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CO-OCCURRENCE OF AFLATOXIN B₁, FUMONISIN B₁, OCHRATOXIN A AND ZEARALENONE IN CEREALS AND PEANUTS IN COTE D'IVOIRE

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CO-OCCURRENCE OF AFLATOXIN B1, FUMONISIN B1, OCHRATOXIN A AND ZEARALENONE IN CEREALS AND PEANUTS IN COTE D'IVOIRE

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Food Types:	Peanuts, Rice, Cereals and grain

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Table 1: Different mycotoxins assayed and detected in the same commodities in Côte d'Ivoire

Mycotoxins	Food samples		
	Maize	Rice	Peanuts
Aflatoxin B1 ($\mu\text{g}/\text{kg}$) Range (positive %)	n=10 <1.5-20 (100%)	n=10 <1.5-10 (100%)	n=10 1.5-10 (100%)
Fumonisin B1 (mg/kg) Range (positive %)	n=10 0.3-1.5 (100%)	Not detected	n=10 <0.3-6 (70%)
Ochratoxin A ($\mu\text{g}/\text{kg}$) Range (positive %)	n=10 0.16-0.86 (100%)	n=10 0.16-0.92 (100%)	n=10 0.0-0.64 (60%)
Zearalenone ($\mu\text{g}/\text{kg}$) Range (positive %)	n=10 20-50 (100%)	n=10 50-200 (100%)	n=10 50-200 (100%)

Table 2: Concentrations of aflatoxin B1, fumonisin B1, ochratoxin A and zearalenone detected in maize samples, (n=10)

Mycotoxins	Maize samples									
	1	2	3	4	5	6	7	8	9	10
Aflatoxin B1 ($\mu\text{g}/\text{kg}$)	1.5	<1.5	1.5	1.5	1.5	<1.5	5	5	1.5	20
Fumonisin B1 (mg/kg)	1.5	0.3	0.3	1.5	1.5	0.3	0.3	1.5	0.3	1.5
Ochratoxin A ($\mu\text{g}/\text{kg}$)	0.67	0.29	0.14	0.39	0.15	0.86	0.50	0.32	0.09	0.23
Zearalenone ($\mu\text{g}/\text{kg}$)	50	50	50	50	50	50	50	50	50	50

Table 3: Concentrations of aflatoxin B1, fumonisin B1, ochratoxin A and zearalenone detected in rice samples, (n=10)

Mycotoxins	Rice samples									
	1	2	3	4	5	6	7	8	9	10
Aflatoxin B1, ($\mu\text{g}/\text{kg}$)	1.5	1.5	5	10	1.5	10	1.5	5	<1.5	<1.5
Fumonisin B1, (mg/kg)	Not detected									
Ochratoxin A ($\mu\text{g}/\text{kg}$)	0.91	0.68	0.53	0.20	0.92	0.16	0.50	0.20	0.9	0.16
Zearalenone, ($\mu\text{g}/\text{kg}$)	50	50	200	50	50	200	50	50	50	50

Table 4: Concentrations of aflatoxin B1, fumonisin B1, ochratoxin A and zearalenone detected in peanuts samples, (n=10), nd= not detected

Mycotoxins	Peanuts samples									
	1	2	3	4	5	6	7	8	9	10
Aflatoxin B1, ($\mu\text{g}/\text{kg}$)	1.5	5	5	5	5	1.5	5	5	5	10
Fumonisin B1, (mg/kg)	<0.3	<0.3	<0.3	<0.3	6	6	3	nd	nd	nd
Ochratoxin A ($\mu\text{g}/\text{kg}$)	0.26	0.56	0.20	nd	nd	nd	nd	0.64	0.25	0.33
Zearalenone, ($\mu\text{g}/\text{kg}$)	50	50	200	50	50	200	50	50	50	200

