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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.

(1988). *Fundam. Appl. Toxicol.* , - . In this study a concentration-response was 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 0.020 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 0.046 mg T-2/kg body weight for the rat and 0.50 mg T-2/kg body weight for the guinea pig. These data show that inhaled T-2 toxin is at least 10 times more toxic to the rat (0.046 mg T-2/kg body weight inhaled vs 1.0 mg T-2/kg body weight systemic) and twice as toxic to the guinea pig (0.5 mg T-2/kg body weight inhaled vs. 1-2 mg T-2 kg body weight systemic) than systemically administered T-2 toxin. Guinea pigs exposed to T-2 aerosols with varied times and aerosol concentration showed that when given the same received dose over a shorter interval of time, T-2 was more toxic. Histopathology of major organs in both the rat and guinea pig after respiratory exposure to T-2 toxin was similar to that after systemic administration of T-2 toxin. Histopathology of the respiratory tract after T-2 aerosol exposure was minimal and could not account for the increased toxicity of inhaled T-2 toxin.



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T-2 toxin, a small non-protein trichothecene mycotoxin, is produced by various species of *Fusarium fungi* and is the main toxic components of the mycotoxins produced by these 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 (Creasia, *et al.*, 1987; Marrs *et al.*, 1985; Ueno, 1984). We have previously reported (Creasia, *et al.*, 1987) that T-2 toxin is at least ten times more toxic to the mouse by inhalation than by systemic administration. We have extended the acute inhalation studies to include the rat and guinea pig, the subject of this paper.

METHODS

Animals. The animals used in this study were 190-200-g, male outbred Hartley Cr1:(HA)BR VAF/Plus™ guinea pigs and 90-100-g, male CDF®(F-344)/Cr1BR VAF/Plus™ rats obtained from Charles River Breeding Labs, Kingston, NY. All animals were held for 1 week for observation and acclimatization before being used in this study. The guinea pigs, held in stainless steel cages, 10 per cage on hardwood chips had free access to food (NIH-34M) and water. The rats, five per open-bottom polycarbonate cage, had free access to food (NIH-07) and water. Animal rooms were maintained at 24°C and 50% relative humidity 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 and for rats, 105 g.

Chemicals. Purified (>99%) T-2 mycotoxin was obtained from Myco Labs, Chesterfield, MO, as a white crystalline powder and was confirmed to be >99% by both HPLC and GC-mass spectrophotometer analysis. Synthetic, non-exchangable, [³H]T-2, dissolved in ethanol (ETOH) (specific activity 11Ci/mmol; 1.1 µmol/ml), was obtained from Amersham International, Amersham U.K., for use as a tracer.

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 1.5 liter aerosol chamber. The chamber was operated dynamically at 2.2 liter/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 mCi (50 μ l) from an ETOH solution of [3 H]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 ETOH solutions of T-2 and was measured by scintillation counting (Beckman L55800 Scintillation Counter, Beckman Instruments, Inc., Irvine CA) of the [3 H]-tracer. Samples for aerosol mass concentration measurements 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, Albuquerque, NM) operated at 100 cm³/min. The average aerodynamic 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 under light microscopy, indicating that the T-2 aerosol in this study was an aerosol of an ETOH solution of T-2. 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 post aerosol exposure were used for LD50 calculations. The slope of the concentration-response curves was analyzed by probit analysis to calculate the LC50's (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 immediately (i.e. within a few minutes) post exposure and were killed by CO₂ asphyxiation. The intact carcasses were then placed in either 250 ml (rats) or 500 ml (guinea pig) 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] (Beckman Instruments, Inc.). The remaining six animals of each species were held and observed for lethality for 48 hr postexposure.

Aerosol concentrations x 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 0.300 or 0.400 mg T-2/liter air. In the other series of exposures, time of exposure was 30 min and guinea pigs were exposed to either 150 or 170 µg of T-2/liter air. Lethality was determined after 24 hr.

Systemic Administration of T-2 Toxin. T-2 toxin was administered to both rats and guinea pigs by intraperitoneal injection (i.p.) 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 pentobarbital (60

mg/kg) 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 carefully removed. The extent of pulmonary edema was then determined by weighing the respiratory tract tissue before and after drying at 190°F for 48 hr. Control animals exposed to either ETOH or air only were processed similarly.

Histopathology. Both rats and guinea pigs designated for histopathological examinations were anesthetized with sodium pentobarbital ip and killed by exsanguination. After detailed gross inspection, the lungs were inflated with 5.0 ml of 10% buffered formalin and the following organs were removed and fixed in 10% buffered formalin: brain, upper respiratory tract, lung, heart, gastrointestinal tract, pancreas, liver, mesenteric lymph nodes, 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 μ m thick and were stained with hematoxylin and eosin (H&E). 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 and clearly show a species difference in susceptibility to inhaled T-2 toxin. 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 became 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 one week later.

All guinea pigs exposed to ≥ 0.40 mg T-2/liter air aerosol concentrations 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 recumbent prior to death.

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 dry-weight of the excised lower respiratory tract of rats exposed to 2.0 mg T-2/liter air was compared, no difference was found from the water content of the lower respiratory tract obtained from control rats (data not shown). Additionally, the lungs of two - four animals from each group of rats or guinea pigs that died when exposed to the higher aerosol concentrations were examined for gross signs of pathology. 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 2. Percent lethality in both species indicate that the aerosol concentrations were in the LC50 range. The total μg equivalents of inhaled T-2 toxin retained by each species exposed to its respective LC₅₀ aerosol concentrations was taken as an LD₅₀ dose for each species.

