ESCOLA UNIVERSITÁRIA VASCO DA GAMA

MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

Exposure assessment of infants to Aflatoxin M1 in breast milk and maternal social-demographical and food consumption determinants

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List of Abbreviations and acronyms

AFB1: Aflatoxin B1

AFM1: Aflatoxin M1

EDI: Estimated daily intake

ELISA: Enzyme-linked immunosorbent assay

EU: European Union

HI: Hazard Index

IARC: International Agency for Research On Cancer

LOD: Limit of Detection

ML: Maximum level

SD: Standard Deviation

UHT: Ultra high temperature

Exposure assessment of infants to Aflatoxin M1 in breast milk and maternal social-demographical and food consumption determinants

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Abstract

Mycotoxins are secondary metabolites of fungi that have toxic effects on both humans and animals. Aflatoxins are mycotoxins produced by some strains of fungus, such as *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxin M1 (AFM1) which can be transmitted to newborns via breast milk, is a hydrolyzed metabolite of Aflatoxin B1 (AFB1) that is ingested along with contaminated food. AFM1 is classified as "possibly carcinogenic agent for Human" (group 2B IARC).

The occurrence of AFM1 in maternal milk and the degree of exposure of infants to this toxin were studied. The correlation between the concentration of AFM1 and basic socio-demographic factors and the consumption of certain categories of food was also aimed. Thus thirty milk samples from nursing mothers living in Portugal central region were collected, in 2016, and analyzed using a competitive commercial ELISA kit, in order to determine the presence of AFM1.

Thirteen samples (43.3%) contained levels of AFM1 above the detection limit (5ng/L), ranging between 5.1 and 10.2ng/L (7.12±1,89ng/L). Statistical analysis showed a moderated correlation between the maternal consumption of dry fruits (r=0.48) and milk (r=0.4) and the concentrations of AFM1 found in the samples. No other studied determinants, whether socio-demographic (age, weight, height, number of children, period of lactation, educational level, professional activity, residence, characteristics of breastfeeding, the infants' weight) or dietary (frequency of food consumption) showed a significant statistical influence. AFM1 estimated daily intake (EDI) was higher for younger babies (1.06ng/kg b.w.; <7kg) as compared with the older ones (≥ 7kg; 0.8ng/kg b.w.), which can be explained by the higher consumption versus weight. The hazard index for both groups (<7kg b.w.: 5.3; ≥7kg; 4.0) were far greater than 1.0, which is the value that indicate risk for consumers.

The results of this study suggest the need to reinforce surveillance of AFB1 occurrence in food, particularly dry fruits and milk, as a protective measure, not only for adults, but ultimately for nursing infants exposed by lactation. Although AFM1 presents an inferior carcinogenic potency, it is noteworthy that when compared with adults, infants feature a lower capacity of carcinogen biotransformation, a fairly restricted diet and a higher consumption in relation to body weight.

Keywords: Aflatoxin M1; breast milk; Aflatoxin B1; infants; exposure;

1. Introduction

One health involves a complex human-animal-environment web. As these three components are associated, a negative impact on one of them can cause consequences on the others. Aflatoxin B1 (AFB1) is a risk for animals that are exposed to this mycotoxin through the consumption of contaminated feed, in particular farm animals. Considering that milk is one of the animal-derived food where a significant carry-over from feed to food occur, it can be regarded as a sentinel matrix for the AFB1 vulnerability of the agro-food system in the One Health perspective (Frazzoli,

Gherardi, Saxena, & Belluzzi, 2017). AFB1 is the most common aflatoxin in food and amongst the most potent genotoxic and carcinogenic mycotoxins, classified as Group 1 by the International Agency for Research on Cancer (IARC, 2012).Given that the main AFB1-related compound present in milk is the hydroxy-metabolite aflatoxin M1 (AFM1), several studies surveyed the occurrence of this biomarker of exposure in ruminant milk, as commercially available to the consumers (Škrbić, Živančev, Antić, & Godula, 2014; Tsakiris *et al.*, 2013; Duarte *et al.*, 2013).

