codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

JOINT OFFICE: Viale delle Terme di Caracalla 00100 ROME Tel: 39 06 57051 www.codexalimentarius.net Email: codex@fao.org Facsimile: 39 06 5705 4593

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DISCUSSION PAPER ON AFLATOXINS IN PISTACHIOS

BACKGROUND

1. The 33rd session of the Codex Committee on Food Additives and Contaminants (CCFAC) agreed that Iran, assisted by Sweden, would prepare a discussion paper providing supporting data and relevant information with a view to the establishment of maximum limits for Aflatoxin B_1 (AFB_1) and total Aflatoxins in pistachio's for further processing and pistachio's for direct consumption for consideration at its next session.[1]

INTRODUCTION

2. The Aflatoxins are produced primarily by some strains of *Aspergillus flavus* and most, if not all, strains of *A. parasiticus*. Aflatoxin B_1 is the most frequent one present in contaminated samples and Aflatoxins B_2, G_1 and G_2 are generally not reported in the absence of AFB_1. The commodities with the highest risk of Aflatoxin contamination include corn, peanuts, cottonseed, Brazil nuts, pistachio nuts, figs, spices and copra. The most important dietary sources of Aflatoxins are maize and groundnuts and their products, which may form an essential part of the food diet in some countries.[1]

3. The pistachio (Lat. *Pistacia vera*, Anacardiaceae) originates from Central Asia. Domestication occurred less than 2000 years ago and traders introduced them throughout the Middle East and the Mediterranean area. Today the major production areas are located in the Middle East, North America and Europe. Iran is the world's largest pistachio producer.

4. The pistachio is a semidry stone fruit consisting of a single kernel enclosed in a thin, bony shell, which is surrounded by the hull. The shell partially splits to varying extents at least a month before maturity and harvest. Normally, the hull does not rupture when the shell splits in the immature pistachio fruit. However, in a small percentage of the pistachios, the shell and the still adhering hull splits together. This hull rupture, often referred to as "early splitting", is a very important event for infection with the Aflatoxin producing fungi *Aspergillus flavus/parasiticus*. The rupture exposes the kernel to airborne fungal spores or insects, like the navel orange worm [*Amyelios transitella* (Walker)], which might carry fungal spores on their bodies. [2-4]

FORMATION AND PREVENTION OF AFLATOXINS IN PISTACHIOS

5. An "early split" nut is characterised by a distinct, dark and smooth-edged split on the hull. The oldest "early splits" have rough and shrivelled hulls and contain the highest levels of Aflatoxin and may contain

[1] ALINORM 01/12A, para. 198.

99% of all Aflatoxin detected. Kernels infested by the navel orange worm had substantial more infections by *A. flavus/parasiticus* and had 84% of all Aflatoxin detected. Studies of the Aflatoxin content in singel nuts have revealed that many of the "early splits" contain more than 20 µg/kg and some above 1000 µg/kg. Normal looking nuts contained less than 2 µg/kg. [5-6]

6. Infection with *A. flavus/parasiticus* before harvest may lead to further build up of Aflatoxin after harvest if drying is slow or if storage and transportation is under high humidity. Prevention of Aflatoxin in pistachios can be achivied in one or two ways: by preventing the infection in the first place or by modifying the environment to inhibit mould growth and mycotoxin production. In the orchard, important preventive measures are those that reduce the amount of "early splits" and the sources of the Aflatoxin producing fungi as far as possible, and those that minimise insect damage.[7]

7. Since the primary infection of the pistachio with *A. flavus/parasiticus* takes place already in the orchard, the most important preventive measure after harvest is to prevent further growth of the fungi and accumulation of toxin. To improve stability and avoid further contamination, the pistachios should be dehulled and dried to a moisture content of 5-7%, corresponding to a water acitivity (A_w) of less than 0.70 at 25°C, as soon as possible after harvest, usually within 48 hours.[8-9]

8. Prevention of Aflatoxin being built up during storage and transportation depends on keeping a low moisture content, the temperature in the environment, and the hygienic conditions. When preventive action can not be achieved, corrective actions, such as sorting, needs to be carried out to ensure that contaminated material does not enter the food chain.

