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REVIEW ARTICLE

Regulatory considerations of aflatoxin contamination of food in Mexico

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ABSTRACT. Aflatoxins are potent mutagenic and carcinogenic compounds produced by some strains of *Aspergillus flavus*, *A. nomius*, and *A. parasiticus* that are commonly present in the environment. Human populations, particularly those whose basic diet includes grains, are in risk to exposure to aflatoxins. It is thus necessary to monitor and control the contamination of food and feed by aflatoxins in both domestic and international trade. As large amounts of corn are imported into Mexico from the United States, this paper stresses the need to develop legislation and enforce standards to ensure trade of corn with the minimal amount of aflatoxin.

Key words: Corn trade, legislation, mycotoxins.

RESUMEN. Las aflatoxinas son potentes compuestos mutagénicos y carcinogénicos producidas por algunas cepas de *Aspergillus flavus*, *A. nomius*, y *A. parasiticus* que comúnmente se encuentran presentes en el ambiente. La población humana, particularmente aquella cuya dieta básica incluye granos, está en riesgo de exposición a estas aflatoxinas. Por consiguiente es necesario monitorear y controlar la contaminación por aflatoxinas en alimentos de consumo humano, así como en aquellos destinados a la alimentación del ganado. Debido a que grandes cantidades de maíz son importadas a México desde los Estados Unidos, este manuscrito hace hincapié en la necesidad de desarrollar legislaciones e imponer estándares que aseguren que sólo maíz con la mínima concentración de aflatoxinas sea comercializado tanto a nivel doméstico como internacional.

Palabras clave: Comercio de maíz, legislación, micotoxinas.

INTRODUCTION

Fungal metabolites called mycotoxins are ubiquitous in the human environment where they are important contaminants of food. Poisoning by mycotoxins frequently occurs in poultry and livestock,[9,21] but these substances may also be dangerous to human health.[30,32] Aflatoxins are an important group of mycotoxins with adverse effects on animal and public health.[26]

Aflatoxins are highly substituted coumarins containing a fused dihydrofurofuran moiety. Aflatoxin B1 (AFB_1), the most toxic, mutagenic and carcinogenic member of the series, is further characterized by the fusion of a cyclopentenone ring to the lactone ring of the coumarin structure, and an unsaturated bond at the 2,3-position on the terminal furan ring.[25] Aflatoxin B_1 is produced when toxigenic strains of *A. flavus*, and *A. parasiticus* grow on a wide variety of substrates when appropriate conditions of moisture content (optimum: 18-19.5%), relative humidity (85%) and temperature (25-400ºC) are met.[19] In addition to the factors that control or influence the growth of *A. flavus* or *A. parasiticus*, other variables affect aflatoxin production including: (i) the strain of the invading fungus; (ii) the genetic susceptibility of the whole host plant (in the case of field contamination) or its products (in the case of contamination during storage); (iii) the chemical composition of these substrates, and; (iv) both biotic and abiotic stress factors (e.g. drought conditions and insect damage) that increase the probability of fungal infection.[33] Thus, aflatoxin production occurs under a wide variety of conditions.

Clearly, humans come into contact with aflatoxins when they consume plant products that have been exposed to toxigenic strains of *A. flavus* or *A. parasiticus* during growth, harvest, or storage. Secondary exposure, through the consumption of products derived from animals that consumed aflatoxin-contaminated feed (i.e. M1-aflatoxins in milk), also occurs.[32] Major food sources of aflatoxins include grains (particularly corn, sorghum, and millet), peanuts, beans, and tree nuts (almonds, pistachios, etc.). Oilseeds and oilseed meal (cottonseed, copra) may also be heavily contaminated, but these products are mostly incorporated into animal feed.[26]

REGULATIONS IN THE USA

In 1983, Labuza[15] examined three aspects of mycotoxins in food in the USA: (a) relevant laws, (b) the Food and Drug Administration (FDA) guidelines with respect to the law and (c) the courts interpretation of these laws.[5] Labuza also presented several cases of regulations applied to interstate shipments of corn. FDA analyzes raw agricultural products for aflatoxin through the compliance program

