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Human next-generation risk assessment of trichothecene toxicity

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ABSTRACT

Trichothecenes are naturally occurring chemicals, produced by fungi, that can be found in contaminated crops. Trichothecenes have the potential to indirectly damage DNA and exacerbate genotoxic effects of genotoxicants. However, genotoxicity data for most trichothecenes are limited and data gaps remain. Here we use the γH2AX/pH3 assay to evaluate DNA damage *in vitro* of 13 trichothecenes. Three human cell lines (SH-SY5Y, ACHN, and HepG2) were exposed to each trichothecene (0.001–100 μM) to assess toxicity as models for the brain, kidney, and liver, respectively. Concentration-dependent induction of DNA damage, illustrated by γH2AX induction, was observed for all trichothecenes. In vitro-in vivo extrapolation (IVIVE) modeling was employed to support *in vivo* equivalent potency ranking and screen for risk potential. Diacetoxyscirpenol, T-2, and HT-2 had the highest genotoxic potency, notably in SH-SY5Y cells. Administered equivalent doses (AEDs) derived from IVIVE were compared against exposure data from French total diet studies to assess risk potential. AEDs derived for T-2 and HT-2 from the SH-SY5Y model were within 100-fold of exposure levels for infants aged one year or less. Overall, the potential for trichothecenes to damage DNA and higher exposures in infants highlights the need to investigate the cumulative effects across the broader trichothecene family.

1. Introduction

Trichothecenes are a family of chemically related mycotoxins produced by toxigenic strains of different mold species. More specifically, trichothecenes are sesquiterpenes that share a common structure including a tetracyclic ring system and a stable epoxide group between C12 and C13. The trichothecene family is divided into four groups (types A-D) with types A and B being a major source of natural contaminants in grains, both before and after harvest (Polak-Śliwińska and Paszczyk, 2021). The trichothecenes most commonly found in foods include type A toxins: T-2 toxin (T-2), HT-2 toxin (HT-2), diacetoxyscirpenol (DAS); and type B toxins: deoxynivalenol (DON) and nivalenol (NIV). Like most mycotoxins, trichothecenes are heat-stable and are resistant to degradation during conventional food processing temperatures (Polak-Śliwińska and Paszczyk, 2021). The pervasive presence of trichothecenes in contaminated food commodities and the myriad adverse effects caused by these exposures has had significant impacts on global health and the economy (Milićević et al., 2010). Understanding the health impacts of trichothecenes is crucial amid rising contamination exacerbated by global warming, necessitating the development of robust risk assessment strategies and the implementation of proactive risk management measures to alleviate their adverse impacts on public health.

Several acute and chronic toxicity concerns associated with trichothecene exposures have been identified in risk assessments (EFSA, 2011, 2013; Knutsen et al., 2017, 2018; World Health Organization, 2023). Furthermore, recent evidence has demonstrated that the most prevalent trichothecenes are ribotoxic in that they can inhibit protein synthesis and subsequently exacerbate the genotoxicity of a broad spectrum of genotoxic agents (Garofalo et al., 2022, 2023; Payros et al., 2017). However, there is uncertainty regarding the direct genotoxic potential of trichothecenes themselves as there have been conflicting results across genotoxic endpoints. Trichothecenes tested using the Ames assay found no evidence of mutagenic activity (Alonso-Jauregui et al., 2022). It has been noted for well-studied trichothecenes, such as T2 and HT-2, that studies with positive results observe clastogenic effects at concentrations expected to inhibit protein/DNA synthesis or induce oxidative stress (EFSA, 2011), suggesting a possible indirect mechanism. In the case of

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DAS and DON, the available evidence was not sufficient to reach a conclusion on its *in vivo* genotoxicity and mode of action information was noted as a source of uncertainty (EFSA et al., 2017; 2018). For many of the other trichothecenes, there is limited toxicity data and their relative genotoxic potency is unknown.

Next-generation risk assessment (NGRA) strategies are needed to address the data-poor trichothecenes, as there are too many structurally related chemicals within the trichothecene family (approximately 200–300 chemicals) to evaluate using traditional approaches. Animal-free toxicity testing approaches have been gaining confidence in NGRAs as alternatives to rodent toxicity tests as they are higher-throughput and have high biological relevance (Kavlock et al., 2018). These approaches rely on new approach methodologies (NAMs), that include high-throughput *in vitro* and/or *in silico* screening data, that are commonly paired with quantitative *in vitro-in vivo* (IVIVE) modeling to estimate human doses expected to lead to adverse health outcomes (Paul Friedman et al., 2020). Recognizing that there are notable differences in toxicodynamics and toxicokinetics between laboratory rodents and humans, NAM studies have the benefit of using human cell models and human absorption, distribution, metabolism, excretion (ADME) models when assessing hazard/risk. Recently, a NAM-based assessment investigated the toxicity of DON using human immune THP-1 cells and a human IVIVE model to derive effect doses for two adverse outcomes: intestinal proinflammatory cytokine secretion and bile acid malabsorption (Wang et al., 2024). The study demonstrated that a NAM-based assessment strategy provides a sensitive measure of DON toxicity, relative to apical endpoints, and identified end points requiring further investigation to protect the health of various populations. NAM-based strategies have also been applied to quantitively assess the genotoxic potential of chemicals and have been shown to be more sensitive than rodent studies (Beal et al., 2022; Kuo et al., 2022).

