



HAL
open science

Determinants of AFM1 in breast milk in a selected group of Egyptian mothers

Nektaria Polychronaki, Paul C Turner, Hannu Mykkanen, Yunyun Gong, Hassan Amra, Mosaad Attia Abdel-Wahhab, Hani El-Nezami

► **To cite this version:**

Nektaria Polychronaki, Paul C Turner, Hannu Mykkanen, Yunyun Gong, Hassan Amra, et al.. Determinants of AFM1 in breast milk in a selected group of Egyptian mothers. *Food Additives and Contaminants*, 2006, 23 (07), pp.700-708. 10.1080/02652030600627222 . hal-00577585

HAL Id: hal-00577585

<https://hal.science/hal-00577585>

Submitted on 17 Mar 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Determinants of AFM1 in breast milk in a selected group of Egyptian mothers

Journal:	<i>Food Additives and Contaminants</i>
Manuscript ID:	TFAC-2005-352.R1
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	02-Feb-2006
Complete List of Authors:	Polychronaki, Nektaria; University of Kuopio, Department of Clinical Nutrition; University of Leeds, Unit of Molecular Epidemiology, LIGHT laboratories Turner, Paul; University of Leeds, Molecular Epidemiology Unit Mykkanen, Hannu; University of Kuopio, Department of Clinical Nutrition Gong, Yunyun; University of Leeds, Molecular Epidemiology Unit Amra, Hassan; National Research Centre, Department of Food Toxicology and Contaminants Abdel-Wahhab, Mosaad; National Research Centre, Department of Food Toxicology and Contaminants El-Nezami, Hani; University of Kuopio, Department of Clinical Nutrition
Methods/Techniques:	Chromatography - HPLC, Statistical analysis, Extraction
Additives/Contaminants:	Aflatoxins
Food Types:	Human milk

SCHOLARONE™
Manuscripts

1
2
3 **1 Determinants of aflatoxin M₁ in breast milk in a selected group of**
4
5
6 **2 Egyptian mothers**
7

8
9
10
11
12
13 Polychronaki Nektaria^{1,2}, Turner C. Paul³, Mykkänen Hannu¹, Gong Yunyun³, Amra Hassan⁴,
14
15 Abdel-Wahhab Mosaad⁴, El-Nezami Hani^{1,2}
16

17
18
19
20 ¹Department of Clinical Nutrition, University of Kuopio, Finland

21
22 ²Food and Health Research Centre (ETTK), University of Kuopio, Finland

23
24 ³Molecular Epidemiology Unit, Centre for Epidemiology and Biostatistics, Leeds Institute of
25
26 Genetics, Health and Therapeutics, University of Leeds, UK, LS2 9JT

27
28 ⁴Department of Food Toxicology and Contaminants, National Research Centre, Dokki, Egypt
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 **Abstract**
4

5 In Egypt there is a paucity of biomarker data on aflatoxin exposure. In this study we assessed
6 the level and frequency of breast milk AFM₁ as a biomarker of maternal exposure. Breast milk
7 samples were collected from a selected group of 388 Egyptian lactating mothers of children
8 attending the New El-Qalyub Hospital, Qalyubiyah governorate, Egypt, during May-September
9 2003. Following aflatoxin extraction, AFM₁ levels were assessed by HPLC with fluorescence
10 detection. Approximately 36 % of mothers tested positive for AFM₁ (median 13.5 pg/ml, IQR
11 10.27 to 21.43). Non-working status (p= 0.018, OR 2.87), obesity (p= 0.004, OR 3.01), high
12 corn oil consumption (p= 0.002, OR 2.21), number of children (>1) (p= 0.025, OR 1.99), and
13 early lactation stage (<1 month) (p= 0.028 OR 3.57), contributed to the occurrence of aflatoxin
14 in breast milk. AFM₁ contamination of breast milk was frequent albeit at moderate levels.
15 Growth and development of the infant is rapid and thus it is possible that aflatoxin exposure
16 through breast milk has a significant health effect.

17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33 **Keywords**
34

35 Egypt, breast milk, aflatoxin M₁, exposure, infants
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1 Introduction

Breast milk is considered to be the ideal food for the infant (Pronczuk et al. 2004; WHO 2003), and the importance of breast-feeding for normal growth and development has become increasingly recognized. However, in addition to its nutritionally and immunologically beneficial components, human milk may contain trace amounts of a wide range of contaminants following maternal dietary exposures (Jensen and Slorach 1991). In some less developed parts of the world a family of highly toxic and carcinogenic naturally occurring compounds, aflatoxins (AFs), have been observed in breast milk (Abdulrazzaq et al. 2003; El-Nezami et al. 1995; IARC 1993; Navas et al. 2005; Zarba et al. 1992). Aflatoxin B₁ (AFB₁), a potent liver carcinogen, is the most commonly occurring and carcinogenic of the aflatoxins, and is classified as a class 1A human carcinogen (IARC 1993). Maternal consumption of aflatoxin contaminated food whilst breast feeding can result in the accumulation of aflatoxins and their metabolites in breast milk. Approximately 95% of AFB₁ metabolites excreted in milk are in the form of AFM₁, though AFM₂, AFG₁ and AFB₁ are also reported (Abdulrazzaq et al. 2003; El-Nezami et al. 1995; Turconi et al. 2004; Zarba et al. 1992). IARC (1993) has classified AFM₁ as a proven animal carcinogen, but as yet there is insufficient data to be classified a human carcinogen, and thus is regarded as a possible human carcinogen (group 2B). In addition to being potent carcinogens, aflatoxins may contribute to early childhood growth faltering (Turner et al. 2003), and particularly strong associations have been reported around the weaning stage in Beninese infants (Gong et al. 2002, 2004). In addition a reduced level of salivary sIgA has been associated with aflatoxin exposure in Gambian children (Turner et al. 2003). More recently the immune status of Ghanaian adults has been reportedly affected by aflatoxin exposure (Jiang et al. 2005).

