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Toxicokinetics of T-2 Mycotoxin and Its Metabolites  
in Cynomolgus Monkeys

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In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources Commission on Life Sciences-National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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Toxicokinetics of T-2 Mycotoxin and Its Metabolites in Cynomolgus Monkeys. MEREISH, K. A., PACE, J. G., NASEEM, S. M., DINTERMAN, R. E., WANNEMACHER, R. W., JR., AND BUNNER, D. L. *Toxicol. Appl. Pharmacol.* \_\_, . The disposition of T-2 mycotoxin and its metabolites was studied in cynomolgus monkeys after an intravenous bolus injection (14.55  $\mu$ g/kg) of tritium-labeled T-2 toxin. Serum, urine, and feces were collected at selected intervals, while tissues were collected 28 days after dosing. T-2 and its metabolites were separated and identified by high-performance-liquid-chromatography (HPLC). Serum and urinary metabolites were identified as HT-2, 3'-OH T-2, 3'-OH HT-2, and T-2 tetraol. Their concentrations in serum declined biexponentially. Only small quantities (< 1.0%) of the parent toxin and HT-2 were detected in urine. In the biotransformation of the toxin, the 3'-hydroxylation of T-2 and HT-2 was favored over ester hydrolysis at the C-4 position. Over 2-weeks, the cumulative renal and fecal excretion represented 68.95% and 31.89%, respectively, of the administered dose. The residual [<sup>3</sup>H]toxin in several organs after 28 days of exposure accounted for only 0.01% of that administered. *Residual [3H]toxin in several organs after 28 days of exposure accounted for only 0.01% of that administered.*

T-2 toxin (4 $\beta$ , 15-diacetoxy-8 $\alpha$ -(3-methylbutyryloxy)-3 $\alpha$ -hydroxy-12,13-epoxytrichothec-9-ene) is a secondary toxic metabolite produced by certain *Fusarium* species. Several laboratories have studied the metabolism of T-2 toxin in various animal models. Mice and rats metabolize T-2 to HT-2, neosolaniol, and several unknowns (Matsumoto et al., 1978), while the lactating cow metabolizes the parent compound to HT-2, neosolaniol, 4-deacetylneosolaniol (4DN), and 3'-hydroxy T-2 and 3'-hydroxy HT-2 (Yoshizawa et al., 1981; 1982). Glucuronide conjugates of T-2, HT-2, 3'-OH T-2 triol, and T-2 tetraol have been identified in guinea pigs (Pace et al., 1985) and swine (Corley et al., 1985). The de-epoxidation products of 3'-OH HT-2 and T-2 tetraol have been identified in rats (Yoshizawa et al., 1985). Pharmacokinetic parameters of T-2 toxin have been described in swine, cattle (Beasley et al., 1986), guinea pigs (Pace et al., 1985), and dogs (Sintov et al., 1986).

Interest in human exposure to trichothecene mycotoxins, their metabolic products, and detection of these compounds in biological fluids has encouraged us to define the time course of occurrence and disappearance of these metabolites in the primate model. The objectives of this study were to evaluate T-2 and its metabolites in serum and urine and to examine the pharmacokinetic and metabolic profile of T-2 toxin in a nonhuman primate model.

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## MATERIALS AND METHODS

*Chemicals.* Tritium-labeled T-2 toxin (labeled in the C-3 position, nonexchangeable radiopurity > 98%, specific activity 16.2 Ci/mmol) was obtained from Amersham Corp., Arlington Heights, IL. Unlabeled T-2 toxin was purchased from Calbiochem - Behring Diagnostics, San Diego, CA. Tritium-labeled HT-2 was prepared by alkaline hydrolysis of [<sup>3</sup>H]T-2 according to the method of Wei and Chu (1985). Tritium-labeled 3'-OH HT-2 and 3'-OH T-2 were obtained from F. S. Chu, University of Wisconsin, Madison, WI.

*Animals.* Four adult male, cynomolgus monkeys (*Macaca fascicularis*), weighing 4 - 7 kg were used for this study. Each monkey was given a complete physical examination; preliminary blood samples were subjected to hematological and clinical chemistry analysis. All monkeys were adapted to metabolic cages for 1 week and were allowed access *ad libitum* to monkey chow and water.

After an overnight fast, each monkey was anesthetized as described previously (McNamee et al., 1984). Halothane (0.7 - 1% in O<sub>2</sub>) was used to maintain anesthesia while iv catheters were inserted in the right atrium and femoral vein by aseptic techniques. The monkeys were fitted with a leather jacket which was attached to a previously described tethering system (McNamee et al., 1984). Patency of the atrial catheter was assured by slow infusion (100 ml/day) of 0.45% sodium chloride containing

4 u/ml of heparin. The femoral venous catheter was flushed daily and maintained with a heparin lock.

Three days after surgery, each monkey was injected via the femoral venous catheter with 0.1 ml/kg of saline containing 500  $\mu$ Ci of [ $^3$ H]T-2 mycotoxin (14.55  $\mu$ g/kg, sp. act. 16.2 Ci/mmol). At 5, 15, 30, 60 min; 2, 6 and 24 hr; and 2, 3, 7, and 14 days after exposure to the labeled T-2 mycotoxin, 5 ml blood samples were collected from the atrial catheters. Each blood sample was immediately placed in a 7 ml sodium fluoride vacutainer, mixed, poured into a 7 ml thrombin vacutainer, mixed, and placed in an ice bath. After 10 to 20 min, the blood samples were centrifuged and serum removed. Sera were stored at  $-80^{\circ}\text{C}$  until used for determination of [ $^3$ H]T-2 mycotoxin and its metabolites.

Urine and fecal samples were collected every 8 hr for 48 hr. The urine was collected in containers containing 1.7 g of sodium fluoride, and the cages washed down with 10 ml of water. Urine volume, pH, oral glucose, protein, and ketones were measured and recorded. After the first 48 hr, urine samples for each 24 hr were pooled and analyzed. A sample of urine was assayed for total radioactivity, and the remainder was stored at  $-80^{\circ}\text{C}$  until subjected to chromatographic analysis of [ $^3$ H]T-2 mycotoxin and its metabolites. Urine collections were continued for 28 days after exposure to the toxin.

Fecal samples were weighed, dissolved in 2N potassium hydroxide, and analyzed for total tritium content in a liquid scintillation counter (Beckman, Fullerton, CA). At 28 days postexposure, the monkeys were killed with T-61 (Am Hoechst

Corp., Somerville, NJ) and necropsied. Various tissues were removed, weighed, and dissolved in 2N potassium hydroxide. A sample of the digest was analyzed for total radioactivity.