However the burden of food items with mycotoxins like AFB1 is also of concern for humans (Hof, 2016). AFB1 is metabolized in the human liver by Cytochrome P450–associated enzymes into AFM1, usually considered a detoxication byproduct of AFB1. Once released into blood, AFM1 can be detected in milk in an extent related with maternal dietary habits (Ghiasain & Maghsood, 2012; Ishikawa *et al.*, 2016). The exposure of nursing infants to AFM1, associated with their high consumption, low body weight, their high metabolism and a low detoxification capacity, renders babies highly susceptible to the adverse effects of this mycotoxin (Cantú-Cornelio *et al.*, 2015). AFM1 is classified by IARC as a group 2B carcinogen (possibly carcinogenic to humans; IARC, 2012). Although its carcinogenic potency is probably one or even two orders of magnitude lower than that of AFB1 the acute toxicity of AFM1 seems to be similar or slightly less than that of AFB1 (EFSA, 2004).

In the European Union, with respect to infant feeding, there are strict regulatory limits in place for complementary or weaning food. The European Commission establishes a maximum level (ML) of 25ng/Kg for AFM1 in infant formulae and follow-on formulae, including infant milk and follow-on milk, which is half of the ML for general consumers (EC, 2006). Austria and Switzerland further lowered the maximum level up to 10ng/Kg for infant food. China instead, establishes a limit of 500mg/Kg (Maleki, Abdi, Davodian, Haghani, & Bakhtiyari, 2015).

Understandably, for human breast milk, no limits are imposed, as it is acknowledged that the health benefits of exclusive breastfeeding likely far surpass the putative health risk from lactation transfer of mycotoxins, especially in highly regulated world regions (Warth *et al.*, 2016). Indeed, human breast milk is considered the best source of nutrition for babies, it contains an optimal balance of fats, carbohydrates and proteins. The benefits of milk include the development of immunity, and growth (Landrigan, Sonawane, Mattison, McCally, & Garg, 2002). But the advantages of breast feeding go beyond the properties of milk itself. A complex of nutritional, environmental, socioeconomic, psychological as well as genetic interactions occur and all combined lead to an enormous list of benefits both to the mother and to the infant (Shamir, 2016). While the limits of AFM1 in infant food are very strictly regulated and controlled by surveillance programs, breast milk is comparably rarely evaluated (Warth *et al.*, 2016). Given the scarcity of studies in Europe and the complete absence of reports in Portugal, along with the potential hazard of AFM1 to breastfed babies, the objectives of the herein reported work were to evaluate the exposure of infants to AFM1 through consumption of breast milk and the main socio-demographical and food consumption determinants of the lactating mothers.

2. Materials and methods

2.1. Sampling

The breast milk samples (5mL; convenience samples) used in this study, were collected from 30 voluntary nursing mothers, in six different municipalities of Portugal: Castro Daire (*n*=10), São Pedro do Sul (*n*=8), Tarouca (*n*=4), Vila Nova de Paiva (*n*=4), Coimbra (*n*=2), Vila Real (*n*=1) and Vouzela (*n*=1), in two different seasons - Summer (September; *n*=4) and Fall (October, November, December; *n*=26).

Immediately after collection (by pump expression) the milk was frozen (-20ºC) in sterile breast milk storage bags, protected from light until analysis in the Laboratory of Molecular Biology of Escola Universitária Vasco da Gama, in Coimbra.

All the procedures in this study were executed according with the Helsinki Declaration of 2013 and the Declaration of Taipei on Ethical Considerations regarding Health Databases and Biobanks of 2016, of the World Medical Association (WMA, n.d.). Thus the participants voluntarily signed a written informed consent, after explaining the objectives of the study and assuring the confidentiality and privacy of the data gathered.

The inclusion criteria established for this study were: the existence of breastfeeding, the nursing mothers' healthy status and the filled socio-demographic and food questionnaires. The defined exclusion criteria were the occurrence of mammary infectious or tumor disease, age under 18 years and childbirth at less than three weeks (colostrum and transition milk).

