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Acute Inhalation Toxicity of T-2 Mycotoxin in the Rat and Guinea Pig

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Acute Inhalation Toxicity of T-2 Mycotoxin in the Rat and Guinea Pig. CREASIA, D. A., THURMAN, J. D., WANNEMACHER, R. W., JR., AND BUNNER, D. L. (1990). Fundam. Appl. Toxicol. 14, 54–59. In this study, concentration-response parameters were determined for rats and guinea pigs systematically exposed to an aerosol of T-2 toxin. The LC50 for a 10-min exposure to T-2 toxin aerosol was 0.02 mg T-2/liter air for rats and 0.21 mg T-2/liter air for guinea pigs. Data from total T-2 deposition in rats and guinea pigs exposed to their respective LC50 aerosol concentration gave an LD50 of 0.05 mg T-2/kg body weight for the rat and 0.4 mg T-2/kg body weight for the guinea pig. These data show that inhaled T-2 toxin is approximately 20 times more toxic to the rat (0.05 mg T-2/kg body wt inhaled vs 1.0 mg T-2/kg body wt ip) and at least twice as toxic to the guinea pig (0.4 mg T-2/kg body wt inhaled vs 1-2 mg T-2/kg body wt ip) than ip administered T-2 toxin. Histopathologic examination of major organs in both the rat and guinea pig after respiratory exposure to T-2 toxin indicated that lesions were similar to those described after systemic administration of the toxin. Gross and microscopic alterations of respiratory tract tissue after T-2 aerosol exposure were minimal and could not account for the increase in toxicity. • e 1990 Society of Toxiclogy.

1-2 foxin, a small nonprotein trichothecene mycotoxin, is produced by various species of Fusai um fungi (Bamburg et al., 1968; Ciegler, 1978; Joffe, 1971; Lutsky et al., 1978). The chemistry and systemic toxicity of T-2 and related trichothecenes have been extensively reviewed (Bamburg and Strong, 1971; Committee on Protection Against Trichothecene Mycotoxins, 1983). There are only a few reports in the literature, however, on the inhalation toxicity of T-2 or related mycotoxins (Pang et al., 1988; Creasia et al., 1987; Marrs et al., 1986; Ueno, 1984) and there are discrepancies in these reports on the degree of acute toxicity from inhaled T-2. We have previously reported that T-2 toxin is at least 10 times more toxic to the mouse by inhalation than by systemic administration

(Creasia *et al.*, 1987). Marrs *et al.* (1986) reported a similar or slightly higher LD50 from inhaled T-2 than from subcutaneously administered T-2 toxin in the guinea pig, and Pang *et al.* (1988) suggests a higher LC50 from inhaled T-2 than from intravenously administered T-2 in the swine. In order to address these discrepancies, we have extended our acute inhalation studies to include the rat and guinea pig and we have assessed the effect of extended duration of aerosol exposure (exposure methodology utilized in the reports by Marrs and Pang) on the acute toxicity of inhaled T-2 in the guinea pig. We report our findings on these subjects in this paper.

METHODS

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Animals. The animals used in this study were 190-200 g, male outbred Hartley Crl:(HA)BR VAF/Plus guinea

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0272-0590/90 \$3.00 Copyright © 1990 by the Society of Toxicology. All rights of reproduction in any form reserved. pigs and 90-100 g, male CDF (F-344)/CrIBR VAF/Plus rats obtained from Charles River Breeding Labs (Kingston, NY). All animals were held in Bio Clean enclosures (Lab Products, Inc. Maywood, NJ) for 1 week for observation and acclimatization before being used in this study. The guinea pigs were kept in stainless-steel cages, 10 per cage on hardwood chips, and had free access to food (NIH-34M) and water. The rats, 5 per open-bottom polycarbonate cage, also had free access to food (NIH-07) and water. Animal rooms were maintained at 24°C, 50% relative humidity, 12-hr light/dark cycle and air flow was 12 room air changes per hour. At the time of the study, the average weight of the guinea pigs was 325 g (range 312-331) and for rats, 105 g (range 99-108).

Chemicals. Purified (>99%) T-2 mycotoxin was obtained from Myco Labs (Chesterfield, MO) as a white crystalline powder and was confirmed to be >99% pure by both HPLC and GC-mass spectrophotometer analysis. Synthetic, nonexchangeable [3 H]T-2, dissolved in ethanol (ETOH) (spec act 11 Ci/mmol; 1.1 μ mol/ml), was obtained from Amersham International, Amersham UK, for use as a tracer for aerosol mass concentrations measurements and to quantitate deposition of T-2.

