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Metabolism, Distribution, and Excretion of Deoxynivalenol with Combined Techniques of Radiotracing, High-Performance Liquid Chromatography Ion Trap Time-of-Flight Mass Spectrometry, and Online Radiometric Detection

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ABSTRACT: Dispositions of deoxynivalenol (DON) in rats and chickens were investigated, using a radiotracer method coupled with a novel γ-accurate radioisotope counting (γ-ARC) radio-high-performance liquid chromatography ion trap time-of-flight tandem mass spectrometry (radio-HPLC-IT-TOF-MS/MS) system. 3β-^{3}H-DON was chemically synthesized and orally administrated to both sexes of rats and chickens as single or multiple doses. The results showed that DON was widely distributed and quickly eliminated in all tissues. The highest concentration was found in the gastrointestinal tract at 6 h post-administration. Substantially lower levels were detected in the kidney, liver, heart, lung, spleen, and brain. Three new metabolites were identified tentatively as 10-deoxynivalenol-sulfonate, 10-deepoxy-deoxynivalenol (DOM-1)-sulfonate, and deoxynivalenol-3α-sulfate. Deoxynivalenol-3α-sulfate was a major metabolite in chickens, while the major forms in rats were DOM-1 and DON. Additionally, a higher excretion rate in urine was observed in female rats than in male rats. The differences in metabolite profiles and excretion rates, which suggested diverse ways to detoxify, may relate to the different tolerances in different genders or species.

KEYWORDS: *metabolism, disposition, deoxynivalenol, tritium labeled*

INTRODUCTION

Deoxynivalenol [DON (Figure 1)], one of the primary trichothecene toxins produced by *Fusarium culmorum* and *Fusarium graminearum* species, is a widespread contaminant in grains, such as wheat, barley, oat, and maize. The presence of DON in grains is considered as an important limit for economic benefits in the livestock industry. It reduces feed intake, body weight, and reproductive capacities of food-producing animals while increases disease incidence and, thus, leads to significant economic losses.[1] Upon consumption of DON-contaminated grains or food products, it is known to trigger a wide range of disorders in humans and animals, including vomiting, diarrhea, and gastroenteritis, by targeting ribosome and endoplasmic reticulum (ER), ultimately leading to inhibition of protein synthesis.[2,3] Because of these mycotoxicosis, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has recently set the limit of group provisional maximum tolerable daily intake (PMTDI) of DON for 1 μg kg^{-1} of body weight (bw) day^{-1}.[4]

Previous studies revealed that DON was widely distributed and rapidly excreted in rats and chickens. Following a single oral administration of 10 mg/kg of bw of [^{14}C] DON to rats, DON was detectable in the gastrointestinal tract, liver, testes, prostate, seminal vesicles, and heart, with negligible radioactivity retained in the above tissues in 96 h. Excretion in the urine and feces accounts for 25 and 64%, respectively, of the administered dose.[5] In chickens, after a single dose of 2.2 mg of [^{14}C] DON per bird, radioactivity was detectable in all tissues, including the gastrointestinal tract, liver, spleen, heart, kidney, fat, and brain in 3 h, and 78.6 ± 21% was excreted within 24 h.[6] DON can be extensively metabolized mainly into deepoxy-deoxynivalenol [DOM-1 (Figure 1)] and DON conjugates.[7] Using unlabeled DON, DOM-1 was identified in rat feces by gas chromatography−mass spectrometry (GC−MS) and shown later to be a major form in rat excreta.[5,8] After *in vitro* incubation with the contents of large intestines or intestinal microbes of chickens, DON was completely converted into DOM-1.[9,10] Glucuronide conjugations appear to be important phase II metabolites in rats, swine, sheep, cows, and humans, including DOM-1-glucuronide and three DON-glucuronide conjugates.[7,11,12] One of the major glucuronide conjugations, DON-3α-glucuronide, could be a biomarker for monitoring DON exposure in human urine.[13] Additionally, minor DON sulfate conjugation was also found, which involved 2% of the recovered dose in sheep urine, following intravenous administration.[14]

Although research on metabolism, distribution, and excretion of DON in rodent and poultry has been partially performed, information on *in vivo* metabolism of DON in poultry is still limited. The gender differences in metabolism, distribution, and excretion were not investigated in rats or any other species. In

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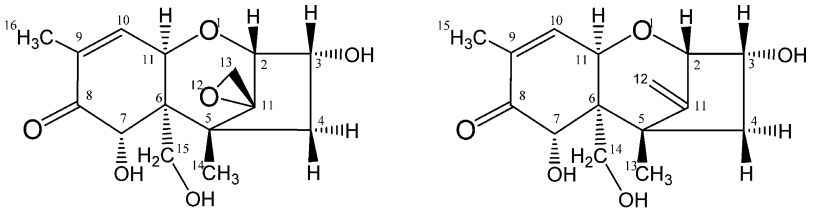

Figure 1. Structures of DON and DOM-1.

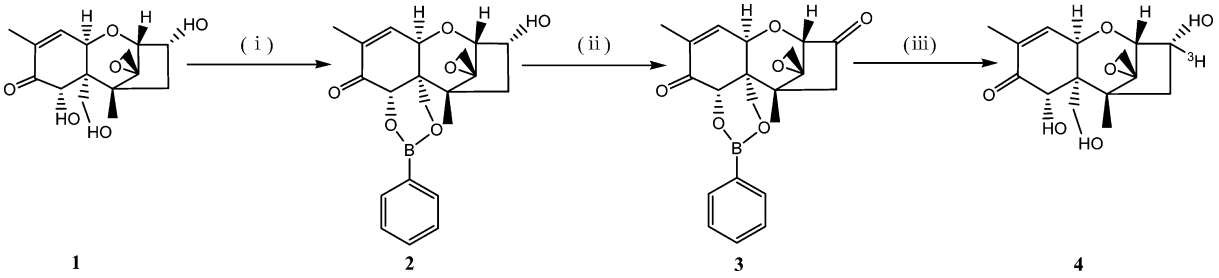

Figure 2. Synthesis of [3β-^{3}H] DON: (i) 1.1 equiv phenylboronic acid, acetone, reflux, 10 h, (ii) Swern oxidation, CH_2Cl_2, −80 °C, 15 min, and (iii) 1/4 equiv NaB^3H_4, isopropanol, room temperature, 30 min.

this study, a comparison study on metabolism, distribution, and excretion of DON in both sexes of rats and chickens was investigated by a radiotracer method, coupled with a novel γ-accurate radioisotope counting (γ-ARC) radio-high-performance liquid chromatography mass spectrometry (radio-HPLC-MS) system. The γ-ARC radio detector was specifically designed for enhanced sensitivity of radioactivity and online radioisotope detection. The full understanding of the dispositions of DON in both sexes of rodents and poultry would provide basis data of DON for future food safety evaluation and toxicological research.

