SMOKE TOXICITY METHODOLOGY

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INTRODUCTION

New generations of aircraft interior material will have to meet new and more rigid standards for flammability and thermal stability. In addition, the toxicity of their pyrolysis products must be within some reasonable limits. To address this latter point, NASA has asked SRI International to evaluate the toxicity of the pyrolysis products from five candidate aircraft materials. (Candidate material #5 was found to be completely resistant to pyrolysis and was therefore replaced by material #6.) Perhaps the most important part of this study was to demonstrate that we could do controlled pyrolysis of material and produce reproducible biological end points.

MATERIALS AND METHODS

Six materials were supplied by NASA, the Lyndon B. Johnson Space Center. For purposes of discussion, these materials (listed in Table 1) have been arbitrarily assigned numbers 1 to 6 according to the order in which they arrived in the laboratory.

Animals

Young adult male Fisher 344 rats were used for these studies. The animals were acclimated for approximately one week prior to exposure. Those used for the behavioral testing were housed individually in hanging wire cages. Those used for toxicity studies were housed in plastic cages, 5 per cage, on hardwood bedding. All animals were provided with food and water *ad libitum*. All animals were fasted overnight prior to exposure.

Table 1

MATERIALS IDENTIFICATION

Material No.	Description
1	Laminated polyimid foam and fiberglass sheets
2	Rigid polyimid foam sheets
3	Resin beads
4	Polyphenylene sulfide beads
5	Dixie cups filled with a white solid material
6	Polyphenyl sulfone molded pods

