# Assessing Global Human Exposure to T-2 Toxin via Poultry Meat **Consumption Using a Lifetime Physiologically Based** Pharmacokinetic Model

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Supporting Information

ABSTRACT: Residue depletion of T-2 toxin in chickens after oral gavage at 2.0 mg/kg twice daily for 2 days was determined in this study. A flow-limited physiologically based pharmacokinetic (PBPK) model was developed for lifetime exposure assessment in chickens. The model was calibrated with data from the residue depletion study and then validated with independent data. A local sensitivity analysis was performed, and 16 sensitive parameters were subjected to Monte Carlo analysis. The population PBPK model was applied to estimate daily intake values of T-2 toxin in different countries based on reported consumption factors and the guidance value of 0.25 mg/kg in feed for chickens by the European Food Safety Authority (EFSA). The predicted daily intakes in different countries were all lower than the EFSA's total daily intake, suggesting that the EFSA's guidance value has minimal risk. This model provides a foundation for scaling to other mycotoxins and other food animal species.

KEYWORDS: food safety, T-2 toxin, physiologically based pharmacokinetic (PBPK) model, chicken, lifetime exposure

## INTRODUCTION

T-2 toxin is a type A trichothecene mycotoxin. It can be produced by different Fusarium species, including F. sporotrichoides, F. poae, F. equiseti, and F. acumninatum. These Fusarium species can grow and invade crops, especially under moist cool conditions prior to harvest.<sup>1</sup> The contamination of foods and feeds with T-2 toxin is a worldwide food safety problem.<sup>2</sup>

T-2 toxin is rapidly metabolized and eliminated in different animal species.  $1^{-3'}$  In vivo studies demonstrated that hydrolysis, hydroxylation, de-epoxidation, and conjugation are the major metabolic pathways, and the main metabolites are T-2 triol and HT-2 toxin.<sup>1-3</sup> The tissue distribution and excretion of T-2 toxin were studied in chickens and ducks.<sup>4,5</sup> In broiler chickens, orally administered T-2 toxin was rapidly metabolized and excreted into the intestine through the biliary excretion system.<sup>4</sup> No plasma levels of T-2 toxin nor HT-2 toxin could be detected after a single oral bolus at 0.02 mg/kg; and T-2 toxin had a short elimination half-life (3.9 min) in chickens after intravenous (IV) administration (0.02 mg/kg).<sup>6</sup> Following multiple oral administrations at 2 mg/kg with 12-h intervals for 2 days, T-2 toxin and T-2 triol were detected, but plasma levels of HT-2 toxin were below the quantification limit.' The elimination half-lives of T-2 toxin and T-2 triol were 23.4 and 87.6 min, respectively, after multiple oral administrations.<sup>7</sup> It was reported that T-2 toxin had a low absolute oral bioavailability of 17.07%.7 The European Food Safety Authority Panel on Contaminants in the Food Chain (CONTAM) established a group Tolerable Daily Intake

(TDI) of 0.1  $\mu$ g/kg b.w. per day for the sum of T-2 and HT-2 toxins.<sup>8</sup> The indicative value for feed of 250  $\mu$ g/kg was recommended by the European Food Safety Authority (EFSA) for T-2 toxin in broiler chickens.<sup>8-10</sup> Although these Health-Based Guidance Values (HBGVs) provide a starting point, additional actions should be undertaken to reduce the risk of T-2 toxin.

Physiologically based pharmacokinetic (PBPK) modeling is a computational process that simulates the absorption, distribution, metabolism, and excretion of compounds in the body based on interrelationships among key physiological, biochemical, and physicochemical determinants using mathematical equations.<sup>11,12</sup> Multiple PBPK models have been developed for environmental contaminates in food animals, wildlife, and humans, including lipophilic pesticides,<sup>13,14</sup> organohalogen contaminants,<sup>15</sup> and mycotoxins.<sup>16</sup> PBPK models were applied to conduct risk assessment of organic pollutants, including polychlorinated biphenyl (PCB), dichlorodiphenyldichloroethylene (DDE), dichlorodiphenyltrichloroethane (DDT), and polybrominated diphenyl ether (PBDE), during the entire life span of marine mammals, such as harbor porpoises and long-finned pilot whales.<sup>17,18</sup> These models provide a new, nondestructive tool that enable the feasible and ethical exposure assessment of environmental contaminates.