*Determination of T-2 and metabolites in serum and urine.* Serum samples were thawed at room temperature. An aliquot (0.5 ml) of each serum sample was mixed with 0.25 ml of acetonitrile and kept in an ice bath for 60 min. Supernatants were collected after samples were centrifuged at 600 rpm at 4°C, for 15 min in an Eppendorf centrifuge (model 5415). Precipitated proteins were washed with 0.25 ml of acetonitrile, centrifuged, and the supernatants pooled. An aliquot of the pooled supernatants was analyzed for T-2 and its metabolites by high performance liquid chromatography (HPLC) (Beckman 450) with a radiometric detector, (Flo-One/Beta model CT, Radiomatic Instruments and Chemicals Co., Inc., Tampa, FL). Urine samples were centrifuged at 800 rpm for 15 min and analyzed by HPLC for T-2 and its metabolites.

T-2 and its metabolites were separated by reverse-phase HPLC with an Altex ultrasphere C-18 (250 x 4.6 mm) column. The solvent system was a gradient of water:acetonitrile. The gradient began with 25% acetonitrile for 3 min and increased linearly thereafter to 100% within 20 min. Flow rate was maintained at 1.0 ml/min. Radiometric detection was set to mix 1 ml of HPLC eluent with 4.0 ml of Flow Scint III (Radiomatic Inst. Inc., Tampa, FL) scintillation fluid. Under these conditions [<sup>3</sup>H]T-2, HT-2, 3'-OH T-2, 3'-OH HT-2, and T-2 tetraol were measured and quantitated by the peak area method by using

[<sup>3</sup>H]labeled standards. The radiometric detector efficiency was between 15-25%.

*Pharmacokinetic analysis.* Serum data are expressed as pmole/ml for T-2 and its metabolites. Urine data for mycotoxins are presented as pmole/hr, ( $\Delta X_u/\Delta t$ ) vs t, where t is the mid-collection time point (Gibaldi and Perrier, 1975).  $X_u$  represents the cumulative amount of unchanged toxin excreted in the urine and  $\Delta X_u/\Delta t$  is the average rate of renal excretion of unchanged toxin over a finite period of time. Urinary metabolites were subjected to the same data analysis.

Data from each monkey were initially analyzed for preliminary estimates of coefficients and exponents as a polyexponential model by a curve-stripping technique (CSTRIP). No assumptions were made with regard to the ratio of the exponents, and data points were all weighted as 1/observation.

The initial parameter estimates were then used as an input for nonlinear regression analysis, using a modified method of Gauss-Newton for minimization of the sum squares, to calculate the terminal linear slope. Toxin half-lives were calculated from the quotient  $0.693/\text{terminal rate constant}$  of the linear phase. The area under the serum concentration-time curve AUC was calculated according to the trapezoidal rule, with extrapolation to infinity, by dividing the last experimental concentration point by the terminal rate constant of the linear phase (Gibaldi and Perrier, 1975).

## RESULTS

Mean serum concentration-time data of T-2 and its metabolites measured after a single iv dose of T-2 are presented in Figure 1. Urinary excretion of T-2 and its metabolites is presented as pmol mycotoxin/hr vs mid-collection time point (Fig. 2). The pharmacokinetic parameters of T-2 and its metabolites in serum and in urine are shown in Tables 1 and 2, respectively. The apparent overall, first-order elimination rate constant ( $k$ ) of T-2 and its metabolites were calculated from the slope of the plot of the elimination phase of serum concentration-time data.

Considering the available information on T-2 metabolism in other species (Corley *et al.*, 1985; Pace *et al.*, 1985; Yoshizawa *et al.*, 1982), and the detectable quantities of T-2 and its metabolites in monkey serum and urine, a simple compartmental model (scheme 1) and metabolic pathway (scheme 2) were proposed.

In the proposed scheme 1, the following assumptions were made:

- a) linear kinetics is observed at administered dose.
- b) all doses and concentrations are in molar terms.
- c) for the parent toxin and its metabolites, distribution is much more rapid than disposition.
- d) all biotransformations are unidirectional.
- e) metabolite formation occurs only in the central compartment.

The apparent first-order elimination rate constants, for urinary excretion of T-2 and its metabolites ( $k_{10} \dots k_{50}$ ), were calculated from the final elimination phase of urinary excretion

data (plotted as  $\log X_u/dt$  vs  $t$ ). The overall elimination rate constant of T-2 and its metabolites ( $k_{le} \dots k_{5e}$ ) from serum is the sum of several constants for different biotransformation and excretion processes which can be expressed by the equations in Appendix 1, and calculated from the final elimination phase of serum concentration-time data (Fig. 1, scheme 1).

The mean values of all rate constants shown in scheme 1 appear in Table 3. The apparent first-order rate constant for [ $^3H$ ]toxin elimination from serum ( $k_{6e} = 0.145 \text{ hr}^{-1}$ ) was found to be similar to that calculated from equation 9 ( $0.141 \text{ hr}^{-1}$ ) (Appendix 1).

All serum metabolites levels (Fig. 1) were below the parent toxin serum levels, and (except for the T-2 tetraol) declined within one half-life of the parent toxin. After 2 hr, the metabolites were the only species measurable in serum. A plot of  $\log$  serum metabolite concentrations vs time eventually became linear with a slope of  $k/2.303$ , which was different from the slope of T-2 decline. The first-order rate constant for elimination of metabolites was smaller than the first-order rate constant for parent toxin. However, it was found that the  $k_{2e}$  ( $0.454 \text{ hr}^{-1}$ ) value of HT-2 was close to the  $k_{le}$  of T-2 ( $0.457 \text{ hr}^{-1}$ ) (Table 3).

The cumulative percent of [ $^3H$ ]label excreted in urine over 14 days represented  $68.95 \pm 7.73\%$  (mean  $\pm$  SE) of the administered dose, while  $31.89 \pm 7.07\%$  was eliminated through the feces (Fig. 3). Thus, approximately 100% of the dose was excreted through urine and feces within 14 days. The amounts of T-2 and HT-2 toxin excreted in urine over 156 hr represented less than 2% of

the total [<sup>3</sup>H]toxin urinary excretion; hydroxylated metabolites of T-2 were the major component (Table 4).