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3 **CO-OCCURRENCE OF AFLATOXIN B₁, FUMONISIN B₁, OCHRATOXIN A AND**
4 **ZEARALENONE IN CEREALS AND PEANUTS IN COTE D'IVOIRE**
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31 **Abstract:**
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34 The present survey examined 30 samples of rice (n=10), maize (n=10) and peanuts (n=10)
35 from Côte d'Ivoire for aflatoxin B₁, fumonisin B₁ and zearalenone using immunoassays, and
36 ochratoxin A using a validated HPLC method with fluorescence detection. Similarly to some
37 other countries it appeared that in Côte d'Ivoire several mycotoxins are present in the same
38 commodities. These mycotoxins are from different structural families: aflatoxin B₁, fumonisin
39 B₁, zearalenone, and ochratoxin A. There are normally produced by fungal species from
40 *Aspergillus*, *Penicilium* and *Fusarium* genera. Some samples contained four mycotoxins
41 (86%). Four peanuts samples did not show ochratoxin A (14%) whereas they contained
42 aflatoxin B₁ concentrations, above the EU regulatory limits. The concentrations of ochratoxin
43 A, zearalenone and fumonisin B₁ were low and may not cause problem *per se* however fears
44 remain that the tolerable daily intake might be exceeded due to eating habits and that
45 synergistic effects might be important with the combination of several mycotoxins.
46 Investigations in this direction are underway together with isolation and characterization of
47 fungal species involved.
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3 **Keywords:** aflatoxin B₁, fumonisin B₁, zearalenone, ochratoxin A, co-occurrence, Côte
4 d'Ivoire.
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8 9 **Introduction**

10
11 The European Union (EU) has established regulatory limits for mycotoxins in crops and
12 foodstuffs, (EU Commission Directive, 2005) this implies that crops exported from any
13 country to Europe have to be analysed for mycotoxins. In Côte d'Ivoire, a preliminary survey
14 conducted by us showed the presence of ochratoxin A in maize, millet rice and peanuts
15 (Sangare-Tigori et al., 2005). Other authors have found aflatoxins in several foods samples,
16 IARC, 1993; Creppy, 2002a). The question was then; do we have multi-mycotoxin
17 contamination in Côte d'Ivoire? The present study aimed to determine the presence and levels
18 of ochratoxin A, aflatoxins, fumonisins and zearalenone in cereals (maize and rice) and
19 peanuts in order to give an answer to the above question.
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35 **Materials and methods**

36 37 **Samples**

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39 Every time the samples were taken according to the state of the art, with four identical
40 sampling sizes at four different levels of each bulk (top to bottom). Five kg samples were
41 taken each time and mixed to form the sample for analysis. 5 kg from the mixture of 20 kg
42 were ground and 200 g of powder were taken after cautious homogenization of the samples,
43 which were then stored at -20°C until subsequent extraction and analysis. Maize, rice and
44 peanuts were obtained from markets in Abidjan, Côte d'Ivoire, especially in Adjamé, Abobo
45 and Treichville. These markets represent the significant areas which provide feeds to other
46 markets in Côte d'Ivoire.
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59 Samples for analysis were assigned a number from 1 to 10 for each type of foodstuffs. Each
60 sample was composed of five sub-samples of equal size of 5 kg mixed carefully prior to

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2
3 grinding. Thus a tochratoxin A1 of 50 samples were analysed simultaneously for each type of
4
5 foodstuffs. All the samples were taken from locally produced crops by the end of November
6
7 2002, they were stored at -20°C and analysed from January 2003.
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9

10 11 12 **Chemicals**

13
14 Toluene (Prolabo), HPLC-grade methanol (Scharlau), HPLC-grade acetonitrile (Scharlau),
15
16 glacial acetic acid (Chromanorm-Prolabo), anhydrous magnesium chloride approx.98%
17
18 (Sigma), sodium acetate (Merck) and hydrochloric acid(Prolabo) were all provided by Merck
19
20 Eurolab France. Ultrapure water, MilliQ R Plus was obtained using Millipore (France) system
21
22 in tandem with USF ELGA Prima (France) system. Sep-Pak Plus Silica Cartridges were from
23
24 Waters (France) (3 cm long, 1 cm diameter). Ochratoxin A was purchased from Sigma-
25
26 Aldrich (USA). The standard solutions of ochratoxin A were made in methanol according to
27
28 the concentration established using a UV spectrophotometer ($\epsilon = 5500 \text{ mol}^{-1} \text{ cm}^{-1}$). The
29
30 required quantity was evaporated to dryness and dissolved in the mobile phase indicated
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32 under chromatographic conditions.
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41 OCHRATOXIN A was assayed by an HPLC method with fluorescence detection and the
42
43 others mycotoxins by immunoassays (Rhône Diagnostic Technologies, France).
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48 Each sample of rice, maize and peanut was analysed separately for each family of mycotoxin.
49
50 Four different extracts were obtained for ochratoxin A, aflatoxin B₁, fumonisin B₁ and
51
52 zearalenone, (Abdelhamid, 1990, Wood, 1992, Shephard et al. 1996, Scott 2002, Creppy
53
54 2002a).
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59

