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Colophon

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Publiekssamenvatting

De inname van mycotoxinen in voedsel geschat via metingen in urine

Schimmels die bijvoorbeeld op granen, noten en vruchten groeien, kunnen giftige stoffen maken die ons voedsel verontreinigen (mycotoxinen). Door de lage concentraties vormen ze doorgaans geen gevaar voor de gezondheid, maar het is wel van belang om te volgen in welke mate mensen deze mycotoxinen via voedsel consumeren. Een tijdrovende manier om dat te doen is door alle voedingsmiddelen door te meten, maar het kan ook in urine en bloed worden gemeten.

RIVM-onderzoek maakt nu duidelijk onder welke voorwaarden via urine kan worden berekend hoeveel mycotoxinen mensen via voedsel binnenkrijgen. De snelheid waarmee mycotoxinen via de urine worden uitgescheiden verschilt namelijk per mycotoxine en is daardoor van invloed op de manier waarop en wanneer de urine moet worden opgevangen.

In dit onderzoek is ingezoomd op twee mycotoxinen die sterk verschillen in de snelheid waarmee ze via de urine worden uitgescheiden. Het ene mycotoxine (afgekort DON) wordt snel, binnen 24 uur, uitgescheiden. Hiervoor moet de urine worden verzameld gedurende 24 uur nadat het verontreinigde voedsel is gegeten. Het maakt daarbij niet uit of de consument DON gedurende lange of korte tijd voorafgaand aan de urinecollectie heeft geconsumeerd.

Het andere mycotoxine (afgekort OTA) wordt gedurende enkele maanden uitgescheiden. Daardoor heeft het geen zin om de concentratie in urine te meten als de consument maar kort aan OTA is blootgesteld, bijvoorbeeld enkele dagen of weken. Meten in urine is alleen zinvol als een consument gedurende enkele maanden dagelijks OTA binnenkrijgt. In dat geval is de manier waarop urine wordt verzameld minder belangrijk dan bij DON: het kan door 24 uursurine of ochtendurine te verzamelen.

Kernwoorden: mycotoxinen, voedsel, inname, biomonitoring, urine, uitscheiding, toxicokinetiek.

Synopsis

Estimation of the dietary intake of mycotoxins by means of measurements in human urine

Moulds that grow on for example grains, nuts and fruits may produce toxic substances that contaminate our food. These toxic substances are called mycotoxins. Because of the low concentrations in food, there is generally no risk for human health but it remains important to monitor to what extent people are exposed to mycotoxins via food intake. A time-consuming way to know how much mycotoxins are ingested is to measure all relevant food products but it can also be measured in urine.

RIVM research points out the conditions that must be met to be able to estimate the dietary intake of mycotoxins by means of measurements in human urine. The rate of renal excretion between the various mycotoxins differs and this rate determines the method and frequency of urine collection.

This research focussed on two mycotoxins that differ greatly in the renal excretion rate. One mycotoxin (abbreviated as DON) is rapidly excreted via urine: within 24 hours. Therefore, it is necessary to collect urine during 24 hours after ingestion of DON. It doesn't matter how long the consumer has been exposed to DON prior to urine collection.

The other mycotoxin (abbreviated as OTA) is excreted slowly via urine: within a few months. Therefore, it is not useful to measure its concentration in urine if the consumer has ingested OTA for a short period (e.g. for a few days or weeks). If the consumer has ingested OTA daily for several months, it is useful to measure its concentration in urine. In this case the method of collecting urine is less important than for DON. Urine may be collected during 24 hours or in the morning.

Keywords: mycotoxins, food, intake, biomonitoring, urine, excretion, toxicokinetics.

Contents

Summary

Mycotoxins are substances produced by micro-fungi growing on plants and plant products during production and storage. Examples of fungi producing mycotoxins are *Aspergillus*, *Penicillium* and *Fusarium*. The production of mycotoxins depends on the plant, the fungal species and climate conditions (temperature and humidity).

Mycotoxins can have detrimental health effects in animals and humans, with toxicity depending on the type of mycotoxin, the ingested dose, the exposure duration and characteristics of the exposed individual. Therefore, assessment of the health risks caused by exposure to mycotoxin is necessary.

Previous mycotoxin exposure assessments in the Netherlands were based on concentration data obtained from monitoring and survey programmes combined with food consumption data, from duplicate diet studies or from a mycotoxin dedicated total diet study.
Another approach, not explored before in the Netherlands for mycotoxins, is human biomonitoring. Human biomonitoring (HBM) measures the levels of substances in body fluids and tissues. HBM can be used to estimate the internal and external exposure of humans to substances. One of the advantages of HBM is that it is not necessary to determine mycotoxin concentrations in food and food consumption data for the estimation of the dietary mycotoxin intake. However, several conditions must be met to be able to estimate the dietary intake by means of measuring renal mycotoxin excretion.

The goal of this research is to determine the feasibility of estimating the dietary mycotoxin intake by determining mycotoxin excretion, including their (major) metabolites, in urine. In other words, to test the applicability of (urinary) biomarkers of (mycotoxin) exposure. Secondly, to develop a calculator in Excel that can be used by people who are not familiar with toxicokinetic models but who are interested to see (visually) how dietary mycotoxin intake is related to mycotoxin levels in urine. The applicability of several methods of urine collection are discussed: morning urine, spot urine and 24-hour urine. The applicability is based on toxicokinetic models that predict urinary levels in humans after consumption of a certain amount of mycotoxin. Different theoretical models have been applied: a model for a rapidly eliminated mycotoxin, namely deoxynivalenol (DON) and a model for a slowly eliminated mycotoxin, namely ochratoxin A (OTA). These different elimination profiles have been chosen to show the consequences for the method of urine collection and its applicability to estimate the dietary mycotoxin intake.

For DON it is necessary to collect urine during 24 hours after ingestion of DON. It doesn't matter how long the consumer has been exposed to DON. For OTA it is not useful to measure its concentration in urine if the consumer has ingested OTA for a short period (e.g. for a few days or weeks). If the consumer has ingested OTA daily for several months it is useful to measure its concentration in urine. In this case the method of collecting urine is less important. Urine may be collected during 24 hours or in the morning.

1   Introduction

Mycotoxins are substances produced by micro-fungi growing on plants and plant products during production and storage. Examples of fungi producing mycotoxins are *Aspergillus*, *Penicillium* and *Fusarium*. The production of mycotoxins depends on the plant, the fungal species and climate conditions (temperature and humidity). The latter conditions are responsible for the frequently observed seasonal differences in mycotoxin concentrations (Marroquín-Cardona *et al.*, 2014).

Mycotoxins can have detrimental health effects in animals and humans, with toxicity depending on the type of mycotoxin, the ingested dose, the exposure duration and characteristics of the exposed individual. Therefore, assessment of the health risks caused by exposure to mycotoxin is necessary. Risk assessment is carried out by comparing the mycotoxin exposure with the health-based guidance value derived for that mycotoxin.

Previous mycotoxin exposure assessments in the Netherlands were based on concentration data obtained from monitoring and survey programmes combined with food consumption data (Boon *et al.*, 2009), from duplicate diet studies (Bakker *et al.*, 2009; Jekel and van Egmond, 2014) or from a mycotoxin dedicated total diet study (Sprong *et al.*, 2015).
Another approach, not explored before in the Netherlands for mycotoxins, is human biomonitoring. Human biomonitoring (HBM) measures the levels of substances in body fluids and tissues (EFSA, 2015a). HBM can be used to estimate the external and internal exposure of humans to substances. One of the advantages of HBM is that it is not necessary to determine mycotoxin concentrations in food and food consumption data for the estimation of the dietary mycotoxin intake. However, several conditions must be met to be able to estimate the dietary intake by means of measuring renal mycotoxin excretion (e.g. toxicokinetic parameter estimation, method of urine collection, etc.).

The goal of this research is to determine the feasibility of estimating the dietary mycotoxin intake by determining mycotoxin excretion, including their (major) metabolites, in urine. In other words, to test the applicability of (urinary) biomarkers of (mycotoxin) exposure. Secondly, to develop a calculator in Excel that can be used by people who are not familiar with toxicokinetic models but who are interested to see (visually) how dietary mycotoxin intake is related to mycotoxin levels in urine. The applicability of several methods of urine collection are discussed: morning urine, spot urine and 24-hour urine. The applicability is based on toxicokinetic models that predict urinary levels in humans after consumption of a certain amount of mycotoxin. Different theoretical models have been applied: a model for a rapidly eliminated mycotoxin, namely deoxynivalenol (DON) and a model for a slowly eliminated mycotoxin, namely ochratoxin A (OTA). These different elimination profiles have been chosen to show the consequences for the method of urine collection and it's applicability to estimate the dietary mycotoxin intake.

The models are made in Excel files that enable the user to edit some input parameters and view the corresponding output: a graph of the simulated urinary mycotoxin levels over time. The user may vary model parameters like dietary mycotoxin intake, body weight, mycotoxin fraction absorbed, absorption and elimination half-life, renal excretion, urine production, etc. The resulting output is depicted in three types of graphs: plasma mycotoxin levels, urinary mycotoxin levels and the cumulative amount excreted in urine over time. The latter is a direct measure for the internal dose (this equals mycotoxin *up*take) and can be used to estimate the dietary *in*take (as long as bioavailability is known or can be estimated).

