solaniol, 36519-25-2; deacetylneosolaniol, 74833-39-9; mixed-function oxidase, 9040-60-2.

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Disposition of T-2 Toxin, a Trichothecene Mycotoxin, in Intravascularly Dosed Swine

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Metabolite profiles in plasma and tissues of two swine were determined following intravascular administration of tritium-labeled T-2 toxin. The plasma elimination phase half-life was 90 min for total tritium residues. At 4-h postdosing (time of death), the greatest amount of radioactivity was located in the gastrointestinal tract (15.5 and 24.1% of the dose for the two swine). The remaining tissues accounted for 5.2 and 4.7% of the dose for the two swine. A total of 21 metabolites was identified by reversed-phase HPLC radiochromatography. Approximately 55% of the extractable radioactivity in the tissues and gastrointestinal tract of both swine corresponded to T-2 toxin, HT-2, deepoxy HT-2, T-2 triol, deepoxy T-2 triol, 3′-OH T-2, 3′-OH HT-2, T-2 tetraol, and deepoxy T-2 tetraol. The major metabolite in tissues, PM-XV, did not correspond to any standard and represented an additional 27% of the extractable radioactivity.

INTRODUCTION

T-2 toxin, 4β,15-diacetoxy-3α-hydroxy-8α-[(3-methylbutyryl)oxy]-12,13-epoxytrichothec-9-ene, is a toxic fungal metabolite produced by several species of *Fusaria* (Bamburg and Strong, 1971; Pathre and Mirocha, 1977). T-2 toxin has been found in naturally contaminated corn, barley, and mixed feeds in the U.S. and Canada at concentrations as high as 25 ppm (Vesonder, 1983). When present in the diets of livestock and poultry, T-2 toxin has been associated with feed refusal, perioral and pharyngeal irritation, intestinal irritation, and possibly hemorrhage, diarrhea, infertility, and lowered immunity (Hsu et al., 1972; Palyusik and Koplik-Kovacs, 1975; Speers et al., 1977; Weaver at al., 1977; Weaver et al., 1978a,b; Rafai and Tuboly, 1972; Hoerr et al., 1982).

Studies on the fate of T-2 toxin in laboratory animals, poultry, and livestock have demonstrated that the parent compound is rapidly cleared from body fluids and tissues. Tritium-labeled T-2 toxin and its metabolites rapidly distributed to tissues of orally dosed mice with maximum levels reached within 30 min, declining thereafter to nondetectable levels by 72 h (Matsumoto et al., 1978). Metabolites were eliminated in a feces to urine ratio of 3:1 over a 72-h time period. Matsumoto et al. (1978) identified T-2 and HT-2 in rat feces at 2.7 and 7.5% of the administered dose, respectively, in addition to two unknown metabolites at 25.8 and 9.1% of the administered dose. No parent T-2 toxin was detected in the urine of rats; however, neosolaniol, HT-2, and three unknowns totaling less than 8% of the administered dose were identified. The chemical structures of T-2 toxin and various metabolites are given in Table I. Rats eliminated metabolites in a feces to urine ratio of 5:1 over a 24-h period. Ueno (1977) reported similar results, finding HT-2 and neosolaniol in rat excreta.

T-2 and its metabolites were eliminated primarily through the bile into the gastrointestinal tract and excreta of orally dosed chickens (Chi et al., 1978). Maximum levels were reached by 4 h in blood, plasma, abdominal fat, liver, heart, kidneys, and carcass and by 12 h in muscle, skin, bile, and gall bladder. Yoshizawa et al. (1980) identified neosolaniol, HT-2, T-2 triol, 4-deacetylneosolaniol, and T-2 tetraol in the excreta of broiler chickens. However, several unknown compounds labeled TB-1 through TB-8 were found to be quantitatively more significant. TB-1 and TB-2 were later identified as 3′-OH T-2; TB-3 (major metabolite) was identified as 3′-OH HT-2, TB-4 as 8-

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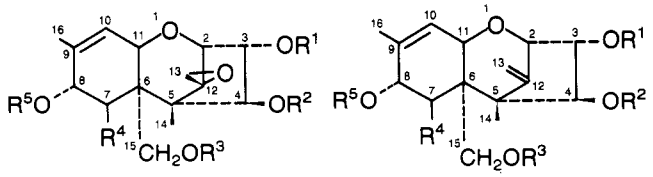
Table I. Chemical Structures of T-2 Toxin and Its Metabolites



skeleton A skeleton B

compd	skeleton	R^1	R^2	R^3	R^4	R^5
T-2 toxin	A	H	Ac	Ac	H	$COCH_2CH(CH_3)_2$
3′-OH T-2	A	H	Ac	Ac	H	$COCH_2COH(CH_3)_2$
HT-2	A	H	H	Ac	H	$COCH_2CH(CH_3)_2$
deepoxy HT-2	B	H	H	Ac	H	$COCH_2CH(CH_3)_2$
3′-OH HT-2	A	H	H	Ac	H	$COCH_2COH(CH_3)_2$
3′-OH-7-OH HT-2	A	H	H	Ac	OH	$COCH_2COH(CH_3)_2$
3-acetoxy-3′-OH HT-2	A	Ac	H	Ac	H	$COCH_2COH(CH_3)_2$
T-2 triol	A	H	H	H	H	$COCH_2CH(CH_3)_2$
deepoxy T-2 triol	B	H	H	H	H	$COCH_2CH(CH_3)_2$
neosolaniol	A	H	Ac	Ac	H	H
4-deacetyl-neosolaniol	A	H	H	Ac	H	H
4-acetoxy T-2 tetraol	A	H	Ac	H	H	H
8-acetoxy T-2 tetraol	A	H	H	H	H	Ac
T-2 tetraol	A	H	H	H	H	H
deepoxy T-2 tetraol	B	H	H	H	H	H

acetoxy T-2 tetraol, and TB-5 as 15-acetoxy T-2 tetraol (also called 4-deacetylneosolaniol) (Visconti and Mirocha, 1985). Visconti and Mirocha (1985) also identified 3′-OH HT-2, HT-2, and T-2 triol in addition to small amounts of T-2, 4-acetoxy T-2 tetraol, 15-acetoxy T-2 tetraol, and T-2 tetraol in the liver of chickens 18 h after administration of toxin.