Four weeks post intoxication, the percent of toxin dose remaining in organs ranged from 0.0037% to 0.0269% with a mean of  $0.0131 \pm 0.0037\%$  (mean  $\pm$  SE). The residual [<sup>3</sup>H]label expressed as pmol/g and pmol/organ is presented in Table 5. Bone marrow contained the highest amounts of radiolabel represented as pmol/g. The remaining organs were ranked by mean value as follows: lymph node > liver > spleen > kidneys > adrenals > lungs > muscle > testes > brain > heart > fat > bile > urine > and blood. However, when the residual dose was expressed as pmol/organ, the liver was found to contain the highest amount.

## DISCUSSION

The linear mammillary disposition model with two compartments was chosen to describe the kinetics of T-2 toxin and its metabolites HT-2, 3'-hydroxy HT-2, and 3'-hydroxy T-2 in monkey serum (Table 1). T-2 tetraol concentration in serum was best described by a three-compartment open model with a lag time of 0.03 hr (Table 1). The urinary excretion data of 3'-hydroxy HT-2 and T-2 tetraol were best described by a two-compartment model (Table 2), while the excretion of 3'-hydroxy T-2 was simply described by a one-compartment model. In the early phase, the decline in T-2, HT-2, and 3'-hydroxylated products was fast, i.e., of short elimination half-life (0.17 - 1.45 hr).

Comparison of either integrated area under the curve (AUC) values (Table 1) or  $k_{12}$  ( $0.037 \text{ hr}^{-1}$ ), to  $k_{13}$  ( $0.419 \text{ hr}^{-1}$ ) (Table 3), indicated that 3'-hydroxylation of T-2 predominated over ester hydrolysis at the C-4 position (scheme 2). Similarly, 3'-hydroxylation of HT-2 ( $k_{24} = 0.454 \text{ hr}^{-1}$ ) was a more favored mechanism than ester hydrolysis of 3'-OH T-2 at the same C-4 position ( $k_{34} = 0.194 \text{ hr}^{-1}$ ). This was also reflected in the serum half-lives (HT-2  $t_{1/2} = 1.53 \text{ hr}$ ; 3'-OH T-2  $t_{1/2} = 5.1 \text{ hr}$ ). The formation constant of T-2 tetraol had a negative value (Table 3). This suggests that the biotransformation to T-2 tetraol may have proceeded through the formation of other metabolites such as 4-DN and/or 3'-hydroxy triol, indicated by a dashed arrow in scheme 2. However, these metabolites were not detected in urine or serum of the monkeys.

The apparent overall elimination rate constant for serum T-2 tetraol ( $k_{5e}$ ) was  $0.0369 \text{ hr}^{-1}$  while the urinary elimination rate constant ( $k_{50}$ ) was  $0.0315 \text{ hr}^{-1}$  (Table 3). This indicated that, although urinary excretion was a slow process, it was the major route for T-2 tetraol elimination. However, structural modification of HT-2 and the parent T-2 toxin was favored over urinary elimination. From equation 7 and 8, it was also concluded that the elimination pathway for T-2 and HT-2 was primarily through biotransformation and distribution, with a short half-life of distribution phase (alpha phase) of 0.14 and 0.22 hr, respectively (Table 1). The metabolic conversion of T-2 through the cleavage of the ester linkage at the C-4 position does not alter T-2 elimination (Table 3) or toxicity (Sato and Uno, 1977).

The short half-life and low serum AUC value for HT-2 (Table 1) suggested that HT-2 formation and biotransformation was so rapid that its detection in serum as an indicator of T-2 intoxication would be useful only within the first 6 hr following exposure. Similarly, the rapid biotransformation of T-2 hindered its late detection in serum. The AUC (Table 1) and serum levels of 3'OH T-2, 3'-OH HT-2, and T-2 tetraol (Fig. 1) were high enough to be detected in serum, even 48 hr after exposure.

A good agreement between the graphically obtained apparent first-order rate constant for [ $^3\text{H}$ ]toxin elimination from serum ( $k_{6e}$ ) ( $0.145 \text{ hr}^{-1}$ ) and that calculated by equation 9 ( $0.141 \text{ hr}^{-1}$ ) indicated that the proposed scheme (scheme 1) was a reasonable approximation of T-2 mycotoxin kinetics in monkeys.

The mean cumulative percent of [<sup>3</sup>H]label excreted in urine up to 6.5 days post intoxication represented insignificant amounts of T-2 (0.058%) and HT-2 (0.558%). The majority of urinary excretion was in the form of 3'-OH T-2 (17.72%), 3'-OH HT-2 (32.15%), and T-2 tetraol (26.17%) (Table 4). The mean elimination half-life of T-2 (1.29 hr) in monkeys (Table 1) was longer than that observed in swine (13.8 min), calves (17.4 min) (Beasley et al., 1986), and dogs (5.3 min) (Sintov et al., 1986). On the other hand, the fraction of T-2 toxin eliminated as parent toxin in monkeys (Table 4), swine, and calves (Beasley et al., 1986) urine was negligible.

The total radioactivity in the urine initially declined sharply, then increased between 24 and 48 hr, and finally declined linearly (Fig. 2). Such a profile may reflect the animal's physiological recovery period from mycotoxin toxic effects. However, if such an explanation was applicable, the curvature would be observed for T-2 and all metabolites. Since it was observed in the case of 3'-OH T-2, 3'-OH HT-2, and T-2 tetraol, and not for T-2 or HT-2, this profile might be due to enterohepatic circulation. Differences between T-2 and HT-2 urinary profiles and those of the other metabolites (Fig. 3), may also be the result of the high biotransformation rate of these two mycotoxins, overwhelming the effect of enterohepatic circulation. The prolonged excretion time of [<sup>3</sup>H]toxin detected 14 days post intoxication also suggests a functional enterohepatic circulation. Moreover, the persistence of

[<sup>3</sup>H]toxin in the body (4 weeks post intoxication) may be a major consequence of its enterohepatic circulation.

Glucuronide conjugates of mycotoxins were identified in the bile and urine of swine and guinea pigs (Corley *et al.*, 1985; Pace *et al.*, 1985) but not that of monkeys. Although conjugates were not detected in monkey serum or urine, it is possible that conjugates were excreted in feces (which were not tested) or underwent hydrolysis. Conjugates can be hydrolyzed to their aglycones by gut bacterial hydrolases; such a reaction would hinder the detection of mycotoxin conjugates in urine and feces.

In monkeys, the cumulative percent of [<sup>3</sup>H]toxin excreted in the urine and feces over 14 days (Fig. 3) represented a mean value of 68.95% and 31.89% of the administered dose, respectively. The maximum cumulative percent of excretion in feces (30%) and in urine (66%) was approached by the fifth and third day, respectively. Thereafter, only an additional 2% excretion in urine and 2% in feces was observed. By contrast, it was found that 88 to 91% of radioactivity was lost via urine within 4 hr after [<sup>3</sup>H]T-2-toxin administration to swine (Corley *et al.*, 1985). In mice, 57% of the total orally administered dose of T-2 was eliminated through the feces while only 12% was eliminated in the urine (Matsumoto *et al.*, 1978). Such species differences in the elimination of T-2 mycotoxin and its slow elimination in primates might be extended to humans.