Usually these kinetic models are based on a fixed dose over time e.g. a daily dose of *x* mg or *y* mg/kg body weight or a fixed dose several times a day (*z* mg every 8 hours, i.e. three times *z* mg per day). However, in real life a person consumes different doses of mycotoxins during the day. For example: DON in breakfast cereals and/or a different dose of DON in bread consumed at lunch and/or another dose of DON in pasta used for the evening meal. Therefore, we also developed a different type of model where the dose can be altered manually per dosing interval or where the dose is varied at random (within a certain range) per dosing interval by the Excel program. Again, the effect of this fluctuating dosage regimen is visually depicted in the above-mentioned types of graphs.

These models enable us to determine the preferred method of urine collection and test its (theoretical) applicability to estimate the internal mycotoxin dose that is relevant for toxicity and to estimate the dietary mycotoxin intake.

2 DON: a model for a fast eliminating mycotoxin

2.1 Introduction

The mycotoxin deoxynivalenol (DON), also known as vomitoxin, is a trichothecene produced by fungi belonging to the *Fusarium* species. These fungi grow on plants and can cause *Fusarium* head blight on living crops. DON is commonly found in cereals and mixed feeds. A survey within the European Union revealed that 44% of food samples tested was contaminated with DON, demonstrating the frequent contamination (EFSA, 2013).
When ingested, DON may cause adverse effects on the gastro-intestinal tract, the nervous system, and the immune system (Maresca, 2013). Based on the most critical effect (decreased weight gain), a tolerable daily intake (TDI) of 1 µg/kg body weight (bw) per day was set based on a 2-year feeding study in mice (JECFA, 2010; SCF, 2002). In 2010, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) extended this TDI to a group provisional maximum tolerable daily intake (PMTDI) including the derivatives 3-actyldeoxynivalenol (3-ADON) and 15-acetyldeoxynivalenol (15-ADON). In addition, a group acute reference dose (ARfD) of 8 µg/kg bw/day for DON, 3-ADON and 15-ADON was established (JECFA, 2010).

2.1.1 Toxicokinetics of DON

The toxicokinetics of DON were studied in several animal species (EFSA, 2015b). In most animals, including farm animals like pigs, DON is well and rapidly absorbed. After absorption, DON can be metabolized albeit to a limited extent, depending on the species, to deepoxy-deoxynivalenol (DOM-1). In most species, DON and DOM-1 are extensively and rapidly glucuronidated (phase 2 biotransformation) and subsequently excreted via the urine.
Unfortunately, well-performed kinetic studies with DON in humans have not been published. Excretion of DON in human urine after consumption of contaminated food has been studied (Turner *et al.*, 2010; Warth *et al.*, 2013) but controlled studies with measured intake and (urinary) excretion over time are lacking. Nevertheless, from biomonitoring studies in humans it is clear that also in humans, DON is extensively glucuronidated and these glucuronides are rapidly excreted (more than 80% of the ingested dose within 24 hours). In our models the default value for the elimination half-life of DON is 3 hours to make sure that 24 hours after ingestion of DON approximately 99% of the dose is renally excreted.
The excretion of DOM-1 was not modelled because it is considered a minor metabolite in humans (EFSA, 2015b).

2.2 Toxicokinetic models applied for DON

2.2.1 Introduction

Based on the limited available information on the toxicokinetics of DON a simple one-compartment model is developed.
A fraction of dietary DON intake is absorbed across the gut wall with a finite absorption half-life, so no instantaneous absorption is assumed. Distribution of DON is assumed to be fast with respect to absorption,

metabolism and excretion kinetics and consequently the human body is considered as one compartment. In this model it is assumed that DON is only metabolised through glucuronidation. Furthermore it is assumed that (a fraction of) non-metabolised DON and DON-glucuronide are only excreted renally. More technical details on this model are found in section 7.1.

2.2.2   *Excel calculator for DON*
A calculator is made in Excel and it is intended for a few exercises to show differences in cumulative excretion of DON and DON-glucuronide when varying some model parameters like body weight, DON absorption and elimination half-life, percentage of non-metabolised DON excretion, DON distribution volume, dietary DON intake, fraction of DON absorbed across the gut wall and urine production. DON is administered once, twice or three times a day (at breakfast, lunch and dinner).
In the Excel file a standard and an actual model are presented. The standard model is a reference model and contains default values that cannot be changed. For the actual model the above-mentioned parameters values can be edited be the user.
The output of the standard and the actual model are compared in one figure. These outputs are: 1) concentration-time curves of DON in plasma, 2) urinary concentrations of DON after every three hours of sampling and 3) respectively 4) cumulative 24-hours excretion of DON and its glucuronides over time as percentage of DON dose.
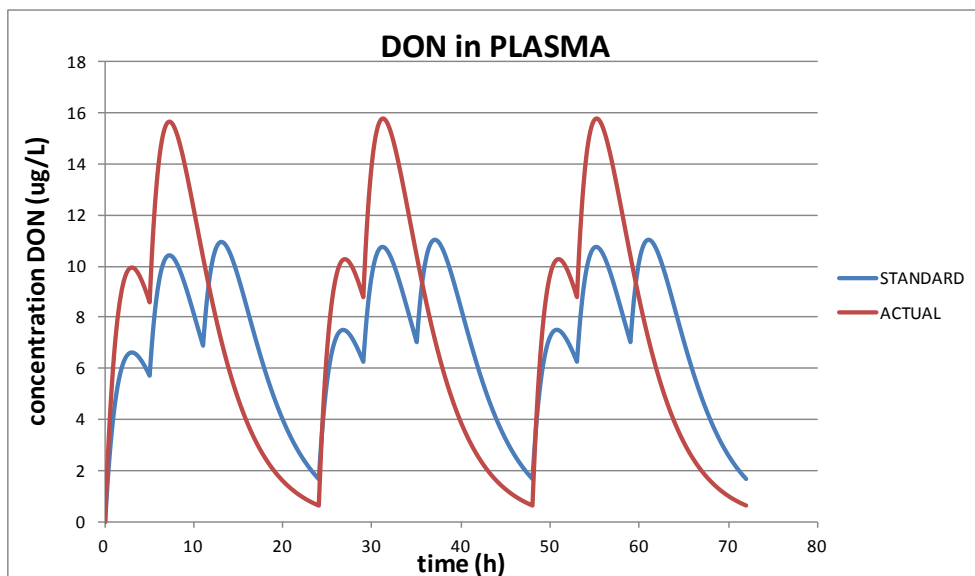The graphical presentations are given in figures 1-4.

Figure 1. Concentration-time curve of DON in plasma after administration of a fixed dose of DON three times a day (standard scenario; blue line) or two times a day (actual scenario; red line) during three consecutive days.
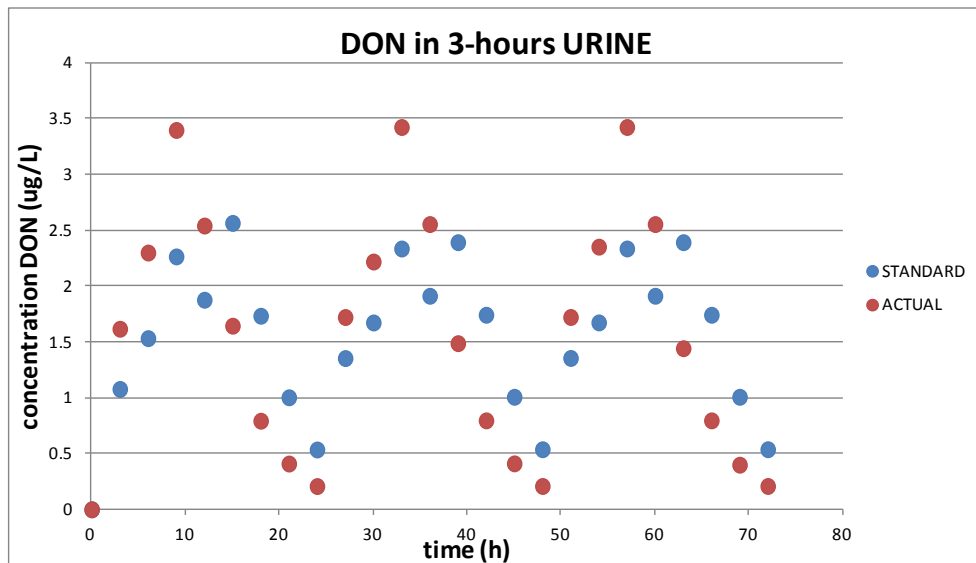
Figure 2. Urinary concentrations of DON per sampling interval (every 3 hours) after administration of a fixed dose of DON three times a day (standard scenario; blue dots) or two times a day (actual scenario; red dots) during three consecutive days.
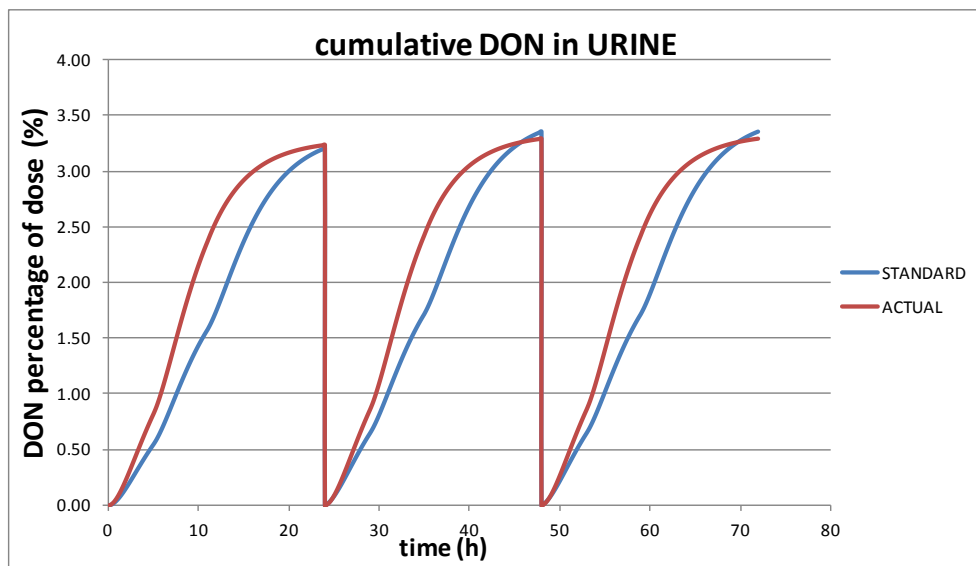