The purpose of this study was to add to the weight of the evidence in the genotoxicity assessment of trichothecenes and to use a NGRA approach to assess the genotoxic potential of data-poor trichothecenes (Supplementary Fig. S1). The *in vitro* γH2AX/pH3 assay (Kopp et al., 2019) was used to evaluate DNA damage following exposures to thirteen trichothecenes. A previous assessment of other mycotoxins (i.e., aflatoxins) using this assay demonstrated good predictivity for genotoxicity (Theumer et al., 2018). Moreover, γH2AX quantification has been demonstrated to be highly predictive of other *in vitro* genotoxicity tests, such as Ames, mouse lymphoma assay, and chromosome aberration assay (Smart et al., 2011), while the combination of γH2AX/pH3 biomarkers offers more effective identification of genotoxic mode of action (Kopp et al., 2019). A human hepatoblastoma cell line (HepG2), human renal adenocarcinoma cell line (ACHN), and human neuroblastoma cell line (SH-SY5Y) were used in the investigation as representative models for liver, kidney, and brain tissues, respectively. Tissue-specific IVIVE modeling was employed to estimate the doses expected to lead to DNA damage for the different trichothecenes in the target tissues to establish their relative potency. Comparisons with dietary levels were made to estimate the risk potential of trichothecenes commonly found in foods. Overall, this study provides a novel approach for hazard and risk assessment of mycotoxins, without using any animals, and supports the identification of priority trichothecenes for future monitoring.

2. Materials and methods

2.1. Chemicals and reagents

T-2 toxin (T-2; CAS: 21259-20-1), deoxynivalenol (DON; CAS: 51481-10-8) and 3-acetyldeoxynivalenol (CAS: 50722-38-8) were purchased from Enzo life science (Lyon, France). HT-2 toxin (HT-2; CAS: 26934-87-2), T-2 tetraol (CAS: 34114-99-3), neosolaniol (CAS: 36519-25-2), NT 2 toxin (CAS: 76348-84-0) were purchased from Analyticon Discovery (Potsdam, Germany). T-2 triol (scirpenol; CAS: 34114-98-2), 15-acetoxyscirpenol (CAS: 2623-22-5), nivalenol (NIV; CAS: 23282-20-4), 15-acetyldeoxynivalenol (CAS: 88337-96-6) and fusarenon X (CAS: 23255-69-8) were purchased from Fermentek (Jerusalem, Israel). Diacetoxyscirpenol (DAS; CAS: 2270-40-8) was purchased from CLUZEAU INFO LABO (Sainte-Foy-La-Grande, France).

Benzo[*a*]pyrene (BaP), etoposide (ETO), nocodazole (NOCO) and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (Saint Quentin Fallavier, France). All compounds (purity >95%) were prepared in DMSO. Penicillin, trypsin, phosphate buffered saline (PBS), RNAse A and Triton X-100 were purchased from Sigma-Aldrich. The blocking solution (Maxblock Blocking Medium) was purchased from Active Motif (Waterloo, Belgium).

2.2. Cell lines and maintenance

HepG2 (ATCC N8HB-8065) and ACHN (ATCC N8CRL-1611) cells were grown in minimum essential medium, alpha modification (αMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 μg/mL streptomycin in a humidified atmosphere with 5% CO_2 at 37 °C. SH-SY5Y cells (ATCC: CRL-2266) were maintained in Dulbecco's Modified Eagle Medium F12 supplemented with 10% FBS, 100 U/mL penicillin and 100 μg/mL streptomycin in a humidified atmosphere with 5% CO_2 at 37 °C.

2.3. Trichothecene treatment

HepG2, ACHN, SH-SY5Y cell lines were grown in 96-well plates, containing 200 μL medium per well, at a cell density of 3.2×10^4 per well. After 24 h, the medium was replaced with 90 μL of medium without serum. Trichothecene (stock solutions of 50 mM in DMSO) were diluted in medium without serum and 10 μL were added to per well. The final culture medium to which cells were exposed contained 0.2% (*v/v*) of DMSO. Cells were incubated for 24 h at different concentrations of trichothecenes including 0.001, 0.01, 0.1, 1, 10, or 100 μM. Positive controls were tested at 0.001–1 μM for BaP, 0.1–10 μM for ETO, and 0.1–1000 μM for NOCO. Experiments were performed at least three times independently on different days (biological triplicates) to calculate mean and SEM.

2.4. γH2AX/pH3 assay

The γH2AX/pH3 Assay was performed as described previously (Khoury et al., 2016, 2020; Theumer et al., 2018). Briefly, after the 24-h exposures to trichothecenes, the cells were fixed with 4% paraformaldehyde (Electron Microscopy Science, Pelanne Instruments, France) in phosphate buffered saline (PBS) and permeabilized with 0.2% Triton X-100. Cells were then incubated in blocking solution (MAXblock Blocking Medium and 0.1 g/L RNAse A) before 2-h incubation at room temperature with primary antibodies (rabbit monoclonal anti-γH2AX [Clone20E3, Cell signaling] or pH3 [Clone CM312, Sigma]) in PST buffer. Infrared fluorescent dye conjugated to goat antibody (CF770, Biotium) was used for detection. RedDot2 (Biotium) was added simultaneously to the secondary antibody for DNA labelling. After a 1-h incubation, fluorescence was measured using Odyssey Infrared Imaging Scanner (Li-CorScienceTec, Les Ulis, France). In a single experiment, the γH2AX/pH3 assay evaluated both cytotoxicity and potential to induce DNA damage and aneugenicity, respectively. In order to calculate cytotoxicity, the DNA content of the treated cells—which is correlated with cell count—was compared to the DNA content of the cells treated with DMSO (the control) and expressed as a percentage, or relative cell count (% RCC). Previous results have indicated that this approach is not vulnerable to generating misleading positive results due to the induction of apoptosis (Khoury et al., 2020). The amount of phosphorylation level change of the histone examined was calculated by dividing the fluorescence of γH2AX or pH3 in treated wells by that of untreated wells.

