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«TRAINING ACTIVITIES ON FOOD CONTAMINATION CONTROL AND MONITORING WITH SPECIAL REFERENCE TO MYCOTOXINS»



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# THE STUDY OF CONTAMINATION OF FOOD PRODUCTS BY SOME MYCOTOXINS IN THE GEORGIAN SSR


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The task of protection of food against a potentially extensive contamination with toxic metabolites of microscopic mould fungi - mycotoxins - is one of the problems of protection of the human internal environment.

Mycotoxins are highly toxic metabolites of microscopic fungi and they are widely spread in nature. At present more than 240 strains of different species of mould fungi are known and these produce approximately 100 toxic compounds. Numerous studies have demonstrated the potential hazard of mycotoxins for the health of man and animals. Thus, a large group of mycotoxins - aflatoxins, sterigmatocystin, cyclochlorotin, luteoskyrin , etc. - have a primarily hepatotropic action; ochratoxine and citrinin manifest nefrotic effects; strong embryotoxic, teratogenic, and carcinogenic properties have been found in patulin; trichothecene mycotoxins and zearalenon - metabolites of microscopic fungi belonging to the Fusarium genus - are responsible for the development of alimentary toxic aleukia in man (1,2,3).

Mycotoxins are found in all countries, primarily in vegetative farm products; wheat, maize, rice, barley, oats, sorghum, peanuts, legumes, some nuts and fruit, and also in the feeds. They may affect products not only of plant but also of animal origin.



Investigators concentrate on mycotoxins since their protential hazard for man's health has been demonstrated. The-

se include aflatoxins - a group of mycotoxins of very strong hepatotoxic and hepatocarcinogenic properties produced by mould fungi such as Aspergillus flavus and Aspergillus parasiticus. Their discovery in 1960 triggered large-scale research into mycotoxins.

Aflatoxins were first discovered in a Brazilian peanut meal which in 1960 caused in England the death of approximately 100,000 turkeys (1,4). Since then aflatoxins were found in many food products and feeds. The group of aflatoxins, besides its main representatives - aflatoxins $B_1$, $B_2$, $G_1$ and $G_2$ - includes a number of their derivatives (aflatoxins $M_1$, $M_2$, $B_{2a}$, $G_{2a}$, $GM_1$, $P_1$, $Q_1$). Aflatoxins also include aflatoxicol, aspertoxin, sterigmatocystin and its derivatives. Out of the four main representatives of aflatoxins, aflatoxin $B_1$ is most toxic and usually it is synthesized in the largest amount, whereas aflatoxin $G_2$ is produced in the last amount.

Aflatoxins have a pronounced hepatotropic action. Most species of mammals are susceptible to aflatoxins' carcinogenic action. Numerous investigators have demonstrated hepatocarcinogenic properties of aflatoxins, their ability to induce tumours in rainbow trout, ducklings, mice, rats, and monkeys. Epidemiological studies in a number of Asian and African countries have revealed a direct correlation between the incidence of cancer of the liver upon the population and the content of aflatoxins in food (3,5,6).

Aflatoxins can contaminate food products in any stage of their production - in the field, at the time of harvesting, during transportation or storage, in the process of cooking at home.

The main pathway of aflatoxins' arrival in human organism is the alimentary route, with contaminated food of vegetative origin and through the alimentary chains (milk and flesh of animals which have consumed contaminated feeds).

Aflatoxins are widely spread in nature. This is shown by information of J.Bainton and B.D. Jones who have reviewed the results of study of mycotoxin content in food and feeds during 1970-1976. Thus, in Africa 1,594 out of 2,965 samples of oil cake or oil-bearing seed meal and 152 out of 13,089 samples of edible nuts contained small amounts of aflatoxins. In North America aflatoxins have been found in one of 70 samples of fodder, in 57 out of 978 samples of nuts and in 184 out of 1,295 samples of oil-bearing plants. In South America, one of 23 samples of feed, 258 out of 461 samples of oil-bearing crops and 12 out of 15 studied samples of vegetative products contained aflatoxins. In Asia, the concentration of aflatoxins in 24.840 out of 25,565 studied samples of oil-bearing crops was as high as 100-1,000 g/kg. In Australia, aflatoxins were found only in feeds. In Europe, 62 out of 547 samples of feeds contained aflatoxin; 3 out of 30 samples of nuts contained aflatoxins at a content of 10-50 g/kg (7).

Aflatoxins, as natural contaminants, have been likewise found in products of animal origin: in milk, in different types of dairy products, eggs,and tissues of animals consuming feeds contaminated with aflatoxins.

Aflatoxins are excreted by cows with milk in the form of highly toxic metabolite, aflatoxin $M_1$. Aflatoxin $M_1$ has been found in 50% of samples of milk from private farms in Iran.

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Contamination of feeds with aflatoxins is a potential hazard for the health of man since aflatoxins accumulate in tissues of contaminated animals (8,9).

Acute intoxication with aflatoxin, in most animals, is distinguished by a rapid development of clinical symptoms of contamination and a high mortality rate (7).

