M1 (AFM1), which collects in the animal's milk and gets passed on to those who consume the milk. In addition, AFM1 can not only be found in raw milk, but it can also be found in sterilized and pasteurized milk, yogurt, cheese, dry milk, infant formula, milk-based consumables and even breast milk. This is because aflatoxins are difficult to eliminate from milk and dairy products, even after repeated processing, including sterilization and pasteurization.

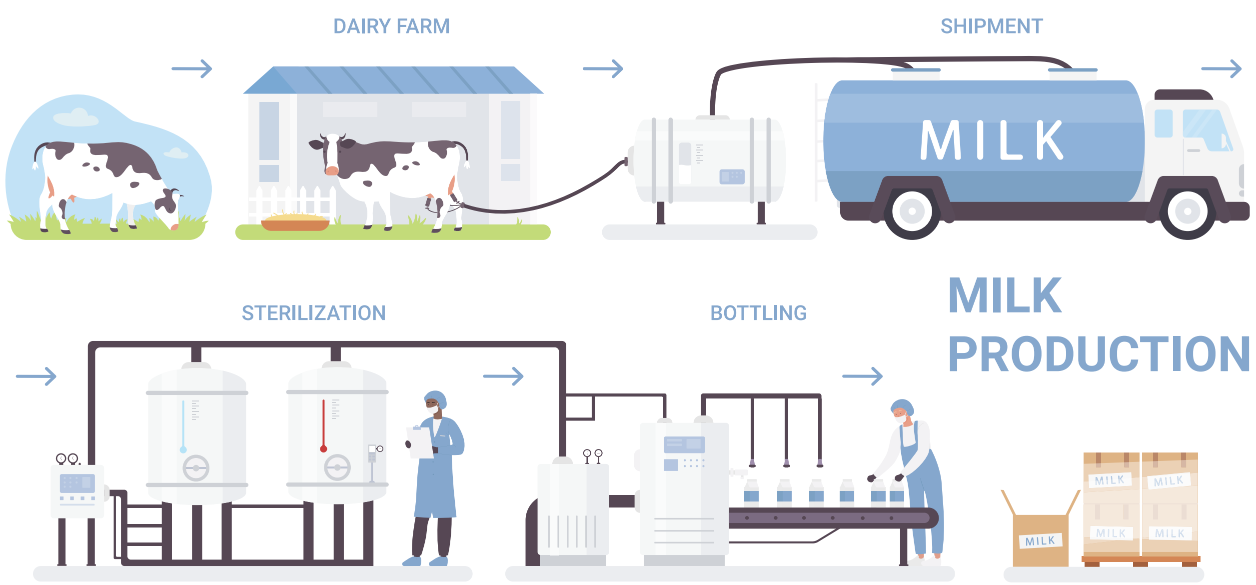
Due to its high toxicity and hepatocarcinogenic potential, the level of AFM1 allowed in milk in dairy products is strictly regulated in developed countries, although threshold values vary depending on country. For example, the regulatory limit for AFM1 in milk and dairy products is 50 ppt in European countries and 500 ppt in the United States. It is even stricter in the EU for milk specially prepared for infants and children (25 ppt). Acceptable levels for other countries are listed in the table below.

Country	AFM1 maximum limit (ppb = µg/kg)	Type of Product
European Union	0.05	Milk/Raw Milk
	0.25	Cheese
United States	0.5	Milk/Raw Milk
Switzerland	0.05	Milk/Raw Milk
	0.25	Cheese
	0.02	Butter
Australia	0.05	Milk/Raw Milk
Germany	0.05	Milk/Raw Milk
Belgium	0.05	Milk/Raw Milk
Sweden	0.05	Milk/Raw Milk
France	0.05	Milk/Raw Milk
Italy	0.05	Milk/Raw Milk
Iran	0.05	Milk/Yogurt/Ice Cream
	0.2	Cheese
	0.02	Butter
China	0.5	Milk/Raw Milk
Turkey	0.05	Milk/Raw Milk/Yogurt
	0.025	Cheese
Netherlands	0.02	Butter
	0.2	Cheese
Brazil	0.5	Milk
Argentina	0.5	Milk/Raw Milk
Peru	0.5	Milk/Raw Milk
Mexico	0.5	Milk/Raw Milk
Ecuador	0.5	Milk/Raw Milk
Chile	0.05	Raw Milk
Japan	0.5	Milk
India/Pakistan	0.5	Milk/Raw Milk
Morocco	0.05	Milk
	0.5	Powdered Milk
Israel	0.05	Milk/Raw Milk
Malaysia/Singapore	0.5	Milk/Raw Milk
Vietnam	0.5	Milk/Raw Milk
South Africa	0.05	Milk/Raw Milk
Kenya	0.5	Milk/Raw Milk

