Bull. Environm. Contam. Toxicol. 29, 371–378 (1982)

Acute Inhalation Toxicity of Ammonia in Mice

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At least one-half million Americans are employed by industries producing millions of tons of NH_3 each year. In addition, millions more will be exposed via atmospheric sinks in certain areas of the country (Mississippi delta, Michigan, northern Maine) which exceed TLV's (Threshold Limit Values) according to a NIOSH report in 1974 (NATIONAL ACADEMY OF SCIENCES 1979). The most serious NH_3 related exposures result from accidents involving transfer of the gas from one storage or transportation tank to another. These acute toxic episodes culminate in numerous deaths and injuries each year (HELMERS et al. 1971).

To date, little information exists concerning the lethal and sublethal sequelae following acute exposure to NH_3 gas in a controlled experimental situation. Of species tested, mice appear to be extremely sensitive to NH_3 toxicity (NATIONAL ACADEMY OF SCIENCES 1979) and thereby provide a good animal model for studying the mammalian response to NH_3 exposure.

Reported here are the results of experiments designed to determine an LC50 in mice from a 1-h exposure to NH_3 followed by a 14-day observation period.

MATERIALS AND METHODS

1. The Exposure System and Analysis:

A dynamic inhalation exposure system (KAPEGHIAN et al. 1980) was utilized to expose each of 4 groups of 12 mice (simultaneously) to one of 3 concentrations of NH_3 gas (MATHESON, East Rutherford, NJ) in air, or to air only. The general schematic of the gas mixing and exposure-chamber system can be seen in Figure 1.

Briefly, the mixing board serves to mix, dilute, and deliver up to 3 concentrations of NH_3 in air in addition to providing a source of uncontaminated air. Each of 4 glass distributing tubes receives a given concentration of NH_3 (or air), and shunts the flow of gas equally to attached chamber tubes housing the experimental animals. A portion of the distributing tube atmosphere is continually routed to a gas-sampling port. NH_3 concentrations were then determined by the method described by KAPEGHIAN et al. (1981). Equilibration time for chamber NH_3 concentrations was 10-15 min.

2. Animals:

Male albino ICR mice weighing 25-30g were purchased from Harlan Industries, Cumberland, IN. The mice were allowed free access to food (Purina Laboratory Chow #5001, Ralston Purina

0007-4861/82/0029-0371 $01.60

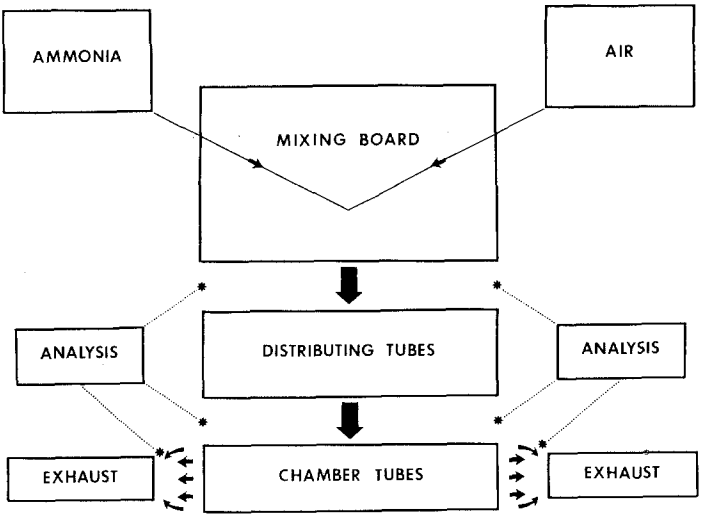

Figure 1
The Exposure System: A General Schematic

(Arrows indicate direction of flow. * indicates positions available for gas sampling.)

Co., St. Louis, MO) and water except while in chamber tubes where neither was available. Sterilized ground corn-cob bedding (SAN-I-CeL®, Paxton Processing Co., Inc., Paxton, IL) was used throughout the study and was replaced daily. All animals were allowed at least 1-week to acclimate to our animal facilities before being employed experimentally. A 12:12-h light:dark cycle at an ambient temperature of 23± 1° C was maintained continually.

