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FOOD MYCOTOXINS SURVEY AND MONITORING PROGRAMS

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Abstract - Traditionally, mycotoxin problems have come to our attention mainly because of outbreaks of animal diseases and in several instances human diseases and these are then related to a mycotoxin as the causative agent. Undoubtedly this will continue to be a very important means for detecting mycotoxin. In recent years, however, now that practical, sensitive analytical methods have become available, we have been able to seek out mycotoxin contamination, monitor problem foods and survey potential problem foods for a number of mycotoxins.

Traditionally, mycotoxin problems have come to our attention mainly because of outbreaks of animal diseases and in several incidents human diseases and these are then related to a mycotoxin as the causative agent. The classic example of an animal disease outbreak leading to the discovery of a mycotoxin is the "turkey X" disease of the early sixties. In this instance, toxic peanut meal being used in turkey rations killed thousands of young turkeys. Details of this episode have been reported elsewhere and it will be sufficient to merely point out that investigations into this disease outbreak led to the discovery of aflatoxin as the causative agent. Undoubtedly, this will continue to be a very important means for discovering mycotoxins; however, in recent years now that practical sensitive analytical methods have become available, it is possible to seek out mycotoxin contaminations, monitor problem foods and survey potential problem foods for a number of mycotoxins. The discussion here will be confined to this latter category.

For our purposes, we will define a survey program as one in which the goal is to determine whether or not a problem or potential problem exists by analyzing selected samples for known mycotoxins, and a monitoring program as one in which a foodstuff is analyzed for a mycotoxin because it has already been established that a mycotoxin problem exists.

There are several things one should expect to get out of a well designed mycotoxin survey program. First - the completed program should show whether or not a problem or a potential problem exists. And then, if contamination is found, there should be an indication of the extent of the contamination. It also, should indicate whether the problem is localized or is of a more general nature. Other pieces of information will usually be produced but these three are the most important.

DESIGN OF A SURVEY

Assuming that the mycotoxin of concern has been designated, the foodstuffs to be examined are selected. This may be for one or a number of foods. It makes little difference because the points to be considered are the same for each situation. In many instances past experience or experience from studies carried out in other parts of the world will give some idea as to foods in which problems may exist and information such as this can be helpful in making wise selections. The number of samples to be analyzed should be large enough to give statistically significant results. This means that the plan should be developed with the assistance of a statistician. The samples should represent the geographical area of concern. Sets of samples should be taken at different seasons of the year. An excellent example for the desirability of evaluating seasonal variations is in surveying milk for aflatoxin M. Large seasonal differences for contamination of milk for some parts of Germany have been reported (Ref. 1). Samples should be taken over a period of several years because some crops have shown considerable year to year variation. Data for pistachio nuts and peanuts are excellent examples of this type of variation (Ref. 2).

The size of the sample taken is of the utmost importance for foods such as grains, oil seeds and nuts in which it has been shown that in many instances only a very few individual units are contaminated. (Ref. 3). If homogenous foods such as milk, cheese and beer are being investigated a sample only large enough for the analysis is completely adequate.

There are well proven aflatoxin methods for the analysis of foods (Ref. 4); it is important however to choose a method which best meets the needs for the particular food being analyzed. The method selected should be sufficiently sensitive to detect levels of concern for the particular food. For example, a method for milk should be capable of readily detecting 0.01 to 0.05 μg M_1 per liter, that is one half but preferably one tenth ppb. On the other hand a method capable of detecting 5 μg/kg in corn and other grains will usually be more than adequate for most purposes. The cost to carry out an analysis both in analyst time and cost of reagents and equipment is an important point to be considered. In many instances, particularly in some parts of the world, the availability of required reagents for the method will be the deciding factor.

It is important to carry out chemical derivative confirmation procedures for the aflatoxin, particularly when initial detections are being made and in every instance where there is any doubt from the TLC analysis. This will prevent the reporting of false positive results. Methods are now available in which the derivatization is carried out quickly and easily directly on the TLC plate for aflatoxins B_1, G_1, and M_1(Ref. 5,6).