In regions that are at high risk for aflatoxin contamination it is important to understand exposure patterns using aflatoxin biomarkers during pregnancy and early infancy to assess the health consequence to the child. Exposure during pregnancy leads to in utero exposures (Wild et al. 1991), and then during early breast feeding AFM₁ is likely to be the major aflatoxin ingested by

1 the infant. In this respect it is important to note that AFM₁ although less carcinogenic than
2 AFB₁, it is reportedly as cytotoxic (Neal et al. 1998), and is being consumed by infants that are
3 rapidly developing and thus highly susceptible to toxic insult. This study aims to assess
4 aflatoxin exposure in a selected group of nursing Egyptian mothers by measuring the level of
5 AFM₁ in breast milk. Aflatoxin exposure is known to occur in Egypt (El-Nezami et al. 2000;
6 Hifnawy et al. 2004; Selim et al. 1996), though there is limited biomarker data from this region.
7 It is possible that aflatoxin could contribute to the frequent growth faltering in this region, and
8 or play a role in the development of liver cancer. However, it is recognised that hepatitis viruses
9 are the major contributors to liver cancer in Egypt (Angelico et al. 1997; Saeed et al. 1991). The
10 presence of aflatoxins in breast milk will indicate the frequency and level of both maternal and
11 to some extent early infant exposure. These data will serve as a baseline of exposure patterns
12 and levels, from which a longitudinal assessment of aflatoxin exposure and infant health can be
13 conducted.

14 **Materials and methods**

15 ***Study population***

16 Breast milk samples were collected from 388 Egyptian mothers that agreed to participate in the
17 study (participation rate 95%) during May-September 2003. The mothers were recruited by the
18 nurses of the New El-Qalyub Hospital, Qalyubiyah governorate, Egypt. Qalyubiyah governorate
19 is located in the eastern Nile region near the head of the Delta. The area is barred from the south
20 by Cairo and Giza governorates. The governorate is famous for its agricultural production of
21 maize, cotton, wheat, and several fruit and vegetables. In the Qalyubiyah governorate
22 approximately two thirds of the population lives in rural areas, and the humid and hot
23 environment promotes mould growth and aflatoxin production (El-Shewy 1987).

24 ***Collection of dietary, socioeconomic, demographic and clinical data***

25 Intake of dietary sources of aflatoxins was assessed using a structured food frequency
26 questionnaire (FFQ) to record mother's usual intake over the last month before sample
27 collection. The FFQ was administered by interview conducted always by the same health centre
28

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1 nurse and thirty minutes were needed for completion. The FFQ focused on foods more likely to
2 contribute to dietary intake of aflatoxins and consisted of two components: a food list and a
3 frequency response section. The data on food use were expressed as frequencies ranging from
4 several times a day to never. The groups of food included grain products (wheat bread, corn
5 bread, pasta, rice, corn), milk and milk products (Romano cheese, feta cheese, cream), legumes
6 (beans, lentils), meat (chicken, beef, lamb), fish, corn oil, cottonseed oil, dried fruits, and
7 peanuts. These food groups include those likely to be aflatoxin contamination in Egypt (El-
8 Nezami et al. 2000; Hifnawy et al. 2004; Selim et al. 1996). Complete dietary data was received
9 from all study subjects.

10
11 Socioeconomic data (working status, income, level of education), demographical data (place of
12 residence, age), and clinical data (health status, miscarriages, medication) were recorded by
13 interview while the anthropometrical data (mother's weight and height, baby's weight and
14 height current and at delivery) were taken from the reports of the New El-Qalyub Hospital
15 where the mothers were followed since the delivery. Child body weight was measured by
16 nursing staff using a Tanita 1584, Precision weighing Balance, Bradford, MA (max weight 20
17 kg, accuracy 50 g). The socioeconomic status of the households was dichotomized in categories
18 of either higher or lower (HSES/ LSES). HSES included families with one or both parents
19 working and at least one work demanding higher education (teachers, nurses, government
20 employees, police officers etc.), and LSES one or none of parents working and the work not
21 demanding higher education (butcher, carpenter, tailor, salesman, conductor, driver, farmer
22 etc.), respectively. For assessing children's growth status, the measured anthropometric values
23 (baby's weight and height) were expressed as standard deviations (Z-scores) below or above the
24 median value of the international reference population recommended by the National Centre of
25 Health Statistics/World Health Organization (WHO) (WHO 1995). Weight for height Z-score
26 (WHZ), weight for age Z-score (WAZ), and height for age Z-score (HAZ) below -2 reflects
27 malnutrition, while a value below -3 severe malnutrition.

28

1 *Breast milk aflatoxin extraction*

2 Breast milk was collected into a sterile plastic container by self-expression prior nursing the
3 baby. The sample was kept at 4 °C and frozen within one day at -20 °C prior to extraction.
4 Extraction of aflatoxins from milk samples was modified from the method of El-Nezami et al
5 (1995). Briefly, 10 ml breast milk samples were warmed to 37°C and were shaken to distribute
6 fat. For defatting, the samples were centrifuged (3000g, 15 min, 5°C) and filtered via glass
7 wool. To facilitate the passage through a C18 cartridge (Strata C18-E, 50 µm, 70A,
8 Phenomenex, USA), the samples were diluted 1:1 with milli-Q water. The cartridge was pre-
9 activated with 10 ml acetonitrile and then 10 ml of water, prior to passage of diluted breast milk
10 at a flow rate of 3.5 ml/ min. The loaded cartridge was then washed with 10 ml water, 10 ml
11 basic acetonitrile/ water (1% ammonia, 10% acetonitrile) and 10 ml acidic acetonitrile/water
12 (1% acetic acid, 10% acetonitrile). AFM₁ was eluted with 5 ml acidic acetonitrile (1% acetic
13 acid, 40% acetonitrile). AFM₁ was extracted twice from the eluent with 2 ml dichloromethane.
14 Following centrifugation (3000g, 15 min) to separate the layers, the two dichloromethane
15 fractions were pooled and dried under nitrogen gas. The residue was dissolved in 0.7 ml of
16 methanol.