Figure 3. Cumulative excretion of DON in urine after administration of a fixed dose of DON three times a day (standard scenario; blue line) or two times a day (actual scenario; red line) during three consecutive days.
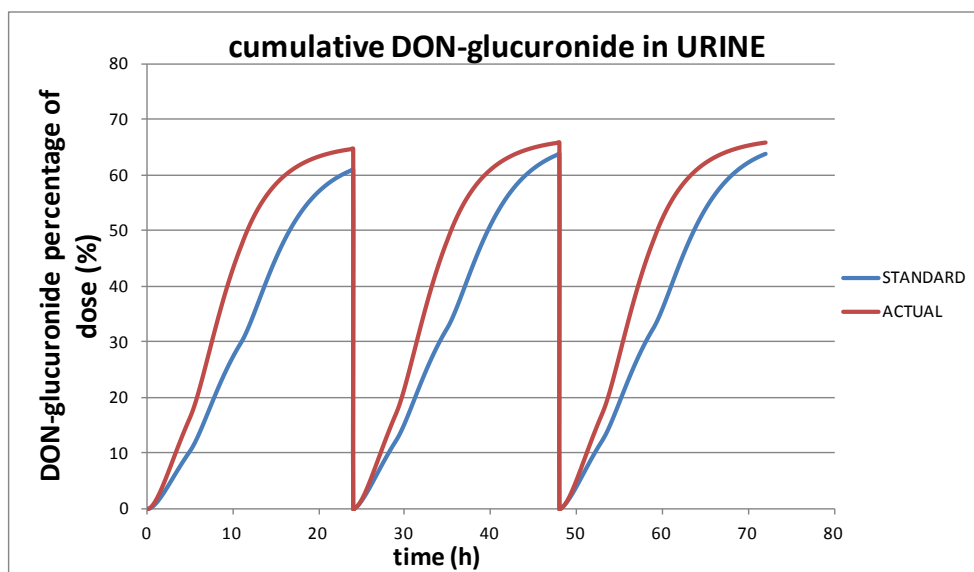
Figure 4. Cumulative excretion of DON-glucuronide in urine after administration of a fixed dose of DON three times a day (standard scenario; blue line) or two times a day (actual scenario; red line) during three consecutive days.

The Excel file contains two sheets, both with standard and actual scenarios. The second sheet "DON-expert" leaves as much freedom as possible to the user with respect to the daily intake scenario, but requires some Excel skills. The first sheet "DON-easy" restricts the freedom, but is easier for the less experienced Excel user. In the "DON-easy" sheet the user can choose for daily intake to be at an actual constant level that is consumed 1, 2 or 3 times a day, or to let it vary randomly between 50% and 150% around an actual mean level. The daily meal scheme is fixed throughout.
The graphical presentations are given in figures 5-8.

Figure 5. Concentration-time curve of DON in plasma after administration of a fixed dose of DON three times a day (standard scenario; blue line) or with a random dose two times a day (actual scenario; red line) during three consecutive days. Mean level of the random dose equals the fixed dose of the standard scenario.
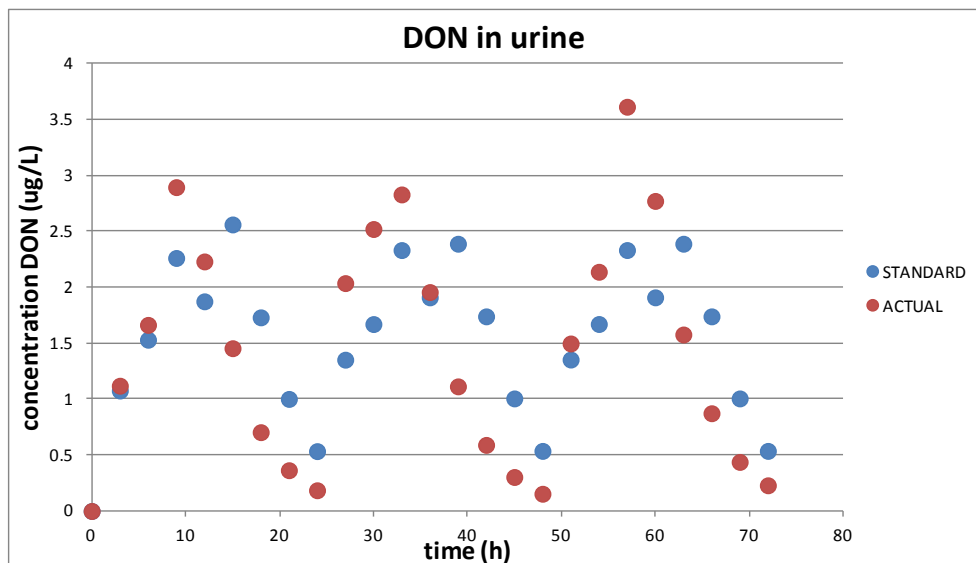
Figure 6. Urinary concentrations of DON per sampling interval (every 3 hours) after administration of a fixed dose of DON three times a day (standard scenario; blue dots) or a random dose two times a day (actual scenario; red dots) during three consecutive days.

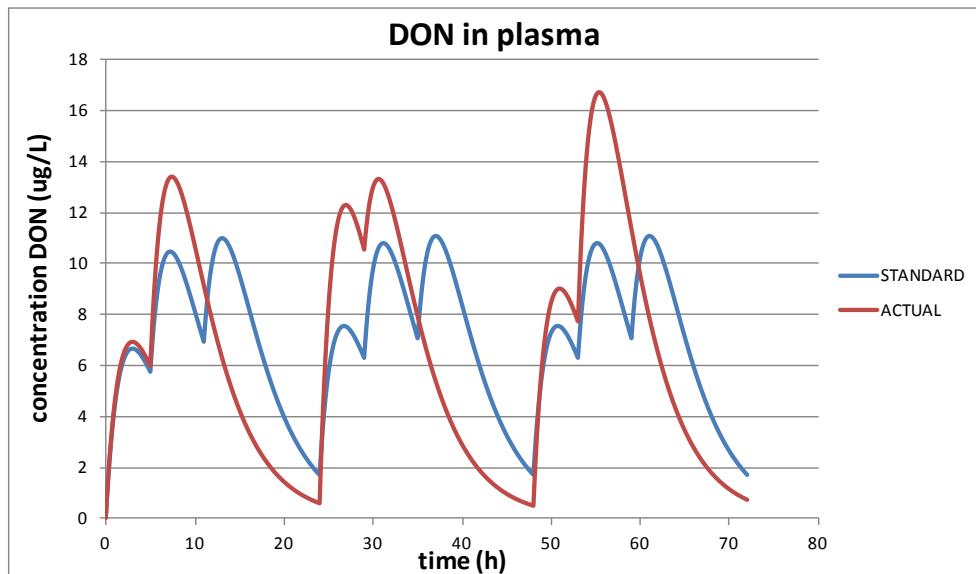*Figure 7. Cumulative excretion of DON in urine after administration of a fixed dose of DON three times a day (standard scenario; blue line) or a random dose two times a day (actual scenario; red line) during three consecutive days.*