9. The pistachio nuts most likely to be infected by mould and contaminated with Aflatoxin, the rough "early splits", have several physical characteristics which distinguish them from normal nuts. The most distinguishing features of "early splits" are shell staining and hull adhesion tendencies. The recognition of the dark stain present on the lips of the shells of "early splits" as basis for machnine sorting has been described.[10-11]

10. The distribution of Aflatoxin in pistachios has been thoroughly investigated. The results from the investigations indicate that sorting for quality (discarding scalpers, stained floaters and hand pick outs) removes a large part of the Aflatoxin present at harvest. Further more, these studies have also evaluated sampling methods and could form the basis for a Codex sampling plan for Aflatoxins in pistachios. The distribution of Aflatoxins are very heterogeneous in a lot of pistachio and consequently the out-line of the sampling plan is critical.[12-18]

CHEMICAL STRUCTURE AND ANALYTICAL METHODS

11. Chemically, Aflatoxins are highly oxygenated naturally occurring heterocyclic compounds and have closely related structures. All Aflatoxins essentially contain a coumarin nucleus fused to a bifuran. A pentanone structure is attached to coumarin nucleus in case of Aflatoxins of B series. This is substituted by a six-membered lactone in Aflatoxins of G series.[19]

12. Various analytical methods for the identification and quantification of Aflatoxins have been developed. For analysis of Aflatoxins in pistachio, thin layer chromatography (TLC), liquid chromatography (LC), and immunochemical methods of analysis have been validated. Early methods were generally based on TLC. Today, methods using HPLC with fluorescence detection are most common. TLC and LC methods for determining Aflatoxins in food are laborious and time consuming. Through advances in biotechnology, highly specific antibody-based tests are now commercially available that can identify and measure Aflatoxins in food in less than 10 min. Only a few of these kits have been evaluated by collaborative studies. The simple, specific, and rapid immunoassays will play an increasing role in monitoring foods and feeds for mycotoxins. [20-21]

TOXICOLOGICAL EVALUATION

13. The Aflatoxins were evaluated by JECFA at its thirty-first, forty-sixth, forty-ninth and its fifty-sixth meetings (Aflatoxin M_1 only). At its forty-ninth meeting in 1997, JECFA considered estimates of the

carcinogenic potency of Aflatoxins and the potential risks associated with their intake. At that meeting, no numerical TDI was proposed since theses compounds are genotoxic carcinogens, but the potency estimates for human liver cancer resulting from exposure to aflatoxin B_1 were derived from epidemiological and toxicological studies. JECFA reviewed a wide range of studies conducted with both animals and humans that provided qualitative and quantitative information on the hepatocarcinogenicity of Aflatoxins. The JECFA evaluated the potency of these contaminants, linked those potencies to estimates of intake, and discussed the potential impact of 2 hypothetical standards on peanuts (10 or 20 ppb) on sample populations and their overall risk.[1] Regarding pistachio, similar information is required.

14. In the evaluation at its forty-ninth meeting, the JECFA noted that the carcinogenic potency of Aflatoxin B1 is substantially higher in carriers of hepatitis B virus (about 0.3 cancers/year/100 000 persons/ng of Aflatoxin B1/kg of body weight per day), as determined by the presence in serum of the hepatitis B virus surface antigen (HBsAg +individuals), than in HBsAg – individuals (about 0.01 cancers/year/100 000 persons/ng of Aflatoxin B1/kg of body weight per day). [1]

15. The JECFA also noted that vaccination against hepatitis B virus would reduce the number of carriers of the virus, and thus reduce the potency of the Aflatoxins in vaccinated populations, leading to a reduction in the risk for liver cancer.[1]

OCCURRENCE OF AFLATOXINS IN PISTACHIOS

16. Among 21 pistachios samples analyzed in Sweden between 1996 to 1998, more than 90% of samples contained Aflatoxin B1 and total lower than 2 and 4 ppb (EU maximum limits for Aflatoxins), respectively, although in two samples, much higher levels, up to 1900 and 2200 ppb, respectively, was reported [22]

