Prediction of deoxynivalenol toxicokinetics in humans by in vitro-to-in vivo extrapolation and allometric scaling of in vivo animal data

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Abstract

Deoxynivalenol (DON) is the most prevalent mycotoxin in cereals worldwide. It can cause adverse health effects in humans and animals, and maximum levels in food and feed have been implemented by food authorities based on risk assessments derived from estimated intake levels. The lack of human toxicokinetic data such as absorption, distribution, and elimination characteristics hinders the direct calculation of DON plasma levels and exposure. In the present study, we have, therefore, used in vitro-to-in vivo extrapolation of depletion constants in hepatic microsomes from different species and allometric scaling of reported in vivo animal parameters to predict the plasma clearance [0.24 L/(h × kg)] and volume of distribution (1.24 L/kg) for DON in humans. In addition, we have performed a toxicokinetic study with oral and intravenous administration of DON in pigs to establish benchmark parameters for the in vitro extrapolation approach. The determined human toxicokinetic parameters were then used to calculate the bioavailability (50–90%), maximum concentration, and total exposure in plasma, and urinary concentrations under consideration of typical DON levels in grain-based food products. The results were compared to data from biomonitoring studies in human populations.

Keywords

Allometric scaling
Deoxynivalenol (DON)
Human exposure
IVIVE
Pig
Toxicokinetics

Electronic supplementary material

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Introduction

Strategies for the prediction of in vivo kinetic data from in vitro biotransformation experiments are well established for pharmaceutical compounds (Obach et al. 1997; Iwatsubo et al. 1997; Ito and Houston 2005). Liver microsomes or hepatocytes of humans and animals are used to metabolise test compounds in vitro under conditions of the first-order kinetics, allowing in vitro-to-in vivo extrapolation (IVIVE) of the hepatic clearance and derived kinetic parameters with the help of species-specific conversion factors (Barter et al. 2007; Smith et al. 2008; Thörn et al. 2011). The determination of essential kinetic parameters such as clearance (CL), plasma half-life ($t_{1/2}$), volume of distribution (V_d), and oral bioavailability (f) is necessary for estimating the exposure and maximum plasma concentration after oral administration of a new drug, which is an important safety issue for the initial trial in human volunteers during phase-I of drug development. The resulting data describing the absorption, distribution, metabolism, and excretion (ADME) of a substance can then be applied as input parameters for *in silico* physiologically based pharmacokinetic (PBPK) modelling, which is used for the simulation of plasma concentration–time profiles (De Buck et al. 2007; Chen et al. 2012). The successful determination of IVIVE data is thus a prerequisite for all subsequent applications, and prediction accuracy within 0.5-fold to twofold of the observed values under consideration of the variation in the observed value is widely regarded as satisfactory (Jolivette and Ward 2005; De Buck et al. 2007; Abduljalil et al. 2014).

The combination of in vitro extrapolation data with in vivo data from animal studies can improve method predictability (Chiba et al. 2009). The empirical observation that anatomical, physiological, and biochemical characteristics in mammals are exponentially correlated with the individual bodyweights (BW) (Boxenbaum 1982) has been successfully employed for the allometric scaling of pharmacokinetic parameters in drug discovery. Data from at least two or three animal species are needed for the reliable extrapolation of, respectively, human V_d, or CL (Mahmood and Balian 1996), and their BW should span as broad a range as possible (Lindstedt and Schaeffer 2002). The same mechanisms apply for the extrapolation of oxidative cytochrome P450 enzymes-catalysed phase-I transformations and phase-II UDP-glucuronosyltransferase (UGT) conjugation reactions, both for IVIVE (Soars et al. 2002; Naritomi et al. 2015) and allometric scaling of in vivo animal data (Deguchi et al. 2011).

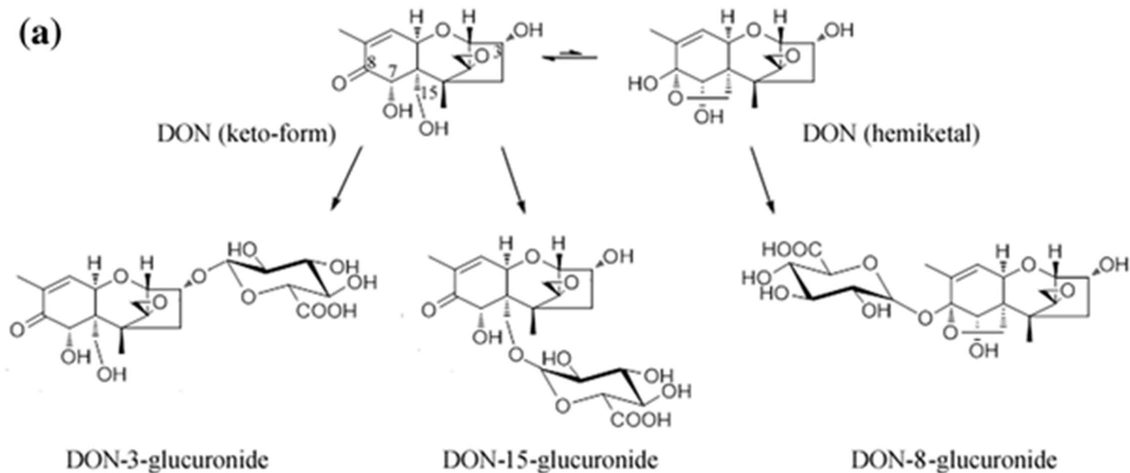
Even if predictive pharmacokinetics has repeatedly proven its power and value, it has not been employed in toxicokinetic studies of natural toxins so far. The kinetics of the most important mycotoxins have been elucidated in a considerable numbers of in vivo studies in laboratory and domestic animals (Wu et al. 2010; Dänicke and Brezina 2013) and provide the opportunity for further data compilation. Several mycotoxins have been

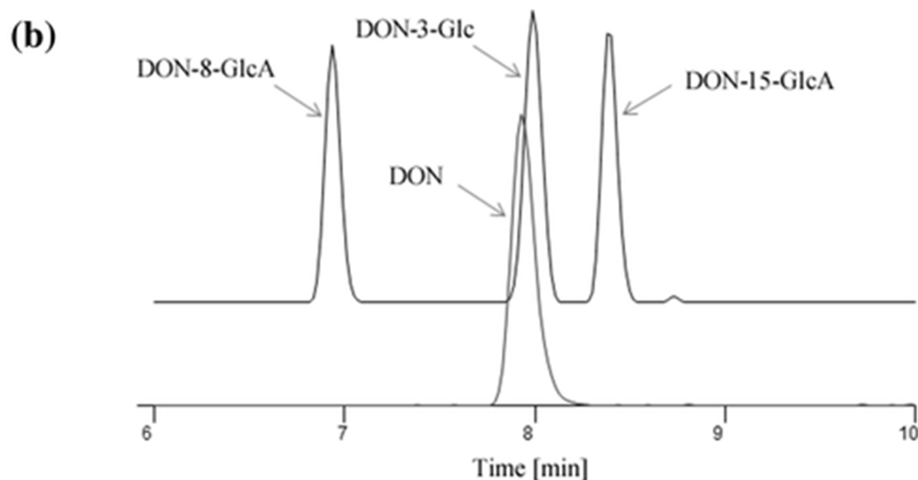
investigated in biotransformation assays, and main metabolites of hepatic metabolism have been identified (Maul et al. 2012). The combination of in vivo and in vitro results and their use in IVIVE and allometric scaling for exposure predictions and risk assessment in humans after unintentional uptake of mycotoxins in food is, however, unexplored. So far, human data are limited to collections of concentrations in urine, faeces, plasma, and breast milk (Waseem et al. 2014) and some in vitro metabolism assays (Warth et al. 2013).

Deoxynivalenol (DON) (Fig. 1a), a B-type trichothecene produced by field-growing *Fusarium* species, is the most prevalent mycotoxin in grain worldwide and occurs regularly in staple food and feed (Nagl and Schatzmayr 2015). The extent of contamination depends on the region of cereal production and is influenced by weather conditions (Bernhoft et al. 2013). Adverse health effects caused by DON in different animals include immunotoxicity, anorexia, damage to the intestinal barrier, neurotoxicity and reproductive toxicity at chronic low-level exposure, and abdominal distress, diarrhoea, and emesis under acute mycotoxicosis (Payros et al. 2016). In humans, several outbreaks of acute DON intoxication have been described with symptoms ranging from nausea, vomiting, and diarrhoea to fevers and bloody stool (Knutsen et al. 2017). Effects of chronical exposure are, however, not sufficiently documented. The main mode of action on the cellular level is the inhibition of protein synthesis by binding to the 60S ribosomal subunit (Pestka 2010). National and international food and feed safety authorities such as the European Food Safety Authority (EFSA) have assessed the risk from DON exposure and implemented maximum levels for DON in feed- and foodstuffs (Knutsen et al. 2017). The tolerable daily intake (TDI) of DON, including its derivatives 3-acetyl-DON, 15-acetyl-DON, and DON-3-glucoside, in humans was set to 1 µg/kg BW/day, and the acute reference dose ARfD to 8 µg/kg BW. Biomonitoring surveys have confirmed that more than 90% of the European population have measurable DON in urine, varying with population group, age, sex, and nationality (Meky et al. 2003; Waseem et al. 2014; Brera et al. 2015). Especially, small children can be exposed to doses that exceed the TDI several times (Sundheim et al. 2017; Knutsen et al. 2017).

Fig. 1

a Molecular structures of the keto and hemiketal conformations of DON and three DON-glucuronide isoforms, **b** UHPLC-HRMS (plasma method) extracted ion chromatograms for DON ($[M\text{-acetate}]^-$, *m/z* 355.1398) and DON-glucuronide ($[M\text{-}H]^-$, *m/z* 471.1508) standards





Animals and humans show different sensitivity to DON exposure (Dänicke and Brezina 2013), depending on species-specific biotransformation pathways. DON is eliminated mainly through conjugation to glucuronic acid and urinary as well as faecal excretion (Turner et al. 2009; Wu et al. 2010; Lattanzio et al. 2011; Warth et al. 2013). Major metabolites are DON 3-glucuronide (DON 3-GlcA), DON 15-glucuronide (DON 15-GlcA) (Fig. 1a), and in addition, glucuronidation products of hemiketal and isomeric DON-conformations such as, respectively, DON 8-glucuronide (DON 8-GlcA), iso-DON 3-glucuronide (iso-DON 3-GlCA), and iso-DON 8-glucuronide (iso-DON 8-GlCA) have been reported (Uhlig et al. 2016; Pestka et al. 2017). Ruminants show increased tolerance to DON due to intra-ruminal microbial detoxification to deepoxy-deoxynivalenol (DOM-1) (Wu et al. 2010), which is subject to further regioselective conjugation reactions. Birds extensively produce the sulphate-conjugated DON 3-sulphate and DON 15-sulphate, and DON-sulphonates (DONS) have been detected in rat and mouse faeces (Wan et al. 2014; Pestka et al. 2017).

DON glucuronides can be produced in in vitro metabolism assays (Maul et al. 2012; Uhlig et al. 2016; Schwartz-Zimmermann et al. 2017). Data on DON toxicokinetics in

humans that would allow the prediction of plasma concentrations and exposure for different doses are, however, not available.
It was, therefore, the aim of the present study to provide essential toxicokinetic parameters of this important mycotoxin in humans by combining IVIVE and allometric scaling. For this reason, we have conducted in vitro glucuronidation assays with substituted liver microsomes of different species under linear kinetic conditions and made a comprehensive search of published animal data. In addition, we have performed an in vivo kinetic study with oral and intravenous administration of DON in Norwegian-crossbred piglets, which allowed the direct comparison to results from our pig microsomal assay.

