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The Role of Key Breakthroughs in Aflatoxin M_1 Testing in the Growth and Profitability of the Dairy Industry

INTRODUCTION

For the dairy industry, the difficulty of adapting to today's rapidly evolving regulatory environment is as undeniable as the urgent need to rise to the challenge. As producers of a dietary staple in the majority of nations around the globe, the dairy industry is an important source of nutritional and economic value for a significant portion of the industrialized and developing world, providing 10 percent of the world's protein supply[1] and generating global revenues projected to reach USD 442.32 billion by 2019.[2] This promising outlook reflects changing consumer tastes and demographics, including aging populations, urbanization, and the rise of a middle class in emerging nations such as India, China, Russia, and Brazil. As these ongoing shifts continue to open the way for new growth opportunities in both mature and emerging markets, they're simultaneously driving public and governmental demand for higher standards of safety and purity across a growing number of geographical locations and product categories. In the midst of these changes, the potential of dairy stakeholders to expand their share of profitable new markets and the continued security of the world's dairy supply increasingly hinges on the success of industry efforts to minimize contamination at every stage of the production chain. One of the greatest threats to these efforts is aflatoxin M_1 (AFM_1), a highly toxic naturally occurring contaminant that dairy regulations in many parts of world restrict to scarcely detectable levels.

As the dairy industry intensifies its commitment to food safety, the adoption of routine AFM_1 monitoring programs is becoming an increasingly common risk reduction strategy at dairy farms and processing facilities around the world. To ensure the effectiveness of these programs, while maximizing the return on their technology investment, an expanding range of dairy industry stakeholders are looking to major test developers for more innovative, practical, and cost-effective analytic solutions. The importance of choosing the right testing system is impossible to overstate. This whitepaper aims to help you do that by explaining the basic facts of AFM_1 contamination and the consumer health, regulatory, technical, and business issues that should factor into your decision.

AFLATOXIN M_1 CONTAMINATION: OVERVIEW

HOW AFLATOXIN M_1 GETS INTO DAIRY FOODS AND WHY IT'S A SERIOUS, PERVASIVE, AND PERSISTENT SAFETY THREAT

AFM_1 is excreted in the milk of cows that have consumed aflatoxin-contaminated feed. Aflatoxins (AFs) are the most widely known and studied classes of mycotoxins, as well as the natural contaminants of greatest concern. These toxic byproducts of common soil- and air-borne molds frequently occur in corn, cottonseed, soybeans, and peanuts grown in warm, humid climates as well as during droughts, hot spells, and other extreme weather conditions in temperate regions. Feed ingredients exposed to insect damage, severe storms, and other forms of plant stress in the field and to extremes in temperature and water activity during post-harvest handling and storage are particularly vulnerable to widespread infection by aflatoxin-producing molds. While these mold-friendly environmental factors are an endemic problem in tropical and subtropical zones, they're becoming increasingly prevalent in temperate regions as a result of climate change. Other post-harvest factors, including mechanical damage and the heat processing used to produce feed ingredients such as distiller's grains with solubles (DDGS), can also increase the aflatoxin content of dairy cow diets.

The four different aflatoxins most commonly found in dairy feed, AFB_1, AFB_2, AFG_1, and AFG_2, not only have the potential to impair livestock performance, but can also increase the risk a variety of serious diseases and health conditions in humans as well as animals. Effects can range from chronic immunosuppression, impaired nutritional status, reduced growth to liver and kidney damage, cancer, and potentially fatal acute toxicity. The most potent of these multi-risk toxins, AFB_1, is the most carcinogenic natural contaminant currently known. Approximately 2 to 6 percent of the AFB_1 consumed by cows is converted in their liver to AFM_1, the toxic metabolite that ends up in their milk.