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In this study, we hypothesized that the pharmacokinetics and tissue depletion of T-2 toxin and its main metabolite in chickens can be simulated using a PBPK model, and the developed PBPK model can be used to assess exposure to T-2 toxin and its metabolites for different populations of humans from different countries. To test this hypothesis, the objective of this study was to determine the tissue depletion profile of T-2 toxin and its main metabolites in chickens following oral gavage twice daily for 2 days, and then we used the newly collected data plus literature data to develop a population PBPK model to simulate the tissue residues of T-2 toxin during the entire lifetime of chickens. This model was validated with independent data from the literature. Finally, we used this validated model to estimate human daily exposure levels of T-2 toxin and its main metabolites for humans in 46 different countries with different meat consumption factors. This population PBPK model contributes to human exposure assessment of T-2 toxin from food products of chicken origin, and it serves as a foundation for scaling to other mycotoxins and other animal species for global human exposure assessment of mycotoxins via consuming animal-derived food products.

## MATERIALS AND METHODS

**Chemicals and Reagents.** T-2 toxin (98.0%), HT-2 toxin (99.9%), and T-2 triol (99.9%) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Acetonitrile, methanol, and ammonium acetate (HPLC grade) were purchased from Fisher Scientific Co. (Pittsburgh, PA, USA). Other reagents were of analytical grade and purchased from Guangzhou Chemical Regent Factory (Guangzhou, China). Ultrapure water was prepared using the Milli-Q water purification system (EMD Millipore, Billerica, MA).

Animals. Thirty 5-week-old healthy chickens (kebao-500, weighted 1.3  $\pm$  0.3 kg), were purchased from Guangzhou Poultry Breeding Farm (Guangzhou, China). The broilers were acclimatized for 1 week under standard environmental conditions ( $25 \pm 2 \degree C$ , 50-60% relative humidity) before the experiment in the Laboratory Animal Center of South China Agricultural University (Guangzhou, China). The broilers were supplied with T-2 toxin free feed and water ad libitum. The composition of the feed was 56.8% corn, 25.0% soybean meal, 8% bran wheat, and other ingredients, which could meet the daily nutrition of the chickens. All procedures were conducted in accordance with Institutional Animal Care and Use Committee (IACUC) protocols of South China Agricultural University.

Residue Depletion Experiment of T-2 Toxin in Chickens. Twenty-five chickens were exposed via oral gavage to 2 mg/kg T-2 toxin twice daily for 2 consecutive days, and five animals were left untreated as control animals. At 1, 2, 3, 4.5, and 6 h after the last dosing, 1 control animal and 5 medicated animals were randomly slaughtered and the tissue samples (liver, kidney, muscle and fat) were collected and immediately frozen at -20 °C in order to prevent degradation at room temperature until further analysis. Sample preparation and determination were adopted from the method reported by our laboratory.<sup>7,19</sup> Briefly, the method involved liquidliquid extraction, purification by solid-phase extraction, and subsequent analysis with liquid chromatography tandem mass spectrometry with an electrospray ionization interface in positive ion mode. In this study, the limit of detection (LOD) was calculated as the analyte concentration that produced a peak signal 3 times that of the background noise, whereas the limit of quantification (LOQ) was the analyte concentration that produced a peak signal 10 times that of the background noise. For both selected tissues (i.e., liver, kidney, muscle, and fat) and plasma, the LOD of the assay for T-2 toxin, HT-2 toxin, and T-2 triol were 0.2, 0.3, and 0.2  $\mu$ g/kg, respectively, and the LOQ for T-2 toxin, HT-2 toxin, and T-2 triol were 0.4, 0.6, and 0.4  $\mu$ g/kg, respectively.

Table 1. Summary of Toxicokinetic Studies of T-2 Toxin in Chickens Used for Calibration and Evaluation of the PBPK Model<sup>a</sup>

study	route	dose	age (week)	BW (kg)	Ν	analyte	matrix	assay	source				
Calibration													
data set 1	IV	0.5 mg/kg BW, single dose	5-week-old	1.3	12	T-2 toxin, HT-2 toxin, T- 2 triol	Р	LC-MS/MS	Sun et al., 2015				
data set 2	oral gavage	2.0 mg/kg BW, twice daily, 2 days	5-week-old	1.4	8	T-2 toxin, T-2 triol	Р	LC-MS/MS	Sun et al., 2015				
data set 3	oral gavage	2.0 mg/kg BW, twice daily, 2 days	5-week-old	1.3	5	T-2 toxin, HT-2 toxin, T-2 triol	L, K, M, F,P	LC-MS/MS	present study				
Evaluation													
data set 4	IV	0.02 mg/kg BW, single dose	3-week-old	NA	8	T-2 toxin	Р	LC-MS/MS	Osselaere et al., 2013				
data set 5	oral gavage	0.5 mg/kg BW, single dose	3-week-old	NA	3	[ <sup>3</sup> H]T-2 toxin	L, K, M	radioassay	Giroir et al., 1991				
data set 6	oral gavage	0.5 mg/kg BW, single dose	6-week-old	NA	4	[ <sup>3</sup> H]T-2 toxin	L, K, F, M, P	radioassay	Chi et al., 1978				

"Notes: The abbreviation for the exposure route: IV, intravenous injection. The abbreviations for the matrix: P, plasma; L, liver; K, kidney; M, muscle; F, fat. The abbreviation for the assay: LC-MS/MS, liquid chromatography tandem mass spectrometry. NA: not available.