Figure 8. Cumulative excretion of DON-glucuronide in urine after administration of a fixed dose of DON three times a day (standard scenario; blue line) or a random dose two times a day (actual scenario; red line) during three consecutive days.
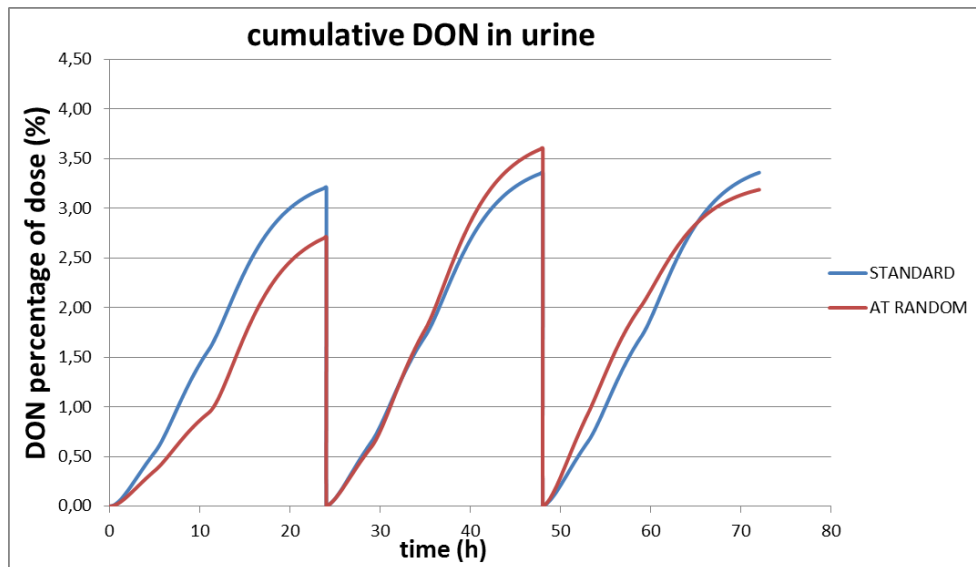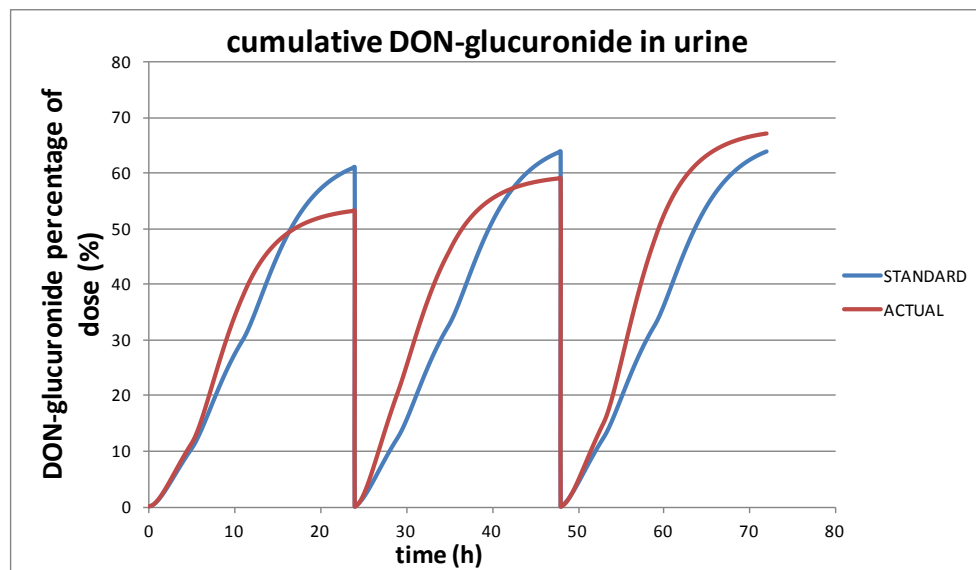
3 OTA: a model for a slow eliminating mycotoxin

3.1 Introduction

The mycotoxin ochratoxin A (OTA) is a pentaketide produced by fungi belonging to the *Aspergillus* and *Penicillium* species. The production of OTA during storage is the most important factor of OTA contamination in food. It has been found in cereals, coffee beans and other plant products.

Various studies in pigs and humans have associated OTA with endemic porcine nephropathy and Balkan endemic nephropathy (Pfohl-Leszkowicz and Manderville, 2007). OTA accumulates in the body, in particular in the kidneys. OTA is teratogenic and the International Agency on Cancer classified OTA in group 2b as possible carcinogen for humans (IARC, 1993).

Based on renal effects observed in a 90-day feeding study in female pigs a tolerable weekly intake (TWI) of 0.12 μg/kg body weight (bw) was derived by EFSA (EFSA, 2006). EFSA applied a composite uncertainty factor of 450 to a LOAEL of 8,000 ng OTA/kg bw per day which would result in a tolerable daily intake (TDI) of 17.8 ng OTA/kg bw per day. Given the relatively long half-life of OTA in humans, EFSA concluded that a tolerable <u>weekly</u> intake would be more appropriate. Therefore, a TWI of 120 ng/kg bw was established. In turn, we used as default value, a tolerable daily dose in our toxicokinetic model of 17.8 ng/kg bw/day.

3.1.1 Toxicokinetics of OTA

Following oral ingestion, OTA is rapidly absorbed and reaches the systemic circulation, where it is extensively bound to plasma proteins. In animal studies the extent of absorption varies between 40% in chickens and 66% in pigs (Galtier *et al.*, 1981). In most animal species, the kinetic behaviour of OTA has been described as a two-compartment open model (Dietrich *et al.*, 2005).

The toxicokinetic profile of OTA was studied in one human volunteer following ingestion of 395 ng ^{3}H-labelled OTA (3.8 μCi) (Studer-Rohr *et al.*, 2000). A two-compartment open model was found to describe the *in vivo* data. This two-compartment model consisted of a fast elimination and distribution phase ($t_{1/2}$ about 20 hours) followed by a slow elimination phase (renal clearance about 0.11 mL/min) and a calculated plasma half-life of 36 days. The major analyte in blood serum is the parent compound, and only small concentrations of OTA metabolites and/or conjugates could be measured (Studer-Rohr *et al.,* 2000). In contrast, analysis of urine samples indicated that only about 50% of the radioactivity in the urine was parent OTA, suggesting the presence of OTA metabolites (particularly ochratoxin α) and/or OTA glucuronic acid conjugates. However, in a recent biomonitoring study carried out in Belgium with 394 volunteers no significant amounts of metabolites of OTA were detected in urine (Heyndrickx *et al.*, 2015).

3.2 Toxicokinetic models applied for OTA

3.2.1 Introduction

Based on the publication of Studer-Rohr on the toxicokinetics of OTA in a human volunteer a two-compartment model is developed (Studer-Rohr *et al.*, 2000).

A fraction of dietary OTA intake is absorbed across the gut wall with a finite absorption half-life, so no instantaneous absorption is assumed. OTA is distributed over a central and a peripheral compartment. In this model it is assumed that the metabolism of OTA is negligible and that OTA is only excreted renally. More technical details on this model are found in section 7.2.

3.2.2 Excel calculator for OTA with a fixed dose

A calculator is made in Excel and it is intended for a few exercises to show differences in cumulative excretion of OTA when varying some model parameters like body weight, OTA distribution and elimination half-life, percentage of non-metabolised OTA excretion, distribution volume, dietary OTA intake and urine production. OTA is administered once a day (at breakfast).

In the Excel file a standard and an actual model are presented. The standard model is a reference model and contains default values that cannot be changed. The actual model contains the above-mentioned parameters and their values can be edited be the user.

The output of each model is presented in separate graphs. These outputs are: 1) concentration-time curves of OTA in plasma, 2) urinary concentrations of OTA and 3) cumulative excretion of OTA over time. Some graphical presentations are given in figures 9-12. In the actual curves the daily dose is given as a bolus at the start of the day and in the standard curves the daily dose is spread over 24 hours. In figure 9 the accumulation during the first seven days is depicted because these details are not clearly visible on a time-scale of 200 days (figure 10).
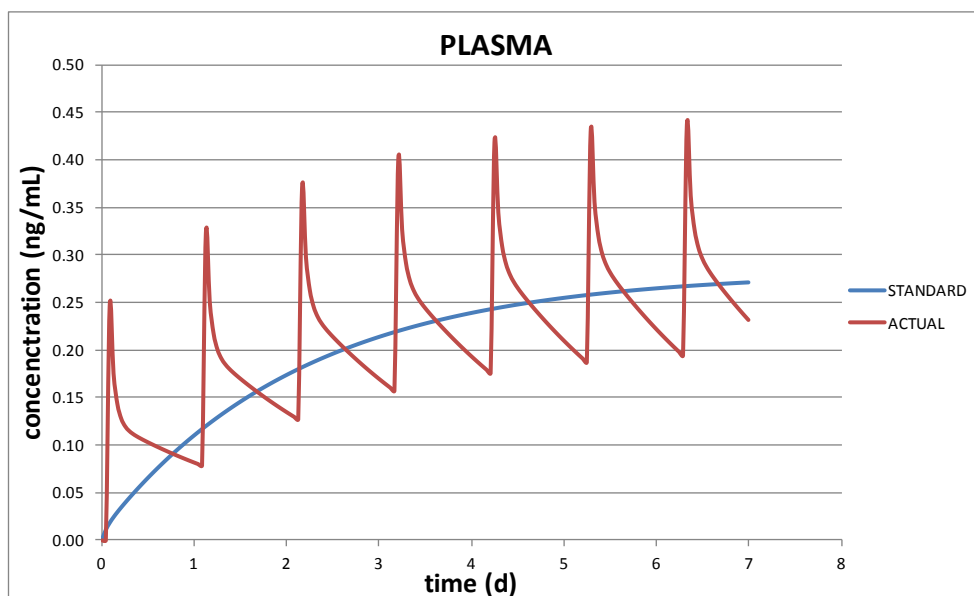
Figure 9. Concentration-time curve of OTA in serum after daily administration of a fixed dose of OTA during seven days. The standard scenario assumes gradual uptake during the whole day and the actual scenario assumes another extreme: instantaneous intake of a bolus dose uptake. The latter clearly shows the daily OTA kinetics.