Aerosol exposure. The inhalation exposure chamber was designed for nose-only exposure. Basically, aerosol exposure for both rats and guinea pigs was accomplished by placing each animal in an open-ended, cone-shaped holder with only the animal's nose protruding into a 1.5liter aerosol chamber. The chamber was operated dynamically at 2.2 liters/min. The test aerosols were generated by atomizing ETOH solutions of T-2 mycotoxin with a Lovelace nebulizer (Intox Products, Albuquerque, NM) operated at 2.2 liters/min (Mercer et al., 1968). The ETOH solutions of T-2 were prepared by first dissolving weighed quantities of crystalline T-2 in measured volumes of pharmaceutical-grade ETOH. Approximately 0.06 mCi (50 µl) from an ETOH solution of [3H]T-2 was added as a tracer for aerosol mass concentration measurements, and 0.6 mCi (0.5 ml) from the same solution was added as a substitute for pharmaceutical-grade ETOH for deposition and retention studies in rats and guinea pigs exposed to the T-2 aerosol. The mass concentration of the T-2 aerosol was varied by atomizing different concentrations of T-2 toxin in ETOH and was measured by scintillation counting (Beckman L55800 scintillation counter, Beckman Instruments, Inc., Irvine, CA) of the ³H tracer. Aerosol mass concentration measure-

ments were obtained from 1.0-liter samples taken on fiberglass filters (Gelman Scientific Inc., Ann Arbor, MI). The range of the aerosol mass concentrations used in this study was 2.0 to 0.001 mg T-2/liter air. Aerosol particle size was determined with a Mercer type (Mercer *et al.*, 1970) cascade impactor (Intox Products, Alburquerque, NM) operated at 100 cm³/min. The average aerodynamic mass median diameter of the aerosol particles was 0.6 μ m with a σ_{g} of 1.6 to 1.8. The aerosol remained as spherical liquid droplets as observed by light microscopy. Unless otherwise noted, all aerosol exposures were for 10 min. Surviving animals were observed at least twice daily for 1 week postexposure, but only those animals dying within 24-hr postaerosol exposure were used for LD50 calculations. The slope of the concentration-response curves was analyzed by probit analysis to calculate the LC50s (Finney, 1977).

Retention of inhaled T-2. Retention of inhaled T-2 was measured in two groups. One group of 12 rats was exposed to 0.023 mg of T-2/liter air (~rat LC50); the other group of 12 guinea pigs was exposed to 0.310 mg T-2 liter air (~guinea pig LC50). Six animals from each species and group were arbitrarily selected within a few minutes postexposure and killed by CO₂ asphyxiation. The intact carcasses were placed in either 250 ml (rats) or 500 ml (guinea pigs) of 2.0 N KOH and incubated for 48 hr at 37°C. Triplicate 1.0-ml aliquots were then taken and assayed for ³H. The remaining six animals of each species were held and observed for lethality for 48 hr postexposure.

Aerosol concentrations \times aerosol exposure time study. The relationship between two variables—T-2 aerosol concentration (C) and length of time of T-2 aerosol exposure (T)—was evaluated in guinea pigs. Twelve guinea pigs were exposed for 10 min to T-2 aerosol concentrations of either 0.3 or 0.4 mg T-2/liter air. In the other series of exposures, time of exposure was 30 min and guinea pigs were exposed to either 0.15 or 0.17 mg of T-2/liter air. Lethality was determined after 24 hr.

Intraperitoneal administration of T-2 toxin. T-2 toxin was administered to both rats and guinea pigs by intraperitoneal injection according to standard laboratory procedure. All injection volumes were at 1.0 ml/kg body weight. The T-2 toxin used in this aspect of the study was obtained from aliquots taken prior to nebulization from the T-2 toxin preparation used for aerosol generation.

Pulmonary edema. Pulmonary edema (lung water content) was quantitated only in rats. These rats were first exposed to an aerosol of 2.0 mg T-2/liter air; 6 hr postexposure the animals were anesthetized with sodium pentobarbitol (60 mg/kg ip) and killed by exsanguination. The lower respiratory tract (larynx, trachea, and intact lung lobes) was then isolated from the carcass and extraneous lymphatic and cardiac tissues were carefully removed. The extent of pulmonary edema was then determined by weighing the respiratory tract tissue before and after drying at 99°C for 48 hr. Control animals exposed to either ETOH or air only were processed similarly.