3. Experimental:

Two preliminary range-finding experiments were performed. During the exposure period, NH_3 gas sampling and analyses were conducted at 3 to 5 min intervals. The average 1-h NH_3 concentrations utilized for this part of the study ranged from zero to 4860 ppm. The following observations were recorded during each exposure: eye irritation (blinking, scratching), dyspnea, frothing, convulsions, excitation/escape behavior, coma and death. Approximate times were recorded for the onset of each of the above responses.

Upon review of the preliminary data, 4 groups of 12 mice

each were employed for the comprehensive lethality study. The same protocol for all parameters was followed as described for the preliminary experiment with the following exceptions: complete necropsy procedures were conducted on all animals (except those dying during the 14-day observation period), and daily body weights were recorded for each animal during the 14-day observation period. The LC50 and 95% confidence interval were determined by the method of LITCHFIELD & WILCOXON (1949).

Mean body weight changes were analyzed using the "t" test for paired observations while mean organ-to-body weight ratios were compared using either the Student "t" test or the one-way ANOVA followed by Duncan's Multiple Range Test (KIM & KOHOUT 1975).

4. Necropsy Procedures:

Animals that died during an exposure were initially examined externally for any visible signs of toxicity. The following tissues were then excised, blotted and weighed: brain, heart, kidneys, liver, lungs, and spleen. Portions of the trachea and jejunum were also sectioned. All tissue samples were placed in fixative, embedded in paraffin, cut and stained with hematoxylin and eosin according to procedures outlined by LUNA (1968).

Following the 14-day observation period, surviving mice were weighed and anesthetized with ether; corresponding tissues from these mice were then examined according to procedures described above.

RESULTS

NH_3 LC50:

Table 1 contains the data for the 1-h lethality of NH_3 in mice; the observation period continued for 14 days. The 1-h LC50 was calculated to be 4230 ppm; the 95% confidence limits were 4070 and 4400 ppm.

TABLE 1
Lethality Following 1-H Exposure of Mice to NH_3

Experiment No.	NH_3 Concentration (ppm)	% Mortality (14-Day)
1	4490	66.6
	3950	25.0
	1190	0
	0	0
2	4860	100
	2130	0
	1340	0
	0	0
3	4860	83.3
	4220	41.6
	3440	0
	0	0

LC50 = 4230 ppm
95% C.L. = 4070-4400 ppm
(n = 12 mice/conc.)

The signs of acute toxicity included tremors and ataxia with spontaneous clonic convulsions progressing towards coma and a final tonic extensor seizure. Irritant effects included excitation/escape behavior, rapid vigorous tail revolutions, eye and nose irritancy (blinking, scratching), and dyspnea. The degree to which these responses occurred depended upon the NH_3 concentration to which each group was exposed. The onset of the irritant effects of NH_3 was immediate in all cases and lasted 5 to 10 min. After the animals returned to a less active state as irritant responses diminished, other observable signs of toxicity increased. Following the onset of clonic seizures, frothing was frequently observed. Animals progressing to a comatose state died immediately following a tonic-extensor convulsion. At high NH_3 concentrations deaths usually occurred within 30 min; 90% occurred in the initial 15-20 min of exposure. Lower lethal concentrations of NH_3 produced death as late as 45 min after initiation of the 1-h exposure. Upon return of the surviving mice to home cages, the usual cage-exploration and grooming activities were diminished. During the 14-day observation period NH_3-treated mice were, in general, dyspneic and lethargic; they lost weight (Table 2) and developed a "humped-back" appearance. It was not uncommon to detect severe rales in many NH_3-treated animals. Delayed deaths were observed up to 14 days following the 1-h NH_3 exposure; however 95% of the mortalities generally occurred within 72-h.

Body Weight Changes:

As demonstrated by the data in Table 2, body weights of all

TABLE 2
Effect of NH_3 Exposure (1-Hour) on Body Weights of Mice

	NH_3 Concentration (ppm)			
	0 (Air-Only)	3440	4220	4860
Control[a] (Exposure Day)	26.7±0.25[b]	28.4±0.44	29.1±0.45	27.0±0.35
Day 1	26.1±0.23	24.5±0.52*	24.0±0.73*	23.3±0.88*
Day 2	26.7±0.27	23.5±0.58*	22.7±0.71*	21.5±1.5*
Day 3	26.9±0.26	23.9±0.57*	23.3±0.64*	22.5±1.5*
Day 4	27.1±0.29	23.8±0.59*	23.6±0.53*	22.5±1.5*
Day 14	26.8±0.59	26.8±0.51*	25.6±0.81*	26.0±0.1

--*Significantly different ($p \leq 0.05$) from exposure day control by "t" test for paired observations.