Approximately 72 and 29% of orally administered tritium-labeled T-2 toxin was eliminated in the feces and urine, respectively, of a lactating cow (Yoshizawa et al., 1981). Only trace amounts of the administered toxin were detected in the milk. Elimination phase half-lifes were 12, 16, and 24 h for urine, plasma, and milk, respectively. In comparison, Beasley (1986) demonstrated a 17.4-min elimination phase half-life for the parent compound, T-2 toxin, from bovine plasma following intravascular administration. Yoshizawa et al. (1981) detected small amounts of HT-2, neosolaniol, and 4-deacetylneosolaniol in the urine of the lactating cow along with significantly greater amounts of unknown compounds designated TC-1 to TC-8. The major metabolites found in feces were TC-3, TC-6, and 4-deacetyolneosolaniol. Neither T-2 nor TC-1 was detected. In plasma, TC-1, TC-3, TC-6, and TC-8 were the major compounds identified. In milk, the major compounds were TC-1, TC-3, and TC-8. Very little unmetabolized T-2 (<0.1 ppb) was detected by 36 h. In contrast, unmetabolized T-2 toxin was found in bovine milk at levels up to 160 ppb (Robison et al., 1979a). Yoshizawa et al. (1982) later identified TC-1 as 3′-OH T-2 and TC-3 as 3′-OH HT-2. The metabolite TC-6 was tentatively identified as 3′-OH, 7-OH HT-2 by Pawlosky and Mirocha (1984).

The distribution of radioactivity in the tissues of swine by 18 h (Robison et al., 1979b) was very similar to that of chickens (Chi et al., 1978) with the exception that the kidneys of swine had a slightly higher level of radioactivity per gram of tissue than liver, just the opposite of chickens. Less than 50% of the administered radioactivity was accounted for with the remainder thought to be in the gastrointestinal tract.

We have already reported the metabolic profile of T-2 toxin in the bile and urine in two female pigs following intravascular administration (Corley et al., 1985). The major free (unconjugated) metabolites were 3′-OH HT-2 and T-2 triol. Glucuronide conjugates accounted for approximately 70% of the total metabolite residues. The major conjugated metabolites were glucuronides of HT-2, 3′-OH T-2, 3′-OH HT-2, and T-2, with T-2-glucuronide accounting for a much greater percentage of metabolite residues in bile (42%) than in urine (11%).

The purpose of this study was to determine the disposition of T-2 toxin in the tissues, including the gastrointestinal tract and contents of the two female pigs. An improved method for the chromatographic separation of tritium-labeled metabolites of T-2 toxin is also discussed.

EXPERIMENTAL SECTION

Reference Standards. Tritium-labeled T-2 toxin (labeled in the C-3 position, radiopurity >99%, sp act. 1.287 mCi/mg) was synthesized by the method of Wallace et al. (1977). Unlabeled standards of T-2 toxin, neosolaniol, HT-2, T-2 triol, 4-deacetylneosolaniol, and T-2 tetraol were produced from cultures of *Fusarium tricinctum* in our laboratory. Additional standards of 3′-OH T-2 and 3′-OH HT-2 were provided by T. Yoshizawa (Yoshizawa et al., 1982).

Tritium-labeled T-2 toxin was used to prepare labeled standards of HT-2, T-2 triol, and T-2 tetraol by alkaline hydrolysis (Wei et al., 1971) and 3′-OH T-2 by S-9 rat liver homogenates (Wei and Chu, 1985). Tritium-labeled 3′-OH HT-2 was prepared from 3′-OH T-2 by alkaline hydrolysis. Deepoxy derivatives of HT-2, T-2 triol, and T-2 tetraol were prepared from tritium-labeled T-2 with bovine rumen microflora (manuscript in preparation).

Animal Treatment. Two 20-kg female crossbred swine (Yorkshire × Hampshire; Thrushwood Farms, Fairbury, IL) were injected with erysipelas bacterin (Rhusigen, Pitman-Moore, Inc., Washington Crossing, NJ) and acclimated to the large animal-holding facility at the College of Veterinary Medicine, University of Illinois. All feeds offered to experimental swine were free from detectable concentrations of trichothecene mycotoxins and aflatoxins. Following preanesthetic administration of atropine sulfate, anesthesia was induced and maintained by halothane and oxygen. In-dwelling catheters for dosing and blood collection were surgically implanted in the aorta via the femoral artery and were tunneled subcutaneously anterior and dorsal to the pelvis. The swine were returned to holding pens and allowed to recover for at least 3 days following surgery.

Immediately prior to dosing, the subcutaneous catheters were exteriorized with Lidocaine hydrochloride local anesthetic, and Foley catheters were inserted into the urinary bladder. Tritium-labeled T-2 toxin was diluted with nonradioactive T-2 toxin in 1.5 mL of 50% ethanol such that each swine received 1 mCi of total radioactivity in a T-2 toxin dose of 0.15 mg/kg body weight intravascularly as a single bolus injection.

During the course of the experiment, the animals were restrained in a plastic-lined sling. Blood was collected in heparinized tubes on ice 10, 20, 30, 45, 60, 90, 120, 150, 180, 210, and 240 min after dosing and centrifuged to obtain plasma. Urine was collected hourly and feces as produced. The animals were killed 4 h after dosing by administration of an anesthetic dose of pentobarbital followed by exsanguination. Bile was immediately collected from the gallbladders. The gastrointestinal tracts were separated into

stomach, duodenum, jejunum, ileum, and large intestine (including cecum). The contents were removed from each section; the mucosa was scraped and rinsed with water (residues added to contents). All tissues were weighed, diced, and flash-frozen in dry ice + 2-propanol.

Determination of Total Radioactivity. The total radioactivity in plasma was determined by adding a 0.2-mL aliquot directly to 5 mL of Aquasol-2 liquid scintillation cocktail (New England Nuclear Corp., Boston, MA). The total radioactivity in tissues, including the gastrointestinal tract and contents, and feces was determined by first homogenizing 5 g of sample in 15 mL of 0.1 M acetate buffer (pH 3.8). A 0.05-mL aliquot of the homogenate was incubated with 0.3 mL of Protosol tissue solubilizer (New England Nuclear Corp., Boston, MA) for 2 h at 60 °C. The samples were then decolorized by adding 0.2 mL of 30% H_2O_2 followed by heating an additional 30 min and counted in 5 mL of Aquasol-2.

Extraction of Plasma. A 1–4-mL volume of plasma was diluted, in duplicate, to 6 mL with 0.1 M acetate buffer (pH 3.8). All samples were heated at 90 °C for 30 min to inactivate enzyme inhibitors. After cooling, 2 mL of either 0.1 M acetate buffer or β-glucuronidase (type L-II from limpets; 4000 units/mL in 0.1 M acetate buffer; Sigma Chemical Co., St. Louis, MO) was added and the samples were incubated with gentle mixing in a 38 °C water bath for 18 h. A positive enzyme control utilizing phenolphthalein glucuronide (0.01 M, pH 7.0; Sigma Chemical Co.) and β-glucuronidase in control plasma was included with each set of samples.

Each sample, after the addition of 10 mL of saturated NaCl, was extracted 4 times with 20 mL of ethyl acetate. Centrifugation was necessary between each partition. The extracts were combined and filtered through funnels containing 2 g of $CuCO_3$ (basic; Fisher Scientific, Itasca, IL) between two layers of anhydrous sodium sulfate. The funnels were rinsed with an additional 20 mL of ethyl acetate, and the extracts were concentrated. The residue was redissolved in 0.1 mL of toluene–acetonitrile (95:5) for TLC radiochromatography according to the methods described previously (Corley et al., 1985).