17. According to a report from Mexico, among 244 pistachios samples analysed during 1993-1996, 2 % contained Aflatoxins higher than 20 ppb.[1]

18. Taguchi et al. did not detect any aflatoxins in 24 pistachios samples analyzed in Osaka from 1988 through 1992. [23]

19. According to the report of Japanese Ministry of Health, among 2422 pistachios samples analysed during 1972-1989, 96.5% contained Aflatoxin B_1 Lower than Limit of detection and 2% contained Aflatoxin B_1 higher than 10 ppb.[1]

20. Among 47361 pistachios samples analysed during March 1998-March 2001 in I. R. Iran, 59.6% contained Aflatoxin B1 lower than limit of detection (LOD), 16.6% contained Aflatoxin B1 between LOD-2 ppb, 13.9% contained Aflatoxin B1 between 2-10 ppb, and 9.9% contained Aflatoxin B1 higher than 10 ppb.[24- 25]

DIETARY INTAKE

21. The primary route of potential human exposure to Aflatoxins is ingestion of contaminated food. Grains, peanuts, tree nuts, and cottonseed meal are among the foods on which Aflatoxin-producing fungi commonly grow. Meat, eggs, milk, and other edible products from animals that consume Aflatoxin – contaminated feed are additional sources of potential exposure. [1]

22. Although commodities such as corn, peanuts, cottonseed, brazil nuts, pistachio nuts and copra all classified as commodities with the highest risk of Aflatoxin contamination, but their associated risk to human varies due to difference in their dietary intake. Hence, widely used commodities such as corn posses a greater risk to human than others. Data published by WHO (GEMS/FOOD) suggests that daily consumption of pistachio in the world is between 0-0.3 g. [25] In general, the dietary intakes of pistachio is at least 7% (0.3 g /4.3 g) of whole Nuts (in shell) consumption.[26]

CONCLUSIONS & RECOMMENDATIONS

23. The present Discussion Paper on Aflatoxins in Pistachios leads to the following broad recommendations for consideration at the 34th Session of the CCFAC:

I Based on all the toxicological evidence available to date, levels of Aflatoxins need to be as low as technologically feasible, taking into account economic and social factors. The ultimate way to protect the consumer from the toxic effect of Aflatoxins, is to encourage and ensure a good agricultural practice by:

- revealing the critical points where the fungi starts growing and producing Aflatoxins during the agricultural production.
- including quality control programs in agricultural production.
- improving the training of individuals involved at all stages of production.
- supporting research on methods and techniques to prevent fungal contamination in the orchard and during harvesting, processing and storage.

II It is recommended that a code of practice be established by Codex for the reduction of Aflatoxins in tree nuts.

III It is recommended that the Committee ask governments to provide data, to supplement the data presented in this document, so that the establishment of maximum levels can be considered.

IV It is recommended that Codex should establish sampling plans and methods of analysis for Aflatoxins in pistachios.