MATERIALS AND METHODS

Chemicals. DON for the animal experiment was produced according to the method by Altpeter and Posselt.[15] DON and DOM-1 for structure confirmation were commercially purchased from Sigma-Aldrich Co. (St. Louis, MO). NaB^3H_4 (500 mCi/mmol) was from American Radiolabeled Chemicals, Inc. (St. Louis, MO). Phenylboronic acid (>98%) and oxayl chloride (>98%) were provided by Aladdin, Inc. (Shanghai, China). Other organic solvents were of analytical grade and from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Tritium-Labeled DON. Tritium-labeled DON (3β-^{3}H-DON) was prepared using a modified method, as illustrated in Figure 2.[16] Briefly, the mixture of DON (**1**) and phenylboronic acid in dry acetone was refluxed for 4 h to give the 7,15-dihydroxyl-protected product (**2**), followed by Swern oxidation to afford a 3-keto-DON-7,15-phenylboronic acid ester (**3**). After reduction with NaB^3H_4 in isopropanol, 3β-^{3}H-DON (**4**) was obtained in moderate yields. Further purification of DON was conducted using model 2695 HPLC with a 2996 detector (Waters, Milford, MA). The separation procedure was performed on a 150 × 21.5 mm inner diameter, 10 μm, Venusil XBP-18 column (Agela Techonologies, Inc., Tianjin, China), eluting with MeOH/water (1:4, v/v) at a flow rate of 5 mL/min at 30 °C. Fractions containing pure ^{3}H-DON were pooled and concentrated under nitrogen, then subjected to a freeze-drying procedure, and finally dissolved in absolute EtOH to afford 110 mCi of 3β-^{3}H-DON (22% yield) with a radiochemical purity of 99%.

Subjects. Wistar rats (8 weeks, 200 ± 10 g) were supplied by the Hubei Provincial Center for Disease Control and Prevention (Wuhan, China). Avian broilers (4 weeks, 1.5 ± 0.3 kg) were from Charoen Pokphand Group (Hubei, China). After routine immunization, both species were placed in all-stainless-steel metabolism cages and fasted overnight with free access to DON-free feed and water. A constant environmental temperature of 25 ± 2 °C was maintained with a relative humidity of 55 ± 5% and 12 h light/dark cycle. All experimental procedures were conducted in accordance with the guidelines of the Committee on the Care and Use of Laboratory Animals of China (permit number SYXK 2007-0044).

Instrumentation. The γ-ARC radio-HPLC-MS system consisted of an online isotope detector system (γ-ARC 2.0, AIM Research Co., Hockessin, DE), an ion trap time-of-flight mass spectrometry (IT-TOF-MS) instrument and high-performance liquid chromatography (HPLC). The online isotope detector (γ-ARC) is fitted with a 750 μL flow cell and a splitter (600-PO10-06). The IT-TOF-MS instrument is equipped with an orthogonal spray electrospray ionization (ESI) source (Shimadzu Corp., Kyoto, Japan), while the HPLC system (Shimadzu Corp., Kyoto, Japan) contains a solvent delivery pump (LC-20AD), an autosampler (SIL-20AC), a DGU-20A3 degasser, a photodiode array detector (PDA, SPD-M20A), a communication base module (CBM-20A), and a column oven (CTO-20AC). This γ-ARC detector uses dynamic flow counting technologies to accurately detect and quantitate radioisotopes and is controlled by LC Solutions software (Shimadzu Corp., Kyoto, Japan). A liquid scintillation counter, Tri-Carb 2900TR, includes a quench standards set, which contains the same amount of nuclide with varying amounts of quenching agent (PerkinElmer, Waltham, MA).

Dosing and Sample Collection. *Excretion and Metabolism of [^{3}H] DON.* Animals were divided into three dose groups (0.5 and 2.5 mg/kg of bw dose rat group and 2.5 mg/kg of bw dose chicken group), with males (n = 3) and females (n = 3) in each group. On the basis of the dose levels, a single dose of [^{3}H] DON (1.1 mCi/mg) was administered orally. The excreta were collected 6, 12, and every 24 h thereafter (24, 48, 72, 96 h, etc.) after administration until radioactivity was no longer detectable in urine or feces. Each feces sample was homogenized in an equal amount of MeOH/water (50:50, v/v), while each urine sample was divided into two parts and stored at −20 °C prior to total radioactivity determination and metabolite profiling.

Distribution and Depletion of [^{3}H] DON. Three groups of animals, including two rat groups and one chicken group (each group with 15 males and 15 females), were given [^{3}H] DON for 5 days. In accordance with the excretion and metabolism tests, rats were dosed 0.5 and 2.5 mg kg^{-1} of bw day^{-1}, respectively, and chickens received 2.5 mg kg^{-1} of bw day^{-1}. According to the above excretion rate of

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DON in these animals, rats were sacrificed at 6, 24, 48, 72, and 96 h after dosing, while chickens were sacrificed at 6, 12, 24, 48, and 72 h. The following samples were obtained from both rats and chicken: gastrointestinal tract, plasma, bile, brain, heart, liver, kidney, lung, spleen, pancreas, urinary bladder, ovary/testis, adipose, muscle, and skin. Above tissues were thoroughly rinsed with phosphate-buffered saline (PBS) to avoid external blood contamination and stored at −20 °C prior to analysis.

Determination of Total Radioactivity and Radioactivity Profiles. Radioactivity was determined on Tri-Carb 2900TR, with samples mixed with 10 mL Ultima Gold scintillation cocktail (PerkinElmer, Waltham, MA) and counted for 5 min unless 2σ of 0.4% was reached. An external standard quench curve was used to correct for differences in counting efficiency and calculate disintegrations per minute (dpm), where dpm = counts per minute (cpm)/efficiency. Urine (200 μL), plasma (200 μL), and bile (10 μL) were directly mixed with Ultima Gold scintillation cocktail and counted on Tri-Carb 2900TR. Air-dried feces (190−210 mg) were powdered and burned in oxygen in an autosampler oxidizer (PerkinElmer, Waltham, MA) to generate ^3H_2O by combustion before analysis. Individual tissues (190−210 mg) were dissolved in a 2 mL tissue Solvable solubilizer (PerkinElmer, Waltham, MA) in unquenched standard vials for 12 h. A total of 0.2 mL of 30% H_2O_2 was added dropwise with swirling to bleach. After standing for 10 min at ambient temperature, samples were warmed to 37 °C for 15 min to decompose peroxides and, thus, minimize chemiluminescence. Quantification of radioactivity in HPLC elutes was performed on the γ-ARC detector, using a dynamic flow mode at a flow of 1.0 mL/min. A linear relationship exists between cpm (X), obtained from the γ-ARC detector, and dpm (Y) from Tri-Carb 2900TR: $Y = 4.37X + 175.93$ ($r = 0.997$).