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Rev Latinoam Microbiol 2005; 47 (3-4): 160-164

which objectives are: the collection and analysis of food and feeds to determine regulatory levels; to remove from interstate commerce food that contains violative aflatoxin levels and to determine potential problems and control measures.[26] Nevertheless, no information on the regulatory issues dealing with mycotoxins in international trade of corn was presented.[7] Furthermore, the USA was one the first countries to introduce legislation regulating on aflatoxin levels in food and feed. It is important to note that most of the corn produced in the USA is used for animal feed. As a consequence, the maximum permitted level of aflatoxins in corn is 20 µg/kg.

REGULATIONS IN OTHER COUNTRIES

Labuza's publication leads to the adoption, expansion, and changes in regulations concerning mycotoxins in many countries. An international inquiry on mycotoxins was initiated by the National Institute for Public Health and the Environment.[29] Dutch Embassies around the world were requested to gather up-to-date information about mycotoxin regulations from local authorities. At least 99 countries had regulations concerning mycotoxins in food and feed in 2003. All of them have at least regulatory limits for aflatoxin B_1 contents and some have limits for the four of them (B_1, B_2, G_1 and G_2). Specific regulations exist for other mycotoxins as well.[29] The maximum tolerated levels for aflatoxin B_1 in food are from 1 to 20 µg/kg. The most frequently occurring limit is 4 µg/kg, and this limit is applied in the 29 countries which follow the harmonized regulations of the European Free Trade Association (EFTA) and the European Union (EU). The 20 µg/kg limit is applied in 17 countries: half of them are in Latin America (where it is also a MERCOSUR Harmonized Limit), the rest in Africa and the United States. Harmonization of tolerance levels is also taking place in some free trade Zones (EU, EFTA, MERCOSUR, Australia/New Zealand). Furthermore, harmonization efforts are being undertaken for goods moving in International commerce. Therefore, standardization of procedures for sampling and analytical methodology is under discussion with the aim of establishing reliable and harmonized regulations all over the world.[29]

Restriction of aflatoxin B_1 levels contributes significantly to the improvement of public health as B_1 is the most important of the aflatoxins both in terms of toxicology and occurrence. Commodities are often contaminated with B_1 but much less frequently with B_2, G_1, G_2. Although most people are at risk of exposure to mycotoxins, the individual effects of consumption are not the same because of differences in dietary habits and levels of contamination. It is known with aflatoxin B_1 that a person of 70 kg who ingests 1 µg/day will have 14-23 ng of the adduct aflatoxin-lysine (AFB1-Lysine) in blood.[16] This chronic exposure to aflatoxin B_1, results in 30-fold higher levels of AFB_1-lysine than that produced by a single dose.[22]

RELEVANCE OF CONTAMINATION OF CORN BY AFLATOXINS IN MEXICO

Aflatoxins are an important health hazard in Mexico for the following reasons: (i) Mexico has one of the highest per capita consumptions of corn in the world (≈ 325 g/day);[6] (ii) Mexico imports 6 million tons of corn per year (often of dubious quality) at a cost of 550 million dollars,[8] representing 11% of total North American exports; (iii) storage conditions for corn in Mexico are insufficiently developed and there is no regular monitoring of aflatoxin contamination;[17] and (iv) laws regulating the domestic and international trade of corn contaminated with aflatoxins have not been formulated.

IMPORTS OF CORN IN MEXICO FROM THE USA

Since the "North American Free Trade Agreement" (NAFTA) came into force, the volume of corn imported from the USA grew by 140%.[8] According to the USA standards for corn, Grade 2 is usually imported which corresponds to grain of the following characteristics: a) Broken corn and foreign material, 3% (maximum limit); b) Damaged kernels, 5% maximum limit; c) Moisture content 15%; d) Aflatoxin content, (20 µg/kg maximum allowed limit). As mentioned above, 20 µg/kg corresponds to the maximum level permitted in the USA for International Trade.

