Distribution of Tritium-Labeled T-2 Toxin in Swine

Two weanling crossbred pigs were intubated with tritium-labeled 4β,15-diacetoxy-3α-hydroxy-8α-(3-methylbutyryloxy)-12,13-epoxytrichothec-9-ene (T-2) toxin to determine the tissue distribution and the excretion pattern of T-2 and/or its metabolites. The percentage of administered radioactivity (18 h after dosing) in one animal intubated with 0.1 mg of [^{3}H]T-2/kg body weight in 50% aqueous ethanol (0.75 mL) was 0.7% (muscle), 0.43% (liver), 0.08% (kidney), 0.06% (bile), 21.6% (urine), and 25.0% (feces). The calculated residue levels for T-2 and/or its metabolites based upon the specific radioactivity of the tissue were as follows: muscle, 3.1 ppb; fat, 0.49 ppb; liver, 13.8 ppb; and kidney, 15.9 ppb. The percentage of administered radioactivity in the other animal intubated with 0.4 mg of [^{3}H]T-2/kg body weight (1.22×10^{10} dpm) was 0.7% (muscle), 0.29% (liver), 0.08% (kidney), 0.14% (bile), 17.6% (urine), and 0.84% (feces). The corresponding residue levels for T-2 toxin in the tissues of this animal were as follows: muscle, 11.5 ppb; liver, 37.7 ppb; and kidney, 61.4 ppb. Since these residue levels were low, it was impossible to chemically identify the metabolites present.

In recent years the trichothecene mycotoxins have come under scrutiny as being involved in and possibly the cause of several disease syndromes common in farm animals in the midwest United States as well as in other temperate climates. The major source of these compounds is *Fusarium tricinctum*. This fungus has been isolated consistently from molded and toxic corn samples (Bamburg et al., 1969; Gilgan et al., 1966). In solid culture at a moisture content of 23% or greater this fungus can produce large quantities of T-2 toxin (4β,15-diacetoxy-3α-hydroxy-8α-(3-methylbutyryloxy)-12,13-epoxytrichothec-9-ene; Bamburg et al., 1968; Smalley and Strong, 1974).

The animal disorders which have been described by several authors and associated with T-2 and other trichothecene toxins have involved mainly the digestive tract, resulting in bloody diarrhea and hemorrhaging in the stomach, heart, intestines, bladder, and kidneys (Grove et al., 1970; Kosuri et al., 1970; Hsu et al., 1972; Petrie et al., 1977). Although our research has shown that purified T-2 toxin (97% by GLC) does not produce hemorrhaging in crossbred swine (Weaver et al., 1978a,b,c) or White leghorn chickens (Chi et al., 1977a,b,c) when administered in either an acute or chronic study, by various routes of administration and at any of several T-2 dose levels; we did verify that T-2 is quite lethal in both swine (IV) and chickens (by intubation into the crop), but not when fed in a ration (refusal factor) (Chi et al., 1977b,c, 1978a; Weaver et al., 1978b,c). The first report of a direct association of T-2 toxin with moldy corn toxicosis was provided by Hsu et al. (1972) in which T-2 toxin at a level of 2 ppm was found in the feed of 35 lactating cows, seven of which died over a period of 5 months.

Because of the possibility of meat contamination by this toxin resulting from ingestion of contaminated grain by food producing animals, we have studied the distribution of tritium-labeled T-2 toxin in two species, chickens, and swine. We have recently reported a comprehensive study of the distribution in broiler chicks (Chi et al., 1978b) and in this paper we report a similar distribution experiment in young swine.

MATERIALS AND METHODS

Two weanling crossbred swine (Yorkshire × Duroc × Hampshire) were obtained from University of Minnesota stock and acclimated overnight in individual stainless steel metabolism cages. The cages were lined with polyethylene film to facilitate cleaning, and the urine was collected separately from the feces. The animals were provided with food and water ad libitum throughout the experiment.

The dose of toxin used in this distribution study was kept low so that it would have minimal caustic or emetic effects on the digestive processes of the animal. Our data concerning the distribution of T-2 toxin in chicks (Chi et al., 1978b) showed that the levels of radioactivity in most tissues peaked between 4 and 12 hours after dosing. Since chickens have a higher rate of metabolism than swine, it was decided that 18 h should be allowed for distribution of the toxin before sacrificing the animal. The distribution experiment in chickens also enabled us to judge which organs and tissues in the swine would be most likely to contain appreciable levels of radioactivity.

The dose of T-2 toxin given to the second animal was adjusted to maximize metabolite and radioactivity levels in the tissues without inducing emesis and concomitant loss of toxin.