Materials and methods

Chemicals and biological materials

Deoxynivalenol (DON; 3α,7α,15-trihydroxy-12,13-epoxytrichothec-9-en-8-one; $C_{15}H_{20}O_6$; 296.32 g/mol) was obtained from Sigma-Aldrich Norway (Oslo, Norway). Deepoxy-DON (DOM-1) was purchased from Romer Labs (Tulln, Austria), while DON-3-*O*-D-glucuronide (DON-3-GlcA), DON-15-*O*-β-D-glucuronide (DON-15-GlcA), and DON-8-*O*-D-glucuronide (DON-8-GlcA) were available from earlier work (Uhlig et al. 2016). Acetonitrile, methanol, and water (Fisher Scientific, Fair Lawn, NJ, USA) were of Optima™ LC/MS quality. Uridine 5-diphosphoglucuronic acid, uridine 5-diphospho-N-acetylglucosamine, and methoxyamine hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO, USA) and UGT Reaction Mix Solution B containing 250 mM Tris–HCl, 40 mM $MgCl_2$, and 0.125 mg/mL alamethicin in water was from BD Biosciences (Woburn, MA, USA). Human liver microsomes (X008068, Lot SBM, mixed pool of 50 male and female donors), rat (pool of 20 male Wistar rats, M00021, Lot LTH), dog (pool of 6 male Beagle dogs, M00201, Lot HSN), and minipig (pool of 3 male Göttingen minipigs, M00061, Lot GNP) liver microsomes were obtained from Bioreclamation IVT (Baltimore, MD, USA) and stored in liquid nitrogen until use. The P450 content, enzyme activity, and total protein concentration had been determined by the manufacturer. In addition, self-prepared microsomes from female Wistar rats (Uhlig et al. 2016) and male chickens (Ivanova et al. 2014) were used.

Preparation of liver microsomes from Norwegian piglets

Livers were harvested from two weaned 6-week old male Norwegian-crossbred piglets (Landrace-Yorkshire/Duroc-Duroc); 13 and 15 kg body weight (BW) that had been operated for hernia at the Norwegian University of Life Sciences (NMBU), Oslo, Norway, in accordance with the guidelines set by the Norwegian Animal Research Authority. After 2-day quarantine allowing the elimination of the drugs used in the hernia operation (azaperone, lidocaine, flunixin, and sulphadiazine–trimethoprim), the animals

were euthanised by captive bolt pistol. The livers were flushed in situ with physiological saline solution (pH 7.0) by cannulation of the vena porta and washing out blood through an incision in the vena cava inferior. Subsequently, the livers were extricated, cut into pieces of about 100 g, and stored immediately at − 80 °C. Microsomes were prepared under cooling from minced livers by manual tissue homogenisation in ice-cold 0.1 M potassium phosphate buffer (PBS, pH 7.5) with a Potter Elvehjem homogeniser and subsequent differential centrifugation. Cell debris, cell nuclei, and organelles were precipitated twice at 16,000*g* (Beckman Instruments, Palo Alto, CA, USA) for 20 min at 4 °C. The resulting supernatants were centrifuged in polyallomer tubes at 100,000*g* in an ultracentrifuge (Beckman Instruments) for 1 h at 4 °C in an SW41Ti swing-out rotor. Microsomes were resuspended from the precipitate by manual homogenisation in 0.1 M PBS pH 7.5 and stored in aliquots at − 80 °C until further use. The total protein content was determined by Lowry Protein Assay (Bio-Rad Laboratories, Hercules, CA).

DON in vitro toxicokinetics with substituted liver microsomes of different species

Substrate depletion assays measuring the concentration–time course of DON were performed with commercially available rat (RLM), dog (DLM), minipig (MPLM), and human (HLM) liver microsomes under conditions of the first-order kinetics to determine assay half-life ($t_{1/2,\ assay}$). In addition, DON was metabolised with in-house made rat (RLMH), chicken (CLMH) and Norwegian-crossbred piglet (PLMH) microsomes. Microsomal protein (2 mg/mL in assay) from the different species was added to incubation buffer containing 7.4 mM uridine 5-diphosphoglucuronic acid, 50 mM Tris–HCl (pH 7.5), 8 mM $MgCl_2$, 0.3 mM uridine 5-diphospho-*N*-acetylglucosamine, and 25 µg/mL alamethicin in a total assay volume of 0.5 mL. After pre-incubation at 37 °C for 2 min, the reaction was started by adding DON in acetonitrile resulting in assay concentrations of 0.05–5 µM. The acetonitrile fraction in the microsomal incubation system did not exceed 0.9% (Busby et al. 1999). Metabolism reactions were performed at 37 °C in a shaking water bath, and 100 µL aliquots were withdrawn at 0, (2.5), 5, 10, 15, 30, and (60 and 90) min, immediately mixed with ice-cold acetonitrile (1:1) and stored on ice until centrifugation for 5 min at 20,000*g* (Eppendorf, Hamburg, Germany). The supernatant was stored at − 20 °C until analysis by UHPLC-MS/MS. Incubations without microsomes or without DON were included for control of compound stability or background noise, respectively.

DON in vivo toxicokinetics in Norwegian piglets

Norwegian-crossbred piglets, 5 weeks old, six of each sex, males castrated, about 20 kg BW, were obtained from a commercial breeder and allowed to acclimatise for 7 days in the production animal facility of the Faculty of Veterinary Medicine at NMBU. Animals were housed individually in boxes with socialisation-friendly openings on sawdust bedding and had free access to water (drinking cups-H_2O (49,014); Domino AS, Tørring, Denmark). The piglets were weighed and earmarked at study start. They had free access

to conventional piglet feed via automatic feeders (K1, Domino AS) until 12 h before study start and 2 h after the application of DON. The feed contained less than 25 µg/kg DON and DON glucosides as measured by UHPLC-HRMS.

Six piglets of both sexes were dosed intravenously with 0.08 mg/kg BW DON in sterile physiological saline solution (0.2 mg DON/mL) via butterfly cannula in the ear vein. Blood was withdrawn at 0, 0.042, 0.083, 0.17, 0.33, 0.67, 1, 1.5, 2, 4, 6, 8, and 24 h from the jugular vein using a 21G cannulae and heparinised 3 mL vacutainers (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). The exact time of each blood withdrawal was recorded. Fresh blood samples (1–2 mL) were stored refrigerated until centrifugation at 1500*g* for 15 min (Eppendorf) and the obtained plasma was stored at − 20 °C until analysis by UHPLC-MS/MS.

Six piglets of both sexes were dosed orally with 0.125 mg/kg BW DON in water (0.1 mg DON/mL) by gavage via an infusion tube behind the tongue. Blood was withdrawn at 0, 0.083, 0.17, 0.33, 0.67, 1, 1.5, 2, 4, 6, 8, and 24 h, and centrifuged and stored as described above. At study end, the animals were euthanised after sedation with azoperone and ketamine by intravenous application of an overdose of pentobarbital.

The in vivo piglet study had been approved by the Norwegian Animal Research Authority.

Preparation of samples from in vitro and in vivo assays for analysis by UHPLC-HRMS

Aliquots from in vitro assays were centrifuged through Costar Spin-X centrifuge Tube Filters 0.22 µm (Corning INC, Corning, NY, USA) at 15,000*g* for 1 min (Eppendorf), transferred to 300 µL fixed- insert HPLC vials (Thermo Fisher Scientific, Waltham, MA, USA) and directly analysed by UHPLC-MS/MS.

Plasma samples (250 µL) were transferred into conical 15 mL plastic tubes (Corning Inc., Corning, NY, USA), mixed with 750 µL acetonitrile, vortexed for 15 s, and sonicated for 5 min. Proteins were precipitated by centrifugation at 2000*g* for 10 min at 4 °C (Beckman Coulter, Brea, CA, USA); supernatants were transferred to 10 mL conical glass tubes and evaporated to dryness at 60 °C using a gentle stream of nitrogen. Dried samples were stored refrigerated, or substituted with 200 µL water, vortexed for 15 s, sonicated for 5 min, transferred to HPLC vials (Thermo Fisher Scientific) and directly analysed by UHPLC-HRMS.

UHPLC-HRMS method for the quantitative analysis of DON and DON metabolites in plasma

Piglet plasma samples were analysed by ultra-high-pressure liquid chromatography high-resolution mass spectrometry (UHPLC-HRMS) on a Q-Exactive™ Hybrid Quadrupole-Orbitrap mass spectrometer equipped with a heated electrospray ion source (HESI-II) and coupled to a Dionex Ultimate 3000 UHPLC system (Thermo Fisher Scientific). The HESI-II interface was operated at 300 °C in the negative ionisation mode, and the

parameters were adjusted as follows: spray voltage 4 kV, capillary temperature 250 °C, sheath gas flow rate 35 L/min, auxiliary gas flow rate 10 L/min, and S-lens RF level 55. Data were acquired in the selected ion monitoring (SIM)/data-dependent MS^2 (dd-MS^2) mode targeting $[M - acetate]^-$ ions of DON and deepoxy-DON (*m*/z 355.1387 and 339.1438, respectively) and $[M - H]^-$ ions of the DON glucuronides (*m/z* 471.1497) with a quadrupole isolation width of 2 *m/z*, and a mass resolution of 70,000 full-width half-maximum (FWHM) at *m/z* 200 for SIM. The presence of a target ion above the set threshold intensity of 5×10^3 triggered an MS^2 scan for analyte verification (dd-MS^2) using a normalised collision energy 35%. The mass resolution during dd-MS^2 was set to 17,500 FWHM. The automatic gain control (AGC) target was 5×10^5 ions including a maximum injection time of 250 ms during SIM, whereas, for dd-MS^2, the AGC target was 5×10^4 and the maximum injection time was 200 ms.
Chromatographic separation was achieved at 30 °C on a 100 × 2.1 mm i.d. Acquity UPLC HSS T3 column (1.8 µm; Waters, Milford, MA, USA) with 5 × 2.1 mm i.d. XSelect HSS T3 VanGuard pre-column (2.5 µm, 100 Å, Waters). The injection volume was 6 µL, and samples were eluted using a water (A)/acetonitrile (B) gradient, both phases containing 5 mM ammonium acetate and 0.1% acetic acid, with a flow rate of 0.5 mL/min. The column was eluted isocratically with 100% A for 1 min, and then, a linear gradient was applied increasing linearly to 15% B in 15 min, and to 100% B in 0.5 min. After flushing the column for 2.5 min with 100% B, the mobile phase composition was returned to the initial conditions, and the column was washed for 2.9 min. Xcalibur version 2.2 was used for data processing (Thermo Fisher Scientific). Buffer-based and matrix-matched calibration curves were constructed with standards in the range of 1.5 to 250 µg/L, and the LOD and LOQ were 0.1 and 0.3 µg DON/L plasma, respectively.
DON-related metabolites in piglet plasma were detected in full-scan (FS) mode with a mass resolution of 70,000 FWHM in both positive and negative ion modes using fast polarity switching (scan range m/z 150–1000 for both ion modes), and all-ion fragmentation (AIF) in the negative ion mode (scan range m/z 80–700). AIF was performed using stepped normalised collision energy set to 35 ± 20%. The AGC target was set to 3×10^6 and 1×10^6 during FS and AIF, respectively, including a maximum IT of 200 ms. Standards of DOM-1, DON-3-GlcA, DON-15-GlcA, and DON-8-GlcA allowed the unambiguous identification of the metabolites.

UHPLC-HRMS method for DON and DON metabolites in in vitro samples

Samples from in vitro assays were analysed using the same UHPLC-HRMS instrument as above with identical interface settings, but operated in full-scan (FS) mode with mass ranges of m/z 200–720 in both negative and positive modes. In FS mode, the AGC target was set to 5×10^5 ions with a maximum injection time of 250 ms, whereas, in dd–MS^2 mode, the AGC target was 1×10^5 and with a maximum injection time of 100 ms. The quadrupole mass filter was operated with an isolation window of *m/z* 2. Chromatographic separation was performed at 30 °C on a 150 × 2.1 mm i.d. Kinetex F5 column (2.6 µm; Phenomenex, Torrance, MA, USA) with KrudKatcherTM Ultra HPLC 0.5 µm in-line

filter (Phenomenex). The injection volume was 1 µL, and samples were eluted using a water (A)/methanol (B) gradient, both containing 0.2% formic acid, with a flow rate of 0.25 mL/min. The column was eluted isocratically with 3% B for 1 min, then a gradient was applied increasing linearly to 15% B in 15 min, and finally to 97% B in 3 min. After washing the column for 2 min with 97% B, the mobile phase was returned to the initial conditions and the column was re-equilibrated for 3 min.