The agency responsible for exports of corn in the USA is the Federal Grain Inspection Service (FGIS). The FGIS was established in the U.S. Department of Agriculture in 1976. The primary task of the Agency is to carry out the provisions of the U.S Grain Standards, to ensure integrity in the inspection, weighing, and handling of American grain. FGIS is responsible for establishing official U.S. standards for grain and other assigned commodities and administrating a nationwide system of official inspection and weighing. In addition to FGIS, private and state agencies may, upon application, be authorized to perform official services under the authority contained in the Act.[27]

Inspection: the U.S. Grain standards Act requires that, with some exceptions, all U.S. export grain be officially inspected. At export locations, inspection is performed by FGIS' or by State agencies that have delegated authority for export inspection by the administrator. For domestic grain marketed at inland locations, the Administrator designates private and State agencies to provide official inspection services on request and payment of a fee. To ensure that the official U.S. grain standards are applied

Rev Latinoam Microbiol 2005; 47 (3-4): 160-164

uniformly nation wide, FGIS field officials provides oversight, guidance, and assistance to non Federal agencies performing inspection activities, both at export and inland inspection points.[28]

Buyers and sellers unsatisfied with inspection results can request appeal inspections, first from a FGIS field office and then, if desired, from the Board of Appeal´s and Review. A quality control program provides for the monitoring of the national inspection system, ensuring that all field locations apply uniformly the U.S. grain standards.

Establishing and maintaining official U.S. standards, when needed, is the responsibility of FGIS. Such standards exist for corn, wheat, rye, oats, barley, flaxseed, sorghum, soybeans, triticale, sunflower seed, and mixed grain. FGIS has been given authority to perform applied research for the purpose of developing methods to improve accuracy and uniformity in grading grain.[28]

FGIS also administers a registration program for all firms that export grain from the United States. In conjunction with the office of the Inspector General, FGIS investigate reported violations and initiate follow-up and corrective action when appropriate.

In international transactions, the quality of the grain sold refers to that at the warehouse in the selling site. What happens to the corn (quality wise) during transportation is the responsibility of the buyer. Time in transport is crucial to maintaining quality, since the level of contamination may significantly increase under poor conditions. Furthermore, there is no agreement among the different laboratories on the use of a single, standardized method to measure aflatoxins. Comparisons therefore are difficult to make, with the result that aflatoxin levels are often underestimated.

This decade-long surge of corn imports into Mexico, the diverse climatic conditions under which corn is stored, as well as the likelihood that the aflatoxin levels increase during transport and storage, has lead to an increased risk of hepato-cellular carcinomas. Thus the only way to ensure a safe supply of corn is to develop regulations and policies that will be applied throughout the processes of purchasing, transportation, distribution, storage and consumption.

AFLATOXINS IN MEXICO

Levels of contamination

Aflatoxins have been found in different commodities at concentrations significantly above levels permitted in the USA. Such commodities include corn, common beans, sorghum, peanuts, tortillas, etc.[11,14] Concentrations of aflatoxins in corn (mostly of corn grown in Mexico) ranged from 15 to 250 µg/kg in 1986 12. Similarly, nixtamal-flour contained aflatoxin levels that ranged from 2.7 to 17% µg/kg in a survey performed in 2004.[20] Given that Mexico has the highest per capita consumption of corn in the world, these data suggest that the Mexican population is constantly exposed to the harmful effects of aflatoxins. Therefore, several actions to decrease the aflatoxin contamination in corn should be undertaken. For international transactions the grain should contain no more than 20 µg/kg of Aflatoxin B1, at the selling site, during transportation, and final storage. This particular period of time is crucial in maintaining the quality of grain, since the level of contamination may significantly increase due to poor management and storage conditions. In regard to national corn production, attention should be paid, during growth and harvest of corn, particularly in those geographic zones in which high aflatoxin contamination has been reported. It is also very important to reinforce the use of "traditional or industrial nixtamalización" as a mean to reduce aflatoxin contamination in corn for human consumption.

