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Metabolism of T-2 Toxin in Rats: Effects of Dose, Route, and Time

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Metabolic profiles of the excreta from rats following iv, oral, and dermal administration of tritium-labeled T-2 toxin at 0.15 and 0.60 mg/kg were determined. The major metabolites in urine were 3′-OH HT-2, T-2 tetraol, and unknown metabolite M5, whereas the major metabolites in feces were deepoxy T-2 tetraol, 3′-OH HT-2, and unknown metabolites M5, M7, and M9. The metabolite labeled M9 (major metabolite) was tentatively identified as deepoxy 3′-OH HT-2. There was no significant effect on metabolic profiles due to dose, but there was a variable effect associated with the route of administration. The increase over time of appreciable levels of deepoxy metabolites as a percentage of extracted radioactivity was both consistent and statistically significant.

The trichothecene mycotoxins are a chemical group of fungal metabolites characterized by a 12,13-epoxytrichothec-9-ene skeleton. T-2 toxin, one of over 40 naturally occurring trichothecenes, is a toxic metabolite produced primarily by species of *Fusarium* (Bamburg and Strong, 1971). Serious mycotoxicoses, including moldy corn toxicosis in the United States and fusariotoxicosis in Canada, have been attributed to T-2 toxin (Hsu et al., 1972; Puls and Greenway, 1976). This toxin was also possibly involved in the bean hull toxicosis of farm animals in Japan (Ueno et al., 1972). In addition, alimentary toxic aleukia, which has been a human health problem in Russia, was found to be associated primarily with the ingestion of moldy cereals infected with T-2 toxin producing strains of *Fusarium* (Joffe, 1971). The signs of trichothecene intoxication included emesis, decreased weight gain, lethargy, diarrhea, feed refusal, necrosis, lowered immunity, hemorrhage, and death (Hsu et al., 1972; Beasley et al., 1986; Kosuri et al., 1970; Osweiller et al., 1981; Boonchuvit et al., 1975; Wyatt et al., 1973; Glavits et al., 1983). Trichothecene mycotoxins, including T-2 toxin, and their effects on humans have received considerable international attention because of their alleged use in chemical warfare as the agent Yellow Rain in Southeast Asia (Watson et al., 1984).

The toxin, when administered to rodents, chickens, cattle, and swine, is rapidly metabolized into various products. T-2 toxin is rapidly metabolized and eliminated in feces to urine ratios of 3:1 in mice, 5:1 in rats, and 1:4 in guinea pigs (Matsumoto et al., 1978; Pace et al., 1985).

The major metabolites isolated from urine and feces of a lactating cow after daily oral administration of T-2 toxin were 3′-OH HT-2, 3′-OH T-2 toxin, and 3′-OH 7-OH HT-2 (Pawlosky and Mirocha, 1984; Yoshizawa et al., 1981, 1982). In a previous study using rats given an oral dose of T-2 toxin, the major metabolites isolated from feces were HT-2 and T-2 toxin at 2.7 and 7.5% of the administrated dose, respectively, whereas two unknown metabolites representing 25.8 and 9.1% of the dose were also present (Matsumoto et al., 1978). In urine from rats administered T-2 toxin, neosolaniol, HT-2, and three unknown metabolites accounting for less than 8% of the administered dose were identified (Matsumoto et al., 1978). The metabolites of T-2 toxin in the excreta of chickens, following oral administration, were T-2 toxin, HT-2, neosolaniol, T-2 tetraol, and several unknown metabolites. These were later identified as 3′-OH T-2 toxin, 3′-OH HT-2, 8-acetoxy and 15-acetoxy T-2 tetraol (Visconti and Mirocha, 1985). In guinea pigs administered T-2 toxin im, the major urinary

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metabolites were identified as T-2 tetraol, 4-deacetylneosolaniol, 3′-OH HT-2. In bile, the major metabolites were HT-2, 4-deacetylneosolaniol, 3′-OH T-2 triol, and 3′-OH HT-2 (Pace et al., 1985). In swine administered T-2 toxin iv, the major urinary metabolites were 3′-OH HT-2 and T-2 triol plus the glucuronide conjugates of HT-2, 3′-OH T-2, 3′-OH HT-2, and T-2 toxin (Corley et al., 1985). Recently, deepoxy metabolites of 3′-OH HT-2 including deepoxy T-2 tetraol, deepoxy 3′-OH HT-2 and deepoxy 3′-OH T-2 triol were identified in rat feces (Yoshizawa et al., 1985a).

It is clear from the literature that the metabolism of T-2 toxin follows four distinct pathways: hydrolysis, hydroxylation, deepoxidation, and glucuronidation (Ohta et al., 1977; Yoshizawa et al., 1984, 1985a; Corley et al., 1985). The purpose of this study was to determine the effects of dose, route of administration, and time on the first three metabolic pathways.

