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INHALATION TOXICOLOGY: VIII. ESTABLISHING HEAT TOLERANCE LIMITS FOR RATS AND MICE SUBJECTED TO ACUTE EXPOSURES AT ELEVATED AIR TEMPERATURES

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NOTICE

The animals used for this experiment were lawfully acquired and treated in accordance with the "Guide for the Care and Use of Laboratory Animals," National Research Council. DHHS Publication No. (NIH) 86-23.

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16. Abstract

Experimental animal subjects are used most commonly to assess the toxicity of thermal decomposition products (smoke) from burning materials. Nascent smoke is obviously quite hot; therefore, the design of smoke toxicity assay systems must provide for adequate cooling of the gases prior to exposure of the animals. This research has addressed the question of how much cooling is required.

Rats and mice were exposed to elevated air temperatures over the range of 38 °C to 110 °C. The exposure duration required to produce hyperthermic collapse (physical incapacitation) was measured for each temperature. A graph of time-to-collapse as a function of exposure temperature was constructed for each species and statistically derived equations were fit to each data set. Times-to-collapse ranged, for the rat, from 60 minutes at 40 °C to less than 4 minutes at 110 °C. For the mouse, they ranged from approximately 60 minutes at 40 °C to 2.5 minutes at 90 °C. The significance of these findings as they relate to smoke toxicity testing is discussed.

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INHALATION TOXICOLOGY: VIII.
ESTABLISHING HEAT TOLERANCE LIMITS FOR HATS AND MICE SUBJECTED TO ACUTE EXPOSURES AT ELEVATED AIR TEMPERATURES

INTRODUCTION

Fire in the environment of an aircraft accident significantly increases the likelihood of serious injury and death for passengers or crew who otherwise might escape unharmed. The burning of spilled fuel and/or nonmetallic cabin materials produces the two elements primarily responsible for this increased risk, namely, an elevated environmental temperature and an atmosphere of toxic and irritating gases. Many laboratories are currently involved in efforts to identify the nature and significance of the tonic hazard associated with the fire environment, but there is little or no research on the thermal hazard.

Thermal effects could be either direct or indirect. A direct effect would be one that resulted from the transfer, from the environment to an individual, of a quantity of heat sufficient to produce physical ,incapacitation or death. Some indirect effects might be: the panic resulting from being surrounded by, or having an exit blocked by, a mass of hot air or a sheet of flames; the increased rate of accumulation of toxic gases brought on by increased respiration rate in a hot environment; or the increased toxicity or irritation of gases inhaled at an elevated temperature.

Almost all assay protocols in combustion toxicology utilize some physiological response of an experimental animal to measure toxicity. If thermal stress to the animal is significant, i.e., the temperature and caloric content of the exposure atmosphere exceed certain critical levels, one will be measuring combined effects of heat and toxic gases. Thus, for meaningful evaluation of test procedures and results, it would be desirable to know the magnitude of any thermal hazard component of a test procedure.

When a small-animal test system for evaluating the toxicity of combustion products was designed and used at the Civil Aeromedical Institute (CAMI), chamber atmosphere temperatures up to 35 "C were shown to have no effect on the toxicity of CO or HCN. The effect of temperatures above 35 "C was not explored since it was quite easy to maintain the atmosphere below that value in this small system.

For those studies that require a larger system, and especially for so-called "full scale" systems, it becomes difficult if not impossible to maintain an atmosphere below 35 °C. To evaluate these and other test procedures, it would be helpful to

know the thermal tolerance limits for the species of experimental animal used. Since this information could not be found in definitive form in the scientific literature, we undertook a limited investigation to define the heat tolerance limits of rats and mice when exposed to elevated atmospheric temperatures for relatively short periods of time.

MATERIALS AND METHODS

An exposure chamber with a removable top was constructed of 0.125-inch plywood sandwiched between 30-mil sheets of aluminum. Inside dimensions were 22 by 24 by 26 inches: the enclosed volume is approximately 225 L. A controlled, electrical resistance heating element (9-in dia) was mounted in front of a 9-in fan in one corner of the floor with the fan output directed toward the diametric corner. Two additional 6-in fans were mounted near the ceiling, blowing parallel to the ceiling, but in opposite directions.

