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## Single case assessment of mould toxins on popcorn kernels

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Ochratoxin A (OTA) is one of those mould toxins often referred to in literature as mycotoxins. Mould fungi of the species *Penicillium* and *Aspergillus* produce OTA. It can be found in a variety of foods or feeds, such as cereals and cereal products, legumes, coffee, grapes or wine, but also in oilseeds such as sunflower and pumpkin seeds. Chronic intake of OTA can cause kidney damage in humans. In addition, carcinogenic effects were identified in animal experiments.

The Federal Ministry of Food and Agriculture has asked the German Federal Institute for Risk Assessment (BfR) for a single case assessment. This consisted of the health assessment of a single sample of popcorn kernels with an OTA concentration of 8.7 micrograms ( $\mu\text{g}$ ) per kilogram (kg).

The BfR reached the following result in the single case assessment: Health impairments are unlikely even with long-term average or high consumption of popcorn kernels with the above-mentioned OTA concentration. However, it must be recognised that adults with a high consumption of popcorn kernels with an OTA concentration of 8.7  $\mu\text{g}$  per kg are already using up 91% of the tolerable weekly intake (TWI) of OTA. For children and adolescents, this figure is 41% and 65% respectively. The TWI may be exceeded through additional OTA intake via other OTA-containing foods. Exceeding the TWI for a prolonged period may lead to health impairments.

It should be noted that this is a single case assessment, meaning that the health assessment was based on only one sample of popcorn kernels. The maximum level of OTA pursuant to Regulation (EC) No 1881/2006 was exceeded in this particular sample of popcorn kernels. The BfR possesses no further data on OTA concentrations in popcorn kernels.

### 1 Subject of the assessment

The Federal Ministry of Food and Agriculture (BMEL) asks the German Federal Institute for Risk Assessment (BfR) to provide a health assessment of a single sample of popcorn kernels (unpopped) with an ochratoxin A (OTA) concentration of 8.7 micrograms ( $\mu\text{g}$ ) per kilogram (kg).

### 2 Results

When looking at realistic scenarios for children (3 to <5 years), adolescents (14 to <18 years) and adults (18 to <65 years), the BfR comes to the conclusion that the occurrence of a health impairment is unlikely, both with short-term high consumption as well as with a long-term average or high consumption of popcorn kernels with an OTA concentration of 8.7  $\mu\text{g}$  per kg.

However, it has to be taken into account that in the case of long-term high consumption of popcorn kernels with an OTA concentration of 8.7  $\mu\text{g}$  per kg by adults, 91% of the TWI for

OTA has already been used up, meaning that the TWI can be exceeded with additional intake of OTA via other food. When exceeding the TWI for OTA over a prolonged period, the occurrence of a health impairment is possible.

The BfR has no data on OTA concentrations in popcorn kernels from food monitoring or the BfR MEAL Study (meals for exposure assessment and analysis of food studies). The toxicological assessment relates to the particular case at hand, which exceeds the maximum level of OTA of 3 µg per kg set out in Regulation (EC) No 1881/2006 in “products derived from unprocessed cereals, including processed cereal products and cereals intended for direct human consumption”.

### 3 Rationale

#### 3.1 Description of the agent

Ochratoxin A (OTA) is a mycotoxin produced by several species of fungi of the genus *Penicillium* and *Aspergillus* and is found in various food and feed such as cereals and cereal products, legumes, coffee, grapes or wine, but also in oilseeds such as sunflower and pumpkin seeds.

Popcorn kernels refers to a special type of maize that is used to make popcorn. Popcorn kernels (unpopped) are characterized by a thin but hard shell unlike other maize varieties. When heated, the water bound inside the kernel turns into water vapour, which has a higher volume than liquid water and therefore causes the shell to burst at about 200 °C. Through heat and pressure, the starch stored inside is released explosively during production and gives the popcorn its characteristic foam-like structure.

#### 3.2 Hazard characterisation of ochratoxin A

LD<sub>50</sub> values between 0.2 milligrams (mg) per kg bodyweight (bw) (for dogs) and 30 mg per kg bw (for male rats) were determined for acute OTA toxicity, depending on the species tested (Pfohl-Leszkowicz 2007). In addition, the half-life (t/2) after oral intake differs greatly between different species. Therefore, the half-life of OTA in male Wistar rats is about five days (Dietrich 2005), while a human study with a volunteer showed a nearly ten-fold higher half-life of about 35 days (Studer-Rohr 1995).

The most sensitive endpoint after chronic intake of OTA is renal toxicity. In addition to immunotoxic and teratogenic effects, carcinogenic effects were also shown in animal experiments (EFSA 2006). In 1993, the International Agency for Research on Cancer (IARC) classified OTA as a substance of the group 2B (possibly carcinogenic to humans) (IARC 1993).