The amount of residual dose (pmol/g) (Table 5) was uniformly distributed to all organs, except for bone marrow and bodily fluids, without tissue binding specificity. Such a distribution

pattern would result if all residuals of T-2 toxin were biotransformed into highly polar metabolites such as T-2 tetraol. This distribution, however, reflects only the toxin remaining after 28 days, which represents 0.013% of the administered dose.

From the intercepts of Y-axis of alpha and beta phase of urinary excretion rate data (Fig. 2), it was found that 3'-OH HT-2 is the major metabolite present in the early excretion phase followed by 3'-OH T-2 and T-2 tetraol. Their relative mean serum half-lives (3'-OH T-2 = 18.85 hr; 3'-OH HT-2 = 43.72 hr; T-2 tetraol = 72.12 hr) (Table 1) indicated that T-2 tetraol was the primary species in the late excretion phase. Therefore, analytical methods designed to detect exposure to T-2 should be capable of detecting 3'-OH HT-2, 3'OH T-2, and T-2 tetraol.

In summary, the distribution and elimination of T-2 toxin in the monkey, a primate model, were different from those in swine, cattle, and dogs. In monkeys, T-2 toxin and its metabolites possessed long half-lives and slow elimination rates similar to those exhibited in guinea pigs (Pace et al., 1985). [<sup>3</sup>H]toxin was found evenly distributed in several organs 28 days following the administration of a nonlethal dose of T-2 toxin. T-2 metabolites were detected in monkey urine samples for 6 days after a single low-dose iv exposure. It is possible that T-2 toxin distribution, biotransformation, and elimination in monkeys may parallel that in humans.

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## REFERENCES

- BEASLEY, V. R., SWANSON, S. P., CORLEY, R. A., BUCK, W. B.,  
KORITZ, G. D., AND BURMEISTER, H. R. (1986). Pharmacokinetics  
of the trichothecene mycotoxin, T-2 toxin, in swine and  
cattle. *Toxicon* 24, 13-23.
- CORLEY, R. A., SWANSON, S. P., AND BUCK, W. B. (1985).  
Glucuronide conjugates of T-2 toxin and metabolites in swine  
bile and urine. *J. Agric. Fd. Chem.* 33, 1085-1088.
- GIBALDI, M., AND PERRIER D. (1975). Pharmacokinetics, Drug and  
the Pharmaceutical Sciences. Vol. 1, pp. 6-9, 16-18. Marcel  
Dekker, Inc., New York and Basel.
- MATSUMOTO, H., ITO, T., AND UENO, Y. (1978). Toxicological  
approaches to the metabolites of *Fusaria*. XII. Fate and  
distribution of T-2 toxin in mice. *Jpn. J. Exp. Med.* 48, 393-  
399.
- MCNAMEE, G. A., WANNEMACHER, R. W., JR., DINTERMAN, R. E.,  
ROZMIAREK, H., AND MONTREY, D. (1984). A surgical procedure  
and tethering system for chronic blood sampling, infusion, and  
temperature monitoring in caged nonhuman primates. *Lab. Anim.  
Sci.* 34, 303-307.

- PACE, J. G., WATTS, M. R., BURROWS, E. P., DINTERMAN, R. E.,  
MATSON, C., HAUER, E. C., AND WANNEMACHER, R. W., JR. (1985).  
Fate and distribution of <sup>3</sup>H-labeled T-2 mycotoxin in guinea  
pigs. *Toxicol. Appl. Pharmacol.* **80**, 377-385.
- SATO, N., AND UENO, Y. (1977). Comparative toxicities of  
trichothecenes. In *Mycotoxins in Human and Animal Health*. J.  
V. Rodricks, C. W. Hesseltine, and M. A. Mehlman (Eds). p.p.  
295-307. Pathotox, Park Forest South, IL.
- SINTOV, A., BIALER, M., AND YAGEN, B. (1986). Pharmacokinetics  
of T-2 toxin and its metabolite HT-2 toxin, after intravenous  
administration in dogs. *Drug Metab. Dispos.* **14**, 250-254.
- WEI, R. D., AND CHU, F. S. (1985). Modification of *in vitro*  
metabolism of T-2 toxin by esterase inhibitors. *Appl. Environ.  
Microbiol.* **50**, 115-119.
- YOSHIZAWA, T., MIROCHA, C. J., BEHRENS, C. J., AND SWANSON, S. P.  
(1981). Metabolic fate of T-2 toxin in a lactating cow. *Food  
Cosmet. Toxicol.* **19**, 31-39.
- YOSHIZAWA, T., SAKAMOTO, T., AYANO, Y., AND MIROCHA, C. J.  
(1982). 3'-Hydroxy T-2 and 3'-hydroxy HT-2 toxins: new  
metabolites of T-2 toxin, a trichothecene mycotoxin in  
animals. *Agric. Biol. Chem.* **46**, 2613-2615.

YOSHIZAWA, T., SAKAMOTO, T., AND KUWAMURA, K. (1985). The structure of deepoxytrichothecene metabolites from 3'-hydroxy HT-2 toxin and T-2 tetraol in rats. *Appl. Environ. Microbiol.* 50, 676-679.

TABLE 1

MEAN KINETIC DATA OF T-2 MYCOTOXIN AND ITS METABOLITES IN MONKEY SERUM

Toxin	A (pmol/ml)	B	t1/2 $\alpha$ (hr)	t1/2 $\beta$ (hr)	AUC (pmol.hr/ml)
T-2	172.13	4.624	0.17	1.29	47.36
	$\pm 42.63$	1.99	0.08	0.83	18.19
HT-2 (pooled)	2.48	0.62	0.22	1.53	2.17
3'-OH T-2	30.42	2.79	0.43	5.10	30.46
	$\pm 12.43$	0.67	0.35	2.91	18.29
3'-OH HT-2 (pooled)	2.67	0.02	1.45	43.76	990.65
T-2 Tetraol <sup><math>\alpha</math></sup>	-13.344	14.10	0.13	0.80	540.05
	$\pm 3.97$	8.69	0.05	0.27	336.36
	P = 11.2 (pmol/ml) $\pm 5.15$	t1/2 $\pi$ = 72.17 (hr) $\pm 33.81$	Lag time = 0.031 (hr) $\pm 0.013$		

<sup>$\alpha$</sup>  Three compartment open model with lag time

Abbreviations are: A = zero time intercept of the first phase, B = zero time intercept of the elimination phase, t1/2 $\alpha$  = half-life of the first phase, t1/2 $\beta$  = terminal biological half-life, P = zero time intercept of the third compartment, t1/2 $\pi$  = half-life of the third compartment, (AUC) = area under the curve from t = 0 to  $\infty$ .