Determination of in vitro toxicokinetic parameters

Toxicokinetic parameters were derived from the substrate depletion rate constants (k_e) determined by the regression analysis of measured peak areas of DON versus time curves ($A_{DON}(t) = b + a \times e^{-k_e t}$). Assay half-life ($t_{1/2,assay} = \ln 2/k_e$) and assay clearances ($CL_{assay} = V_{assay} \times k_e$) were calculated under consideration of the assay volume (V_{assay}). Disregarding potential protein binding of DON in the reaction mixture [assuming that the fraction unbound in the assay ($f_{u,assay} \sim 1$)], the determined assay clearances approximated the intrinsic assay clearances ($CL_{int,assay}$), which is a measure of enzyme activity and described by the Michaelis–Menten equation parameters' maximal velocity ($v_{max,assay}$) and reaction constant ($K_{M,assay}$) under the condition that the substrate concentration is well below the K_M value ($CL_{int,assay} = v_{max,assay}/K_{M,assay}$). The individual $K_{M,assay}$ were determined from depletion experiments with different initial DON concentrations by plotting the determined depletion rate constants versus the respective concentrations. The inflection point of the curve in a lin-log plot represented the K_M value, occurring when k_e is half of the theoretical maximum k_0 at infinitesimally low DON concentrations [$k_e = k_{0[DON]\rightarrow 0} \times (1 - [DON]/([DON] + K_M)$] (Obach and Reed-Hagen 2002).
The $CL_{int,assay}$ for RLM, RLMH, CLMH, DLM, MPLM, PLMH, and HML were upscaled to the assay-independent, intrinsic liver clearances ($CL_{int} = CL_{int,assay} \times MRI \times RLW/Prot_{assay}$) by considering the amounts of microsomal protein in the assays ($Prot_{assay}$), species-specific relative liver weights (RLW), and microsomal recovery indexes (MRI) (Table 1). In vitro-to-in vivo extrapolation (IVIVE) was performed by applying the well-stirred liver model (Obach et al. 1997; Ito and Houston 2005) and calculating systemic blood clearances ($CL_{b,vitro}$) from the CL_{int}, while considering the hepatic blood flow (Q) of the different species [$CL_{b,vitro} = Q \times CL_{int} \times f_{u,b}/(Q + CL_{int} \times f_{u,b})$]. The fraction unbound ($f_{u,b}$) in blood of DON in pig, rat, and sheep was higher than 90% (Prelusky et al. 1987, 1988; Meky et al. 2003) and was, therefore, not considered for the calculation of $CL_{b,vitro}$ ($f_{u,b} \approx 1$). The maximal bioavailability (f_{max}) after oral application was calculated under the assumption of complete absorption from the gastrointestinal tract ($f_a = 1$) as $f_{max} = 1 - CL_{b,vitro}/Q$ and used for estimating low (< 10%), intermediate (10–30%) and high (> 30%) exposure (Obach et al. 1997). Plasma clearances (CL_p) were calculated from the blood clearances ($CL_p = CL_{b,vitro} \times c_p/c_b$) using the blood–plasma partition coefficient (c_b/c_p) of DON, which is close to unity in pig (Prelusky et al. 1987). Maximum exposure after iv application was calculated as $AUC_{iv,norm,max} = 1/CL_p$, and maximum exposure after po application was

calculated as $AUC_{po,norm,max} = f_{max}/CL_p$. Comparison of in vitro- and in vivo-derived toxicokinetic data allowed the evaluation of the prediction quality. Twofold differences between predicted and animal experimental clearances were considered as extrapolative inliers fulfilling the success criterion (Jolivette and Ward 2005).

Table 1

Toxicokinetic parameters of DON for different species derived from in vitro microsomal assays

Parameter	RLM[a]	RLMH[a]	CLMH[b]	DLM[c]	MPLM[d]	PLMH[e]	HLM[f]
k_e (min^{-1})[g]	0.083	0.085	0.010	0.010	0.014	0.014	0.015
$t_{1/2,assay}$ (min)	8.4	8.2	69	69	50	50	46
$K_{M,assay}$ (µM)[h]	0.40	0.46	1.12	0.13	0.73	0.48	0.21
$CL_{int,assay}$ (mL/min)	0.042	0.043	0.005	0.005	0.007	0.007	0.008
CL_{int} [L/(h × kg)]	6.08	6.22	0.37	0.53	0.41	0.46	0.39
$CL_{b,vitro}$ [L/(h × kg)][i]	2.48	2.51	0.33	0.42	0.35	0.37	0.30
f_{max} (%)	41	40	88	80	87	82	79
$AUC_{iv,norm,max}$ (h × kg/L)	0.40	0.40	3.07	2.36	2.82	2.70	3.33
$AUC_{po,norm,max}$ (h × kg/L)	0.17	0.16	2.66	1.90	2.49	2.21	2.63

[a]MRI = 61 mg/g; RLW = 40 g/kg bw; $Prot_{assay}$ = 1 mg; Q_{rat} = 4.2 L/(h × kg)

[b]MRI = 35 mg/g; RLW = 35 g/kg bw; $Prot_{assay}$ = 1 mg; $Q_{chicken}$ = 2.7 L/(h × kg)

[c]MRI = 55 mg/g; RLW = 32 g/kg bw; $Prot_{assay}$ = 1 mg; Q_{dog} = 2.1 L/(h × kg)

[d]MRI = 41 mg/g; RLW = 24 g/kg bw; $Prot_{assay}$ = 1 mg; $Q_{minipig}$ = 2.6 L/(h × kg)

[e]MRI = 34 mg/g; RLW = 32 g/kg bw; $Prot_{assay}$ = 1 mg; Q_{pig} = 2.1 L/(h × kg)

[f]MRI = 40 mg/g; RLW = 22 g/kg bw; $Prot_{assay}$ = 1 mg; Q_{human} = 1.4 L/(h × kg)

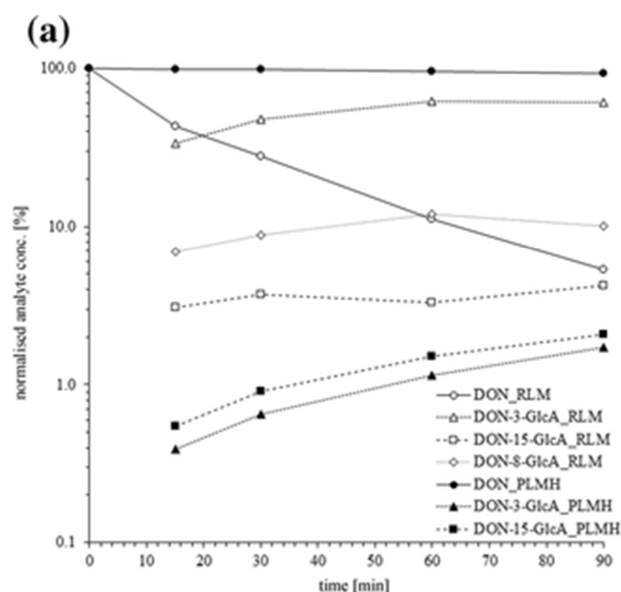
[g]Determined by regression of DON depletion plots (Fig. 2d)

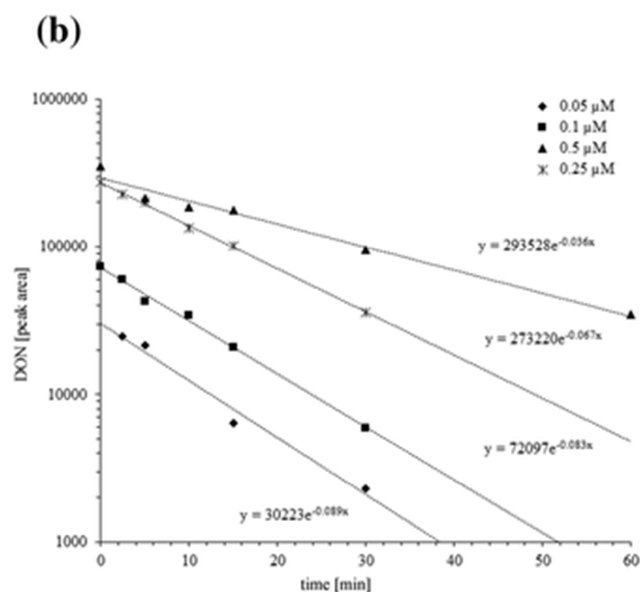
[h]Derived from depletion plots at different DON concentrations (Fig. 2b)

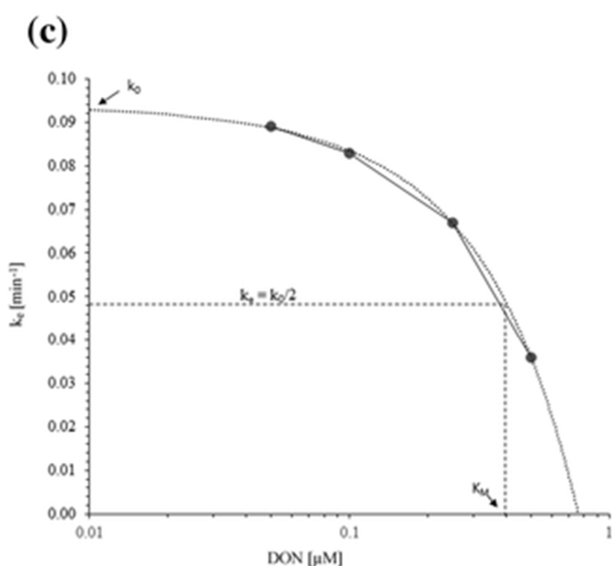
[i]Equivalent to CL_p since $c_b/c_p \approx 1$

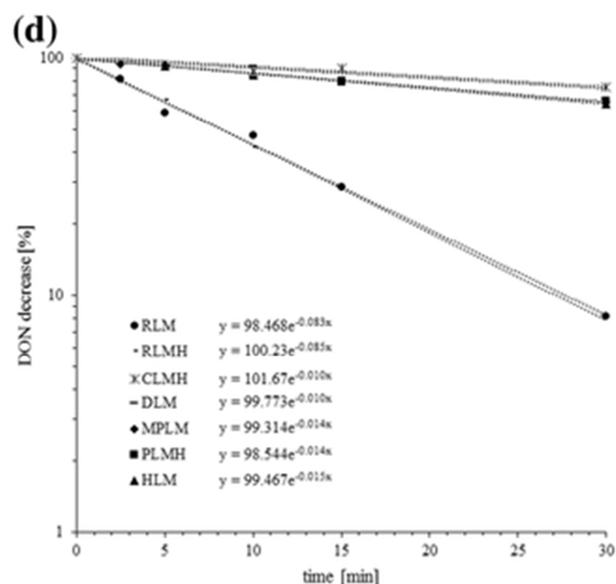
Fig. 2

a Formation of DON glucuronides in rat (RLM) and pig (PLMH) liver microsome assays, **b** depletion of DON in rat liver microsomes (RLM), **c** determination of K_M in RLM assay, and **d** DON depletion kinetics in liver microsomes of different species









Determination of in vivo toxicokinetic parameters

Toxicokinetic analysis of the piglet study was performed by non-compartmental analysis using Phoenix 64 WinNonlin 7.0 (Pharsight, St. Louis, MO, USA). Parameters calculated for intravenous or extravascular administration of DON were area under the plasma concentration–time curve (AUC), plasma clearance (CL_p), volume of distribution (V_d), elimination half-life ($t_{1/2}$), time to maximal plasma concentration (t_{max}), and maximal plasma concentration (c_{max}). The absolute bioavailability was calculated from the dose-normalised AUCs as $f = AUC_{po,norm}/AUC_{iv,norm}$. Data were expressed as means of six animals per dose group ± standard deviations (SD). The exact sampling time points were used for each pig and slopes were adjusted individually for the calculation of elimination parameters. The AUCs for DON 3-GlcA and DON 15-GlcA were determined for all pigs, and means and SDs were calculated.