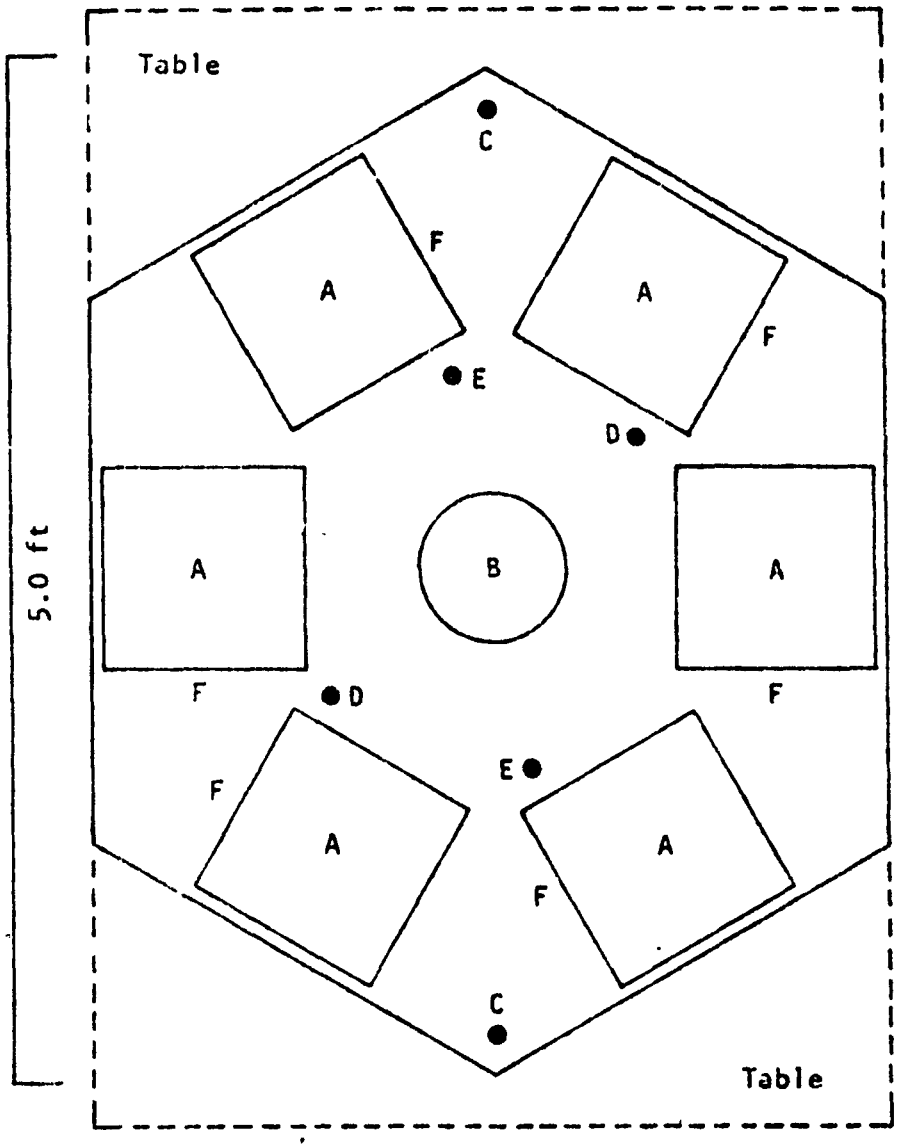
Exposure/Pyrolysis Facility

The animal exposure chamber is constructed on top of a 4 ft x 6 ft table. The chamber is hexagonal in shape and is approximately 24 in. high. It can accommodate six stainless-steel behavioral cages or several wire cages. Figure 1 is a diagram of the chamber arrangement. The cages (A) are arranged around the entry port for the smoke/pyrolysis products (B). On two opposite sides of the chamber are exhaust ports (C) for evacuating the chamber. There are two sampling ports (D) for continuous monitoring of CO, CO_2, and O_2. Two multiple thermocouple arrangements (E) are located on opposite sides of the smoke entry port. These thermocouples indicate whether temperature layering, and consequently pyrolysis product layering, is occurring in the chamber. In addition, individual thermocouples (F) next the each animal exposure chamber measure the temperature to which the animals are being exposed.

Figure 2 shows the arrangement beneath the chamber that permits continuous monitoring of CO, CO_2, and O_2. The atmospheric sample is drawn through a filter to remove particulate matter and through a moisture trap to protect the instruments from damage. The sample passes through the O_2, CO, and CO_2 monitors, through a flow meter and pump, and then is returned to the chamber so that no volume is lost from the chamber.

Figure 3 shows the multiple thermocouple arrangement that is located at each of two positions (E) in Figure 1. The thermocouples are 15 cm apart and the top one is 15 cm from the chamber top.

Figure 4 is a diagram of the pyrolysis apparatus, which is located beneath the chamber. Mounted on top of a laboratory jack so that it can be moved in and out, the apparatus is sealed against the bottom of the smoke entry port (B in Figure 1) when operating. The pyrolysis/combustion chamber is a Pyrex glass cylinder 17 cm in diameter. It sits on an aluminum base that contains a load cell, which measures the weight loss of the sample during pyrolysis. Two air-inlet ports are also located in the base so that the atmosphere in which pyrolysis and/or combustion occurs can be regulated. The atmospheres enter through



A = Behavioral chambers or animal cages
B = Entry port for smoke/pyrolysis products
C = Venting ports
D = Sampling ports
E = Multiple thermocouples to measure temperature layering
F = Individual thermocouples at each cage