Values are mean  $\pm$  SE, n = 4

TABLE 2

KINETIC DATA OF T-2 METABOLITES CALCULATED FROM URINARY  
EXCRETION IN THE MONKEY

Toxin	A (nmol/ml)	B	t1/2 $\alpha$ (hr)	t1/2 $\beta$ (hr)	AUC (nmol)
3'OH T-2	3.77			7.50	41.72
( $\alpha$ )	$\pm 0.79$			0.23	13.13
3'OH HT-2	3.68	0.58	8.31	14.70	52.81
	$\pm 1.60$	0.32	0.93	2.06	19.95
Tetraol	2.58	0.11	8.92	28.59	40.33
	$\pm 0.67$	0.06	0.79	10.13	9.75

Note: T-2 and HT-2 were detected, but there were insufficient data for kinetic analysis.

$\alpha$  Data were fitted with one-compartment open model.

Abbreviations are: A = zero time intercept of the first phase; B = zero time intercept of the elimination phase; t1/2 $\alpha$  = half-life of the first phase; t1/2 $\beta$  = terminal biological half-life; AUC = area under the curve. Values are mean  $\pm$  SE, n = 4.

TABLE 3

ELIMINATION AND BIOTRANSFORMATION RATE  
CONSTANTS FOR T-2 AND ITS METABOLITES IN THE MONKEY

Apparent Rate Constant	Mean Value (hr <sup>-1</sup> )
k1e	0.457
k2e	0.454
k3e	0.259
k4e	0.008
k5e	0.0369
k6e	0.145
k10	0
k20	0
k30	0.0659
k40	0.0433
k50	0.0315
k12	0.037
k13	0.419
k24	0.454
k34	0.194
k45	-0.043

TABLE 4

CUMULATIVE PERCENT OF [<sup>3</sup>H]TOXIN EXCRETED IN MONKEY URINE (0-6.5 DAYS)

Toxin	Mean	SD	Range
T-2	0.058	0.084	0 - 0.176
HT-2	0.558	0.644	0.01 - 1.49
3'-OH T-2	17.720	12.004	0.78 - 27.03
3'-OH HT-2	32.153	11.94	15.51 - 42.12
T-2 Tetraol	26.170	4.94	21.35 - 33.10

Values are mean  $\pm$  SD, n = 4.

TABLE 5

DISTRIBUTION OF [<sup>3</sup>H]TOXIN IN MONKEY ORGANS 28 DAYS AFTER  
INTOXICATION WITH AN iv DOSE (14.55 µg/kg) OF T-2

Organ/Tissue	pmol/organ X10E-3	pmol/g X10E-6
Liver	11.34 ± 2.42	112.17 ± 21.44
Brain	4.37 ± 0.79	66.14 ± 10.54
Lungs	3.77 ± 1.20	79.96 ± 21.25
Testes	3.03 ± 0.65	72.02 ± 13.31
Kidneys	1.92 ± 0.45	98.50 ± 27.84
Heart	1.24 ± 0.26	58.00 ± 14.13
Spleen	0.91 ± 0.11	103.25 ± 15.36
Adrenal	0.07 ± 0.02	93.94 ± 19.60
Lymph nodes	N.D.	119.19 ± 37.35
Fat (scrotal)	N.D.	47.18 ± 13.59
Muscle	N.D.	76.73 ± 15.3
Bone marrow	N.D.	204.55 ± 82.02
Bile <sup>a</sup>	N.D.	35.33 ± 10.63
Urine <sup>a</sup>	N.D.	27.75 ± 12.93
Blood <sup>a</sup>	N.D.	22.51 ± 5.69

<sup>a</sup> Data represented as pmol/ml

N.D. not determined. Values are mean ± SE, n = 4.

FIG. 1. Mean T-2, HT-2, 3'-OH T-2, 3'-OH HT-2, and T-2 tetraol serum levels in the monkey. Each point represents the mean, n = 4.

FIG. 2. Mean urinary excretion rate of [<sup>3</sup>H]mycotoxins T-2, HT-2, 3'-OH T-2, 3'-OH HT-2, and T-2 tetraol in monkeys. Each point represents the mean, n = 4.

FIG. 3. Cumulative percent of dose excreted after administration of [<sup>3</sup>H]T-2 toxin. Each point is the mean  $\pm$  SE, n = 4.

Scheme 1: Pharmacokinetic model of T-2 and its metabolites in monkeys.

Scheme 2: Metabolic pathway of T-2 toxin in monkeys.

## APPENDIX 1

The apparent rate constants in Scheme 1 represent:

- k0 zero-order rate constant of T-2 iv bolus input.
- k10 apparent first-order rate constant of T-2 urinary excretion.
- k20 apparent first-order rate constant of HT-2 urinary excretion.
- k30 apparent first-order rate constant of 3'-OH T-2 urinary excretion.
- k40 apparent first-order rate constant of 3'-OH HT-2 urinary excretion.
- k50 apparent first-order rate constant of T-2 tetraol urinary excretion.
- k12 apparent first-order rate constant for the loss of T-2 from serum by metabolism into HT-2.
- k13 apparent first-order rate constant for the loss of T-2 from serum by metabolism into 3'-OH T-2.
- k24 apparent first-order rate constant for the loss of HT-2 from serum by metabolism into 3'-OH HT-2.
- k34 apparent first-order rate constant for the loss of 3'-OH T-2 from serum by metabolism into 3'-OH HT-2.
- k45 apparent first-order rate constant for the loss of 3'-OH HT-2 from serum by metabolism into T-2 tetraol.