Special attention should be paid to the findings concerning the possible correlation between the level of food contamination with aflatoxins and the incidence of primary liver cancer in humans. According to Austwick and Martin, there is a direct dependence between the incidence of primary cancer of the liver and the consumption of aflatoxin-containing products (11,12).

Information in the literature concerning other manifestations of aflatoxin toxicity is very limited. R.Schoental has found experimentally a teratogenic effect of aflatoxin $B_1$ in hamsters and rat embryos (13).

One of the most important factors facilitating the prevention of mycotoxicoses is the arrangement of supervision over contamination of food and feeds with mycotoxins and also the control of the levels of contamination.

Some countries have introduced stringent limitations for the content of aflatoxins in food products; thus, in the USA the limit is 20 $\mu$g/kg for the sum of $B_1+G_2$ aflatoxins and 0.5 $\mu$g/kg) for aflatoxin $M_1$. In Brazil the maximum allowable level fixed for aflatoxins is 30 $\mu$g/kg, in Sweden 20 $\mu$g/kg (9).

The principal part in the prevention of mycotoxicoses in

man is played by a system of supervision aimed at the prevention of contamination of food with mycotoxins or the lowering of the contamination to a level which is safe for man's health.

An important link in the system of control over the contamination of food with mycotoxins, specifically with aflatoxins, is the analysis of samples. As it is, a large number of methods of determination of aflatoxins in different food products have been worked out. In the main, the search is conducted along two lines: the development of chemical techniques of analysis; the creation of biological methods of identification.

All chemical techniques of detection of aflatoxins are based on a characteristic fluorescence of these compounds in the ultraviolet light. The main technique of identification and of quantitative determination of aflatoxins is thin-layer chromatography (1, 14, 15). However, along with this method, many investigators have recently suggested highly sensitive methods of liquid chromatography. H.Soweighardt, P.J.Colley, G.E.Neal, Gregory, D.Manley, and others believe that the methods of detection and qualitative determination of aflatoxins by high-performance liquid chromatography is quite reliable (16, 17, 18,19).

Very simple screening methods (such as direct fluorescence or the application of minicolumns) (3, 20, 21, 22) have been worked out to simplify the analytical procedure and to detect rapidly the level of contamination in a large number of samples.

W.O.Harder and F.S. Chu suggest the radioimmunochemical technique of identification of aflatoxin $M_1$. S.J.Langone and V.H. Vunakis suggest the radioimmunological technique for the identification of aflatoxin $B_1$ in blood serum, urine, in non-purified extracts of maize and peanut oil with the use of aflatoxin-specific antibodies. The sensitivity of this method does not differ from other analytical techniques, but unlike them, it does not require the additional stage of purification of extracts for the analysis.

The biological techniques of detecting aflatoxins, unlike chemical ones, take more time and are poorly specific, therefore, their use is limited. At present they are applied as tests confirming the presence of aflatoxins in the studied objects and in the analysis of materials for which no specific chemical techniques have been developed.

We, at our Institute have studied the content of aflatoxins making use of the "Methodical Recommendations for the detection, identification, and determination of aflatoxin content in food products, drawn up at the Institute of Nutrition of the USSR Academy of Medical Sciences and approved by the USSR Ministry of Health.

To determine the extent of contamination of food products of local production with aflatoxins, we analyzed 1,240 samples of maize, wheat, oats, barley, peas, beans, rye, soya beans, and mixed fodder representing 47 different areas and towns of the Republic.

The analyses conducted from 1979 to 1982 have shown that 21 samples contained afkatoxins, i.e. the average level of

contamination with aflatoxins was comparatively low- about 2% (Table 1).

According to the literature reports the average indicator of the extent of contamination with aflatoxins in many countries is much higher (7, 23, 24).

Eleven of the contaminated samples had a content of aflatoxins which exceeded the allowable concentration adopted in the USSR (5 μg/kg). The maximum contamination level was 600 μg/kg.

It follows from Table 1 that out of 308 samples of maize, aflatoxins were found in 11 samples (3.5%). This corresponds to the results of the examination of maize in 8 Mid-Western states of the USA (26). Only in one out of 11 contaminated samples of maize aflatoxins $B_1$,$B_2$,$G_1$,$G_2$ were detected. The maximum content of aflatoxin $B_1$ detected in maize was 600 μg/kg.

Table 1

CONTENT OF AFLATOXINS IN SAMPLES

| Product | Total number of samples | Number of samples with aflatoxins | Aflatoxin content, µg/kg | | | | Detection frequency, % |
|---|---|---|---|---|---|---|---|
| | | | $B_1$ | $B_2$ | $G_1$ | $G_2$ | |
| Maize | 308 | 11 | 0.5-600 | 0 - 8 | 0 - 80 | 0 - 8 | 3.5 |
| Wheat | 210 | 2 | 0 - 13 | - | - | - | 0.9 |
| Barley | 136 | 2 | 0 - 1.4 | - | - | 0 - 2.5 | 1.4 |
| Oats | 107 | 1 | 0 - 4.6 | - | - | - | 0.9 |
| Rye | 17 | - | - | - | - | - | - |
| Beans | 169 | 2 | - | - | - | 0 - 3 | 1.1 |
| Peas | 62 | 1 | 0 - 3.75 | - | - | - | 1.5 |
| Soya beans | 110 | - | - | - | - | - | - |
| Mixed fodder | 121 | 2 | 0 - 56.4 | - | - | - | 1.6 |
| Total | 1,240 | 21 | | | | | 2 |