**PBPK Modeling for T-2 Toxin and Its Major Metabolite in Chickens.** The present model was designed to include two submodels for T-2 toxin and T-2 triol, respectively, with each submodel consisting of 6 compartments, including plasma, liver, kidney, muscle, fat, and the rest of body (Figure 1). The other minor metabolites, including HT-2 toxin, were pooled together and modeled as a single compartment. The liver was modeled as an individual compartment in the T-2 toxin submodel because liver is the major metabolic organ. The liver, kidney, muscle, and fat were modeled as individual compartments because these organs are common edible tissues and relevant to food safety. Additionally, it was necessary to include a lumped compartment to account for disposition of T-2 toxin and T-2 triol to the rest of body. All compartments were assumed to be blood-flow-limited and well-stirred. Administrations of T-2 toxin via IV administration and oral gavage were included in the model.

IV administration was described with a single rate of administration and directly added into venous blood, with the dose multiplied by body weight and then divided by the duration of the infusion period (Timeiv, set Timeiv = 0.01h).<sup>20</sup> The oral gavage of T-2 toxin was described with a two-tissue compartment model based on Buur et al.<sup>21</sup> and Lin et al.<sup>22</sup> Repeated oral exposure paradigms were described with the PULSE function as detailed in the PBPK model for the herbicide atrazine.<sup>23</sup> The present study considered lifetime exposure to T-2 toxin in feed, and the lifetime exposure paradigm in chickens was based on a recently published lifetime PBPK model for monensin in chickens.<sup>24</sup> The growth of the animal (body weight and organs or tissues) and daily feed intake were described with equations fitted with field data.<sup>24</sup> The rate of change for T-2 toxin and T-2 triol in each tissue compartment was described using mass balance differential equations as described previously.<sup>22,25</sup> Urinary elimination of T-2 toxin and T-2 triol was described with a first-order elimination rate equation in the kidney compartment. Berkeley Madonna (Version 8.3.23.0, University of California at Berkeley, CA) was used to develop the model and run all simulations. Model code is provided in the Supporting Information and will also be available on our Web site (http://iccm.k-state.edu/).

**Model Parametrization.** Physiological parameters (Table S1, Supporting Information), such as cardiac output, blood flow rates, and tissue volumes were from the literature, and the body weight was calculated as an average of experimental animals. For the chemical-specific parameters, oral absorption rate constants, hepatic metabolic rate, hepatic and urine elimination rate constants were estimated by visually fitting to the experimental data from the residue depletion studies (listed in Table 1, detailed below). The tissue:plasma partition coefficients (PCs) for T-2 toxin and T-2 triol were calculated using the areas under the concentration–time curve (AUC) method (AUC<sub>tissue</sub>/AUC<sub>plasma</sub>).<sup>26,27</sup> The chicken growth and exposure parameters, including body weight at the beginning (BW<sub>BEGIN</sub>) and

the end  $(BW_{END})$  of the growth curve, age at the beginning  $(AGE_{BEGIN})$  and the end  $(AGE_{END})$  of the growth curve, coefficient of the Gompertz equation for growth (Bgomp), Slope<sub>X</sub> and intercept<sub>X</sub> for the regression line of tissues (liver, fat, and muscle) on the body weight, slope (a\_feed) and intercept (b\_feed) for the regression of feed intake on body weight, T-2 toxin concentration in feed (Feed<sub>SupplLevel</sub>), time at begin of the treatment (TreatmentStart), duration of the light period in a day (LightingPeriod), and light restored every 24 h (PulseInterval), were based on recent studies.<sup>9,24</sup> All chemical-specific parameters are provided in Table S2.

**Model Calibration and Evaluation.** The PBPK model was calibrated using the concentration-time data from the residue depletion experimental data from the present study and from our earlier study<sup>7</sup> (Table 1). The model was further evaluated by comparing model simulations with concentration-time data from literature.<sup>4–6</sup> Key information on all selected toxicokinetic data sets used in the model calibration and evaluation is provided in Table 1. Based on World Health Organization (WHO) PBPK modeling guidelines,<sup>28</sup> if the simulations were generally within a factor of 2 of the measured values, the model was considered reasonable and validated. The goodness-of-fit between observed and predicted concentrations were evaluated by model convergence, visual inspection, and further analyzed with linear regression analysis using GraphPad Prism 6 (GraphPad Software Inc., La Jolla, CA).<sup>25</sup>

**Sensitivity Analysis.** A normalized sensitivity analysis was performed to determine which parameters had high impacts on critical model outputs, including 24-h area under the concentration curves (AUCs) of T-2 triol in liver, kidney, muscle, fat and plasma. The normalized sensitivity coefficient (NSC) was calculated using the following equation.<sup>23,29</sup>

$$NSC = \frac{\Delta r}{r} \times \frac{p}{\Delta p}$$

where *p* is the original parameter value,  $\Delta p$  is 1% of the original parameter value, *r* is the model output derived from the original parameter value, and  $\Delta r$  is the change of the model output resulting from 1% increase in the parameter value. Parameters with values of | NSC|  $\geq 0.5$  were considered highly sensitive<sup>22</sup> (Table S3, Supporting Information).

