

CHANGING FEED INDUSTRY IMPERATIVES CALL FOR A CLOSER LOOK AT ON-SITE AFLATOXIN TEST METHODS

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INTRODUCTION

A RISING CALL TO ACTION

As government-mandated and industry-driven hazard identification and prevention efforts become standard operating procedure for an expanding cross-section of the global feed industry, the need of its diverse stakeholders for fast, easy, cost-effective access to reliable aflatoxin data continues to intensify. Recognition of aflatoxins as a chemical hazard of very high concern spans the gamut of national and international regulatory agencies, standards bodies, and public health organizations from the U.S. Federal Drug Administration (FDA), the Canadian Food Inspection Agency, and the Pan American Health Organization to the World Trade Organization (WTO), the World Health Organization (WHO), and the Codex Alimentary Commission. This broad consensus coincides with surging public demand for food production practices that protect humans and animals from dietary contaminants. Their interplay has heightened awareness of the industry's key role in the agricultural sphere and the weighty responsibilities it entails. As a linchpin of the dairy and other livestock sectors and thus both a major contributor to animal welfare and a vital link in the global food supply chain, the feed industry is increasingly viewed as one of the logical starting points for strict control of a universally acknowledged threat to animal well-being, public health, and food security.



This perspective informs the sweeping food safety laws that have emerged in the North America, Europe, and parts of Asia over the last decade. Under the 2011 Food Safety Modernization Act (FSMA), for example, U.S. manufacturers of feed ingredients, finished feeds, and pet food; grain processors; producers of biofuel co-products like distillers dried grains (DDGs); and grain elevators that house imported grain or engage in processing are required to implement hazard analysis and risk-based preventive controls to help limit the spread of toxic contaminants across the production chain. FSMA rules also hold feed transporters accountable for hazardous shipping conditions. In practice, these requirements mean that each of these operations needs to monitor aflatoxin levels in the commodities within their purview, either by collecting samples of incoming ingredients and products for lab testing or testing samples in house.

While laboratory analysis remains a prerequisite for export certification, the significant expense and long lead times entailed in this approach to hazard control render it impractical for regularly monitoring quality and safety and for determining the acceptability of shipments and sorting lots at buying points. Rapid on-site immunoassays, such as commercial ELISA kits and quantitative strip tests, offer feed businesses faster, less expensive alternatives. Yet as closer scrutiny from government agencies, feed customers, and the public drives an intensifying focus on product testing, test options that afford ever-greater time and cost savings are becoming an increasingly urgent industry priority.

RISKIER ENVIRONMENTAL CONDITIONS

Toxic metabolites of *Aspergillus* and *Penicillium* molds, the four major aflatoxins (AFB₁, AFB₂, AFG₁, and AFG₂) pose a threat to the quality, safety, and value of virtually every feed ingredient, including whole grains (e.g., corn, wheat, rice, sorghum, millet), milling byproducts, oil meals, and ethanol co-products. Aflatoxin outbreaks are an endemic risk in tropical and subtropical climate zones and irrigated deserts, where high temperatures and extremes of water activity (i.e., heavy rainfall and high humidity/drought) feature prominently in regional weather patterns. The escalating levels of CO₂ that now pervade the global atmosphere work in concert with these climate extremes to optimize conditions for rapid aflatoxin synthesis in grain and fodder crops both before and after harvest. Violent storms, such as hurricanes and tornadoes, which often severely damage pre-harvest crops, also predispose feedstuffs to aflatoxin contamination in the field and during storage.