--[a]n=12 animals for all groups on exposure day. For animals exposed to 4220 ppm NH_3, n decreased to 8 by Day 1 and to 7 for Days 2-14. For animals exposed to 4860 ppm NH_3, n decreased to 3 by Day 1 and to 2 for Days 2-14.

--[b]Mean body weight (g) ± S.E.

NH_3-treated groups decreased significantly on Day 1 and had declined maximally by Day 2. On Day 3, the progressive decrease in body weights of all treated groups began to reverse; however, as evidenced by body weights on Day 4, the process was not rapid. In fact, by Day 14 only one NH_3-treated group had returned to the control level of body weight; this was unexpectedly, the high NH_3-concentration group. Body weights of animals receiving air-only dropped slightly by Day 1, but this change was not significant. This group had not gained weight by Day 14; however, daily fluctuations in weight following Day 4 produced an even maintenance of weight throughout the observation period.

Organ-to-Body Weight Ratios:

Mean organ-to-body weight ratios (± S.E.) for the lungs (13.8±1.14 g/kg), liver (60.1±3.89 g/kg), and heart (6.17±0.41 g/kg) were significantly elevated ($p<0.05$, Students "t-test") in animals that died during the 1-h exposure to 4860 ppm NH_3 (n=4) compared to respective control values (6.88±0.28 g/kg-lungs; 43.2±2.07 g/kg-liver; 5.15±0.16 g/kg-heart) in animals sacrificed immediately following a 1-h exposure to air-only (n=6). Likewise lung (11.6 g/kg), liver (53.3 g/kg), and heart (5.52 g/kg) weights were elevated in the animal that died during the 1-h exposure to 4220 ppm NH_3, however, this was not statistically evaluated since n=1. By Day 14 (see Table 3) only liver weights were significantly elevated (compared to respective control values) in survivors of groups exposed for 1-h to either 4860 ppm or 4220 ppm NH_3.

TABLE 3
Selected Organ-to-Body Weight Ratios of Mice Surviving 14 Days Following Exposure to NH_3 (1-Hour)

	NH_3 Concentration (ppm)			
	0 (Air-Only)	3440	4220	4860
Brain	16.4±0.29[a]	16.7±0.39	17.0±0.31	17.4±0.56
Heart	4.89±0.06	4.94±0.15	4.91±0.11	5.38±0.39
Kidneys	7.71±0.19	7.46±0.24	8.21±0.16	8.23±0.68
Liver	48.2±1.21	50.5±0.99	54.8±1.82*	57.8±1.82*
Lungs	6.51±0.21	7.07±0.18	8.07±1.16	6.69±0.23
Spleen	4.08±0.25	3.63±0.15	4.02±0.35	3.19±0.19
	(n=12)	(n=12)	(n=7)	(n=2)

--*Significantly different from air-only control ($p<0.05$) by one-way ANOVA followed by Duncan's Multiple Range Test.
--[a]Mean organ-to-body weight ratio (g/kg) ± S.E.

Pathology:

On macroscopic examination, lungs from mice that died during NH_3 exposure were observed to be diffusely hemorrhagic. No other remarkable changes were observed in these animals. Mice sacri-

ficed on Day 14 showed no significant gross pathologic changes.

Histologically, the lungs of animals that died during NH_3 exposure revealed acute vascular congestion and diffuse intra-alveolar hemorrhage. These changes were most severe in mice that had received the highest NH_3 concentration (4860 ppm). Disruption of alveolar septal continuity was also evident at that concentration. In livers from animals that died during NH_3 exposure only acute congestion of hepatic sinusoids and blood vessels were observed.

The lungs of animals from every treatment group sacrificed on Day 14 (including controls) displayed a mild to moderate degree of chronic focal pneumonitis histologically. The severity of these chronic changes appeared to increase slightly with increasing NH_3 concentrations. Focal atelectatic changes were also evident in the lungs of the survivors of the group receiving the highest NH_3 concentration (4860 ppm).