Aerosol Concentration x 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 3 and clearly show that the effect of a faster dose-rate from inhaled T-2 toxin aerosol is to increase mortality in guinea pigs.

Systemic Administration of T-2. The LD50 of T-2 toxin for rats and guinea pigs i.p. injected was 1.5 mg T-2/kg body weight (0.98 - 1.9, 95% C.I.) and 1.2 mg T-2/kg body weight (0.86 - 1.53, 95% C.I.) respectively. This data compares favorably with that already published. (Fairhurst et al., 1987; Feuerstein et al., 1985).

Histopathology. No significant lesions were observed by light microscopy in either the upper respiratory tract or lungs in any of the animals exposed to aerosols of T-2 mycotoxin. In other organs, examined microscopically, 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 ~ 90% of lymphocytes in the thymic cortex by 24 hr postexposure. Cellular necrosis was also observed in spleen lymphocytes of these animals but the necrosis 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 were required to produce the thymic or splenic cell lesions.

DISCUSSION

Using data from T-2 aerosol retention studies in both rats and guinea pigs exposed to their respective T-2 aerosol LC50, we calculated an LD50 from inhaled T-2 of 0.046 mg T-2/kg body weight for the rat and an LD50 of 0.50 mg T-2/kg body weight for the guinea pigs. Thus, data from this laboratory show that the potency of inhaled T-2 was at least 10 times greater in the rat (0.05 mg/kg inhaled vs 1.0 mg/kg i.p.) and twice as great in the guinea pig (0.5 µg/kg inhaled vs. 1-2 µg/kg i.p.) when compared to i.p. administered T-2.

Data on the comparative toxicity of inhaled vs systemically administered T-2 presented in this paper and in a previous report (Creasia *et al.*, 1987) from this laboratory are in contrast to data reported by Marrs *et al.*, (1986). These investigators reported no difference in toxicity between inhaled and systemically (sc) administered T-2 toxin to guinea pigs. Different aerosol exposure methodology employed between the two laboratories may explain the variation in the results. In the report by Marrs *et al.*, inhalation toxicity data for inhaled T-2 were developed by maintaining aerosol concentrations essentially constant and incrementally increasing the time of aerosol exposure. This methodology assumes at least a linear relationship between 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; Phelan, 1984), however, indicating that this assumption is not necessarily correct. Phelan (1984) suggests that an overloading of a defense mechanism by a faster dose-rate may account for this phenomenon. We believe Phelan's explanation to be also true for inhaled T-2 toxin (Table 3) and believe that this explains the difference in toxicity from inhaled T-2 toxin between the two laboratories. Regardless, the data presented in Table 3

clearly documents the lack of linearity for potency of inhaled T-2 toxin with time of exposure as the variable.

Data from this study, however, does 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. O_2 , CO_2) 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 evidence of acute pulmonary injury by either gross or by histological examination with the light microscope. Additionally, the observed clinical signs of animals that died following T-2 toxin aerosol exposure were not consistent with animals dying from asphyxiation.

Release of shock inducing mediators from the lung might be an explanation for the increased potency of inhaled T-2 toxin, but this has not been tested for.

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TABLE 1

RAT AND GUINEA PIG MORTALITY FROM INHALED

T-2 MYCOTOXIN

<u>Rats</u>		<u>Guinea Pigs</u>	
Aerosol Mass	No. Dead/	Aerosol Mass	No. Dead/
Concentration	No. Exposed	Concentration	No. Exposed
(mg/liter)		(mg/liter)	
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)	
24 hr LC ₅₀ : 0.020 mg T-2/liter air		24 hr LC ₅₀ : 0.209 µg T-2/liter air	
(0.012 - 0.027; 95% C.I.)		(0.133 - 291; 95% C.I.)	

^aA 10 min aerosol exposure

TABLE 2

RETENTION OF INHALED T-2 MYCOTOXIN BY THE
RAT AND GUINEA PIG^a

Species	Aerosol Mass Concentration (mg T-2/liter air)	No. Dead/ No. Exposed	µg Equivalents of T-2 ^{b,c} (whole body retention)
Rat	0.023	4/6	4.6 ± 1.2
Guinea Pig	0.310	4/6	126 ± 14.4

^aA 10 min aerosol exposure

^bN = 6

^cMean ± S.E.

TABLE 3

MORTALITY FROM INHALED T-2 IN THE GUINEA PIG:

EFFECT OF TIME OF EXPOSURE

Aerosol Mass Concentration (mg T-2 liter air)	Time of Exposure (min)	Dose-Rate ^a (mg-min T-2/liter air)	No. Dead/ No. Exposed
0.300	10	3000	7/12
0.150	30	4500	0/12
0.400	10	4000	10/12
0.170	30	5100	0/12

^aDose-rate was obtained by multiplying aerosol mass concentration x time of exposure (i.e. CxT)

TABLE 4

EFFECT OF INHALED T-2^a ON PULMONARY
WET/DRY WEIGHT IN RATS

Treatment	N	<u>Lung Weight (mg)</u>		
		Wet	Dry	Difference
Air Only	6	959 ± 47	225 ± 13	734 ± 33
ETOH Only	6	964 ± 53	224 ± 15	740 ± 41
T-2 & ETOH	12	960 ± 53	225 ± 11	745 ± 22

^aT-2 aerosol mass concentration = 2.0 mg T-2/liter air