EXPERIMENTAL SECTION

Reference Standards. Tritium-labeled T-2 toxin (labeled in the C-3 position, radiopurity >98%, specific activity 500 mCi/mmol) was obtained from Amersham Corp., Arlington Heights, IL. 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.

Tritium-labeled T-2 toxin was used to prepare labeled standards of HT-2, T-2 triol, and T-2 toxin by alkaline hydrolysis (Wei et al., 1971). Tritium-labeled 3′-OH HT-2 and 3′-OH T-2 triol were prepared by alkaline hydrolysis from 3′-OH T-2 toxin, which was prepared by incubation of labeled T-2 toxin with S-9 rat liver homogenates (Knupp et al., 1986). Radiolabeled deepoxy derivatives of HT-2, T-2 triol, and T-2 tetraol were prepared from tritium-labeled T-2 toxin with bovine rumen microflora (Swanson et al., 1987a).

Animal Treatment. Male Sprague–Dawley rats weighing 200–250 g were obtained from Harlan Sprague–Dawley Inc., St. Louis, MO. Rats were individually housed in metabolic cages (Nalge Co., Rochester, NY) and allowed to acclimate to a 12-h day/night cycle for 7 days prior to dosing. Feed and water were provided ad libitum except for 12 h predosing when it was removed. Six days following dosing all animals were killed by cervical dislocation.

Animal Dosing. Each rat was given 10 μCi of tritium-labeled T-2 toxin at either 0.15 or 0.60 mg/kg of body weight. Intravenous doses were dissolved in 0.25 mL of 50% ethanol/water and administered through a tail vein. The oral doses were dissolved in 0.25 mL of 50% ethanol/water and administered by gavage. Dermal doses were applied to an area approximately 1 cm^2 located between the scapulas in 0.1 mL of 90% DMSO/water. Each dose and route combination was repeated three times for a total of 18 rats.

Sample Handling. Feces and urine were collected every 6 h and stored at −20 °C until analysis.

Determination of Total Radioactivity. The total radioactivity in urine was determined by adding 0.1 mL plus 0.4 mL of water directly to 5 mL of Aquasol-2 liquid scintillation cocktail (New England Nuclear Corp., Boston, MA). Quench correction was done by spiking another aliquot of urine with tritium-labeled T-2 toxin. The total radioactivity in feces was determined by first homogenizing 1.0 g in 20 mL of 0.1 M acetate buffer (pH 3.8). A 0.1-mL aliquot was removed and added to a 7-mL glass vial containing 0.1 mL of perchloric acid (60%) and 0.2 mL of hydrogen peroxide (30%). The vials were capped tightly and heated at 60 °C for 24 h. Following heating, the vials were allowed to cool, and 5 mL of Aquasol-2 was added. Spiked feces samples were treated identically to allow for correction of the quenching effect from perchloric acid. Urine and feces samples were counted on a Packard Tri-Carb 300M liquid scintillation counter (Packard Instruments, Chicago, IL).

Urine Extraction. A 1.0–10.0-mL volume depending on the amount of total radioactivity present was diluted to 15 mL with water, followed by the addition of 5 g of NaCl and 15 mL of acetonitrile. Upon centrifugation at 2000 rpm, the top layer was removed. The extraction was repeated three times with acetonitrile and a fourth time with acetonitrile/acetone (1:1). The top layers were combined, and 100 mL of methylene chloride was added to drive residual water out of solution. Anhydrous sodium sulfate (approximately 50 g) was added to remove water (solutions appeared clear). Cupric carbonate (2.5 g) was added, and the samples were filtered through ash-free analytical filter pulp (Schleicher and Schuel, Inc., Keene, NH). The pulp and flasks were rinsed three times with 20 mL of ethyl acetate. Samples were then concentrated to dryness and redissolved in 1–2 mL of methanol, diluted with an equal volume of water, and filtered through a disposable membrane filter (Arco LC25, 0.2 μm; Gilman Sciences, Ann Arbor, MI) for HPLC radiochromatographic analysis.

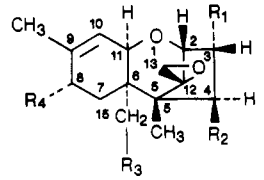
Feces Extraction. To a 0.5–3.0-g sample were added 10 mL of 0.1 M acetate buffer (pH 3.8) and 10 mL of acetonitrile. Each sample was mixed well and centrifuged at 2000 rpm, and the aqueous acetonitrile layer was transferred to a 30-mL plastic centrifuge tube. The feces were extracted three times with 10 mL of acetonitrile/water (1:1). To the combined aqueous acetonitrile portions was added 5 g of NaCl followed by shaking and centrifugation to allow the phases to separate. The acetonitrile layer was removed, and the extraction was repeated three times with 10 mL of acetonitrile and a fourth time with acetonitrile/acetone (1:1). The acetonitrile extracts were diluted with methylene chloride and processed as described above for urine.