Based on a lowest observed effect level (LOEL) of 8 µg per kg bw per day for kidney toxicity in pigs as the most sensitive species, and using a safety factor of 450, the European Food Safety Authority (EFSA) adopted in 2006 a TWI (tolerable weekly intake) of 120 nanograms (ng) per kg bw as health-based guidance value for the chronic effects of OTA in humans (EFSA 2006). In 2010, EFSA assessed new studies on the toxicology of OTA, but the TWI remains the same (EFSA 2010).

An acute reference dose (ARfD) as health-based guidance value for the acute effects of OTA in humans has not been derived by EFSA.

### 3.3 Exposure assessment

#### 3.3.1 Description of a single sample of popcorn kernels with an OTA concentration of 8.7 µg per kg

In an official sample of popcorn kernels (unpopped) taken in Baden-Württemberg, a concentration of ochratoxin A of 8.7 ( $\pm$  2.7) µg per kg was found.

The BfR has no data on OTA concentrations in popcorn kernels from food monitoring or the BfR MEAL Study (meals for exposure assessment and analysis of foods), so that it is not possible to compare this particular sample to other samples.

The exposure assessment described in the following and subsequent toxicological assessment relates to the particular case at hand which exceeds the maximum level of OTA of 3 µg per kg set out in Regulation (EC) No 1881/2006 in “products derived from unprocessed cereals, including processed cereal products and cereals intended for direct human consumption”.

#### 3.3.2 Data used for determination of consumption data

##### 3.3.2.1 Consumption data for children according to the VELS study

Data on the consumption of popcorn kernels for children were taken from the VELS study (nutrition survey to obtain consumption data for infants and young children for the assessment of an acute toxicity risk from residues of plant protection products) (Heseker et al., 2003; Banasiak et al., 2005). Between 2001 and 2002, the study was conducted on 816 infants and toddlers between the ages of 6 months and less than 5 years all over Germany. The parents recorded all consumed food in two three-day nutritional protocols for each child. The consumption data of the children between 3 and under 5 years were used for the present intake calculation. Due to the presence of single-day consumption data, two three-day nutritional protocols are suitable for exposure assessments for both acute and chronic risks.

##### 3.3.2.2 Consumption data for adolescents and adults in accordance with NVS II

The National Nutrition Survey II (NVS II) is currently the most recent representative study on the consumption of the population in Germany. The study, which surveyed about 20,000 individuals between the ages of 14 and 80 using three different survey methods (dietary history, 24-hour recall and weighing protocol), took place between 2005 and 2006 throughout Germany (MRI 2008). The evaluations presented here are based on the data of the two independent 24-hour recalls of NVS II, which were collected in a computer-assisted interview using “EPIC-SOFT” (MRI 2008, Krems et al., 2006). Data was evaluated from 13,926 people for whom both interviews were available. Due to the presence of single-day consumption data, the method of 24-hour recall is suitable for exposure assessments for both acute and chronic health risks. The intake assessments were based on the individual bodyweight of the respondents.

#### 3.3.3 Assessment of short-term intake of OTA via the consumption of popcorn kernels

Popcorn kernels (indicated as “Puffmais” in the VELS study) are consumed by approximately 12.5% (N = 37) of the group of children from 3 to <5 years. For an estimation of short-term intake, the maximum consumption for all survey days is determined on an individual level and

the 95<sup>th</sup> percentile of the amounts consumed is determined in relation to all popcorn consumers which, according to the VELS study, totals 5.0 grams (g) per kg bw per day (Tab. 1).

For children of 3 to <5 years, with an OTA concentration of 8.7 µg per kg in the consumed popcorn kernels, an intake of 5.0 g of popcorn kernels per kg bw per day resulted in an intake of 43.5 ng of OTA per kg bw per day (Tab. 1).

**Tab. 1: Short-term intake of OTA via short-term high consumption of popcorn kernels in children (3 to <5 years) according to the VELS study and for adolescents (14 to <18 years) and adults (18 to <65 years) in accordance with NVS II with an OTA concentration of 8.7 µg per kg**

	Short-term high consumption of popcorn kernels [g per kg bw per day]	Short-term intake of OTA [ng per kg bw per day]
Children	5.0	43.5
Adolescents	2.5*	21.8
Adults	3.6*	31.3

\* Consumption data from the European food consumption database of EFSA

For adolescents and adults, the BfR has not carried out its own evaluation, but refers to the evaluations of the consumption data from NVS II in the European food consumption database of EFSA, which contains the German data from NVS II. Accordingly, popcorn kernels are consumed by 1.6% (N = 16) and 0.6% (N = 59) of adolescents (14 to <18 years) and adults (18 to <65 years) respectively. For EFSA, each day of consumption was considered as a case for each test subject, from which the 95<sup>th</sup> percentile is determined which, according to NVS II, is 2.5 g per kg bw per day for adolescents and 3.6 g per kg bw per day for adults (Tab. 1).