Metabolite Profiling. The Strata-X column (Phenomenex, Torrance, CA) was conditioned with 2 mL of MeOH followed by 2 mL of H_2O. A total of 0.4 mL of urine sample was diluted with 1.5 mL of 2% phosphoric acid in 10 mM ammonium acetate, mixed, and applied to the preconditioned Strata-X column. After washing with 2 mL of 0.1% phosphoric acid, followed by 2 mL of 5% aqueous ammonia in MeOH, the eluate was concentrated under nitrogen at 50 °C, followed by reconstitution with 0.1 mL of mobile phase (10% MeOH, containing 5 mM ammonium acetate). A total of 2 g of the homogenized feces was extracted twice with an equal volume of MeOH and centrifuged at 8000 rpm at 25 °C for 10 min. The combined extracts were concentrated under nitrogen at 50 °C, followed by reconstitution with a mobile phase up to 0.1 mL. Recovery was determined by detection of radioactivity in an equal amount of samples before and after above procedures on Tri-Carb 2900TR. The chromatographic separation was performed on a 150 × 2.1 mm inner diameter, 5 μm, Thermo Hypersil Gold (Thermo Fisher Scientific, Inc., Waltham, MA) using a linear gradient at a flow rate of 0.2 mL/min at 40 °C. The mobile phases were 5 mmol/L ammonium acetate (A) and MeOH (B). The gradient was programmed as follows: 0−30 min, 1−20% B; 30−50 min, 20% B; 50−52 min, 20−95% B; 52−53 min, 95−1% B; and 53−60 min, 1% B. A total of 20 μL of sample was injected each time. The eluate from the chromatographic separation was divided into two portions. The portion for metabolic quantification was applied to a γ-ARC 2.0, and the rest was analyzed and identified by ion trap time-of-flight tandem mass spectrometry (IT-TOF-MS/MS). To obtain the abundant signal for the product ion scan, mass spectrometric analysis was carried out on a full-scan mass spectrometer, operated in both positive and negative modes with a mass range of m/z 150−650. The MS/MS spectra were produced by collision-induced dissociation (CID) of the selected precursor ions using argon as the collision gas with a relative energy of 50%. Prior to data acquisition, external mass calibration was carried out using a direct infusion of reference standards from 50 to 2000 Da, including 0.25 mL/L trifluoroacetic acid and 0.1 g/L sodium hydroxide. Mass errors (5 mDa) were allowed after calibration.

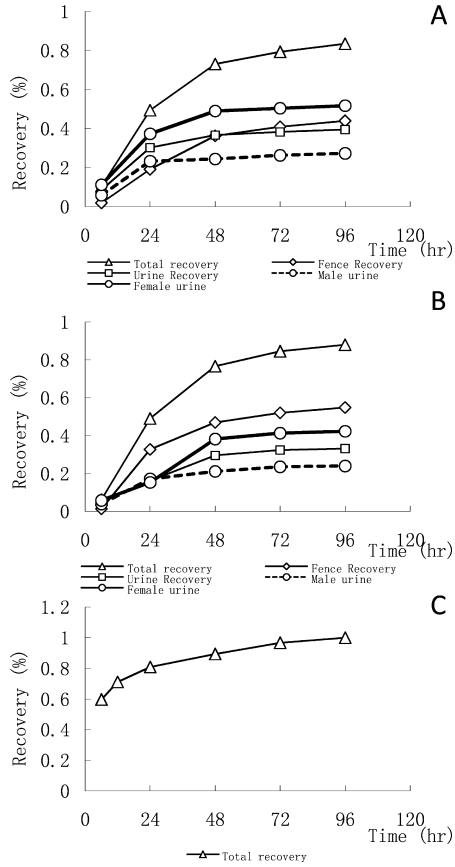

Figure 3. Mean cumulative excretion of total radioactivity in rat and chicken excreta: (A) 0.5 mg/kg of bw rat group, (B) 2.5 mg/kg of bw rat group, and (C) 2.5 mg/kg of bw chicken group.

RESULTS AND DISCUSSION

Synthesis of the Tritium-Labeled DON. Tritium-labeled DON was partially synthesized from DON via a three-step route. Protection of 7,15-dihydroxyl groups with phenylboronic acid and oxidation of the secondary hydroxyl of compound **2** to the 3-ketone derivative (**3**) were preceded smoothly in good yields as reported earlier.[17,18] Afterward, tritiated sodium borohydride was used for reduction of the 3-keto group, and tritium was introduced to DON with high specific radioactivity. A previous study on the synthesis of [^{3}H] T-2 toxin revealed a mixture of 3α- and 3β-hydroxyl epimeric products in a ratio of 4:1.[16] In this study, the absence of a 4β-hydroxyl group in 3-keto DON has resulted in a unique 3α-hydroxyl product when reduced with $NaBH_4$; no β epimer was found. The stereoselectivity and regioselectivity of this reduction of compound **3** with $NaBH_4$ have been described previously.[19] Thus, the desired compound 3β-^{3}H-DON was obtained. To ascertain the "exchange risk" of radioactive samples quantitatively, total radioactivity in plasma and urine was measured on Tri-Carb 2900TR before and after freeze-drying. Because no tritiated water was formed, the "exchange risk" could be ignored[20] (data not shown).