INCIDENCE OF CANCER

In apparent contradiction to the aflatoxin intake, liver cancer is only the 13th leading-cause of death in women and the 3rd in men.[23] However, reports of Mexican patients with viral hepatic disease and high levels of aflatoxin B_1 in urine, have been published.[1,2] Undoubtedly, the lime treatment given to corn in the process of tortilla making, known as "nixtamalización", which has been practiced in Mexico since pre-Hispanic times, reduces by 95% the concentration of aflatoxin in the final product.[13,18] Unfortunately, "traditional nixtamalización" is not used in modern procedures in which corn is directly used to make flour, flakes, food additives, etc.

Nevertheless, data from Kenya, Mozambique, The Philippines, Swaziland, Thailand and Uganda, show a positive association between high intakes of aflatoxins and high incidence rates of liver cancer,[31,32] especially in adult men. Indeed, the incidence of liver cancer in many of these studies is a linear function of the log of dietary aflatoxin intake.[30]

REGULATION OF AFLATOXIN LEVELS IN MEXICO

Several points need to be stressed in regard to the regulation of aflatoxins in Mexico. First, Mexican agricultural authorities have accepted the USA standard (20 µg/kg) of aflatoxins in corn as the maximum allowed level.[4] This level contrasts starkly with the 2 µg/kg established by the EU and EFTA for most of foods.[29] Second, epidemiological data concerning liver cancer/aflatoxin ingestion in Mexico is missing. Furthermore, estimates of aflatoxin intake, its excretion in urine, etc. need to be made. These data are impor-

Rev Latinoam Microbiol 2005; 47 (3-4): 160-164

tant since it is commonly accepted that it takes about 6 years of exposure to low levels of aflatoxins for tumor initiation.[32]

Perhaps there is little reason to worry, since Mexicans have been consuming corn for millennia, yet the incidence of liver cancer remains low. During the last decades however, the international and domestic trade in corn has increased dramatically. Studies are needed to determine whether cause-effect relationships between aflatoxin intake and liver cancer exist in different regions of the country. Similarly, the establishment of new food industries that do not include "traditional nixtama1ización" as part of their production processes enhances the need for serious studies. After all, the lowered growth rates of chicken, pigs and cattle, fed with contaminated feed, may be related to aflatoxin intake,[21] underlining the need for stricter regulation of aflatoxin levels. Mexico has the requisite infrastructure (qualified personnel, properly equipped laboratories, etc.) to monitor aflatoxins in food and feed and to advise the Government on appropriate legislation.

LEGISLATION AND REGULATION OF AFLATOXIN LEVELS IN MEXICO

The following changes in the legislative and regulatory environment in Mexico need to be made:

1. Adoption of a standard method for measuring aflatoxin contents at both the national and international levels. Many different methods have been reported to determine the presence of aflatoxins.[24] These range from simple chemical analyses to sophisticated monoclonal antibodies methods.[10] Similarly, the cost, equipment, and time consumed per determination vary considerably. It is important nevertheless, to select and establish a single official technique which needs to be sensitive, reliable and reproducible but also simple and cost-effective.
2. Establishment (or adoption) of maximum levels of aflatoxins in food (especially corn) that is designed for human consumption.
3. Introduction of legislation to ensure that the newly established maximum levels of aflatoxins are not exceeded.
4. Epidemiological studies on aflatoxin intake and its effect on human health.
5. To designate laboratories on a regional basis (Northeast, Center and South), that should be certified to measure aflatoxin levels. The laboratories could belong to the Departments of Agriculture and Health, to Universities, etc.