Extraction of Tissues, Gastrointestinal Tract, and Contents. To each homogenate prepared for determination of total radioactivity was added 5 g of NaCl, and the metabolites were extracted three times with 20 mL of acetonitrile. A fourth extraction using acetonitrile acetone (1:1) was necessary to improve recoveries of T-2 tetraol. Extracts were combined and 80 mL of methylene chloride was added to drive residual water out of solution. Anhydrous Na_2SO_4 (approximately 40 g) was added to remove water (solutions should appear clear). Cupric carbonate (2.5 g) was added to remove pigments, and the samples were filtered through Whatman No. 1 filter paper. The flasks and filters were rinsed with methylene chloride–acetonitrile (1:1) and the samples were concentrated. Each sample was redissolved in 0.25 mL of methanol, diluted with an equal volume of water (solutions became very cloudy), and filtered through a disposable membrane filter (ACRO LC13, 0.2 μm; Gelman Sciences, Ann Arbor, MI) for HPLC radiochromatographic analysis.

It was important that the extracts were clear prior to the addition of $CuCO_3$ since the presence of water in solution decreased the ability of the $CuCO_3$ to decolorize the extracts. Differences were also noted between brands of basic $CuCO_3$ in their ability to provide adequate cleanup of tissue extracts.

HPLC Radiochromatography. An HPLC system (Perkin-Elmer Series 4, Norwalk, CT) was equipped with a 15 cm × 4.6 mm i.d. column packed with 5 μm C18 (Econosphere, Alltech Associates, Deerfield, IL). A 20–90% methanol in water linear gradient over 30 min at a flow rate of 1.0 mL/min was used to separate metabolites. Fractions (0.2 mL) were collected and assayed for radioactivity in Scinti Verse LC liquid scintillation cocktail (Fisher Scientific Co., Itasca, IL).

Sample Preparation for Capillary GLC and GC/MS. A 70-mL volume of urine from the two swine was diluted with an equal volume of 0.1 M acetate buffer (pH 3.8) and heated at 90 °C for 30 min. After cooling, β-glucuronidase was added and the samples were incubated at 38 °C for 18 h. Equal volumes of the incubation mixture were added to three Chem Tubes (CT 2050, Analytichem International, Harbor City, CA) for the extraction of metabolites. The metabolites were eluted from the Chem Tubes with ethyl acetate, combined, and concentrated. The extract was redissolved in 20 mL of acetonitrile and diluted with an equal volume of methylene chloride. Cupric carbonate (1 g) was added, and the samples were filtered and concentrated. The residue was redissolved in chloroform, and equal amounts were added to two Florsil columns (2.5 g, 60–100 m; Fisher Scientific, Ithaca, IL) slurry-packed in chloroform. Metabolites were eluted from each column with 150 mL of chloroform–acetone (7:3). The eluates were combined, concentrated, and redissolved in 2 mL of methanol. Water (8 mL) was added, and the samples were filtered through a 0.2 μm filter (Acro LC25; Gelman Sciences, Ann Arbor, MI). HPLC purification was accomplished on a preparative C_{18} column (25 cm × 10 mm i.d., 10 μm C_{18}; Alltech Associates, Deerfield, IL) and a 20–90% methanol in water linear gradient over 60 min at a flow rate of 2 mL/min. Fractions containing the major metabolites were suitable for analysis by capillary gas chromatography with electron capture detection following derivatization. Additional cleanup using preparative thin-layer chromatography (Whatman PLK-5; developed in 9:1 chloroform–methanol) was necessary for GC/MS analysis.

Gas Chromatography. Sample extracts were dissolved in 1.0 mL of toluene–acetonitrile containing 2 mg/mL of 4-(dimethylamino)pyridine (Sigma Chemical Co., St. Louis, MO). Derivatization was accomplished by adding 0.05 mL of trifluoroacetic acid anhydride (Pierce Chemical Co., Rockford, IL) followed by heating at 60 °C for 20 min. After cooling, 1.0 mL of 5% aqueous sodium bicarbonate was added. The samples were vortexed and centrifuged, and an aliquot of the organic layer was diluted with isooctane for GC analysis.

Capillary gas chromatography was carried out on a Hewlett-Packard 5790 equipped with a ^{63}Ni electron capture detector and a 25 m × 0.25 mm i.d. fused silica capillary column (DB-1701, 0.25-μm film coating; J & W Scientific, Rancho Cordova, CA). A column temperature program from 90 °C (hold 1.0 min) to 270 °C (hold 10 min) at 10 °C/min was used. Other operating conditions: injector, 275 °C; detector, 340 °C; hydrogen carrier gas at 45 cm/s. Retention times were 13.41, 15.57, 16.61, 16.76, 16.94, 17.99, and 20.20 min for TFA esters of T-2 tetraol, deepoxy T-2 triol, neosolaniol, T-2 triol, deepoxy HT-2, HT-2, and T-2, respectively. The 3′-hydroxy metabolites of T-2 and HT-2 yielded split peaks following derivatization with TFAA (Pawlosky et al., 1984) with retention times of 18.09 and 18.74 min for 3′-OH HT-2 and 20.32 and 21.21 min for 3′-OH T-2.

GC/MS. Aliquots of metabolites purified by preparative TLC were concentrated and derivatized in 0.05 mL of TFAA at 60 °C for 10 min. The TFAA was evaporated

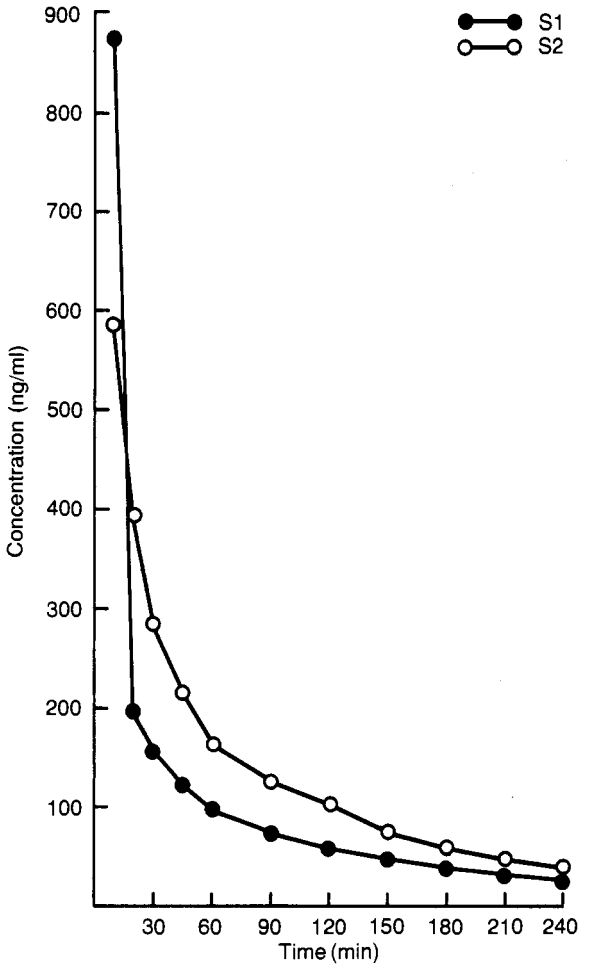


Figure 1. Concentration (ng/mL) of T-2 and metabolites (total radioactivity) in plasma over time from two swine (S1 and S2) administered 1.0 mCi of tritium-labeled T-2 toxin intravascularly at 0.15 mg/kg body weight.