Excretion of the Radioactivity. DON was rapidly eliminated in rats and chickens. In rats, 73.08 ± 11.92% (n = 6; 0.5 mg/kg of bw) and 76.63 ± 8.91% (n = 6; 2.5 mg/kg of bw) of the radioactive dose were recovered from urine and feces in 48 h. By 96 h, they were accumulated to 87.99 ± 10.30% (n = 3; 0.5 mg/kg of bw) and 83.44 ± 7.88% (n = 3; 2.5 mg/kg of bw), respectively. In chickens, 80.90 ± 6.24% (n =

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Table 1. Tissue Distribution and Depletion Rates of Radioactivity in Wistar Rats Following a Multi-dose of [^{3}H] DON at 2.5 mg kg^{-1} of bw Day^{-1} for 5 Days

	concentration (μg/kg)					elimination kinetics[a]	
tissues	6 h	24 h	48 h	72 h	96 h	K_e ($\times 10^{-2}$)	$t_{1/2}$ (h)
plasma	160.4 ± 22.1	80.8 ± 7.5	30.2 ± 6.6	4.5 ± 3.7	ND[b]	5.33	13.0
muscle	132.0 ± 29.3	81.6 ± 9.0	23.4 ± 9.0	17.8 ± 4.9	7.4 ± 6.3	3.18	21.8
abdominal fat	50.1 ± 7.0	25.8 ± 1.3	8.9 ± 2.7	5.3 ± 1.3	3.3 ± 1.7	2.06	33.7
stomach	1795.9 ± 43.6	753.0 ± 19.2	33.9 ± 15.1	23.5 ± 5.6	6.3 ± 3.7	6.48	10.7
large intestine	5226.2 ± 38.8	1970.8 ± 28.7	53.0 ± 6.3	22.3 ± 2.9	3.7 ± 0.9	8.35	8.3
small intestine	1473.7 ± 38.3	642.8 ± 17.5	43.2 ± 15.9	15.6 ± 2.7	3.7 ± 2.2	5.13	13.5
liver	776.6 ± 34.0	365.4 ± 20.3	50.8 ± 10.0	20.8 ± 1.8	5.6 ± 2.0	5.59	12.4
kidney	418.7 ± 40.3	220.9 ± 20.0	52.0 ± 8.6	30.5 ± 8.9	11.2 ± 1.8	3.98	17.4
heart	211.3 ± 36.8	134.3 ± 16.0	50.8 ± 9.9	24.2 ± 4.8	8.8 ± 1.3	3.69	18.8
lung	254.0 ± 46.0	157.5 ± 19.0	65.0 ± 14.2	36.5 ± 9.8	20.0 ± 3.1	2.46	28.2
skin	194.0 ± 23.4	123.1 ± 12.3	49.9 ± 2.1	27.2 ± 5.2	5.0 ± 3.7	3.89	17.8
spleen	235.4 ± 55.4	161.7 ± 25.4	66.5 ± 19.4	38.3 ± 6.5	15.9 ± 4.1	3.00	23.1
brain	100.0 ± 43.9	73.5 ± 13.6	43.7 ± 19.5	27.9 ± 19.5	11.3 ± 7.8	2.35	29.5
testes	218.4 ± 56.9	118.7 ± 12.5	40.7 ± 12.3	23.0 ± 8.4	8.9 ± 4.8	3.52	19.7
ovary	171.1 ± 23.5	115.5 ± 22.3	53.1 ± 9.6	34.9 ± 7.9	15.4 ± 6.7	2.63	26.3
adrenals	172.9 ± 22.2	94.1 ± 9.9	36.1 ± 7.4	ND	ND	3.7	18.5

[a]The terminal slope (β) for determining the elimination constant (K_e) and half-life ($t_{1/2}$) of tissues was calculated by linear regression analysis using points by WinNonlin Enterprise, version 5.2.1. [b]ND = not detectable.

Table 2. Tissue Distribution and Depletion Rates of Radioactivity in Wistar Rats Following a Multi-dose of [^{3}H] DON at 0.5 mg kg^{-1} of bw Day^{-1} for 5 Days

	concentration (μg/kg)					elimination kinetics[a]	
tissues	6 h	24 h	48 h	72 h	96 h	K_e ($\times 10^{-2}$)	$t_{1/2}$ (h)
plasma	41.8 ± 2.4	ND[b]	ND	ND	ND	—[c]	—
muscle	31.0 ± 8.4	ND	ND	ND	ND	—	—
abdominal fat	11.5 ± 4.6	ND	ND	ND	ND	—	—
stomach	775.1 ± 7.8	123.9 ± 9.2	ND	ND	ND	—	—
large intestine	760.8 ± 38.8	123.3 ± 28.7	26.7 ± 6.3	8.7 ± 2.8	3.7 ± 0.9	4.13	16.8
small intestine	834.4 ± 30.3	87.8 ± 17.5	10.6 ± 8.9	4.3 ± 2.7	ND	7.97	8.7
liver	249.5 ± 30.2	35.1 ± 20.3	50.8 ± 10.0	ND	ND	4.50	15.4
kidney	154.9 ± 35.6	22.7 ± 21.1	11.3 ± 8.6	6.8 ± 6.0	ND	2.52	27.5
heart	211.4 ± 13.8	163.4 ± 24.5	97.5 ± 18.7	18.9 ± 5.0	ND	4.50	15.4
lung	107.4 ± 35.4	38.6 ± 18.9	19.3 ± 14.2	4.8 ± 2.2	ND	4.53	15.3
skin	47.3 ± 8.9	6.4 ± 5.8	ND	ND	ND	—	—
spleen	95.4 ± 55.4	47.4 ± 25.4	31.3 ± 19.4	15.0 ± 6.5	ND	2.68	25.9
brain	26.8 ± 6.1	14.7 ± 12.6	ND	ND	ND	—	—
testes	80.3 ± 10.9	17.7 ± 4.5	9.4 ± 5.5	4.8 ± 3.4	ND	2.72	25.5
ovary	52.7 ± 13.6	19 ± 6.8	11.9 ± 5.6	8.2 ± 3.8	2.7 ± 1.5	2.95	23.5
adrenals	39.34 ± 6.56	ND	ND	ND	ND	—	—

[a]The terminal slope (β) for determining the elimination constant (K_e) and half-life ($t_{1/2}$) of tissues was calculated by linear regression analysis using points by WinNonlin Enterprise, version 5.2.1. [b]ND = not detectable. [c]— = not determined.