**Monte Carlo Analysis.** Monte Carlo analysis was implemented to evaluate the impact of uncertainties of sensitive parameters on tissue residue predictions. Normal distribution was assumed for physiological parameters, including blood flow rates, and liver volume, daily feed intake; while chemical-specific parameters were assumed to be log-normally distributed (Table S4, Supporting Information). Probabilistic distributions (variability) of model parameter values were derived from previous reported interindividual variability.<sup>24,30,31</sup> The coefficients of variation (CV) of 10% and 15% were assumed for

Table 2. Concentrations of T-2 Toxin and T-2 Triol in Tissues and Plasma after Oral Gavage to 2 mg/kg T-2 Toxin Twice Daily for 2 Consecutive Days in Chickens

		concentration <sup><i>a</i></sup> ( $\mu$ g/kg)							
residue	withdrawal time (h)	liver	kidney	muscle	fat	plasma			
total residues <sup>b</sup>	1	64.8 ± 11.9	111.2 ± 29.5	18.8 ± 6.1	$26.2 \pm 7.1$	93.0 ± 26.3			
	2	39.0 ± 11.1	$70.6 \pm 17.3$	$13.0 \pm 4.2$	$17.0 \pm 4.9$	60.4 ± 14.8			
	3	$20.2 \pm 5.2$	38.8 ± 12.1	$7.4 \pm 3.6$	8.8 ± 2.6	33.4 ± 11.1			
	4.5	$7.8 \pm 2.8$	14.8 ± 6.0	$3.0 \pm 1.1$	$3.4 \pm 1.5$	$11.2 \pm 3.1$			
	6	$2.4 \pm 0.5$	$5.6 \pm 2.2$	$0.9 \pm 0.3$	$1.4 \pm 0.6$	$4.2 \pm 2.0$			
T-2 toxin	1	$27.2 \pm 3.4$	$23.4 \pm 6.4$	$11.4 \pm 4.3$	$10.8 \pm 2.6$	$21.6 \pm 4.5$			
	2	$16.4 \pm 2.7$	$13.4 \pm 2.1$	$7.4 \pm 2.1$	6.4 ± 1.9	$14.6 \pm 3.0$			
	3	$8.6 \pm 2.2$	8.6 ± 2.2	4.6 ± 1.6	3.6 ± 1.0	$7.6 \pm 2.2$			
	4.5	$3.4 \pm 1.6$	$3.4 \pm 1.9$	$1.5 \pm 0.5$	$1.2 \pm 0.4$	$2.8 \pm 1.3$			
	6	$1.0 \pm 0.2$	$1.2 \pm 0.3$	$0.5 \pm 0.2$	$0.4 \pm 0.2$	$1.0 \pm 0.4$			
T-2 triol	1	$37.6 \pm 8.5$	87.8 ± 23.1	7.4 ± 1.9	15.4 ± 4.6	$71.4 \pm 21.9$			
	2	$22.6 \pm 8.4$	$57.2 \pm 15.3$	$5.6 \pm 2.2$	$10.6 \pm 3.0$	45.8 ± 11.9			
	3	$11.6 \pm 3.0$	$30.2 \pm 9.9$	2.8 ± 1.9	$5.2 \pm 1.6$	25.8 ± 9.0			
	4.5	$4.4 \pm 1.2$	$11.4 \pm 4.1$	$1.5 \pm 0.6$	$2.2 \pm 1.2$	8.4 ± 1.9			
	6	$1.4 \pm 0.3$	$4.4 \pm 1.9$	$0.4 \pm 0.1$	$0.9 \pm 0.4$	$3.2 \pm 1.6$			
<sup>a</sup> Each value represen	ts the mean $\pm$ SD for five	chickens. <sup>b</sup> Value re	epresents the summat	tion of T-2 toxin a	nd T-2 triol.				