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. A sample volume of 100 μL containing approximately 100 000 cpm was injected onto the column. Fractions (0.2 mL) were collected and assayed for radioactivity in Scinti Verse LC liquid scintillation cocktail (Fisher Scientific Co., Itasca, IL). Due to the efficient cleanup from HPLC, the effect of quench was not evident in the fractions assayed for radioactivity.

TLC Chromatography. Aliquots of samples were spotted into the outer channels of a precoated silica gel TLC plate (5 × 20 cm, 0.25-mm gel thickness, J. T. Baker), which was activated for 1 h at 110 °C. Plates were developed in chloroform/methanol (9:1) and allowed to air-dry. To obtain radiochromatographic profiles, 1–2-mm bands were scraped from the TLC plate directly into scintillation vials. A 0.1-mL volume of water was added to each vial, followed by 0.25 mL of methanol. Samples were then counted in 5 mL of Aquasol-2. Standards were visualized under a long-wave (365-nm) UV lamp after having been sprayed with 30% sulfuric acid in methanol and heated at 110 °C for 35 min.

Statistical Analysis. Analysis of variance (ANOVA) using the percentage of extracted radioactivity (perofext)

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



name	R_1	R_2	R_3	R_4	R[a]
T-2 toxin	OH	OAc	OAc	$OCOCH_2CH(CH_3)_3$	-O-
3'-OH T-2	OH	OAc	OAc	$OCOCH_2C(OH)(CH_3)_3$	-O-
neosolaniol	OH	OAc	OAc	OH	-O-
HT-2	OH	OH	OAc	$OCOCH_2CH(CH_3)_3$	-O-
3'-OH HT-2	OH	OH	OAc	$OCOCH_2COH(CH_3)_3$	-O-
T-2 triol	OH	OH	OH	$OCOCH_2CH(CH_3)_3$	-O-
T-2 tetraol	OH	OH	OH	OH	-O-
deepoxy 3'-OH HT-2	OH	OH	OAc	$OCOCH_2C(OH)(CH_3)_3$	$=CH_2$
deepoxy T-2 tetraol	OH	OH	OH	OH	$=CH_2$

[a] Represents the epoxide group at the 12,13-position or its replacement following deepoxidation.

as the dependent variable was used. A value of $p < 0.05$ was used to indicate significance. Tukey's studentized range test ($p < 0.05$) was used for comparisons between route, dose, and time.

RESULTS AND DISCUSSION

Methodology. The analytical procedures developed and applied in this study were well suited for the analysis of tritium-labeled T-2 toxin and metabolites, which have a wide range of polarities as shown in Table I. Previously published methods for the analysis of T-2 toxin and metabolites in excreta involved the use of Amberlite XAD-2 for the extraction of metabolites and Florisil and/or C-18 for the cleanup of samples prior to thin-layer chromatography (Yoshizawa et al., 1980). In these studies, a solvent partition into acetonitrile followed by a cupric carbonate cleanup step was used. The use of cupric carbonate in this study provided a simple and rapid alternative to the use of Florisil and C-18. Significant cleanup of urine and feces extracts was also achieved by precipitating the oily residue after cupric carbonate cleanup with aqueous methanol, followed by filtration through 0.2-μm filters. The replacement of TLC with reversed-phase HPLC greatly increased reproducibility and resolution.

Percent recoveries from spiked urine samples were 46.0 ± 1.2, 74.6 ± 2.0, 73.4 ± 1.3, and 75.1 ± 2.0 ($\bar{x}$ ± SE, n = 4) for T-2 tetraol, T-2 triol, HT-2, and T-2 toxin, respectively. Percent recoveries from spiked fecal samples were 42.6 ± 1.7, 71.2 ± 2.1, 70.6 ± 1.9, and 73.1 ± 2.3 ($\bar{x}$ ± SE, n = 4) for T-2 tetraol, T-2 triol, HT-2, and T-2 toxin, respectively. The reproducibility of the extraction procedure for overall radioactivity was tested on a representative urine sample from a treated animal. The percent recovery of radioactivity was 59.0 ± 2.1 ($\bar{x}$ ± SE, n = 12). Considering the wide range of polarity between T-2 toxin and its metabolites, this standard error (SE) was quite low.

Excretion of Total Radioactivity. The total cumulative excretion of radioactivity in rat urine and feces is shown in Figure 1. The excretion of radioactivity was rapid and nearly complete (greater than 95%) 72 h after administration of labeled T-2 toxin in orally dosed rats. This rapid excretion of T-2 toxin and metabolites was consistent with that reported in other species (Matsumoto et al., 1978; Pace et al., 1985; Yoshizawa et al., 1980, 1981).