CONCLUSIONS

Under Mexican Law, it is the responsibility of the National Institute of Agricultural Forestry and Livestock Research (INIFAP) to monitor the quality of agricultural commodities. Consequently this institute should coordinate the following actions: to determine the levels of aflatoxin in food and feed; to remove from commerce, food with violative aflatoxin levels, and to determine potential problems of aflatoxins in Mexico. Economic analyses, in which the evaluation of losses caused by aflatoxin contamination, should be included.

REFERENCES

1. Alvarez, M.T., Carvajal, M., Lisker-Melman, M., Rojo, F., Mendez, I. 1998. Aflatoxin B1 in urine of humans with chronic B and C Hepatitis and Cirrhosis in Mexico. In: Mycotoxins and Phycotoxins: Developments in Chemistry, Toxicology and Food Safety. Miraglia, M., van Egmond H., Brera, C., Gilbert, J. (eds.). Printed in the USA.
2. Álvarez-Bañuelos, M. T., Carvajal-Moreno, M., Ruisánchez, N., Rojo, F. 2000. Aductos-ADN-Aflatoxina como biomarcadores de exposición en grupos de riesgo de cáncer de hígado. Revista Cubana de Oncología 16:35-39.
3. Cardwell, K.F., Desjardins, A., Henry, S.H., Munkvold, G., Robens, J. 2001. Mycotoxins: The cost of Achieving Food Security and Food Quality. APS net August. pp 1-25.
4. DOF (Diario Oficial de la Federación). 1999. Diciembre 10. Nom 188-SSA1. México.
5. Duggan, R.E. 1970 Controlling Aflatoxins. FDA papers. April. pp. 13-18.
6. Elías-Orozco, R., Castellanos-Nava, A., Gaytan-Martinez, M. Figueroa-Cardenas, J.D., Loarca-Piña, G. 2002. Comparison of nixtamalización and extrusion processes for a reduction in aflatoxin content. Food Additives and Contaminants 19:878:885.
7. Fischbach, H.1984. Coping with the aflatoxin problem in the early years. J. Assoc. Off. Anal. Chem. 67:1-3.
8. Gonzáles, R. 2005. La importación de maíz de EU creció 15 veces con el TLCAN. La Jornada. Marzo 05 2005.
9. Glahn R.P., Beers, K.W., Bottje, W.G., Wideman, R.S., Huff, W.E., Thomas, W. 1991. Aflatoxicosis alters avian renal function, calcium and Vitamin D metabolism. J. Toxicol Environ Health 34: 309-321.
10. Groopman, J.D., Busby, W.F., Donahue, P.R. Wogan, G.N. 1986. Aflatoxins as risk factors of liver cancer: an application of monoclonal antibodies to monitor human exposure. In: Biochemical and molecular epidemiology of cancer. Alan R.L. Inc. pp 233-256.
11. Guzmán-de-Peña D. 1989. Micotoxinas en el Bajío Guanajuatense. Avance y Perspectiva 40: 8-15.
12. Guzmán-de-Peña, D., Anguiano, R.G.L., Medina Arredondo, J.J. 1992. Modification of the method 1 AOAC (CB method) for the detection of aflatoxins. Bulletin of Environmental Contamination and Toxicology 49:485-489.
13. Guzmán-de-Peña, D., Trudel, L.T. Wogan, G.N. 1995. Fate of 3H-aflatoxin during "nixtamalización" Bulletin of Environmental Contamination and Toxicology 55: 858-864.
14. Guzmán-de-Peña, D. 1997. El Estudio de las aflatoxinas en Mexico. In: Perspectivas de la Microbiología en México. Ruiz-Herrera, J., Guzmán-de-Peña, D., y Peña-Cabriales, J.J. (eds.).Talleres gráficos de la Dirección de Publicaciones y Materiales educativos del Instituto Politécnico Nacional. México, D.F.
15. Labuza, T.P. 1983. Regulation of Mycotoxins in Food Journal Protection 46: 260-265.
16. Liang-Shang G., Skipper, P.L., Peng, X., Groopman, J.D., Chen, J.S., Wogan, G. N., Tannenbaum, S. R. 1988. Serum albumin adducts in the molecular epidemiology of aflatoxin carcinogenesis: Correlation with aflatoxin B1 intake and urinary excretion of aflatoxin M1. Carcinogenesis 9: 1323-1325.