Histologic examination of livers from mice sacrificed on Day 14 following acute NH_3 exposure demonstrated increasing cellular damage with increasing NH_3 concentrations. At 3440 ppm NH_3, there was evidence of sublethal cellular injury, i.e., swelling and increased cytoplasmic granularity of hepatocytes. Livers from animals surviving 4220 ppm NH_3 also demonstrated hepatocyte swelling but with marked cytoplasmic vacuolization and frank cellular necrosis in scattered foci. These changes were also seen at 4860 ppm NH_3. However, the extent of spotty necrosis was greatly increased.

In animals surviving NH_3 exposure there was no histological evidence of damage to the heart with the exception of the occasional observation of serous atrophy of pericardial fat.

Animals from each group which were sacrificed on Day 14 often displayed a mild degree of follicular hyperplasia of the spleen. This change seemed to be absent in mice that died during NH_3 exposure.

No other remarkable histopathological changes were seen in animals that died during the NH_3 exposure or those sacrificed on Day 14.

DISCUSSION

The LC50 reported here (4230 ppm) is slightly lower than the value (4837 ppm) cited by CHRISTENSEN et al. (1974). However, the relative "time until death" from NH_3 exposure in mice reported herein agrees with the results of WEEDON et al. (1940) (cited by NATIONAL ACADEMY OF SCIENCES 1979). The sudden onset and then progressive decrease in body weights during the days immediately following NH_3 exposure represent an extremely significant and untoward response to the gas. The overall impression from the body weight declinations and emaciated appearance of many NH_3-treated mice was that these animals were in a state of starvation. This apparent anorectic effect following NH_3 exposure has not been previously reported in rodents; however, it has been observed in several other species (NATIONAL ACADEMY OF SCIENCES 1979).

The lungs of animals that died during the exposure were all

extremely congested with evidence of hemorrhage on both gross and microscopic examination. The shifting of blood and fluid into alveolar spaces could theoretically account for the increased weight of the lungs compared to controls. Although the pulmonary changes described in the study presented here were not viewed at the ultrastructural level, they are in accord with the observations of NIDEN (1968) who reported disruption of both Type I pneumocytes and terminal bronchiolar cells following exposure to lethal concentrations of NH_3 in mice. Similar destructive effects at the alveolar level have also been reported by BOYD et al. (1944) in cats and rabbits after lethal concentrations of NH_3.

The livers from animals that died during exposure to 4860 ppm in the study presented here were significantly elevated in weight and were congested; however there were no other pathological changes observed. After 14 days, however, the livers from all NH_3-treated groups revealed marked hepatocyte swelling with vacuolization (possibly fat), and focal necrotic changes which increased in severity as NH_3 concentration increased. These changes are typical responses which occur in the liver during starvation or hypoxia (ROBBINS & ANGELL 1976). Although previously unreported in mice, fatty changes in the liver following continuous exposure to NH_3 have been reported in other species (COON et al. 1970). WEATHERBY (1952) also reported congestion in livers from guinea pigs exposed repeatedly to 140-200 ppm NH_3; no changes in lung tissue were noted however.

Even though heart weights were increased in animals that died during the 1-h exposure to NH_3 at 4860 ppm, no changes in the cardiac musculature were observed histologically. By Day 14 however, histologic examination revealed serous atrophy of the fat tissue surrounding the heart in animals surviving lethal concentrations of NH_3. Such atrophic changes are commonly observed during starvation (MOREHEAD 1965).

In summary, the study on NH_3 lethality in mice presented here yields the following: 1) the mouse is a species sensitive to NH_3 toxicity and appears to respond in this study as in other related reports, making it useful as an animal model in these studies; 2) the most immediate untoward effect of acute NH_3 gas exposure seen microscopically is congestion and hemorrhage of lung tissue with alveolar disruption and loss of septal continuity, and 3) the most significant late sequelae of acute NH_3 exposure are the degenerative changes in the liver which appear to be strongly related to the precipitous decline in the nutritional state of the animal.

ACKNOWLEDGMENTS: The authors wish to thank Ms. Margaret Tangeman for her careful typing of the manuscript. This project was supported in part by the Research Institute of Pharmaceutical Science, School of Pharmacy, University of Mississippi, University, MS 38677, and by NIH Training Grant 5 T32-GM 07099-05.

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Accepted July 6, 1982