2.2. Participant basic information and food consumption

The volunteers were instructed to fill a socio-demographic questionnaire (Annex 1) to obtain information regarding the age, weight, height, number of children, period of lactation (date of birth), educational level, professional activity and local of residence). Additional data was collected on the characteristics of breastfeeding (exclusive or complemented with baby commercial formulas) and the infants' weight at birth and at the time of milk collection.

Participating mothers also filled a semi-quantitative food questionnaire, about their consumption habits in the previous week (recall period of 7 days). This document (Annex I) included the following food categories: Azores milk, milk, yogurt, coffee, rice, bread, chocolate, cereals, cookies, cakes and dry fruits.

2.3. Determination of AFM1

The determination of Aflatoxin M1 was carried out through a competitive Enzyme-linked immunosorbent assay (ELISA) using a commercial Kit (RIDASCREEN ® Aflatoxin M1 R-Biopharm AG®, Germany) and following the enclosed manufacturer's instructions.

Briefly, the milk samples were thawed and then centrifuged (Sigma 3K15 centrifuge, Reagente 5, Porto) for 10 minutes at 3500*g* and 10ºC, for degreasing. The top cream layer, containing the fat part of the milk was removed, and the defatted supernatant was used directly in the test.

After recording standard and sample positions (duplicated wells), 100 µL of antibody were added to the bottom of each well. The plate was gently shacked manually before a 15 minute incubation at room temperature. After a washing procedure, repeated three times, 100 µL of each standard and sample were added to separate duplicate wells. After a 30 minute (room temperature) incubation and a subsequent washing step, the AFM1 enzyme conjugated (100 µL) was added. Free and enzyme conjugated AFM1 competed for the antibody binding sites (competitive enzyme immunoassay) in a 15 minute room temperature incubation (at dark). The unbounded enzyme conjugate was removed by a washing step. Substrate/chromogen (100 µL) was added to the wells and a 15 minute incubation was carried out in the dark. The bound enzyme conjugate converted the chromogen into a blue product. After completing the final incubation, the stop solution was added to the wells, and the color changed from blue to yellow. The measurement of absorbance was made immediately after this procedure at 450nm.

The standard curve was drawn with the mean values of each of the six duplicated concentration levels (0; 5; 10; 20; 40; 80 ng/L). AFM1 quantification in milk samples was determined thought the formula: [absorbance standard (or sample)/ absorbance zero standard] x 100 = % absorbance. The absorptions were inversely proportional to the AFM1 concentration. The zero was made equal to 100% and the absorbance values were quoted in percentages. The values calculated for the standards were entered in a system of coordinates on semi-logarithmic graph against the AFM1 concentration (ng/L). According to the manufacturer, the cut-off value was 5 ng/L.

2.5 Estimated daily intake and Hazard Index of AFM1 for infants

The estimated daily intake (EDI) of AFM1 was calculated through a deterministic method combining the average body weight of the babies [b.w.], the babies' daily consumption of milk [milk consumption] and the average concentration of AFM1 in the positive samples (≥ 5 ng/L; [toxin]), and for the worst case scenario, as follows:

EDI (ng/kg b.w. /day) = [toxin] x [milk consumption]/ [b.w.]

The two daily breast milk consumption estimates (Ministério da Saúde, n.d.) were considered: 150mL/kg (for babies with weight up to 7kg) and 1L (for infants with weight equal or higher than 7kg). The average body weight and AFM1 concentration were calculated for both groups.

The Hazard Index (HI) was calculated through the Tolerable Daily Intake (TDI) of AFM1, as originally proposed by Kuiper-Goodman (1990) and also reported by Tsakiris *et al.* (2013). The proposed value of TDI (0.2 ng/kg b.w.) corresponded to a risk level of 1:100.000 and was calculated by dividing the TD_{50} (threshold dose per body weight) by an uncertainty factor of 5000 (safety value). The HI could thus be determined by dividing EDI by the proposed value of TDI. A HI higher than 1 indicates risk to consumers.