a\_feed and b\_feed, respectively, while a CV of 30% was assumed for the sensitive physiological parameters (blood flow rates, and liver volume), and 20% was assumed for the sensitive chemical-specific parameters (partition coefficients, hepatic metabolic rate, hepatic and urine elimination rate constant). Each Monte Carlo analysis included 1000 iterations. To ensure the physiological plausibility of randomly selected physiological parameters, that is, the sum of the fractional blood flows equals to 100% and the sum of the fractional tissue volumes equals to 100%, randomly selected physiological model parameters were adjusted in a fractional manner to maintain mass balance. Berkeley Madonna (Version 8.3.23.0, Berkeley Madonna, Inc., Berkeley, CA) was used to run Monte Carlo analysis based on the method we recently reported.<sup>32,33</sup>

**Model Application.** Coupled with Monte Carlo analysis, the PBPK model was employed to estimate the residue depletion profiles of T-2 and T-2 triol in muscle for 1000 chickens exposed to T-2 toxin at the EFSA's guidance value of 0.25 mg/kg feed for 33.25 consecutive days.<sup>9</sup> Next, the human daily intakes of T-2 toxin were calculated based on the model-predicted maximum muscle concentrations of total residues (i.e., T-2 toxin plus T-2 triol) and based on the chicken meat consumption factors in different countries reported by Organisation for Economic Co-operation and Development (Table S5, Supporting Information).<sup>34</sup> The calculated human daily intakes of T-2 toxin total residues in different countries were then compared to the TDI of 100 ng/kg/day from the EFSA to assess the possible risk of the current EFSA's guidance value.

## RESULTS AND DISCUSSION

The present PBPK model properly describes tissue depletion of T-2 toxin and its major metabolites T-2 triol in chickens following multiple oral gavage at 2 mg/kg twice daily for 2 days. The model has been evaluated and validated with multiple independent data sets.<sup>4–6</sup> Our model was able to adequately simulate independent data that have not be used in our model calibration. This is considered model validation in the field of PBPK modeling. Since PBPK modeling is a physiologically based approach, one advantage of PBPK models is the ability to conduct extrapolation across doses, exposure paradigms, and even species. In addition, the impact of parameter uncertainties on model predictions was assessed through sensitivity analysis and Monte Carlo simulations. Finally, the model was extrapolated to predict the tissue concentrations of chickens exposed to T-2 toxin via guidance value at 0.25 mg/kg feed for 33.25 consecutive days. In the Monte Carlo simulation with 1000 chickens (each iteration represented one chicken), the parameters of each chicken were randomly sampled, so the results represented a summary of results from a diverse population of 1,000 subjects. The model-derived TDIs of T-2 toxin total residues were calculated by multiplying the model-predicted maximum residue concentration in muscle with the chicken meat consumption factors in different countries. The model-derived TDIs for different countries were all 100-fold lower than EFSA's TDI (100 ng/kg/day). These results suggest that the EFSA's guidance exposure level of 0.25 mg/kg T-2 toxin in feed for chickens has minimal risk to human consumers.

Residue Depletion Study. In the residue depletion study, chicken plasma, liver, kidney, muscle, and fat were collected and quantified. The measured average concentrations of T-2 toxin, T-2 triol, and total residues in tissues are shown in Table 2. Based on the comparison of total residue concentrations in various tissues, the highest residue concentrations were 64.8  $\pm$ 11.9, 111.2  $\pm$  29.5, 18.8  $\pm$  6.1, and 26.2  $\pm$  7.1  $\mu$ g/kg in liver, kidney, muscle, and fat, respectively. Among all tissues, the depletion profile of total residues in the kidney was slower than in other tissues, and total residues were still detected consistently at 6 h after the last dosing at concentrations of  $2.4 \pm 0.5$ ,  $5.6 \pm 2.2$ ,  $0.9 \pm 0.3$ , and  $1.4 \pm 0.6 \,\mu g/\text{kg}$  in liver, kidney, muscle, and fat, respectively. An increasing number of studies have been focusing on T-2 toxin toxicity, metabolism, disposition, and analytical method, but information about the tissue residue depletion profiles of T-2 toxin following repeated oral gavage is still quite limited.<sup>4–7,35</sup> Chi et al. (1978) investigated the excretion and distribution of radioactivity in chickens following single oral gavage to 0.5 mg/kg tritiumlabeled T-2 toxin.<sup>4</sup> The authors reported that the amounts of T-2 toxin or its metabolites in edible portions of the carcass were 60 and 40  $\mu$ g/kg at 24 and 48 h, respectively.<sup>4</sup> The bile, including the gall bladder, contained the highest specific radioactivity among organs and tissues (except GI tract) during the 48 h period after exposure, indicating that T-2 toxin and/or its metabolites were excreted into the intestine through the bile and that liver was a major organ for excretion.<sup>4</sup> The metabolism, tissue retention, and excretion of T-2 toxin were