3; 2.5 mg/kg of bw) of the radioactive dose was recovered in 24 h and accumulated to 94.52 ± 3.59% (n = 6) in 72 h from excreta (Figure 3). The urine radioactivity excretion rate was 39.51 ± 13.41% (n = 6) of the radioactive dose in the 0.5 mg/kg of bw group and reduced to 33.12 ± 10.31% (n = 6) in the 2.5 mg/kg of bw group. Significant differences in the dose- and sex-dependent excretions were found in rats. For female rats, urine radioactivity excretion rates were 57.13 ± 1.18% (0.5 mg/kg of bw; n = 3) and 42.26 ± 3.07% (2.5 mg/kg of bw; n = 3), while for males, they were 27.29 ± 0.67 and 23.97 ± 2.26%, respectively. This difference in the urine excretion rate may relate to the previous report that the female rats were less sensitive than male rats in response to DON.[21]

Distribution and Depletion of Total Radioactivity in Tissues. After DON was administered to rats and chickens for 5 days, the animal body weight, urine volume, feces weight, and general appearance of the animals were not affected, except for a slight reduction in food intake in rats at the high dosage. The concentrations of the DON-related substance in the tissues at each time point was shown in Tables 1−3. The terminal slope (β) for determining the elimination constant (K_e) and half-life ($t_{1/2}$) of tissues was calculated by linear regression analysis using points by WinNonlin Enterprise, version 5.2.1. DON was widely distributed in 6 h and quickly depleted in all tissues from rats and chickens. In rats, the highest concentration of radioactivity was detected at 6 h post-administration in the gastrointestinal tract at 5226.2 and 834.4 μg/kg, which declined to 50.3 and 10.6 μg/kg in 48 h, for the 2.5 and 0.5 mg/kg of bw groups, respectively; substantially lower levels were detected in the kidney, liver, heart, lung, spleen, and brain. In the 0.5 mg/

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Table 3. Tissue Distribution and Depletion Rates of Radioactivity in Chickens Following a Multi-dose of [^{3}H] DON at 2.5 mg kg^{-1} of bw Day^{-1} for 5 Days

	concentration (μg/kg)					elimination kinetics[a]	
tissues	6 h	12 h	24 h	48 h	72 h	K_e ($\times 10^{-2}$)	$t_{1/2}$ (h)
bile	822.5 ± 261.1	111.2 ± 27.2	89.8 ± 45.5	4.0 ± 1.3	ND[b]	11.36	6.1
plasma	209.6 ± 63.3	94.4 ± 7.7	35.9 ± 13.1	5.5 ± 4.7	ND	7.88	8.8
muscle	295.9 ± 14.9	132.9 ± 32.3	91.4 ± 23.4	56.1 ± 9.4	ND	2.34	29.6
abdominal fat	492.2 ± 14.4	146.3 ± 30.1	86.3 ± 21.9	56.8 ± 19.3	ND	2.50	27.7
gizzard	1009.6 ± 39.6	316.2 ± 21.7	164.7 ± 52.2	44.7 ± 19.1	24.7 ± 13.4	4.33	16
large intestine	1776.2 ± 53.8	187.3 ± 23.0	294.0 ± 19.1	70.0 ± 11.9	50.0 ± 37.4	3.69	18.8
small intestine	1666.3 ± 208.1	200.9 ± 12.8	96.3 ± 27.2	39.3 ± 10.3	19.3 ± 15.0	3.35	20.7
crop	1928.7 ± 24.4	569.5 ± 88.1	225.1 ± 44.6	69.0 ± 5.0	28.9 ± 1.1	4.28	16.2
bursal	1726.7 ± 88.1	300.0 ± 13.8	112.5 ± 10.1	47.2 ± 19.5	ND	4.91	14.1
liver	531.9 ± 14.0	165.3 ± 36.5	90.2 ± 12.2	54.1 ± 5.3	ND	2.96	23.4
kidney	1025.5 ± 24.4	262.3 ± 65.0	104.1 ± 22.6	55.7 ± 8.0	ND	4.08	17
heart	565.1 ± 33.2	131.0 ± 46.7	81.3 ± 14.0	50.5 ± 8.7	ND	2.56	27.1
lung	649.2 ± 32.3	154.5 ± 57.6	79.8 ± 19.7	54.3 ± 10.6	ND	2.72	25.5
skin	148.9 ± 79.9	138.6 ± 57.8	72.7 ± 36.6	13.2 ± 4.1	ND	6.60	10.5
spleen	408.9 ± 107.0	146.4 ± 54.8	79.0 ± 8.6	53.9 ± 12.4	ND	2.61	26.6
pancreas	230.8 ± 22.3	123.2 ± 35.1	42.2 ± 32.6	21.5 ± 4.9	ND	5.46	12.7
brain	133.6 ± 32.1	99.8 ± 23.7	78.8 ± 13.7	42.8 ± 6.1	ND	3.24	21.4
testes	2353.3 ± 1134.9	296.1 ± 41.2	130.1 ± 51.6	72.1 ± 10.7	ND	3.71	18.7
ovary	1015.0 ± 452.5	303.0 ± 32.5	131.8 ± 32.0	56.8 ± 9.5	ND	4.50	15.4

[a]The terminal slope (β) for determining the elimination constant (K_e) and half-life ($t_{1/2}$) of tissues was calculated by linear regression analysis using points by WinNonlin Enterprise, version 5.2.1. [b]ND = not detectable

Table 4. Measured Masses, Predicted Masses, Mass Errors, and Major Fragment Ions of Identified DON Metabolites

compounds	retention time (min)	measured mass (Da)	predicted mass (Da)	error (mDa)	error (ppm)	elemental composition	major fragment ions	identification
MD1	5.6	377.1005	377.0912	9.3	24.6	$[M - H]^-$ $C_{15}H_{21}O_9S^-$	347, 295, 265, 247, 217, and 138	10-deoxynivalenol-sulfonate
MD2	7.5	471.149	471.1508	1.8	3.8	$[M - H]^-$ $C_{21}H_{27}O_{12}^-$	411, 307, 265, 247, 229, and 217	DON-3α-glucuronide
MD3	9.2	361.0991	361.0963	2.8	6.6	$[M - H]^-$ $C_{15}H_{21}O_8S^-$	331, 261, 249, 231, and 215	10-DOM-1-sulfonate
MD4	12.3	375.0714	375.0755	4.1	10.9	$[M - H]^-$ $C_{15}H_{19}O_9S^-$	345, 327, 265, 247, 229, and 217	deoxynivalenol-3α-sulfate
MD5	18.8	355.135	355.1398	4.8	13.5	$[M + CH_3COO]^-$ $C_{16}H_{23}O_8^-$	295, 265, 247, and 138	DON
MD6	27.5	339.1426	339.1449	2.3	6.8	$[M + CH_3COO]^-$ $C_{16}H_{23}O_7^-$	279, 261, 249, 231, and 215	DOM-1

kg of bw group in rats, the radioactivity in the plasma, muscle, fat, and adrenals was not detectable at 24 h, while at 96 h after administration, the radioactivity was not detectable in most tissues, except the large intestine and ovary. Nevertheless, in the 2.5 mg/kg of bw group in rats, at 96 h after administration, the radioactivity can be detected at very low levels (<20 μg/kg) in most of the tissues, except the plasma and adrenals. In chickens, the radioactivity was eliminated even faster. The highest concentration was also found at 6 h post-administration in the gastrointestinal tract at 1928.7 μg/kg, and it declined to 225 μg/kg in 24 h. At 72 h after administration, radioactivity was detectable in the gastrointestinal tract only and the concentrations were less than 50 μg/kg. Initially, higher specific radioactivity in the gastrointestinal tract appeared to undergo fast, with $t_{1/2}$ ranging from 8.3 to 20.7 h. Estimated clearance rates for radioactivity varied slightly among the various tissues examined, with the elimination half-life values occurring between 6 and 34 h. The gastrointestinal tract, the first barrier against food contaminants, is highly sensitive to mycotoxins, especially DON. Moreover, the results of the distribution showed that DON could be detected in the brain, which suggested that DON or its metabolites may deliver across the blood−brain barrier in rats and chickens.