2.6. Statistical analysis

Statistical analysis was carried out with the Software R®. A descriptive statistics was made. Whenever two independent samples were compared regarding the AFM1 levels, the non-parametric test Wilcoxon Mann Whitney Test was applied. Correlation between variables were also studied searching for possible tendencies between AFM1 levels and one or two determining factors. Given the non-parametric nature of practically all data, the Spearman test was used.

3. Results and discussion

The exponential equation y=105.21E (-0,02x), from the standard curve obtained by two determinations of the six concentration levels was used to calculate the content of AFM1 in the analyzed samples. The correlation coefficient (r^2) was 0.9940, thus featuring a linear trend.

Despite the use of a convenience sample, the enrolled lactating mothers attempted to represent the Portuguese nursing population. The 30 mothers that participated in this study were between 21 and 39 years old (average 32.2years old), with 1 up to 5 children (average 1.9 children per mother). The time of breastfeeding was also considered, ranging from 1.16 months up to 27 months (average 7.03 months).

From all 30 samples collected in this study, 13 (43%) contained levels of AFM1 above the detection limit (5 ng/L). The concentrations within the positive samples ranged between 5.1 and 10.2 ng/L, with an average level of 7.12±1.89 ng/L. When compared with the recent studies summed up in Table 1, the average level determined in the present study was comparable with the ones previously reported in Cyprus (Kunter *et al.*, 2016) and México (Cantú-Cornelio *et al*, 2016; Maleki *et al.*, 2015). It was, however, ten times lower than the ones reported in Jordan (Omar, 2012) and Egypt (El-Tras, El-Kady, & Tayel, 2011). In fact, if maximum levels are compared with the two latter studies, the present work determined a 14- (Jordan; Omar, 2012) and 32-times (Egypt; El-Tras, El-Kady, & Tayel, 2011) lower value. None of the breast milk samples analyzed in the current study surpassed the EU established maximum limit for commercial infant milk and follow-on milk (25 ng/L), as opposed to the reports in Jordan (96.25%; Omar, 2012), Egypt (52%; El-Tras, El-Kady, & Tayel, 2011), as well as in Turkey (21.21%; Kılıç Altun *et al.*, 2016). Comparison of the incidence rates showed an AFM1 contamination of the Portuguese samples more widespread than previously reported in Brazil (Ishikawa *et al.*, 2016), Iran (Ghiasain & Maghsood, 2012; Jafari, Fallah, Kheiri, Fadaei, & Amini, 2017), and Turkey (Atasever, Yildirim, Atasever, & Tastekin, 2014).

Previous studies reported the influence of some socio-demographical determinants for the AFM1 content in breast milk samples. In Egypt, the major determinants for the AFB1 occurrence in breast milk samples were the non-working status, obesity, number of children (above one) and the early stage of lactation (Polychronaki et al., 2006). However in the present study, no statistical differences were observed regarding the local of residence, season of collection, age of the nursing mother, number or children, date of birth (stage of lactation) or educational level.

Table 1. Reported worldwide occurrence and levels of AFM1 in human breast milk samples.