FIGURE 1 DIAGRAM LOOKING DOWN ON THE TOP OF THE EXPOSURE CHAMBER

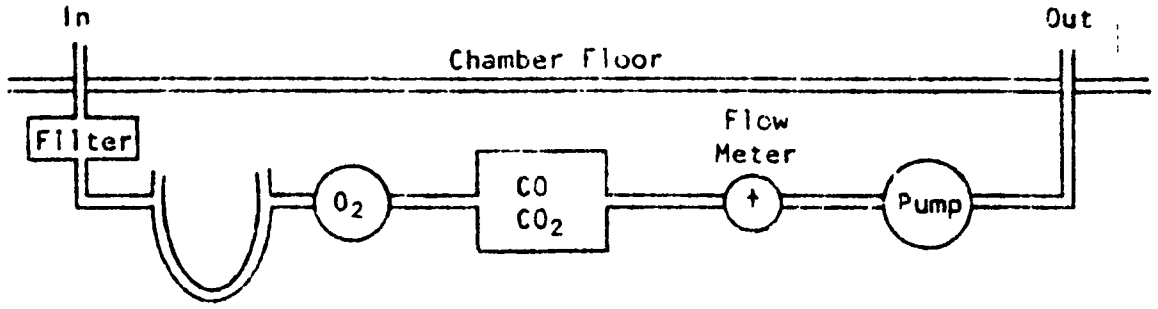


FIGURE 2 ARRANGEMENT FOR CONTINUOUS MONITORING OF O_2, CO, AND CO_2

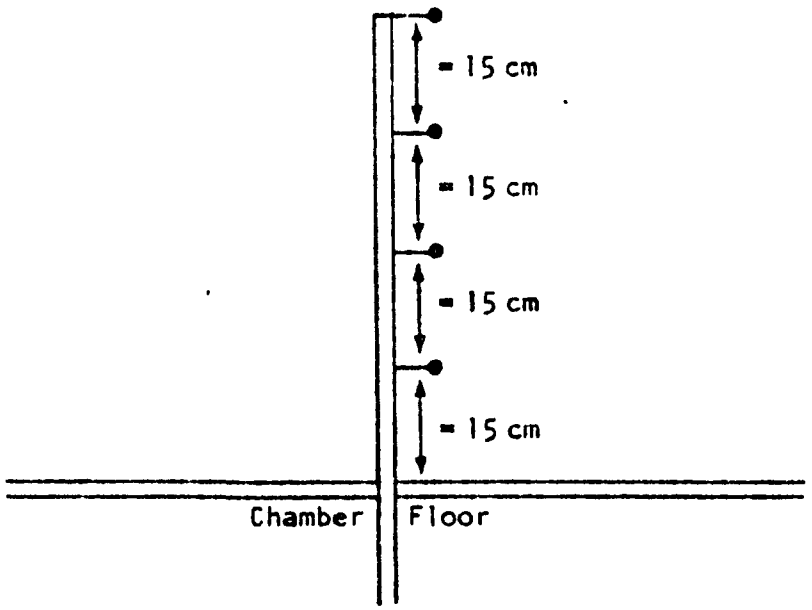


FIGURE 3 MULTIPLE THERMOCOUPLE PROBES

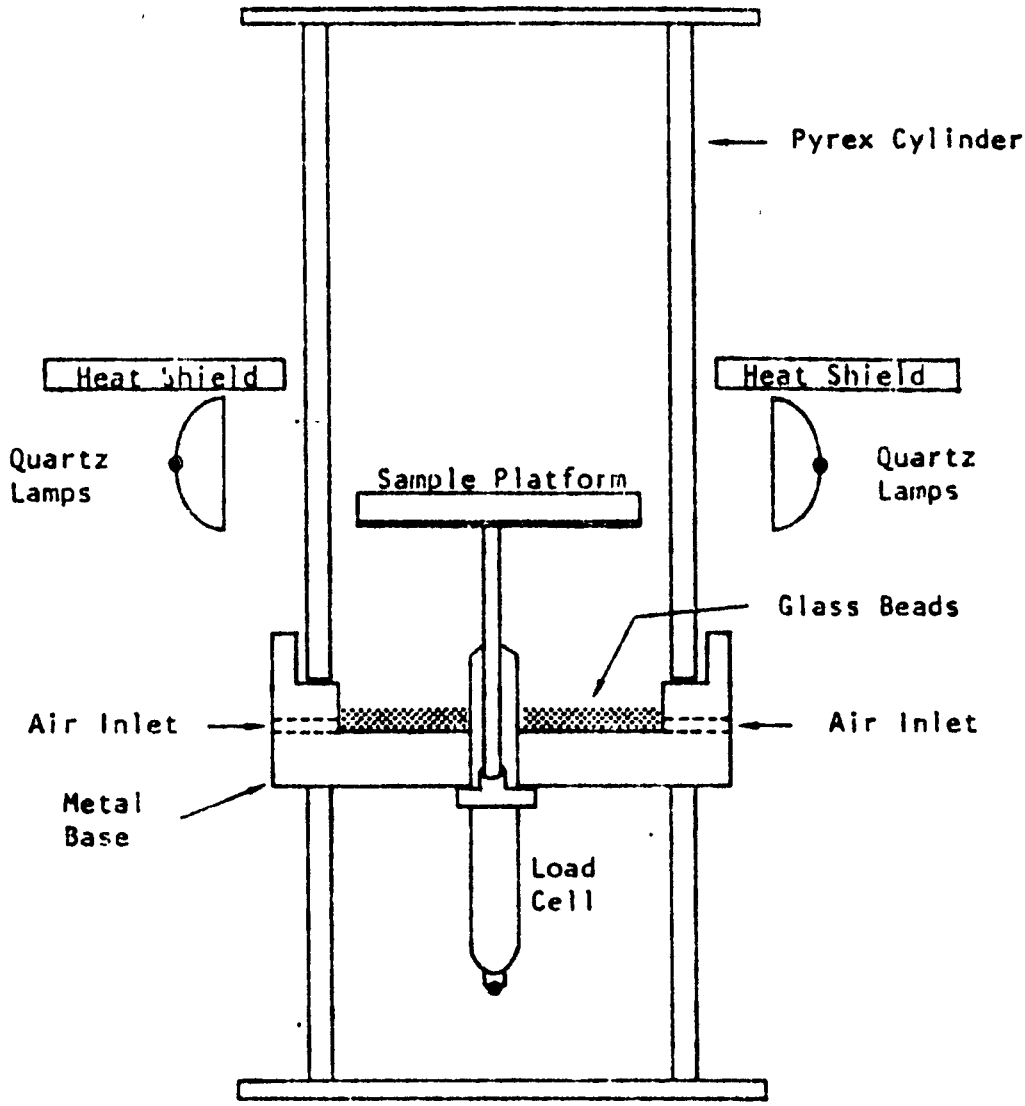


FIGURE 4 DIAGRAM OF PYROLYSIS APPARATUS

the base and pass through glass beads that disperse and mix the atmospheres. The mixture then passes up around the sample area and along the inner surface of the glass and into the chamber. Three banks of quartz lamps are arranged around the pyrolysis/combustion chamber to provide a heat source for pyrolysis. By varying the number of quartz lamps in each bank and their distance from the sample, a wide range of energies of radiant flux is available. The banks of quartz lamps are shielded from the bottom of the chamber by an asbestos heat shield so that they contribute no heat to the animal exposure chamber.

Acute Toxicity Studies

During the acute toxicity exposures, rats are housed two per cage in five open mesh (9.6 mm x 9.6 mm) wire cages, each measuring 22.3 cm x 22.9 cm x 27.9 cm. Additional rats can be placed in