Compilation of toxicokinetic data of DON from published in vivo studies

A literature search was conducted to identify the published DON in vivo studies presenting toxicokinetic data. Using the following search string [deoxynivalenol (Title/Abstract)] AND (dose OR food OR feed OR diet) AND (half-life OR bioavailability OR exposure) in PubMed and Google Scholar numerous studies containing miscellaneous collections of toxicokinetic parameters were identified. In addition, several studies not captured by the search but found in reference lists of included studies, reviews or meta-analyses, or obtained by specific searching were considered. The search was not limited to recent years. All available toxicokinetic data from relevant studies were sorted by species and year of publication.

Allometric scaling and prediction of DON toxicokinetic data in humans

$CL_{b,vitro}$ of different species were correlated through BW-dependent allometric scaling (log CL ~ log BW) (Obach et al. 1997). The same was performed with the available clearance data from in vivo studies and the values were extrapolated to humans. The used BW were either acquired from the respective in vivo studies, or average BW of 0.2 kg for Wistar rats, 1.5 kg chicken, 11 kg for Beagle dogs, 19.6 kg for Göttingen minipigs, 25 kg for growing pigs, and 70 kg for humans were used for scaling of the in vitro data.
Human plasma clearance was calculated from the determined blood clearance using the blood–plasma partition coefficient (c_b/c_p) of DON in pig (Prelusky et al. 1988). Half-life in humans was calculated from the volume of distribution and the estimated plasma clearance, both, determined from IVIVE and from allometric scaling ($t_{1/2} = \ln 2 \times V_d/CL_p$). Tentatively, c_{max} after oral application ($c_{max} = f \times D \times V_d^{-1} \times e^{(-\ln 2 \times t_{max}/t_{1/2})}$) and exposure ($AUC_{po} = D \times AUC_{po,norm}$) were calculated using typical doses (D) in food and the predicted f, V_d and $t_{1/2}$. The concentration of total DON in 24 h urine ($c_{urine_24\ (total\ DON)} = excretion_{urine_24\ h} \times D \times BW/V_{urine_24\ h}$) was estimated based on published renal excretion rates and mean daily urine excretion in adults. An excretion calculator (http://www.clinicalculator.com/english/nephrology/excrea/excrea.htm) was used for normalisation by creatinine content.

Results

Analysis of DON and DON glucuronides by UHPLC-HRMS

Samples from the in vitro kinetic assays and the in vivo pig study were analysed by high-resolution mass spectrometry. The baseline separation of the signals allowed the positive identification of DON and the metabolites DON-3-GlcA, DON-15-GlcA, and DON-8-GlcA (Fig. 1b). The different microsomal preparations produced species-specific metabolite profiles. While all three DON glucuronides were detected in rat microsome incubations, both DON-3-GlcA and DON-15-GlcA were found in pig (Fig. 2a) and human microsomes, whereas dog microsomes produced only DON-3-GlcA. Small amounts of DON-3-GlcA and DON-15-GlcA were found in chicken microsomes (data

not shown). The yields for the respective glucuronides varied with the species. In the rat microsome assay, DON-3-GlcA was the most important metabolite, in contrast to the human microsome assay, where DON-15-GlcA was dominating. In pig microsomes, both metabolites were produced with comparable rates.

Determination of in vitro toxicokinetic parameters of DON for different species

Depletion assays at different DON concentrations were performed with seven microsomal preparations and elimination constants (k_e) were determined by regression analysis for each incubation (Fig. 2b). Using the individual k_e, reaction constants, $K_{M,assay}$ were defined for each microsome species (Fig. 2c). The $K_{M,assay}$ of DON in the chicken glucuronidation assay was notably higher than for the other species and indicated lower enzyme affinity. The k_e from the in vitro assays run under optimised conditions and with DON concentrations below the K_M (Fig. 2d) were subsequently used for the calculation of species-specific kinetic parameters (Table 1). There was a clear difference between the metabolism rates in the rat microsome assays and the other assays. As a result, DON was predicted to be a substance with intermediate clearance in rats, and with low clearance in chickens, dogs, minipigs, pigs, and humans.

Determination of in vivo toxicokinetic parameters in piglets after iv and po application of DON

The toxicokinetic in vivo study in Norwegian-crossbred piglets was performed as a benchmark for the in vitro experiments. The pig liver microsomes had been prepared from animals of the same breed and age. The doses used for intravenous (iv) and oral (po) administration were chosen following published reports. The aim was achieving measurable plasma concentrations of DON and its metabolites, while at the same time avoiding symptoms of acute toxicosis. A single event of marginal emesis occurred at 0.5–1 h after po application in two of six piglets, and at 0.2–1.5 h after iv application in five of six piglets. Due to the negligible extent, it was considered insignificant for the outcome of the study, especially since further feed consumption was normal.
The plasma concentration–time profiles (Fig. 3a, b) were used for the calculation of toxicokinetic parameters by non-compartmental analysis (Table 2). The half-life in plasma was 2.6 h after iv administration. Plasma clearance was low and comparable to the value that was predicted in the in vitro study (Table 1). The volume of distribution of DON was approximately ten times the plasma volume in pig (Setiabudi et al. 1976), indicating moderate dispersion to lower body compartments and/or protein binding. The maximal plasma concentration after po administration was reached after 3.5 h, and absolute bioavailability was determined as 53%.

Fig. 3

Plasma concentration–time profiles of DON and DON glucuronides in piglets ($n = 6$) after **a** intravenous application of 0.08 mg/kg BW or **b** oral application of 0.125 mg/kg BW DON

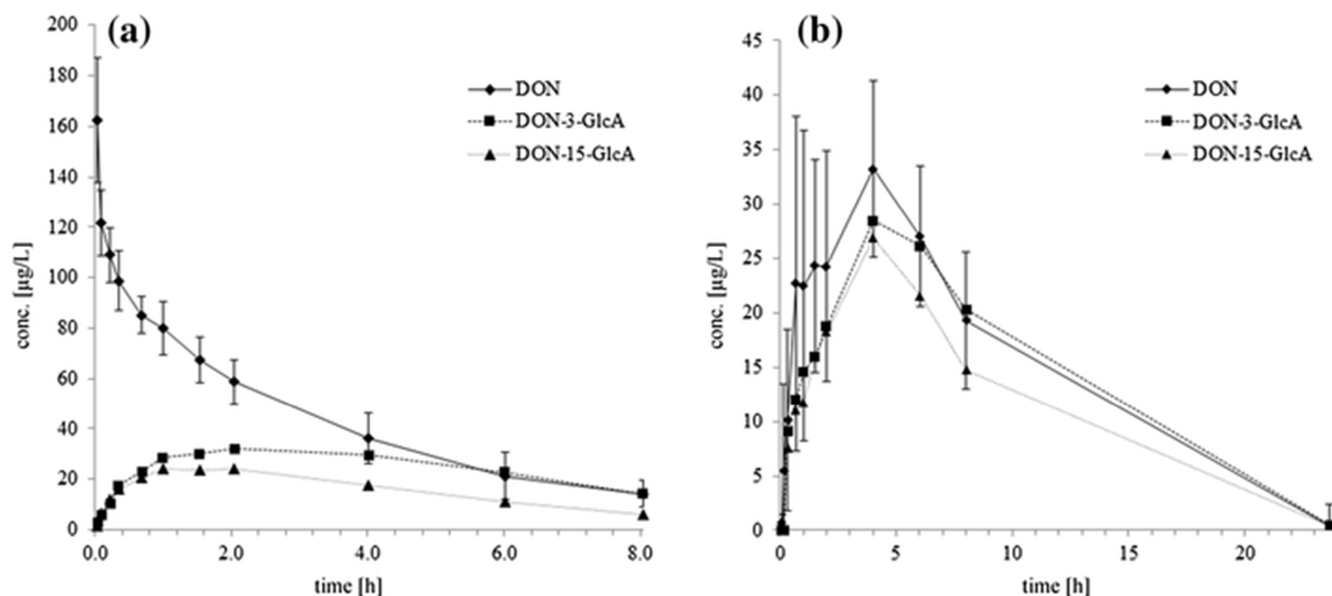


Table 2

Toxicokinetic parameters (means ± SD) of DON and the metabolites DON-3-GlcA and DON-15-GlcA in piglets after intravenous and oral application of DON

Parameter	po ($n = 6$)						iv ($n = 6$)					
	DON		DON-3-GlcA		DON-15-GlcA		DON		DON-3-GlcA		DON-15-GlcA	
		SD		SD		SD		SD		SD		SD
Dose (mg/kg)	0.125						0.08					
AUC_{iv} (µg × h/L)							407	97.1				
AUC_{po} (µg × h/L)	335	60	316	147	261	172			242	122	202	121
c_{max} (µg/L)	35.7	6.2	29.9	19.1	27.9	19.5			32.4	12.5	30.0	12.8
t_{max} (h)	3.5	1.7	4.3	0.7	4.0	1.2			1.8	1.0	1.3	0.4
$t_{1/2}$ (h)	3.8	1.0	3.9	1.2	3.7	1.3	2.6	0.6	2.7	0.9	3.3	1.5
CL_p [L/(h × kg)]							0.21	0.05				
V_d (L/kg)							0.70	0.05				
$AUC_{iv,norm}$ (h × kg/L)							5.09	1.21				
$AUC_{po,norm}$ (h × kg/L)	2.68	0.48										
f (%)	52.7											
AUC_{met}/AUC_{DON} (%)			94.3			77.9			59.5		49.6	

The DON metabolites DON-3-GlcA and DON-15-GlcA were detected with the same 10:8 ratio of exposures ($AUC_{DON-3-GlcA}$:$AUC_{DON-15-GlcA}$) both after iv and po application. In comparison to the measured DON exposure (AUC_{DON}), however, the exposure to the DON

glucuronide metabolites (AUC_{met}) was 78–94% after po and 50–60% after iv application (Table 2).

Review of published in vivo toxicokinetic parameters of DON in different animal species

A thorough search of in vivo study reports containing toxicokinetic data was performed and the results were listed according to species and publication date (Table 3). In total, information on 13 animal species was collected. The available data were, however, rather fragmented, heterogeneous and incomplete. In many cases, only the excretion of total DON, i.e., the sum of DON and its metabolites, via the urine had been studied. The excretion ratios within 24 h after uptake ranged from < 18% in cows to > 90% in pigs. Measurable urine concentrations were detected in all species, depending strongly on the given dose and route of application. Maximum plasma concentrations after oral application appeared in mice, rats, and birds at less than 1 h, in sheep, pigs, cows, and horses at 2–4 h, and in fish at 8 h. Absolute bioavailabilities calculated from connected iv and po experiments were only determined in a few studies. The recorded values were in the range of < 3% in cow and 8% in sheep, 6–30% in chicken, turkey and pigeon, and 50–100% in pig and piglet. Plasma clearances have been reported for chicken, turkey, pigeon, pig, and sheep. In addition, we have calculated an approximated CL_p for mice from the given values for dose and AUC after intranasal instillation (Table 3). The clearances were low-to-intermediate in comparison with the respective species-specific liver blood flows. Half-lifes in plasma after iv administration ranged from about 0.3 h in birds to 1 h in sheep and 3 h in pig, while half-lifes were prolonged after oral uptake. The described volumes of distribution varied considerably between studies for some species. Mean values were at 0.2 L/kg in sheep ($n = 1$), 2.5 L/kg in chicken ($n = 3$), 3.9 L/kg in turkey ($n = 2$), 1.0 L/kg in piglet ($n = 4$), and 1.9 L/kg in pig ($n = 4$) (Table 3).