60 **Apparatus**

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2
3 The rotatory evaporator, Heidolf WB 2000, was from Prolabo (France). The chromatographic
4 system consisted of a Bischoff pump Model A 2200 and an Alcott Autosampler Model RF-
5 551 Fluorescence Monitor. To measure the peak area, a PIC3 ® software from ICS was used.
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7
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9
10 A pre-packed stainless steel column(250 x 4.6 mm) with 10 µm Spherisorb ODS1 and a
11 guard column (30 x 4.6 mm) packed with 10 µm Lichrosorb C-18 were used.
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15

16 17 **Extraction and purification of OCHRATOXIN A:**

18
19 A solution containing 10 g of mixed ground sample, 50 ml of 0.4 M MgCl₂ and 30ml of 2N
20 hydrochloric acid were extracted twice with 50 ml of toluene. The two phases were separated
21
22 by centrifugation (5 min at 3000 g). The organic phase was evaporated to dryness under
23
24 vacuum and evaporated extract was dissolved in 1 ml of methanol and loaded onto the
25
26 cartridge. The cartridge was then washed with 10 ml toluene-acetic acid (90:10; v/v). The
27
28 flow rate during washing and elution of the Sep Pak cartridge was maintained at 1 ml/min.
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30
31 The elute was evaporated to dryness and dissolved in 1ml of the mobile phase (Ospital et
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33 al.,1998).
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41 **Chromatographic conditions for ochratoxin A:**

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43 The mobile phase (0.45 µm filtered and degassed) consisted of 48% acetonitrile and 52%
44
45 4mM sodium acetate acetic acid (19:1 v/v). The flow rate was 1 ml/min, the column was at
46
47 room temperature and the injection volume was 50 µl. The run time for one cycle was 12 min,
48
49
50 fluorescence detection wavelengths were excitation 330 nm and emission 470 nm. The
51
52 retention time of OCHRATOXIN A under the conditions described was approximately 7 min.
53
54 Analytical data were collected stored and processed using the PIC 3 software developed by
55
56 ICS. The quantification of OCHRATOXIN A was achieved automatically by the computer
57
58 according to the areas under the curve of four standard solutions in the range of 0.6 ng/ml to
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1
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3 60 ng/ml of OCHRATOXIN A, run sequentially, during the course of analysis. They were
4
5 linearly related to the amount of OCHRATOXIN A injected. The correlation coefficient was
6
7 always between 0.997 and 0.998.
8
9

10 11 12 ***OCHRATOXIN A confirmation*** 13

14
15 Out of the 1 ml final extract, 0.5ml was evaporated to dryness and 0.4 ml of the buffer 0.04M
16
17 Tris in 1 M NaCl adjusted to pH 7.5 with HCl was added to the evaporated extract. To this
18
19 solution 100 µl of carboxypeptidase (Sigma, Ref C-0261, 100 U/ml) was added and the
20
21 mixture incubated for 3h at 37°C. Fifty µl of this solution was injected into the
22
23 chromatographic system for separation and quantification of OT alpha, under the same
24
25 conditions as described for OCHRATOXIN A. The quantification of OT alpha was achieved
26
27 automatically by the computer according to the areas under the curve of five standards of OT
28
29 alpha (elution time 5.1min). All the positive samples were confirmed using this procedure that
30
31 shows moreover disappearance of the peak of OCHRATOXIN A..
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39 **Other toxins:** 40 41 42