In recent years, climate scientists and public health officials have voiced concern about mounting evidence that aflatoxin-conducive conditions are increasing in intensity and frequency and spreading to more temperate regions of the globe. (See "Climate Data" sidebar.) This situation calls for increased vigilance among buyers and sellers of feed commodities in affected regions, especially during the post-harvest period. Whether raw materials are stored in grain elevators, housed in grain mill storerooms, or en route to these venues, the onset of extreme weather patterns can lead to contamination levels far exceeding those that typically occur in the field. Laboratory studies suggest just how dramatic the impact of a humid heat wave during storage or shipment can be. For example, researchers found that when corn and peanut samples were tested after spending 7 days in storage at 31°C (88°F) and 100 percent humidity, the contamination levels were 1,000-fold higher than those in freshly harvested corn.¹ Hot, humid weather can likewise spell trouble for finished feeds at any point in the post-harvest continuum from manufacturing plants to distribution warehouses to retail outlets to storage bins on farms and ranches.

In addition to assessing pre-harvest and post-harvest weather, a comprehensive hazard analysis should account for high-risk storage conditions resulting from maintenance lapses, such as leaky bins and malfunctioning ventilation systems, as well as for the potential impact of supply chain issues on storage and transportation timelines.

INCREASINGLY CONSEQUENTIAL SUPPLY CHAIN ISSUES

The majority of today's feed manufacturers and distributors rely on an ever-more complex and widely dispersed network of foreign and local suppliers. This consequence of an increasingly globalized marketplace means that feed commodities tend to travel greater distances and spend more time in shipping and storage containers, where a gradual buildup of heat and moisture can create favorable conditions for widespread mold growth. Feedstuffs with a high moisture content, such as wet brewer grains (WBGs) and wet corn are particularly prone to this problem. The fallout of the ongoing COVID-19 pandemic has magnified the impact of this hazard. With production slowdowns, plant closures, and other pandemic-related restrictions still impeding supply chain flows, lengthy transit delays and storage durations have become the norm, expanding the window for severe post-harvest contamination.

By systematically assessing these potential influences on contamination levels, businesses can develop a road map for risk-based testing logistics. Deciding which lots to target for intensive testing based on the extent of their exposure to high-risk conditions helps minimize expensive guesswork. By allocating testing resources where logic dictates, companies can lay the groundwork for a cost-efficient means to a broadly beneficial end: an aflatoxin control strategy that protects the quality of their own inventory while mitigating the threat of contaminated feedstuffs traveling farther down the supply chain and ultimately entering animal diets.

To fully capitalize on that strategy, however, a feed operation must first negotiate any business and technical roadblocks standing in the way of timely access to verifiable evidence of its products' fitness for food-producing animals in its target market.

THE RELATIONSHIP BETWEEN FEED INDUSTRY QUALITY CONTROL AND THE SAFETY AND ABUNDANCE OF THE GLOBAL FOOD SUPPLY

The far-reaching implications of aflatoxins for human and animal health and efficient, sustainable food production are rooted in their toxicological properties. The most commonly occurring aflatoxin in food and feed, AFB₁ is the most potent carcinogen currently known, as well as a major risk factor in the development of other severe health problems including liver, kidney, and neurological damage and chronic immunosuppression. While acute, potentially fatal toxicosis can result from a single dose of heavily contaminated food or feed, the cumulative effects of low doses of AFB₁ consumed over time can increase the risk of cancer and other life-threatening illnesses in humans and animals. The effects of chronic AFB₁ ingestion can prove equally detrimental to livestock productivity, inducing symptoms such as feed refusal and decreased feed efficiency, growth rates, and egg and milk production. (See Table 1). Very young humans and animals, as well as those whose immune systems are compromised due to disease or other stresses, face a heightened risk of toxic effects from aflatoxin exposure.

Compelling Consumer Concerns

Toxicological studies indicate that the damaging effects AFB₁ are exacerbated by its interactions with other mycotoxins present in the same plant matrix. Commodities that contain AFB₁ usually contain the other three related aflatoxins, and all four frequently occur together with one or more of other major mycotoxins of concern such as ochratoxin A, fumonisins, deoxynivalenol, zearalenone, and T-2/HT-2 toxins. Statistical analyses of the evidence to date suggests that the

potential of AFB₁ and these other toxicologically significant mycotoxins to cause chronic or acute conditions in humans and animals is far greater when they're ingested as a mixture than when they're consumed individually. The fact that even legally compliant levels of aflatoxins can inflict harm when they occur in commodities that contain other mycotoxins calls for an abundance of caution when screening feedstuffs for animals in high-risk categories. In addition to immature livestock, this classification includes pets which may be frail, elderly, or subject to breed sensitivities. (See "Pet Food Industry" sidebar.) For feed sectors whose target markets comprise animals of this kind, customer trust often hinges on test data that confirms their products exceed minimum safety standards.