17. Méndez-Albores, J.A., Arambula, V.G., Vázquez, BME., Mendoza, E.M., Preciado, O.R.F., and Moreno-Martínez, E. 2003. Effect of high moisture Maize storage on tortilla quality. Journal of Food Science 68:1878-1881.
18. Méndez-Albores, J.A:, Arambula-Villa, G., Loarca-Piña, M.G., González-Hernández, J. Castaño-Tostado, E., Moreno-Martínez, E. 2004. Aflatoxins'fate during the nixtamalización of contaminated Maize by two tortilla-making processes. Journal of Stored Products Research 4: 87-94.
19. Payne, G.A. 1992. Aflatoxin in Maize. Crit Rev. Plant Sci 10: 423-440.
20. Reyes-Velásquez, W.P., Landeros-Ramirez, P., Anguiano-Ruvalcaba, G.L. y Guzmán-de-Peña, D. 2005. Presencia de aflatoxinas y fumonisinas en harinas de maíz nixtamalizado comercializadas en la zona Metropolitana de Guadalajara, 2004. V Congreso del Noroeste I Nacional en Ciencias Alimentarias y Biotecnología. Nov. Hermosillo Sonora, México.
21. Robens, J.F. and Richard, J.L. 1992. Aflatoxins in Animal and Human Health. Reviews of Environmental Contamination and Toxicology 127:69-85.
22. Sabbioni, G., Skipper, P.L., and Tannenbaum, S.R. 1987. Isolation and characterization of the major serum albumin adduct formed by aflatoxin B1 in vivo in rats. Carcinogenesis 8:819-824.
23. Salud Pública de México. 2005. Estadística de Mortalidad en México: muertes registradas en el año 2003. Salud Pública de México 47:171-187.
24. Scott, P.M., 1990. Mycotoxins-General referee reports. J Assoc. Off. Anal. Chem. 73:98.
25. Steyn, P.S., Vleggaar, R., and Wessels, P.L. 1980. The biosynthesis of aflatoxins and its congeners. In: The Biosynthesis of Mycotoxins: A study in secondary metabolism. (Ed) Pieter S. Steyn. Academic Press. New York London. pp 105-150.
26. Task Force Report 139. 2003. Mycotoxins: Risks in Plant, animal, and human systems. Council for Agricultural Science and Technology. pp 1-199.
27. United States Government Manual. 1985. Federal Grain Inspection Service. Food Safety and Inspection Service. Department of Agriculture. pp 112-113.
28. United States Department of Agriculture. Federal Grain Inspection Service. 1986. Program directive 906.2 Implementation of the FIGS-FDA memorandum.
29. van Egmond, H.P. and Jonker, M.A. 2004. Worldwide regulations for mycotoxins in food and feed in 2003. FAO Food and Nutrition paper 81. Food and Agriculture Organization of the United Nations. Rome.
30. Wang, J.S. and Groopman J.D. 1999. DNA damage by mycotoxins. Mutation Research 424:167-181.
31. Wild, C.P., Jiang, Y.Z., Allen, S.J., Jansen, L.A.M., Hall, A.J. and Montesano, R. 1990. Aflatoxin- Albumin adducts in human sera from different regions of the world. Carcinogenesis 11:2271-2274.
32. Williams, J.H., Phillips, D.T., Jolly, P.E., Stiles, J.K., Jolly, C.M: Aggarwal D. 2004. Human Aflatoxicosis in developing countries: A review of toxicology, exposure, potential health consequences, and interventions. Am. J. Clin. Nutr. 80:1106-1122.
33. Widstrom, N.W. 1996. The aflatoxin problem with corn grain. In: Advances in Agronomy. Sparks, D. (eds.). Academic Press New York. pp 219-280.

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