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# Occurrence and exposure assessment of aflatoxins in Catalonia (Spain)

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ABSTRACT

The main objective of this study was to assess the exposure of Catalonian (Spain) population to AFs. Thus, two sub-objectives were considered: (1) to assess the occurrence of AFs in food marketed in Catalonia, and (2) to assess the consumption of those foods susceptible to AFs contamination by Catalonian population. AFs were analysed in a total of 603 samples considering special commodities as free-gluten, ethnic or baby foods. Analytical method consisted of an extraction and clean-up of aflatoxins step using immunoaffinity columns, and determination by HPLC with post-column photochemical derivatization and fluorescence detection. Food dietary intake was assessed using a food frequency questionnaire, administered to 1387 individuals by trained interviewers. Contamination and consumption raw datasets were combined by means of a direct method and a stochastic method, building the pseudo-parametric bootstrap confidence intervals of the main outputs. Margins of exposure (MoE) and cancer incidence were estimated for the different collectives. The highest percentages of positive samples were found in red pepper, pistachios and peanuts. Considering our results, the most exposed group was the celiac sufferer collective followed by the adolescents; however health concern should not be expected in the population groups.


## 1. Introduction

Aflatoxins $B_1$, $B_2$, $G_1$ and $G_2$ are mycotoxins that can be mainly produced by moulds of *Aspergillus* species, like *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius*, and can occur in a wide range of important raw food commodities including cereals, nuts, spices, figs and dried fruits (Malone et al., 2000; Otta et al., 2000).

Occurrence of AFs in foods from Spanish market has been previously reported in several studies where corn-based products and other cereals, pulses, dried fruits and nuts, snacks, breakfast cereals, bread, herbs or spices were analysed (Sanchis et al., 1986, 1995; Santamarina et al., 1986; Jiménez et al., 1991; Blesa et al., 2004), nevertheless, exposure assessment of Spanish population has not been conducted yet.

The main problem to assess mycotoxins intake is related to few representative available dietary data regarding food bearing mycotoxins contamination. There are some methods developed to assess dietary intake overall known as market basket, 24-h dietary recall and food record methods, food-frequency methods or dietary history. Given that corn-based products consumption is considered as sporadic or casual, food-frequency methods should be chosen because it may be advantageous to sacrifice precise intake measurements in exchange for more crude information related to an extended period of time (Walter, 1998).

Risk characterization is the estimation of the severity and probable occurrence or absence of known and potential adverse health effects on an exposed population (Kuiper-Goodman, 1995). Evaluation of toxicological data carried out by the Scientific Committees of the European Community results commonly in the estimation of a tolerable daily intake (TDI) derived from the No-Observed-Adverse-Effect-Level (NOAEL) from an animal study, applying a 100-fold uncertainty factor. However, the NOAEL approach is not appropriate for genotoxic carcinogens, because no threshold can be assumed in this case. Alternatively, the margin of exposure (MoE), defined as the ratio between a point on the dose–response curve for the adverse effect and human intake, has been proposed to characterise the risk of these contaminants. Mathematical analysis of the dose–response data from animal bioassays can be used to define the intakes necessary to produce a given level of response, such as 10% or 25% cancer incidence. The most commonly used methods are the T25 approach (chronic daily dose which give tumours in 25% of the animals above background at a specific tissue site) and $BMD_{10}$ approach (the 95% lower confidence interval on a benchmark dose (BMD) for a 10% increase in tumour incidence determined by fitting dose–response data to various mathematical models) (EFSA, 2005, 2007).

In the framework of the Project to Assess the Exposure of Catalonian Population to the Mycotoxins, and following the line of our previous studies (Cano-Sancho et al., 2009, 2010, 2011a,b), the main objective of this study was to assess the exposure of

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Catalonian population to aflatoxins combining raw contamination and consumption data. Thus, two sub-objectives were considered: (1) to assess the occurrence of aflatoxins in food marketed in Catalonia; and (2) to assess the consumption of those foods susceptible to aflatoxin contamination by Catalonian population.