Table 3

Published toxicokinetics data of deoxynivalenol in different species

Species	Route	Dose (mg/kg BW) or (mg/kg diet/day)[b,c]	*F* (%) AUC_{po} (µg × h/L)	Excretion (%) CL_p [L/(h × kg)]	V_d (L/kg)	$t_{½}$ (h)	t_{max}[e] (h)	c_{max}[e] (µg/L) c_{urine}[g] (µg/L)	References[k]
Mouse	po	5[a]	600 (3 h)	–	–	7.6	0.5	1300	Azcona-Olivera et al. (1995)
	po	25	–	–	–	–	0.5	–	Yordanova et al. (2003)
	po	5	3100	–	–	–	0.25	1100[d]	Amuzie et al. (2008)
	in	5	7200	0.69	–	–	0.5	3300[d]	Amuzie et al. (2008)
	po	25	–	–	–	11.8	5–15	12000[d]	Pestka et al. (2008)

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Published toxicokinetics data of deoxynivalenol in different species

Species	Route	Dose (mg/kg BW) or (mg/kg diet/day)[b,c]	F (%) AUC_{po} (μg × h/L)	Excretion (%) CL_p [L/(h × kg)]	V_d (L/kg)	$t_{½}$ (h)	t_{max}[e] (h)	c_{max}[e] (μg/L) c_{urine}[g] (μg/L)	References[k]
	po	5	3150	–	–	4	0.25	1248[d]	Pestka and Amuzie (2008)
	ip	1	–	–	–	–	1	690–806[d]	Clark et al. (2015a)
	ip	1	–	–	–	–	1	770–1141[d]	Clark et al. (2015b)
	ip	1	–	79–90% (24 h)	–	–	–	–	Pestka et al. (2017)
Weanling	po	5	4380	–	–	4	0.25	2228	Pestka and Amuzie (2008)
Rat	po	6	–	15% (72 h)	–	–	–	–	Yoshizawa et al. (1983)
	po	10[a]	–	89% (72 h)[d]	–	–	–	–	Lake et al. (1987)
	po	10[a]	–	89% (72 h)[d]	–	–	–	–	Worrell et al. (1989)
	po	5[a]	–	37% (72 h)	–	–	8	291	Meky et al. (2003)
	Diet	3.57 /kg bw (4d)	–	–	–	–	–	1900–4900[g]	Lattanzio et al. (2011)
	po	2	–	28% (48 h)	–	–	–	–	Nagl et al. (2012)
	po	0.09	–	–	–	–	0.28	2.0	Versilovskis et al. (2012)
	po	0.5[a]/2.5[a]	–	75% (48 h)[d]	–	–	6	42/160[a]	Wan et al. (2014)
	po	2	–	75% (24 h)[d]	–	–	–	–	Schwartz-Zim. et al. (2014)
Fish									
Carp	Diet	1.0 (4w)	–	–	–	–	8	0.7	Pietsch et al. (2014)
Salmon	Diet	6 (8 w)	89.4 (8 h)	–	–	15.1	–	5.7	Bernhoft et al. (2017)
Birds									
Chicken	po	1.3–1.7[a]	< 1	99% (72 h)[d]	–	3.1	2.3	–	Prelusky et al. (1986a)
	po	0.7 (1d)	–	74% (24 h)[d]	–	–	–	–	Lun et al. (1988v

Table 3

Published toxicokinetics data of deoxynivalenol in different species

Species	Route	Dose (mg/kg BW) or (mg/kg diet/day)[b,c]	*F* (%) AUC$_{po}$ (μg × h/L)	Excretion (%) CL$_p$ [L/(h × kg)]	V_d (L/kg)	$t_{½}$ (h)	t_{max}[e] (h)	c_{max}[e] (μg/L) c_{urine}[g] (μg/L)	References[k]
	Diet/po	5.8 (1w)/0.1[a]	–	75% (24 h)[d]	–	(1.5)	1	–	Lun et al. (1989)
	po	2.2[a]	–	79% (24 h)[d]	–	–	–	–	Prelusky et al. (1989)
	Diet	2.5 (35d)	–	–	–	–	–	< 2	Dänicke et al. (2007a)
	Diet	0.095 /kg bw	–	–	–	3.8	2	0.9	Yunus et al. (2010)
	po/Diet	0.15/7.5 (1w)	–	–	–	–	0.7	6.5/3.9	De Baere et al. (2011)
	Diet	0.87/5.0 (5w)	–	–	–	–	–	< 0.7	Awad et al. (2011)
	po	2.8	–	–	–	–	1/5	27/2.6	Yunus et al. (2012)
	Diet	7.5 (1w)	–	–	–	–	–	4	Osselaere et al. (2012)
	po	0.75	–	–	–	0.7	0.6	8–14	Devreese et al. (2012a)
	iv	0.75	–	7.2	5.0	0.5	–	–	**Osselaere et al**. (**2013**)
	po	0.75	19%	–	–	0.6	0.5	26	**Osselaere et al**. (**2013**)
	po	2.5[a]	–	81% (24 h)[d]	–	–	6	210[a]	Wan et al. (2014)
	Diet	0.4–1 (70w)	–	–	–	–	–	0.2–0.6	Ebrahem et al. (2014)
	po	0.5	11%	–	–	–	–	–	Broekaert et al. (2014)
	iv	0.5	–	–	–	–	–	–	Broekaert et al. (2014)
	po	0.75	–	–	–	–	0.6	27	Devreese et al. (2015)
	iv	0.75	–	–	–	–	0.4	–	Devreese et al. (2015)
	iv	0.5	–	6.1	1.0	0.3	–	–	**Broekaert et al**. (**2015**)
	po	0.5	11%	–	–	–	0.5	4.2	**Broekaert et al**. (**2015**)
	Diet	1.7 (1d)	–	80% (24 h)[d]	–	–	–	–	Schwartz-Zim. et al. (2015)

Table 3

Published toxicokinetics data of deoxynivalenol in different species

Species	Route	Dose (mg/kg BW) or (mg/kg diet/day)[b,c]	F (%) AUC_{po} (μg × h/L)	Excretion (%) CL_p [L/(h × kg)]	V_d (L/kg)	$t_{½}$ (h)	t_{max}[e] (h)	c_{max}[e] (μg/L) c_{urine}[g] (μg/L)	References[k]
	po	0.5	5.6%	–	–	–	0.5	7.4	**Broekaert et al**. (**2017**)
	iv	0.5	–	5.2	1.4	0.3	–	–	**Broekaert et al**. (**2017**)
Turkey	iv	1, (1[a])	–	0.41	0.3	0.7	–	–	Gauvreau (1991)
	po	1/5, (5[a])	1%	–	–	–	0.2/1	6.7/24	Gauvreau (1991)
	iv	5[a]	–	97% (48 h)[d]	–	–	–	–	Gauvreau (1991)
	Diet	(5.4 po) (2w)	–	–	–	–	–	< 2	Dänicke et al. (2007b)
	Diet	5.2 (12w)	–	–	–	–	–	3	Devreese et al. (2014)
	iv	0.75	–	8.2	7.4	0.6	–	–	Devreese et al. (2015)
	po	0.75	21%	–	–	0.9	0.6	13	Devreese et al. (2015)
	Diet	1.5 (1d)	–	52% (24 h)	–	–	–	–	Schwartz-Zim. et al. (2015)
Pekin duck	Diet	6–7 (49d)	–	–	–	–	–	< 6	Dänicke et al. (2004b)
Pigeon	iv	0.3	–	12.5	5.7	0.3	–	–	**Antonissen et al**. (**2016**)
	po	0.3	30%	–	–	0.6	0.5	10	**Antonissen et al**. (**2016**)
Pig	Diet	2.8–5.9 (14w)	–	67% (5d)	–	–	–	–	Friend et al. (1986)
	po	1[a]	64%	–	–	–	3.8	367	**Prelusky et al**. (**1990**)
	iv	1[a]	–	0.19	1.1	4.1	–	–	**Prelusky et al**. (**1990**)
	iv	1	–	–	–	3.9	–	–	Prelusky and Trenholm (1991)
	Diet	0.23 (/BW; 16d)	–	–	–	–	–	580[g,h]	Razzazi et al. (2002)
	Diet	2.5 (2.5d)	–	–	–	2.4	2.8	52	Eriksen et al. (2003)

Table 3

Published toxicokinetics data of deoxynivalenol in different species

Species	Route	Dose (mg/kg BW) or (mg/kg diet/day)[b,c]	F (%) AUC_{po} (μg × h/L)	Excretion (%) CL_p [L/(h × kg)]	V_d (L/kg)	$t_{½}$ (h)	t_{max}[e] (h)	c_{max}[e] (μg/L) c_{urine}[g] (μg/L)	References[k]
	Diet	4.2 (7d)	–	–	–	5.8	4.1	–	Dänicke et al. (2004a)
	Diet	1.2–4.6 (10w)	–	57% (7d)	–	–	–	14	Dänicke et al. (2004c)
	Diet	9.6 (35d)	–	–	–	–	(1.5)	22	Dänicke et al. (2005a)
	Diet	6.5 (12w)	–	–	–	–	(1.5)	18	Goyarts et al. (2005)
	Diet	5.7 (28d)	89%	–	–	6.3	1.6	22	Goyarts and Dänicke (2006)
	iv	0.053	–	0.23	3.8	4.2–31	–	–	**Goyarts and Dänicke** (**2006**)
	Diet	5.7 (0.07 po)	54%	–	–	5.2	1.7	15	**Goyarts and Dänicke** (**2006**)
	po	6.7 (12w)	–	–	–	–	2.2	13–30	Goyarts et al. (2007)
	Diet	9.6 (35d)	–	68% (5 h)[d]	–	–	1.0	9	Dänicke et al. (2007c)
	po	0.05	–	–	–	–	0.4	8.6	De Baere et al. (2011)
	iv (inf)	0.1/h (1 h)	–	0.71[f]	2.1	2.3	1.0	77	Dänicke et al. (2012)
	po	0.05	–	–	–	–	–	30	Devreese et al. (2012b)
	po	0.044	87%	–	–	3.7	1.8	17	Rohweder et al. (2013)
	Diet/inf	3.1(37d)/0.1/h	–	–	–	–	–	7.5/19	Dänicke et al. (2014)
	po	0.075	–	85% (24 h)[d]	–	–	–	–	Nagl et al. (2014)
	iv	0.05	–	0.22	0.6	2.0	–	–	**Paulick et al**. (**2015**)
	po (Diet)	0.07	99%	–	–	6.1	4.9	28.8	**Paulick et al**. (**2015**)
	Diet	1–5 (7d)	–	–	–	–	–	0.4–5.3[g,i,h]/c	Thanner et al. (2016)
Piglet	iv	0.5	–	< 57% (24 h)	–	2.1–3.7	–	–	Coppock et al. (1985)
Piglet	iv	0.3[a]	–	93%[d], 0.11	1.3	1.6	–	–	Prelusky et al. (1988)

Table 3

Published toxicokinetics data of deoxynivalenol in different species

Species	Route	Dose (mg/kg BW) or (mg/kg diet/day)[b,c]	*F* (%) AUC_{po} (μg × h/L)	Excretion (%) CL_p [L/(h × kg)]	V_d (L/kg)	$t_{½}$ (h)	t_{max}[e] (h)	c_{max}[e] (μg/L) c_{urine}[g] (μg/L)	References[k]
	po	0.6[a]	48–65%	95% (14 h)[d]	–	–	0.8	135–322[a]	Prelusky et al. (1988)
	Diet	0.2–3.9 (35d)	–	–	–	–	–	0.4–12/56–380[g]	Döll et al. (2003a)
	Diet	0.2–3.9 (37d)	–	–	–	–	4.0	1–11/56–380[g]	Döll et al. (2003b)
	Diet	3.2 (12d)	–	–	–	–	–	15.5	Dänicke et al. (2005c)
	Diet	0.55–1.23 (11w)	–	–	–	–	–	5–7	Döll et al. (2008)
	Diet	2.3 (28d)	–	–	–	–	–	11	Dänicke et al. (2010)
	po	0.05	–	–	–	–	1.3	30	Devreese et al. (2014
	iv	0.036	–	0.91	1.2	2.9	–	–	**Broekaert et al. (2015)**
	po	0.036	100%	–	–	–	1.8	6.3	**Broekaert et al. (2015)**
	Diet/po	0.9 (10d)/0.3	–	–	–	–	2.0	168	Alizadeh et al. (2015)
	iv	0.25/0.75	–	–	–	–	0.5	9.2/26.8	Deng et al. (2015)
	iv	0.036	–	0.32	0.6	2.7	–	–	**Broekaert et al. (2017)**
	po	0.036	81%	–	–	–	0.7	24	**Broekaert et al. (2017)**
	Diet	0.09/5.36 (6w)	–	–	–	–	–	< 0.2/31	Paulick et al. (2018)
	Diet	0.09/5.36 (6w)	–	–	–	–	–	3100[g,i,h]/c	Tran et al. (2018)
	iv	0.08	–	0.21	0.7	2.6	–	–	**Fæste et al. (2018**[j])
	po	0.125	53%	–	–	–	3.5	36	**Fæste et al. (2018**[j])
Sheep	iv	0.5	–	–	–	1.1	–	–	**Prelusky et al. (1985)**
	po	5	7.5%	–	–	1.9	4.7	–	**Prelusky et al. (1985)**