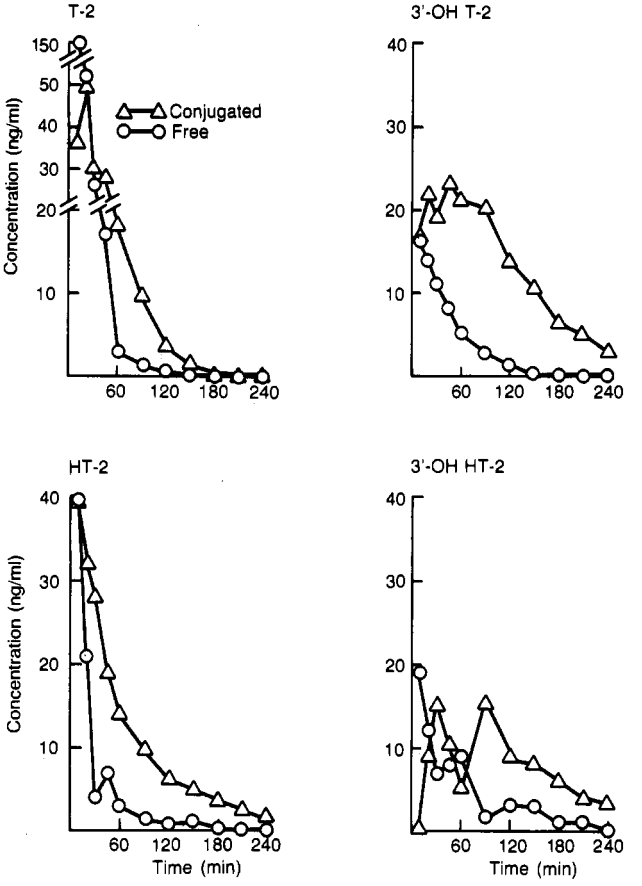


Figure 2. Concentration (ng/mL) of T-2 toxin, 3′-OH T-2, HT-2, and 3′-OH HT-2 in the free and conjugated forms in plasma over time from a pig (S2 only) administered 1.0 mCi of tritium-labeled T-2 toxin intravascularly at a dose of 0.15 mg/kg body weight.

under nitrogen at room temperature and the residue dissolved in 0.05 mL of benzene. GC/MS analysis was carried out on a Hewlett-Packard 5985 by both positive chemical ionization in methane plasma and electron impact at 70 eV. Gas chromatography was performed on a 1.8 m × 2 mm i.d. borosilicate glass column packed with 3% OV-17 (100/120 m Supelcoport; Supelco, Inc., Bellefonte, PA) and a column temperature program of 190–280 °C at 8 °C/min.

RESULTS

Plasma. The total concentrations of T-2 and metabolites in plasma over time are given in Figure 1. The plasma elimination phase half-life was approximately 90 min for T-2 metabolites. The major free (unconjugated) metabolites identified by one- and two-dimensional TLC were 3′-OH T-2, HT-2, and 3′-OH HT-2 in addition to the parent compound. These four compounds accounted for 60% of the total radioactivity in plasma samples taken 10 min after toxin administration but decreased to less than 10% by 4 h, when the swine were euthanized. Other free metabolites detected at much lower concentrations (<6 ppb) included neosolaniol, T-2 triol, 4-deacetylneosolaniol, and T-2 tetraol. When plasma samples were incubated with β-glucuronidase, an average of 89.2% of the total extractable radioactivity (extraction efficiency (mean ± SE); 72.8 ± 3.1%, n = 11) in plasma at all time periods sampled was associated with T-2, 3′-OH T-2, HT-2, and 3′-OH HT-2. The concentrations and percentages of these four compounds in the free and conjugated form are given in Figures 2 and 3. Although 3′-OH HT-2 was the predominant free metabolite in plasma, 3′-OH T-2 and 3′-OH HT-2 were nearly equal in concentration following enzyme hydrolysis.

Tissues and Gastrointestinal Tract and Contents. The distribution of T-2 and its metabolites (total radioactivity) in the tissues and gastrointestinal tract and contents is summarized in Table II. The greatest amount of radioactivity was located in the contents of the gastrointestinal tract (10.1 and 16.1% of the dose for S1 and S2, respectively) followed by the gastrointestinal tract itself (5.3 and 8.2%) and remaining tissues sampled (5.2 and 4.9%).

Within the gastrointestinal tract and contents of both swine, the ileum and its contents contained the greatest total amount of radioactivity and the duodenum and its contents the least. In the remaining tissues sampled, the muscle contained the greatest total amount of radioactivity followed by the liver, kidneys, pancreas, lungs, heart, mesenteric lymph nodes, spleen, bone marrow, and brain.

Although the total metabolite residues in many tissues of both swine 4 h after dosing were present at very low concentrations, several metabolites were identified following HPLC separation. The concentrations of 21 metabolites, designated PM-I to PM-XXII (PM-XXI represents T-2) are given for each tissue and section of the gastrointestinal tract and contents in Tables III and IV. Representative radiochromatograms are given in Figure 4.

The total metabolite residues identified in all tissues represented 5.4 and 8.0% of the dose in swine S1 and S2, respectively. These free, extractable metabolites account for only 26.0 and 27.9% of the total radioactivity present in all tissues. The recovery (percent) of radioactivity after

Table II. Total Metabolite Residues[a] in Tissues, Including the Gastrointestinal Tract and Contents from Two Swine (S1 and S2) 4 h following the Intravascular Administration of 1.0 mCi of Tritium-Labeled T-2 Toxin at a Dose of 0.15 mg/kg Body Weight