Based on these consumption levels, an OTA concentration of 8.7 µg per kg in the consumed popcorn kernels results in a short-term intake of 21.8 ng of OTA per kg bw per day in adolescents and 31.3 ng per kg bw per day in adults (Tab. 1).

### 3.3.4 Assessment of long-term intake of OTA via the consumption of popcorn kernels

Popcorn kernels are consumed by approximately 12.5% (N = 37) of the group of children from 3 to <5 years. For the estimation of long-term intake, the average consumption over all survey days (two three-day periods) is determined on an individual basis and from this the median of the consumption levels was calculated for average consumers, whilst the 95<sup>th</sup> percentile is calculated for high consumers, in each case based on all popcorn consumers according to the VELS study. This results in consumption levels of 0.04 g per kg bw per day for average consumption and 0.8 g per kg bw per day for high consumers (Tab. 2).

**Tab. 2: Long-term intake of OTA via long-term consumption (differentiated by average and high consumption) of popcorn kernels in children (3 to <5 years) according to the VELS study and for adolescents (14 to <18 years) and adults (18 to <65 years) in accordance with NVS II with an OTA concentration of 8.7 µg per kg**

	Long-term consumption of popcorn kernels [g per kg bw per day]		Long-term intake of OTA [ng per kg bw per week]	
	Average consumers	High consumers	Average consumers	High consumers
Children	0.04	0.8	2.4	48.7
Adolescents	0.31	1.27	18.7	77.4
Adults	0.36	1.79	21.9	109.0

For children of 3 to <5 years, this results in intake levels of 2.4 ng of OTA per kg bw per week for average consumers and 48.7 ng of OTA per kg bw per week for the high consumers with the application of the present intake levels and the assumption of an OTA concentration of 8.7 µg per kg within the consumed popcorn kernels (Tab. 2).

According to NVS II, popcorn kernels are consumed by 1.6% (N = 16) and 0.6% (N = 59) of adolescents (14 to <18 years) and adults (18 to <65 years) respectively. For the estimation of long-term intake, the average consumption over both survey days is determined on an individual basis and from this the median of the consumption levels was calculated for average consumers, whilst for high consumers the 95<sup>th</sup> percentile is calculated, in both cases based on all popcorn consumers. This results in an average intake of 0.31 g per kg bw per day for average consumption and a high consumption of 1.27 g per kg bw per day in adolescents (Tab. 2). Amongst adults, a similar method results in consumption levels of 0.36 g per kg bw per day for average consumption and 1.79 g per kg bw per day for high consumers (Tab. 2).

For adolescents of 14 to <18 years, this results in intake levels of 18.7 ng of OTA per kg bw per week for average consumers and 77.4 ng of OTA per kg bw per week for the high consumers with the application of the present intake levels and the assumption of an OTA concentration of 8.7 µg per kg within the consumed popcorn kernels (Tab. 2). For adults of 18 to <65 years, this results in intake levels of 21.9 ng of OTA per kg bw per week for average consumers and 109 ng of OTA per kg bw per week for high consumers due to the higher intake per kg bw (Tab. 2).

### 3.4 Risk characterisation

Potential **short-term high consumption** (95<sup>th</sup> percentile of maximum consumption) of popcorn kernels at 5.0 g per kg bw per day with an OTA concentration of 8.7 µg per kg would result in an OTA intake of 43.5 ng OTA per kg bw per day for a **child** of 3 to <5 years. When consumed once, 36% of the TWI for OTA of 120 ng per kg bw is used up (Tab. 3).

Potential **short-term high consumption** (95<sup>th</sup> percentile of maximum consumption) of popcorn kernels at 2.5 g per kg bw per day for an **adolescent** (14 to <18 years) or 3.6 g per kg bw per day for an **adult** (18 to <65 years) with an OTA concentration of 8.7 µg per kg would result in an OTA intake level of 21.8 ng of OTA per kg bw per day or 31.3 ng per kg bw per day. When consumed once, 18 % or 26% of the TWI for OTA of 120 ng per kg bw are used up, respectively (Tab. 3).