REFERENCES

1. JECFA, 1998: Forty-ninth meeting of the Joint FAO/WHO Expert Committee on Food Additives. Safety evaluation of Certain Food additives and Contaminants: Aflatoxins. WHO Food Additives Series 40 (Geneva WHO), pp 359-469.
2. Crane J. C. 1989: Pistachios In: CRC Handbook of fruit set and development, CRC Press, Boca Raton, 389-399.
3. Mojtahedi H, Rabie C J, Lübben A, Styen M & Danesh D, 1979: Toxic Aspergilli from pistachio nuts. Mycopathologia 67 (2), 123-127.
4. Rice R E, 1978: Navel orange worm: a pest of pistachio nuts in California, Entomological Society of America 71 (5), 822-824.
5. Sommer N F, Buchanan J R & Fortlage R J, 1986: Relation to early splitting and tattering of pistachio nuts to Aflatoxin in the orchard. Phytopathology 76, 692-694.
6. Doster M A & Michailides T J, 1994(a): Aspergillus moulds and Aflatoxin in pistachio nuts in California, Phytopathology 84 (6), 583-590.
7. Doster M A & Michailides T J, 1994 (b): Development of *Aspergillus* moulds in litter from the pistachio trees. Plant disease 78 (4), 393-397.
8. Kader A A & Thompson JF, 1992: Postharvest handling systems: Tree nuts. In: Postharvest Technology of Horticultural Crops (Ed. A.A. Kader), University of California, Division of Agriculture and Natural Resources, Davis, U.S.A. Publication No. 3311.
9. Beuchat L R, 1978: Relationship of water activity to moisture content in tree nuts. J. Food Sci. 43, 754-755.Pitt J D & Hocking A. D, 1997: Fungi and Food Spoilage (2nd edition). Blackie Academic & Professionals, London, 375-383.
10. Pearson T C, Slaughter D C and Studer H E, 1994: Physical properties of pistachio nuts. Transaction of the ASAE 37 (3), 913-918.
11. Pearson T C & Schatzki T F, 1998: Machine Vision System for automated detection of Aflatoxin-contaminated pistachios. J. Agric. Food. Chem. 46, 2248-2252.
12. Schatzki T F, 1995a: Distribution of Aflatoxin in pistachios. 1. Lot distribution. J. Agric. Food. Chem. 43, 1561-1565.

13. Schatzki T F, 1995b: Distribution of Aflatoxin in pistachios. 2. Distribution in freshly harvested pistachios. J. Agric. Food. Chem. 43, 1566-1569.
14. Schatzki T F & Pan JL. 1996: Distribution of Aflatoxin in pistachios. 3. Distribution in pistachio process streams. J. Agric. Food. Chem. 44, 1076-1084.
15. Schatzki T F & Pan JL. 1997: Distribution of Aflatoxin in pistachios.4. Distribution in small pistachios. J. Agric. Food. Chem. 45, 205-207.
16. Schatzki T F, 1998: Distribution of Aflatoxin in pistachios. 5. Sampling and testing U.S. pistachios for Aflatoxin. J. Agric. Food. Chem. 46, 2-4.
17. Schatzki T F, 1999: Distribution of Aflatoxin in pistachios. 6. Seller's and buyer's risk. J. Agric. Food. Chem. 47, 3771-3775.
18. Schatzki T F, 2000: Distribution of Aflatoxin in pistachios. 7. Sequential sampling. J. Agric. Food. Chem. 48, 4365-4368.
19. Salunkhe D K, Adsule R N and Padule D N, 1987: Aflatoxins in foods and feeds, Metropolitan Book Co. Pvt. Ltd., New Dehli, India, p. 18.
20. Official Methods of Analysis, 17th Edition, Vol II, 2000, Horwitz W (ed)., AOAC Int, Arlington, VA, Chapter 49.
21. Trucksess M W and Wood G E, 1994: Recent methods of analysis for aflatoxins in foods and feeds, In: The Toxicology of Aflatoxins: Human Health Veterinary and Significance. Groopman J D (ed) Eagan Press, pp 409-431.
22. Thuvander A, Moller T, Enghardt Barbieri H, Jansson A, Salomonsson A-C and Olsen M, 2001: Dietary intake of some important mycotoxins by the Swedish population. Food Addit. Contam. 18(8), 696-706.
23. Taguchi S, Fukushima S, Sumimoto, T, Yoshida, S and Nishimune T, 1995: Aflatoxins in Foods Collected in Osaka, Japan, from 1988 to 1992. J. AOACI. 78, 325-327.
24. Iranian Ministry of Health and Medical Education, Food and Drug Control Labs (2001) "The situation of Aflatoxin contamination in pistachio during March1998-March 2001".
25. Institute of Standards and Industrial Research of Iran, Food and Microbiology Labs (2001) "The situation of Aflatoxin contamination in pistachio during March1998-March 2001".
26. WHO Food Safety Issues, GEMS/FOOD Regional Diets, pp. 4-5, 1998.