An 8-in-diameter, three-compartment rotating cage, as used in the CAMI toxicity test protocol [1], was mounted near the ceiling and driven at 6 rpm by a powered shaft that extended through one wall; the subjects were thus forced to walk at a linear velocity of 150 in/min. The ceiling of the chamber has a pane of thermally resistant glass mounted over the cage area to permit visual observation of the test animals. The animals were shielded from direct thermal radiation of the heater and heater shroud by the placement of an aluminum-foil-covered asbestos board between the rotating cage and the hot surfaces. Relative humidity (RH) within the chamber was neither controlled nor measured during a test; however, the RH at the start *of* each test has been 20 to 30 percent.

Animals were obtained from the Charles River Breeding Laboratories, Wilmington, MA. Rats were male albinos (Sprague-Dawley derived) and mice were randomly bred male albinos, CD-1 strain. Both species were inspected by a veterinarian on receipt and then held in isolation for 8 days. All were maintained for 4 days on drinking water containing 1.5 g/L of sulfathiazole, then normal tap water for the remaining 4 days of isolation. All were fasted overnight prior to use but were allowed access to water.

The exposure chamber was preheated to the desired temperature and maintained for 5 minutes *tu* allow thermal equilibrium of all components. The cage was then removed, an animal was placed in each of the three compartments, and the cage was replaced in the chamber. The rotation motor, the temperature recorder chart, and a timer were all turned on simultaneously. The elapsed time at which each animal became physically incapacitated, as determined visually by the observer, was recorded as the time-to-incapacitation, t_i.

For the rat study, a minimum of two replications were conducted at all but two temperatures; only one run (three rats;) was conducted at 38 °C and one at 48 °C. A total of 59 rats were exposed at 11 different temperatures.

Some laboratories utilize mice for fire-hazard testing: therefore, we felt it was desirable to conduct a few experiments with mice even though the CAMI inhalation toxicity protocol utilizes rats exclusively. The same chamber and techniques were used for the mouse study as were for the rat study. Exposures were conducted at five temperatures with a total of 13 mice.

The justification for any amount of this type of animal research--necessarily accomplished with unanesthetized subjects--is the desire to reduce fire hazards for humans. We felt that the need for a meaningful smoke toxicity assay protocol, that is, one for which the contribution from hyperthermia would be negligibl'e, was a powerful justification in itself; however, we also felt compelled to ask how the animal responses might relate to those for humans in similar thermal environments. We therefore conducted an in-depth survey of the literature concerned with human thermal tolerance limits.

RESULTS AND DISCUSSION

The results of the exposures with rats are in Table 1; those for mice are in Table 2. These data, for each of the two species, were used to derive by nonlinear, least squares regression techniques an equation that best fit each data set. Since thermal radiant energy flux is a function of the fourth power of the temperature in degrees Kelvin (K^4), the exposure temperature appears in the fitted equations in that form. Those equations are:

$$\text{(a) Rat:} \quad t_i = 1.0 + \frac{3.4 \times 10^{10}}{(K^4 - 308.7^4)} ,$$

$$\text{(b) Mouse:} \quad t_i = 1.0 + \frac{1.4 \times 10^{10}}{(K^4 - 310.7^4)} ,$$

where t_i = time-to-incapacitation, in minutes, and
K = exposure temperature, degrees Kelvin.

Figures 1 and 2, respectively, illustrate the correspondence between the data set and the derived equation for rats and for

TABLE 1. RAT INCAPACITATION TIME AS A FUNCTION OF AIR TEMPERATURE, FOR TOTAL-BODY EXPOSURE

Run No.	Air Temp., °C	Time-To-Incapacitation, min. (1)	(2)	(3)
1	38	>240	>240	>240
2	40	67.5	83.9	67.5
3	40	52.9	52.6	83.3
4	45	35.6	28.8	28.9
5	45	25.0	28.5	----
6	48	20.4	25.6	24.6
7	50	18.2	18.5	17.9
8	50	16.6	17.0	17.4
9	60	11.0	13.2	12.6
10	60	10.8	11.5	11.8
11	70	8.2	9.5	8.5
12	70	8.1	8.3	8.2
13	80	6.6	6.7	6.2
14	80	6.6	7.2	6.4
15	90	4.9	4.9	4.7
16	90	4.9	5.0	5.2
17	100	4.6	4.6	4.7
18	100	4.4	4.5	4.3
19	110	4.0	3.9	3.4
20	110	3.8	3.8	3.9