17 *HPLC analysis*

18 Reverse phase HPLC (model LC-10ADvp solvent delivery system; model SIL-10Advp auto
19 injector, Shimadzu, Japan; ODS-5 C18 Brownlee reverse phase column (220 x 4.6 mm, particle
20 size 5 µm) with a C18 guard column (Perkin Elmer, Norwalk, Conn.) was used to determine the
21 level of AFM₁ in breast milk extracts. An isocratic system with water: methanol: acetonitrile
22 66:17:17, flow rate 1 ml/ min, oven temperature 40 °C and injection volume of 30 µl were used.
23 Detection was by excitation at 360 nm and emission at 440 nm (Fluorescence-10A XL,
24 Shimadzu detector) (Figure 1). The concentrations of AFM₁ in milk were estimated from a
25 standard curve 0.04-10 ng/ ml methanol, prepared from AFM₁ in chloroform (9.93 µg/ ml)
26 reference material RM 423 (LGC Promochem AB, Borås, Sweden). An AFM₁ standard was
27 injected every 10 injections as a quality control, AFM₁ was stored at -20 °C in a silylated vial
28 wrapped in aluminium foil. Since AFM₁ is a possible carcinogen, care was exercised to avoid

1 personal exposure and proper decontamination procedures with 10 % sodium hypochlorite were
2 used. HPLC grade methanol, acetonitrile and dichloromethane were purchased from
3 Sigma/Aldrich Chemical Co. (Kemia Oy, Helsinki, Finland).

4
5 [Insert Figure 1 about here]

6 ***Validation and repeatability of the measurements***

7 Validation and repeatability of the measurement of breast milk aflatoxin was carried out using
8 breast milk samples of Finnish mothers kindly provided by the Breast Milk Centre of Kuopio
9 University Hospital (Kuopio, Finland). These breast milk samples were extracted and analyzed
10 by the method described above. AFM₁ was not detected in these samples. For the recovery
11 studies seven aflatoxin non detect breast milk samples (triplicates) were spiked with 12.5, 25,
12 50, 125, 250, 375, 500 pg AFM₁/ ml milk. One water sample (triplicates) was spiked with 250
13 pg AFM₁/ ml water. AFM₁ was extracted from the samples as previously described. The
14 average percentages of aflatoxin recovered were 88 ±28 and 94 ±8 from breast milk and milli-Q
15 water respectively. Correlation coefficient between AFM₁ added and AFM₁ recovered from
16 spiked breast milk was 0.993. The identity of AFM₁ was confirmed through spiking of positive
17 breast milk samples with AFM₁, and by derivatization of AFM₁ to form AFM_{2a} according to the
18 method of AOAC international (AOAC 1995).

19
20 To assess the repeatability of the method, breast milk samples were spiked with 250 pg AFM₁/
21 ml milk (4 replicates). The analysis gave a mean value of 205 ±15 pg/ ml milk and 8%
22 coefficient of variation.

23
24 The limit of detection was 0.06 ng AFM₁ /ml methanol or 4.2 pg AFM₁/ ml of breast milk based
25 on a 10-ml breast milk sample and reconstitution of extracts with 0.7 ml of methanol, and was
26 defined as the lowest concentration of AFM₁ that could be reliably detected (>3:1 signal to
27 noise ratio) and quantified (% CV <20) for triplicate extractions.

28

1
2
3 1 ***Ethical considerations***
4

5 2 This study was approved by the Ethics Committee of the University of Kuopio and Kuopio
6
7 3 University Hospital in Finland and by the National Research Centre Ethical Committee in
8
9 4 Egypt. All subjects were made aware of the content of the study and if they agreed to
10
11 5 participate, a written informed consent was obtained.
12

13
14 6 ***Statistical analysis***
15

16 7 SPSS 11.0 for windows was used in the statistical analysis of the data. AFM₁ values did not
17
18 8 follow normal distribution even after logarithmic transformation (p= 0.005, Kolmogorov-
19
20 9 Smirnov normality test). Therefore, to identify possible associations between AFM₁ levels and
21
22 10 related factors, the non-parametric Mann-Whitney U (or Kruskal-Wallis) test or Spearman
23
24 11 correlation were used for continues variables. The mothers were dichotomized depending on the
25
26 12 presence/absence of AFM₁ in their breast milk, and chi-square test was used to associate the
27
28 13 presence of AFM₁ in breast milk with categorical variables. Logistic regression models were
29
30 14 used to analyse the association of each potential factor and AFM₁ presence/ absence. For the
31
32 15 FFQ data analysis, the food-consumption data were either regrouped into the two categories of
33
34 16 “daily consumption” and “weekly or monthly consumption” (the category “never” was included
35
36 17 to the “weekly or monthly consumption” category); or treated as continuous variable, i.e. the
37
38 18 reported frequencies of consumption were expressed as a number representing the frequency of
39
40 19 consumption. eg. 1/ month, 1/ fortnight, 1/ week or 3-4 times/ week equal 1/ 30 or 0.03, 1/ 15 or
41
42 20 0.06, 1/ 7 or 0.21, 3.5/ 7 or 0.5 respectively (Willet 1990). Missing data points in the results
43
44 21 presented reflect individuals for whom in one or more variable was missing for that analysis
45
46 22 being reported.
47
48
49
50
51
52

53 24 **Results**

54
55 25 ***Descriptive characteristics and food consumption data***
56

57 26 Descriptive data of 388 mothers and their infants are presented in Tables 1 and 2. The median
58
59 27 age of the mothers was 25 years (range 15- 47), most mothers (71.6%) already had one or more
60
28 children, and 14% had previously had a miscarriage. Most households (76%) belonged to LSES

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1 category and 89% of the mothers were not employed and were either at home or involved in
2 farming. Corn bread, wheat bread, corn oil, cotton seed oil and beans were used in a daily basis
3 by most of the mothers, but mothers belonging to the LSES category more frequently consumed
4 beans ($p= 0.009$), corn oil ($p= 0.001$), and cotton seed oil ($p= 0.001$) than mothers belonging to
5 the HSES category. Fifteen percent of the mothers were obese ($BMI>30$), and obese mothers
6 were consumers of corn oil more frequently than the non obese mothers (84 vs 67%, $p= 0.020$).
7 A few of mothers (6.2%) reported a health problem associated with liver or intestine function,
8 while two percent of the mothers reported intake of medicines such as blood pressure reducing
9 tablets and anti-depressant tablets, or dietary supplements such as iron and calcium tablets.