Once a significant amount of AFM_1 gets into a batch of milk, the only universally proven way to get rid of it completely is to dump the batch. While several detoxification methods exist, including the use of probiotics, questions remain about the stability and safety of the deactivated compounds they produce. As a heat-resistant chemical, rather than a microorganism, AFM_1 is also relatively impervious to the processing methods typically used to kill bacteria and molds. This chemical stability, as well as its overall hardiness, enables AFM_1 not only to withstand sterilization and pasteurization and the acidic conditions used to produce dairy foods such as yogurt, cheese, cream, butter, and ice cream, but also to persist in milk byproducts, like the whey proteins found in many brands of infant formula and nutrition supplements. Recent studies indicate that the large quantities of milk that go into most processed dairy foods, as well as the tendency of aflatoxins to bind with milk proteins, can actually concentrate the aflatoxin content of milk in the products derived from it. In fact, several studies have found aflatoxin levels in dairy products that were 2 to 5 times higher than in the milk they were made from.[3]

THE RISING TREND IN GLOBAL REGULATORY REQUIREMENTS: MORE NUMEROUS, MORE DIVERSE, MORE COMPLICATED, MORE DEMANDING

Both the difficulty of preventing AFM_1 contamination and the public health implications of failing to do so have shaped a highly exacting regulatory scene for dairy suppliers and manufacturers in every product and geographical sector of the market. Since 2002 at least 60 nations have established AFM_1 regulations. Their stringency and complexity varies considerably across different regions of the world. Maximum allowable limits across the globe generally range from 0 to 0.5 ppb (0 to 500 ppt). While a single AFM_1 regulation for milk prevails in many countries, separate limits for a wide variety of dairy products exist in a number of others. These wide disparities reflect variations among different nations in the level of public and government concern about AFM_1; economic and trade considerations; and the prevalence of risk factors for contamination and AFM_1-related diseases, including AF occurrence rates in dairy feed, dairy food consumption patterns, and the age and nutritional and health status of key consumer segments.

Table 1: AFM_1 Regulations for Dairy Food in Africa, Asia, Europe, Latin America, the Middle East, and North America[4, 5, 6]

Country	Products	AFM_1 Limits in ppbs
Argentina	Milk-based infant food; liquid and powdered milk	0.05
	Other milk products	0.5
Austria	Liquid whey products	0.025
	Pasteurized fresh milk; milk for children	0.01
	Dried and condensed milk; whey powder	0.4
Barbados	Milk	0.05
Belgium	Milk; powdered and condensed milk	0.1
Brazil	Milk (products)	0.5
	Imported milk products	0.1
Bulgaria	Liquid and powdered milk; cheese	0.01
	Powdered milk for infant feeding and special diets	0
Canada	Milk	0.5
China	Milk and milk products	0.5
Czech and Slovak Republics	Liquid milk	0.5
Egypt	Milk and dairy products	0
EU	Milk	0.05
	Infant formula	0.025
France	Milk for children	0.05
	Milk and dairy food for infants (under 3 yrs.)	0.03
	Milk powder	0.5
	Milk powder for infants (under 3 yrs.)	0.3
Honduras	Milk and its products; cheeses	0.25
India	Milk	0.5
Israel	Milk; milk powder	0.05
Japan	Milk	0.05
Romania	Milk and dairy products	0
Saudi Arabia	Food for infants/children	0.05
	Dried milk not for infants	0.5
	Liquid milk and its products	0.3
South Africa	Milk and milk products	0.05
South Korea	Milk	0.05
Sweden	Liquid milk products	0.05
Switzerland	Infant and baby food	0.02
	Milk and dairy products	0.05
	Cheese	0.25
Turkey	Milk and milk products	0.05
U.S.	Milk	0.5
Uruguay	Milk, milk products, and butter	0.5

AFLATOXIN CONTAMINATION IN FEED: THE CHALLENGES OF CONTROLLING THE KEY RISK FACTOR

In the absence of an industry-standard decontamination solution, the first line of defense against AFM_1 is to cut off its source. To that end, the FDA currently restricts total aflatoxin levels in dairy feed to 20 ppb. In the European Union (EU), where aflatoxin regulations are more extensive and stringent, legislators have set a separate legal limit of 5 ppb for AFB_1 in dairy feed. The key to complying with these strict standards lies in the consistent practice of proven aflatoxin prevention and control strategies such as mold and pest reduction and routine product monitoring. While this upstream management strategy substantially decreases the cost and complexity of ensuring milk safety, it comes with certain limitations. The first and most serious is the impact of aflatoxin risk factors that lie beyond human control. For instance, studies have shown that seasonal weather variations, such as high temperatures in the summer or frigid winters followed by rainy springs, tend to increase the AFB_1 content of grain, which in turn results in higher AFM_1 concentrations in the dairy foods produced at different times of year. According to many climate scientists, these seasonal variations are likely to become more extreme in the wake of climate change, further compounding the risk of non-compliant levels of AFM_1 in milk.