Research revealing the presence of residues of AFB₁ and its co-contaminants in the meat, eggs, and milk of animals fed contaminated feed has fueled consumer demand for stronger reassurances that animal products are free of toxic contaminants. Although the level of aflatoxins in these products is generally very low, the recognition of AFB₁ as a Group 1 liver carcinogen (i.e., a known cause of liver cancer in humans) by the International Agency for Research on Cancer (IARC), together with animal and human studies confirming the buildup of aflatoxins in the liver from chronic exposure, underscores the importance of minimizing their presence in these foods. To date, regulatory activity surrounding this issue has focused mainly on the presence of the AFB₁ metabolite AFM₁ in milk, which can cause severe health damage in infants and children. The sale of milk that contains more than 0.5 ppb AFM₁ is banned in the United States, and even tighter restrictions apply in the EU and some Latin American and Asian countries. Efforts by developed and transitional countries to preempt the serious health and economic repercussions of contaminated milk include stringent regulation of aflatoxin levels in dairy feeds.

Proactive Risk-Based Regulatory Policies

In addition to concerns about the carryover of aflatoxins in animal products, feed regulations in a growing number of countries aim to preserve animal health and productivity by accounting for variations in aflatoxin sensitivity among different species, as well as for the impact of each species' age, weight, and breeding status on its vulnerability. (See Table 1.) Susceptibility to both acute and chronic effects is most pronounced in poultry, followed by swine. Cattle and other ruminants are somewhat more resistant to many of the negative effects of aflatoxins due to microbes in their gut that deactivate mycotoxins.

The second-leading feed-producing country, with an annual output of more than 284 million tons of finished feed and pet food,² as well as a major hub of the international feed trade, the United States bases their aflatoxin regulations on the research findings of FDA scientists. The agency's detailed roster of action levels* targeting total aflatoxins (i.e., the sum of AFB₁, AFB₂, AFG₁, and AFG₂) in animal diets serves as a model for many of its trading partners, including Canada and much of Latin America. (See Table 1) Aflatoxin limits in other regions, such as the Pacific Rim and Japan, align more closely with the even stricter regulations set by EU legislators. For instance, while the U.S. aflatoxin limit for dairy feeds is 20 ppb for total aflatoxins, EU regulations for that feed category specify an AFB₁ limit of 5 ppb. Both sets of regulations factor in the extent of the exposure risk presented by different feed commodities and types of feed.

**Limits at or above which the FDA will take legal action to remove products from the market.*

Table 1: FDA action levels for total aflatoxins in livestock feed; health/performance effects of unsafe levels

Animal	Action Level for Corn/ Peanuts	Action Level for Cottonseed Meal	Health/Performance Effects from Chronic Exposure to Unsafe Levels*
Immature animals	20 ppb	300 ppb	Liver damage, Hemorrhage, Depressed disease resistance
Breeding animals	100 ppb	300 ppb	Impaired reproductive function, Embryo death, Birth defects, Reduced reproductive efficiency
Poultry	100 ppb	300 ppb	Increased cancer risk Liver/kidney damage, Immune suppression, Hemorrhage, Contaminated eggs, Decreased feed efficiency/ growth/ meat/ egg production/quality
Swine (> 100 lb)	200 ppb	300 ppb	<i>Increased cancer risk Immune suppression Liver damage Internal bleeding Reduced growth/feed efficiency</i>
Cattle	Dairy Cows	20 ppb	Contaminated milk Decreased milk production
	Finishing Beef Cattle	300 ppb	300 ppb Reduced growth/feed efficiency Liver damage

* All classes of animals that experience acute aflatoxicosis (poisoning from a single or brief exposure to high doses of aflatoxins) may experience generalized hemorrhage, bloody diarrhea, and death in 1-3 days. Young animals and pregnant sows are the most susceptible to these effects.