the sixth cage for blood-gas analysis upon completion of the exposure. Usually 30 minutes after the time the pyrolysis has begun, the chamber is purged with fresh air. During the exposure, the animals are observed through two viewing ports until the smoke density makes this impracticable.

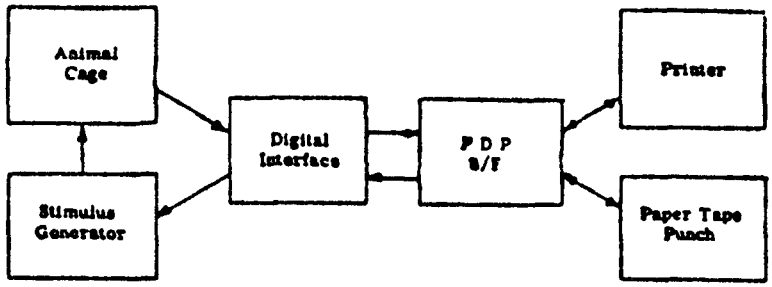
Animals sacrificed for blood-gas analysis are injected with sodium pentobarbital, and blood is taken by syringe from either the inferior vena cava or the descending aorta just inferior to the branching of the renal arteries. Sampling times are 5 to 7 minutes and 30 minutes after termination of the exposure. Carboxyhemoglobin, oxyhemoglobin, and total hemoglobin are determined with an Instrumentation Laboratories Model 182 co-oximeter calibrated for rat blood. Blood gases are determined with an Instrumentation Laboratories Model 713 blood-gas analyzer.

Incapacitation Studies

Apparatus

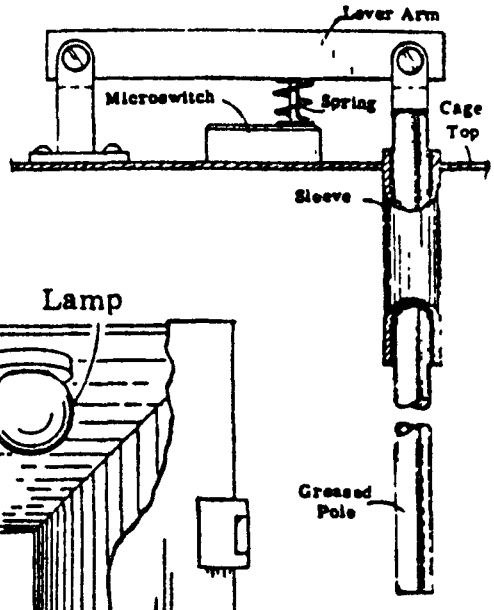
Each test chamber measures 30.2 cm x 30.2 cm x 35.6 cm and is constructed of stainless-steel (see Figure 5). Brass rods (3 mm diameter) spaced 1.27 cm apart serve as the floor. The rods can be electrified