Testing Methods

As a result, there must be robust, sensitive analytical methods in place for detecting mycotoxins, especially aflatoxins. This is especially critical for any facilities that process milk and milk products – the major source of aflatoxin introduction into humans. Fortunately, there are several methods that can be used to identify the presence of mycotoxins and aflatoxins in food. The preferred methods for the analysis of aflatoxin in milk remain lab-based and include enzyme-linked immunosorbent assays (ELISA), fluorescence spectrometry and high-performance liquid chromatography (HPLC). Each of these methods provides advantages and shortcomings.

Determining which technology to use depends on many factors, including the level of sensitivity needed and where the testing needs to take place. In the field, lateral flow tests are often used. However, at the creamery, as milk is processed into multiple products, ELISA tests are the preferred method. For further confirmation by 3rd party labs, HPLC is often used as it can separate many organic analytes in milk and detect toxins by UV absorption, fluorescence, or mass spectrometry to levels of parts per trillion (ppt). Nevertheless, the method of choice is often dictated by the matrix – fresh milk versus pasteurized milk, powdered milk, or cheese. Methods need to be validated as well. Often, chromatography is reserved for detailed analysis after running rapid screening tests.



Testing process

Raw milk is tested for a wide range of contaminants, including AFM1, before it even leaves the tanker truck. This can be done at the farm or at the point of reception for a creamery. Testing is typically performed using a lateral flow strip. Often, if high levels of contaminants are detected (above a set threshold), the raw milk will be rejected at this stage. The challenges at this stage are accurate detection as raw milk contains a significant amount of fat which can interfere with testing results.

If the raw milk passes initial testing, it is then tested by the dairy processor by ELISA for quantitation of AFM1 levels. This data confirms if the milk is suitable for processing into final milk products. ELISA tests are relatively inexpensive and easy to perform on site. Following processing, the final product must also be tested before release to ensure it meets regulatory guidelines. If any flavorings are added to create the final product (flavored milks, yogurts, etc.), it must also be tested for total aflatoxin along with AFM1 to ensure no additional contaminant was introduced from the flavoring agent(s).

How Hygiena Can Help

Hygiena™ offers an extensive portfolio of aflatoxin testing kits. All are ELISA based and are highly sensitive, reliable, easy to use and are capable of measuring aflatoxin (B1, B2, G1, G2, and M1) in various food and beverages. Total aflatoxin kits are available for detection in corn and corn products, grains, nuts, cereals and other commodities including animal feeds, food and beverages, cannabis flowers, and urine. Specifically, for AFM1, Hygiena offers two very sensitive assays. The **Aflatoxin M1 Low Matrix (High Sensitivity) ELISA** is a competitive enzyme immunoassay designed to quantitate AFM1 levels in milk, milk powder, butter, whey powder, and cheese with a validated detection range of 5 – 100 ppt (parts per trillion), meeting regulatory testing needs for even EU countries. The **Aflatoxin M1 Ultra ELISA** is a second enzyme-linked immunoassay designed to quantitate levels of AFM1 in milk, skim milk powder, and yogurt with detection limits of 5 ppt for milk and 20 ppt for yogurt. This second test allows for a broader range of detection from 5 to 500 ppt and complies with ISO guidelines.

Conclusion

No matter the technology, each facility must determine which technology works best for them by considering the level of sensitivity required, the type of matrix being tested, the location for testing and the level of test complexity acceptable for internal use. While lateral flow strips work well in the field, for lab testing on site, ELISAs are the best option as they offer high sensitivity, relative ease of use, and detect levels as low as ppt. If additional confirmation testing is needed, facilities can send samples to third-party labs which typically use HPLC for confirmatory testing and may also use mass spectrometry when multiple analytes need identified and quantitated.

Learn more at hygiena.com