In the U.S. and many other developed countries, violations of the stringent safety standards governing the marketability of domestic and imported feed commodities can trigger costly consequences that may jeopardize a business’s brand image, market reach, and profitability over the long term. For instance, lapses in FSMA compliance such as failure to carry out and document efforts to identify and address hazardous contamination levels can result in warning letters, fines, or even more financially painful regulatory actions such as product recalls, all of which can provoke lingering negative perceptions of that facility’s offerings among feed buyers, supply chain partners, and the public. This brand damage, in turn, can lead to reductions in sales volume and the prices those offerings command. The need to discard or divert noncompliant to shipments to less lucrative markets can likewise threaten a company’s bottom line. In cases where contaminated feed or pet food causes an animal’s death or impairs its health or productivity, the responsible parties may face a lawsuit claiming damages for the owner’s losses. Conversely, a comprehensive audit trail of aflatoxin test results testifies to the quality and value of a business’s products or services and can serve as a compelling competitive differentiator in an ever-more complex, demanding, and compliance-driven feed market.

A 2021 United Nations Food and Agricultural Organization (FAO) report points to both the significance of the revenues at stake in the feed industry's quest to manage this high-risk contaminant and the importance of that effort to the abundance and safety of the global food supply. The report noted that by 2050 the demand for food is expected to grow by 60 percent, driving a rise in the production of animal proteins of approximately 1.7 percent per year, with production of meat and dairy projected to increase by almost 70 percent and 55 percent respectively.³ As a central player in meeting this burgeoning demand, the feed industry is predicted to reach a global value of \$607 billion by 2026.³ Minimizing the occurrence of contaminants that impair livestock health, growth, and productivity promises to enhance the competitiveness of feed manufacturers and their agribusiness partners, while mitigating a threat to the availability of a major source of essential nutrients.

CUTTING COSTLY COMPLEXITIES FROM THE TEST PROCESS TO FIT TIGHTER REQUIREMENTS

Patricia Jackson noted that feed operations striving to balance the demands of evolving regulations and public perceptions with the realities of the current business environment need to carefully evaluate their aflatoxin testing approach. "The key to successfully navigating this high-risk/high-reward landscape lies in testing technology that simplifies end-to-end compliance and quality management," she said. "That technology must provide internal quality inspectors with real-time, actionable data that can be easily shared across the production chain as well as securely stored and readily accessed to verify that products meet regulatory requirements and contract specifications. Equally important, it must do all that without disrupting the business's core operations or breaking its budget."

The social and economic upheaval wrought by the COVID-19 pandemic has brought the importance of a lean approach to feed testing into even sharper focus. Already in steep decline before the Great Resignation of disaffected, COVID-wary workers in 2020–2021, the current workforce participation rate still hovers near its lowest point in several decades. Technically skilled workers remain in particularly short supply. At the same time, the pressing challenges of manufacturing cost management are more acute than ever for feed producers facing exorbitant shipping charges and higher prices for difficult-to-source ingredients from lagging supply chains. As this scarcity of operational and investment resources converges with mounting pressure to ramp up production to pre-pandemic levels, test methods that require significant time and effort or specialized expertise to deliver accurate results can create more problems than they solve.