10
11 [Insert Tables 1 and 2 about here]

12
13 There were 227 boys and 159 girls in the study (mean age 5.9 months: range 0.2- 33 months).
14 There were no reported illnesses within any of the infants. Anthropometric measures revealed
15 the prevalence rate for stunting ($HAZ\leq-2$) was 35.7%, for being underweight ($WAZ\leq-2$) 14.4%,
16 and for wasting ($WHZ\leq-2$) 0.6%. A total of 78 (23.4%) children had a z score of ≤ -3 in at least
17 one parameter (extreme malnutrition). Median z-scores (IQR) for the infants were below zero
18 for stunting and being underweight, but not for wasting (-1.49 (-2.63- 0.02), -0.45 (-1.47- 0.43)
19 and 0.82 (0.17- 1.66) respectively). When divided into quartiles by age a significant inverse
20 trend was observed with age for both stunting ($p= 0.001$) and being underweight ($p= 0.004$)
21 (Table 3). For both stunting and being underweight infants <2 months had the highest median Z-
22 scores (-0.73 (-1.98- 0.14) and -0.29 (-1.16- 0.78) respectively). HAZ scores were lower in all
23 other age groups with lowest values in the 5- 8 month age group (-2.25 (-3.00- -0.96), whereas
24 WAZ scores were lowest in the oldest (>8 months) age group (-1.06 (-1.93- 0.13). WHZ was
25 positively associated with age ($p< 0.001$), however the median Z-scores remained within normal
26 growth limits (-2 to 2) for all the age categories.

27
28 [Insert Table 3 about here]

Breast milk AFM₁ levels and determining factors

AFM₁ was detected in the breast milk of 138 from the 388 mothers (35.5%). The median level of positives was 13.5 pg/ ml (IQR 10.3 to 21.4). AFM₁ in breast milk were detected significantly more frequently (p= 0.016) in mothers consuming corn oil on a daily basis (39.7%) compared to less frequent consumers (27.2%) (Table 4). The frequency of having detectable AFM₁ in breast milk varied greatly by duration of lactation, though there was no clear trend with time. AFM₁ was observed most frequently in breast milk in the first month of lactation (14/24, 58%). The frequency of detection in the first month was significantly greater (p= 0.017) than that in subsequent months combined (123/ 360, 34%). Mothers who did not work were found to have detectable AFM₁ in breast milk more frequently (129/ 339, 38%) than working mothers (7/43, 16%). AFM₁ was detected more frequently (25/ 51, 49%) in obese women (BMI >30) compared to those who were either overweight (BMI>25) (58/ 171, 34%) and those whose BMI was <25 (34/ 114, 30%) (p= 0.031). In multivariable models, however BMI and corn oil consumption were collinear to each other which restricted from being present in the same model. Therefore, separate models including either of these two factors were generated. When BMI was taken into consideration, mum's age, non working status, having more than one child, early lactation stage (<1 month), and obesity appeared to be strong predictors of the AFM₁ presence; non working mothers were 2.87 times more probable to be AFM₁ positive than the working ones (p= 0.018), having more than one child almost doubled the risk of being AFM₁ positive (p= 0.025) and early lactation stage (<1 month) seemed to be a strong predictor of the presence of AFM₁ with an OR of 3.57 (p= 0.028). BMI had a significant overall effect to the AFM₁ presence (p= 0.011). There was no difference in AFM₁ frequency for overweight mothers (BMI 25-30) compared to those with BMI ≤25 (p= 0.57), though obese mothers (BMI ≥30) had three times greater risk of being positive to AFM₁ than those with BMI ≤25 (p= 0.004). In the multivariate corn oil consumption model (excluding BMI), mothers with daily corn oil consumption had 2.21 times greater risk of being AFM₁ positive than the non frequent

1 consumers ($p= 0.002$). Replacing BMI with corn oil in the model didn't alter significantly the
2 strengths of the associations between the other variables and AFM₁ presence.

3 Consumption of other grain products, milk and milk products, meat, fish, legumes, cotton seed
4 oil, dried fruits and peanuts did not show any significant associations with AFM₁ in breast milk
5 ($p>0.05$).

6
7 [Insert Table 4 about here]

8 9 **Discussion**

10 Children are a highly susceptible population group for exposure to environmental toxicants for a
11 variety of reasons, including lower detoxification capacity, rapid growth, higher intakes of air,
12 food and water per kg body weight (Weaver et al. 1998) and therefore, early childhood exposure
13 to the carcinogenic aflatoxins may be critical determinants of later health effects. Exposures in
14 utero and through early infancy may additionally be important. AFM₁ in breast milk is a marker
15 of dietary exposure of the mother to AFB₁ and consequently aflatoxin exposure to the breast-
16 feeding infant (Zarba et al. 1992). In this study 35.5% of mothers were positive for breast milk
17 AFM₁. The frequency is higher than that previously reported for this region (El-Shewy 1987),
18 though this may reflect advances in analytical sensitivity. In our study the median level was
19 13.5 pg/ ml, range 5- 5000pg/ ml compared to that of El-Shewy et al (1987) who reported 22/
20 200 (11%) of samples were positive with a mean of those positive of 160 pg/ ml, range 120-200
21 pg/ ml. The AFM₁ levels in the breast milk of the Egyptian mothers are lower than the levels
22 reported elsewhere. AFM₁ was observed in breast milk from 129/ 140 (92%) of samples in the
23 United Arab Emirates with median level for positives of 560 pg/ ml (range 5- 3400 pg/ ml)
24 (Abdulrazzaq et al. 2003). El Nezami et al (El-Nezami et al. 1995) reported the median breast
25 milk AFM₁ levels in 11/ 73 (15%) to be 71 pg/ ml (range 28- 1031pg/ ml) in Victoria, Australia
26 and 5/ 11 (45%) to be 664 pg/ ml (range 39-1736 pg/ml) in Thailand. In the Gambia, West
27 Africa all five breast milk samples analysed were positive for AFM₁ with total milk values of
28 2100- 9200 pg reported (Zarba et al. 1992).