43 Aflatoxins, fumonisin and zearalenone were purchased from Sigma-Aldrich (USA). They
44
45 were dissolved in methanol and utilized similarly to the standards solutions provided with the
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47 kits.
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53 **Aflatoxin B₁** 54 55 56

57 AFLAPLATE ® (Rhône Diagnostics, France) was used. It is an immunoassay based on a
58
59 direct competition ELISA provided with multi-wells plates. It is a very specific, sensitive and
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1
2
3 fast test. The reaction with antibody develops a colour inversely proportional to the
4
5 concentration of aflatoxin B₁. The development of the colour is stopped by 1 N sulfuric acid
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7 solution and the absorbance of each sample measured at 450 nm in a plate reader.
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10 11 12 **Fumonisin B₁**

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17 FUMONIPLATE ® (Rhône Diagnostics, France) was used to assayed fumonisin B₁.
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24 25 **Zearalenone**

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27
28
29 ZEARAPLATE ® (Rhône Diagnostics, France) was used to assayed zearalenone. It is similar
30
31 to AFLAPLATE ® or FUMONIPLATE ®, but highly specific to zearalenone.
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36 37 **Quality control**

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39 Each matrix analysed in this survey was used for validation, i.e. ochratoxin A and aflatoxin B₁
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41 free maize, rice, peanuts were spiked in the ranges of regulatory limits up to three times these
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43 limits and extracted afterward to evaluate the recovery rates. This was in addition performed
44
45 three times for each matrix using uniformly tritiated toxins (isotopic mixture 1/1000 with a
46
47 minimum of 1µCi/ assay). However, higher concentration of ZEA and fumonisin B₁, 10, 40
48
49 80 and 100 µg/kg, were used to spiked toxins free matrixes that were extracted afterwards.
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52

53 For every five samples of cereals and peanuts, OCHRATOXIN A-free foods provided in
54
55 France and previously analysed for the mycotoxins concerned were spiked with
56
57 OCHRATOXIN A at levels of 0.2; 0.6; 2; 6; 20; 40 and 60 ng/g (µg/kg) were extracted and
58
59 analysed sequentially. The recovery was in the range of 85 to 95% for peanuts and 90 to 95%
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3 for maize and rice samples. This was confirmed by using [³H]-OCHRATOXIN A randomly
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5 during the course of extractions, at least one time for each matrix (n=5)
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10 Similar quality control was performed for the other toxins without radioactivity however. The
11 recoveries were in the range of 89 to 99% for aflatoxin B₁ (spiked at levels of 1.5, 5, 10 and
12 20 µg/kg) and zearalenone (spiked at levels of 20, 50, 200, and 500 µg/kg) and 85 to 95% for
13 fumonisin B₁ (spiked at levels of 0.3, 0.5, 1.5 and 3 mg/kg).
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20 Negative control samples from toxin-free crops samples were routinely analysed along with
21 spiked samples
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29 **Statistical analysis of data**

30 Results are presented as individual values in µg/kg (ppb) for each type of matrix. For the
31 same mycotoxin, concentrations are compared to those found in different matrix. In order to
32 evaluate the contribution of each matrix in human contamination, all individuals values were
33 compared from one matrix to the other using the non-parametric Wilcoxon Rank Sum Test 21
34 (WRST) with p fixed at p=0.01.
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45 **Results and Discussion**

46 Concerning matrix effects, the lowest recovery was always obtained for peanut samples
47 (n=10), whereas the highest was obtained for rice and maize, 80±5% in peanuts and 98±3%
48 for OCHRATOXIN A, fumonisin B₁, aflatoxin B₁ and zearalenone. The mycotoxins assayed
49 were all detected at variable levels in most of the samples. The main results are summarized
50 in Table 1. The different concentrations of mycotoxins in each type of foodstuffs are given in
51 details in Table 2, Table 3 and Table 4 for respectively maize, rice and peanuts. It appears that
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3 the contribution for mycotoxins exposure is due in decreasing order to maize > peanuts > rice
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5 (p=0.01)
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10 **Maize contamination by ochratoxin A:**