	concn,[b] ng/g		% of admin dose[b]	
	S1	S2	S1	S2
liver	107 ± 4	39 ± 3	1.7 ± 0.1	0.7 ± 0.1
kidney	74 ± 2	68 ± 4	0.2 ± 0.0	0.2 ± 0.0
spleen	29 ± 1	42 ± 1	<0.1 ± 0.0	0.1 ± 0.0
MLN[c]	33 ± 1	46 ± 2	0.1 ● 0.0[d]	0.1 ± 0.0[d]
muscle[e]	18 ± 1	19 ± 1	2.9 ± 0.1	3.2 ± 0.2
lung	21 ± 1	24 ± 2	0.1 ± 0.0	0.2 ± 0.0
heart	23 ± 1	26 ± 1	0.1 ± 0.0	0.1 ± 0.0
brain	8 ± 1	13 ± 1	<0.1 ± 0.0	<0.1 ± 0.0
pancreas	NA[f]	159 ± 5	NA[f]	0.2 ± 0.0
bone marrow	18 ± 1	25 ± 2	<0.1 ± 0.0[d]	<0.1 ± 0.0[d]
stomach	91 ± 3	86 ± 4	0.4 ± 0.0	0.5 ± 0.0
contents	399 ± 8	79 ± 2	1.7 ± 0.0	1.1 ± 0.0
duodenum	32 ± 1	101 ± 3	<0.1 ± 0.0	0.2 ± 0.0
contents	55 ± 1[g]	144 ± 2[h]	<0.1 ± 0.0[g]	0.1 ± 0.0[h]
jejunum	180 ± 3	149 ± 5	1.3 ± 0.0	1.4 ± 0.1
contents	303 ± 6[g]	316 ± 7	1.1 ± 0.0[g]	1.5 ± 0.0
ileum	554 ± 34	498 ± 16	3.3 ± 0.2	5.0 ± 0.2
contents	1644 ± 112[g]	1406 ± 49	6.7 ± 0.5[g]	10.8 ± 0.4
lg intestine	48 ± 4	142 ± 6	0.3 ± 0.0	1.1 ± 0.1
contents	68 ± 2	181 ± 4	0.6 ± 0.0	2.6 ± 0.1

[a] All values based upon the specific activity of administered tritium-labeled T-2 toxin. [b] $\bar{X}$ ± SE, n = 9. [c] MLN = mesenteric lymph nodes. [d] Percent of administered dose based on the amount of tissue collected. [e] Total muscle mass estimated at 25% of body weight. [f] NA = not analyzed. [g] n = 6. [h] n = 3.

extraction and cleanup was consistent within a given tissue (coefficients of variation approximately 10%) yet varied greatly between tissues, ranging from a low of 17% for brain (animal S2) to a high of 77% for large intestinal contents (animal S1). These differences are likely due to differences in the percent of metabolites in tissues present as glucuronides, which are not extracted by the procedure used in this study. Recovery data are summarized in Table V. The percent recovery for the HPLC separation step was 98.6 ± 0.7% ($\bar{X}$ ± SE, n = 115).

A total of 56.1 and 52.8% of the extracted radioactivity in tissues and gastrointestinal tract and contents from animals S1 and S2, respectively, had HPLC retention times (see Figure 4) identical with those of authentic tritium-labeled standards of T-2 (PM-XXI), deepoxy HT-2 (PM-XX), HT-2 (PM-XIX), deepoxy T-2 triol (PM-XVIII), T-2 triol (PM-XVII), 3′-OH T-2 (PM-XVI), 3′-OH HT-2 (PM-XIII), deepoxy T-2 tetraol (PM-III), and T-2 tetraol (PM-I). One major metabolite, PM-XV, which represented

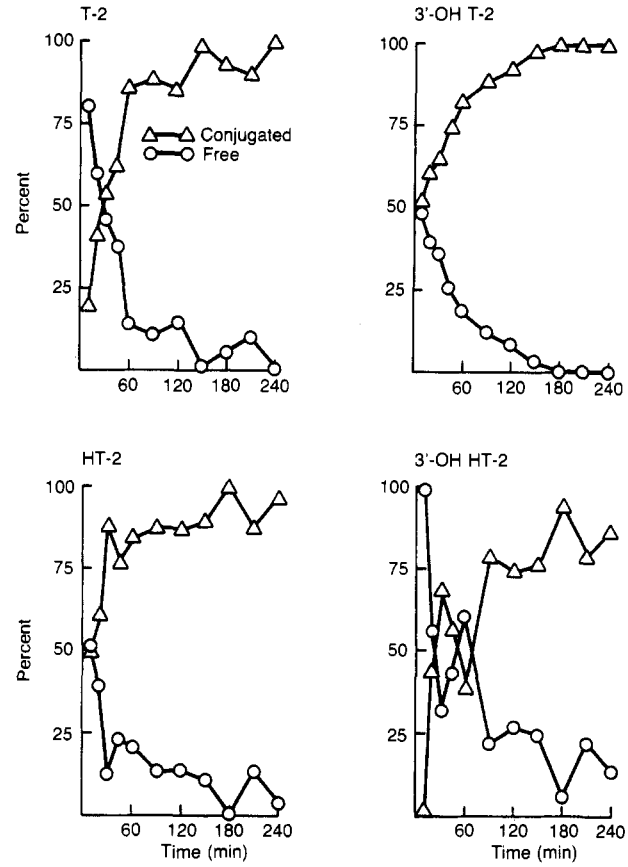


Figure 3. Percentages of T-2 toxin, 3′-OH T-2, HT-2, and 3′-OH HT-2 in the free and conjugated forms in plasma over time from a pig (S2 only) administered 1.0 mCi of tritium-labeled T-2 toxin intravascularly at a dose of 0.15 mg/kg body weight.

Table III. Concentration (ng/g) of the Metabolites of T-2 Toxin in Swine Tissues following HPLC Radiochromatography