1
2
3 1 Our data suggest that maternal aflatoxin exposure, and consequently infant exposure, is
4
5 2 occurring at moderate levels compared to some of the higher risk regions of Africa and south
6
7 3 east Asia. It is difficult to determine the average daily aflatoxin intake of the infant based on
8
9 4 breast milk exposure. Firstly, the maternal intake of aflatoxins will vary from day to day, and
10
11 5 thus breast milk levels will fluctuate. Aflatoxin or its metabolites may also be stored in fat
12
13 6 reserves though the levels and contribution, if any, to breast milk levels at a given stage of
14
15 7 lactation are not known. Secondly, the fat content and total milk volume will vary within a feed
16
17 8 and between feeds, and this will likely impact on the levels of aflatoxins observed. Thirdly, the
18
19 9 average volume an infant will consume in a day will vary. Despite these difficulties we can
20
21 10 make some estimates of aflatoxin intake in the 138 mothers with detectable AFM₁ in breast
22
23 11 milk. Assuming average daily milk volume 500 ml, the average intake of AFM₁ by the infant
24
25 12 can be estimated to be 6.75 ng/ day. Assuming AFM₁ in human milk represents 0.09% to 0.43%
26
27 13 of dietary intake (Zarba et al. 1992), maternal intake is estimated at 1570 to 7500 ng/ day. The
28
29 14 calculated average daily intake by all women in two high risk regions for aflatoxin exposure
30
31 15 were 8 µg AFB₁ in Zhuqing, China (Wang et al. 2001) and 15.7 µg total aflatoxins in The
32
33 16 Gambia, West Africa (Groopman et al. 1992). Given that about 65% of mothers in our study
34
35 17 were not included in this estimate, the overall levels of these Egyptian mothers is modest by
36
37 18 comparison. Wean foods are introduced from about 4- 6 months which are likely to also be
38
39 19 contaminated if parental food is contaminated, thus the overall contribution to aflatoxin
40
41 20 exposure from breast milk will decline with time. However, breast feeding in Egypt, like other
42
43 21 African countries, often continues until infants are two years old, and thus over this extended
44
45 22 period the contribution made by breast milk may be important.
46
47
48
49
50
51
52

53 24 In Egypt the sources of aflatoxin exposure are less well defined than those of West Africa
54
55 25 (Turner et al., 2005). Non-working status, high BMI and/or corn oil consumption, number of
56
57 26 children (>1), and early lactation stage contributed to the occurrence of aflatoxin in breast milk
58
59 27 reported in this study. The non-working mothers were not employed but mostly belonged in
60
28 small farmer communities, they were involved with farming and family work. In small farmer

1 communities, the crops after being harvested are left to dry over plastic or synthetic sheets,
2 which may promote *Aspergillus* growth and toxin production (Turner et al. 2005). Most of these
3 crops are consumed locally by either the household or closer communities. High BMI seemed to
4 act as an indicator of the amount and type of food consumed including corn oil, with obese
5 mothers consuming corn oil more frequently than the non-obese mothers. Worldwide two of the
6 major sources of aflatoxin exposure are groundnuts and corn, thus corn oil is a potential source
7 of aflatoxin exposure in this region (El-Sayed et al. 2003; El-Tahan et al. 2000; Hifnawy et al.
8 2004). The fact that obese mothers had higher AFM₁ levels than the non-obese, possibly reflects
9 the frequency of consumption of corn oil, and thus weakened contribution of corn oil to breast
10 milk AFM₁ level in our analysis when both factors were considered.

11
12 The observed frequency of AFM₁ positivity and child number was not expected, though a higher
13 number of children may act as indicator of lower socioeconomic status and therefore restricted
14 diet. However, in our analysis did not reveal an association between socioeconomic status and
15 AFM₁ positivity. The mothers at early lactation stage (<1 month) had given birth no more than
16 15 days before the breast milk sample was taken. The relative proportions of milk components
17 vary significantly with the stage of lactation, especially in the beginning of lactation (Lawrence
18 1999), and it is possible that changes in total fat content are important in terms of accumulation
19 and mobilization of aflatoxins.

20
21 In developing countries growth faltering is often associated with the quantity and/ or poor
22 quality of foods, in addition to multiple infectious hazards (WHO 2003). However, high levels
23 of aflatoxin-albumin adducts have been associated with growth faltering in Beninese infants
24 (Gong et al., 2002; 2004). In common with many developing countries (Shrimpton et al. 2001)
25 Egyptian infants had a high prevalence of stunting and a moderate frequency of being
26 underweight, based on WHO criteria (WHO 1995). In this study we do not have biomarkers of
27 infant aflatoxin exposure, though the modest maternal breast milk levels of AFM₁ would
28 predict overall exposures from milk or weaning foods to be considerably lower than in the

1
2
3 1 Beninese study. In our follow up longitudinal study we will assess infant biomarkers of
4
5 2 aflatoxin exposure which may be more informative in this respect.
6
7 3 In Egypt, weaning foods are those consumed by the rest of the household, and the fact that
8
9 4 mothers are exposed ensures the exposure of their children. Overall the breast milk
10
11 5 contamination with aflatoxin is moderate, compared to some high exposure regions. However,
12
13 6 for genotoxic carcinogenic substances such as aflatoxins, a threshold value below which the risk
14
15 7 value for human health is equal to zero does not exist. The Joint FAO/WHO Expert Committee
16
17 8 on Food Additives (JEFCA) does not establish a tolerable daily intake (TDI), but strongly
18
19 9 recommends that the level of aflatoxin should be as low as possible. Therefore, the toxicological
20
21 10 significance of the presence of aflatoxin in maternal milk should not be overlooked.
22
23 11 Consequently, a reduction of mother's exposure to aflatoxins and thus, a reduction of children's
24
25 12 exposure through the breast milk is desirable. Reductions in aflatoxin exposure can be
26
27 13 envisaged by several approaches. Where the source of contamination is clearly defined such as
28
29 14 in Guinea, West Africa, post harvest changes to drying and storage of at risk foods is effective
30
31 15 (Turner et al. 2005). Where dietary sources are less well defined such as in Egypt, reduction in
32
33 16 uptake of aflatoxins may be achieved by dietary modulation, eg. with chlorophyllin (Egner et al.
34
35 17 2001) or probiotics (El-Nezami et al. in press).
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60**References**