Aflatoxins were also detected in 2 out of 210 samples of wheat. Out of 136 samples of barley, two were found to contain aflatoxin $B_1$ at a concentration of 1.4 μg/kg and aflatoxin $G_2$ at a concentration of 2.5 μg/kg. Aflatoxin $B_1$ was found in one sample out of 107 samples of oats (0.9%). Two samples of mixed fodder out of 121 samples contained aflatoxin $B_1$ at a concentration up to 56.4 μg/kg.

We should note specifically the comparatively low frequency of detection of aflatoxins in legumes. Out of 341 samples of beans, peas, and soya beans, only two samples of beans were found to contain aflatoxin $G_2$ at a concentration of 3 μg/kg and one sample of peas was found to contain aflatoxin $B_1$ (3.75 μg/kg). Our findings coincide with those of Japanese investigators who in their analysis of 52 samples of beans contaminated with toxigenic strains of fungi did not detect mycotoxins.

Table 2 shows annual data concerning content of aflatoxins in analyzed food products.

It follows from the Table that the highest percentage of detection of aflatoxins was in 1980 (2.9%). Samples with the content of aflatoxins much in excess of the maximum allowable concentration fixed for aflatoxins in the USSR were also found in 1980.

The concentrations of aflatoxins in contaminated samples are given in Table 3. The highest concentrations of aflatoxins were found in maize and mixed fodder. Out of 11 maize samples contaminated with aflatoxins, the concentration of aflatoxins in four samples exceeded 50 μg/kg and in two samples it was higher than 100 μg/kg (147 μg/kg and 600 μg/kg).

Table 2

RESULTS OF ANALYSES OF FOOD PRODUCTS FOR AFLATOXINS

| Product | 1979 | | 1980 | | 1981 | | 1982 | |
|---|---|---|---|---|---|---|---|---|
| | Number of samples | Number of detections | Number of samples | Number of detections | Number of samples | Number of detections | Number of samples | Number of detections |
| Maize | 35 | 1 | 91 | 5 | 166 | 5 | 16 | - |
| Wheat | 27 | - | 65 | 2 | 101 | - | 17 | - |
| Barley | 10 | - | 61 | 2 | 54 | - | 11 | - |
| Oats | 13 | - | 47 | 1 | 37 | - | 10 | - |
| Rye | 5 | - | 4 | - | 5 | - | 3 | - |
| Beans | 21 | - | 22 | - | 108 | 2 | 18 | - |
| Peas | 19 | - | 19 | - | 17 | 1 | 7 | - |
| Soya beans | 23 | - | 20 | - | 57 | - | 10 | - |
| Mixed fodder | 15 | - | 23 | - | 72 | 2 | 11 | - |
| Total: | 168 | 1 | 352 | 10 | 617 | 10 | 103 | - |

Among food products under study, maize proved to be particularly strongly affected by aflatoxin contamination. This finding is confirmed by many investigators who detect aflatoxins in peanuts and maize and in highest concentrations most frequently (3,9).

It was believed until recently that the spread of mycotoxins depends largely upon climatic conditions. However, the studies of recent years have demonstrated that mycotoxins (aflatoxins specifically) may develop in different food products and feeds not only in countries with tropical and subtropical climate but anywhere, with the exception of particularly cold areas of the North (1,8).

Our information confirms this fact. It is well known that Georgia is a republic with a varied climate. The republic includes districts with subtropical, continental, and mountainous climate. The samples which we found to be contaminated with aflatoxins have been grown in and brought from the subtropical and other climatic zones of the Georgian Republic.

The main factors which influence the formation of aflatoxins are humidity and temperature, as confirmed by our data indicating that the highest number of cases of detection of products contaminated with aflatoxins is noted in districts with high humidity and temperature. This is also supported by the fact that in 1980, when the highest frequency of aflatoxin detection was found, the total annual rainfall, compared with other years which we have surveyed, was higher than the average level and the average annual temperature was also higher than the mean annual figure for many years

Table 3

AFLATOXIN CONCENTRATION IN CONTAMINATED SAMPLES

| Sample | Aflatoxin concentration, µg/kg | | | |
|---|---|---|---|---|
| | 5-10 | 10-50 | 50-100 | 100 |
| Maize | 2 | 3 | 4 | 2 |
| Wheat | - | 2 | - | - |
| Barley | 2 | - | - | - |
| Oats | 1 | - | - | - |
| Rye | - | - | - | - |
| Beans | 2 | - | - | - |
| Peas | 1 | - | - | - |
| Soya beans | - | - | - | - |
| Mixed fodder | - | 1 | 1 | - |
| Total: | 8 | 6 | 5 | 2 |

(27).

Thus, our information testifies to a high potential possibility of contamination of food products with aflatoxins in the Georgian Soviet Socialist Republic and proves once again their ubiquity.

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