metabolite	liver	kidney	spleen	MLN	muscle	lung	heart	brain	pancreas	bone marrow
TOL	1.73, 0.71[a]	0.26, TR[b]	0.05, 0.04	ND,[c] 0.09	ND, ND	0.06, ND	0.04, 0.04	ND, ND	–, 0.82	0.04, ND
PM-II	1.61, 0.33	0.88, 0.69	0.29, 0.34	TR, 0.37	TR, ND	0.29, 0.30	0.17, 0.33	0.04, 0.04	–, 1.83	0.30, 0.36
DE TOL	1.72, 1.66	4.30, 4.03	0.92, 1.52	1.51, 1.05	0.98, 1.13	1.03, 1.28	0.88, 1.38	0.28, 0.32	–, 3.07	0.66, 0.74
PM-IV	TR, TR	TR, TR	0.16, TR	TR, 0.14	TR, ND	TR, TR	0.14, TR	ND, 0.09	–, ND	0.21, 0.13
PM-V	0.23, ND	0.20, ND	ND, ND	ND, ND	ND, ND	ND, ND	ND, ND	ND, ND	–, ND	ND, ND
PM-VI	0.11, ND	ND, ND	ND, ND	ND, ND	ND, ND	ND, ND	ND, ND	ND, ND	–, ND	ND, ND
PM-VII	ND, ND	0.19, ND	ND, ND	ND, ND	ND, ND	ND, ND	ND, ND	ND, ND	–, 0.56	ND, ND
PM-VIII	0.54, 0.30	0.17, 0.17	ND, ND	ND, ND	ND, ND	ND, ND	ND, ND	ND, ND	–, 0.90	ND, ND
PM-IX	0.38, 0.15	ND, 0.21	ND, ND	ND, ND	ND, ND	ND, ND	ND, ND	ND, ND	–, 0.74	ND, ND
PM-X	1.31, 0.37	0.34, 0.24	ND, ND	ND, ND	ND, ND	ND, ND	ND, ND	ND, ND	–, 3.16	ND, ND
PM-XI	1.03, 0.30	0.31, 0.25	ND, ND	ND, ND	ND, ND	ND, ND	ND, ND	ND, ND	–, ND	ND, ND
PM-XII	TR, 0.37	0.89, 0.49	ND, ND	ND, 0.09	ND, ND	ND, 0.08	ND, 0.07	ND, ND	–, 3.73	0.08, 0.07
3′-OH HT-2	4.63, 2.42	5.41, 4.96	3.44, 2.74	4.02, 3.03	1.90, 1.10	2.15, 1.24	1.23, 0.80	0.22, 0.07	–, 17.79	2.51, 1.67
PM-XIV	ND, ND	ND, ND	ND, ND	ND, ND	ND, 0.14	ND, 0.12	ND, 0.24	ND, ND	–, ND	ND, 0.15
PM-XV	6.37, 4.21	10.12, 5.66	2.41, 2.14	2.53, 2.12	2.88, 1.98	2.32, 1.68	2.67, 2.32	0.68. 0.44	–, 5.67	1.84, 1.12
3′-OH T-2	0.67, 0.78	0.81, 0.47	0.54, 1.78	0.63, 1.69	0.48, 1.07	0.27, 0.87	0.19, 0.58	0.07, 0.10	–, 3.13	0.40, 1.32
TRIOL	0.35, 0.16	0.14, 0.16	0.11, 0.26	0.22, 0.27	0.14, 0.18	0.11, 0.17	0.04, 0.14	0.03, 0.05	–, 0.65	0.07, 0.15
DE TRIOL	0.23, 0.07	0.06, ND	0.03, ND	ND, ND	ND, ND	ND, ND	ND, ND	ND, ND	–, ND	ND, ND
HT-2	2.66, 1.47	1.08, 1.45	2.16, 2.25	5.21, 3.76	2.06, 1.69	0.85, 1.69	0.48, 0.68	0.10, 0.13	–, 11.68	1.16, 1.35
DE HT-2	0.26, 0.14	0.08, ND	ND, ND	ND, ND	ND, ND	0.13, ND	ND, ND	ND, ND	–, ND	ND, ND
T-2	0.71, 0.27	0.05, 0.08	2.73, 1.04	1.81, 1.37	0.64, 0.85	0.13, 0.85	0.06, 0.32	0.02, 0.05	–, 0.52	0.38, 0.58
PM-XXII	0.20, 0.06	ND, ND	ND, ND	ND, ND	ND, ND	0.04, ND	ND, ND	ND, ND	–, 0.16	ND, ND

[a] Values expressed as the mean of three replicates for the two swine (S1, S2) based upon the specific radioactivity of administered tritium-labeled T-2 toxin. [b] TR = trace (less than 0.01 ng/g of [^{3}H]-T-2 equivalents). [c] ND = none detected. Detection limit 0.005 ng/g of [^{3}H]-T-2 equivalents.

Table IV. Concentration (ng/g) of the Metabolites of T-2 Toxin in the Gastrointestinal Tract (Lining and Contents) of Two Swine following HPLC Radiochromatography

metabolite	stomach	stomach contents	duodenum	duodenum contents	jejunum	jejunum contents	ileum	ileum contents	lg intestine	lg intestine contents
TOL	2.09, TR[a,b]	11.86, 0.11	0.19, 0.69	0.13, 0.09	3.78, 2.67	7.52, 3.37	13.36, 4.29	41.75, 13.66	ND,[c] ND	ND, ND
PM-II	0.57, 1.04	TR, 1.03	0.40, 0.93	0.61, 1.54	1.62, 0.99	1.88, 2.34	5.30, 5.61	10.44, 18.64	0.25, 0.54	0.22, 0.58
DE TOL	1.23, 1.36	1.87, 0.64	0.62, 1.59	0.95, 3.27	1.70, 1.82	3.40, 4.57	5.98, 1.76	14.99, 13.57	1.02, 3.06	1.10, 2.60
PM-IV	TR, 0.10	TR, 0.12	0.10, TR	0.14, 0.36	ND, ND	ND, 0.69	TR, 2.89	TR, ND	TR, TR	TR, 1.93
PM-V	ND, 0.15	1.59, 0.15	ND, ND	TR, ND	ND, ND	TR, ND	TR, ND	TR, ND	ND, 0.74	0.73, 1.48
PM-VI	ND, ND	TR, ND	ND, ND	ND, ND	1.02, ND	1.27, 0.87	4.71, ND	9.36, 9.30	ND, ND	ND, ND
PM-VII	ND, ND	1.43, ND	ND, 0.33	ND, 0.45	ND, ND	ND, ND	7.46, ND	21.13, TR	ND, ND	ND, ND
PM-VIII	0.17, 0.24	0.87, 0.29	ND, 0.16	ND, ND	1.66, 1.19	2.58, 2.37	6.27, 2.38	5.25, 14.39	ND, 0.38	ND, 0.50
PM-IX	0.25, 0.30	3.68, 0.19	ND, 0.65	ND, 1.19	1.71, 0.51	3.41, 1.02	1.57, 1.59	7.87, TR	ND, 0.46	ND, 0.62
PM-X	0.94, 0.89	4.87, 0.90	ND, 1.98	ND, 2.44	TR, 2.98	1.58, 4.77	TR, 6.97	TR, 35.14	0.13, 0.91	0.24, 1.50
PM-XI	0.40, 0.67	1.39, 0.23	ND, TR	ND, ND	1.38, TR	2.37, 2.05	4.05, 4.64	9.15, TR	0.21, 1.11	0.36, 1.23
PM-XII	0.44, 0.45	0.70, 0.51	ND, 0.81	ND, 1.53	TR, 3.28	0.60, 3.76	1.13, 9.77	2.78, 26.12	1.54, 2.23	1.54, 5.48
3′-OH HT-2	3.45, 2.53	8.68, 1.47	2.87, 5.83	4.08, 7.76	7.36, 7.38	12.23, 15.85	29.27, 24.83	66.70, 67.56	3.56, 13.99	7.02, 13.49
PM-XIV	0.68, 2.35	ND, 1.30	ND, ND	ND, ND	ND, 1.50	ND, ND	ND, ND	ND, ND	0.42, ND	ND, ND
PM-XV	5.76, 5.17	13.56, 6.16	2.04, 6.04	3.01, 8.61	5.45, 7.52	11.52, 14.97	24.16, 16.33	45.92, 40.40	10.25, 23.23	38.04, 45.44
3′-OH T-2	1.11, 2.09	2.77, 1.62	0.51, 1.42	0.55, 2.10	0.99, 1.71	0.64, 2.42	1.41, 3.02	6.64, 5.43	1.45, 3.65	ND, 4.71
TRIOL	0.20, 0.28	1.17, 0.29	ND, 0.40	0.13, 0.33	0.39, 0.36	0.40, 0.54	0.67, 0.86	6.24, 3.63	0.33, 1.38	0.55, 2.17
DE TRIOL	0.10, 0.09	0.50, ND	ND, ND	ND, 0.18	ND, ND	0.44, ND	0.75, 0.55	ND, 1.97	ND, ND	ND, 0.76
HT-2	3.21, 2.86	2.80, 0.76	1.80, 2.84	1.76, 3.75	3.54, 3.58	4.22, 4.25	7.09, 9.75	6.77, 17.69	1.50, 7.20	1.32, 8.91
DE HT-2	0.62, 0.28	1.15, 0.51	ND, ND	ND, ND	ND, 0.40	ND, ND	ND, 1.72	2.06, 3.12	0.17, ND	0.31, ND
T-2	3.34, 0.66	3.02, 0.46	0.30, 0.61	0.22, 0.68	1.32, 1.04	1.17, 1.40	7.32, 5.02	12.61, 10.64	0.42, 5.82	0.31, 9.87
PM-XXII	0.21, 0.13	1.34, 0.22	ND, ND	ND, ND	0.35, ND	0.39, ND	ND, ND	2.98, 3.19	0.09, ND	0.17, ND