"As the backbone of many manufacturers' quality control programs," said Jackson "the ELISA approach to aflatoxin monitoring may limit the feed industry's return on its testing investment by slowing the progress of its adaptation to these increased stresses and evolving market demands." She explained that this downside stems largely from the complexity of ELISA procedures. "Their numerous steps and exacting requirements present multiple opportunities for human error and raise the risk of testing bottlenecks that can interfere with workflows and delay access to essential decision support data. The cumulative impact of these pitfalls can undermine a business's safety, quality, and operational goals." She added another caveat for companies keeping a close eye on the balance sheet. "While ELISAs offers economies of scale for batch testing, the price per sample can skyrocket to as much as \$20–\$30 when used to test incoming commodities individually."

VICAM™'s Nancy Collette Zabe elaborated on this point. "Whether ELISA users are testing thirty samples or just one, they need to generate a calibration curve that can serve as a baseline for calculating the aflatoxin concentration of the test sample. This laborious procedure requires setting up and running a test on a full set of calibrants, which entails a painstaking, step-by-step serial dilution process and repeated washes of the multiwell ELISA plate after every step," she said. "In addition to all this hands-on time, each ELISA run involves several incubation periods as well as the use of expensive, highly toxic chemical standards."

"Performing all the steps correctly can be quite tricky," added Jackson. "The user's pipetting skills need to be up to the demands of precisely measuring and dispensing the standards and reagents into a series of very tiny wells without letting the pipette tip touch the bottom of the well. The slightest deviation from the correct amount of liquid or proper pipetting technique can compromise the accuracy and reliability of the results. And because the standards used for calibration consist of real aflatoxins, a pipetting misstep that causes these liquids to spill or splash outside the well takes a toll on workplace safety."

She noted that good mental concentration is also a must. "The tester needs to keep track of four separate wells for the calibration plus a well for each sample. If the wrong calibrant is added to a well or one of the wells is missed, all the wells, which can cost anywhere from \$2.50 to \$5 each, as well as all the sample materials, are reduced to hazardous waste, and the entire test, including the calibration steps, needs to be repeated."

"Given that even when the process goes smoothly, the total turnaround time for an ELISA test can range from 15 minutes to several hours," said Jackson, "it's no surprise that buyers who need to make real-time decisions about inbound shipments of raw materials tend to opt for simpler methods."

One of the most widely used methods of food contaminant testing, quantitative lateral flow immunoassays (i.e., antibody-based strip test systems that deliver numerical results) offer a significantly easier, faster, and less expensive, yet highly reliable alternative to on-site ELISA analysis. This pared-down approach enables users with no laboratory training and minimal instruction to quickly and accurately detect and measure toxin concentrations by inserting a prepared test strip into an optical reader that translates the color changes on the strip into a number that's displayed on its screen.

Jackson noted that ongoing advances in these test devices continue to build on the inherent advantages of their ease of use and versatility. She cites VICAM's most recent addition to its strip test portfolio as an example. "VICAM's new AFLA-V™ ONE fills a long-standing gap in the range of high-efficiency, cost-effective solutions for industry-wide aflatoxin control," said. "Until VICAM scientists adapted its strip test technology for the demands of exceptionally complex sample matrices, the ELISA method was the only viable non-laboratory test option for manufacturers of finished feeds and pet food." She explained that the numerous, diverse chemical constituents of these multi-ingredient formulations comprise a vast array of properties that can interfere with the capacity of the detection antibodies in the test to strongly and exclusively bind with the target analytes, potentially reducing the sensitivity and accuracy of detection. "The water-based extraction methods developed for strip tests are highly effective at isolating and recovering target analytes from the somewhat simpler matrices of discrete ingredients, like corn or peanuts," she said. "But water methods proved much less efficient at separating the aflatoxins in these formulations from the much wider variety of interfering compounds they contain."



VICAM developers eliminated this performance barrier by devising a streamlined, high-powered methanol-based extraction procedure. The use of a strong methanol solution for the extraction process maximizes aflatoxin recoveries while minimizing interferences, boosting sensitivity and accuracy without adding extra steps. While a typical ELISA methanol extraction takes 3 minutes, the procedure for AFLA-V ONE reduces the extraction time to just 90 seconds. Users can choose from two procedures, one with a 2 ppb limit of detection (LOD) and a range extending to 80 ppb, and the other for testing levels from 5 ppb to 300 ppb. With a range/LOD that averages about 5 ppb to 150 ppb, ELISAs fall short of offering a total solution to the intricacies of assessing the suitability of feeds for diverse market segments.