## 2. Material and methods

### 2.1. Samples

Food samples were selected to be the most susceptible commodities to aflatoxin contamination and to be commonly consumed in Catalonia (Serra-Majem et al., 2003). Between 2008 and 2009, samples of peanuts ($n = 212$), pistachios ($n = 169$), dried figs ($n = 49$), sweet corn ($n = 181$), breakfast cereals ($n = 167$), corn snacks ($n = 213$), dried red pepper ($n = 165$) and baby food ($n = 154$, powdered infant formula) were obtained in six hypermarkets and supermarkets from 12 main cities (Tortosa, Tarragona, Reus, Vilanova i la Geltrú, l'Hospitalet de Llobregat, Barcelona, Terrassa, Sabadell, Mataró, Girona, Manresa and Lleida) of Catalonia, Spain, representative of 72% of the population. From each supermarket or hypermarket, 3 samples (if present) of each product were randomly taken. The level of AFs was determined in a total of 72 composite samples obtained by pooling the 3 items taken from each store if were available (12 cities × 6 stores/city = 72 samples/category). However, in some cases, 3 items were not available in the same store, thus, less than 72 composites could be obtained. In case case of the 49 dried figs samples, 18 gluten-free foods (mainly maize-based bread and pasta intended for celiac sufferers) and 35 ethnic food samples, such as Mexican tortillas, corn flour or cuscus (purchased in retail stores specialized in food import), were analysed individually. Regarding brands, we finally obtained 47 brands of peanut, 23 brands of pistachio, 35 of dried figs, 31 of sweet corn, 62 of breakfast cereals, 79 of corn snacks, 40 of red pepper and 21 of baby food, which can be considered the majority of market share in Catalonia of these products, as well as in the rest of Spanish market. The samples were transported and stored in freeze at −20 °C until analysis.

### 2.2. Analytical method

Easi-extract® Aflatoxin immunoaffinity cleanup columns (R-Biopharm, Rhône LTD Glasgow, UK) were used to extract $AFB_1$, $AFB_2$, $AGB_1$ and $AFG_2$ from all analytical samples. Ten grams of homogenised composite was mixed with 15 mL of extractant solution (60% acetonitrile, 40% water) for 20 min and filtered with a Whatman N°4 paper filter. Ten milliliters of filtered solution was diluted with 48 mL of phosphate buffer solution (PBS; 0.8% NaCl, 0.12% $Na_2HPO_4$, 0.02% $KH_2PO_4$, 0.02% KCl) and drained through the immunoaffinity column. After this, the columns were washed with 20 mL of PBS and AFs eluted with 1.5 mL of methanol grade HPLC and 1.5 mL of milli-Q water. Fluorescent derivatives of AFs were obtained using a post-column photochemical derivatizator instrument (UVE™ Derivatizer LC Tech, Germany). Chromatography equipment consisted of a separations module Alliance 2695 Waters®, analytical column Waters Spherisorb® 5 μm ODS2, 4.6 × 250 mm, Multi λ Fluorescence Detector Waters 2475®. Excitation wavelength was 365 nm, and emission wavelength at 0–13 and 13–25 min were 455 and 425 nm, respectively. Mobile phase consisted in a solution of water, methanol and acetonitrile (70:17:17). Aflatoxin concentration was expressed in μg of aflatoxin per kg of each assayed product.

#### 2.2.1. Validation of the method

Recovery data, repeatability, limit of detection (LoD) and limit of quantification (LoQ) are shown in Table 1 for some matrices according to the performance criteria established by Commission Regulation (EC) N° 401/2006 (European Commission, 2006).

The analytical method used for AFs was assessed for selectivity, linearity, and precision. Selectivity was checked by injecting 100 μL of mycotoxin standard solution three times before injecting extracted samples and comparing the peak retention times and the fluorescence spectra of the substances that produced these peaks. Standard curves were generated by linear regression of peak areas against concentrations.

Precision and recovery were established by determination of $AFB_1$, $AFB_2$, $AFG_1$ and $AFG_2$ levels, spiked in each food category by triplicate. Recovery was determined by comparing the absolute responses of each AF, with the absolute responses of calibration standards. It ranked between 72 ± 6 and 118 ± 1% for $AFB_1$, 70 ± 1 and 100 ± 6% for $AFB_2$, 74 ± 2 and 108 ± 1% for $AFG_1$ and between 61 ± 9 and 100 ± 6% for $AFG_2$. Repeatability was expressed as relative standard deviation (RSDr), it ranged between 4% and 20% in the cereal-based matrix and between 5% and 20% in the dried fruit matrix. The limit of detection (LoD) was considered to be three fold the signal of blank noise, and the limit of quantification (LoQ) was considered equal to 3 × LoD. The mean limit of detection (LoD) was 0.03 μg $kg^{-1}$ for $AFB_1$ and $AFG_1$, and 0.008 for $AFB_2$ and $AFG_2$ μg $kg^{-1}$.