Figure 10. Concentration-time curve of OTA in plasma after daily administration of a fixed (standard) dose of OTA and a fixed, repetitive dose (changing every 50 days) varying from 50% to 150% of the TDI of OTA during 200 days using the standard default values.
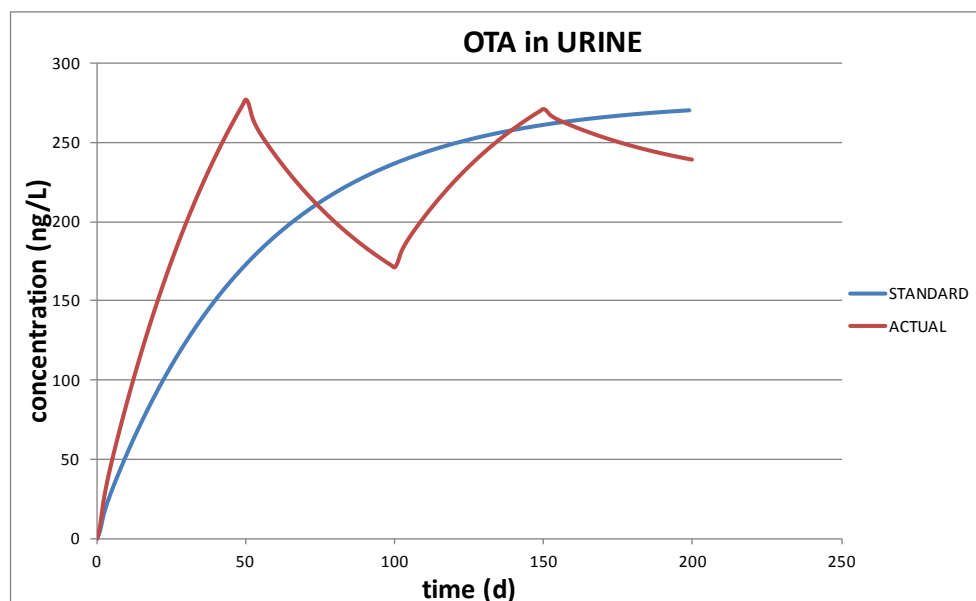
Figure 11. Urinary concentrations of OTA after daily administration of a fixed dose of OTA and a fixed, repetitive dose (changing every 50 days) varying from 50% to 150% of the TDI of OTA during 200 days using the standard default values.
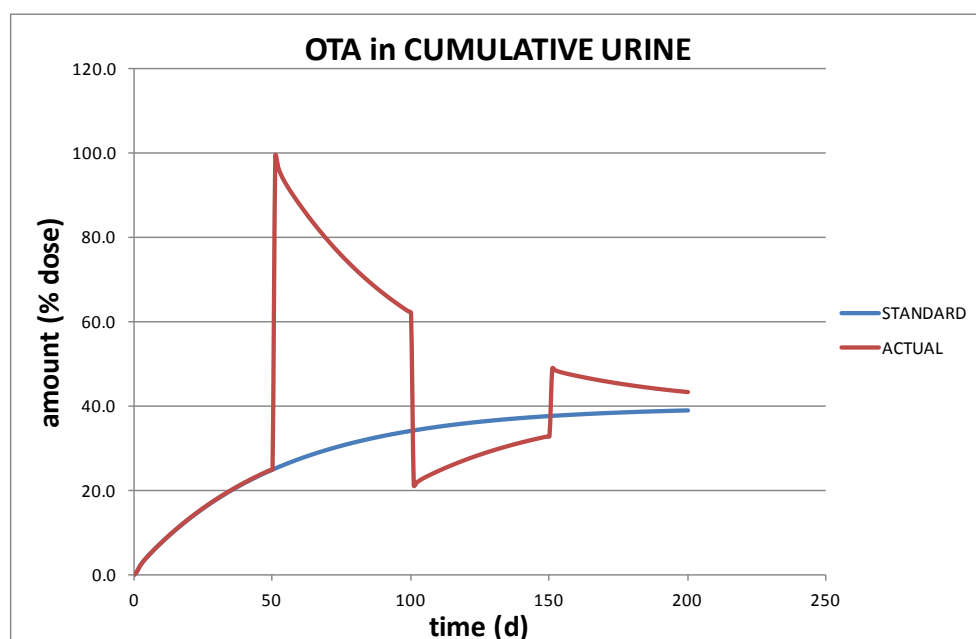
Figure 12. Cumulative excretion of OTA in urine after daily administration of a fixed (standard) dose of OTA and a fixed, repetitive dose (changing every 50 days) varying from 50% to 150% of the TDI of OTA during 200 days using the standard default values.

The Excel file contains two sheets, both with standard and actual scenarios. The second sheet "OTA-manual" leaves as much freedom as possible to the user with respect to the daily intake scenario, but requires some Excel skills. The first sheet "OTA-easy" restricts the freedom, but is more easy for the less experienced Excel user: the user can choose

for a daily intake to be at an actual fixed level or to let it vary between 50 and 150% around an actual fixed mean level.
The graphical presentations are given in figures 13-15.

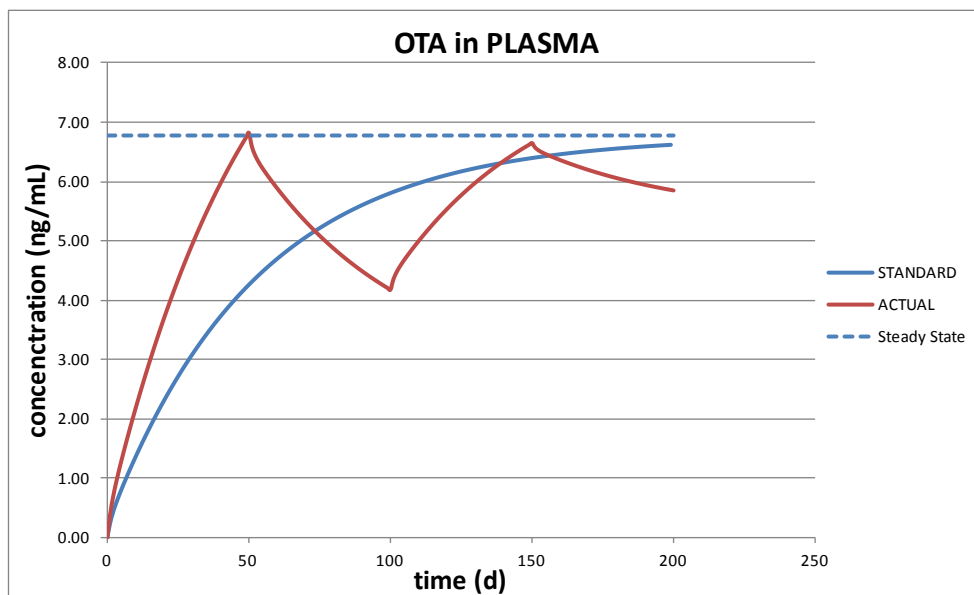*Figure 13. Concentration-time curve of OTA after daily administration of a randomly selected dose of OTA during 200 days using the standard default values.*

Figure 14. Urinary concentrations of OTA after daily administration of a randomly selected dose of OTA during 200 days using the standard default values.