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12 In a previous survey ochratoxin A was detected in cereals, millet, maize and rice and in
13
14 peanuts from Côte d'Ivoire (Sangare-Tigori et al., 2005). The present data thus confirm that
15
16 ochratoxin A is a frequent contaminant of foodstuffs in this country. All maize samples were
17
18 ochratoxin A-contaminated and the concentrations ranged from 0.09 to 0.86 µg/kg, Table 2.
19
20 These concentrations are far less than those found formerly (Dem 1999; Creppy, 2002b).
21
22 Ochratoxin A concentrations in the range of 27 to 57 µg/kg were found in 1998 in cereal
23
24 samples including rice. At present ochratoxin A in rice ranges from 0.16 to 0.92 µg/kg (Table
25
26 1). The statistics of Côte d'Ivoire show that the total rainfalls in 1998 were of about 1512 mm
27
28 versus 1933 mm in 2002 in Abidjan city. The climate in Abidjan city in 2002 was clearly
29
30 rainier than in 1998 but the harvest period was much drier. This could not explain why maize
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32 samples collected in 1998 were unexpectedly more contaminated than samples collected in
33
34 2002. The discrepancy between ochratoxin A concentrations in 2002 compared to 1998 can
35
36 be explained by several reasons linked to political troubles in Côte d'Ivoire. Storage could
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38 have been for shorter time before sampling as compared to 1998, influencing other known
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40 factors in mycotoxins production such as integrity of grains, rate of fungal growth, prevalence
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42 of toxinogenic species, interactions with micro organisms and insects as suggested (Beti et al.,
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44 1995). We just do not know the most relevant reason for this low OCHRATOXIN A
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46 contamination in the year 2002. But, we suspect that in 2002 the cereal traders imported from
47
48 neighbour countries maize usually found and eaten locally. This is actually in line with
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50 information given by the ministry of agriculture and the ministry of trade in 2003, which also
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52 indicated that freshly imported yellow corn was available for human consumption. This
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3 yellow corn was not available in market because it was directly distributed to populations in
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5 need.
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10 **Rice contamination by ochratoxin A:**

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12 Rice samples of the year 2002 (November) were analysed for ochratoxin A and the
13 concentration ranged from 0.16 to 0.912 $\mu\text{g}/\text{kg}$ with an overall mean value of 0.44 ± 0.032 .
14
15 These values are lower than those reported (Abdelhamid 1990) from Egypt where several
16
17 samples of foods, including rice, had been assayed and ochratoxin A was found at
18
19 concentrations ranging from 4 to 577 $\mu\text{g}/\text{kg}$ with an average of $58.2 \pm 22.9 \mu\text{g}/\text{kg}$.
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27 **Peanuts contamination by ochratoxin A:**

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29 In four peanuts samples, ochratoxin A was not detected at the limit of detection of the
30
31 method. Ochratoxin A concentrations ranged from 0.203 to 0.642 $\mu\text{g}/\text{kg}$ in the other samples
32
33 (Table 4). In Tunisia for example, ochratoxin A contaminations ranged from 5 to 46000 $\mu\text{g}/\text{kg}$
34
35 (Bacha et al., 1999). Tunisia has a warm and humid climate that favours fungal development
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37 especially because peanuts are stored for long periods since Tunisia does not produce peanuts.
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39 This could explain extremely high values such as 46000 $\mu\text{g}/\text{kg}$ in this country.
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46 In fact ochratoxin A production depends on conditions of humidity and temperature (Pitt,
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48 1987). The optimal temperature of ochratoxin A production by *Aspergillus ochraceus* is 28°.
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50 This production decreases at 15° or 37° (Trenk et al., 1991). Besides, *Penicillium verrucosum*
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52 formerly *viridicatum* grows at temperatures ranging from 4 to 30° with water activity of about
53
54 22% (Mislivec et al., 1970; Pitt, 1987). In winter times this large variation of temperature may
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56 be encountered in Tunisia rendering the situation more favourable to fungi adapted to these
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58 conditions. It is then assumed that ochratoxin A is more produced in winter times by
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3 *Penicillia* in Tunisia and in Europe and that in continuously warm regions like Côte d'Ivoire
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5 OCHRATOXIN A is produced by *Aspergilli* mainly (Pohland et al., 1992 and Varga et al.,
6
7 1996). Thus, combination of all these factors may explain the high concentrations
8
9 exceptionally found in Tunisia.
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15 After confirmation of the presence of ochratoxin A in most of the samples let us examine the
16
17 case of other mycotoxins, successively aflatoxin B₁, fumonisin B₁ and zearalenone.
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22 **Aflatoxins**