### 2.3. Treatment of left-censored data

In the present study we have applied two different methods to obtain a mean level of contamination for each food dataset, taken into account the left-censored data.

1. Substitution method. Non detected samples were assumed to be the value of the LoD divided by 2. This is the most commonly used procedure in exposure assessment of populations to mycotoxins, thus we used this approach as starting point.
2. Non-parametric method: Kaplan–Meier estimation (KM). This is the standard non-parametric technique for censored data, based on the empirical cumulative distribution function. We address the readers to the report of Tressou (2006) to better understand the mathematic basis of the KM estimator, and its application in food risk assessment studies. With the KM method, the weight of the censored data is distributed over the different observed values below the censoring values. In order to obtain the mean and standard deviation of each contamination dataset, we have applied the syntax to fit the KM model based on the LIFETEST procedure from SAS® also included in the Appendix G in EFSA report (2010).

### 2.4. Dietary intake assessment

In this study, food dietary intake was assessed through a specific Food Frequency Questionnaire (FFQ) developed for Catalonian population including those foods typically consumed in the region which may be potentially contaminated with these mycotoxins. According to World Health Organization (WHO) advices, studies to assess dietary intake of chemical contaminants should show the significant intake among standard population, with all population groups that could have different dietary patterns. Therefore, the population groups considered were: infants (0–3 years), children (4–9), adolescents (10–19 years), adult males (20–65 years), adult females (20–65 years), elders (>65 years), immigrants (17–51 years), celiac sufferers (16–75 years). FFQ consisted of 38 items of specific foods worldwide known to be the most important food contaminated by mycotoxins, excluding those foods not consumed in the region. Concerning frequency of consumption, 5 response options were considered (never, annually, monthly, weekly and daily). Quantities were assessed by portion size with the aid of a series of colour photograph models. Finally, 79 elders, 70 celiac sufferers, 56 immigrants, 336 adult males, 384 adult females, 235 adolescents, 69 children and 164 infant parents were interviewed during 2008 and 2009 in Lleida region ($n = 1393$) by trained interviewers.

### 2.5. Exposure assessment

In this section we recall some general aspects concerning the exposure calculations, already given in Gauchi and Leblanc (2002).

If we assume independency between consumption ($C_{\pi,j}$) and contamination ($T_j$), as well as between their products, we can estimate the mean exposure of the population $\pi$ with the population sample $\pi_0$ as follows:

**Table 1**
Summary of the method performance characteristics for aflatoxin $G_1$, $B_1$, $G_2$ and $B_2$.

| | Matrix | LoD/LoQ (μg $kg^{-1}$) | Spiking level (μg $kg^{-1}$) | $n$ | Recovery (%) | RSDr (%) |
|---|---|---|---|---|---|---|
| $AFG_1$ | Cereal-based foods | 0.033/0.1 | 0.8–1.7 | 5–7 | 108 ± 1–80 ± 7 | 9–12 |
| | Dried fruits | | 0.4–4 | 5–6 | 74 ± 1–98 ± 2 | 13–20 |
| $AFB_1$ | Cereal-based foods | 0.033/0.1 | 0.8–3.3 | 5–6 | 72 ± 6–84 ± 7 | 8–11 |
| | Dried fruits | | 0.4–4 | 5–6 | 96 ± 2–118 ± 1 | 5–15 |
| $AFG_2$ | Cereal-based foods | 0.008/0.025 | 0.2–0.7 | 5–7 | 61 ± 9–91 ± 4 | 4–15 |
| | Dried fruits | | 0.4–1 | 5–6 | 88 ± 9–100 ± 6 | 6–10 |
| $AFB_2$ | Cereal-based foods | 0.008/0.025 | 0.2–0.8 | 5–7 | 70 ± 1–100 ± 2 | 9–20 |
| | Dried fruits | | 0.4–1 | 5–6 | 88 ± 9–100 ± 6 | 6–10 |