[a] Values expressed as the mean of three replicates for the two swine (S1, S2) based upon the specific radioactivity of administered tritium-labeled T-2 toxin. [b] TR = trace (less than 0.01 ng/g of [^{3}H]-T-2 equivalents). [c] ND = none detected. Detection limit 0.005 ng/g of [^{3}H]-T-2 equivalents.

Table V. Recovery (Percent) of Total Metabolite Residues from Tissues, Including the Gastrointestinal Tract and Contents, of Two Swine (S1 and S2) Prepared for HPLC Radiochromatography

tissue	% rec radioactivity[a] S1	S2
liver	24.6 ± 2.8	38.4 ± 4.6
kidney	33.1 ± 2.5	29.5 ± 4.2
spleen	47.6 ± 2.9	31.2 ± 1.5
MLN[b]	52.5 ± 1.6	33.1 ± 4.7
muscle	56.7 ± 0.5	48.4 ± 4.4
lung	37.4 ± 1.1	35.4 ± 1.9
heart	31.1 ± 5.7	29.3 ± 1.2
brain	21.7 ± 4.3	16.9 ± 9.2
pancreas		35.9 ± 0.7
bone marrow	40.4 ± 2.8	33.5 ± 7.6
stomach	30.2 ± 3.0	27.1 ± 4.5
contents	17.0 ± 0.2	23.0 ± 1.2
duodenum	31.1 ± 1.8	26.3 ± 0.7
contents	23.5[c]	26.2[d]
jejunum	19.9 ± 0.3	28.0 ± 2.4
contents	19.1 ± 1.5	22.3 ± 2.3
ileum	23.2 ± 4.3	23.5 ± 1.2
contents	18.9 ± 3.6	23.3 ± 4.3
lg intestine	47.0 ± 5.6	46.4 ± 5.9
contents	77.1 ± 1.7	48.5 ± 1.4

[a] $\bar{X}$ ± SE, n = 3. [b] MLN = mesenteric lymph nodes. [c] n = 2. [d] n = 1.

sented 29.1 and 25.0% of the extracted residues in the tissues and gastrointestinal tract and contents of animals S1 and S2, respectively, did not correspond to any standard. Radiolabeled standards of neosolaniol, 4-deacetylneosolaniol, 4-acetoxy and 8-acetoxy T-2 tetraol, 3′-OH-7-OH HT-2, and 3-acetoxy-3′-OH HT-2 were not available for comparison to unknown metabolites.

DISCUSSION

The time course, dose, and route of administration used in this study were selected to maximize the bioavailability of T-2 toxin, maintain adequate urine output and facilitate tissue metabolite identification. Previous work demonstrated that swine are particularly sensitive to the emetic action of T-2 toxin at an intravascular dose of 0.3 mg/kg (Beasley, 1986) and at oral doses greater than 0.5 mg/kg

Table VI. Distribution of Radioactivity in Two Swine (S1 and S2) 4 h after Intravascular Administration of 1.0 mCi of Tritium-Labeled T-2 Toxin at a Dose of 0.15 mg/kg Body Weight

sample	% admin dose S1	S2	sample	% admin dose S1	S2
blood[a]	0.9	1.4	remaining tissues	5.2	4.7
urine[b]	17.9	42.5	feces	ND	ND
bile[b]	13.1	1.3	total	52.6	74.0
gastrointestinal tract and contents	15.5	24.1			

[a] Total blood volume estimated of 6% of body weight. Concentration of radioactivity in whole blood was assumed to be equivalent to plasma. [b] Corley et al. (1986).

(Robison et al., 1979b). In addition, a significant decrease in urine output (oliguria), a major route for the elimination of T-2 and its metabolites in swine (Corley et al., 1985; Robison et al., 1979b), occurred following intravascular administration of a lethal dose (Beasley, 1986). An intravascular dose of 0.15 mg/kg was well below the threshold for emesis and oliguria. The swine were killed 4 h after toxin administration since the parent compound, T-2, could not be detected after 2 h in plasma or after 4 h in tissues of swine administered 1.2 mg/kg intravascularly (Beasley et al., 1986).

A total of 52.6 and 74.0% of the administered radioactivity in the two swine has been accounted for (Table VI). The assumptions that muscle and blood represented 25 and 6% of the total body weight, respectively, were used to calculate the total amount of radioactivity in these samples. The total mass of bone marrow and mesenteric lymph nodes could not be estimated. Therefore, the total radioactivity determined in these tissues was based on the amount collected at necropsy. The remaining radioactivity was assumed to be in the carcass.

Congestion and hemorrhage were reported to progress aborally in the small intestines, with the duodenum appearing near normal in swine administered lethal doses of T-2 toxin intravascularly (Beasley, 1986; Weaver et al., 1978). The distribution of radioactivity also progressed