In some crops one may be interested in several mycotoxins; for example aflatoxin, zearalenone and ochratoxin in corn (maize). A multi-mycotoxin detection method is available for these three mycotoxins (Ref. 7). The method of Wilson (Ref. 8) will detect sterigmatocystin, patulin and citrinin in addition to these three. This method was in fact used in the survey of corn in which the first natural contamination of a foodstuff with ochratoxin was reported. The savings in time and materials are large when this type of method can be used.

When a survey involves a number of foods, the versatility of a method, that is, its applicability to more than one food, can be an important consideration. Of all of these points discussed, the most important and the one which should overrule other considerations is the first one; that is, the method must have adequate sensitivity for the purposes of the survey.

An analysis and evaluation of the data is the last step of a survey, however it is preferable that the data be evaluated on a continuing basis because this will allow for adjustments and readjustments from the original plan and in this manner produce the best possible study for a given amount of resources.

For example if the resources allow for the analysis of two thousand samples in a multi-food survey, the number of samples for each food will be decided upon by the information available. If the analysis of the first five hundred samples indicate a problem in a food and there is an indication that one or more foods are not apt to be contaminated, the number of samples for the problem food can be adjusted upward and the number for those for which there most likely is no problem can be adjusted downward. Such adjustments should be made in consultation with the statistician so that the final results will still be statistically significant.

A number of surveys have been conducted on a number of foods in many countries of the world. Results of many of these have recently been published by Stoloff (2) so this information can be used as a guide for setting up surveys in other areas.

Peanuts have been surveyed in many parts of the world and undoubtably there is much more information available on this crop than for any other crop. These surveys indicate that peanuts are generally contaminated throughout the world. Cotton seed has been surveyed in the United States (Ref. 9,10) and India (11). In both instances the problem was found to be localized and appears to be correlated with irrigation and perhaps some other agronomic practices. In the United States, surveys have been carried out on Brazil nuts, pistachio nuts, walnuts, almonds and pecans (Ref. 2). In each instance some degree of contamination with aflatoxin was found and monitoring programs were started for these foods; several of these will be discussed later in this presentation. The initial surveys for corn indicated that there was no problem with this crop (Ref. 12). These were large scale surveys involving several thousand samples taken from the corn belt in the United States, the area where the major part of the corn is produced. Some time after these initial surveys were conducted an occasional contamination with aflatoxin involving farm animals was observed but these were usually from outside of the corn belt. This brought about the initiation of surveys in other areas and the results were quite different from the earlier corn belt surveys (Ref. 13). This points out the importance for a survey to be designed so that it will be geographically representative.

A broad spectrum multi-food mycotoxin survey was started last year in Tunisia. The plan calls for the analysis of about 4 thousand samples over a two year period and covers most of the foodstuffs which might become contaminated with aflatoxin in this country. The survey not only covers domestically produced foods but imports as well. Boutrif (14) reports the first year findings.

Similar broad coverage food surveys have recently been started in Egypt, in India and a smaller scale survey has been conducted in Colombia, South America. The latter is expected to be expanded in the near future.

It will be interesting to learn the results of these surveys so that the data can be added to the present world data base (Ref. 2). The surveys mentioned here by no means covers all of those which have been carried out or those which are ongoing but are given as examples of types of surveys which have been conducted. Compilation and dissemination of data from such surveys is important so that the information can be put to use in the control of aflatoxin in foods and feeds throughout the world.

MONITORING PROGRAMS

From our definition, this type of program will have been initiated because it has already been shown that a problem exists or in some instances in which reliable reports indicate a problem.

There are a number of points or places in the harvesting and processing of a crop in which effective monitoring can be carried out. Some of these are: 1) at the point of purchase of the crop as it comes from the farm; 2) ports of entry into a country; 3) export points; 4) receiving points of raw material for manufacturing; 5) in-process control points, that is, critical points in the processing chain at which effective control of the product can be brought about and 6) analysis of the finished product.

