

## **SURVEY OF EDIBLE NUTS FOR AFLATOXINS**

### **Summary**

A survey of aflatoxins in a variety of retail nuts and nut products was carried out by the Agency between November 2003 and March 2004. The purpose of the survey was to determine levels of aflatoxins and to monitor the effectiveness of the controls in place to limit consumer exposure to these toxins.

In the survey a total of 197 samples of retail nuts and nut products were analysed for aflatoxin B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub>. The 154 nut samples analysed included Brazil nuts, peanuts, pistachios, walnuts, hazelnuts, pecans, cashews, macadamias and almonds. The remaining 43 samples were nut products e.g. peanut butter and marzipan.

Levels of aflatoxin B<sub>1</sub> and total aflatoxins in the majority of samples analysed (70%) were so low that they were not quantifiable. The limit of quantification for B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub> was 0.05, 0.03, 0.05 and 0.04 µg/kg respectively. Forty-nine samples (25%) were found to have low levels of aflatoxins, below the regulatory limits of 2 µg/kg for B<sub>1</sub> and 4 µg/kg for total aflatoxins. However, aflatoxins were quantified above the regulatory limits in 10 samples. These were 5 samples of pistachio nuts, 4 samples of Brazil nuts and 1 sample of almonds. None of the nut product samples exceeded the regulatory limits.

Samples for the survey were purchased by Ventress Technical Services Ltd from various types of retail outlets across the UK, covering a wide range of brands. All the samples were analysed at Leatherhead Food International (LFI) using a validated method of analysis.

The results from this survey show an improvement in the situation compared to the previous survey published in 2002, when 13% of samples from the main survey and 9% of samples from an additional survey were found to exceed the EC limits for aflatoxins. This compares with 5% in this latest survey.

The consumption of a very small amount of a mycotoxin on a single occasion such as consumption of a small amount of contaminated nuts is unlikely to cause ill effects. Thus, the results from this survey do not raise any new safety concerns and consequently consumers do not need to change their diets as a result of these findings.

### **Background**

Aflatoxins (aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>) are a group of naturally occurring compounds called mycotoxins, produced by certain moulds growing on some food crops during production and storage. Mycotoxins are found in a wide range of foods around the world. Aflatoxins are produced by *Aspergillus flavus*, *A. parasiticus* and *A. nominus*, mould species that grow in warm humid conditions. They occur mainly in commodities imported from the tropics and sub-tropics, in particular groundnuts (peanuts), edible nuts, dried figs, spices and maize, and products thereof. Studies have demonstrated that aflatoxins and other mycotoxins are seldom uniformly

distributed throughout most food and feed commodities, their occurrence being uneven and sporadic.

Aflatoxins are considered to be genotoxic carcinogens and have been shown to cause cancer in laboratory animals by reacting with DNA. In humans, aflatoxins have been linked to liver cancer in a number of developing countries, where it is common for some foods that are an important part of the diet to contain high levels of aflatoxins. Aflatoxin B<sub>1</sub> is the most toxic aflatoxin. The UK Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) has, since 1981, recommended that aflatoxin concentrations in food should be reduced to the lowest levels technologically achievable. The EC's Scientific Committee for Food (SCF) also concluded that there was a need to reduce aflatoxin exposure to the lowest level achievable.

## **Legislation**

There is currently harmonised EC legislation on mycotoxins. In England this legislation is implemented by The Contaminants in Food (England) Regulations 2003 [S.I. 2003 No. 1478], and sets limits for aflatoxins in food including peanuts, edible nuts and their products. Parallel legislation exists for Scotland, Wales and Northern Ireland. The regulatory limits are 2 µg/kg for B<sub>1</sub> and 4 µg/kg for total aflatoxins. In addition to these regulations, there are currently Emergency Control Regulations for aflatoxins in pistachio nuts from Iran and Turkey, Brazil nuts in shell from Brazil and peanuts from China and Egypt. These controls impose special conditions on the import of these products into the EU. These measures expand and reinforce the legislation already in place to protect public health.

## **Sampling**

In this survey, 198 samples of retail nuts and nut products were analysed. Of these, 155 samples were Brazil nuts (21), peanuts (33), pistachios (26), walnuts (14), hazelnuts (14), pecans (14), cashews (14), almonds (13), macadamias (5) and tiger nuts (1). The 43 nut product samples were peanut butter (30), marzipan (10), pickled walnuts (1), cashew nut butter (1) and hazelnut and almond butter (1).

The samples were purchased between November 2003 and January 2004 by Ventress Technical Services Ltd. The samples were selected from a variety of retail outlets, including supermarkets, smaller shops and market stalls across the UK. A wide range of brands was covered in order to ensure that the survey was representative of the supply of the products to consumers in the UK. The absence of a particular brand means only that the product was not included in this survey. Mixed nut products were excluded from the survey, as it would not have been possible to identify the source of contamination had aflatoxins been found in a sample.

Sampling was carried out in accordance with the Agency's survey guidelines. For mycotoxins surveys carried out by the Agency, a representative retail sample, in this case of approximately 1 kg in weight, is taken. Where the product was sold in packs of less than 1 kg, a number of retail packs were purchased, ensuring that all came from the same batch, and these were mixed thoroughly before taking a sample for analysis.

Details of the samples taken for this survey are given in Annex 1.

## **Methodology**

All the samples were delivered to LFI at ambient temperature and, to ensure homogeneity prior to analysis, all in-shell and shelled nut samples (except marzipan and nut butters) were homogenised to a slurry. Aqueous homogenates of the samples were prepared by homogenising the samples with deionised water. Prior to analysis, all samples were mixed thoroughly to ensure homogeneity.