Another major obstacle to minimizing the aflatoxin content of dairy cow diets lies in the technical challenges of accurately and reliably measuring trace levels of aflatoxins in massive grain shipments. The notorious variability of aflatoxin test results stems largely from improper sampling techniques and error-prone testing procedures. The subsequent risk of misclassified grain lots threatens the safety and value of raw materials and products at every stage of dairy production. Fortunately, it's possible for dairy farmers and their suppliers to not only substantially lower the chances of costly analytic errors, but also mitigate the far-reaching negative impact of unavoidable environmental risk factors by adopting statistically valid grain sampling plans and testing systems that incorporate major advances in aflatoxin determination.

AFLATOXIN M_1 TOXICITY AND ITS IMPACT ON VULNERABLE POPULATIONS

The present impossibility of totally eliminating AFB_1 from the world's feed supply has serious implications for dairy food safety. Because of the extreme toxicity of AFM_1, which is comparable to that of AFB_1, even parts per trillion (ppt) doses consumed over time, can add up to a significantly greater lifetime risk of cancer, chronic immunosuppression, and other serious aflatoxin-related diseases and health problems. Research also suggests that carryover in milk of other mycotoxins that commonly occur in dairy feed such as ochratoxin A (OTA) can dramatically boost the toxic potential of both the AFM_1 and the other mycotoxin residues ingested.

AFM_1 contamination poses the greatest health risk to two populations for whom milk is a particularly important source of nutrition: children and the elderly. A variety of different factors can increase an individual's chances of suffering AFM_1-related health damage. One of the most salient of these variables is the amount of milk a person consumes. Children drink the most milk of any age group, with many formula-fed newborns and young infants relying entirely on fortified milk-based products for their nutrient intake. Breast-fed babies whose mothers consume milk and dairy products that contain AFM_1 or foods contaminated with AFB_1 are also at increased risk of exposure to potentially significant amounts of AFM_1. Chronic ingestion of minute quantities of AFM_1 by infants and children puts them at greater risk of not only severe illness, but also malnourishment and stunted growth. The heightened risk that AFM_1 poses to children's health and development also stems from the immaturity of their immune systems. Until a child's immune system is fully developed it may be unable to muster an effective defense against a potent toxic invader like AFM_1. Children with additional immune challenges or serious health problems, including premature infants and children with HIV-AIDS, liver or kidney disease, and cancer, have even less ability to detoxify aflatoxins.

Inefficient immune function plays equally important role in the elderly population's increased susceptibility to the toxic effects of AFM_1. Aging immune systems are not only slower to respond to toxic health threats, but also less able to detect and repair cell defects, a deficit that increases this rapidly expanding population's sensitivity to carcinogens. Like children with serious ailments, elderly adults with immune disorders, cancer, organ damage, and other debilitating health challenges are particularly vulnerable to AFM_1 toxicity. Older adults also share children's need for nutrient-dense, protein-rich foods such as milk to support muscle and bone health. Regular milk consumption is especially valuable for elderly individuals because of its contribution to improved bone density and a reduced risk of frailty. However, the loss of appetite, dental and swallowing issues, and ill health that commonly occur among elderly adults often result in inadequate nutrient intake from whole foods. For this reason, milk-based nutritional supplements, including products specifically formulated for various health conditions, such as cancer, diabetes, and lung disease, often feature prominently in the diets of the elderly population. The frailest members

of this age group – tube-fed nursing home and hospital patients – not only consume a diet that consists solely of these supplements, but are also the least able to resist the toxic effects AFM_1 exposure.

The challenges of AFM_1 compliance extend beyond conforming to maximum limits. In the U.S. and the EU, dairy processors are required to adopt a preventive approach to minimizing the levels of biological and chemical contaminants in their products. This proactive approach requires processors to develop and implement contamination control measures including product testing, as well documenting the use and effectiveness of these measures. In cases where a hazard needs to be managed at the supplier level, processors are also responsible for verifying their suppliers' adherence to accepted safety practices.