Country (year)	Incidence rate (%)	Mean level ± SD (ng/L)	Range (ng/L)	Analytical (LOD, ng/L)	Reference
Portugal (2016)	13/30 (43.3%)	7.12±1,89	5.1-10.2	ELISA (5)	Present study
Cyprus (2017)	40/50 (80%)	7.84±1.72	5.36-28.44	ELISA (5)	Kunter *et al.* (2016)
Iran (2017)	39/250 (15.6%)	4.54±0.47	11.1-39.3	ELISA (2.3)	Jafari, Fallah, Kheiri, Fadaei, & Amini (2017)
Brazil (2016)	5/94 (5.3%)	18±5	13-25	HPLC-FD (0.021)	Ishikawa *et al.* (2016)
Turkey (2016)	66/74 (89.2%)	19.0±13.0	9.6-80	ELISA (5)	Kılıç Altun *et al.* (2016)
Iran (2015)	85/85 (100%)	5.91±2.03	2.0-10	ELISA (n.a.)	Maleki *et al.* (2015)
Mexico (2015)	100/112 (89%)	Winter:12.78 Spring:12.09 Summer:7.91	3.01-34.24	ELISA (0.92)	Cantú-Cornelio *et al.* (2015)
Turkey (2014)	18/73 (24.6%)	3.01±1.42	1.3-6	ELISA (10)	Atasever, Yildirim, Atasever, & Tastekin (2014)
Jordan (2012)	80/80 (100%)	67.78±4.6	9.71-137.18	ELISA (5)	Omar (2012)
Egypt (2011)	87/125 (69.6%)	74.413±7.070	7.3-328.6	ELISA (5)	El-Tras, El-Kady, & Tayel (2011)
Iran (2012)	8/132 (6.06%)	9.45±1.50	7.1-10.8	ELISA (5)	Ghiasain & Maghsood (2012)

(LOD, Limit of detection; n.a., not available; SD, Standard deviation)

With respect to the educational level, 46.15% of the positive women had the twelve grade or an inferior educational level, 46.15% had a college graduation and 7.69% had a master's degree or a Ph.D. The local of residence of the mothers with AFM1-positive milk breast samples were Castro Daire (9; 69.2%), Tarouca (3; 23.1%) and Coimbra (1; 7.69 %), which means that (92.3%) lived in a rural areas (PRODER, n.d.). Already previously, Jafari, Fallah, Kheiri, Fadaei, & Amini (2017) reported higher AFM1 occurrence and levels in rural areas. With respect to the season of collection, only three (23.1%) of the positive samples were collected in the summer whereas the 10 (76.9%) remaining positive samples were collected in the fall.

As the content of mycotoxins in milk is related with the maternal dietary habits, food questionnaire was associated with the analytical determination of AFM1 in breast milk samples. According to the semi-quantitative consumption data collected in the food questionnaire, AFM1 positive milk samples featured a moderate correlation with a higher consumption of dry fruits (r=0.48) and milk (r=0.4).

Roughly half (46.15%) of the mothers with AFM1-positive milk feature a weekly consumption of dry fruits (3-4 units) ranging from one to three times, on the recall period. Considering the 475 notifications related with mycotoxins in food as reported by the Rapid Alert System for Food and Feed (RASFF) in the 2015 Annual report, 88.6% (421) were related to aflatoxins, which was also the underlying reason for the increasing number of notifications. Aflatoxins were detected in dry fruits such as peanuts, hazelnuts, almonds, also in spices (nutmeg), fruits (dry figs and chilies), originating from Turkey, Iran, Egypt, India, Indonesia, Gambia, Australia and the United States (RASFF, 2016). When considering the sampling period only (September 14th until December 10th 2016), the RASFF Portal reports 124 notifications related with mycotoxins in food, of which 61 involved nut, nut products and seeds. It is noteworthy that all these 61 samples were contaminated with AFB1 content ("RASFF Portal," n.d.). National surveys also reported high AFB1 contamination of dry fruits. In neighboring Spain, 10% of 50 samples of pistachio nuts collected from commercial stores of Catalonia, were contaminated with AFB1 and all of these contaminated samples exceeded the maximum legal limit (2.0 ng/g; Fernane, Cano-Sancho, Sanchis, Marín, & Ramos, 2010). Likewise, in Zambia, the levels of AFB1 present in peanuts, ranged up to 11100 ng/g. In all the groups of samples collected throughout a sampling period of 2 years, the average concentration of AFB1 surpassed the EU maximum level (2.0 ng/g), ranging from 2.93 ng/g to 499 ng/g (Njoroge *et al.*, 2017).