23 **Aflatoxins in maize:**

24
25 Maize samples were contaminated by aflatoxin B₁ and the levels ranged from <1.5 µg/kg to
26
27 20 µg/kg, Table 2. These values are similar to those reported in foodstuffs from Egypt (3 to
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29 12 µg/kg), from Europe and Tunisia (11.5- 180 µg/kg) in corn, wheat, oats, and, corn and
30
31 barley from Korea (Abdelhamid, 1990, Bacha et al., 1999; Scudamore and Patel, 2000;
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33 Creppy, 2002a; Park and Troxell, 2002). These values are interestingly in the range of those
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35 reported from a very similar west-African country from the viewpoint of the climate, Bénin,
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37 (Bouraima et al, 1993).
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46 **Rice contamination by aflatoxins:**

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48 Aflatoxin B₁ concentrations ranged from <1.5 µg/kg to 10 µg/kg in rice samples, Table 3.
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50 According to the EU regulatory limits for aflatoxin B₁ four of these samples (40%) would not
51
52 be accepted for human consumption. Aflatoxin B₁ belongs to the mycotoxins family the most
53
54 studied in the world. In an eastern African country like Kenya, rice may be contaminated at
55
56 levels ranging from 294 to 1050 µg/kg (Jelinek et al., 1989, IARC, 1993, Creppy et al.,
57
58 2002a). In USA, aflatoxin B₁ contamination in rice was about 280 µg/kg, in India, 180 µg/kg,
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3 in Philippines, 16 µg/kg and in Thailand 98 µg/kg. It appears that in Côte d'Ivoire, rice is less
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5 contaminated than in some other countries including developed countries. However in the
6
7 present case all samples are contaminated by aflatoxin B₁ and other mycotoxins that could
8
9 elicit some synergism with AFB₁ whereas in other countries aflatoxin B₁ is not necessarily
10
11 contaminated by some other toxins. So it remains to understand the cause of this multi-
12
13 contamination, if any.
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20 **Peanuts contamination by aflatoxin B₁:**

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22 Ten samples of peanuts from Côte d'Ivoire were assayed for aflatoxins and results showed
23
24 that aflatoxin B₁ concentrations ranged from 1.5 to 10 µg/kg, Table 4. All the peanuts samples
25
26 were contaminated with an average of 4.8 µg/kg. These concentrations are similar to those
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28 found in Egypt: 3 to 12 µg/kg, (Abdelhamid, 1990) and were far less than those encountered
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30 in Sudan, 250 µg/kg, (Jelinek et al., 1989; Bacha et al., 1999; Scudamore et al., 2000; Creppy,
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32 2002a; Park and Troxell, 2002).
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39 Aflatoxins are produced by at least three fungal species including, *Aspergillus flavus* which
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41 produces aflatoxins B₁ and B₂ and *Aspergillus parasiticus* which produces all four aflatoxins
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43 B₁, B₂, G₁ and G₂ and *Aspergillus niger*. The dryness, temperature, type of grain and their
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45 contaminants may influence the growth of the mould. After the harvest, the conditions of
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47 storage and transportation of grains or foods may enhance aflatoxin B₁ concentrations
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49 suddenly. The most favourable conditions for growth and production aflatoxins by *A. flavus*
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51 are quite similar to that leading to production of ochratoxins by *A. ochraceus*, *aliaceus* or other
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53 *Aspergilli* (Christensen et al., 1973). It is thus not surprising that most of samples collected in
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55 the present survey are contaminated by aflatoxin B₁ and ochratoxin A.
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Fumonisin

All maize, rice and peanuts samples were analysed for fumonisin B₁.