Structural Determination of Identified Ions. *DON (MD5) and DOM-1 (MD6).* As shown in Table 4, MD5 (t_R = 18.8 min) gave a deprotonated ion $[M + CH_3COO - H]^-$ and its molecular formula was deduced as $C_{15}H_{20}O_6$. A product ion scanning spectrum provided its characteristic fragment ions at m/z 295 $[M - H]^-$, 265 $[M - H - 30]^-$, 247 $[M - H - 48]^-$, and 217 $[M - H - 78]^-$, suggesting that it was the parent product (DON). The molecular form of metabolite MD6 (t_R = 27.5 min) was calculated as $C_{15}H_{20}O_5$ by IT-TOF-MS ($[M + CH_3COO - H]^-$). The difference of the 16 Da mass between m/z 339 and 355 indicated the neutral loss of oxygen based on DON. Its major product ions were m/z 279 $[M - H]^-$, 261 $[M - H - 18]^-$, 249 $[M - H - 30]^-$, and 231 $[M - H - 48]^-$, suggesting that it was DOM-1, a de-epoxy product of DON. The structures of those two compounds were confirmed by comparison of their retention times and MS/MS spectra on liquid chromatography−mass spectrometry (LC−MS) to the standards.

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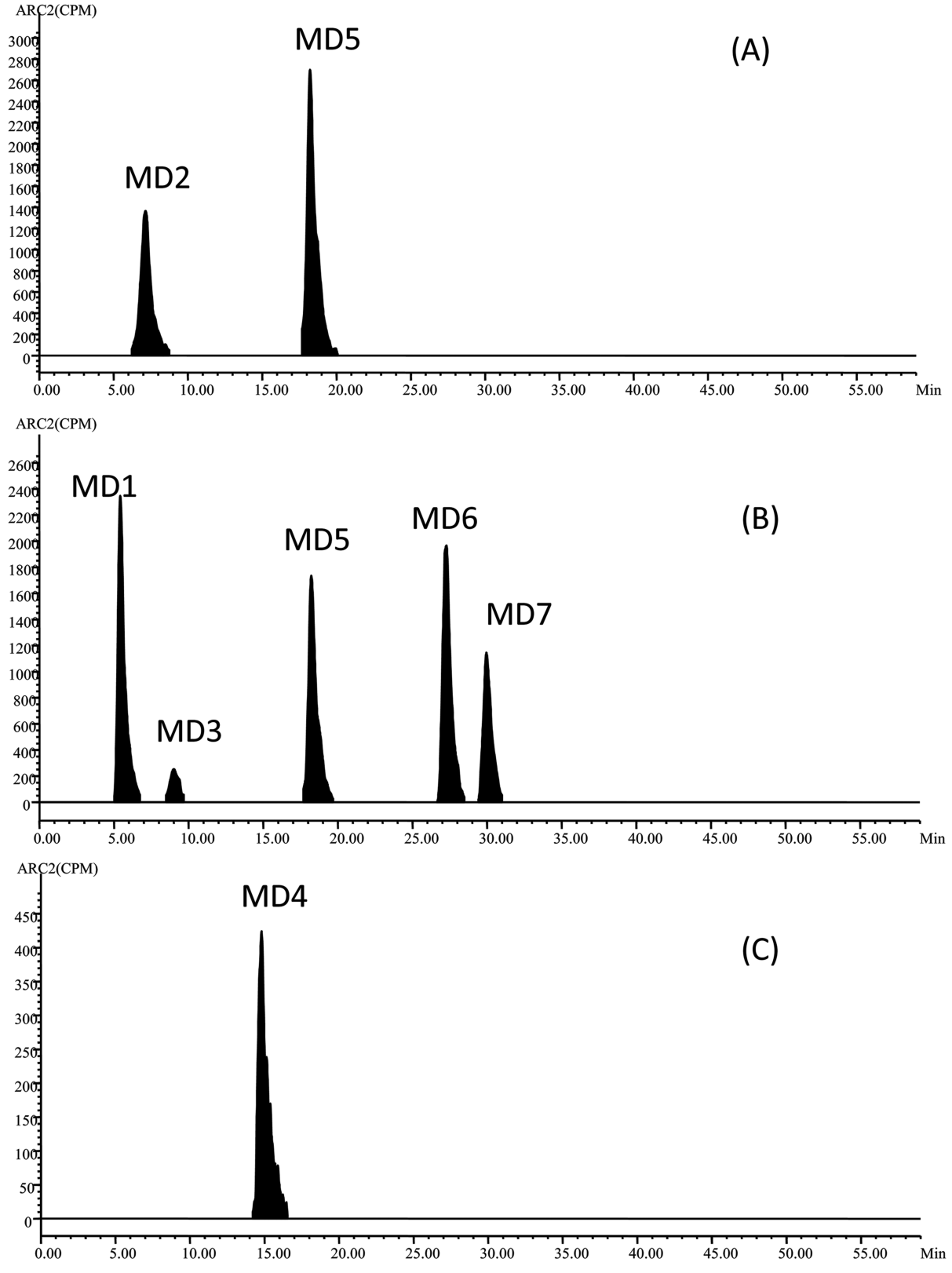

Figure 4. Representative radio-HPLC chromatograms of excreta from [^{3}H]-DON-treated animals: (A) 0−6 h urine from [^{3}H]-DON-treated rats, (B) 6−24 h feces extracts from [^{3}H]-DON-treated rats, and (C) 0−48 h excreta from [^{3}H]-DON-treated chickens.