Histopathology. Both rats and guinea pigs designated for tissue examinations were anesthetized with sodium pentobarbitol (60 mg/kg ip) and killed by exsanguination. After detailed gross inspection, the lungs were inflated with 5.0 ml of 10% buffered formalin, tied off, and, along with the following organs, were placed in the same fixative: brain, upper respiratory tract, heart, gastrointestinal tract, pancreas, liver, mesenteric lymph nodes,

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Rats		Guinea pigs		
Aerosol mass concentration (mg/liter)	No. dead/ No. exposed ^h	Aerosol mass concentration (mg/liter)	No. dead/ No. exposed ^c	
1.0	12/12	0.92	6/6	
0.1	12/12	0.76	6/6	
0.05	11/12	0.66	6/6	
0.03	8/12	0.40	5/6	
0.02	5/12	0.30	3/6	
0.01	2/12	0.25	3/6	
0.001	0/12	0.15	0/6	
Control	0/12	0.075	1/6	
(ETOH only)		0.025	0/6	
		Control	0/6	
		(ETOH only)		

TABLE I		
RAT AND GUINEA PIG MORTALITY FROM INHALED ^a T-2 Mycotoxii	N	

" A 10-min aerosol exposure.

^h Twenty-four hour LC50 for the rat: 0.020 mg T-2/liter air (0.012-0.027; 95% CI).

^c Twenty-four hour LC50 for the guinea pig: 0.209 mg T-2/liter air (0.133-291; 95% Cl).

spleen, thymus, kidneys, adrenal glands, and testes. All tissues were fixed a minimum of 48 hr prior to embedding in paraffin. Sections were cut $5 \,\mu$ m thick and stained with hematoxylin and eosin. All tissue sections were examined by light microscopy.

RESULTS

The results of exposing both rats and guinea pigs to various aerosol mass concentrations of T-2 toxin are summarized in Table 1. All rats exposed to either 0.1 or 1.0 mg T-2/liter air died within 12 hr. None of the

rats exposed to T-2 toxin showed any overt clinical signs of toxicity immediately postexposure, but they did become lethargic about 2 hr before death. All of the rats that survived for 24 hr (i.e., those exposed to lower aerosol concentrations) were still alive 1 week later.

All guinea pigs exposed to aerosol concentrations ≥ 0.40 mg T-2/liter air died within 18 hr. Clinical signs were similar to those observed in rats, except that guinea pigs, in addition to becoming lethargic, tended to become prostrate and laterally recombent prior to death.

TABLE	2
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EFFECT OF INHALED T-2" ON PULMONARY WET/DRY WEIGHT IN RATS				
			Lung weight (mg) ^b	
Treatment	N	Wet	Dry	Difference
Air only	6	959 ± 47	225 ± 13	734 ± 33
ETOH only	6	964 ± 53	224 ± 15	740 ± 41
T-2 and ETOH	12	960 ± 53	225 ± 11	745 ± 22

"T-2 aerosol mass concentration = 2.0 mg T-2/liter air.

" Mean ± SE.

INHALED T-2 TOXIN

RETENTION OF INHALED 1-2 MYCOTOXIN BY THE RAT AND GUINEA PIG"				
Species	Aerosol mass concentration	No. dead/	mg equivalents of T-2 ^{b,c}	
	(mg T-2/liter air)	No. exposed	(whole body retention)	
Rat	0.023	4/6	0.0046 ± 0.0012	
Guinea pig	0.310	4/6	0.126 ± 0.0144	

TABLE 3

"A 10-min aerosol exposure.

^b N = 6 (different animals than in dead/exposed group).

" Mean ± SE.

No fulminating pulmonary edema was observed in any of the rats or guinea pigs exposed to T-2 toxin aerosol. When the wet weight and the dry weight of the excised lower respiratory tract of rats exposed to 2.0 mg T-2/liter air were compared to tissue from control rats, no difference was found in the water content (Table 2). Additionally, the lungs of two to four animals from each group of rats or guinea pigs that died when exposed to the higher aerosol concentrations were examined for gross changes. No evidence of gross pulmonary hemorrhage was observed in any of these animals and the lungs readily collapsed when the diaphragm was punctured.

Retention of inhaled T-2. Data on the retention of inhaled T-2 by rats and guinea pigs are summarized in Table 3. Percentage lethality in both species indicates that the aerosol concentrations were in the LC50 range. The total milligram equivalents of inhaled T-2 toxin retained by each species exposed to its respective LC50 aerosol concentrations were taken as an LD50 dose for each species.

Aerosol concentration \times aerosol exposure time study. In these experiments we evaluated the effect of dose rate on mortality in guinea pigs that inhaled T-2 toxin aerosol. These data are summarized in Table 4 and clearly show that the effect of a faster dose rate from inhaled T-2 toxin aerosol is to increase mortality in guinea pigs.