The radiolabeled T-2 toxin used in this study was prepared by oxidation of T-2 in the C-3 position with dimethyl sulfide/*N*-chlorosuccinimide, followed by reduction with high specific activity sodium [^{3}H]borohydride as described by Wallace et al. (1977).

Scintillation counting was performed in triplicate on a Packard Tri-Carb liquid scintillation spectrometer, Model 3375. Quenching was determined by the external standards method. Background samples were prepared for all liquid scintillation work using control tissue which was subjected to the same sample preparation procedures and reagents as were the radioactive samples.

A. Distribution Studies. One animal (7.5 kg body weight) was intubated with 0.75 mg (0.1 mg/kg body weight, 2.41×10^9 dpm) of [^{3}H]T-2 toxin in 50% aqueous ethanol (0.75 mL) ([^{3}H]T-2 toxin: 3.213×10^9 dpm/mg of T-2). After 18 h the animal was sacrificed by exsanguination, and fresh tissue samples were obtained. The heart, liver, and kidneys were removed intact; the bile was aspirated from the gall bladder and most of the skeletal muscle was removed. The small amount of abdominal fat present was also collected. Urine remaining in the bladder was removed by syringe. Abdominal muscle was not collected as it contained a significant quantity of interstitial adipose tissue.

Tissue samples for scintillation counting were prepared as described earlier (Chi et al., 1978b). Urine was counted directly without digestion using a dioxane based liquid scintillation cocktail (Aquafluor, New England Nuclear).

B. Metabolism Study. For this experiment, one pig (9.5 kg body weight) was intubated with 3.8 mg (0.4 mg/kg body weight; 1.22×10^{10} dpm) of [^{3}H]T-2 toxin in 50% aqueous ethanol (3.8 mL) ([^{3}H]T-2 toxin: 3.213×10^9 dpm/mg of T-2). The animal was sacrificed as above, and tissue samples were obtained for scintillation counting and metabolite extraction. The distribution pattern of radioactivity in this animal is presented along with that from

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Table I. Distribution of Radioactivity in Weanling Swine after Intubation with [^{3}H]T-2 Toxin

	0.1 mg/kg body weight (2.41×10^9 dpm)			0.4 mg/kg body weight (1.22×10^{10} dpm)		
tissue	dpm/mg of tissue	dpm/tissue	% of admin. activity	dpm/mg of tissue	dpm/tissue	% of admin. activity
muscle[a]	10.1	1.8×10^7	0.7	36.8	8.8×10^7	0.7
heart	12.4	4.0×10^5	0.02	not counted		
blood	12.8			not counted		
fat	15.7			not counted		
spleen	15.9	2.3×10^5	0.01	not counted		
liver	44.2	1.0×10^7	0.4	121.0	3.6×10^7	0.3
kidney	51.1	2.0×10^6	0.1	197.1	9.5×10^3	0.1
bile	1.6×10^3	1.4×10^6	0.1[b]	6.7×10^3	1.7×10^7	0.1[b]
urine	3.2×10^3	5.2×10^8	21.6	1.3×10^4	2.2×10^9	17.6
feces	5.8×10^3	6.0×10^3	25.0	7.0×10^2	1.0×10^8	0.8

[a] Total muscle weight was estimated as 25% of the body weight. [b] The total amount of bile recovered was very low and hence the recovery of radioactivity is low; however, the specific activity (dpm/mg tissue) of the bile that was recovered was very high.

part A. For both animals (parts A and B), the gavage tube was rinsed with 15 mL of water after intubation of the toxin.

RESULTS AND DISCUSSION

The distribution of T-2 in major tissues is shown in Table I. The value for the percentage of radioactivity in the muscle is approximate as not all of the muscle could effectively be removed and weighed. The fraction of blood and fat obtained could not be estimated reliably; therefore, the total recovery of radioactivity could not be determined for these tissues. It is particularly interesting to note that the kidney had a slightly higher specific activity (dpm/mg of tissue) than the liver. This is the opposite of what was found in the distribution studies in chickens (Chi et al., 1978b). It is also apparent that the urine is a major excretory pathway in swine. (Since the urine and feces are voided together in chickens, the contribution of the urine to the excretion of radioactivity was not determined.) Since the bile was collected at necropsy and not continuously before it entered the small intestine, it is unclear what percentage of the total radioactivity actually passed through the liver and bile and what fraction may have passed directly to the intestine in the feces without being absorbed. The urine, however, provided direct evidence that at least 20% of the radioactivity was absorbed. Approximately 50% of the administered radioactivity was recovered in the organs and tissues shown in Table I (low dose animal). The remainder was thought to be in the gastrointestinal tract, which was not assayed.