2.5. Statistical analysis

The findings for at least three biological independent experiments are shown as a mean ± standard error of the mean (SEM). Using a two-sided Student's *t*-test (*P ≤ 0.05; **P ≤ 0.01), statistically significant increases in biomarkers after treatment were compared with the vehicle (DMSO) control. If the measured biomarker increased by 1.5 times compared to the DMSO control, the result was concluded as positive. The lowest observed effect concentration (LOEC) and all other statistically significant results (* denotes $p < 0.05$) at non-cytotoxic concentrations were reported. Benchmark concentration (BMC) modeling were performed using a modified version of the PROAST R package (version 70.0) that allowed for batch modeling (Beal et al., 2022). The critical effect size for deriving BMCs was chosen to be 0.4 and this was based on the within-group variation of the dataset (i.e., one standard deviation) as recommended by Beal et al. (2023).

2.6. High-throughput toxicokinetics modeling

IVIVE was performed in R using the HTTK v2.2.1 R package (Pearce et al., 2017). The HTTK physiologically based toxicokinetic (pbtk) model, containing separate tissue compartments for the gut, liver, lungs, arteries, veins, kidneys, and rest of the body, was used to estimate the steady-state concentration (C_{ss}) reached in various tissues after simulating a constant dose rate of 1 mg/kg bw/day. Under the assumption of a linear relationship between C_{ss} and dose rate, the AED/BMC ratio is proportional to the dose rate divided by simulated dose (i.e., AED/BMC = 1 mg/kg bw/day/C_{ss} or AED = BMC/C_{ss} at 1 mg/kg bw/day). The C_{ss} values for liver, kidney, and rest of the body were used to estimate AEDs from the HepG2, ACHN, and SH-SY5Y LOECs/BMCs, respectively.

Input parameters (Supplementary Table S1) for the trichothecenes were added to the HTTK database using the add_chemtable(overwrite = F) function. The collection of input parameters followed the same strategy as applied previously (Beal et al., 2021). Briefly, toxicokinetic input parameters (fraction unbound in plasma protein and hepatic clearance) were based on *in silico* predictions (ADMET Predictor version 10). The log of the partition coefficient of a solute between octanol and water (LogP) was obtained from the PubChem database (Kim et al., 2023) for most chemicals. LogP values were not available for 3-Acetyldeoxynivalenol and 15-Acetoxyscirpenol and instead were estimated using the ChemmineOB package (version 1.34) in R. Additional predictions for toxicokinetic parameters were available for 12 out the thirteen trichotecenes and where extracted from the MycoCentral database (www.mycoCentral.eu (Habauzit et al., 2024)) and the potential impacts on the results were explored. Briefly, these parameters were predicted with online free ADME tools (ADMETSAR, PKCSM, and ADMETLAB2) using SMILES obtained from PubChem (Dong et al., 2018; Pires et al., 2015; Xiong et al., 2021; Yang et al., 2019).

To run the pbtk model, the R function calc_mc_css(output.units = "uM", model = "pbtk") was used with the "tissue" parameter set to "liver" for HepG2 data, "kidney" for ACHN data, and "brain" for SH-SY5Y data. The AED was estimated by dividing the LOEC or the BMC_{40} by the reported C_{ss} value for each chemical at each respective tissue.

2.7. Estimation of bioactivity exposure ratios (BERs)

The lower confidence levels for AEDs for each chemical were divided by the respective exposure values to estimate the BERs. Dietary exposure values were obtained from the French Total Diet Study (Etude de l'alimentation totale française 2 (EAT 2) - 2006–2010 (ANSES, 2011)). The 95th percentile upper bound estimate for both adults and infants was chosen for the BER derivation. Additionally, dietary exposure values were also available from a French Infant Total Diet Study that had children divided into four age groups: 1–4 months (n = 124 individuals), 5–6 months (n = 127), 7–12 months (n = 195), and 13–36 months (n = 259)(Vin et al., 2020). The 90th percentile upper bound estimate was used for BER derivation. The 90th percentile lower bound estimate for the 1–4 month age group was zero, and thus, for presentation in the figures the lower bound estimate of the mean was used instead. The dietary exposure for DON was reported as the sum of DON and its acetylated compounds in both diet studies; this was used as a conservative estimate of DON exposure levels as the acetylated forms are all metabolised to DON and absorbed at the same extent as DON (EFSA et al., 2017). The AEDs for each chemical were divided by the respective exposure values.