MD2 and MD4 (3α-Hydroxy Conjugates). Metabolites MD2 and MD4 were expected for phase II metabolites, because the skeleton was identical with DON according to the MS/MS fragments (Figure 5A). MD2 (t_R = 7.5 min) gave a $[M - H]^-$ molecule. The product ion spectrum of MD2 displayed a fragment ion at m/z 411, 265, 247, 217, and 175. The mass loss of 206 Da (176 + 30 Da) and the fragment ion m/z 175 indicated that it is a DON-3α-glucuronide conjugate. MD4 (t_R = 12.3 min) showed a $[M - H]^-$ molecule with a series of product ions at m/z 345, 327, 265, 247, 229, and 217.

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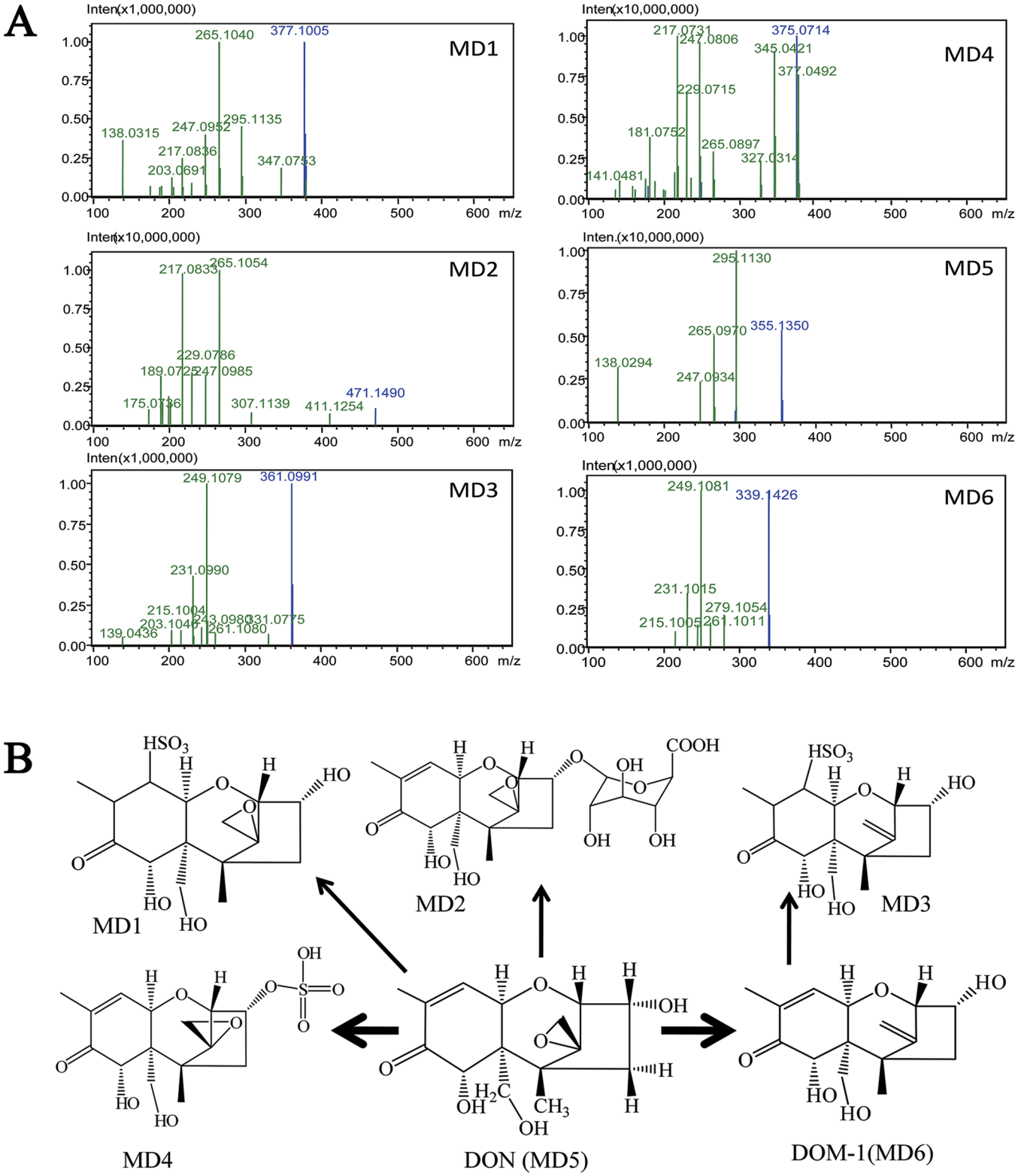

Figure 5. MS and MS^2 of the metabolites of DON and the potential metabolism pathway in the excreta of rats and chickens. (A) MS and MS^2 of the identified metabolites. (B) Structure of the identified metabolites and the potential metabolism pathway.

Subsequent neutral loss of 80 Da from fragment ions m/z 345 and 327 yielded fragment ions at m/z 265 and 247, respectively, which is a specific signature for sulfate conjugates. The product ion at m/z 345 because of the loss of 30 Da ($-CH_2O$) indicated that the sulfate is conjugated at the 3α-hydroxy position. For deoxynivalenol-sulfate, first reported in sheep urine,[14] the details of structural elucidation are missing. Here, the fragmentation patterns (m/z 345, 327, 265, 247, and 217) in MS/MS confirmed that the sulfate was conjugated with a 3α-hydroxyl group.

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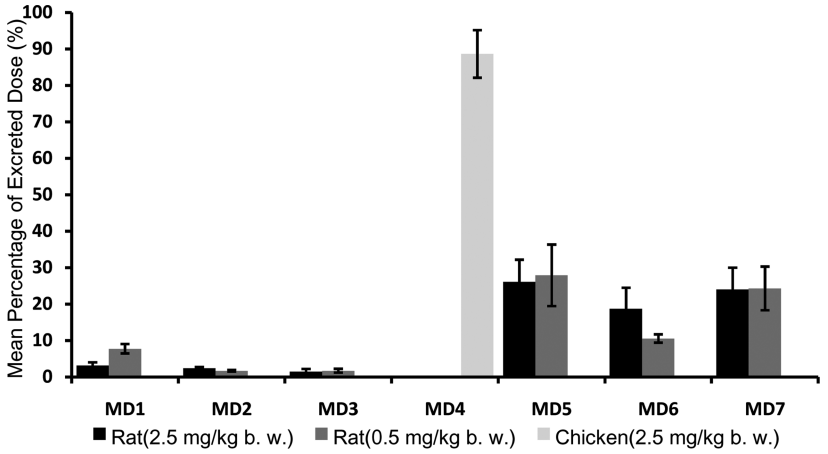

Figure 6. Total excretion of metabolites from rats and chickens (n = 6) 0−96 h after single-dose administration [means ± standard deviation (SD)].

