Occurrence of mycotoxins in a total diet study (TDS) for adults and children in the Netherlands

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Background

A total diet study (TDS) may be a valuable approach to assess the exposure of consumers to mycotoxins. A TDS study was performed on the risk associated with mycotoxin intake in cooperation with the Dutch National Institute for Public Health and the Environment (RIVM) and the Netherlands Food and Consumer Product Safety Authority (NVWA). The average Dutch diet was divided into the main separate food categories, and a sample representative for each food category for both adults' and childrens' diets was prepared from various products proportionally to their intake within this food category. All products were prepared as they are usually consumed. In total, more than 1300 individual food products were carefully processed to 88 homogenized samples.

Objective

Determination of the occurrence of 59 selected mycotoxins, including aflatoxins, ochratoxin A (OTA), trichothecenes, patulin, fumonisins, *Alternaria* toxins (AOH, AME), ergot alkaloids, pyrrolizidine alkaloids and several emerging mycotoxins, such as enniatins and beauvericin, in the 88 samples homogenized from the TDS study.

Preparation of TDS samples

- Average Dutch diet was divided into 54 main food groups.
- Each group was sampled relative to the average consumption of their individual subgroups and brand diversity was taken into account.
- Each subgroup consisted of 6 (minor products) or 12 (frequent consumption) similar products (brands).
- Products were prepared "as eaten":
  - Manual removal of non-edible parts and washing
  - Preparation according to standard recipes (cooking, baking, frying, brewing, etc.).
- After preparation, products were combined on prepared weight basis according to their consumption proportions and homogenised.
- Samples were lyophilised before analysis.

Figure 1. Preparation of the samples for the TDS study.

Methodology and results

- Patulin: extraction with ethyl acetate and analysis by LC-MSMS.
- Aflatoxin M1 (AFM1): extraction with dichloromethane, clean up with immunoaffinity columns and analysis by HPLC-Fluorescence (FLD).
- AFB1, AFB2, AFG1, AFG2: extraction with chloroform, clean up with immunoaffinity columns and analysis by HPLC-Kobra® Cell FLD.
- Trichothecenes: extraction with ACN/water (84:16), clean up with MycoSep#227, silanization with Tri-Sil TBT and analysis by GC-MS/MS.
- Multimethod for mycotoxins: extraction with ACN/water/HCOOH (84:16:1) and analysis by LC-MS/MS.

Table 1. Concentration (µg/kg) of the detected mycotoxins in the positive TDS samples.

	Tomato pure / sauce	Legume adults	Legume children	Appel sauce	Other fruit 2 adults	Dried fruit adults	Dried fruit children	Peanuts adults	Peanuts children	Other nuts adults	Other nuts children	Cream, ice cream and coffee Cream, ice cream adults	Cream, ice cream and coffee Cream, ice cream children	Blue cheese adults	Blue cheese children	Rice	Pasta	Bread adults	Bread children	Rye and cornproducts adults	Rye and corn products children	Breakfast cereals adults	Breakfast cereals children	Oils	Chocolate adults	Chocolate children	Liquorice adults	Liquorice children	Dry cake / biscuits	Coffee	Apple juice	Juices 2 adults	Juices 2 children	Beer	Wine
Aflatoxin B1	<1.0	<1.0	<1.0	<0.2	<0.2	<0.2	<0.2	+	+	<0.2	<0.2	<1.0	<1.0	<1.0	<1.0	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<1.0	<1.0	<1.0	<1.0	<0.2	<0.2
DON	<100	<2.5	<2.5	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<2.5	35	28	37	30	28	29	116	<100	<100	<100	<100	<100	55	<100	<100	<100	<100	9.1	<2.5
T2	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<5.0	<5.0	<5.0	<5.0	<5.0	<1.0	<5.0	14	+	+	<1.0	<1.0
HT2	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<20	<20	<20	<20	<20	<1.0	<20	+	<20	<20	<1.0	<1.0
AOH	8.5	<1.0	<1.0	1.2	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	2.7	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	2.9	+	1.3	2.8	+	+	<1.0	<1.0	+	+	+	<1.0	1.0
AME	+	<2.0	<2.0	+	<2.0	8.1	<2.0	5.4	3.9	4.1	5.2	<2.0	<2.0	<2.0	<2.0	3.4	3.2	<2.0	<2.0	<2.0	<2.0	3.2	7.6	+	+	+	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
BEAU	2.4	<2.0	<2.0	<2.0	2.7	2.4	<2.0	2.3	2.1	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	+	+	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
ENN B	+	<5.0	<5.0	6.6	<5.0	<5.0	<5.0	+	+	<5.0	<5.0	+	+	<5.0	<5.0	<5.0	8.7	16	17	51	58	28	16	+	+	+	<5.0	<5.0	15	<5.0	+	<5.0	<5.0	<5.0	<5.0
ENN B1	+	-	-	+	<5.0	<5.0	<5.0	+	+	<5.0	<5.0	+	+	<5.0	<5.0	<5.0	+	7.6	8.3	15	15	12	8.3	+	+	+	<5.0	<5.0	6.7	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0
FB1	<10	<10	<10	<10	<10	10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	18	+	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Micophenolic acid	<10	<10	<10	<10	<10	140	327	<10	<10	<10	<10	<10	<10	120	14	<10	<10	<10	<10	<10	<10	<10	<10	<10	+	+	24	22	<10	<10	<10	<10	<10	<10	<10
OTA	<0.5	1.7	1.0	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	1.3	1.1	<0.5	1.1	<0.5	<0.5	<0.5	<0.5	<0.5
Roquefortine C	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	552	172	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0
Sterigmatocystin	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	+	0.5	+	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Scopolamine	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	2.6	2.3	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Ergocornine+(-inine)	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	5.0	6.0	3.7	3.7	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	5.5	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Ergocristine+ (-inine)	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	+	8.4	11	7.3	4.2	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	9.9	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Ergocryptine+(inine)	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	+	6.1	5.5	5.4	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	5.5	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Ergometrine+(-inine)	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Ergosine+(-inine)	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	+	7.7	7.6	3.7	2.4	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	6.6	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Ergotamine+(-inine)	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<2.0	8.2	10	<2.0	<2.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	+	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0

(+):Peak found, but concentration lower than LOQ.

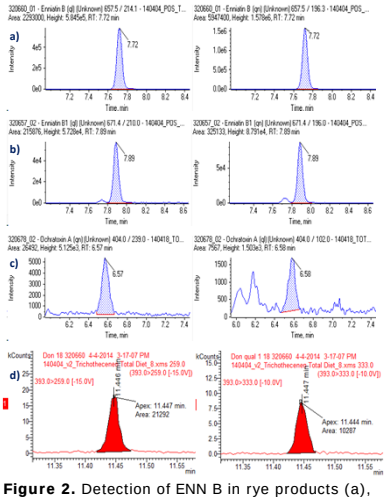

Figure 2. Detection of ENN B in rye products (a), ENN B1 in pasta (b), OTA in liquorice (c) and DON in breakfast cereals for children (d).

Conclusions

- 35 out of 88 positive samples for one or more mycotoxins.
- Grains (grain-based foods) were positive for ergot alkaloids, enniatins (ENNs) and DON.
- OTA was detected at low levels in legumes, liquorice and coffee.
- *Alternaria* toxins (AOH, AME) occurred for a wide variety of sample types: tomato sauces, nuts, grain-based products, chocolate and wine.

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