**Tab. 3: Extent to which the TWI for OTA of 120 ng per kg bw is used up with short-term high consumption of popcorn kernels with an OTA concentration of 8.7 µg per kg in children (3 to <5 years), adolescents (14 to <18 years) and adults (18 to <65 years)**

	Short-term intake of OTA [ng per kg bw per day]	Extent to which the TWI for OTA is used up [%]
Children	43.5	36
Adolescents	21.8	18
Adults	31.3	26

Potential **long-term average consumption** (median of average consumption) of popcorn kernels with an OTA concentration of 8.7 µg per kg, would result in an OTA intake of 2.4 ng per kg bw per week for a **child** of 3 to <5 years, 18.7 ng per kg bw per week for a an **adolescent** of 14 to <18 years and 21.9 ng per kg bw per week for an **adult** of 18 to <65 years (Tab. 4). This results in rates of 2%, 16% and 18% of the TWI being used up respectively (Tab. 4).

Potential **long-term high consumption** (95<sup>th</sup> percentile of average consumption) of popcorn kernels with an OTA concentration of 8.7 µg per kg results in an OTA intake of 48.7 ng per kg bw per week for a **child** of 3 to <5 years, 77.4 ng per kg bw per week for an **adolescent** of 14 to <18 years and 109.0 ng per kg bw per week for an **adult** of 18 to <65 years (Tab. 4). This results in rates of 41 %, 65 % and 91 % of the TWI being used up respectively (Tab. 4).

**Tab. 4: Extent to which the TWI for OTA of 120 ng per kg bw is used up with long-term consumption (differentiated by average and high consumption) of popcorn kernels with an OTA concentration of 8.7 µg per kg in children (3 to <5 years), adolescents (14 to <18 years) and adults (18 to <65 years)**

	Long-term intake of OTA [ng per kg bw per week]		Extent to which the TWI for OTA is used up [%]	
	Average consumers	High consumers	Average consumers	High consumers
Children	2.4	48.7	2	41
Adolescents	18.7	77.4	16	65
Adults	21.9	109.0	18	91

The consumption data used and therefore also the associated results of the health assessment are limited in scope to popcorn kernel consumers, as the proportion of consumers amongst the total population is 12.5% in children (3 to <5 years), 1.6% in adolescents (14 to <18 years) and 0.6% in adults (18 to <65 years). As a consequence, only these consumers are exposed to OTA in popcorn kernels.

The health assessment of the BfR is based on the consideration of conservative exposure scenarios which generally lead to an overestimation rather than an underestimation of exposure. The toxicological assessment relates to the particular case at hand, which exceeds the maximum level of OTA of 3 µg per kg set out in Regulation (EC) No 1881/2006 in “products derived from unprocessed cereals, including processed cereal products and cereals intended for direct human consumption”. Therefore, it cannot be assumed that, in the case of long-term consumption of popcorn kernels, all the consumed popcorn kernels will have an OTA concentration at this particular level.

No acute reference dose (ARfD) for OTA was derived as health-based guidance value for the assessment of acute effects after a single, high short-term intake, therefore health assessment can be performed only in comparison with the TWI, which was derived for the assessment of long-term chronic effects. In the case of high-consumption (single high, short-term intake), all three age groups under consideration remain well below the TWI. Therefore, the BfR concludes that the occurrence of a health impairment after a single intake of popcorn kernels with an OTA concentration of 8.7 µg per kg is unlikely.

When looking at realistic scenarios for children (3 to <5 years), adolescents (14 to <18 years) and adults (18 to <65 years), the BfR comes to the conclusion that the occurrence of a health impairment after long-term average or high consumption of popcorn kernels, with an OTA concentration of 8.7 µg per kg, is unlikely.

However, it should be taken into account in the health assessment of the long-term intake of OTA that popcorn kernels are not the only entry pathway for OTA and that OTA can also be taken up by consuming other foods. In the case of adults, long-term high consumption of popcorn kernels with an OTA concentration of 8.7 µg per kg leads to 91% of the TWI for OTA being used up, meaning that the TWI can be exceeded with additional intake of OTA via

other food. When exceeding the TWI for OTA over a prolonged period, the occurrence of a health impairment is possible.

#### Further information on this subject on the BfR website...

The effects of processing on mycotoxin concentrations in cereal-based food and feed (in German):

<https://www.bfr.bund.de/cm/343/auswirkungen-der-verarbeitung-auf-mykotoxingehalte-in-getreidebasierten-lebens-und-futtermitteln.pdf>

Mould toxins in food - how to protect yourself (in German):

<https://www.bfr.bund.de/cm/350/schimmelpilzgifte-in-lebensmitteln-so-koennen-sie-sich-schuetzen.pdf>



BfR "Opinions app"

## 4 References

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### **About the BfR**

The German Federal Institute for Risk Assessment (BfR) is a scientifically independent institution within the portfolio of the Federal Ministry of Food and Agriculture (BMEL) in Germany. It advises the Federal Government and Federal Laender on questions of food, chemical and product safety. The BfR conducts its own research on topics that are closely linked to its assessment tasks.

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