3. Results

The thirteen trichothecenes were evaluated for cytotoxicity and indicators of genotoxicity in three selected human cell lines. All trichothecenes showed some levels of cytotoxicity with the exception of 3-acetyldeoxynivalenol, which did not reduce viability below the threshold 50% limit in any of the cell lines at the tested concentrations (1–100 μM). The SH-SY5Y cell line was the most sensitive to the cytotoxic effects of trichothecene exposures, with trichothecene concentrations as low as 0.1 μM reducing viability below the threshold limit (Supplementary Table S2; Supplementary Fig. S2). Viability was also reduced below the threshold limit in the ACHN cell line for four chemicals (DAS, NIV, NT 2 toxin, and T-2 triol), but this was only at the top concentration of 100 μM. Although there was an association between increasing concentrations of trichothecenes and cytotoxicity in HepG2 cells, none of the chemicals decreased viability below the fixed threshold limit of 50%.

The genotoxic potential was assessed at concentrations below 50% cytotoxicity using the γH2AX/pH3 assay (Supplementary Table S3; Fig. 1). The results suggest that all of the trichothecenes tested have potential for inducing DNA damage in at least one cell line with most of the trichothecenes showing positive induction of γH2AX in all three cell lines. There were only two exceptions: 3-acetyldeoxynivalenol was only genotoxic in HepG2 at the top concentration of 100 μM, and NIV was positive in ACHN and HepG2 but not significantly different from the solvent control in SH-SY5Y cells at the concentrations tested. The lowest active concentration for γH2AX induction to occur was at 0.01 μM for HT-2, T-2, and DAS in SH-SY5Y cells (Fig. 2). Based on the LOEC values (Supplementary Table S3; Fig. 2), the potencies for inducing γH2AX foci of trichothecenes were in the following order: DAS = T-2 = HT-2 > 15-acetoxyscirpenol > NT 2 toxin = fusarenon X = Neosolaniol = T-2 triol (scirpenol) > 15-acetyldeoxynivalenol = DON = T-2 tetraol = NIV > 3-acetyldeoxynivalenol. The potency ranking based on BMC_{40} confidence intervals was highly similar (Supplementary Table S4; Fig. 3). The trichothecenes did not induce the pH3 biomarker, related to aneugenicity, above solvent controls (Supplementary Table S3) and therefore, only the γH2AX results were analyzed further. The positive controls, BaP, ETO, and NOCO, all produced expected results in the γH2AX/pH3 assay (Supplementary Table S3).

IVIVE was used to model AEDs for each trichothecene based on the LOEC and BMC_{40} values to support a quantitative hazard assessment. The potency ranking based on AEDs did not differ from the LOEC and BMC_{40} from which they were derived (Figs. 2 and 3). This is expected as the trichothecenes all had very similar input toxicokinetic parameters (Supplementary Table S1) and the C_{ss} for the trichothecenes were all within 2.5-, 2.0-, and 2.0-fold of each other for brain, kidney, and liver, respectively. Other toxicokinetic parameters were available for most of the trichothecenes and their use had a minimal impact on C_{ss}; specifically, the different C_{ss} values for a each trichothecene were all on the same order of magnitude of each other (largest difference was 1.5×). To be consistent with the remaining trichothecenes and previous studies, these alternate TK parameters were not used in the hazard assessment or potency comparisons.

To support a quantitative risk assessment, BERs were estimated using the AEDs and total diet exposure values (Supplementary Table S5). Total diet exposure values were available for NIV, DON, HT-2, and T-2 to