The second food item that featured a moderate correlation with AFM1 occurrence in breast milk was milk (0.4). More than three quarters (76.92%) of lactating mothers with AFM1 positive-milk samples consumed 1-3 cups of milk daily. Of these, only one women (7.69%) consumed Azores milk. The differentiated origin of the milk was studied on the basis of a previous study, conducted in Portugal (Duarte *et al.*, 2013), that showed that Azorean bovine milk commercial brands were the only ones presenting AFM1 concentration higher than the EU maximum levels.

In many studies it was reported a linear relationship between the amount of AFB1 in animal feed and the levels of AFM1 in the corresponding milk. Roughly 1 to 6% of the AFB1 present in the animals' feedstuff appears in milk between 12 to 24 hours after the AFB1 ingestion (Prado,

Oliveira, Lima, & Moreira, 2008). In a recent study, conducted in Tanzania, AFB1 on feed (sunflower seedcakes or sunflower based seedcake) used in dairy farms and AFM1 in milk were determined. The authors reported that 83.8% of the milk samples were contaminated with AFM1, all above the EU maximum level (50 ng/L) whereas 65% of the feed was contaminated with AFB1 and 61.53% exceeded the EU maximum level for feed (5 ng/g) (Mohammed, Munissi, & Nyandoro, 2016). The thermostablility of aflatoxins was demonstrated in a Brazilian AFM1 survey (Santos, França, Katto, & Santana, 2015) of ultra-high temperature (UHT) milk and powder milk. The totality of the analyzed samples were contaminated, up to 810 ng/L. In a survey of a dairy product intended to infant consumption (flavored milk) it was observed that 10 out of 30 samples exceeded the EU maximum level.

Although without statistical significance it is relevant the high consumption of other food items by the respondent nursing mothers with AFM1-positive milk samples: once daily 53.85% consumed yogurts, 61.64% coffee, 23.08% chocolate, and 23.08% cookies. Cereals and cereal-derived foods were also greatly consumed. Once to twice a day rice was consumed by 53.84% of the mothers with AFM1-positive milk, whereas bread by 84.61% and cereals by 30.77%. In previous studies, the consumption of bread and cereals was a determinant for AFM1 occurrence in breast milk among Iranian lactating mothers (Jafari, Fallah, Kheiri, Fadaei, & Amini, 2017).

It is important to notice however that, as recently reviewed by Warth *et al.* (2016) besides the major metabolite, AFM1, the parent mycotoxin AFB1 as well as other aflatoxins and their metabolites may also be present in milk. Nevertheless, the determination of AFM1 in the breast milk samples analyzed in the present study, allowed the calculation of the AFM1 EDI. According to the two consumption estimates, the values of EDI were higher for younger infants (<7kg b.w.; 1.06 ng/kg b.w./day) as compared with older ones (≥7kg; 0.8 ng/kg b.w.). As no statistical significance was found regarding the stage of lactation, this difference is probably justified by the higher consumption versus weight (Tsakiris *et al.*, 2013). Higher EDIs were previously reported in Mexico (2.35 ng/Kg b. w. / day; Cantú-Cornelio *et al.*, 2015) and Western Iran (4.56-6.88 ng/kg b.w./ day; Ghiasain, 2012).

Regarding the HI, both groups surpassed the 1.0 ng/Kg b.w. / day, considered the level above which there is a risk for the consumers. Again, the HI was higher for younger infants (<7kg b.w./ day; 5.3) as compared with older ones (≥7kg; 4.0). The calculated HI for both groups were much higher than the one previously reported in Brazil (0.35; Ishikawa *et al.*, 2016). However, if only the AFM1 highest level (10.2 ng/L) found in the present study was used, the corresponding EDI would be even higher (1.53 ng/Kg b.w./ day), then we calculated the HI for this EDI and the value was 7.65 a value by far superior to 1, which is the one that indicates risk for consumers. Although the AFM1 concentration present in this sample is below 25 ng/L (EU maximum level for infant formula), the HI is very high. Also the concentration found in this sample exceed the maximum level for AFM1 allowed for baby food in Austria and Switzerland (10 ng/L) (Maleki, Abdi, Davodian, Haghani, & Bakhtiyari, 2015).