Table 3

Published toxicokinetics data of deoxynivalenol in different species

Species	Route	Dose (mg/kg BW) or (mg/kg diet/day)[b,c]	*F* (%) AUC_{po} (µg × h/L)	Excretion (%) CL_p [L/(h × kg)]	V_d (L/kg)	$t_{½}$ (h)	t_{max}[e] (h)	c_{max}[e] (µg/L) c_{urine}[g] (µg/L)	References[k]
	iv	0.5	–	68% (7 h)[d]	–	–	–	–	Prelusky et al. (1986b)
	po	5	–	72% (14 h)[d]	–	–	–	–	Prelusky et al. (1986b)
	iv	4[a]	–	97% (24 h)[d]	–	–	–	–	Prelusky et al. (1987)
	iv	1[a]	–	0.12	0.2	1.1	–	–	Prelusky et al. (1990)
	Diet	0.01 po (7d)	–	–	–	–	1	0.4	Lohölter et al. (2012)
Cow	po	1.9	< 1%	–	–	(4.0)	4.1	90–200	Prelusky et al. (1984)
	Diet	66 (5d)	–	18% (72 h)	–	–	–	–	Côté et al. (1986)
	Diet	3.5 (28d)	< 3%	–	–	–	–	–	Dänicke et al. (2005b)
	Diet	8.2 (3w)	–	96% (24 h)[d]	–	–	2	< 0.5	Seeling et al. (2006)
	Diet	5.3 (8w)	–	–	–	–	3	3.5	Keese et al. (2008)
	Diet	2.6–5.2 (13w)	–	–	–	–	–	1.3/3.6	Winkler et al. (2014)
	Diet	2.6–5.2 (13w)	–	–	–	–	–	1.2/1.3	Winkler et al. (2015a)
	Diet	2.6–5.2 (13w)	–	–	–	–	–	131–242[g,i,h]/c	Winkler et al. (2015b)
	Diet	0.274 /kg bw (9w)	–	–	–	–	–	< 0.8	Dänicke et al. (2016)
Horse	Diet	12 (10d) (0.08 po)	–	–	–	–	7.3	38	Setyabudi et al. (2012)
	Diet	7.9 (21d) (0.05 po)	–	–	–	–	3	5.2	Schulz et al. (2015)
Human	Diet	0.3–1.4 × 10^{-3}/d/kg	China	–	–	–	–	12[g,h,d]	Meky et al. (2003)
	Diet	1.1–7.4 × 10^{-3}/d/kg	China	–	–	–	–	37[g,h,d]	Meky et al. (2003)
	Diet	26 g cereals	UK	–	–	–	–	0.6[g,i,h,d]/c	Turner et al. (2008a)
	Diet	322 g cereals	UK	–	–	–	–	7.2[g,i,h,d]/c	Turner et al. (2008a)

Table 3

Published toxicokinetics data of deoxynivalenol in different species

Species	Route	Dose (mg/kg BW) or (mg/kg diet/day)[b,c]	F (%) AUC_{po} (µg × h/L)	Excretion (%) CL_p [L/(h × kg)]	V_d (L/kg)	$t_{1/2}$ (h)	t_{max}[e] (h)	c_{max}[e] (µg/L) c_{urine}[g] (µg/L)	References[k]
	Diet	0.3×10^{-3} in 197 g	UK	–	–	–	–	8.9[g,i,h,d]/c	Turner et al. (2008b)
	Diet	107–300 g cereals	UK	–	–	–	–	5.4–9.3[g,i,h]/c	Turner et al. (2008c)
	Diet	180 g cereals	UK	–	–	–	–	7.5[g,h,d]	Turner et al. (2009)
	Diet	20×10^{-3} in 400 g	France	–	–	–	–	0.5–28.8[g,d]	Turner et al. (2010a)
	Diet	11×10^{-3} in 206 g	UK	72% (24 h)[d]	–	–	–	10[g,i,h,d]	Turner et al. (2010b)
	Diet	206 g cereals	UK	–	–	–	–	2.4[g,h]	Turner et al. (2011a)
	Diet	45 g cereals	China	–	–	–	–	5.9[g,i,h,d]/c	Turner et al. (2011b)
	Diet	$0.5–1 \times 10^{-3}$/d/kg	Austria	–	–	–	–	30[g,h,d]	Warth et al. (2011)
	Diet	–	Italy	–	–	–	–	3.0–8.0	Lattanzio et al. (2011)
	Diet	–	Italy	–	–	–	–	3.7[g,h]	Solfrizzo et al. (2011)
	Diet	–	Iran	–	–	–	–	1.5[g,i,h,d]/c	Turner et al. (2012)
	Diet	$0.2/2.4 \times 10^{-3}$/d/kg	UK	–	–	–	–	0.5–43[g,i,d]/c	Hepworth et al. (2012)
	Diet	ca. 200 g cereals	Austria	–	–	–	–	22[g,h,d]	Warth et al. (2012)
	Diet	–	Egypt	–	–	–	–	0.5–59.9[g,i]/c	Piekkola et al. (2012)
	Diet	–	Portugal	–	–	–	–	16.3[g,h,d]	Cunha and Fernandes (2012)
	Diet	–	Belgium	–	–	–	–	0.7–68.3[g,i]/c	Ediage et al. (2012)
	Diet	2.3×10^{-3}/d/kg	Austria	68% (24 h)[d]	–	–	–	8–11, 39[g,d]	Warth et al. (2013)
	Diet	$2.5–5.4 \times 10^{-3}$/d/kg	Sweden	–	–	–	–	0.5–178[g,i,d]/c	Wallin et al. (2013)
	Diet	$0.9–33 \times 10^{-3}$/d/kg	Croatia	–	–	–	–	7–903[g,i,d]/c	Šarkanj et al. (2013)

Table 3

Published toxicokinetics data of deoxynivalenol in different species

Species	Route	Dose (mg/kg BW) or (mg/kg diet/day)[b,c]	F (%) AUC_{po} (μg × h/L)	Excretion (%) CL_p [L/(h × kg)]	V_d (L/kg)	$t_{½}$ (h)	t_{max}[e] (h)	c_{max}[e] (μg/L) c_{urine}[g] (μg/L)	References[k]
	Diet	0.8–33 × 10^{-3}/d/kg	S. Africa	–	–	–	–	20.4[g,i,h,d]/c	Shepard et al. (2013)
	Diet	0.2–2.6 × 10^{-3}/d/kg	Cameroon	–	–	–	–	6.0[g,i,h,d]/c	Abia et al. (2013)
	Diet	0.2–0.6 × 10^{-3}/d/kg	Europe	–	–	–	–	> 1[g] (0–5%)	EFSA (2013)
	Diet	0.2–0.5 × 10^{-3}/d/kg	UK	–	–	–	–	7.2–20[g,i]/c	Gratz et al. (2014)
	Diet	0.3 × 10^{-3}/d/kg	Nigeria	–	–	–	–	2.0[g]	Ezekiel et al. (2014)
	Diet	< 4.2 μg/kg maize	Ivory Coast	–	–	–	–	< 0.8–10[g,d]	Kouadio et al. (2014)
	Diet	0.5 × 10^{-3}/d/kg	Germany	68% (24 h)[d]	–	–	–	21[g,i,h,d]/c	Gerding et al. (2014)
	Diet	0.4 × 10^{-3}/d/kg	Spain	–	–	–	–	15–32[g,i,d]/c	Rodriguez-Carr. et al. (2014)
	Diet	0.6 × 10^{-3}/d/kg	Italy	–	–	–	–	12[g,h]	Solfrizzo et al. (2014)
	Diet	–	Thailand	–	–	–	–	7.2[g,i,h,d]/c	Warth et al. (2014)
	Diet	49 × 10^{-3}/d/kg	Spain	72% (24 h)	–	–	–	17.1[g,i,h]/c	Rodriguez-Carr. et al. (2015)
	Diet	0.3–4.4 × 10^{-3}/d/kg	Haiti	–	–	–	–	20.2[g,h,d]	Gerding et al. (2015)
	Diet	111–135 g cereals	Sweden	–	–	–	–	4.4[g,i,h]/c	Wallin et al. (2015)
	Diet	397–456 g maize	Tanzania	–	–	–	–	40–49[g,i,d]/c	Gong et al. (2015)
	Diet	–	Europe	–	–	–	–	4.6–39[g,i,d]/c	Brera et al. (2015)
	Diet	0.06–10 × 10^{-3}/d/kg	Belgium	–	–	–	–	6.1[g,i,h]/c	Heyndrickx et al. (2015)
	Diet	–	Belgium	–	–	–	–	59[g,h,d]	Huybrechts et al. (2015)
	Diet	0.05–7.2 × 10^{-3}/d/kg	Bangladesh	–	–	–	–	0.2–7.2[g,d]	Ali et al. (2015)
	Diet	–	Germany	–	–	–	–	1.1–13.4[g,i]/c	Föllmann et al. (2016)
	Diet	–	Sweden	–	–	–	–	0.5–135[g,d]	Turner et al. (2016)

Table 3

Published toxicokinetics data of deoxynivalenol in different species

Species	Route	Dose (mg/kg BW) or (mg/kg diet/day)[b,c]	*F* (%) AUC_{po} (μg × h/L)	Excretion (%) CL_p [L/(h × kg)]	V_d (L/kg)	$t_{½}$ (h)	t_{max}^{e} (h)	c_{max}^{e} (μg/L) c_{urine}^{g} (μg/L)	References[k]
	Diet	–	UK	–	–	–	–	38–40[g,i,h,d]/c	Wells et al. (2016)
	Diet	6×10^{-3}/d/kg	Bangladesh	–	–	–	–	0.2–1.8[g]	Ali et al. (2016)
	Diet	268–975×10^{-3}/d/kg	Germany	–	–	–	–	0.2–38.4[g]	Ali et al. (2016)
	Diet	0.4–1.5×10^{-3}/d/kg	Spain	–	–	–	–	1.1[g,i,h]/c	Vidal et al. (2016)
	Diet	–	UK	–	–	–	–	12.7–40[g,i,h,d]/c	Wells et al. (2017)
	Diet	0.5–1.0×10^{-3}/d/kg	UK	–	–	–	–	19–25[g,i,h,d]/c	Papageorgiou et al. (2018)
	Diet	1.0–1.4×10^{-3}/d/kg	China	–	–	–	–	27.8–45.1[g,h,d]	Deng et al. (2018)
	Diet	0.3×10^{-3}/d/kg	Nigeria	–	–	–	–	0.1–6.2[g]	Šarkanj et al. (2018)
	Diet	1 μg/kg BW bolus	Belgium	45–73%(24 h)[d]	–	–	–	–	Vidal et al. (2018)
Children	Diet	0.1–2.9×10^{-3}/d/kg	Cameroon	–	–	–	–	0.1–77[g]	Ediage et al. (2013)
	Diet	0.5–1.0×10^{-3}/d/kg	Europe	–	–	–	–	> 1[g] (0.4–46%)	EFSA (2013)
	Diet	0.1–0.3×10^{-3}/d/kg	Tanzania	74% (24 h)[d]	–	–	–	2.3–5.7[g,h,d]	Srey et al. (2014)
	Diet	0.8×10^{-3}/d/kg	Spain	–	–	–	–	28[g,i,h,d]/c	Rodriguez-Carr. et al. (2014)
	Diet	257 g maize	Tanzania	–	–	–	–	48[g,i,h,d]/c	Gong et al. (2015)
	Diet	–	Europe	–	–	–	–	14–42[g,i,h,d]/c	Brera et al. (2015)
	Diet	0.1–20×10^{-3}/d/kg	Belgium	–	–	–	–	5.5[g,i,h]/c	Heyndrickx et al. (2015)
	Diet	1.0–2.0×10^{-3}/d/kg	UK	–	–	–	–	32–56[g,i,h,d]/c	Papageorgiou et al. (2018)
	Diet	2.1–3.1×10^{-3}/d/kg	China	–	–	–	–	63–73[g,h,d]	Deng et al. (2018)
	Diet	0.3×10^{-3}/d/kg	Nigeria	–	–	–	–	1.5–5.0[g]	Šarkanj et al. (2018)