In rats given an iv injection of T-2 toxin at 0.15 mg/kg, the excretion of radioactivity was very rapid and nearly

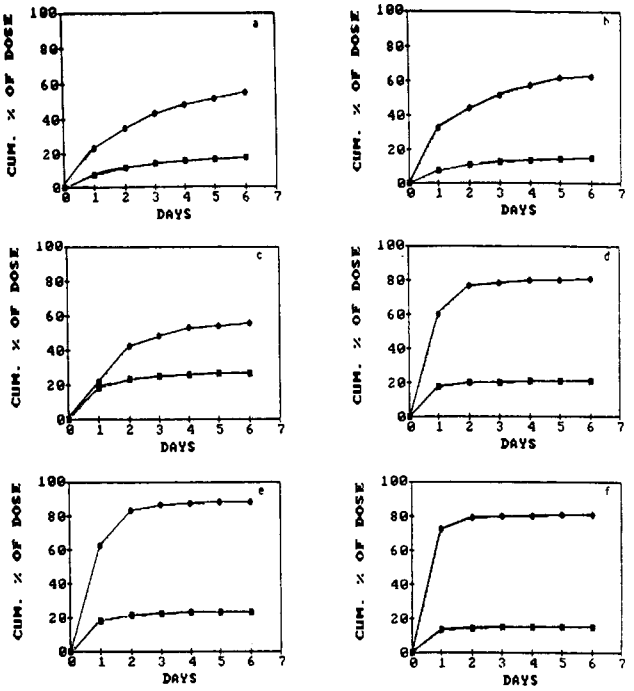


Figure 1. Cumulative excretion of radioactivity in urine (■) and feces (●) from rats administered tritium-labeled T-2 toxin at 0.6 mg/kg dermally (a), iv (c), and orally (e) or 0.15 mg/kg dermally (b), iv (d), and orally (f).

complete after 72 h, but in rats given 0.6 mg/kg, the excretion was less than 80% after 72 h. Extensive vascular damage was noted in rats given T-2 toxin at this concentration, possibly resulting in decreased absorption of T-2 toxin.

In dermally dosed rats, less than 60% of the radioactivity was excreted 72 h after administration. Excretion studies using swine as a model have shown that the skin and surrounding fat act as a depository for T-2 toxin (Pang et al., 1987). This may account for the decreased excretion of radioactivity in dermally dosed rats. As in iv-dosed rats, the excretion of radioactivity was less in the high-dose (0.6 mg/kg) rats than the low-dose rats.

Metabolite Determination. HPLC analysis of urine and fecal extracts resulted in 16 different radioactive peaks. A total of 68.3% of the extracted radioactivity in urine averaged over dose, route, and time had HPLC retention times identical with those of standards of T-2 toxin (5.6%), HT-2 (8.9%), 3'-OH HT-2 (29.3%), 3'-OH T-2 toxin (3.2%), and T-2 tetraol (21.3%). Two unknown metabolites labeled M5 (7.5%) and M9 (13.8%) accounted for an additional 21.3% of the extracted radioactivity. Metabolite M9 was further characterized by its hydrolysis to deepoxytetraol and its comigration on TLC with 3'-OH HT-2. On the basis of retention time data, it was postulated that M7 was deepoxy 3'-OH T-2 triol, M5 was 3'-OH T-2 triol, and M9 was deepoxy 3'-OH HT-2.

In feces, a total of 29.1% of the radioactivity averaged over dose, route, and time had HPLC retention times identical with those of deepoxy HT-2 (3.4%), 3'-OH HT-2 (15.1%), and deepoxy T-2 tetraol (10.6%). Three unknown metabolites accounted for an additional 61.4% of the extracted radioactivity. These metabolites were labeled M5 (5.2%), M7 (9.7%), and M9 (46.5%).

Chromatographic analysis of the radioactivity in the excreta of rats revealed that the major metabolites regardless of dose or route were 3'-OH HT-2, HT-2, T-2 tetraol, deepoxy T-2 tetraol, and several unknowns. The hydrolysis of T-2 toxin to T-2 tetraol via several intermediates, as well as the hydroxylation of T-2 toxin and

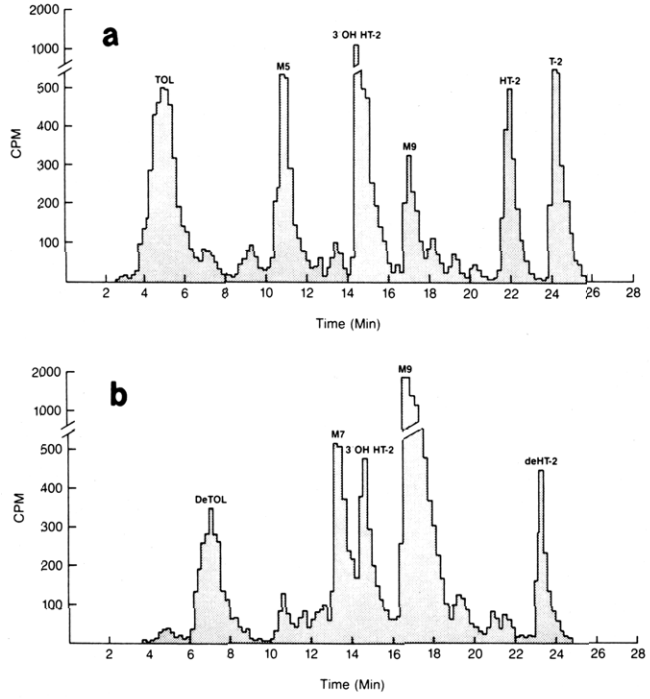


Figure 2. Representative HPLC chromatograms of urine (a) and feces (b) from rats administered tritium-labeled T-2 toxin.