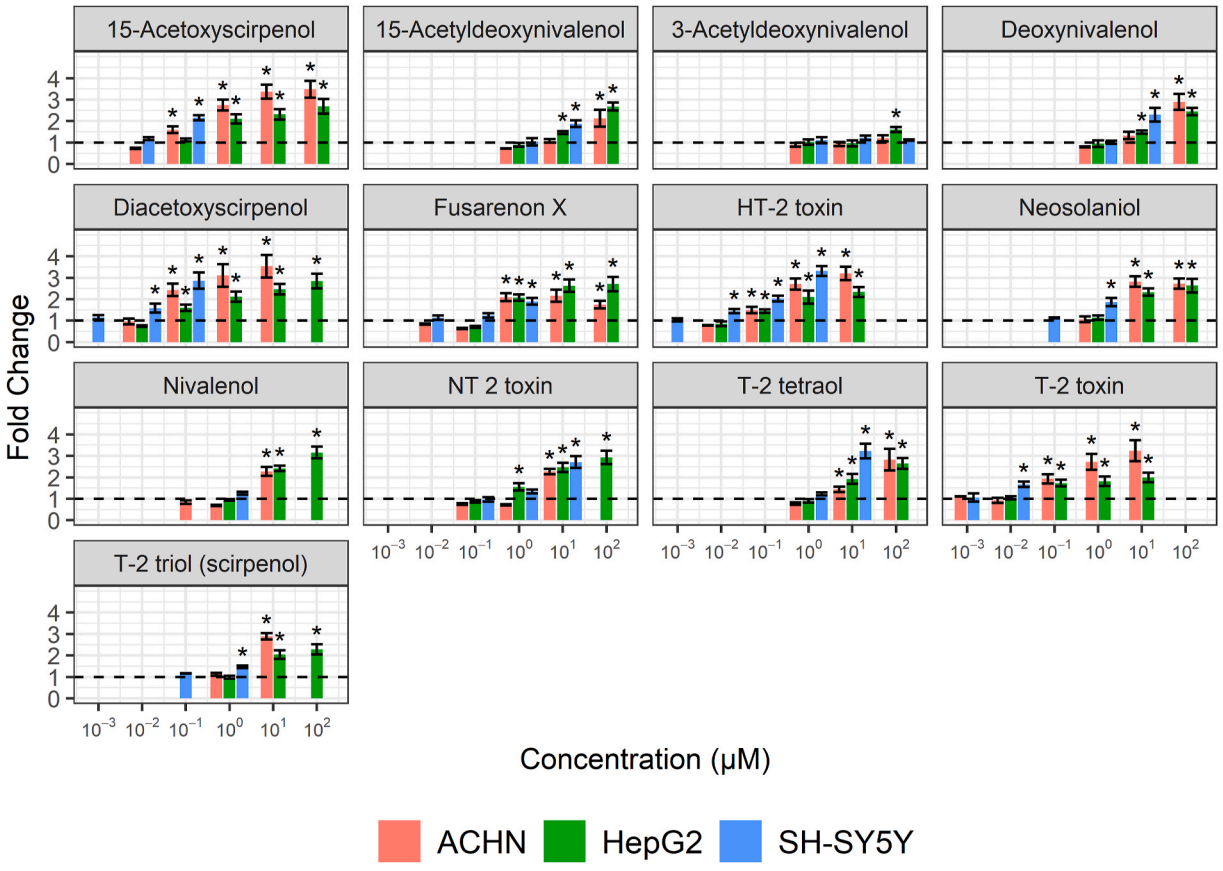

Fig. 1. Genotoxicity of trichothecenes with the γH2AX/pH3 ICW assay in ACHN, HepG2, and SH-SY5Y cell lines. Each value represents the mean fold change increase above background ± SEM after 24 h of treatment; dashed line indicates the control level. Only results with viability values above 50% are shown. Statistically significant increases in γH2AX relative to solvent control were noted (*, $P < 0.05$).

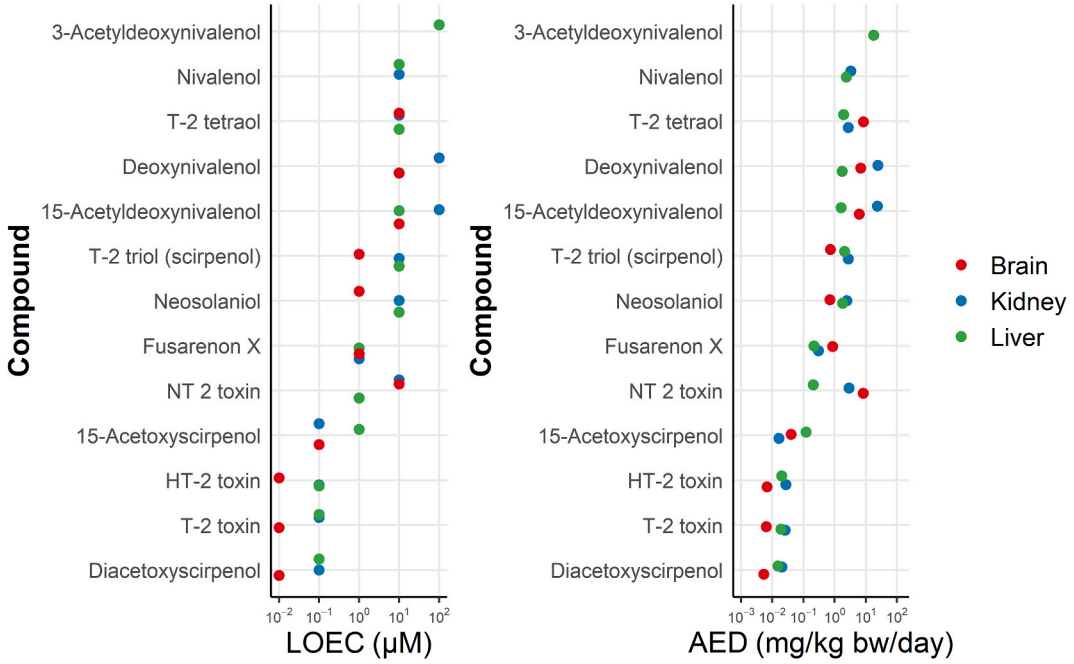

Fig. 2. Potency ranking of trichothecenes based on LOEC (left; units in μM; IVIVE not applied) and based on AED (right; units in mg/kg bw/day; IVIVE applied).

estimate BERs (Fig. 4). NIV had a BER above 1000, with the lowest BER estimate of 4858.6 based on the 95th percentile infant exposure level and the AED for liver. DON had a BER between 100 and 1000, with the lowest BER estimate of 589.8 based on the 95th percentile infant exposure level and the AED for liver. HT-2 and T-2 toxins had BERs between 1 and 100 as a result of low AEDs in both liver and brain. HT-2