Table 3

Published toxicokinetics data of deoxynivalenol in different species

Species	Route	Dose (mg/kg BW) or (mg/kg diet/day)[b,c]	F (%) AUC_{po} (µg × h/L)	Excretion (%) CL_p [L/(h × kg)]	V_d (L/kg)	$t_{½}$ (h)	t_{max}[e] (h)	c_{max}[e] (µg/L) c_{urine}[g] (µg/L)	References[k]

[a]Radiolabelled (^{14}C)-DON, total radioactivity measured

[b]Units for po or iv studies: mg/kg BW; units for DON application via the diet (feeding studies): mg/kg feed/d; units for human studies: g food/d or mg DON/ food or estimated daily exposure (PMTDI): mg DON/d/kg BW

[c]Duration of feeding studies: d = days, w = weeks

[d]Total amount (all DON species)

[e]Plasma concentration, mean of study individuals

[f]Endotoxaemic pigs

[g]Concentration in urine

[h]Mean concentration (of n study participants) in urine: first-voided morning, 24 h-collected or 48 h-collected urine

[i]ng DON/mg creatinine in urine (/c)

[j]This manuscript

[k]References are listed in Supplement; references in bold type include toxicokinetic studies with complete data sets

Comparison of in vitro predicted and in vivo determined plasma clearances

The heterogeneity of the publishedin vivo data (Table 3) and the limitation of the in vitro data to available liver microsomes (Table 1) was a handicap for the comparison. Nevertheless, the performance of the piglet toxicokinetics study (Table 2) in the present project allowed the direct alignment with the predicted parameters from incubations with microsomes prepared from the same pig breed. The in vitro $CL_{b,vitro}$ = 0.37 L/(h × kg), equal to the $CL_{p,vitro}$ under consideration of $c_p/c_p \approx 1$, was within the 0.5-fold to twofold range of the in vivo CL_p = 0.21 L/(h × kg) (Jolivette and Ward 2005). This match demonstrated the applicability of the substituted liver microsomal assay and the IVIVE computations to imitate DON biotransformation in pigs, which gives reason to expect a similar outcome for other species with glucuronidation as the main metabolisation pathway of DON. In contrast, the prediction of the clearance in chicken was unsuccessful. While the in vitro assay predicted a low $CL_{b,vitro}$ = 0.33 L/(h × kg) (Table 1), DON was cleared in vivo very efficiently with CL_p = 6.12 L/(h × kg) (mean of n = 3 studies) (Table 3). This mismatch indicated that glucuronidation is not the predominant metabolism pathway of DON in chicken.

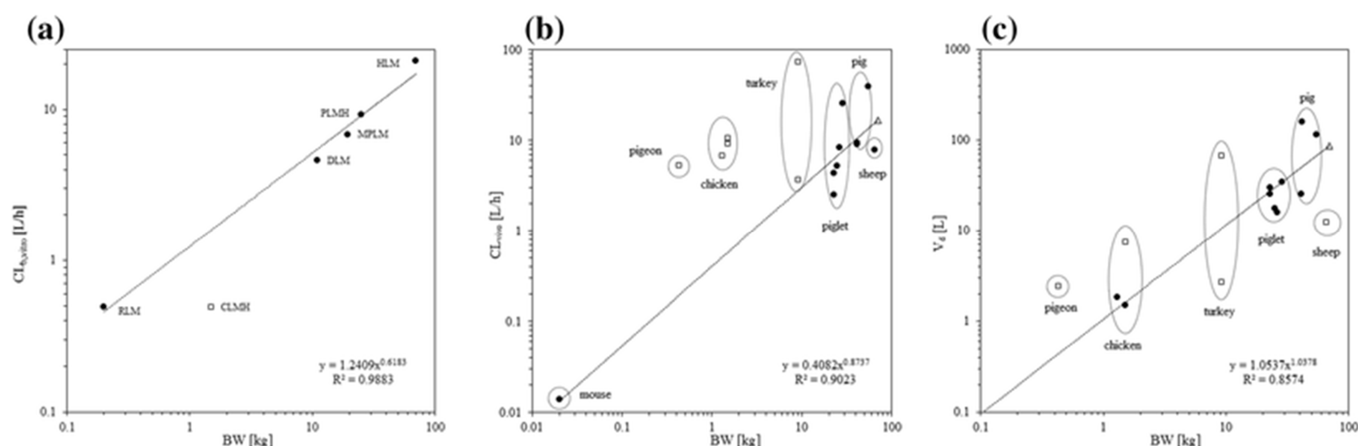
Extrapolation of human DON toxicokinetics using in vitro and in vivo data from different species

The DON depletion data obtained in the human microsome assay were used for the direct prediction of human clearance [$CL_{b,vitro}$ = 0.30 L/(h × kg)] and maximal bioavailability (f_{max} = 79%) by IVIVE (Table 1). Allometric scaling of the in vitro predicted $CL_{b,vitro}$ from six species (Fig. 4a) showed good correlation (R^2 = 0.9883) with the exception of the

chicken value, which was consequently excluded. This divergence pointed at alternative biotransformation pathways in this species, in line with the detected lower affinity (increased $K_{M,\mathrm{assay}}$, Table 1) of DON to chicken glucuronosyltransferases and the lack of correspondence between predicted in vitro and measured in vivo clearance.

Fig. 4

Allometric scaling of published DON: **a** in vitro clearances, **b** in vivo clearances, and **c** in vivo volumes of distribution. Filled circles indicate values that were included into the correlation, unfilled squares show unconsidered data, and the unfilled triangle represents the predicted human value



The allometric scaling of plasma clearances from in vivo studies (Table 3) showed a similar deviation for chicken, turkey, and pigeon (Fig. 4b). When the data from the three published chicken studies were considered, the correlation coefficient dropped to $R^2 = 0.5119$ (data not shown). We decided, therefore, to exclude the chicken, pigeon and turkey clearances, the latter coming from two each other contradicting studies. This approach resulted in $R^2 = 0.9023$ and a predicted human $CL_p = 0.24$ L/(h × kg) (Fig. 4b; Table 4) that was close to the CL_p predicted by IVIVE. The allometric scaling of the published volumes of distribution resulted in a human $V_d = 1.24$ L/kg with correlation $R^2 = 0.8574$ (Fig. 4c; Table 4). It was performed with all available data (Table 3), excluding, respectively, one chicken, turkey, pigeon, and sheep study.

Table 4

Predicted DON toxicokinetics in humans and exposure from grain consumption

Parameter	Humans	Sources
CL_b [L/(h × kg)]	0.30/0.24	$CL_{b,\mathrm{vitro}}$ (Table 1)/allom. scal. (Fig. 4b)
c_b/c_p (blood/plasma coeff.)	1.01	From pig (Prelusky et al. 1988; Table 3)
CL_p [(L/(h × kg)]	0.30/0.24	Calculated from CL_b (Table 4)
V_d (L/kg)	1.24	Allom. scal. (Fig. 4c)
$t_{1/2}$ (h)	2.9/3.6	Calculated from CL_p and V_d (Table 4)

Table 4

Predicted DON toxicokinetics in humans and exposure from grain consumption

Parameter	Humans	Sources
t_{max} (h)	2.0	Estimated from pig (Table 3)
f (%)	50–90	f_{max} (Table 1) and estimated from pig (Table 3)
$AUC_{po,norm(,max)}$ (h × kg/L)	2.63/2.08–3.75	CL_p, f_{max} (Table 1)/CL_p allom. scal., f (Table 4)
$excretion_{urine_24\,h}$ (%, total DON)	70	Exposure studies (Table 3)
dose (mg/kg)	0.2-2.0 × 10^{-3}	Exposure studies (Sundheim et al. 2017; Table 3)
c_{max} (µg/L)	0.1-1.0	Calculated from $t_{1/2}$, f, dose, V_d, t_{max} (Table 4)
c_{max_ARfD} (µg/L)	2.0-5.8	Calculated from $t_{1/2}$, f, ARfD[a], V_d, t_{max} (Table 4)
$AUC_{po,max}$ (µg × h/L)	0.5–5.4	Dose (Table 4), $AUC_{po,norm,max}$ (Table 1)
AUC_{po} (µg × h/L)	0.4–7.5	Dose (Table 4), $AUC_{po,norm}$ (Table 4)
$c_{urine_24\,h\,(total\,DON)}$ (µg/L)	4.9–49	Dose, $excretion_{urine_24\,h}$, V_{urine_24h}[b], BW (Table 4)
$c_{urine_24\,h\,(total\,DON)}$/c (ng/mg creatinine)	3.0–30	$c_{urine_24\,h\,(total\,DON)}$, creatinine $excretion_{_24\,h}$[c] (Table 4)

[a]8 µg/kg/day (Knutsen et al. 2017)

[b]2.0 L/day, mean daily urine excretion for adult

[c]1610 mg/day for adult male, 70 kg BW

Prediction of human exposure from consumption of cereals

The determination of the fundamental toxicokinetic parameters CL_p and V_d by IVIVE and allometric scaling was the prerequisite for the prediction of depending parameters such as $t_{1/2}$ and c_{max} (Table 4). Using both values for human CL_p and V_d, half-life in human plasma was estimated as 2.9 to 3.6 h, which is comparable to $t_{1/2}$ in pig studies (Table 3). The absolute bioavailability of DON in humans was assumed to be high and in the range of f = 50% to 90%, in congruence with the in vitro determined maximal bioavailability f_{max} = 79%. The combination of the different values for CL_p and $f_{max)}$ yielded a dose-independent AUC after oral uptake in the range of $AUC_{po,norm(,max)}$ = 2.08–3.75 h × kg/L (Table 4).

Human exposure (AUC_{po}) to DON from the consumption of cereal-based food products was estimated using doses derived from mean low and mean high intake in a risk assessment on DON for different age groups in Norway that was based on typical occurrence and food consumption data (Sundheim et al. 2017; Table 3). The predicted exposures were AUC_{po} = 0.4–0.5 µg × h/L for low consumers and AUC_{po} = 5.4–7.5 µg × h/L for high consumers of cereals (Table 4). Accordingly, maximum DON concentrations in plasma were calculated as c_{max} = 0.1 – 1.0 µg/L, under consideration of two doses, the predicted range for $t_{1/2}$ and an estimated t_{max}. Using the acute reference dose, ARfD = 8 µg/kg, as intake resulted in c_{max_ARfD} = 2.0–5.8 µg/L.

The excretion of total DON via the urine within 24 h after low and high intake via the diet was predicted for Norwegian adults using a mean excretion ratio (70%) that was derived from published human biomonitoring studies (Table 3). Both, the calculated

absolute $c_{\text{urine_24 h (total DON)}}$ = 4.9–49 µg/L and the creatinine content-normalised $c_{urine\text{_24h (total DON)}}/c$ = 3.0–30 ng/mg (Table 4) were in the range of data observed in human studies in Europe (Table 3; Brera et al. 2015).

Discussion

Mycotoxins are omnipresent in food and feed, posing an immanent risk for human and animal health. Occurrence and toxic potential of the most prevalent mycotoxins have been studied, and levels for maximum concentrations in consumables have been implemented by authorities in many countries. Tolerable daily intakes (TDI) are based on animal toxicity data and the application of a safety margin (Knutsen et al. 2017). Toxicokinetic data that would directly link intake, exposure and toxic effects, are, however, not available for humans and cannot be determined in vivo due to ethical reasons.

This gap can be bridged by the application of in vitro-to-in vivo extrapolation (IVIVE) and allometric scaling, which are well-established concepts for the prediction of kinetic parameters in humans in preclinical drug discovery (Iwatsubo et al. 1997; Chiba et al. 2009; Chen et al. 2012). In the present study, we have, therefore, ventured to transfer this approach to mycotoxins. Using the extensively studied deoxynivalenol (DON), the most common mycotoxin in cereals, as model compound, we could draw on experiences and data from numerous in vivo animal experiments. They delivered the database for the allometric scaling, while we developed the format for in vitro metabolism assays under kinetic conditions for IVIVE for this project.