HT-2 at the 3′-position, have been previously reported (Yoshizawa et al., 1980, 1984; Visconti and Mirocha, 1985). The deepoxidation pathway in vivo has been reported with 3′-OH HT-2 and in vitro with T-2 toxin (Yoshizawa et al., 1985a,b). This study presents evidence indicating that deepoxidation is an important in vivo metabolic pathway for T-2 toxin in rats.

Deepoxidation results in significant detoxification of T-2 toxin. The deepoxy derivatives of HT-2, T-2 tetraol, and T-2 triol exhibited no toxicity (LD_{50} greater than 5000 mg/mL) to brine shrimp (Swanson et al., 1987b).

The metabolic profiles (HPLC radiochromatograms) for representative urine and fecal extracts are shown in Figure 2. The metabolic profiles expressed as a percent of the extracted radioactivity for route and dose over time are given in Table II and III.

The extracted radioactivity represented 52.4 ± 2.4 and 43.9 ± 1.4% ($x \pm SE$, $n = 58$) of the total radioactivity in urine and feces, respectively. Although no differences were noted in the extracted radioactivity between doses and routes, a decrease in extracted radioactivity was observed over time. This decreasing trend was due possibly to an increased amount of nonextracted polar metabolites, such as glucuronide conjugates, and/or binding. Although conjugated metabolites were not investigated in this study, they have been reported in swine (Corley et al., 1985).

Statistical Interpretation for Urine. There were no significant differences in metabolic profiles expressed as a percent of extracted radioactivity due to dose. However, route and time and their interactions were significant for several metabolites as shown in Figures 3–5. The percentage of HT-2 averaged over dose and time in orally dosed rats was significantly lower than in dermally dosed and iv-dosed rats. This effect was also significant but reversed for metabolite M9. The percentage of metabolite M9 averaged over dose and time in orally dosed and iv-dosed rats was significantly higher than for dermally dosed rats.

For T-2 tetraol, the percentage averaged over dose and time was greatest for iv-dosed rats, but for 3′-OH T-2 toxin, the percentage was less for iv-dosed rats than orally or dermally dosed rats (see Figure 3).

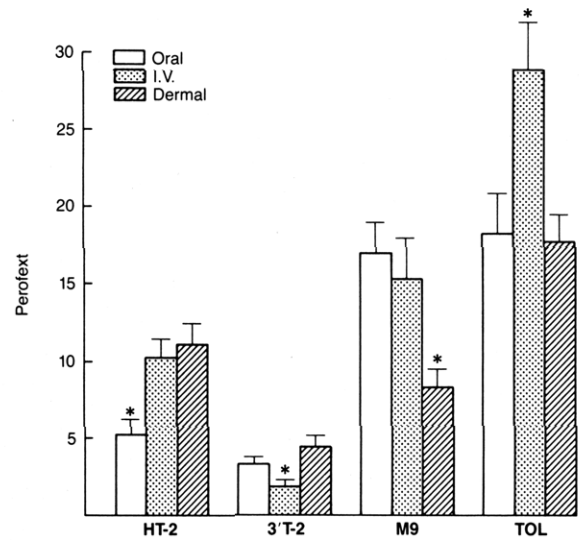


Figure 3. Effect of route on selected metabolites in rat urine (* indicates $p < 0.05$) expressed as percent of extracted radioactivity (perofext).

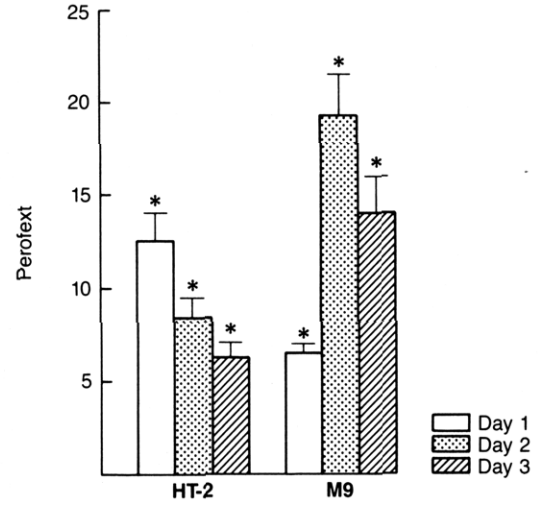


Figure 4. Effect of time on selected metabolites in rat urine (* indicates $p < 0.05$) expressed as percent of extracted radioactivity (perofext).