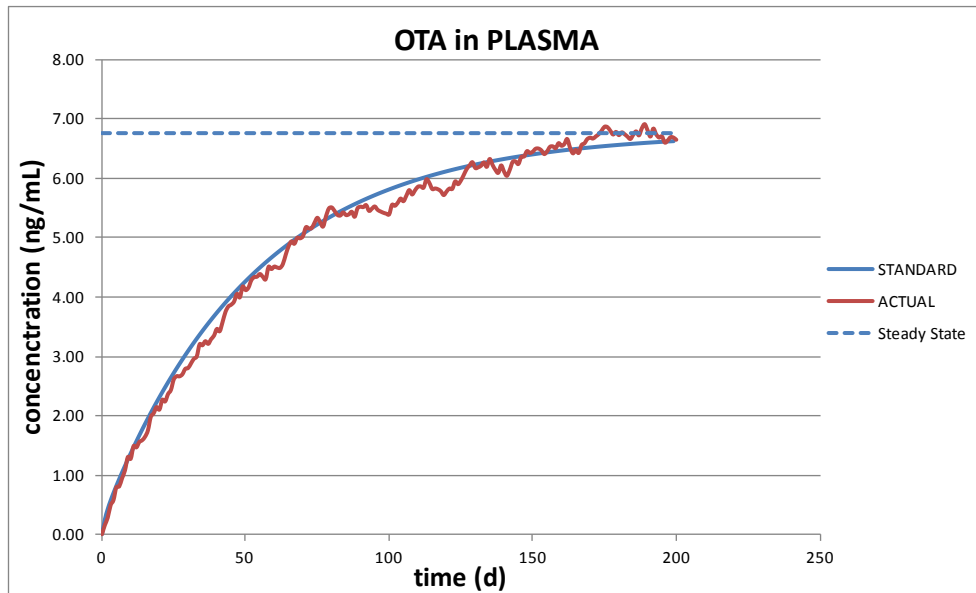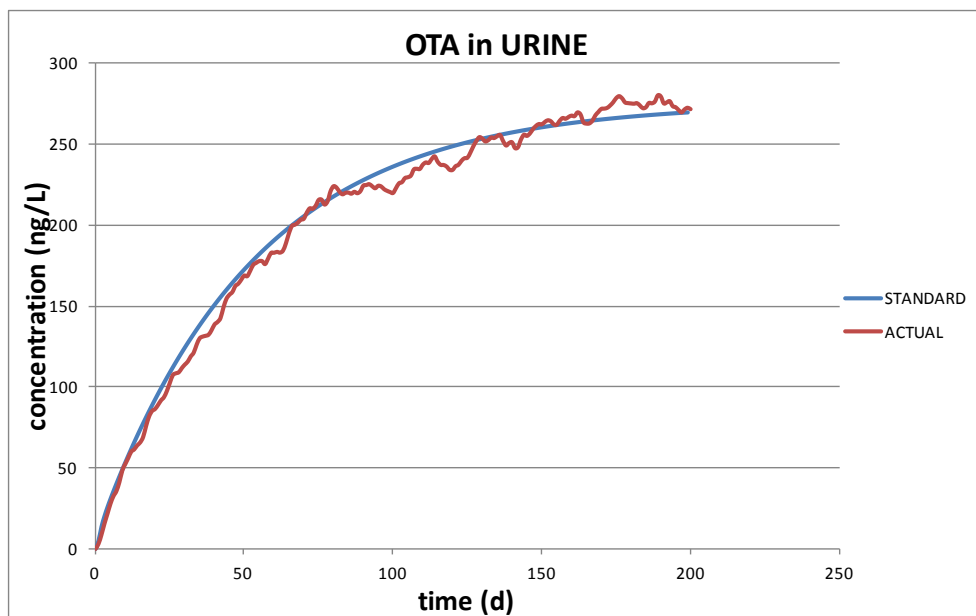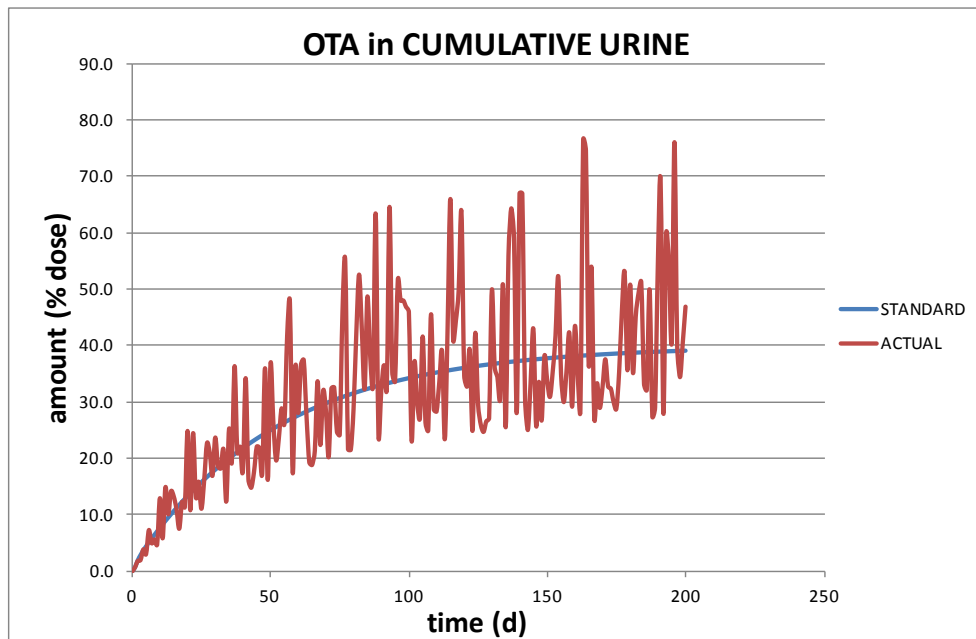*Figure 15. Cumulative excretion of OTA in urine after daily administration of a randomly selected dose of OTA during 200 days using the standard default values.*

4   Conclusions and discussion

Based on the toxicokinetic models presented in this report, the following items will be discussed:
1. the feasibility of estimating the dietary mycotoxin uptake of DON and OTA by determining renal mycotoxin excretion
2. the various methods for urine collection with respect to their applicability to estimate the dietary intake of DON and OTA.
3. The practical use of the applied models in the Excel calculator.

4.1   DON

The fast elimination of DON (elimination half-life of 3 hours, default value) results in an almost complete excretion of the dose in 24 hours when applying a fixed dose (see figure 1). During the first day of consumption there is a small difference in the percentage of dose excreted between two or three times a day ingestion of DON (see figure 3). After three days of consumption this small difference hasn't changed between the different dosage regimens. The same is true for the excretion of DON-glucuronide (see figure 4). Therefore, a quantitative collection of urine during 24 hours should give a good estimate of the DON intake during that particular dosing day. Due to the fast elimination of DON and its glucuronide(s) no conclusions can be drawn regarding the dietary daily DON intake over a longer period (it's a snapshot and in a way this is comparable to the dietary intake determined with a duplicate diet study).
Collection of spot urine (a sample of urine taken at any time of the day) will give a large variation in urinary concentrations of DON and its glucuronide(s), depending of the time of collection in relation to time of intake and individual variation (see figure 2). The obtained urinary biomarker concentrations can be normalized in function of the creatinine content to correct for variable dilutions (Turner *et al.*, 2010; EFSA, 2015b). However, as Heyndrickx pointed out, the use of creatinine-adjusted biomarker concentrations in order to accurately compare exposures among volunteers remains a subject of research (Heyndrickx, 2015). Alternatively, estimating the daily excretion of DON and its glucuronides by correcting for daily urine production may over- or underestimate the dietary DON intake. A sample of morning urine most likely will result in lower urinary levels (depending on time and size of the last dose) when compared to spot urine. In the event of lower but detectable urinary levels it may result in a smaller variation in the urinary biomarker concentrations when compared to spot urine. As is the case for spot urine, biomarker concentrations in morning urine need to be corrected (somehow) for variable dilutions.
Obviously, a random dose shows a greater fluctuation in the excretion of DON and its glucuronide when compared to a fixed dose. Nevertheless, after three days of consumption, a quantitative collection of urine during 24 hours is still the best option for a reasonable estimate of the dietary DON intake for that day. This option has also been recommended by a group of researchers who conducted an experimental study for the account of EFSA (EFSA, 2015b). The random dose gives an insight in the

extent of the (theoretical) uncertainty of the estimate of the dietary DON uptake.

4.2 OTA

The slow elimination of OTA (elimination half-life of 36 days, default value) results in accumulation of OTA in the human body when ingested daily (see figures 9 and 10). This accumulation continuous for several months and after approximately 180 days a plateau is reached (see figure 10, standard dose). This plateau is characteristic of a so-called 'steady state' situation which means that the overall intake of a substance is (fairly) in dynamic equilibrium with its elimination. In practice, it is generally considered that steady state is reached after 4 to 5 times the elimination half-life for a substance after regular dosing is started. This also means that the total amount of OTA excreted during 24 hours is constant whenever this plateau is reached (see figure 11, standard dose). Obviously, after dosing there still will be some fluctuation in urinary biomarker concentrations. This can be seen in figure 10, where the dose was varied manually from 50% to 150% of the TDI of OTA. Therefore, at steady state and assuming a fixed dose, quantitative collection of urine during 24 hours should give a good estimate of the OTA intake. However, whenever the dose is randomly varied from 0.5 to 1.5 times the TDI of OTA, the estimated percentage of the dose may vary from 2 (inverse of 0.5) to 0.67 (inverse of 1.5) times the average percentage of the dose excreted (see figure 15). The practical value of a (fairly) constant daily intake during months is rather limited. An example for such a situation would be e.g. the daily consumption of contaminated coffee which (in many cases) could lead to a fairly constant intake.

Collection of spot urine at steady state will give a smaller variation in urinary concentrations of OTA when compared to DON. It will also be less influenced by the time of collection but individual variations will still occur. As applies for DON, the obtained urinary biomarker concentrations can be normalized in function of the creatinine content to correct for variable dilutions. Again, estimating the daily excretion of OTA by correcting for daily urine production may over- or underestimate the dietary OTA intake. A sample of morning urine at steady state may result in lower urinary levels (depending on time and size of the last dose) when compared to spot urine but in practice this difference may be very small. As is the case for spot urine, biomarker concentrations in morning urine need to be corrected (somehow) for variable dilutions. The further a consumer is away from this steady state, i.e. exposed during a short period, the more the calculation of the dietary intake, based on renal excretion, will lead to an underestimation.