Since glucuronidation has been shown to be the major biotransformation pathway for DON in most species (Payros et al. 2016), we decided to base our IVIVE approach on this phase-II conjugation reaction and established an incubation system with liver microsomes substituted with UDP-glucuronosyltransferase (UGT) co-substrates. The use of microsomes instead of primary hepatocytes in in vitro metabolism studies has the advantage of greater availability, practicability (storage), and comparability (reduced inter-individual variability through pooled livers from several individuals) but reduces the number of observable conversions. The formation of DON-sulphates, as reported in birds, depends on cytosolic sulphotransferases and was, therefore, not detectable in the microsomal, membrane-bound system. Likewise, the production of DOM-1 through reductive de-epoxidation by microbiota was not considered. In vivo DON sulphonation, which has been observed in rodents (Wan et al. 2014; Pestka et al. 2017), is a rare metabolic pathway with unclear mechanism. It was potentially associated with the addition of glutathione to an unsaturated ketoaldehyde in the substrate, and was thus not covered by thein vitro experiments in the present study.

The hepatic glucuronidation pattern of DON varies between species, which has been shown consistently in in vivo (Lattanzio et al. 2011; Schwartz-Zimmermann et al. 2017) and in vitro (Maul et al. 2012; Uhlig et al. 2016) studies. In addition, there are differences related to sex, age, health status, diet, or environment (Pestka et al. 2017;

Chen et al. 2016). The DON-metabolising UGTs belong to an enzyme superfamily that is found ubiquitously in living organisms and has a widely ranging spectrum of endogenous and exogenous substrates. Genetic polymorphism is known for several UGTs and isoforms are expressed tissue-specifically (Guillemette 2003). Using recombinant human UGTs, it was demonstrated that DON-15-GlcA is predominantly produced by UGT2B4 and DON-3-GlcA by UGT2B7, one of the most important hepatic UGTs (Maul et al. 2015). Both enzymes occur in different variants in Caucasian, Hispanic, African, and Asian populations (Guillemette 2003), which might be one reason for the observed geographical variability in DON metabolism (Chen et al. 2016). However, considering this diversity, the data on animal and human DON glucuronides from different reports are astonishingly congruent, and were also confirmed in this study by our in vitro and in vivo experiments. DON-3-GlcA is the main glucuronidation product in mouse, rat, fish, and dog, whereas DON-15-GlcA is predominant in humans, and the levels of both metabolites are similar in pig (Maul et al. 2012; Nagl et al. 2014; Uhlig et al. 2016; Schwarz-Zimmermann et al. 2017). In contrast, only traces of DON-3-GlcA have been found in chicken and turkey, where the main biotransformation product is DON-3-sulphate (Maul et al. 2012; Devreese et al. 2015).

Although we followed the formation of DON glucuronides in the different in vitro metabolism assays, with equivalent outcome as in a previous study (Maul et al. 2015), our main objective was the determination of species-specific elimination constants k_e as basis for the IVIVE calculations. The assays were run under linear conditions with DON start concentrations below the individual $K_{M,assay}$, preventing an underestimation of the intrinsic enzyme activities, i.e., the assay clearances $CL_{int,assay}$, which is especially critical for high-affinity (low $K_{M,assay}$) substrates (Iwatsubo et al. 1997). The $K_{M,assay}$ were comparable for all species with the exception of chicken, indicating a lower affinity of DON to avian UGTs, and reflecting the in vivo situation. The inclusion of microsomes from two separate preparations of, respectively, male and female Wistar rats in the experiment showed that there was no sex-related difference in the in vitro DON-elimination rates. Similarly, in a mouse study, the slight sex-dependant disparity observed in the formation of individual DON glucuronides became insignificant, when the sum of all metabolites was considered (Pestka et al. 2017).

The depletion half-life in the liver microsomal assays were used for the prediction of hepatic clearances by IVIVE using well-established parameters and models for the upscaling of data from enzyme activity in the assay to the intact organism (Iwatsubo et al. 1997; Ito and Houston 2005; Naritomi et al. 2015). The extrapolated blood clearances ($CL_{b,vitro}$) were low for chicken, dog, minipig, pig, and human, and intermediate for rat. Consequently, the predicted maximum bioavailabilities after oral uptake were very high for all species aside from rat. Reviews on the success rate of extrapolations from human hepatic microsomes in drug discovery, however, have shown a systematic underprediction of in vivo clearances that is particularly relevant for substances with low $CL_{b,vitro}$ (Iwatsubo et al. 1997; Soars et al. 2002; Chiba et al. 2009; Naritomi et al. 2015).

Factors contributing to the bias are on one hand assay-related such as non-specific binding, enzyme inhibition, or loss of metabolic activity, and, on the other hand, dependent on variations of CL_b in humans due to genetic polymorphism, dietary habits, smoking, alcohol consumption, or medication. Prediction accuracy can also be compromised if a substance is extensively metabolised in extrahepatic tissues or is substrate to multiple biotransformation pathways. Nevertheless, we considered DON as a good choice for exploring the applicability of IVIVE in mycotoxinology, because the molecule is hydrophilic, binds little to plasma proteins (Prelusky et al. 1987, 1988), and eliminates mainly through glucuronidation. Furthermore, the existence of toxicokinetic data from in vivo animal studies allowed direct comparison of predicted and measured parameters for several species without being restricted to humans.
In this context, the in vivo study in Norwegian-crossbred piglets was performed to allow direct comparison with results from the in vitro assay that was carried out with microsomes prepared from exactly the same breed. In this way, we excluded several of the factors threatening success in IVIVE. Comparison of the results showed agreement of the predicted and measured clearances, proving the suitability of the approach (De Buck et al. 2007; Chen et al. 2012; Abduljalil et al. 2014). The toxicokinetic data determined in the present experiment fitted well to findings in previous pig studies (Dänicke and Brezina 2013). DON is highly bioavailable ($f > 50\%$) and cleared slowly ($CL_p < 0.5$ L/(h × kg); $t_{1/2} > 2.5$ h), with small differences between piglets and adult pigs. The considerable conformity between studies performed in several European countries indicated that DON toxicokinetics in pigs was little influenced by the different breeds used in the respective experiments. Assuming that the same is valid for in vitro metabolism methods, this could imply extended applicability of IVIVE for DON.
The comprehensive survey of animal and human studies containing in vivo toxicokinetic parameters of DON was performed to generate a database for comparison with IVIVE data and for interspecies allometric scaling. Regarding the reported high-clearance values in several chicken studies, it was evident that the prediction of $CL_{b,vitro}$ from the chicken microsomal assay had failed. This was, however, not surprising, since, in avian species, DON is cleared mostly as DON sulphate (Devreese et al. 2015). In this sense, the missed match between results from in vitro glucuronidation and in vivo sulphation confirmed the specificity and reliability of the microsomal UGT assay. Allometric scaling of the IVIVE-predicted $CL_{b,vitro}$ from different species confirmed that DON is metabolised differently in chicken. Whereas correlation was good for rat, dog, minipig, pig, and human data, the predicted chicken $CL_{b,vitro}$ did not fit.
Allometric scaling of the compiled in vivo CL_p from different species showed that the data for chicken, turkey, and pigeon had to be removed to achieve good correlation. Including only animals with glucuronidation as predominant elimination pathway for DON, we were able to extrapolate to human clearance using the standard power-law equation $CL_p = a \times BW^b$ with the allometric exponent $b = 0.87$. This value is in the expected range, since metabolism-related body functions such as oxygen consumption,

cardiac output, and minute ventilation at rest have been shown to scale in average with $b = 0.75$ in species with bodyweights from 4 g to 4000 kg (Lindstedt and Schaeffer 2001). Allometric scaling of clearances takes advantage of the finding that fundamental physiological mechanism in mammals are size-dependent, so that human data can be derived (Boxenbaum 1982; Mahmood and Balian 1996; Deguchi et al. 2011). The good allometric fit of the DON in vivo clearances was remarkable, because the underlying data set actually fulfilled the criteria of including at least three species but was clearly imbalanced by the great number of pig studies. The predictability of a substance's human CL_p from allometry can be obstructed by several determinants, including high lipophilicity (octanol–water partition coefficient, ClogP > 2) and great differences in plasma binding between species (Jolivette and Ward 2005; Tang and Mayersohn 2006). Both factors are, however, unproblematic in case of DON. Moreover, even if the DON in vivo clearance values were low, the CL_p correlation was apparently not compromised, although allometric scaling works generally better for high clearance than for low clearance substances.

The allometrically determined human CL_p for DON [0.24 L/(h × kg)] was close to the $CL_{p,vitro}$ predicted by IVIVE [0.30 L/(h × kg)], fulfilling the success criterion of < twofold deviation (De Buck et al. 2007; Abduljalil et al. 2014). We decided, therefore, to stay with the basic BW-based allometric scaling and to not include correction factors such as brain weight (BrW), maximum lifespan potential (MLP), or glomerular filtration rate (GFR), which have been applied in different studies with varying outcomes (Nagilla and Ward 2004).

The prediction of a substance's volume of distribution in humans is based on animal V_d allometry or on in silico physiologically based modelling under consideration of in vitro determined tissue–plasma partitioning coefficients (Mahmood and Balian 1996; De Buck et al. 2007; Chen et al. 2012). V_d can be divided into small < 0.7 L/kg, moderate 0.7–3.5 L/kg, and large > 3.5 L/kg categories (Jolivette and Ward 2005), reflecting to which extent the substance is dispersed into the different body compartments. Since distribution is mainly dependent on the substance's physical properties, resulting in typical tissue binding properties, it correlates with tissue mass and body weight, and it is unaffected from potential differences in the metabolism. Thus, V_d categorisation is generally stable in different species, and the exponent of the allometric equation for V_d is close to unity ($b \approx 1$) (Mahmood and Balian 1996; Lindstedt and Schaeffer 2001).

Regarding the published in vivo studies on DON toxicokinetics, the reported V_d in pigs and chicken were of moderate size. Allometric scaling predicted likewise a moderate human V_d (1.24 L/kg) with the allometric exponent $b = 1.04$, fulfilling the condition of unity. The number of species included met the minimum requirement (Mahmood and Balian 1996), and the correlation was sufficient, although pig data were dominant. Sheep, pigeon, and turkey V_d had to be excluded from the allometry as they were either inconclusive (two diametrically different values for turkey) or disproportionally high (pigeon) and low (sheep), indicating dissimilar binding properties of DON in these

species. However, pigs are regarded as the most suitable animal model for DON exposure in humans (Nagl et al. 2014), which supported our approach and gave confidence in the predicted V_d.

Surveys in different human populations in Europe have shown that the amount of cereal consumption is the strongest significant determinant of urinary DON levels (Turner et al. 2009; Brera et al. 2015). In consequence, DON levels in urine have been used to estimate exposure from food by applying different dietary models (Heyndrickx et al. 2015). Due to the lack of human toxicokinetic data on DON, there are, however, many uncertainties regarding the assessment of risk from dietary intake. Using our predicted values for human CL_p and V_d, we have calculated plasma half-life and bioavailability after oral uptake of DON. Furthermore, we estimated total exposure (AUC_{po}) and the maximum plasma concentration under consideration of a range of typical DON concentrations in grain-based food products (Sundheim et al. 2017; Knutsen et al. 2017). The resulting figures were in the same range as parameters reported in pigs that had received comparable doses; reference values from human studies have never been recorded. Applying the same dose range and the mean urinary excretion rate from human biomonitoring experiments, we calculated the expected DON concentrations in adult urine, which fitted well to observed values.

In conclusion, the combination of IVIVE and allometric upscaling of in vivo animal data allowed the prediction of important human toxicokinetic parameters of DON that were successfully applied to calculate plasma and urine concentrations after oral dietary exposure.

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical standards

This article does not contain clinical studies or patient data. This article does not contain any studies with human participants performed by any of the authors. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution at which the studies were conducted.

Electronic supplementary material

Below is the link to the electronic supplementary material.
Supplementary material 1 (DOCX 35 KB)

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Supplement

References for deoxynivalenol toxicokinetics and exposure data in different species (as listed in Table 3)

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