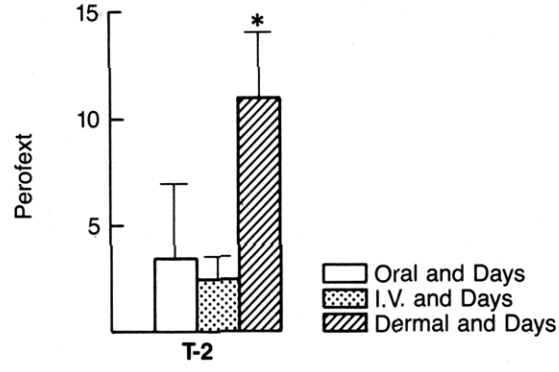


Figure 5. Effect of route and time averaged over dose (* indicates $p < 0.05$) for T-2 toxin in rat urine expressed as percent of extracted radioactivity (perofext).

A significant difference in the percentage of extracted radioactivity over time was noted as shown in Figure 4. For HT-2, the percentage averaged over route and dose for day 1 was greater than for day 3. For M9, the percentages for days 2 and 3 were greater than those for day 1.

Table II. Metabolic Profiles Expressed as Percent of Extracted Radioactivity in Urine from Rats Administered Tritium-Labeled T-2 Toxin[a]

		oral		iv		dermal	
metabolite	day	0.15 mg/kg	0.6 mg/kg	0.15 mg/kg	0.6 mg/kg	0.15 mg/kg	0.6 mg/kg
HT-2	1	3.9 ± 0.1	6.1 ± 1.0	19.8 ± 0.4	11.7 ± 1.8	13.2 ± 1.7	18.6 ± 0.5
	2	4.4 ± 1.8	9.1 ± 4.2	8.8 ± 1.0	6.3 ± 0.7	8.0 ± 1.1	12.1 ± 0.2
	3	4.2 ± 1.4	3.7 ± 1.9	7.6 ± 1.7	7.3 ± 1.7	6.3 ± 0.1	8.6 ± 1.1
3′-OH T-2	1	4.6 ± 1.3	3.3 ± 1.0	2.7 ± 0.6	3.2 ± 0.3	4.6 ± 0.2	6.2 ± 1.0
	2	5.5 ± 0.6	0.3 ± 0.3	1.1 ± 1.1	1.8 ± 0.9	4.2 ± 0.8	3.7 ± 0.9
	3	3.5 ± 2.8	2.1 ± 1.0	3.0 ± 0.6	0.8 ± 0.8	4.0 ± 0.8	3.5 ± 0.4
M9	1	7.5 ± 1.0	5.6 ± 1.1	6.9 ± 0.7	5.1 ± 1.4	6.4 ± 0.8	16.9 ± 1.2
	2	26.5 ± 1.3	21.2 ± 3.9	27.3 ± 2.6	30.3 ± 3.4	9.4 ± 0.3	12.7 ± 1.5
	3	16.4 ± 4.0	19.4 ± 8.0	13.7 ± 3.8	17.2 ± 5.8	3.8 ± 0.3	10.7 ± 1.8
3′-OH HT-2	1	32.1 ± 3.6	42.4 ± 2.6	24.6 ± 1.2	22.6 ± 4.4	30.3 ± 1.4	26.2 ± 2.9
	2	27.0 ± 2.2	33.9 ± 7.3	23.2 ± 0.7	22.4 ± 1.2	36.3 ± 1.8	34.1 ± 2.5
	3	28.7 ± 4.2	36.7 ± 3.6	22.5 ± 5.3	25.4 ± 6.7	29.2 ± 3.4	30.1 ± 1.6
M5	1	12.8 ± 2.8	14.6 ± 1.5	6.9 ± 1.3	12.3 ± 3.7	11.6 ± 1.6	7.1 ± 0.7
	2	5.0 ± 2.3	5.2 ± 2.5	2.4 ± 0.7	4.3 ± 1.2	8.3 ± 1.8	4.7 ± 1.4
	3	7.0 ± 0.6	10.1 ± 1.9	3.9 ± 2.0	7.2 ± 2.7	5.4 ± 1.4	6.1 ± 1.1
T-2 tetraol	1	18.0 ± 4.7	20.3 ± 3.1	29.0 ± 1.6	29.2 ± 3.9	21.7 ± 1.2	22.6 ± 1.6
	2	19.4 ± 1.3	18.0 ± 3.2	28.7 ± 3.1	24.8 ± 3.6	15.0 ± 1.8	16.3 ± 0.6
	3	24.7 ± 0.9	13.0 ± 3.7	31.6 ± 4.0	23.0 ± 8.9	14.4 ± 2.9	17.0 ± 1.0

[a] Values reported as the mean ± SE (n = 3).