MD1 and MD3 (Sulfonate Acid Conjugates). Two new metabolites, MD1 (t_R = 5.6 min) and MD3 (t_R = 9.2 min), were found in radiometric HPLC (Figure 4B). MD1 gave a $[M - H]^-$ molecule in IT-TOF-MS, and its molecular formulas was deduced as $C_{15}H_{22}O_9S$ (Table 4). Its mass fragments (m/z 295 $[M - H - 82]^-$, 265 $[M - H - 82 - 30]^-$, 247 $[M - H - 82 - 48]^-$, 217 $[M - H - 82 - 78]^-$, and 138 $[M - H - 82 - 157]^-$) were identical to those of DON. The mass neutral loss of 82 Da was deduced as H_2SO_3 (calculated 81.9725) by IT-TOF-MS (Figure 5A). The keto group or the 9,10 double bond of DON could potentially undergo an addition with H_2SO_3, which suggested that MD1 was generated from a rare sulfonation metabolic pathway. Further comparison of MS fragmentation patterns and retention time of MD1 with 10-deoxynivalenol-sulfonate, a reference substance that was synthesized on the basis of the protocol by Beyer et al.,[22] confirmed that it was 10-deoxynivalenol-sulfonate. Similarly, MD3 (m/z 361, 331, 279, 249, 261, and 231) was proposed to be 10-DOM-1-sulfonate.

Profiles of Excreted Metabolites. The radioactivity in excreta was completely recovered, as the recovery efficiencies were 98.7 ± 2.4% in rat urine, 96.1 ± 3.6% in rat feces, 99.6 ± 4.1% in chicken excreta. Seven peaks were observed in HPLC radiochromatograms (Figure 4), and six of them were identified as deoxynivalenol-sulfonate, DON-3α-glucuronide, DOM-1-sulfonate, deoxynivalenol-3α-sulfate, DON, and DOM-1. An unexpected metabolite (MD7), which was found in rat urine and feces, was not identified because of its weak ion response in this high-performance liquid chromatography ion trap time-of-flight tandem mass spectrometry (HPLC-IT-TOF-MS/MS) system. In rats, deoxynivalenol-sulfonate, DON-3α-glucuronide, DOM-1-sulfonate, DOM-1, and DON were detected in both dose groups and both genders. DON existed in all samples, while DOM-1 was detected in urine and feces after 24 h post-administration, DON-3α-glucuronide was found only in urine during 0−6 h after dosing, and deoxynivalenol-sulfonate and DOM-1-sulfonate were found only in feces after 24 h post-administration. In chickens, the toxin residue mainly existed in the form of deoxynivalenol-3α-sulfate and DON but DOM-1, deoxynivalenol-sulfonate, DOM-1-sulfonate, and DON-glucuronide were not detectable. In total, the new metabolites, deoxynivalenol-sulfonate and DOM-1-sulfonate, comprised 10% or less of the total radioactivity, while DON-3α-glucuronide involved 2% of the radioactivity. The major compounds, DON, DOM-1, and MD7, accounted for 27.89 ± 8.44, 10.55 ± 1.14, and 24.28 ± 5.99% of the original dose in the 2.5 mg/kg of bw rat group and 26.08 ± 6.09, 18.73 ± 5.74, and 24.02 ± 5.96% in the 0.5 mg/kg of bw rat group, respectively (Figure 6). The new metabolite deoxynivalenol-3α-sulfate, a major metabolite in chickens, accounted for about 88.63 ± 6.53% of the dose. This O-sulfate conjugation was considered to be a detoxification or deactivation pathway.[23] The specific capability of chicken to biotransform DON to deoxynivalenol-sulfate effectively seems to be a rational reason why poultry is less sensitive than rodents to the toxin DON.[24]

A rare sulfonation metabolic pathway, which led to new [M + 82] metabolites of DON and DOM-1 in rats, was found for the first time. Earlier studies have reported that a few chemicals can be converted into [M + 82] metabolites in rats, such as (*R*)-(+)-menthofuran, gambogic acid, and xanthohumol.[25−27] These compounds, including DON, containing an α,β-unsaturated carbonyl group, might result in this biotransformaition *in vivo*. To date, these metabolites were not identified in chicken and swine. It seems that it is a specific metabolic pathway for rats. Although the enzymes involved in this metabolic pathway were not clear, several hypotheses were proposed. One possible mechanism involves an addition of glutathione (GSH) to the unsaturated ketoaldehyde, followed by further metabolism to give the sulfonic acid conjugates.[25] Thus, it can be hypothesized that, if *in vivo* reversibility of the GSH adduct of DON could occur, it could represent a mechanism for DON delivering and regenerating to the toxicological target. The addition of GSH to the double bond of DON was found in wheat, for which nuclear magnetic resonance (NMR) evidence was provided, most likely a 1,4 addition of α,β-unsaturated ketones.[28] In that case, the proposed structures of the sulfonates, the GSH conjugate-processing products, were 10-deoxynivalenol-sulfonate and 10-DOM-1-sulfonate. Alternatively, it might also result from microbial metabolism. However, the action of gut flora was not to catalyze the sulfonation directly but to transform inorganic sulfate to sulfite. The sulfur-containing chemical substance reacted with DON from bile excretion by a well-known Michael addition reaction. In any case, it is a detoxification or deactivation pathway, because the increased polarity of deoxynivalenol-sulfonate is associated with fast excretion of the molecule.[17]

In summary, the metabolism, distribution, and excretion of DON in rats and chicken were investigated in the present study. DON and its metabolites were quickly distributed to the entire body and eliminated from all tissues. Gender and species

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appear to play a role in the excretion rate and metabolic pathway. In addition, to some known metabolites, sulfate-conjugated DON and sulfones were identified as important detoxification routes in chickens and rats. This quick elimination and extensive detoxification of DON in chicken strongly suggested that DON residues from poultry meat would probably be non-toxic to humans, as long as these poultry were exposed to feeds contaminated with a low level of DON 24 h before sacrifice. Metabolites in other species need to be established *in vivo*, as indicated by different metabolism profiles in chickens and rats from this study.

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ABBREVIATIONS USED

DON, deoxynivalenol; ARC, accurate radioisotope counting; bw, body weight; ER, endoplasmic reticulum; JECFA, Joint FAO/WHO Expert Committee on Food Additives; PMTDI, provisional maximum tolerable daily intake; DOM-1, deepoxy-deoxynivalenol; dpm, disintegrations per minute; cpm, counts per minute; CID, collision-induced dissociation; ESI, electrospray ionization

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