- Abdulrazzaq YM, Osman N, Ibrahim A. 2002. Fetal exposure to aflatoxins in the United Arab Emirates. *Ann Trop Paediatr.* 22: 3-9.
- Abdulrazzaq YM, Osman N, Yousif ZM, Al-Falahi S. 2003. Aflatoxin M1 in breast-milk of UAE women. *Ann Trop Paediatr.* 23: 173-179.
- Angelico M and Renganathan E and Gandin C and Fathy M and Profili MC and Refai W et al. 1997. Chronic liver disease in the Alexandria governorate, Egypt: contribution of schistosomiasis and hepatitis virus infections. *J Hepatol.* 26: 236-243.
- AOAC. 1995. Aflatoxins M1 and M2 in fluid milk (Scott PM, ed) In: *Natural Toxins*. Arlington, VA: Association of Official Analytical Chemists. 49: 34-35.
- Egner PA, Wang JB, Zhu YR, Zhang BC, Wu Y, Zhang QN et al. 2001. Chlorophyllin intervention reduces aflatoxin-DNA adducts in individuals at high risk for liver cancer. *Proc Natl Acad Sci U S A.* 98: 14601-14606.
- El-Nezami H, Ma J, Polychronaki N, Zhu H, Ling W, Salminen EK et al.. Probiotic supplementation reduced biomarker for increased risk of liver cancer in young Chinese males from Southern China. *Amr J. Clin Nutri.* In press.
- El-Nezami H, Mykkänen H, Kankaanpaa P, Suomalainen T, Salminen S, Ahokas J. 2000. Ability of a mixture of *lactobacillus* and *propionibacterium* to influence the faecal aflatoxin content in healthy Egyptian volunteers: a pilot clinical study. *Bioscience Microflora.* 19: 41-45.
- El-Nezami H, Nicoletti G, Neal GE, Donohue DC, Ahokas JT. 1995. Aflatoxin M1 in human breast milk samples from Victoria, Australia and Thailand. *Food Chem Toxicol.* 33: 173-179.

- 1
2
3 1
4
5 2 El-Sayed AM, Soher EA, Sahab AF. 2003. Occurrence of certain mycotoxins in corn and corn-
6
7 3 based products and thermostability of fumonisin B1 during processing. *Nahrung*. 47: 222-225.
8
9 4
10
11 5 El-Shewy EA. 1987. Some Toxicological Studies on contaminated breast milk by aflatoxins
12
13 6 [PhD thesis]. Egypt, Faculty of Medicine: Zagazig University, Benha Branch
14
15 7
16
17 8 El-Tahan FH, El-Tahan MH, Shebl MA. 2000. Occurrence of aflatoxins in cereal grains from
18
19 9 four Egyptian governorates. *Nahrung*. 44: 279-280.
20
21 10
22
23 11 Gong Y and Cardwell K and Hounsa A and Egal S and Turner PC and Hall AJ et al. 2002.
24
25 12 Dietary aflatoxin exposure and impaired growth in young children from Benin and Togo: cross
26
27 13 sectional study. *BMJ*. 325: 20-21.
28
29 14
30
31 15 Gong Y and Hounsa A and Egal S and Turner PC and Sutcliffe AE and Hall AJ et al. 2004.
32
33 16 Postweaning exposure to aflatoxin results in impaired child growth: a longitudinal study in
34
35 17 Benin, West Africa. *Environ Health Perspect*. 112: 1334-1338.
36
37 18
38
39 19 Groopman JD and Hall AJ and Whittle H and Hudson GJ and Wogan GN and Montesano R et
40
41 20 al. 1992. Molecular dosimetry of aflatoxin-N7-guanine in human urine obtained in The Gambia,
42
43 21 West Africa. *Cancer Epidemiol Biomarkers Prev*. 1: 221-227.
44
45 22
46
47 23 Hifnawy MS and Mangoud AM and Eissa MH and Nor Edin E and Mostafa Y and Abouel-
48
49 24 Magd Y et al. 2004. The role of aflatoxin-contaminated food materials and HCV in developing
50
51 25 hepatocellular carcinoma in Al-Sharkia Governorate, Egypt. *J Egypt Soc Parasitol*. 34: 479-488.
52
53 26
54
55 27 IARC. 1993. Some naturally occurring substances - food items and constituents, heterocyclic
56
57 28 aromatic amines and mycotoxins. *IARC Monogr Eval Carcinog Risks Hum* 27.
58
59
60

- 1
2
3 1
4
5 2 Jensen AA and Slorach SA. 1991. Chemical contaminants into human milk. Florida, United
6
7 3 States.
8
9 4
10
11 5 Jiang Y, Jolly PE, Ellis WO, Wang JS, Phillips TD, Williams JH. 2005. Aflatoxin B1 albumin
12 6 adduct levels and cellular immune status in Ghanaians. *Int Immunol.* 17: 807-814.
13
14 7
15
16 8 Lawrence RA. 1999. *Breastfeeding: a guide for the medical profession.* 5th ed. St.Louis,
17 9 London, Mosby.
18 10
19
20 11 Lunn PG. 2000. The impact of infection and nutrition on gut function and growth in childhood.
21 12 *Proc Nutr Soc.* 59: 147-154.
22 13
23
24 14 Navas SA, Sabino M, Rodriguez-Amaya DB. 2005. Aflatoxin M(1) and ochratoxin A in
25 15 a human milk bank in the city of Sao Paulo, Brazil. *Food Addit Contam.* 22: 457-462.
26 16
27 17 Neal GE, Eaton DL, Judah DJ, Verma A. 1998. Metabolism and toxicity of aflatoxins M1 and
28 18 B1 in human-derived in vitro systems. *Toxicol Appl Pharmacol.* 151: 152-158.
29 19
30
31 20 Pronczuk J, Moy G, Vallenas C. 2004. Breast milk: an optimal food. *Environ Health Perspect.*
32 21 112: 722-723.
33 22
34
35 23 Saeed AA and Al-Admawi AM and Al-Rasheed A and Fairclough D and Bacchus R and Ring C
36 24 et al. 1991. Hepatitis C virus infection in Egyptian volunteer blood donors in Riyadh. *Lancet.*
37 25 338: 459-460.
38 26
39
40 27 Selim MI, Popendorf W, Ibrahim MS, El-Sharkawy S, El-Kashory ES. 1996. Aflatoxin B1 in
41 28 common Egyptian foods. *J AOAC Int.* 79: 1124-1129.