**Figure 2.** Model calibration of multiple oral gavage with tissues and plasma data for total residues. Comparison of model predictions (solid line) and observed data (squares) for total residue concentrations in liver (a), kidney (b), muscle (c), fat (d), and plasma (e) of chickens exposed to T-2 toxin via multiple oral gavage at 2.0 mg/kg twice daily for 2 consecutive days. Result of regression analysis between model predictions and observed data is shown in f. The determination coefficient  $R^2$  value is 0.99. Observed data are from data set 3 listed in Table 1 collected as a part of the present study.

compared between chickens and ducks after single oral gavage to 0.5 mg/kg tritium-labeled T-2 toxin.<sup>5</sup> There were few significant differences between the two species and the results were consistent with earlier tissue distribution and residue studies in poultry.<sup>5</sup> However, all these earlier studies did not separate T-2 toxin and its metabolites, and only determined the excretion and distribution of radioactivity in plasma and tissues.<sup>4,5</sup> Sun et al. (2015) evaluated toxicokinetics of T-2 toxin and its major metabolites in the plasma of broiler chickens after a single IV (0.5 mg/kg b.w.) and multiple oral gavage (2.0 mg/kg b.w., every 12 h for 2 days). The results showed that T-2 toxin was rapidly absorbed and extensively transformed to metabolites, with the longest elimination halflife of T-2 triol being 87.6 min after oral gavage.

Model Calibration. The plasma concentration-time data from the previous toxicokinetic study after IV and oral administration (data sets 1 and 2 listed in Table 1) were utilized to calibrate the present model. As shown in Figure 2, Figure S1, and Figure S2 (Supporting Information), the model well simulated the kinetic profiles of total residues, T-2 toxin, and T-2 triol in plasma after both IV and oral exposure. The measured concentrations of total residues, T-2 toxin and T-2 triol in liver, kidney, muscle, fat, and plasma from the residue depletion study were compared to simulated data (Figure 2, Figure S3, and Figure S4). Overall, the model-simulated concentrations correlated with the measured data very well for all tissues, especially at later time points (37-42 h). Results of linear regression analyses between model-simulated and measured plasma and tissue concentrations of total residues, T-2 toxin, and T-2 triol were shown in Figure 2f, Figure S3f, and Figure S4f, respectively. The determination coefficient  $(R^2)$  values were 0.99, 0.99, and 0.99, respectively, indicating excellent overall goodness-of-fit.

Currently, PBPK models are available in chickens for midazolam,<sup>36</sup> lipophilic pesticides,<sup>14</sup> marbofloxacin,<sup>37</sup> dano-floxacin,<sup>27</sup> and monensin.<sup>24</sup> Compared with these existing models, the present model structure was designed to include two submodels for T-2 toxin and T-2 triol, which is novel with the context of PBPK modeling in chickens. In addition, these were consistent with the facts that total residues were excreted into the intestine through the bile and that liver was a major organ for excretion. The present modeling approach was consistent with PBPK models for other drugs (e.g., mequindox, ceftiofur, enrofloxacin, flunixin, and sulfamethazine).<sup>25,38</sup>

Model Evaluation. After model calibration, three independent pharmacokinetic data sets (Table 1) were utilized to evaluate the model performance.<sup>4-6</sup> As shown in Figure S5, the model properly simulated T-2 toxin kinetic process in the plasma of chickens following single IV (0.02 mg/kg) exposure.<sup>6</sup> Although the model predictions were lower at the earlier time points than the observed data, the differences were very minor, generally within 1.5-fold, which was considered validated according to the WHO model precision criteria.<sup>24</sup> Likewise, the model estimations were in reasonable agreement with measured data from Giroir et al.<sup>5</sup> for total residue concentrations in liver, kidney, and muscle of chickens exposed to T-2 toxin via single oral gavage at 0.5 mg/kg (Figure S6). In addition, following single oral gavage, model simulations matched the experimental data from Chi et al.<sup>4</sup> up to 48 h after exposure fairly accurately (Figure S7). These simulation results suggest that the model is validated and can be used to simulate the tissue distribution profiles of both T-2 toxin and its main metabolite T-2 triol after different exposure paradigms.

**Sensitivity Analysis.** NSCs for all 33 model parameters were calculated for 5 dose metrics (24-h AUCs for T-2 triol in the liver, kidney, muscle, fat, and plasma), resulting in a total of



**Figure 3.** Model simulation results of lifetime exposure at EFSA's indicative value for feed (2013/165/EU). Model predictions (blue line, 99th percentile; black line, Mean) for total residues concentrations in liver (a), kidney (b), muscle (c), fat (d), and plasma (e) of chickens exposed to T-2 toxin at guidance value of 0.25 mg/kg in feed for 33.25 consecutive days. LOD, limit of detection.