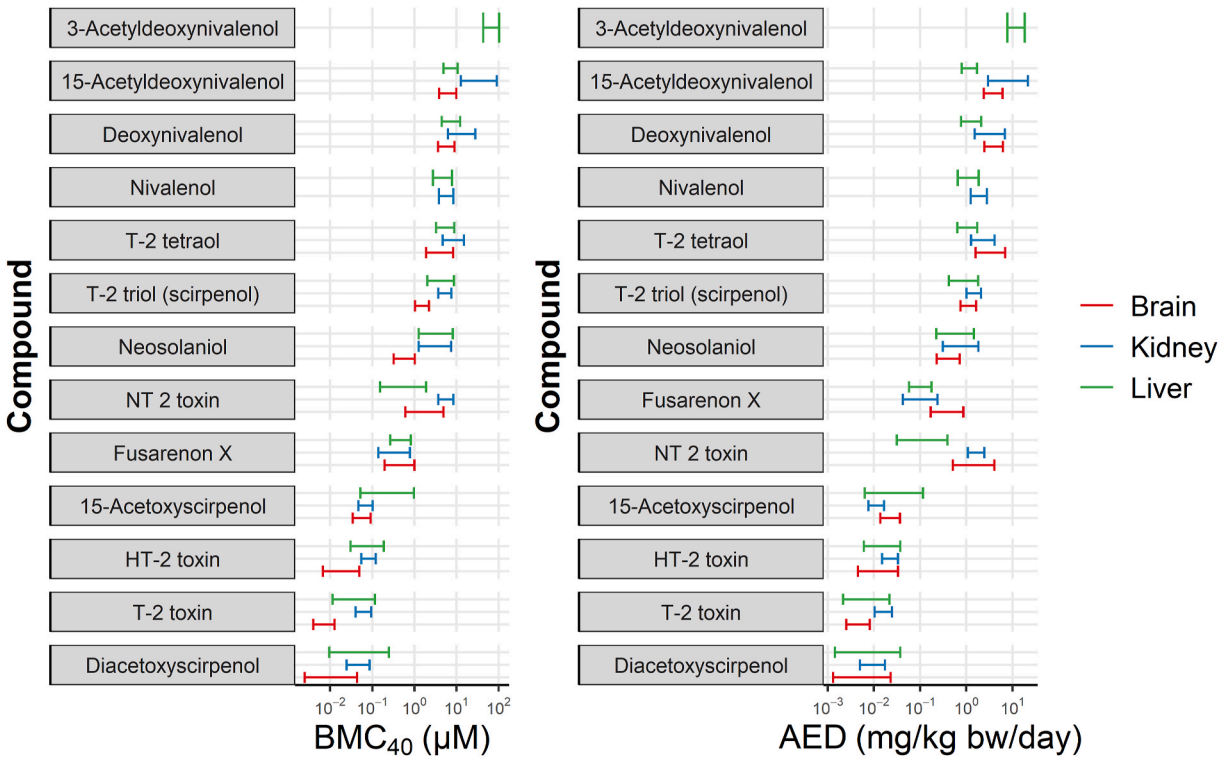

Fig. 3. Potency ranking of trichothecenes based on benchmark concentration modeling (left; units in μM; IVIVE not applied) and benchmark concentration modeling coupled with in vitro-in vivo extrapolation (right; units in mg/kg bw/day).