Vertu TOUCH
Reader



Vertu PREP
mixer



Designed for use with VICAM's newly launched Vertu™ TOUCH Reader and Vertu PREP mixer, this ultra-lean test system incorporates a full range of time- and labor-saving innovations. In addition to intuitive touch screen operation, the Vertu Reader offers users the multiple benefits of fail-safe automated calibration. Instead of having to conduct a new calibration experiment each time they run a test, users simply insert the barcode card provided with the strip tests into the device and its software does the rest, setting up the instrument to accurately interpret every strip in the lot based on a lot-specific calibration curve of toxin concentrations. By archiving a list of up to 99 method calibrations, the system's memory affords convenient access to a traceable record of the test specifications for future reference and auditing purposes. The software also stores and seamlessly shares detailed test data across the extended enterprise, enabling corporate team members to provide government inspectors, supply chain partners, and customers with hard evidence of the quality and reliability of its testing and hazard control practices. In addition to transferring test data directly from the device to a printer for hand-off to internal staff or regulatory officials, users can upload the information to an Excel spreadsheet for easy tracking of emerging sources of risk such as shipments from a particular supplier or region.

Still more reductions in hands-on time derive from the high-performance Vertu mixer, which eliminates the need to shake the sample solution by hand for 2–3 minutes or to hold it in place on a vortex mixer. Its speed and ease of use are complemented by its power to optimize the homogeneity of the test sample, fully capitalizing on the value of a statistically valid sampling approach. In combination, proper sampling and sample preparation techniques, such as mixing and grinding together multiple samples from different portions of a lot and thoroughly blending the material with a solvent, work together to minimize the chances of inaccurate test results caused by the natural tendency of mycotoxins to cluster in tiny, isolated hotspots in multi-ton grain loads. The risk of false positives and false negatives is further reduced by the superior ability of the monoclonal antibodies on the strip to isolate the target analytes from complex sample matrices.

“The entire test process can be completed in approximately 7 minutes and eliminates the need for toxic chemical standards and other costly supplies required for ELISA analysis.” said Jackson. (See Figure 1.).