165 NSCs. Of these NSCs, only 16 parameters with at least one absolute value of NSC more than or equal to 0.5 were presented in Table S3. Blood flow rates of liver, kidney, and muscle (QLC, QKC, and QMC), liver PC (PL), hepatic metabolic rate of T-2 toxin (KmC), fraction of T-2 toxin metabolized to T-2 triol (Frac), and bile elimination rate constant of T-2 toxin and T-2 triol (KbileC and Kbile1C) had high influence on all selected dose metrics with NSCs values of 1.55, 1.10, 1.55, 0.83, 0.83, 1.00, -0.80, and -0.96, respectively. Liver, kidney, muscle, and fat dose metrics were only highly sensitive to their own PCs with NSC values of 1, 1, 1, and 1, respectively. As expected,  $slope_{Liver}$ , intercept\_Liver a feed, and b feed had high impact on all dose metrics with NSCs values of -2.37, -3.01, 1, and 0.95, respectively. Overall, the sensitive parameters identified in the model were mainly those that were associated with the distribution and elimination of T-2 toxin.

**Monte Carlo Analysis.** Monte Carlo analysis was performed to account for the interindividual variability across the population. Only sensitive parameters were subjected to Monte Carlo analysis in this study, which is consistent with the approach used in previous PBPK models.<sup>21,24,27,39</sup> The Monte Carlo analysis did not consider the correlation or covariance between parameters. A better approach to do population PBPK analysis by considering correlation and covariation between parameters is to use more advanced Bayesian method with Markov chain Monte Carlo (MCMC) simulation.<sup>17</sup> The MCMC approach will be applied to improve our models in our future studies.

A Monte Carlo simulation with 1000 iterations is a commonly used and generally accepted approach in the field of PBPK modeling to generate a population simulation results.<sup>24,33,37,38</sup> We have tried a larger number of 10 000 iterations in our recent PBPK modeling study for penicillin G in dairy cows.<sup>33</sup> The results showed that there were no considerable differences in the Monte Carlo simulation results between 1000 and 10 000 iterations. In addition, the present model simulations were performed using the software Berkeley

Madonna. The maximum number of iterations per batch run in Berkeley Madonna is 1000. Monte Carlo analysis was conducted to simulate the concentrations of total residues and T-2 triol in tissues and plasma of individual chickens exposed to T-2 toxin at EFSA's guidance exposure level of 0.25 mg/kg in feed for 33.25 consecutive days as presented in Figure 3 and Figure S8, respectively. The 99th percentiles of the predicted concentrations of total residues in all tissues and plasma were higher than the LODs (0.2  $\mu$ g/kg). The 99th percentiles of the predicted concentrations of T-2 triol in liver, kidney, and plasma was higher than the LODs (0.2  $\mu$ g/kg), except in muscle and fat.

T-2 toxin has a short elimination half-life (3.9 min) in the plasma after intravenous administration (0.02 mg/kg),<sup>6</sup> and chickens typically do not eat during the dark period meaning a daily resetting of tissue exposure. Thus, there is no substantial accumulation of T-2 toxin after daily exposure to T-2 toxin throughout the lifetime. This is why the model-predicted concentrations at the end of the lifetime exposure is close to the model-predicted concentrations on the first day of exposure (Figure 3). However, it has been reported that the edible portions of the carcass contained detectable concentrations of 0.06 and 0.04 ppm of T-2 or its metabolites at 24 and 48 h, respectively, after oral dosing with 0.5 mg of radioactivity-labeled T-2/kg body weight, indicating detectable tissue residues of T-2 toxin or its metabolites at 48 h after oral administration.<sup>4</sup> This result suggests that even though there are no substantial accumulation of T-2 toxin, a moderate amount of residue accumulation, primarily due to the metabolites is possible. Therefore, we think that our lifetime PBPK model is still useful as it can be used to predict the concentrations of T-2 toxin and its metabolite T-2 triol in the edible tissues. Regarding the model validation, we have validated our model using independent data sets that were reported by other research groups.<sup>4-6</sup> This is a well-accepted approach for validating a PBPK model. In the future, it will be more robust if we can validate our model structure for another chemical that



**Figure 4.** Model predictions of human T-2 toxin daily intakes after consuming meat from chickens exposed to T-2 toxin at guidance value (2013/ 165/EU) of 0.25 mg/kg in feed for 33.25 consecutive days. Different bars represent results in different countries based on different meat consumption factors reported from OECD (OECD, 2018). The total daily intake (TDI) value for T-2 toxin from European Food Safety Authority (EFSA) is 100 ng/kg b.w. per day (CONTAM, 2011).

has a longer elimination half-life with progressive accumulation in the edible tissues. This is a subject of our future studies.