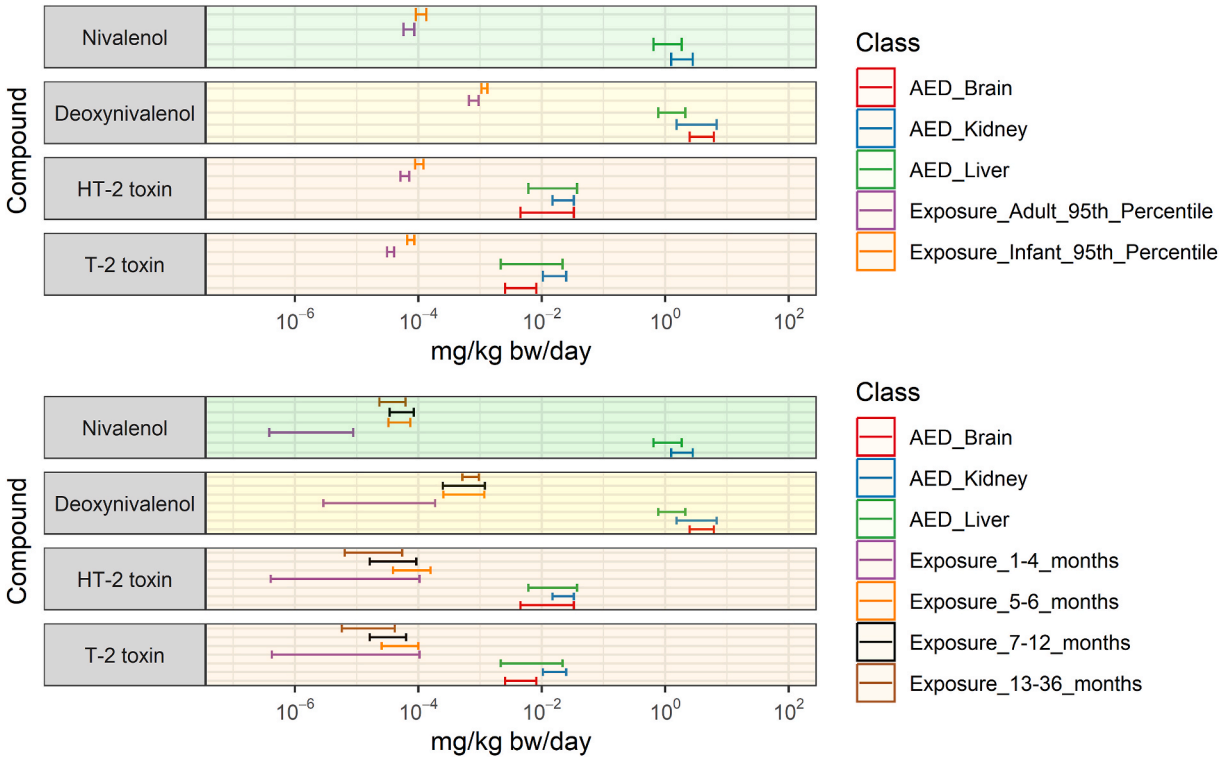

Fig. 4. Bioactivity exposure ratios for T-2, HT-2, DON, and NIV as estimated using total diet studies and administered equivalent doses. Top panel exposure values are based on the French Total Diet Study (Etude de l'alimentation totale française 2 (EAT 2) 2 - 2006–2010) (ANSES, 2011) and the bottom panel exposure values are based on an infant total diet study (Vin et al., 2020). The green shaded area indicates a BER >1000 (NIV), the yellow shade dare indicates a BER of 100–1000 (DON), and the orange shaded area indicates a BER of 1–100 (HT-2 and T-2). AEDs are based on the 90 percent confidence intervals of the BMC_{40}. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

had a lowest BER estimate of 28.6 based on infant exposure (5–6 months) and the AED for brain. T-2 had a lowest BER estimate of 20.8 based on infant exposure (1–4 months) and AED for liver, with a similarly low BER based on the AED for brain (24.3).

4. Discussion

Trichothecenes have been shown to be associated with various toxicities, and the underlying mechanism is believed to be primarily through inhibition of protein synthesis (Li et al., 2022). A better understanding of this inhibitory effect and it's association with chromosomal damage is required to inform risk assessments and address the uncertainties regarding the genotoxic potential of trichothecenes. In the case of most data-poor trichothecenes, there is minimal to no toxicity data to assess their genotoxic potential. In this study, we aimed to augment the weight of evidence in trichothecene genotoxicity assessment by measuring the *in vitro* induction of the γH2AX biomarker as a quantifiable indication of DNA damage (Kopp et al., 2019).

All of the thirteen trichothecenes tested appear to induce some level of DNA damage (i.e., γH2AX induction), and IVIVE analyses provided a means to conduct *in vivo* equivalent potency ranking. The induction of γH2AX by DON was consistent with previous 24-h exposure studies in human gastric epithelial cells (Hou et al., 2023) and four different human prostate adenocarcinoma cell lines (Habrowska-Górczyńska et al., 2019). However, the trichothecene-induced DNA damage is in contrast to recent publications, where no induction was seen after shorter durations of exposure (4–8 h) in intestinal epithelial cells (Garofalo et al., 2022, 2023; Payros et al., 2017). In the current study, DAS had the highest potential to induce DNA damage compared to the other trichothecenes, and this was consistent across the cell models. The AEDs for DAS, based on BMC_{40} confidence intervals, ranged from 1.3 μg/kg bw/day (i.e., $BMCL_{40}$ from SH-SY5Y) to 37 μg/kg bw/day (i.e, $BMCU_{40}$ from HepG2) as shown in Fig. 3. The AED range was above the tolerable daily intake (TDI) of 0.65 μg/kg bw/day and overlapping with the acute reference dose (ARfD) of 3.2 μg/kg bw/day established by EFSA (EFSA et al., 2018). T-2, and its major metabolite HT-2, were the next two chemicals with the highest potential to induce DNA damage. The AED confidence intervals for T-2 and HT-2 overlapped with the AEDs confidence intervals for DAS, and thus, these three chemicals could be considered as equipotent based on the data. EFSA established a TDI of 0.1 μg/kg bw/day for the sum of T-2 and HT-2 (EFSA, 2011), and the AED range for these chemicals is at least one order of magnitude above the TDI (T-2: 2.2–24.7 μg/kg bw/day; HT-2: 4.5–37.1 μg/kg bw/day). NIV, DON, and the acetylated derivatives of DON had the lowest DNA damage potential of the trichothecenes tested. This is reflected in the higher TDI values for these chemicals. EFSA established a TDI of 1.2 μg/kg bw/day for NIV (EFSA, 2013) and a group TDI of 1 μg/kg bw/day for the sum of DON, 3-acetyl-DON, 15-acetyl-DON, and DON-3-glucoside (EFSA et al., 2017). Given that the potency of the data-poor trichothecenes ranked between the chemicals with the highest potential to induce DNA damage (DAS, T-2, HT-2) and lowest potential (NIV, DON), a reasonable estimate of the TDI range, in the absence of other data, would approximate between 0.1 and 1 μg/kg bw/day for these chemicals.

A quantitative risk analysis was conducted by comparing AEDs to chemicals with dietary exposure values from the French total diet studies. Exposure data in the French population was available for the more prominent trichothecenes including NIV, DON, T-2, and HT-2. The BER, which is analogous to the margin of exposure (MOE), can be used to establish whether chemicals are of concern from a public health perspective and if they should be considered as priorities for risk management. T-2 and HT-2 toxins had the lowest BERs in this study and would be flagged as having the highest potential for concern using this approach. There were no dietary exposure values for DAS in the French total diet studies; however, a BER based on the EFSA dietary exposure estimates of 185 ng/kg bw/day and 491 ng/kg bw/day (highest maximum UB observed in "toddlers" and "other children", respectively (EFSA, 2011)) would yield BERs below 100 (results not shown), similar to T-2 and HT-2 toxins. DON and NIV had BERs of $\geq$589 and $\geq$ 4859, respectively, indicative of a lower potential for concern.