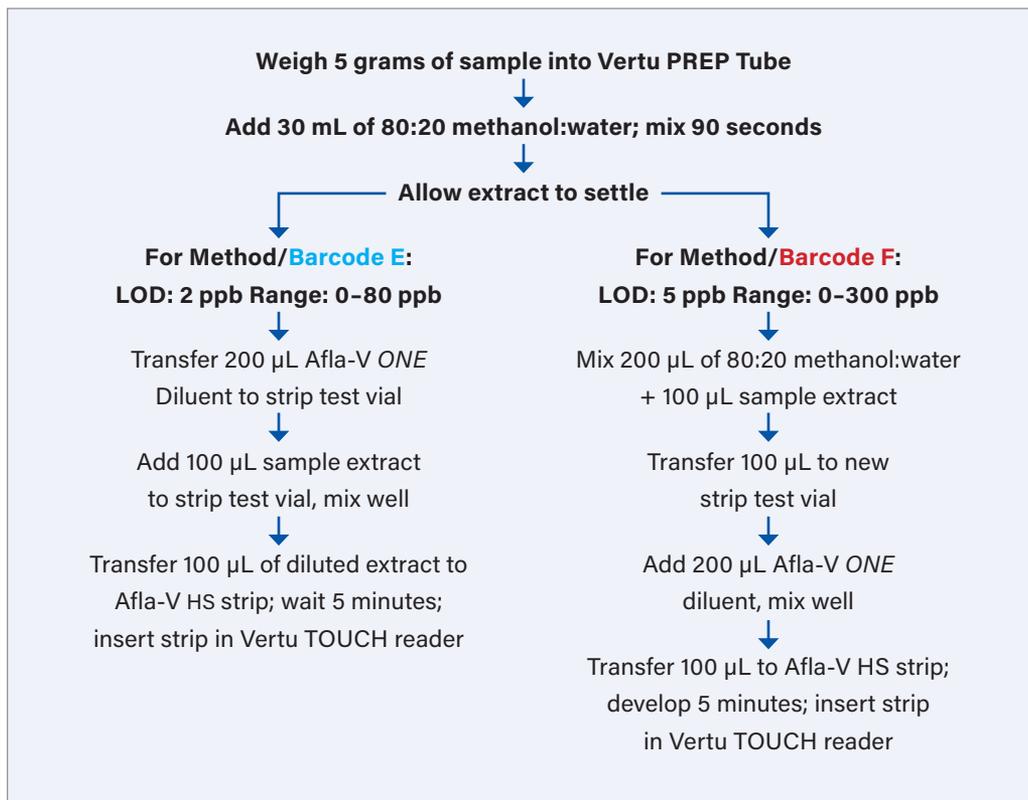


Figure 1: With two super-efficient procedures, Afla-V ONE offers feed producers a testing strategy with the power and flexibility to meet the requirements of multiple target markets.

“As the first quantitative strip test for finished feeds and pet food in world, AFLA-V ONE speaks to issues of major concern to the 21st century feed industry,” she said, noting the importance of continued progress in the development of tests that reduce the economic and technical barriers to compliance with its highest ideals. With a simple, affordable means of farm-to-feedlot aflatoxin control, the test stands to empower every member of the value chain to meet their obligation to advance animal welfare and safeguard the world’s food supply.

CLIMATE DATA SIGNALS GROWING THREAT TO FEED SAFETY

Reports of accelerating environmental changes in the Americas and Europe point to an escalating risk of aflatoxin contamination in feedstuffs across a widening swath of the globe:

- CO₂ levels have increased by 12 percent since 2000.¹
- The past 20 years have brought some of the most severe and persistent drought conditions on record in the western U.S. and Canada.²
- Heat waves are occurring three time more frequently in the U.S. than they did in the 1960s.³
- The frequency, strength, and duration of tropical storms in the Atlantic, Caribbean, and Gulf of Mexico has increased over the last 20 years.³
- Average summer temperatures in Europe have increased by 33°F (0.46°C) per decade since 1950, increasing the intensity of heat waves.⁴
- The previously low-risk areas of Italy and Central Europe experienced severe outbreaks of aflatoxin contamination in maize (corn) crops in 2003 and 2012, respectively.⁵

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AFLATOXIN AWARENESS AND THE PET FOOD INDUSTRY

Despite extensive industry efforts to screen pet food ingredients for aflatoxins before processing, unsafe levels of contamination in the final product can and do slip past quality inspectors. A large-scale 2021 recall linked to the deaths of more than 100 dogs from aflatoxicosis sparked widespread negative publicity at a uniquely complicated time for the industry. While the pandemic has subjected pet food companies to many of the same pressures afflicting other feed sectors, it's fostered a boom in pet adoptions, expanding the industry's customer base and driving an uptick in 2021 global sales of 6.8 percent.¹ Meanwhile consumer interest in the purity and wholesomeness of their pets' diets continues to trend upward and food laws are evolving toward ever more rigorous controls on pet food quality and safety.

The current FDA action level for aflatoxins in pet food is 20 ppb, reflecting the acute sensitivity of many pet species, particularly dogs, to their toxic effects. Because pet foods tend to contain even more ingredients than livestock feeds, accurately determining trace levels of aflatoxins in these formulations presents a particularly tough challenge. New developments like VICAM's AFLA-V ONE quantitative strip test promise to make it easier for conscientious pet food manufacturers to shoulder heavier responsibilities and live up to their customers' rising expectations.

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