Table III. Metabolic Profiles Expressed as Percent of Extracted Radioactivity in Feces from Rats Administered Tritium-Labeled T-2 Toxin[a]

		oral		iv		dermal	
metabolite	day	0.15 mg/kg	0.6 mg/kg	0.15 mg/kg	0.6 mg/kg	0.15 mg/kg	0.6 mg/kg
deepoxy HT-2	1	3.0 ± 1.0	1.6 ± 0.8	5.1 ± 1.7	2.8 ± 1.6	6.8 ± 1.6	5.9 ± 0.8
	2	2.3 ± 1.0	1.7 ± 0.5	2.3 ± 0.8	1.7 ± 1.2	3.8 ± 1.4	4.3 ± 0.4
	3	3.0 ± 1.0	0.6 ± 0.1	1.8 ± 1.1	1.7 ± 0.6	6.8 ± 1.6	5.0 ± 0.8
M9	1	49.7 ± 3.8	14.5 ± 2.4	40.3 ± 3.7	44.2 ± 2.8	43.0 ± 5.8	40.9 ± 7.3
	2	53.6 ± 3.5	60.1 ± 5.1	50.9 ± 2.3	45.3 ± 1.3	51.2 ± 4.4	56.2 ± 2.8
	3	46.6 ± 2.7	50.5 ± 6.8	42.1 ± 1.4	42.7 ± 3.3	48.5 ± 3.8	55.9 ± 1.2
3′-OH HT-2	1	14.2 ± 1.0	50.2 ± 4.2	15.6 ± 5.0	7.7 ± 0.8	15.2 ± 5.1	19.1 ± 7.6
	2	8.9 ± 1.6	12.4 ± 3.3	12.8 ± 1.4	9.8 ± 1.3	8.5 ± 1.5	8.8 ± 1.8
	3	11.6 ± 0.2	18.9 ± 5.6	18.0 ± 2.7	14.0 ± 0.5	15.2 ± 2.9	10.1 ± 1.9
M7	1	9.7 ± 0.5	5.0 ± 3.0	9.4 ± 2.1	18.1 ± 3.1	6.2 ± 1.0	7.6 ± 2.6
	2	12.3 ± 0.7	8.3 ± 1.5	10.4 ± 1.9	13.3 ± 2.6	10.3 ± 1.4	11.0 ± 1.5
	3	10.0 ± 1.4	8.3 ± 2.5	9.2 ± 1.1	8.7 ± 3.8	6.4 ± 2.0	9.6 ± 1.3
M5	1	7.5 ± 1.1	9.1 ± 3.6	9.7 ± 2.0	4.3 ± 1.1	8.7 ± 2.4	6.8 ± 2.4
	2	4.4 ± 1.3	3.0 ± 1.3	4.7 ± 0.6	3.1 ± 1.0	3.7 ± 0.9	2.3 ± 0.8
	3	5.1 ± 1.7	4.2 ± 2.0	4.1 ± 0.5	3.7 ± 0.8	1.8 ± 0.1	1.5 ± 0.2
deepoxy Tol	1	6.7 ± 1.8	4.4 ± 1.4	9.1 ± 1.9	2.3 ± 0.3	2.2 ± 1.0	2.3 ± 0.5
	2	8.4 ± 1.0	9.8 ± 1.7	11.9 ± 0.5	20.3 ± 1.7	9.8 ± 0.5	10.3 ± 1.2
	3	10.4 ± 0.4	9.7 ± 0.8	12.6 ± 0.9	20.9 ± 0.1	8.0 ± 0.6	8.7 ± 0.3

[a] See footnote, Table II.

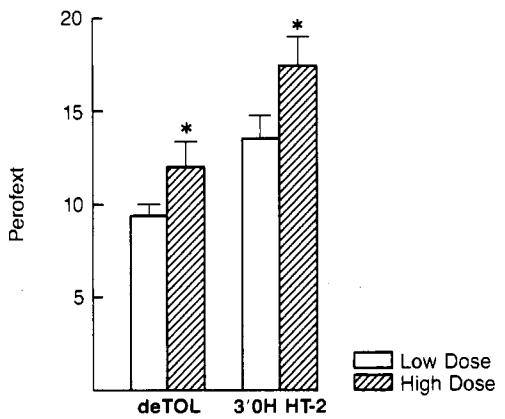


Figure 6. Effect of dose on selected metabolites in rat feces (* indicates $p < 0.05$) expressed as percent of extracted radioactivity (perofext).

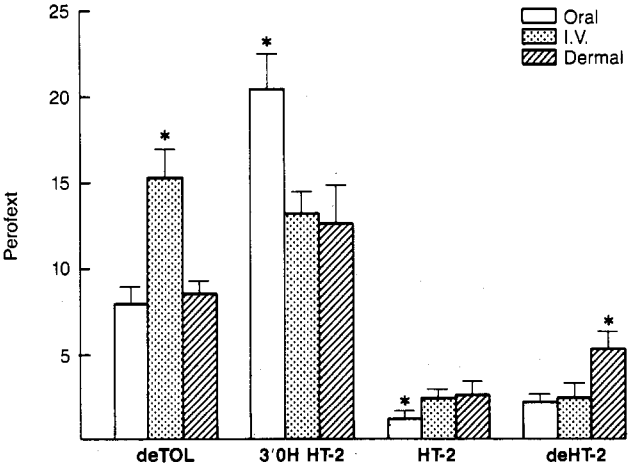


Figure 7. Effect of route on selected metabolites in rat feces (* indicates $p < 0.05$) expressed as percent of extracted radioactivity (perofext).