It also can be seen in Table I that for those tissues that were counted in the high dose animal (0.4 mg/kg), the specific radioactivity (dpm/mg of tissue) of the tissues were in all cases four times higher than the activity in the corresponding tissues of the low dose animal (0.1 mg/kg). Thus, at least within the given dose range, the quantities of T-2 and/or metabolites appearing in the tissues were directly proportional to the dose level. The distribution pattern was not greatly affected by the increased dose. The feces of the high dose animal, however, had a particularly low activity. This simply suggests that a much larger fraction of the activity was retained in the gastrointestinal tract tissue and/or ingesta and fecal material of the high dose animal at the time of sacrifice.

In general the distribution described here paralleled the distribution of radiolabeled T-2 in chickens (Chi et al., 1978b). However, the total radioactivity present in the bile of the swine was lower due to the small volume of bile; the specific radioactivity was very high and in this respect corresponds quite well with the distribution of T-2 in chickens. Since the percentage of total radioactivity in the muscle, organs, and bile in the present study was quite low, it is possible that 18 h (time of sacrifice) was not the optimum period for distribution of the toxin.

Table II. Residue of T-2 and/or Its Metabolites in Selected Tissue and Blood of Weanling Swine Intubated with Radiolabeled T-2 Toxin

tissue	amount of T-2 intubated, mg/kg body weight[a] (ppm)	μg of T-2 and/or metabolites/100 g of wet tissue[b] (ppb)
muscle	0.1 (1.25)	0.31 (3.1)
	0.4[c] (5)	1.15 (11.5)
fat	0.1 (1.25)	0.49 (4.9)
liver	0.1 (1.25)	1.38 (13.8)
	0.4 (5)	3.77 (37.7)
heart	0.1 (1.25)	0.39 (3.9)
kidney	0.1 (1.25)	1.59 (15.9)
	0.4 (5)	6.14 (61.4)
blood	0.1 (1.25)	0.40 (4.0)

[a] The values in parentheses are the equivalent toxin levels had the toxin been mixed with the feed, assuming that animals of this age would consume 600 g of feed per day. [b] These values were calculated from the specific radioactivity levels of the tissues shown in Table I. Also, these figures are given with the assumption that the residues are T-2 toxin or metabolites with molecular weights comparable to T-2 toxin. It was not possible, because of the small quantities, to chemically identify the radioactive metabolites. The corresponding concentration is shown in parentheses. [c] This dose (0.4 mg/kg) was estimated to be the highest dose which could be administered to swine without causing emesis.

Table II gives the equivalent levels of T-2 and/or metabolites which would be found in 100 g of tissue based upon the specific activities of those tissues shown in Table I. Concerning human consumption of contaminated pork, it can be seen that 200–500 g of meat from an animal ingesting T-2 at a level of 2.5 ppm in the feed (a representative level found in natural feed contamination) should result in a meat residue level of 1.2–3.0 μg. Chickens and swine regardless of age are not affected by these small amounts of T-2 toxin when it is administered daily for short periods of time (unpublished results). We do not know, however, if these amounts of toxin will influence human health as we do not know the sensitivity of humans toward this material.

The extremely low levels of toxin found in the muscle, even in the animal receiving 0.4 mg of T-2/kg body weight, precludes direct analysis for T-2 by any methods currently available, including gas chromatography and combined gas chromatography–mass spectroscopy. Previous experience shows that the maximum dose which can be administered to swine without causing emesis is low (ca. 0.5 mg/kg body weight); thus, T-2 residues resulting from such a dose are

too low for detection by present methods. We are currently in the process of developing satisfactory analytical methods for these compounds in tissues. Even with a dose of T-2 toxin approaching that which will cause emesis in swine, the resulting radioactive levels in the edible tissues are very small.