- 1
2
3 1
4
5 2 Shrimpton R, Victora CG, de Onis M, Lima RC, Blossner M, Clugston G. 2001. Worldwide
6
7 3 timing of growth faltering: implications for nutritional interventions. *Pediatrics*. 107: 75.
8
9 4
10
11 5 Turconi G and Guarcello M and Livieri C and Comizzoli S and Maccarini L and Castellazzi
12
13 6 AM et al. 2004. Evaluation of xenobiotics in human milk and ingestion by the newborn-an
14
15 7 epidemiological survey in Lombardy (Northern Italy). *Eur J Nutr*. 43: 191-197.
16
17 8
18
19 9 Turner PC, Moore SE, Hall AJ, Prentice AM, Wild CP. 2003. Modification of immune function
20
21 10 through exposure to dietary aflatoxin in Gambian children. *Environ Health Perspect*. 111: 217-
22
23 11 220.
24
25
26
27 12
28
29 13 Turner PC, Sylla A, Gong YY, Diallo MS, Sutcliffe AE, Hall AJ et al. 2005. Reduction in
30
31 14 exposure to carcinogenic aflatoxins by postharvest intervention measures in west Africa: a
32
33 15 community-based intervention study. *Lancet*. 365: 1950-1956.
34
35
36 16
37
38 17 Wang JS and Huang T and Su J and Liang F and Wei Z and Liang Y et al. 2001. Hepatocellular
39
40 18 carcinoma and aflatoxin exposure in Zhuqing Village, Fusui County, People's Republic of
41
42 19 China. *Cancer Epidemiol Biomarkers Prev*. 10: 143-146.
43
44 20
45
46 21 Weaver VM, Buckley TJ, Groopman JD. 1998. Approaches to environmental exposure
47
48 22 assessment in children. *Environ Health Perspect*. 106 Suppl 3: 827-832.
49
50
51 23
52
53 24 WHO. 1995. *Physical status: the use and interpretation of anthropometry*. Geneva 854.
54
55 25
56
57 26 WHO. 2003. *Global strategy for infant and young child feeding*. Geneva
58
59 27
60

- 1
2
3 1 Wild CP, Rasheed FN, Jawla MF, Hall AJ, Jansen LA, Montesano R. 1991. In-utero exposure to
4 aflatoxin in west Africa. *Lancet*. 337: 1602.
5
6 2
7 3
8
9 4 Willet W. 1990. *Nutritional Epidemiology*. New York Oxford University Press 15.
10
11 5
12
13 6 Zarba A, Wild CP, Hall AJ, Montesano R, Hudson GJ, Groopman JD. 1992. Aflatoxin M1 in
14 human breast milk from The Gambia, west Africa, quantified by combined monoclonal
15
16 7
17 antibody immunoaffinity chromatography and HPLC. *Carcinogenesis*. 13: 891-894.
18
19 8
20
21 9
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1 **Table 1. Descriptive maternal data.**

Mother		% (Number)	Median (IQR)	Range
AFM ₁ (pg/ml)	negative	64% (250)		
	positive	36% (138)	13.5 (10.3-21.4)*	5.6-5131*
Lactation stage**			5 (2-8)	0.2-33.0
Age (years)		100 (381)	25 (22-30)	15-47
Number of miscarriages [#]	Total	100 (383)	-	-
	0	86 (331)		
	1-3	14 (52)		
Number of children ^{##}	Total	100 (384)	-	-
	1	28.4 (109)		
	2-7	71.6 (275)		
Maternal BMI	Total	100 (336)	26.0 (24.0-28.5)	15.8-68.9
	<20	2.4 (8)		
	20-<25	31.5 (106)		
	25-<30	50.9 (171)		
	>30	15.2 (51)		
Employed (%)	Total	100 (382)	-	-
	unemployed	89 (339)		
	employed	11 (43)		
Socioeconomic status	Total	100 (384)	-	-
	LSES [§]	76.3 (293)		
	HSES [§]	23.7 (91)		

2 *for positive samples, **number of months that mother has been breastfeeding index child

3 [#]prior to index child, ^{##}including index child4 [§](LSES) Low socioeconomic status, (HSES) High socioeconomic status

5

1 **Table 2. Descriptive infant data.**

Child		% (Number)	Median (IQR)	Range
sex	boys	58 (227)	-	
	girls	42 (159)		
age (months)			5 (2 to 8)	0.2 to 33.0
HAZ		100 (322)	-1.49 (-2.63 to 0.02)	- 4.2 to 2.9
	> -2	64.2 (207)		
	-3 to -2	17.4 (56)		
	≤ -3	18.3 (59)		
WAZ		100 (375)	-0.45 (-1.47 to 0.43)	- 4.5 to 4.6
	> -2	85.6 (321)		
	-3 to -2	9.3 (35)		
	≤ -3	5.1 (19)		
WHZ		100 (351)	0.82 (0.17 to 1.66)	-2.3 to 4.8
	> -2	99.4 (349)		
	-3 to -2	0.6 (2)		
	≤ -3	0 (0)		

2 Malnutrition status is based on World Health Organization Guidelines.

3 Z score less than -2 for HAZ (stunting), WAZ (being underweight) or WHZ (wasting) is defined

4 as malnutrition, while less than -3 as severe malnutrition.