Maize contamination by fumonisin B₁:

All the samples were contaminated and the concentrations ranged from 0.3 to 1.5 µg/kg with a mean value of 0.9 µg/kg. Fumonisin are the most prevalent mycotoxins in the world (Shepard et al., 1996) and these mycotoxins contaminate essentially maize. The real problem is the fact that maize cobs, although very contaminated, have the appearance of non-contaminated cobs. And some people for example from Transkei in South Africa prefer mouldy maize. Some other people do prefer traditional beers made at home with mouldy maize, because of its particular taste (Norred, 1993). Fumonisin are essentially produced by fungi called *Fusarium moniliforme* and *Fusarium proliferatum* and *Fusarium culmorum* which contaminate the cobs without any physical sign. In South Africa sometimes 100% of maize samples are found contaminated with maximal concentrations reaching 1890 µg/kg (Sydenham et al., 1994). In Benin, a western African country, 9 of 11 samples of maize were fumonisin-contaminated and the maximal concentrations were 2.3 µg/kg (Doko et al., 1995). In France 23 of 25 samples were contaminated with a maximal concentration of 3.35 µg/kg (De Nijs et al., 1999b). The concentrations found in maize in Côte d'Ivoire are not apparently different from those found in Bénin or in France.

Rice contamination by Fumonisin B₁

Unexpectedly rice samples collected in November 2002 were not contaminated by fumonisin B₁. No explanation could be given for that at present except that this rice could be a freshly imported one, however some other mycotoxins such as aflatoxin B₁ and ochratoxin A were found.

Peanuts contamination by fumonisin B₁

Only seven samples analysed were found with appreciable concentrations of fumonisin B₁,

Table 4. The concentrations ranged from <0.3 to 6 µg/kg.

Zearalenone

Zearalenone, (ZEA) also called F2-toxin, is a non-steroidal estrogenic mycotoxin produced as a secondary metabolite by several species of *Fusarium*, particularly *F.graminearum* or *Gibberella zea* (Stob et al.,1962), *F. semitectum*, *F.equiseti*, *F.crookwellense* and *F.culmorum* (IARC, 1993). This mycotoxin may be also produced by *F.tricinatum*, *F.moniliforme*, *F.oxysporum*, *F.sporotrichoides* and *F.laterium* (Mirocha et al., 1971).

The production of zearalenone by these *Fusarium* species is favoured by low temperatures ranging from 10 to 15° (Caldwell et al., 1970) at certain period of fungal infestation of crops.

In fact, the maximum of mycotoxin is produced at such low temperatures that are considered stressful to fungi (Sherwood and Peberdy, 1972, 1974). The water activity in grains is also a crucial factor in zearalenone production by *Fusarium* species. The presence of oxygen is important too, for example *F.graminearum* cannot grow neither produce zearalenone in anaerobia conditions of storage (Escoula, 1979). For all these reasons and especially because of low temperature required during fungal development on crops the presence of ZEA was questionable in foodstuffs collected in Côte d'Ivoire.

Maize contamination by zearalenone:

All the maize samples were contaminated by zearalenone at levels up to 50 µg/kg, Table 2.

These zearalenone concentrations in maize could not endanger people's health considering zearalenone alone (Castegnaro, 1995). Indeed these concentrations are under the EU