There are a number of commodities for which monitoring programs are in use. Some of these are: peanuts, Brazil nuts, pistachio nuts and peanut meal. Both Poland (Ref. 15) and Japan have extensive programs for the importation of peanut meal. In the Japanese program the lots are selected on the basis of analysis in India and these lots are sampled and analyzed again at the point of entry to Japan.

The aflatoxin monitoring plan for peanut processing in the United States is the most highly developed one in use to date. In this plan the peanuts are analyzed as they leave the farm and at various stages of process through to the finished food products. As an illustration, a typical monitoring program for the production of peanut butter will be described. Similar programs are in use for the processing of other peanut products.

Each truck or wagon load is sampled for grading purposes as the peanuts come from the farm. In addition, this sample is shelled and the kernels inspected under low power magnification for the presence of the mold Aspergillus flavus (Ref. 16) and if any contaminated kernels are observed, the lot is not purchased for processing into food. Experience over the years has shown a high correlation between aflatoxin contamination in the lot with the presence of Aspergillus flavus contaminated kernels. By diverting these unacceptable lots from those allowed into the food processing chain, the task of producing an acceptable food product is greatly reduced. The acceptable lots move onto the "sheller," the designation for the manufacturing process in which the nuts are cleaned, shelled and sorted (usually by means of electronic sorters) to produce "raw shelled nuts." Experience has shown by means of analysis that the sorted out nuts tend to contain the bulk of the aflatoxin contamination (Ref. 17). At the final stages of processing of the shelled nuts, samples are removed from a given lot by automatic samplers or by removing samples from every fourth bag of the lot. A lot is usually forty thousand pounds, a truck load, or one hundred thousand pounds, a rail car load. A sample of one hundred and forty four pounds is removed and divided into three forty eight pound portions. A sequential analysis plan (Ref. 18) is used in which only one, two, or three of these forty eight pound samples are ground and analyzed depending upon the results of the first or succeeding analyses. All analyses are carried out by an approved laboratory.

From results of the first analysis the decision is made to: 1) reject the lot; 2) accept the lot or 3) to proceed with sample preparation and analysis of the second portion. In this instance the results of the analyses from the first and second portions are averaged and the decision is made to reject the lot, accept the lot, or proceed with sample preparation and analysis of the third portion. If this is the case the analytical results from the three analyses are averaged and the decision is made to accept or reject the lot. A unique feature of this sampling and analysis plan is that one has the advantage of a very large sample but only enough of the total sample is ground and analyzed to give the necessary information upon which to base a decision for acceptance or rejection of the lot (Ref. 19).

Acceptable lots of "raw shelled peanuts" are shipped to the peanut butter manufacture where the lot is sampled and analysis carried out for raw material quality control purposes. Many processors use the same sampling plan as described above for the sheller. If their results agree with the sheller the lot goes into storage for processing. If the processor's results do not agree with the sheller and the lot is unacceptable by his analysis, the lot is resampled by the original plan and analyzed by a "referee" laboratory. These results determine whether or not the lot is acceptable.

The processor then proceeds with his normal steps of blending, blanching (that is, the removal of the skins), sorting, splitting the nuts into halves, roasting and electronic sorting to remove discolored nuts. The roasted nuts are then ground along with other ingredients to make peanut butter by a continuous process and finally packaged into marketable containers. The processor usually analyzes at one or more stages of the continuous process and unacceptable product is diverted before packaging. Not all processors have the capability for quality control analysis at this step of the process. The finished product is then analyzed before accepting the lot for shipment and sale to consumers. From this description, it is readily seen that the peanuts destined for such use are actually monitored at a number of control points in the process which are: 1) at the point of purchase from the farmer; 2) at least once by the sheller; 3) by the processor on the incoming raw material and at various points in the manufacturing process; and 4) finally the finished product. A system such as this pretty much assures the production of an acceptable product.