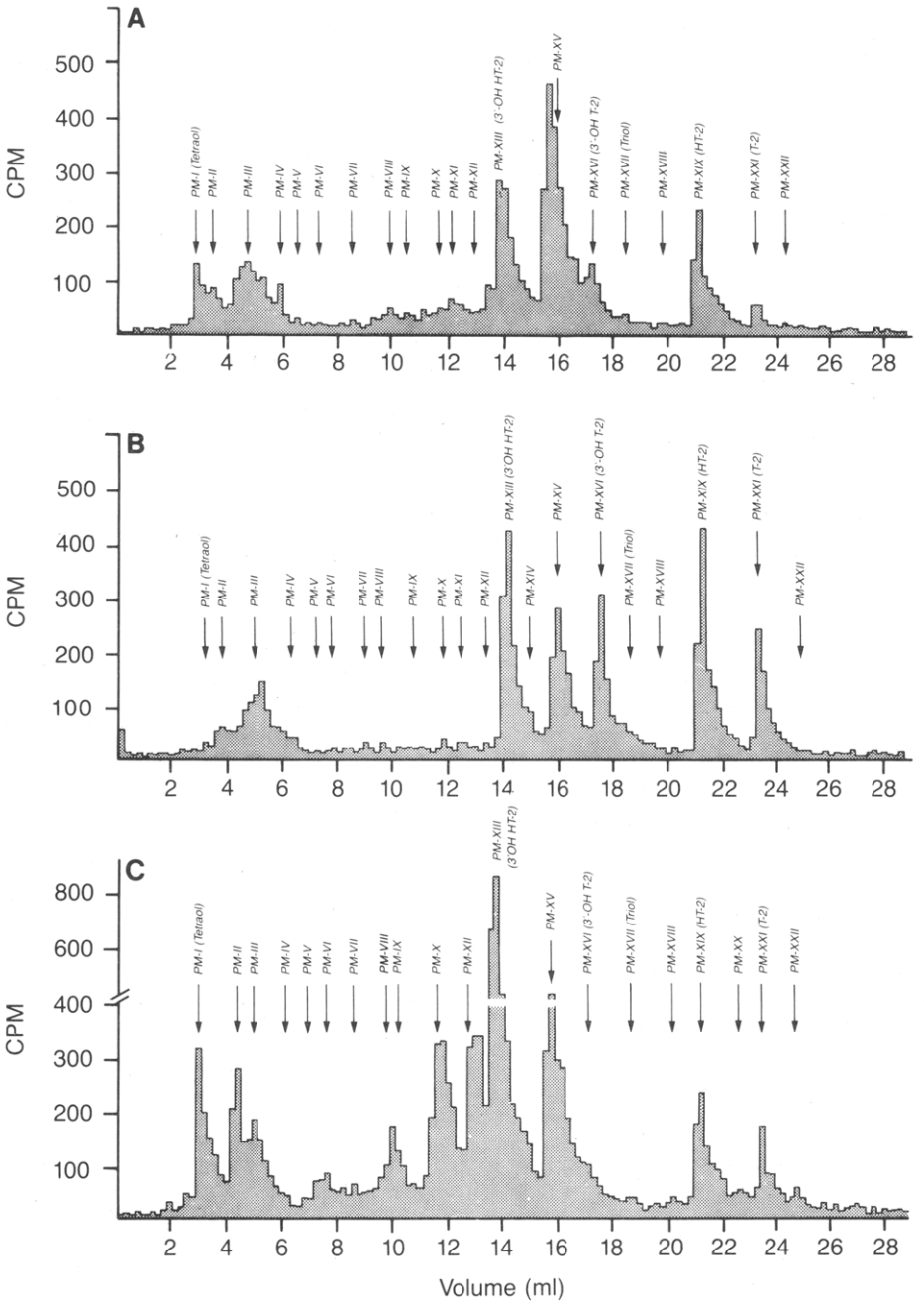


Figure 4. Reversed-phase HPLC radiochromatograms of extracts from liver (A), spleen (B), and ileum contents (C) from a pig (S2 only) administered tritium-labeled T-2 toxin intravascularly at a dose of 0.15 mg/kg body weight.

aborally in the small intestine, with the greatest amounts in the ileum, followed by the jejunum and duodenum. Congestion and hemorrhage were also previously reported in the stomach (Beasley, 1986) and the large intestine (Beasley, 1986; Weaver et al., 1978b). In this study, large amounts, of radioactivity were also detected in these tissues.

The source of the radioactivity in the gastrointestinal tract was assumed to be primarily from the bile, with some contribution from blood flow to these tissues. Since an average of 77% of the metabolites in the bile of these swine was present as glucuronides (Corley et al., 1985), no more than 23% of the total metabolite residues in the gastrointestinal tract should have been extracted and purified for HPLC radiochromatography. The recovery percentages for the extraction of metabolites were generally in agreement with this figure for the stomach and small intestines but increased in the large intestines to as much as 77%, indicating that gut microflora may be involved in the hydrolysis of glucuronides that may result in enterohepatic recirculation and potentiation of the toxic effects of T-2 and its metabolites.

Following HPLC separation, 21 metabolites were identified in tissues and the gastrointestinal tract at concentrations ranging from less than 0.01 to 67.56 ng/g. One of the major free matabolites, T-2 triol, previously identified by thin-layer radiochromatography in bile and urine (Corley et al., 1985), was found to be a minor metabolite by HPLC. A new major metabolite, PM-XV, which did not separate from T-2 triol by silica TLC, was identified by HPLC radiochromatography. The other major metabolites in bile and urine, 3′-OH T-2, HT-2, and 3′-OH HT-2, were also major metabolites in plasma, tissues, and gastrointestinal tract. These compounds were also major metabolites identified in the excreta of chickens (Visconti and Mirocha, 1985) and a lactating cow (Yoshizawa et al., 1981). The presence of T-2 toxin as a major compound in the spleen, mesenteric lymph nodes, stomach (and

contents), and ileum (and contents) is of particular interest since these tissues in swine became necrotic, congested, and/or hemorrhagic following intravascular administration of a lethal dose of T-2 toxin (Beasley, 1986).

The conjugation of T-2 and its metabolites occurred very rapidly. Even in blood taken 10 min after toxin administration, 50% of 3′-OH T-2 and HT-2 were conjugated (Figure 3).

Several metabolites have been tentatively identified by comparing HPLC retention times of the unknowns with tritium-labeled standards. The metabolites 3′-OH HT-2 and HT-2 were present in urine in sufficient quantities to be confirmed by GC/MS as the trifluoroacetic acid derivatives. Capillary gas chromatography with electron capture detection was necessary to detect and confirm 3′-OH T-2. Metabolites such as T-2 tetraol and T-2 triol were not available in sufficient quantities in these 2 pigs to allow confirmation by gas chromatography but have been confirmed in other species (Yoshizawa et al., 1980; Visconti and Mirocha, 1985). The deepoxy derivatives of T-2 tetraol, T-2 triol, and HT-2 were also not present in sufficient quantities for confirmation, and their identification is, therefore, tentative. Deepoxy metabolites of several other trichothecenes have been reported. Deepoxy DON (DOM-1) was detected in the urine and feces of rats (Yoshizawa et al., 1983) and recently in the urine, milk, and feces of dairy cattle (Côté et al., submitted for publication). Deepoxy tetraol and deepoxy 3′-OH HT-2 were identified in rat excreta (Yoshizawa et al., 1985). In our laboratory, deepoxy derivatives of monoacetoxyscirpenol and scirpenetriol were identified in rat excreta (Sakamoto et al., submitted for publication). Work is currently in progress to structurally identify the major unknown metabolite and to confirm the presence of deepoxy metabolites of T-2 toxin in swine.

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Registry No. T-2 toxin, 21259-20-1; HT-2, 26934-87-2; DEHT-2, 99285-99-1; T-2 triol, 2270-41-9; DE F2 triol, 103668-89-9; 3′-OH T-2, 84474-35-1; 3′-OH HT-2, 78368-54-4; T-2 tetraol, 34114-99-3; DE T-2 tetraol, 98896-89-0.

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