Under the sweeping policy changes ushered in by the Food Safety Modernization Act (FSMA) in the U.S., dairy farms, warehouses, and shipping companies, as well as processors, will be subject to much stricter FDA scrutiny, including plant inspections as frequent as every 3 years. As producers of a commodity designated a "high-risk" food, dairy processors will also face more extensive traceability and recordkeeping requirements.

The costly consequences of violating these regulations run the gamut from serious to disastrous. Revenue losses from discarded products are compounded by the cost of disposing of contaminated shipments and denial of access to lucrative markets in North America, Europe, and Asia. In the EU, a failed inspection in one member state automatically triggers an EU-wide "rapid-alert" system that publicly identifies the country of origin as the potential source of a food safety hazard, casting a shadow of suspicion on other dairy exports from that region. With the implementation of FSMA, the FDA has gained the authority to not only reject exports from foreign dairy facilities that refuse to submit to inspections, but also to take action against U.S. companies based on a "reasonable suspicion," rather than compelling evidence, that their products pose a significant health risk. Depending on the severity of the violation's impact on public health and safety, sanctions can range from stiff re-inspection fees, plant shutdowns, and mandatory recalls to suspension of a facility's license to ship or import food, and criminal prosecution of corporate officials. In cases that involve fatalities, executives deemed responsible can face up to 1 year of imprisonment and fines as large as half a million dollars, even if they were unaware of the violation.

As providers of a food that is often one of the first fed to infants and young children, dairy companies are uniquely vulnerable to the reputational damage that results from product recalls and other official sanctions. In fact, dairy brands that want to sustain a bulletproof reputation in the eyes of parents may have to do more than just stick to the letter of the law. The mere perception that the brand of formula, milk, baby food, or other dairy products that their child eats is anything less than 100 percent pure and safe can spark panic in parents and completely destroy their trust in that brand. In the age of social media, even an unfounded rumor about toxic ingredients can do major damage to a company's image. Brands that combine an unblemished safety record with a demonstrated commitment to exercising a higher level of vigilance over their products not only significantly reduce this risk but also tend to command higher prices and lasting loyalty. The knowledge that a dairy provider's products are rigorously tested and conform to the strictest safety standards is deeply reassuring to parents as well as to other highly health– and safety–conscious consumer groups.

THE POTENTIAL IMPACT OF GAME-CHANGING TESTING SOLUTIONS ON THE LONG-TERM HEALTH OF THE DAIRY INDUSTRY

The ability of today's dairy companies to provide that level of reassurance, without impeding their business goals, increasingly rests on continued innovation in AFM_1 test methods. According to Patricia Jackson, Market Development Manager at the Massachusetts-based test developer VICAM,® A Waters Business, revolutionary advances in lateral flow immunoassay technology promise to help a full range of dairy producers develop frequent monitoring programs that are not only practical and affordable, but also uniquely fit for today's toughest food safety challenges. "Thanks to continuous improvement in the sensitivity of this economical, user-friendly method," says Jackson, "a truly preventative approach to dairy safety is now within reach of every segment of the industry."

VICAM's versatile line of AFM_1 strip tests are a case in point. Designed for routine monitoring applications in markets with toughest safety standards, the high-sensitivity version of the test, Afla M_1-V™^{HS} enables onsite users and lab techs with no special training to accurately detect and measure AFM_1 concentrations as low as 0.02 ppb (20 ppt) in as little time as 8 minutes. For testing over a wide dynamic range, VICAM's Afla M_1-V™, combines the same ease-of-use with sensitive detection of levels ranging from 0.1 ppb (100 ppt) to .75 ppb (750 ppt). Sample preparation for these next-generation

immunoassays requires just one filtration step, minimizing the use of reagents and disposable supplies and eliminating the need for a centrifuge. In addition to these cost–and time–saving advantages, strip tests do away with the guesswork involved in methods that entail visual interpretation. Instead, an optical reader clearly displays numerical readings on a digital screen. This combination of benefits enables dairy companies and testing labs across the economic and technical spectrum to reap the dividends of informed real-time buying and selling decisions, efficient high-throughput sample screening, and routine quality and safety checks.