Recent work examining mutagenicity-derived MOEs demonstrated that the regulatory decisions derived from genotoxicity MOEs would be consistent to those based on carcinogenicity-derived MOEs for most chemicals (Chepelev et al., 2022). Thus, it is scientifically justifiable to apply a BER/MOE approach to genotoxicity data, but there is currently no consensus on what would be an appropriate BER for risk assessment activities. Previous work has demonstrated that a lower BER relative to the MOE (up to 10,000 for DNA-reactive carcinogens (Barlow et al., 2006; Benford, 2016)) may be more appropriate given the inherent conservatism built into the models (Health Canada, 2021). There are several justifications for this in our current study: the γH2AX biomarker is an early indicator of DNA damage and may be induced at lower concentrations than those that lead to measurable chromosomal damage, the use of human cell models may preclude the need to use interspecies uncertainty factors (UFs), the HTTK IVIVE model applies a lot of conservative assumptions (Beal et al., 2023) and human interindividual variability is already built into the IVIVE model (Ring et al., 2017), and BMC modeling allows for a robust estimate of the concentration leading to a biologically relevant increase in toxicity (Beal et al., 2023). A BER of 100 has been demonstrated to be useful in identifying chemicals classified as toxic to human health or the environment (Health Canada, 2021) and identifying genotoxic chemicals that pose the highest risk to human health (Beal et al., 2022). Similarly, a recent NAM-based case study on 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) developmental neurotoxicity demonstrated that a UF composite of 100 based on subchronic-chronic extrapolation and 10 for interindividual variability could be used to establish biomonitoring equivalents or TDIs protective of human health (Bloch et al., 2024). Overall, a gradient BER approach could be useful for prioritizing the chemicals with the highest potential for concern in the immediate term, but additional work is required to establish rules around applying UFs in BER derivation and determine if safety thresholds could be established using NAM data.

A striking result from this study was that trichothecenes were most cytotoxic and had the highest potential to induce DNA damage in the SH-SY5Y brain model, and the BERs for the most potent trichothecenes were lowest in infants under the age of 3. This age represents a critical window for brain development as this period sees a large increase in total brain volume, elaboration of new synapses, and rapid myelination; thus, environmental insults during this period have the potential to exert long-lasting or permanent effects on the structure and function of the brain (Knickmeyer et al., 2008). Previous work has shown that trichothecenes, such as DON and T-2, are capable of inducing neurotoxic effects (Dai et al., 2019; Zhang et al., 2020). Similar to our study, T-2 and DON have been shown to induce cytotoxicity in brain cell models including human astrocytes (Weidner et al., 2013a), human IMR-32 neuroblastoma cells (Agrawal et al., 2015), mouse N2A neuroblastoma cells (Maroli et al., 2019), primary porcine brain capillary endothelial cells (Behrens et al., 2015), and both human and rat microglia and astrocytes (Razafimanjato et al., 2011). In most cases, these cytotoxic effects occurred at relatively low concentrations. Investigations have also shown that these trichothecenes can cross the blood-brain barrier, by reducing its integrity, and can cause oxidative damage or pathological lesions in the brain (Behrens et al., 2015; Chaudhary and Lakshmana Rao, 2010; Guo et al., 2018; Ravindran et al., 2011; Wang et al., 1998; Weidner et al., 2013b). The neurotoxic effects of trichothecenes remains an adverse outcome of concern, especially for infants with high dietary exposures, and further work is needed to examine the neurotoxic mechanisms of action for the broader members of the trichothecene family.

5. Conclusions

In conclusion, trichothecenes are capable of inducing DNA damage *in vitro*, possibly as a secondary effect of ribosome toxicity. The higher sensitivity observed in the brain cell model supports previous findings of trichothecene neurotoxicity. The hazard potential of many natural toxins, their metabolites, and subproducts still remains unknown. In the case of trichothecenes, there is a high likelihood of co-exposure in food given the high degree of similarity within this family; thus, evaluations of mixtures and cumulative risk assessments for the broader group are needed. There is also a further need for exposure data to support these types of assessments. This study only used exposure data from French total diet studies and the risk level may be higher in climates that are more conducive to toxigenic fungal pathogens or in regions with less regulation and controls on mycotoxins in food and feeds. Exposure assessment is also complicated by the changing climate which could see mycotoxins appearing in new regions that were not previously considered to be at risk (Casu et al., 2024; Kos et al., 2023; Perrone et al., 2020). It is envisioned that high-throughput screening data, like the γH2AX/pH3 indicator assay for genotoxicity, can be paired effectively with monitoring efforts to form the basis of a pragmatic NGRA strategy to respond to newly emerging mycotoxins of concern and other food contaminants.

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CRediT authorship contribution statement

Marc A. Beal: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Denis Habauzit:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Laure Khoury:** Writing – review & editing, Supervision, Methodology, Formal analysis, Data curation, Conceptualization. **Marc Audebert:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

Marc Audebert and Laure Khoury are co-founders of Preditox SAS, a company specialized in genotoxicity prediction. Laure Khoury is CEO and Marc Audebert serves as consultant to Preditox SAS.

Data availability

Raw data provided as supplementary material.

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Appendix A. Supplementary data

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