Intraperitoneal administration of T-2. The LD50 of T-2 toxin for rats and guinea pigs ip injected was 1.5 mg T-2/kg body weight (0.98–1.9, 95% CI) and 1.2 mg T-2/kg body weight (0.86–1.53, 95% CI), respectively. This datum compares favorably with that already published (Fairhurst *et al.*, 1987; Feuerstein *et al.*, 1985).

Histopathology. No evidence of respiratory disease nor significant lesions were observed by light microscopy in either the upper respiratory tract or lungs in any of the control ani-

Aerosol mass concentration (mg T-2/liter air)	Time of exposure (min)	Exposure-rate ^a (mg-min T-2/liter air)	No. dead/ No. exposed
0.300	10	3.0	7/12
0.150	30	4.5	0/12
0.400	10	4.0	10/12
0.170	30	5.1	0/12

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* Exposure-rate was obtained by multiplying aerosol mass concentration \times time of exposure (i.e., $C \times T$).

mals or animals exposed to aerosols of T-2 mycotoxin. In other organs, we routinely observed lesions characteristic of systemic administration of T-2 toxin, which included necrosis of crypt epithelial cells in the small and large intestine and necrosis of lymphocytes in the thymus and spleen. The lymphocytes in the cortex of the thymus were the cells most sensitive to the toxin. Exposure to the higher aerosol mass concentrations resulted in necrosis of $\sim 90\%$ of the lymphocytes in the thymic cortex by 24 hr postexposure. Necrosis of spleenic lymphocytes of these animals was quantitatively less than that observed in the thymic cortex. Also, higher aerosol mass concentrations were required to produce intestinal crypt epithelial cell necrosis than the thymic or splenic cell lesions.

DISCUSSION

Using total deposition data from T-2 aerosol retention studies in both rats and guinea pigs exposed to their respective T-2 aerosol LC50 and using the average animal body weight at the time of aerosol exposure, we calculated an LD50 from inhaled T-2 of 0.046 mg T-2/kg body weight for the rat and an LD50 of 0.388 mg T-2/kg body weight for the guinea pig. Thus, data from this laboratory show that the potency of inhaled T-2 was approximately 20 times greater in the rat (0.05 mg/kg inhaled vs 1.0 mg/kg ip) and 2 to 3 times greater in the guinea pig (0.4 mg/kg inhaled vs 1-2 mg/kg ip) when compared to ip administered T-2.

Data on the comparative toxicity of inhaled vs systemically administered T-2 presented in this paper and in a previous report from this laboratory (Creasia *et al.*, 1987) are in contrast to data reported by Marrs *et al.* (1986) and Pang *et al.* (1988). Marrs reported no difference in toxicity between inhaled and subcutaneously administered T-2 toxin in guinea pigs. Differences in aerosol exposure methodology employed by the two laboratories may explain the variation in results, and is the reason we decided to assess the effect of duration of aerosol exposure on the acute toxicity of inhaled T-2 in the guinea pig. In the report by Marrs et al. (1986), inhalation toxicity data for T-2 were developed by maintaining aerosol concentrations essentially constant and incrementally increasing the duration of aerosol exposure. This method assumes at least a linear relationship among response (R), aerosol concentration (C), and time of exposure (T) (i.e., R = C $\times T$). There are reports in the literature (Amdur, 1980; Creasia, 1978; Phalen, 1984), however, indicating that this assumption is not necessarily correct. Phalen (1984) suggests that an overloading of a defense mechanism by a faster dose rate may account for a lack of linearity. The data presented in Table 4 of this report clearly document a lack of linearity for potency of inhaled T-2 toxin with time of exposure as the variable, and show that the effect of prolonged duration of aerosol exposure time is to reduce the apparent acute toxicity of inhaled T-2 in the guinea pig. Pang et al. (1988) used a similar aerosol exposure methodology in assessing concentration-response parameters to inhaled T-2 toxin in the swine. This could explain the differences in toxicity from inhaled T-2 toxin reported by the different laboratories.

Data from this study, however, do not explain the enhanced toxicity of inhaled vs systemically administered T-2 toxin. Normally, one would expect that increased mortality after inhalation of a toxic substance would be the result of asphyxiation from pulmonary injury and subsequent impairment of respiratory gas (i.e., O₂, CO₂) exchange. Pulmonary injury sufficient to produce acute impairment of respiratory gas exchange is usually readily apparent, even under gross pathological examination. However, we found no gross or histologic evidence of acute pulmonary injury. In addition, the observed clinical signs of animals that died following T-2 toxin aerosol exposure were not consistent with animals dying from asphyxiation.

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