Jackson empathizes the enormous value of highly practical and exceptionally sensitive rapid quantitative test methods to the dairy industry. "The availability of a budget-friendly strip test that tests at such low levels is a major leap forward in proactive AFM_1 control," she says, noting that this method enables dairy producers to quickly identify and respond to an incipient contamination problem. Armed with actionable data, dairy companies at every point in the value chain can take the right steps to limit the impact of an uptick in AFM_1 levels: dairy farmers can reduce the AFB_1 content in an affected cow's feed ration; manufacturers can remove contaminated milk from the production stream; and producers in both segments can spot supplier quality lapses before they damage their brand reputation. Timely corrective actions like these help fulfill the true purpose of a preventative approach to safety by empowering dairy suppliers and processors to address manageable problems before they spiral out of control.

To meet all their compliance requirements, dairy companies that do business in tightly regulated markets also need to incorporate officially validated instrumental methods into their AFM_1 test strategy. One of first major breakthroughs in mycotoxin testing has since become the gold-standard method for regulatory applications and other critical purposes. With the integration of immunoaffinity (IA) column sample cleanup into advanced laboratory methods, test results that provide convincing evidence of product safety and purity became widely achievable. Performed by highly trained analytical chemists in accredited third-party laboratories, sophisticated instrumental methods such as high or ultra performance liquid chromatography (HPLC/UPLC®) or liquid chromatography–mass spectrometry (LC-MS) meet the exacting requirements for domestic and export market certifications, supplier verification, and proof of compliance with contractual demands. Regulators and high-end processors around the world stipulate the use of these methods because they ensure the most accurate, precise, and reliable measurements of the minute AFM_1 maximum levels legislated by the EU and many of their trading partners. By documenting compliance and traceability across the dairy chain, the certifications issued with these tests also play a vital role in helping suppliers and processors build a solid reputation for responsible production practices in the minds of not only their customers, but also customs inspectors and regulatory officials.

VICAM's offering in this category, Afla M_1™ HPLC, detects AFM_1 levels ranging from 0.01 ppb (10 ppt) to 3 ppb (3,000 ppt) in as little time as 30 minutes. These AOAC-approved immunoaffinity (IA) columns reduce the risk of analytic errors by simplifying sample preparation, removing impurities that interfere with analysis, and harnessing the enhanced capacity of monoclonal antibodies to selectively bind with target analytes. This method can be used to verify the safety of a variety of dairy foods, including fluid milk, milk powder, curd, whey, and cheese.

The simplified format of the company's rapid fluorometric method extends the accuracy and efficiency gains of IA sample cleanup to non-technical personnel. The Afla $M_1^{FL+®}$ test kit incorporates a portable fluorometer that accurately measures AFM_1 concentrations as low 0.0125 ppb in as little time as 15 minutes, while reducing the risk of procedural errors and easing the task of documenting preventive controls by storing test protocols, calibration levels, and up to 200 test results. Although less sensitive and precise than HPLC and LC-MS analysis, the combination of optimized sample preparation and fluorometric detection offers a robust balance of speed, performance, ease-of-use, and affordability for routine screenings onsite, at shipping points, and before traditional instrumental analysis.

REAPING THE REWARDS OF A PREVENTATIVE APPROACH TO DAIRY SAFETY

One of the most tightly regulated sectors in the food industry, the dairy industry now faces a compliance burden of increasing weight and complexity. Yet with this outsized burden comes an equally big opportunity for industry members to demonstrate not only the strength of their dedication to consumer safety but also the human qualities of their brand. A company that meets the highest standards of purity and safety reflects the values and attitudes of ethical, responsible people who care about their customers. Recent marketing statistics confirm the truth of long-standing economic common sense. Brands that demonstrate these human virtues have the power to forge an genuine emotional bond with their audience that frequently results in more

repeat business and more lasting and profitable relationships with customers and business partners.[7, 8] Companies that act responsibly and show concern for their customers also stand to enhance their public image the consumers' perception of the value of their products.[7, 8] As innovative new aflatoxin test systems continue to lower the limits of detection and eliminate the barriers to preventing contamination across the dairy chain, producers who capitalize on these up-to-the minute technologies will gain the regulatory certifications, branding advantages, and business efficiencies they need to grow their share of the world's most demanding markets. And the global reputation of milk and dairy products as safe, wholesome foods will acquire an extra measure of credibility.

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