After homogenisation, all samples were split equally into 3 containers ('A', 'B' and 'C') and stored at -18°C. Sample A was used for the analyses and samples B and C were retained frozen, one for the analysis by the manufacturer/retailer at their request and the other for referee purposes.

The analysis was carried out by LFI using their validated UKAS accredited in-house method. The method involved extraction of samples with 60% aqueous acetonitrile, filtration, dilution of the extract with phosphate buffered saline (PBS) clean-up using immunoaffinity column, HPLC separation and quantification by post column derivatisation with piridynium hydrobromide perbromide (PBPB) and fluorescence detection.

Confirmation of the initial result was carried out by re-extraction of the sample using an HPLC column with phenyl-hypersil as the stationary phase, aqueous acetonitrile as the mobile phase and post column derivatisation with PBPB and fluorescence detection. Details of the limit of quantification (LOQ) are summarised in Annex 2. All results were corrected for recovery and these values are also detailed in Annex 2.

## **Quality Assurance**

Quality assurance for mycotoxin analysis is monitored using naturally contaminated in-house reference materials and by participation in UK and EU proficiency schemes, collaborative and inter-comparison trials. All analyses were conducted with spiked samples; i.e. a known amount of aflatoxins was added to each batch of samples of each matrix, prior to extraction. These samples were used to assess recovery with recoveries between 60 and 120% classed as valid.

## **Measurement uncertainty**

The analytical results have a variability known as measurement uncertainty (MU). For any analytical method each result is reported as the best estimate for the sample and it is always qualified by a measurement of uncertainty, eg x micrograms/kilogram (ig/kg) ± y micrograms/kilogram. For this survey the calculation of MU was carried out using Leatherhead Food International's (LFI) in-house data and a standard coverage factor of 2, equivalent to a confidence factor of 95%.

## Results

The full results of the survey are detailed in Annex 3.

This survey found that aflatoxins were not quantifiable in the majority of samples (70%) and were found at low levels in 49 samples (25%). Ten samples (5%) were found to contain aflatoxin levels above the EC and UK regulatory limits. These were 5 samples of pistachios, 4 samples of Brazil nuts and one sample of almonds. These results are summarised in Annex 4. Confirmatory analysis was carried out on each of these samples. None of the nut products analysed exceeded the regulatory limits.

All results are given as the best estimate value, corrected for recovery, but do not take into account the measurement uncertainty. Food Standards Agency surveys of contaminants are usually reported in this manner since the best estimate values are used to calculate dietary intakes of the various contaminants. Where surveys are conducted for other purposes, the data are reported differently. In their own surveys, for statutory enforcement purposes, public analysts and enforcement authorities always take the uncertainty associated with an analytical measurement into account when considering whether a product breaches regulatory limits. As a result of this they only take action when they are sure that levels of contaminants are above the regulatory limits. The Agency has followed their practice and taken action where the best estimate, less measurement uncertainty, exceeded the regulatory limit.

In accordance with the Agency survey guidelines, companies whose products were found with aflatoxins levels above the regulatory limits were asked to comment on the results reported. Comments received can be seen in Annex 5.

### **Action taken for samples exceeding regulatory limits**

Ten samples contained aflatoxins above the statutory limits ranging from 9.9 µg/kg to 710 µg/kg (see Annex 3). The Agency contacted Local and Home Authorities with responsibility for the companies that produce the 10 brands in question and in some cases contacted the companies directly.

Where appropriate a voluntary product withdrawal was carried out for those samples that were still available at retail level. Analysis was carried out (by either the Local/Home Authority or the company in possession of the nuts) to ensure that any remaining nuts from the affected batches complied with the Regulations. However, it was determined that, in most cases, batches of the affected products were no longer on sale and no further withdrawal action could be taken in these cases.

The traceability of the 10 affected samples was also investigated. The brand owners of these samples were asked to provide information on their suppliers (importers/wholesalers). The importers/wholesalers of the original consignments were then contacted and were requested to inform other companies who were also supplied with nuts from the same consignments.

In the course of tracing the origins of consignments of nuts and tracking their movement after being imported in the UK, it became evident that there was often a lack of traceability data and in some cases poor record keeping. The movement of consignments of nuts after importation into the UK may be complex, as a number of

importers, suppliers, traders or packers may be involved in the chain before the consignments reach the retail level. Thus, full and accurate recording keeping is essential to identify the origins of nuts throughout the supply chain.

Following Agency investigations it also became apparent that some importers/traders misinterpreted the sampling and analytical requirements laid down in the EC Directive 98/53. To address this, the Agency will draft additional explanatory guidelines.

The European Commission was also notified of those samples that exceeded the regulatory limits and the Commission in turn, informed other Member States where relevant. This was done via the Commission's Rapid Alert System for Food and Feed (RASFF).

As a result of the findings, the Agency held a meeting to discuss traceability issues with industry organisations. The Agency will continue discussions to seek assurances that record keeping practices are improved to ensure that, in future, the origins of these products can be traced at any point in the supply chain from when they enter the European Community to when they are sold at retail level.

## **Conclusion**

This survey was intended to determine levels of aflatoxins and to monitor the effectiveness of controls intended to limit consumer exposure to these toxins. The overall picture is improved compared to results published in 2002. Action was taken with regard to the ten samples that exceeded the regulatory limits. The results from this survey do not raise any new safety concerns and consequently consumers do not need to change their diets as a result of these findings.