Obviously, a variable dose shows a greater fluctuation in the renal excretion of OTA when compared to the fixed dose. At steady state, this fluctuation in urinary OTA concentration is not largely affected when a single day of OTA ingestion is close to zero (see figure 14). In general, at steady state, a quantitative collection of urine during 24 hours is still the best option for a reasonable estimate of the dietary OTA intake. However, the difference between 24-hour urine, spot or morning urine could in practice be small. In that case, because of time, budgetary and compliance reasons spot or morning urine may be preferred.

Again, a randomly chosen dose gives an insight in the extent of the (theoretical) uncertainty of the estimate of the dietary OTA uptake.

4.3 Summary and recommendation

The developed models help to interpret how dietary mycotoxin intake is related to mycotoxin levels in urine. The calculator is a tool that can be used by people who are not familiar with toxicokinetic models and it is useful to visualise the above-mentioned relationship.
In case of DON, a quantitative collection of urine during 24 hours is the best option for a good estimate of the dietary DON intake. The frequency and duration of the DON intake is not very important.
In case of OTA, any kind or urine collection can be used whenever the duration of exposure is long enough (several months) and the daily dose is fairly constant. The frequency of OTA intake is not really important as long as intake is not discontinued for several days. Because of time, budgetary and compliance reasons, spot or morning urine may be preferred.

Whereas for OTA there is toxicokinetic evidence from a human study to support the presented model, this is not the case for DON. Therefore, it is recommended to study the renal excretion of DON in human volunteers to validate the assumed model for DON. This can simply be done by administering a single dose of DON to volunteers and collect urine quantitatively at different time intervals during 24 hours after administration.

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6   Acknowledgements

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7 Technical explanations of the toxicokinetic models

The technical details of the applied Excel calculator and the toxicokinetic models for DON and OTA are given in section 7.1 (DON) and 7.2 (OTA).

7.1 Excel calculator and toxicokinetic model for DON

7.1.1 *Excel calculator for DON*
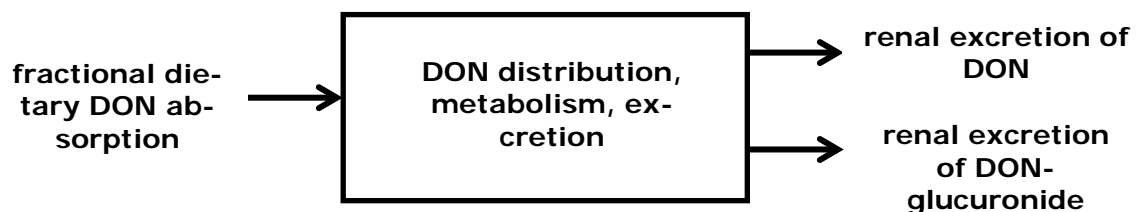The Excel sheet for DON is called: DON in Human Urine Calculator (DONHumUriCal).

PROBLEM:
Human food products and beverages contain, at least background levels of deoxynivalenol.

QUESTION:
Given the dietary intake of DON, what are the corresponding levels in urine.

TOXICOKINETIC MODEL:

A fraction of dietary DON intake is taken up across the gut wall with a finite absorption half-life, so no instantaneous absorption is assumed. Distribution of DON is assumed to be fast with respect to absorption, metabolism and excretion kinetics and consequently the human body is considered as one compartment. In this model it is assumed that DON is only metabolised through glucuronidation. Furthermore it is assumed that (a fraction of) non-metabolised DON and DON-glucuronide are only excreted renally.

DONHumUriCal EXCEL Sheet:
The calculator is intended for a few exercises to show differences in cumulative excretion of DON and DON-glucuronide when varying some model parameters like body weight, DON absorption and elimination half-life, percentage of non-metabolised DON excretion, DON distribution volume, dietary DON intake, fraction of DON absorbed across the gut wall and urine production (green fields). A standard model is presented of which parameters (standard values; yellow fields) cannot be changed. To compare, actual values can be accessed, but some of the model parameter values are the result of calculations with "green fields" parameters that have been input. These resulting values (blue fields) cannot be accessed, too. Resulting concentration-time curves of concen-

trations in serum and, most important, urine are shown graphically for standard and actual values.

DON is administered once, twice or three times a day (7:00, 12:00 and 18:00 hours) and administration cannot be changed by the user(s).

Explanation of model parameters

Parameters that can be edited by the user:

- Human body weight (kg): standard value for an adult is given: 74 kg.
- DON absorption half-life (h): half-life for absorption is assumed to be shorter than for elimination. Standard value for absorption is set at half the value for elimination.
- DON elimination half-life (h): half-life for elimination is assumed to be longer than for absorption. Standard value for elimination is set at double the value for absorption.
- Percentage DON renal excretion (%): this is the percentage of the amount of DON excreted unchanged in the urine. The default value is set at 5%. As a consequence, 95% is glucuronidated.
- DON distribution volume (L/kg bw): the distribution volume (Vd) of DON is assumed to be higher than the total body water (comparable to the Vd of DON in pigs). The default value is set at 1.3 L/kg bw.
- Total daily DON intake (µg/kg body weight): The default value is set at the TDI: 1 µg/kg bw/day.
- DON-easy sheet only: fixed dose value: if =1, then the dose is fixed to the value chosen above; if =0, then the dose is randomly chosen with an average equal to the value chosen above and varying uniformly between 50% and 150% of that mean value.
- DON-easy sheet only: number of meals per day containing DON (1, 2 or 3)
- Fraction DON absorbed (%): equivalent of the bioavailability. Default value is set at 70%.
- Urine volume voided (L/3 hours): the default value is set at 200 mL and is based on an average daily urine production of 1.6 L for an adult.

Parameters that are fixed or derived from editable parameters:

- DON absorption rate (/h): is calculated from the absorption half-life.
- DON total elimination rate (/h): is calculated from the elimination half-life. The total elimination rate is the sum of the renal and the metabolic rate of DON.
- DON renal excretion rate (/h): is set at 5% of the DON total elimination rate.
- DON glucuronidation rate (/h): is set at 95% of the DON total elimination rate.
- Total daily DON intake (µg): calculated from total daily dietary DON per kilogram body weight and human body weight.
- Total daily DON absorbed (µg): calculated from the total daily DON intake and the fraction DON absorbed.
- Meals: The total daily DON absorbed is evenly divided over three meals. These meals are consumed at three time points: 7:00, 12:00 and 18:00 hours (0, 5 and 11 hours and time with respect to first daily intake).

- Urine sampling interval (h): the default value is set at a theoretical value of 3 hours. Obviously this is not true during night rest.
- *Parameter assumption:*
- It is assumed that renal excretion of DON-glucuronide is fast with respect to DON absorption and elimination: it is not an explicitly defined model parameter.

7.1.2 *DON model implementation*
Consider the mono-exponential kinetics of a substance:

$$\frac{dA}{dt} = F \cdot D(t) - kA$$

where *A* denotes the amount of substance, *k* the exponential rate of elimination, *F* the fraction of dose absorbed and *D* the time dependent dosing regimen. In the following three cases of the last will be considered: instantaneous uptake of the total dose applied, *e.g.* when absorption kinetics is fast as compared to elimination kinetics, constant uptake of the total dose applied during the day, *e.g.* when there is no clear time point associated with oral intake, and, in between those two, finite kinetics uptake.

Most often, the kinetic problem described above is solved from time $t = 0$ onwards, but in this case, the problem will be considered from some time *t* onwards which is typically a time point at which the daily (or whatever the time unit) dose changes. It is assumed that there will be no change during a time interval Δt, so during this interval

$$\frac{dA(t+\tau)}{dt} = F \cdot D(t+\tau) - kA(t+\tau), \quad 0 \leq \tau < \Delta t, \quad A(t) \text{ given}$$

The analytical solution to this problem is standard.
In case of instantaneous absorption, the solution is given by

$$A(t+\tau) = e^{-k\tau}\left(A(t) + FD(t)\right), \quad 0 \leq \tau < \Delta t$$

Note that at the time point *t*, caused by instantaneous absorption, the amount of substance jumps discontinually from $A(t)$ to $A(t) + FD(t)$.
In case constant uptake of the total dose, the solution is given by

$$A(t+\tau) = e^{-k\tau}\left(A(t) + \frac{FD(t)}{k}\left(e^{k\tau} - 1\right)\right) = A(t)e^{-k\tau} + \frac{FD(t)}{k}\left(1 - e^{-k\tau}\right), \quad 0 \leq \tau < \Delta t$$

and from time point *t* onwards the amount is gradually rising until, if the time interval Δt would be large enough, a steady value would be reached.