Although no main effects were seen for T-2 toxin, the interaction between route and day was significant. The percentage of T-2 toxin in the urine of orally dosed and iv-dosed rats was significantly less than in dermally dosed rats as shown in Figure 5.

Statistical Interpretation for Feces. Animals given the high dose had significantly greater fractions of the dose metabolized to deepoxy T-2 tetraol and 3′-OH HT-2 than animals given the low dose. However, the interactions

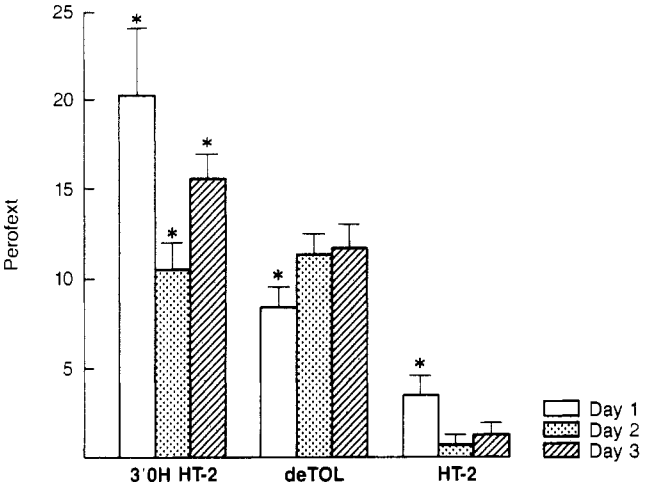


Figure 8. Effect of time on selected metabolites in rat feces (* indicates $p < 0.05$) expressed as percent of extracted radioactivity (perofext).

between route, time, and dose were complex for these two metabolites. The statistical results for feces are summarized in Figures 6–8. Examination of the percentage of extracted radioactivity over dose and time indicated that the percentage of deepoxy T-2 tetraol was significantly higher in feces from iv-dosed rats than orally or dermally dosed rats; but for 3′-OH HT-2, the percentage from orally dosed rats was higher than from iv-dosed and dermally dosed rats. For HT-2 and deepoxy HT-2, the percentages averaged over dose and time were significantly higher in dermally dosed rats than iv-dosed and orally dosed rats (Figure 7).

The effect of time on the metabolic profiles was the most significant effect detected in feces. For 3′-OH HT-2 and HT-2, the percentages averaged over dose and route for day 1 were higher than for day 2; but for deepoxy T-2 tetraol, days 2 or 3 were significantly higher than day 1 (see Figure 8).

The application of HPLC as evidenced by this study was an excellent analytical tool for the isolation, separation, and identification of trichothecene metabolites. The capabilities of HPLC far outweigh those procedures such as TLC that have been used in previous studies.

Evidence from this study suggests that several significant interactions of route and time are important, whereas the effect of dose was not. The major metabolic pathways, hydrolysis, hydroxylation, and deepoxidation, were not effected by the two dose levels used. The effect of route was significant for several metabolites. The higher levels of T-2 tetraol and deepoxy T-2 tetraol in iv-dosed rats indicate that hydrolysis and deepoxidation serve as the major metabolic pathways by this route. The lower value of 3′-OH HT-2 in iv-dosed rats supports this conclusion. The increase of T-2 toxin over time as a percent of extracted radioactivity in urine from rats administered T-2 toxin dermally is not fully understood but may arise from the slow release of toxin from the site of application. In swine topically administered T-2 toxin, the skin and fat acted as a reservoir for the toxin; residues of the parent toxin and the acylation modulus were detected at the application site 14 days after exposure (Pang et al., 1987).

The most significant effect was that of time. The percentage of extracted radioactivity associated with deepoxy metabolites increased significantly over time, regardless of the route or dose. Although the epoxy ring in a trichothecene nucleus seems to be an important moiety for the toxic action of T-2 toxin, the toxicological estimation of deepoxytrichothecenes remains to be established. Deepoxidation has been reported to be a detoxification pathway, and in vitro studies suggest that intestinal microflora are responsible (Yoshizawa et al., 1985a). Alterations of intestinal microflora may result in an increased toxicity of animals to T-2 toxin.

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Registry No. T-2 toxin, 21259-20-1; HT-2, 26934-87-2; 3′-OH T-2, 84474-35-1; T-2 tetraol, 34114-99-3; 3′-OH HT-2, 78368-54-4; deepoxy HT-2, 115827-79-7; deepoxy T-2 tetraol, 98896-89-0; deepoxy-3′-OH HT-2, 98896-86-7.

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