LITERATURE CITED

Bamburg, J. R., Marasas, W. F., Riggs, N. V., Smalley, E. B., Strong, F. M., *Biotech. Bioeng.* **10**, 445 (1968).
Bamburg, J. R., Strong, F. M., Smalley, E. B., *J. Agric. Food Chem.* **17**, 443 (1969).
Chi, M. S., Mirocha, C. J., Kurtz, H. J., Weaver, G., Bates, F., Shimoda, W., Burmeister, H. R., *Poultry Sci.* **56**, 103 (1977a).
Chi, M. S., Mirocha, C. J., Kurtz, H. J., Weaver, G., Bates, F., Shimoda, W., *Poultry Sci.* **56**, 306 (1977b).
Chi, M. S., Mirocha, C. J., Kurtz, H. J., Weaver, G., Bates, F., Shimoda, W., *Poultry Sci.* **56**, 628 (1977c).
Chi, M. S., Robison, T. S., Mirocha, C. J., Reddy, K. R., *App. Environ. Microbiol.* **35**, 636 (1978a).
Chi, M. S., Robison, T. S., Mirocha, C. J., Swanson, S. P., Shimoda, W., *Toxicol. Appl. Pharmacol.* **45**, 391 (1978b).
Gilgan, M. W., Smalley, E. B., Strong, F. M., *Arch. Biochem. Biophys.* **114**, 1 (1966).
Grove, M. D., Yates, S. G., Tallent, W. H., Ellis, J. J., Wolff, I. A., Kosuri, N. R., Nichols, R. E., *J. Argic. Food Chem.* **18**, 734 (1970).
Hsu, I.-C., Smalley, E. B., Strong, F. M., Ribelin, W. E., *Appl. Microbiol.* **24**, 684 (1972).
Kosuri, N. R., Grove, M. D., Yates, S. G., Tallent, W. H., Ellis, J. J., Wolff, I. A., Nichols, R. E., *J. Am. Vet. Med. Assoc.* **157**, 938 (1970).
Petrie L., Robb, J., Stewart, A. F., *Vet. Rec.* **101**, 326 (1977).
Smalley, E. B., Strong, F. M., "Mycotoxins", Purchase, I. F. H., Ed., Elsevier, New York, 1974, pp 199–228.
Wallace, E. M., Pathre, S. V., Mirocha, C. J., Robison, T. S., Fenton, S. W., *J. Agric. Food Chem.* **25**, 836 (1977).
Weaver, G. A., Kurtz, H. J., Mirocha, C. J., Bates, F. Y., Behrens, J. C., Robison, T. S., Gipp, W. F., *Can. Vet. J.* **19**, 72 (1978a).
Weaver, G. A., Kurtz, H. J., Mirocha, C. J., Bates, F. Y., Behrens, J. C., Robison, T. S., *Can. Vet. J.* **19**, 310 (1978b).
Weaver, G. A., Kurtz, H. J., Bates, F. Y., Chi, M. S., Mirocha, C. J., Behrens, J. C., Robison, T. S., *Vet. Rec.* **103**, 531 (1978c).

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Simultaneous Extraction and Analysis of Trifluralin and Nitrofen Residues in Crucifers

A very sensitive analytical method has been developed for determination of trifluralin and nitrofen residues in kohlrabi, radish, rutabaga, and turnip. The method utilizes simultaneous extraction and purification of both herbicides and detection by electron-capture gas chromatography. Recoveries were greater than 90% at fortification levels of 10–40 ppb.

Application of trifluralin (α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine) and nitrofen (2,4-dichlorophenyl 4-nitrophenyl ether) is an effective treatment for selectively controlling various weeds in kohlrabi, radish, rutabaga, and turnip. Since residue data were not available for registration of this use of herbicides, it was necessary to develop a residue method for quantifying chemical residues in these crops.

Trifluralin and nitrofen are readily detectable in the picogram range by electron-capture gas chromatography. Thus the primary objective of the method development was to provide a single extraction and cleanup procedure which would be suitable for both herbicides.

Simultaneous extraction of more than one pesticide residue has been applied to soil residues of trifluralin and three other herbicides (Smith, 1974) and to various herbicide and insecticide residues in crop plants (Luke et al., 1975). The foregoing methods were not employed because of the necessity of handling toxic solvent or because of the more time consuming procedures required in a screening method. Thus the present paper describes a simplified method for the simultaneous extraction, purification, and analysis of trifluralin and nitrofen residues in crucifer crops.

MATERIALS AND METHODS

Sample Extraction. In order to obtain a representative sample, crucifer tubers were diced into small pieces weighing 2–3 g. After thorough mixing, a 25-g sample of the pieces was macerated with methanol in a food blender. The resulting slurry was poured into a Soxhlet thimble and extracted overnight in a Soxhlet extractor with methanol. After cooling, the extract was concentrated to about 50 mL on a rotary evaporator and poured into a 1-L separatory funnel. A 100-mL portion of hexane was used to rinse the residue in the evaporator flask into the separatory funnel.

Partial purification of the extract was achieved by solvent partitioning. Thus 750 mL of 0.1 M KOH containing 5% KCl was shaken with the hexane–methanol extract. After removal of the hexane phase, the aqueous methanol was reextracted with two additional 100-mL portions of hexane. The combined hexane extracts were boiled down to 25 mL on a steam bath.

Silica Gel Cleanup. Purification of soil extracts was not necessary for trifluralin analysis using the electrolytic conductivity detector (Payne et al., 1974). However, preliminary work on crucifers showed cleanup was necessary for analysis on the electron-capture detector. Silica gel (60–200 mesh, Bakers Analyzed Reagent) was activated by heating overnight at 110 °C and deactivated by the addition of 4.6% water. The cleanup column was prepared by adding 4 g of silica gel to a 1 × 20 cm chromatography column containing a glass wool plug and a column of hexane. The column was wet packed to prevent entrapment of air bubbles.

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