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3 regulatory limits for zearalenone.
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8 **Rice contamination by zearalenone:**
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10 All rice samples were zearalenone-contaminated and the concentrations ranged from 50 ppm
11 to 200 µg/kg, Table 3. In France and in Europe, the maximal concentration admitted for
12 human foods and animals feed are 200 µg/kg, (Castegnaro, 1995, EU Commission Directive,
13 2005).
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22 **Peanuts contamination by zearalenone:**
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24 All peanuts samples were contaminated by zearalenone and the concentrations ranged from
25 50 to 200 µg/kg. Although zearalenone was detected in samples collected in Côte d'Ivoire,
26 concentrations do not exceed EU regulatory limits and were rather low in cereals produced
27 and commercialized in Côte d'Ivoire. This country is located in western Africa where
28 temperatures being always between 24 to 34 ° do not permit *Fusarium* species to grow and
29 produce zearalenone in large amounts. However it is present and could worsen the toxic
30 effects of other mycotoxins such as aflatoxin B₁, ochratoxin A and fumonisin B₁.
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43 The literature already reported cases of combination of ochratoxin and aflatoxin or ochratoxin
44 and fumonisin. Cases of combination of four to five mycotoxins in the same commodities
45 have been reported with combined effects in vivo and/or synergistic effects (Dawlatana et al.,
46 2002; Diaz et al., 2001; Gobel and Lusky, 2004; Park and Troxell, 2002). It is known that
47 combination of mycotoxins in general lead to combined, additive or synergistic effects. This
48 may concern organ toxicity but also carcinogenesis, especially considering aflatoxin B₁ and
49 ochratoxin A in the present case, respectively classified in group 1 and 2B by IARC. Since
50 zearalenone is the major mycotoxin implicated in hormonal disruption, and the nephrotoxic
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3 ochratoxin A increasingly involved in hormonal disorders, one may fear that whenever
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5 zearalenone is combined with ochratoxin A and/or fumonisin B₁ this could enhance hormonal
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7 disorders in relation with zearalenone and nephrotoxic effect of ochratoxin A, (Benesis et al.,
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9 2000).

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15 Concerning the possibility that this mixture of mycotoxins, some of them being genotoxic
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17 such as aflatoxins, ochratoxins and zearalenone, could affect immune response in human, the
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19 situation should be carefully evaluated through epidemiological studies. One possibility is the
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21 morbidity linked to infectious diseases such as bacterial or viral ones. It is believed that
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23 mycotoxins may participate in premature death of children in case of bacterial-induced
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25 diarrhoea. In this purpose the childhood seems to be a crucial period when the food available
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27 should be preserved from high contamination since this may worsen malnutrition,
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29 kwashiorkor and possible Reye's syndrome. It seems to be a valuable duty to survey for these
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31 diseases and to determine the severity of malnutrition and human exposure to mycotoxins
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33 mainly for pregnant women and children during the period of lactation since several toxins
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35 including those detected in the present study are known to contaminate breast milk of exposed
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37 people. The fungi that produce the mycotoxins detected should be further isolated cultured
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39 and characterized by mycologists.
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48 The weekly diet of inhabitants of Cote d'Ivoire contains approximately 500g of maize, 3kg of
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50 rice and 150g of peanuts. Using data in table1 the weekly intake can be calculated for
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52 aflatoxin B₁, ochratoxin A, fumonisin B₁ and zeralenone which are 41.5µg (Daily Intake, DI=
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54 99 ng/kg bw/day), 3.29 µg (DI= 7.8 ng/kg bw/day), 1.65 mg (DI= 3.9 µg/kg bw/ day) and 655
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56 µg (DI= 1.56 µg/kg bw/day) respectively. These features appear to be largely above the
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58 tolerable daily intake set by WHO for respectively aflatoxins (< 1ng/kg bw/day), fumonisin
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3 B₁ (2 µg/kg bw/day) and zearalenone (500 ng/ kg bw/day), JECFA 1999, JECFA 2001.
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7 8 **Conclusions** 9

10 The combinations of the four mycotoxins assayed in commodities consumed almost daily
11 endanger people's health. This study reveals that mycotoxins multi-contamination occurs in
12 Côte d'Ivoire in most of maize, rice and peanuts samples collected by the end of November
13 2002, in which ochratoxin A, aflatoxin B₁, fumonisin B₁ and zearalenone were found in
14 appreciable amounts, some of which exceeded EU regulatory limits such as aflatoxin B₁ that
15 mostly poses problems. This situation is expected to occur in tropical countries and suggests
16 that the crops analysed may have been produced in this geographical area. The calculation of
17 daily intake with the highest concentrations found shows that fumonisin B₁ and zearalenone
18 are also of concern due to eating habits although found at concentrations below regulatory
19 limits. The main question is at present what health hazard could be linked to this co-
20 occurrence of aflatoxin B₁, ochratoxin A, fumonisin B₁ and zearalenone? Further
21 investigations are under way.
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