The present model included growth to describe exposure throughout the life of the chicken. The equations used and the parameters were extracted from Ross chickens farms, which were suitable for the chickens used in the study. Regarding edible tissues, the simulations suggested that leg muscles, liver, and fat represent, respectively, 14%, 2.4%, and 2.1% of the body weight on the 30th day. For other avian PBPK models, the fractions are 2.4% or 2.24% of body weight for liver, 5% of body weight for fat, and 40% of the body weight for total muscle. These proportions are in agreement with field data. Additionally, the feeding behavior of chickens was implemented in the model as a continuous oral administration of the daily intake distributed during the hours of the lighting period, which is also consistent with the poultry behavior in the industry.

Model Application. In the present human exposure assessment, we have considered different chicken meat consumption factors between different countries. We used Monte Carlo simulation to try to cover the general variability in human exposure due to various factors within the human population. Therefore, the model-predicted human exposure for each human subpopulation in each country has a range with a lower bound and higher bound (e.g., 99%). However, in the present study, we did not have experimental data that were granular enough to assess the effects of other factors, such as the quality of chicken meat consumed by humans in different countries, the feed storage, feed content, and raising approaches (organically raised or not) on human exposure assessment. The model-derived TDIs were equal to the product of the model-predicted maximum residue concentration in the muscle and the chicken meat consumption factors in different countries. As presented in Figure 4, the calculated TDI values in all studied countries were 100-fold lower than EFSA's TDI (100 ng/kg/day). According to the EFSA criteria, our results suggest that the EFSA's guidance exposure level of 0.25 mg/kg T-2 toxin in feed for chickens has minimal risk to human consumers.

In term of risk assessment, using the most sensitive end points of immunological or hematological effects seen in pigs, EFSA CONTAM Panel established a group tolerable daily intake (TDI) of 100 ng/kg b.w. per day for the sum of T-2 toxin and HT-2 toxin. In poultry, a No Observed Adverse Effect Level (NOAEL) has not be established for poultry, but LOAELs of 40  $\mu$ g/kg b.w. per day was set for broiler chickens (lesions in the oral cavity) and the indicative value for feed is 250  $\mu$ g/kg.<sup>9</sup> Although these were a good starting point, there was no human health risk assessment from the exposure to T-2 toxin via food products of chicken origin. In this study, the estimated human daily intake values of T-2 toxin using the present population PBPK model was 100-fold lower than the group TDI established by EFSA CONTAM Panel. These results indicate that the human daily intake amounts of T-2 toxin from foods derived from chickens exposed to EFSA CONTAM's indicative value of 250  $\mu$ g/kg via feeding appear to be without appreciable risk to humans. These results further support that the use of PBPK modeling coupled with Monte Carlo analysis is useful in risk assessment of environmental contaminants or food contaminants. Additionally, it should be noted that the focus of the present study was to assess human exposure to T-2 toxin via consuming chicken-derived meat products, but in reality, humans may also be exposed to T-2 toxin via consuming other food products, which should be considered in the estimation of total human intake of T-2 toxin in future studies.

In conclusion, we have successfully developed a population PBPK model for T-2 toxin and its major metabolite T-2 triol in chickens following oral gavage and intravenous injection. Evaluation with multiple independent data sets suggests reliable predictive ability of plasma and tissue depletion profiles of this model. Monte Carlo analysis was successfully incorporated into the PBPK model to simulate farming conditions for lifetime exposure assessment in chickens. This model can be used to estimate T-2 toxin residue levels in chickens fed with T-2 toxin contaminated feed. The results can be used to calculate human daily intake of T-2 toxin after consuming chicken-derived foods, thereby helping risk assessment of T-2 toxin residues in chicken-derived food products. The present study confirms our hypothesis that the pharmacokinetics and tissue depletion of T-2 toxin and its main metabolite in chickens can be simulated using a PBPK model and the developed PBPK model can be applied to assess exposure to T-2 toxin and its metabolites for different populations of humans with different meat consumption factors. This model also serves as a foundation for scaling to other mycotoxins and other animal species for global exposure assessment of mycotoxins via consuming contaminated animal-derived meat products.

## ASSOCIATED CONTENT

## **S** Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.8b07133.

Supplementary tables listing physiological parameters and chemical-specific parameters used in the PBPK model, sensitivity analysis results, and OECD meat consumption factors; (2) supplementary figures for other PBPK model simulation results; and (3) PBPK model codes (PDF)

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#### Notes

The authors declare no competing financial interest.

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#### ABBREVIATIONS USED

AUC:area under the time concentration curve; CONTA-M:European Food Safety Authority Panel on Contaminants in the Food Chain; EFSA:European Food Safety Authority; HBGVs:Health-Based Guidance Values; IV:intravenous injection; LOAEL:Lowest Observed Adverse Effect Level; LOD:limit of detection; LOQ:limit of quantification; NOAEL:No Observed Adverse Effect Level; NSC:normalized sensitivity coefficient; PBPK:physiologically based pharmacokinetic; PC::tissue:plasma partition coefficient; TDI:Tolerable Daily Intake; UF:uncertainty factor.

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