The overall elimination rate constants of T-2 and its metabolites ( $k_{1e}$ ... $k_{5e}$ ) from serum are:

$$\text{T-2:} \quad k_{1e} = k_{10} + k_{12} + k_{13} \quad \text{Eq. 1}$$

$$\text{HT-2:} \quad k_{2e} = k_{20} + k_{24} \quad \text{Eq. 2}$$

$$3'\text{OH T-2:} \quad k_{3e} = k_{30} + k_{34} \quad \text{Eq. 3}$$

$$3'\text{OH HT-2:} \quad k_{4e} = k_{40} + k_{45} \quad \text{Eq. 4}$$

$$\text{T-2 Tetraol:} \quad k_{5e} = k_{50} \quad \text{Eq. 5}$$

$$^3\text{H-Toxin (total):} \quad k_{6e} = k_{10} + k_{20} + k_{30} + k_{40} + k_{50} \quad \text{Eq. 6}$$

$k_{12}$ ,  $k_{13}$ ,  $k_{24}$ ,  $k_{34}$ , and  $k_{45}$  values in scheme 1 were determined by substituting the corresponding terms in Eq. 1 through 5.

Since the amounts of T-2 and HT-2 excreted in urine were 0.058% and 0.558%, respectively, of the total urinary [ $^3\text{H}$ ]toxin

excretion during 6.5 days,  $k_{10}$  and  $k_{20}$  were approximated to a value of zero. Consequently, equation 1, 2 and 6 were simplified to:

$$k_{1e} = k_{12} + k_{13} \quad \text{Eq. 7}$$

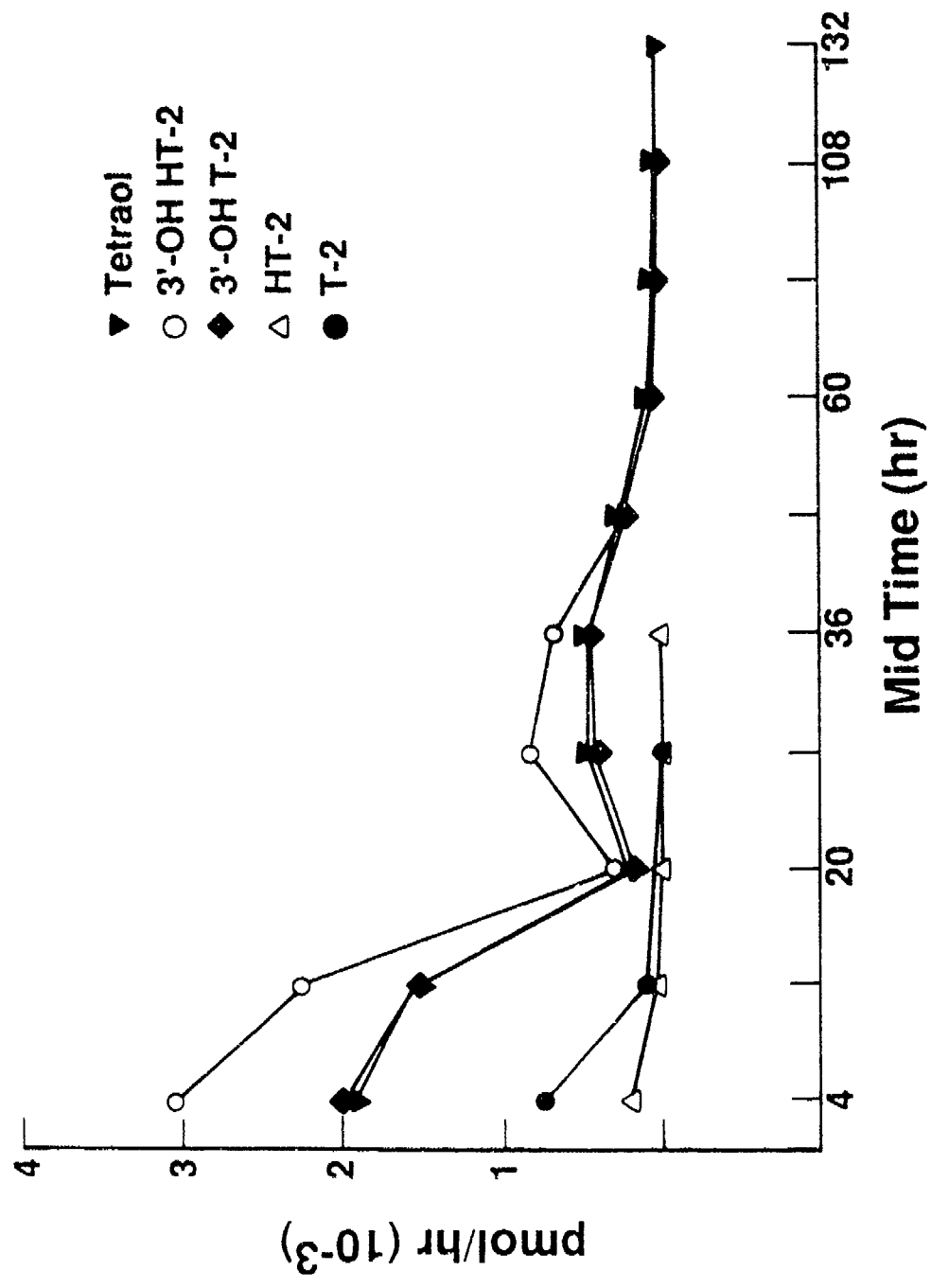
$$k_{2e} = k_{24} \quad \text{Eq. 8}$$

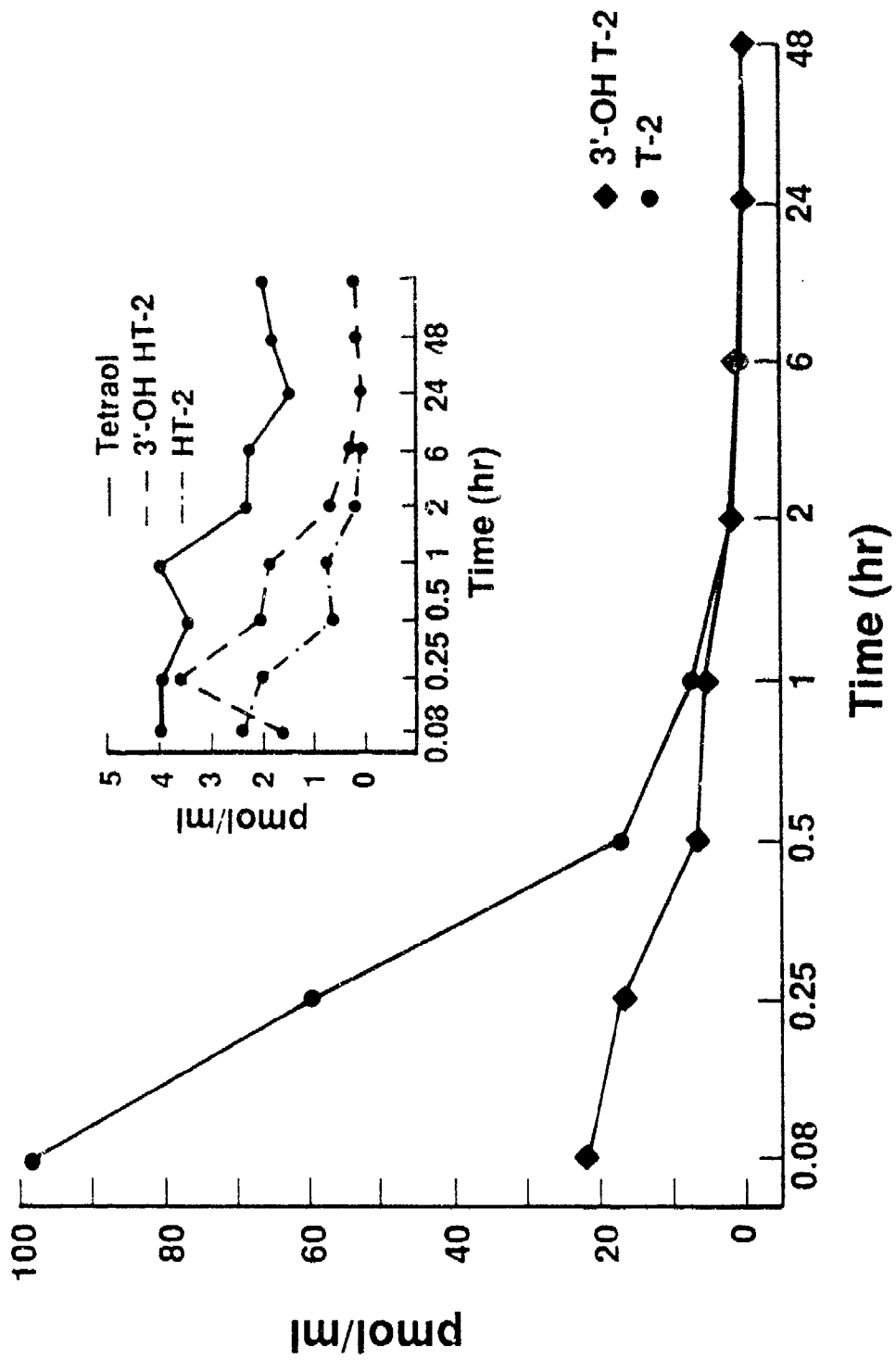
$$k_{6e} = k_{30} + k_{40} + k_{50} \quad \text{Eq. 9}$$

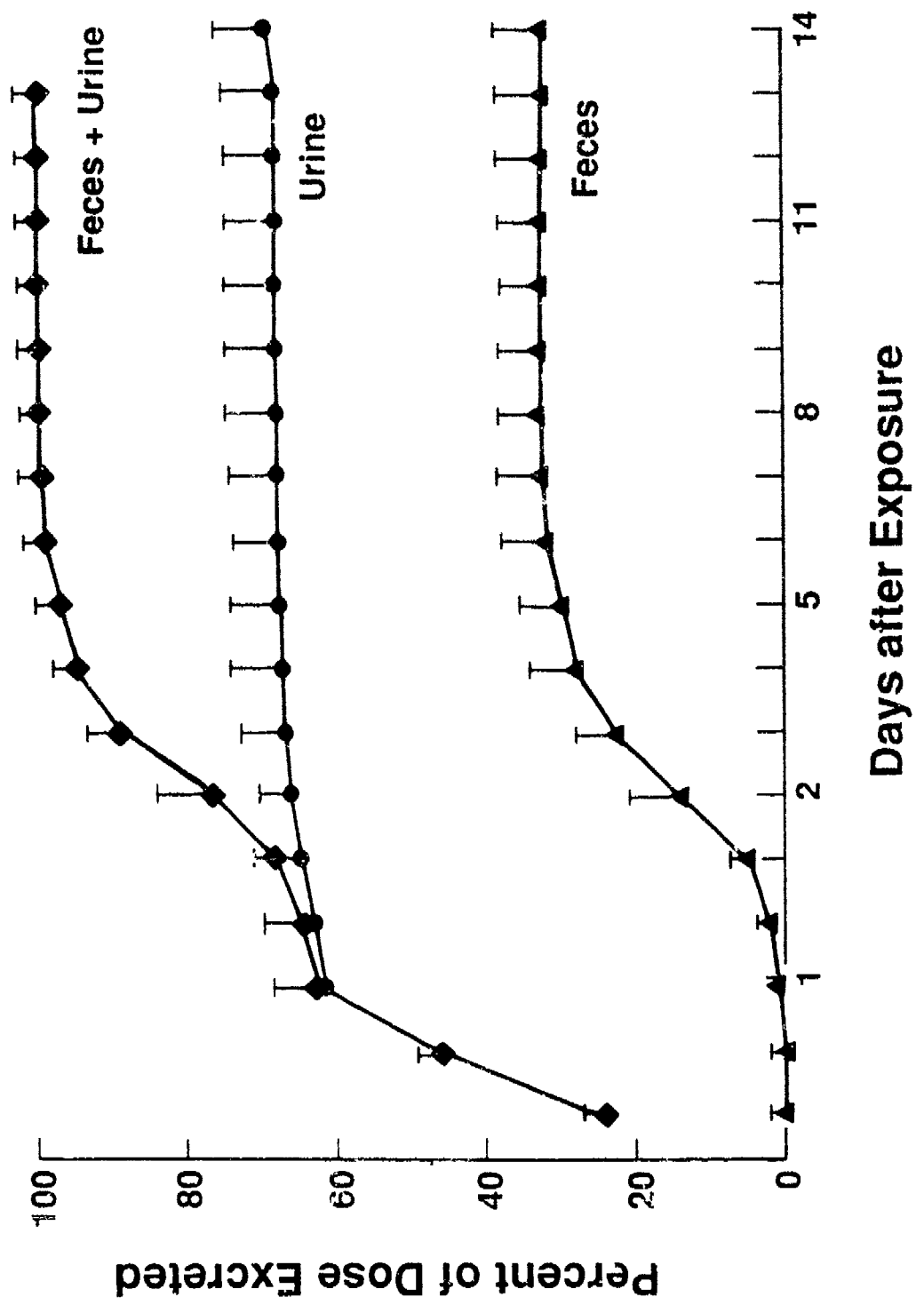
The  $k_{1e}$  (equation 7) value is known from the terminal elimination phase of T-2 and serum data. The ratio of  $k_{12}/k_{13}$  is represented by the serum AUC ratio as:

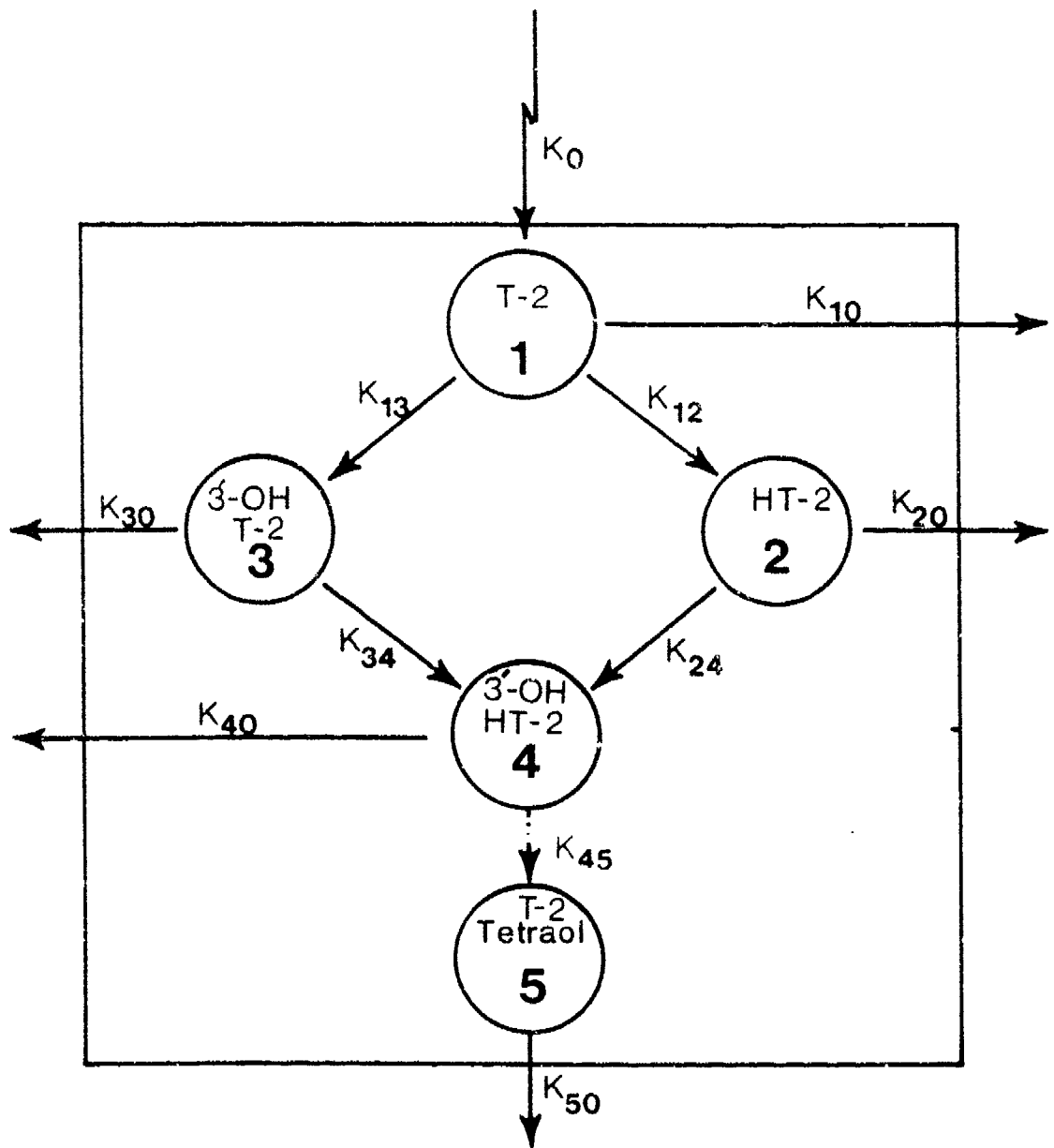
$$\frac{k_{12}}{k_{13}} = \frac{\text{AUC (HT-2)}}{\text{AUC (3'-OH T-2)}} \quad \text{Eq. 10}$$

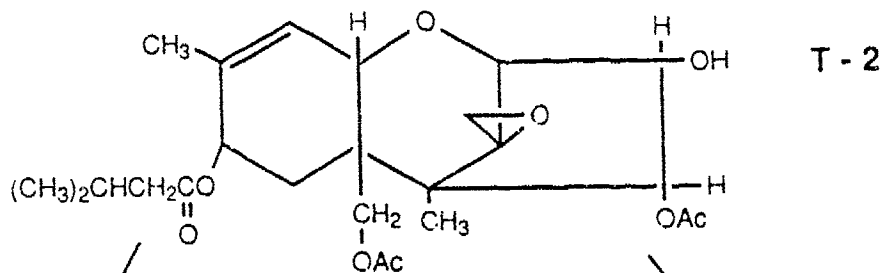
The value of  $k_{12}$  and  $k_{13}$  was obtained by solving equation 7 and 10.



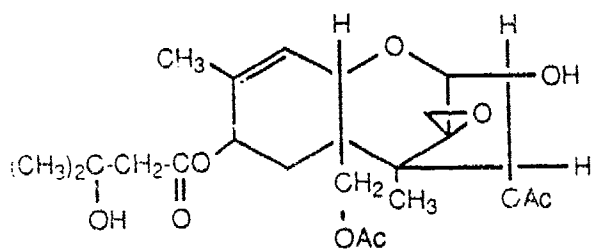




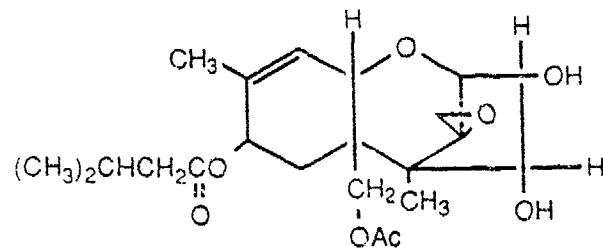




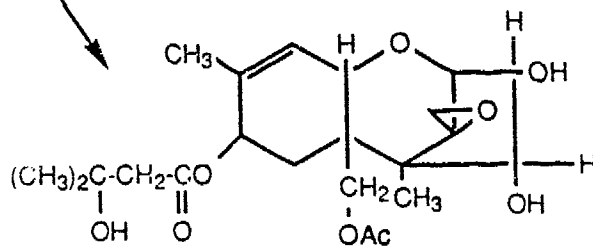
**3' HYDROXY T-2**



**HT-2**



**3' HYDROXY HT-2**



**T-2 TETRAOL**