Another example of a monitoring program is the one for Brazil nuts used in the United States. As mentioned earlier, surveys showed that a problem existed in Brazil nuts and a conserted effort to correct it was initiated by the importers, the processors, the exporters, and Brazilian and United States government agencies. The monitoring program that evolved is a voluntary agreement made between the importers, the Food and Drug Administration (FDA) and the U.S. Department of Agriculture (USDA) in which each lot of imported nuts are sampled and analyzed by an approved USDA laboratory before the lot is accepted. This program has been in effect for six or seven years. At the start of the program approximately twenty five percent of the lots were unacceptable. In several years this was reduced to about three percent and in one year was less than one percent (Ref. 2). The processors in the United States have developed effective methods for "reconditioning", that is, the removal of contaminated nuts from the unacceptable lots.

The monitoring program for pistachio nuts in Iran in conjunction with a volunteer agreement for analyzing imports in the United States, similar to the Brazil nut program, is another example of an effective monitoring program. In many respects the processing procedures are similar to the peanut monitoring program described here which is in use in the United States. The nuts are sampled and analyzed as they are purchased from the growers. Contaminated lots are diverted from the main processing stream. The inprocess lots are also analyzed and only acceptable lots are bagged for export. Five hundred bag lots are sampled and analyzed by a government laboratory and only acceptable lots are certified for shipment (Ref. 20).

This program for pistachio nuts offers an excellent illustration of the manner in which a problem was brought under control in an area where modern quality control tools were not available so it was necessary to fabricate equipment locally, to employ a column detection aflatoxin method for lot purchase and manufacturing control, and to initiate a crash training and laboratory updating program to establish the certification program.

The nuts come in from the growers for sale to the processors in lots as small as several bags (70 kg.) to as many as 400 bags; the usual quantity being less than 100 bags. Each lot is given a designated space on the receiving warehouse floor and it is sampled by taking "hand grabs" from each bag as it is being dumped onto the floor. The "hand grabs" are composited, blended, quartered, ground, and analyzed using a column detection method (Ref. 21). Acceptable lots are allowed to enter the main processing stream but the contaminated lots are set aside and segregated so that they will not contaminate the processing stream.

The nuts are cleaned, size separated, unsplit nuts are cracked and processed in lots up to 2000 bags in size. These process lots are sampled by means of a sampling tool (commonly known as a trier, thief, bomboo) to give at least a 70 kg sample which is blended, quartered and analyzed by the column detection method. Then 500 bag size lots are made up for sale and shipment from acceptable process lots. Before the bags are closed, they are sampled under the supervision of a government inspector by means of a trier to give a 70 kg. sample which is blended, quartered and analyzed in a government laboratory by the official AOAC method (Ref. 4). Acceptable lots are certified for shipment.

Useable triers (sampling tools) were fabricated in a local machine shop for in-process lot and lot certification sampling. Effective blenders were fabricated from open-end 55 gallon drums by installing three baffels to the sides. Blending is achieved by slowly rolling the drum around the yard or warehouse. Essential laboratory equipment was designated for purchase, a proven analytical method was adapted to meet local conditions, and several chemists were given training in laboratories which were competent in aflatoxin analysis.

Under a voluntary agreement between the importers, the USDA, and the FDA, the lots are sampled at the port of entry by Department of Agriculture inspectors and analyzed in a USDA laboratory using FDA designated methods. Acceptable lots are allowed entry for shipment to nut processors; the unacceptable lots are detained and only allowed entry after they have been reconditioned to reduce the aflatoxin to an acceptable level.

The pistachio monitoring program as described here has effectively reduced the number of unacceptable import lots of nuts.

In conclusion it can be readily seen from the examples presented here that effective mycotoxin survey and monitoring programs have been developed and put to use in various parts of the world, and that adequate tools and techniques are available to institute similar programs for other feed and foodstuffs when justified.

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