1 **Table 3. Relationship between infant age and HAZ, WAZ and WHZ.**

Age months (quartiles)	HAZ Median (IQR)	WAZ Median (IQR)	WHZ Median (IQR)
=<2	-0.73 (-1.98 - 0.14)*,**	-0.29 (-1.16 - 0.78)*,#	0.37 (-0.20 - 1.10)*,#
3-5	-1.67 (-2.87 - -0.91)*,#	-0.53 (-1.49 - 0.16)#	1.16 (0.37 - 1.71)*,##
6-8	-2.25 (-3.00 - -0.96)**,*,#	-0.34 (-1.37 - 0.33)	1.29 (0.65 - 1.96)**
>8	-1.29 (-2.55 - 0.35)	-1.06 (-1.93 - 0.13)*	0.74 (-0.11 - 1.53)#,##
	[§] p= 0.001	[§] p= 0.004	[§] p <0.001

2 [§]p values based on Kruskal-Wallis One Way Analysis of Variance on Ranks

3 *,** Statistical significance p <0.001

4 #,## Statistical significance p <0.05

1 Table 4. Factors significantly associated with the presence of AFM₁ in breast milk.

Mothers (n)	AFM ₁ positive n (%)	Univariate		Multivariate model		
		Unadjusted p-values	Adjusted p-values	All variables		Without corn oil
				Adjusted OR (95%CI)	Adjusted p-values	Adjusted OR (95%CI)
Mother's age	-	0.460	0.014	0.94 (0.89-0.99)	0.014	0.94 (0.89-0.99)
Working status						
non-working (339)	129 (38.1)	0.005	0.016	2.99 (1.22-7.01)	0.018	2.87 (1.2-6.87)
working (43)	7 (16.3)					
Number of children						
2-7 (275)	105 (38.2)	0.104	0.039	1.89 (1.03-3.46)	0.025	1.99 (1.09-3.64)
1 (109)	32 (29.4)					
Lactation stage						
<1month (24)	14 (58.3)	0.017	0.016	4.25 (1.30-13.86)	0.028	3.57 (1.15-1.09)
≥1 months (360)	123 (34.2)					
Corn oil consumption						
daily (262)	104 (39.7)	0.016	0.172	1.47 (0.84-2.57)	-	-
weekly/ monthly (125)	34 (27.2)					
BMI						
≤25 (not overweight) (114)	34 (30)	0.031	0.019		0.011	
25-30 (overweight) (171)	58 (34)		0.684	1.12 (0.65-1.92)	0.578	1.16 (0.68-1.99)
≥30 (obese) (51)	25 (49)		0.008	2.79 (1.31-5.91)	0.004	3.01 (1.43-6.33)

2

1
2
3 **1 Figure legends**
4

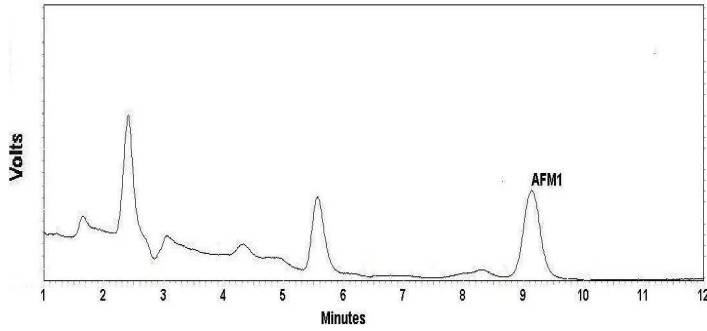
5
6 **2**

7 **3 Figure 1. HPLC chromatograms of AFM₁.** Mobile phase H₂O: CH₃OH: CH₃CN, 66:17:17;
8 flow rate: 1ml/min; column: ODS-5 C18 220 x 4.6, fluorescence detection (ex. 360 nm, em. 440
9 nm). (a) AFM₁ extracted from an Egyptian breast milk sample (1004 pg/ ml breast milk or 14
10 ng/ml methanol); (b) AFM₁ standard 10 ng/ ml methanol; (c) AFM₁ extracted from spiked water
11
12
13
14
15
16
17 (250 pg/ ml water or 5 ng/ ml methanol).
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

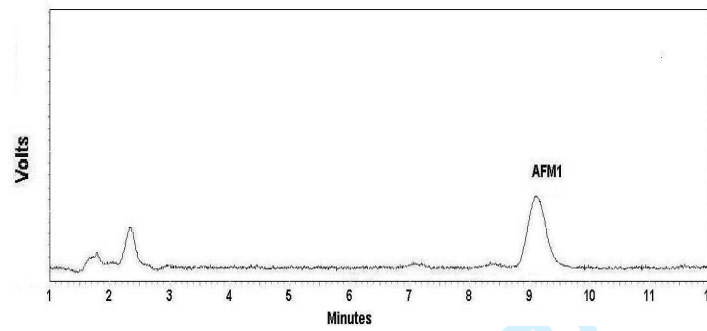
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1 **Figure 1**

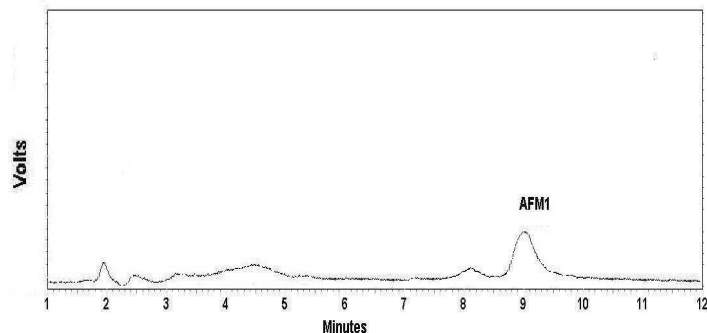
2 (a)



3
4
5 (b)



6
7
8 (c)



9
10
11