TABLE 2. MOUSE INCAPACITATION TIME AS A FUNCTION OF AIR TEMPERATURE, FOR TOTAL-BODY EXPOSURE

Run No.	Air Temp., °C	Time-To-Incapacitation, min. (1)	(2)	(3)
1	40	63.9	44.7	63.5
2	50	9.0	9.3	10.1
3	60	6.2	6.3	5.5
4	70	3.0	3.9	----
5	90	2.5	2.4	----

mice. The figures, as well as the tabulated data, demonstrate the difference in resistance to thermal stress that exists between the rodent species. This difference most likely reflects the differences in body masses and in the surface area/mass ratios.

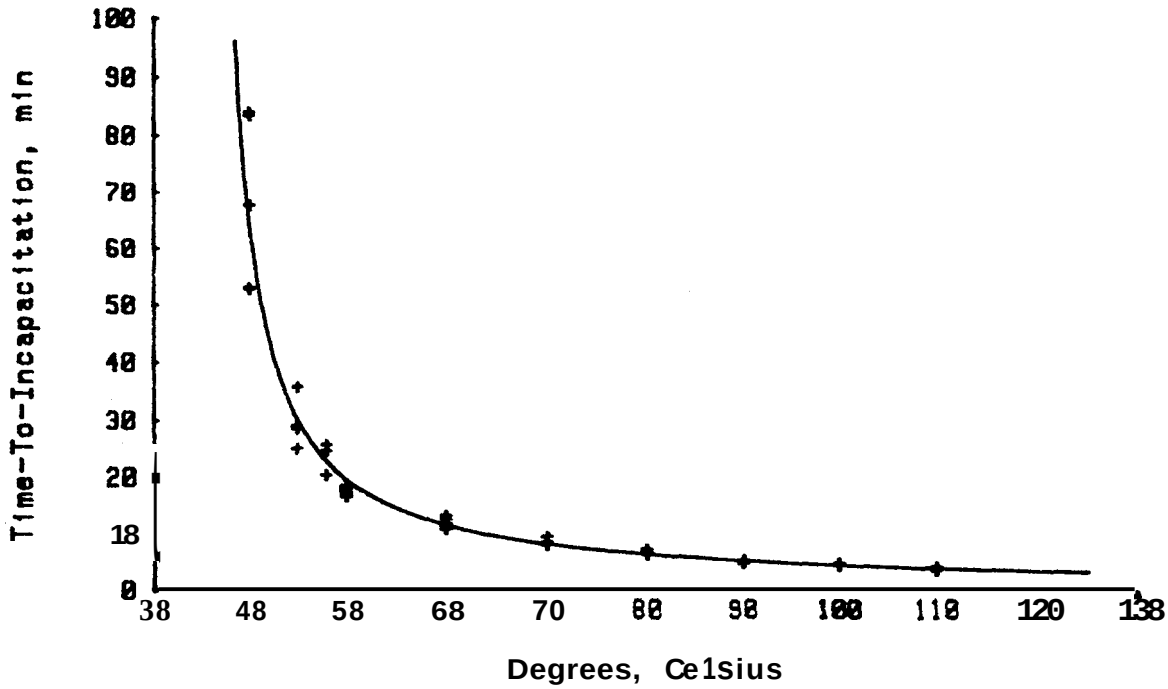

FIGURE 1. TIME-TO-INCAPACITATION AS A FUNCTION OF EXPOSURE TEMPERATURE, FOR RATS. Each datum point represents one animal, n=56.