This study suffered from some limitations associated with the nature of the analyzed samples that compromised the number and integrity of samples collected. Despite the use of an electrical

pump, during breast milk collection it was difficult to collect the total amount of milk of one breastfed or the same fraction in all of the participating mothers to assure an uniform sampling. In addition it was not possible to carry out the collection in the same period of the day or to make pooled samples collected in different moments of the day. Finally, it was only possible to gather most of the samples during the fall due to greater availability of participating mothers in this period.

4. Conclusions

The obtained results should contribute to an increase awareness regarding the presence of AFM1 in human breast milk and the ensuing exposure of the nursing babies. Of the thirty analyzed samples, 13 (43.3%) were AFM1-positive, with an average value of 7.12±1.89 ng/L. None of the samples exceeded the EU maximum limit for AFM1 for commercial babies' and infants' formula (25 ng/L), as the maximum value detected was 10.2 ng/L. The consumption of dry fruits (r=0.48) and milk (r=0.4) by the nursing mothers showed a moderate correlation with higher levels of AFM1 in the breast milk produced. No other determinants, whether socio-demographic or dietary showed a significant statistical influence.

The higher consumption versus weight could be the probable reason for the higher exposure of younger babies (<7 kg) in comparison with the older ones (≥7 kg), as assessed by the calculated EDIs (1.06 vs. 0.8 ng/kg b.w. / day) and HI (5.3 vs. 4.0).

The findings of the present study indicate the need to reinforce surveillance programs and studies, as well as control measures to decrease AFM1 presence in human breast milk and the resulting exposure of newborns.

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AFM1 -_____

DECLARAÇÃO

Eu, ……, declaro que dou o meu consentimento para a recolha de leite materno e posterior determinação do teor de **Aflatoxina M1**.

Coimbra, …… de …… de 2016

Assinatura: …….

Este questionário tem como objectivo avaliar uma potencial correlação entre o consumo de determinados alimentos, normalmente associados à presença de **Aflatoxina B1**, e o teor de **Aflatoxina M1** no leite materno

Características Individuais
Data:
Nome:
Idade:
N° filhos:
Formação escolar:
Profissão:
Data parto:
Peso do bebé à nascença:

Características da Amamentação
Mista (Peito/outro)
Só Peito
Tempo de amamentação (m)
Idade do bebé (m)
Peso da criança (kg)

NOTAS PRÉVIAS

Procure responder às questões de uma forma sincera, indicando a frequência de consumo dos alimentos referidos na tabela.

O questionário pretende identificar o consumo de alimentos associados à presença de Aflatoxina B1 previamente à recolha do leite. Assim para cada alimento, deve assinalar (preenchendo com um X a respectiva opção) quantas vezes por dia ou por semana comeu em média, cada um dos alimentos referidos nesta lista, ao longo do último mês.

No **último mês** qual foi a **frequência** de consumo (assinale com X):

Alimento/Quantidade	Frequência diária			Frequência semanal				Mês
	1*dia	2*dia	>3*dia	1 a 2*	3 a 4*	5 a 6*	Nunca	1 a 3*
Leite açoriano (1copo)								
Leite (1copo)								
Iogurte (1emb)								
Café (1 chávena)								
Arroz (1 un)								
Pão (20gr)								
Chocolate (1 chávena)								
Cereais (100gr)								
Bolachas (2 a 3)								
Bolos (1 fatia)								
Frutos secos (3 a 4 unid)								

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REGISTO DE CASUÍSTICA

	Pesquisa de resíduos de antibióticos em água por espectrofotometria de massa	Pesquisa de Ocratoxina A em cerveja por HPLC-FD	Determinação de Aflatoxina M1 em leite materno Humano por ELISA	**Total**
Validação do método de pesquisa	18	4	6	28
Extração da amostra	90	1	30	121
Aplicação a amostras reais	0	0	30	30
Total	108	5	66	**179**