In case of finite kinetics uptake the analytical approach has to be a little bit more subtle to account for the case that the interval Δt is not sufficiently long to deplete the total amount of substance that was taken in and there is still a non-negligible amount left into the gut. Note that for

the finite uptake kinecs model it is tacitly assumed that the total amount of substance taken in enters the gastro-intestinal tract as a bolus and from that tract is taken up. So, trace should be kept of the amount of substance still left into the gastro-intestinal tract. This amount is denoted by G and actually denotes the amount that will be taken up in due course. The analytical solution to the gastrointestinal sub-model is simply

$$G(t+\tau)=e^{-k_a\tau}\left(G(t)+FD(t)\right)$$

where k_a is the finite exponential rate of absorption. With this quantity, the solution to the kinetic model is

$$A(t+\tau)=A(t)e^{-k\tau}+\frac{k_a}{k-k_a}\left(G(t)+FD(t)\right)\left(e^{-k_a\tau}-e^{-k\tau}\right)$$

Note that the approach outlined above gives completely freedom for the interval lengths Δt, which may vary from interval to interval, and for the intra-interval time points to output the solution. Also note that in case of instantaneous absorption, for pictural reason, it would be preferable to output both the values $A(t)$ and $A(t)+FD(t)$ at the time point of oral intake. The approach lends itself most conveniently to the implementation in Excel-sheets.

7.2 Excel calculator and toxicokinetic model for OTA

7.2.1 Excel calculator or OTA
The Excel sheet for OTA is called: OTA in Human Urine Calculator (OTA-HumUriCal).
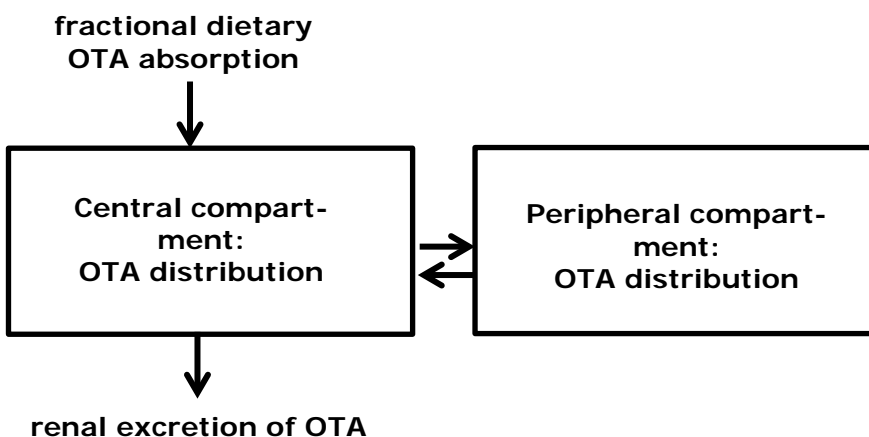
PROBLEM:
Human food products and beverages contain, at least background levels of ochratoxin A.

QUESTION:
Given the dietary intake of OTA, what are the corresponding levels in urine.

TOXICOKINETIC MODEL:

A fraction of dietary OTA intake is taken up across the gut wall into the central compartment. Distribution of OTA occurs between this and a peripheral compartment. It is assumed that OTA is, metabolized or not, excreted renally.

OTAHumUriCal EXCEL SHEET:
The calculator is intended for a few exercises to show differences in cumulative excretion of OTA and possible metabolites when varying some model parameters like body weight, elimination half-life, OTA distribution volume, dietary OTA intake, fraction of OTA absorbed across the gut wall and urine production (green fields). A standard model is presented of which parameters (standard values; yellow fields) cannot be changed. To compare, actual values can be accessed, but some of the model parameter values are the result of calculations with "green fields" parameters that have been input. These resulting values (blue fields) cannot be accessed, too. Resulting concentration-time curves of concentrations in plasma and, most important, urine are shown graphically for standard and actual values.

Explanation of model parameters
Parameters that can be edited by the user:
- Human body weight (kg): standard value for an adult is given: 62 kg.
- OTA distribution half-life (h): half-life for distribution between the compartments.
- OTA initial distribution volume (L): distribution volume of the central compartment.
- OTA elimination half-life (h): half-life for elimination is assumed to be longer than for distribution.
- OTA distribution volume (L): distribution volume of the terminal elimination phase.
- Fraction OTA absorbed (%): equivalent of the bioavailability. Default value is set at 70%.
- OTA-easy sheet only: fixed dose value: if =1, then the dose is fixed to the value chosen above; if =0, then the dose is randomly chosen with an average equal to the value chosen above and varying uniformly between 50% and 150% of that mean value.
- Daily OTA intake (µg/kg body weight): mean or fixed value
- Urine volume voided (L/days): the default value is set at 1.6 L for an adult.
- Percentage of OTA excreted: standard set at 50%.

Parameters that are fixed or derived from editable parameters:
- Distribution phase exponential rate: $\log(2)$/absorption half-live.
- Elimination phase exponential rate: $\log(2)$/elimination half-live.
- Transfer rate from central to peripheral compartment.
- Transfer rate from peripheral to central compartment.
- Rate of elimination from the central compartment.

7.2.2   *OTA model implementation*
Consider the bi-exponential kinetics of a substance:

$$\frac{d}{dt}\begin{pmatrix} A_c \\ A_p \end{pmatrix} = \begin{pmatrix} FD(t) \\ 0 \end{pmatrix} - \begin{pmatrix} -(k_c + k_e) & k_p \\ k_c & -k_p \end{pmatrix}\begin{pmatrix} A_c \\ A_p \end{pmatrix}$$

where A_c, A_p denote the amounts of substance in the central and periph-
eral compartments, k_e is the exponential rate of elimination from the
central compartment, k_c the exponential rate of transfer from the cen-
tral to the peripheral compartment, k_p the exponential rate of transfer
from the peripheral to the central compartment, F the fraction of dose
absorbed and D the time dependent dosing regimen that enters the cen-
tral compartment. Also here, three cases of the last will be considered:
instantaneous uptake of the total dose applied, *e.g.* when absorption
kinetics is fast as compared to elimination kinetics, constant uptake of
the total dose applied during the day, *e.g.* when there is no clear time
point associated with oral intake, and, in between those two, finite ki-
netics uptake.

Instead of 1 function of time A a vector function of time is in order as
well as a 2 × 2 matrix K instead of a constant k. Also this case has
standard solutions with exponents, but to this the matrix K has to be
written as the product of matrices

$$K = -S \Lambda S^{-1}$$
where

$$\Lambda = \begin{pmatrix} \lambda_1 & 0 \\ 0 & \lambda_2 \end{pmatrix}, S = \begin{pmatrix} k_p & k_p \\ \lambda_1 + k_c + k_e & \lambda_2 + k_c + k_e \end{pmatrix}, \begin{cases} \lambda_1 = k_c + k_e + k_p - \sqrt{(k_c + k_e + k_p)^2 - 4k_e k_p} \\ \lambda_2 = k_c + k_e + k_p + \sqrt{(k_c + k_e + k_p)^2 - 4k_e k_p} \end{cases}$$

are the diagonal matrix of eigenvalues, the matrix of eigenvectors and
λ_1, λ_2 the two eigenvalues expressed in terms of the kinetic model's pa-
rameters. For the diagonal matrix Λ, its exponentiation is

$$\exp(-\tau \Lambda) = \begin{pmatrix} e^{-\lambda_1 \tau} & 0 \\ 0 & e^{-\lambda_2 \tau} \end{pmatrix}$$

After these preliminaries, the solution to the kinetic problem proceeds as
above.
In case of instantaneous absorption, the solution is given by

$$\begin{pmatrix} A_c \\ A_p \end{pmatrix}(t + \tau) = S \exp(-\tau \Lambda) S^{-1}\left(\begin{pmatrix} A_c \\ A_p \end{pmatrix}(t) + \begin{pmatrix} FD(t) \\ 0 \end{pmatrix}\right), \quad 0 \le \tau < \Delta t$$

Note that at the time point t, caused by instantaneous absorption, the
amount of substance in the central compartment jumps discontinually

from $A_c(t)$ to $A_c(t)+FD(t)$. Also note that, even if interest is only in the amount in the central compartment, trace should be kept of the amount in the peripheral compartment as well.

In case constant uptake of the total dose, the solution is given by

$$\begin{pmatrix} A_c \\ A_p \end{pmatrix}(t+\tau) = S\exp(-\tau\Lambda)\left(S^{-1}\begin{pmatrix} A_c \\ A_p \end{pmatrix}(t) + \Lambda^{-1}\left(\exp(\tau\Lambda)-I\right)S^{-1}\begin{pmatrix} FD(t) \\ 0 \end{pmatrix}\right)$$

In case of the finite kinetics absorption model, the solution is

$$\begin{pmatrix} A_c \\ A_p \end{pmatrix}(t+\tau) = S\exp(-\tau\Lambda)\left(\begin{pmatrix} A_c \\ A_p \end{pmatrix}(t) + k_a\cdot\left(\Lambda-k_aI\right)^{-1}\cdot\left(\exp\left(\tau\left(\Lambda-k_aI\right)\right)-I\right)\cdot S^{-1}\begin{pmatrix} G(t)+FD(t) \\ 0 \end{pmatrix}\right)$$