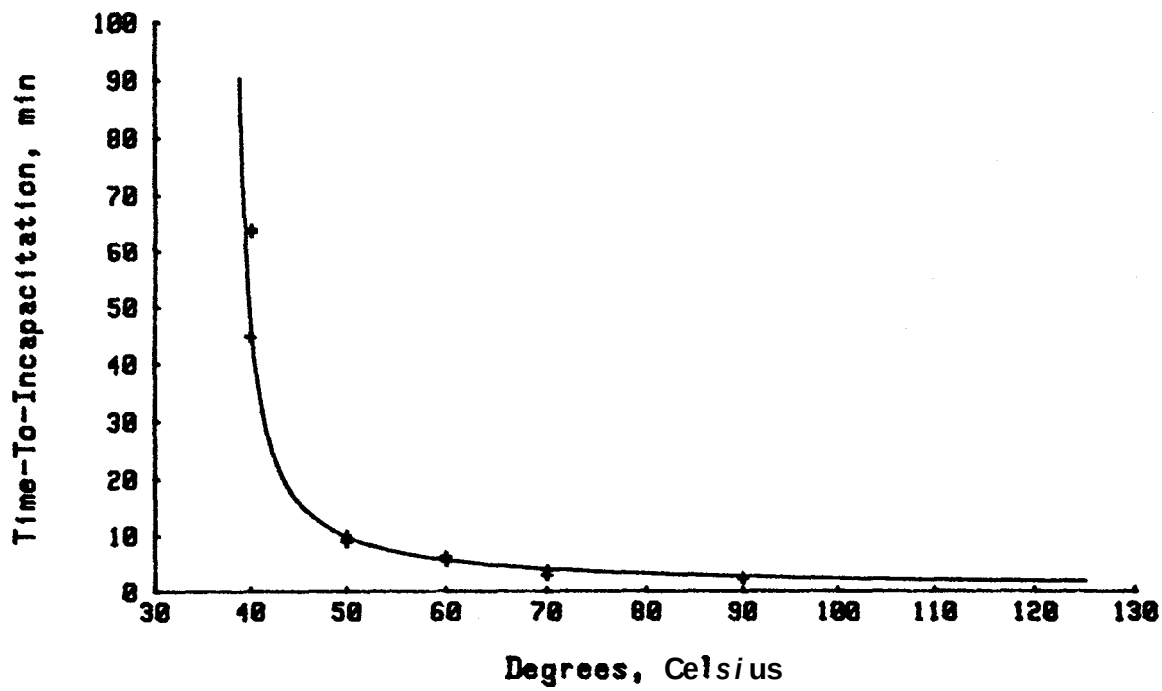

FIGURE 2. TIE-TO-INCAPACITATION AS A FUNCTION OF EXPOSURE TEMPERATURE, FOR MICE. Each datum point represents one animal, n=13.

The statistically derived constants for the equations suggest that the shortest time in which thermal collapse can be produced for either species would be about 1 minute. For the rat, the maximum air temperature for which the body's thermoregulatory system can compensate would be 308.7 K (35.5 °C), while the corresponding value for the mouse is 310.7 K (37.5 °C). At all air temperatures above these respective values, the rodent would experience an increase in total body heat content, leading eventually to a nonsurvivable core temperature.

One may conclude from these data that test animals (rats or mice) used to assay the toxicity of combustion products may not be exposed to air temperatures of 45 "C or higher without risking incapacitation (and death) within 30 minutes because of the thermal stress alone. In addition, one also risks the possibility that a given toxic atmosphere will be rendered still more toxic at elevated temperatures--a circumstance for which we have preliminary evidence in the case of CO at moderately elevated air temperatures. It would seem, therefore, that the upper temperature limit of 35 "C for an exposure atmosphere, as specified in the CAMI protocol[1] and recommended by the National Academy of Sciences", has been justified by the results obtained from this study.

Our survey of the literature addressing the topic of human thermal tolerance limits has convinced us that no actual human exposures have been conducted--or, at least, none have been reported--under conditions that achieved or even approached imminent physical collapse from hyperthermia. A myriad of studies have been conducted for which the endpoint was voluntary tolerance (discomfort), some degree of performance decrement, or a limiting value for some physiological parameter such as core temperature, blood pressure, heart or respiration rate, etc. We, however, are not convinced that these endpoints are sufficiently close to that of hyperthermic collapse to be of real value.

SUMMARY AND CONCLUSIONS

Rats and mice were found to be surprisingly susceptible to physical incapacitation (hyperthermic collapse) when exposed to moderately elevated air temperatures. For rats, thermal collapse occurs in about 60 min at 40 °C, 30 min at 45 °C, and 5 min at 85 "C. Mice are somewhat more sensitive than rats, with a 5-min collapse produced by exposure at about 65 "C.

The results of this study strengthen our previous belief that smoke toxicity tests should never subject rodents to temperatures above 40 "C--preferably not above 35 "C. If these temperatures are exceeded, then the observed endpoint is apt to be the result of both toxic and thermal insults, rather than a measure of toxic potency alone.

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