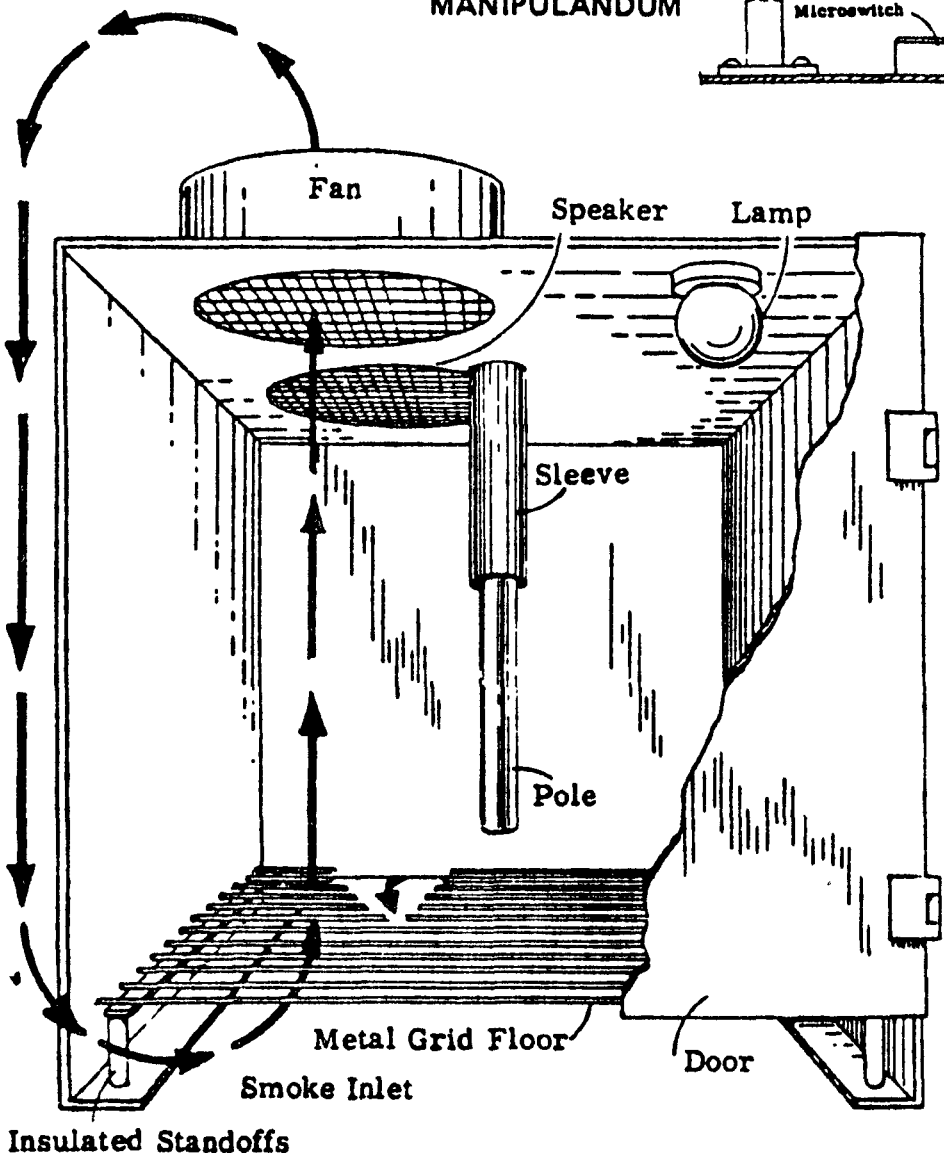
FIGURE 5 CONDITIONED AVOIDANCE RESPONSE APPARATUS



(a) SYSTEM COMPONENTS AND INTERFACES

(b) ANIMAL RESPONSE MANIPULANDUM





(c) C.A.R. CAGE

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with scrambled, constant-current shock. A 19.4-cm aluminum pole (1.27 cm in diameter) is suspended from the center of the ceiling. The pole is lubricated with Vaseline to discourage the rat from remaining on it. Downward displacement of the pole closes a microswitch that signals a response. A 7-watt light, a whisper fan, and an 8-Ω, 10.2-cm loudspeaker are also mounted in the ceiling. The light provides ambient illumination. The fan provides air and smoke circulation by drawing from the open floor, through the chamber, and out the top. Six such chambers are positioned around the table above the smoke generation system. A single hood encloses all the chambers. The test chambers are interfaced with a DEC PDP 8/F computer that provides automatic stimulus presentation and data collection. Data are recorded on a teletype and punched paper tape for offline processing.

CAR Training

Fischer 344 rats are trained to perform the conditioned avoidance response (CAR) in an apparatus similar to the one described above but located in another section of the building. They are first given 30 trials to learn to escape a 1-mA footshock by climbing a 20-cm pole. On each trial, the footshock remains on for 30 seconds unless the rat responds sooner, in which case the trial is terminated. The trials are presented randomly, but once every 1.5 minutes on the average. The rats are then given three daily 60-trial sessions to learn to avoid the footshock by climbing a 13-cm pole in the presence of each of three conditioned stimuli (CS) that precede the 1-mA footshock by 10 seconds. If the rat responds during this interval, the trial is terminated and an avoidance response is recorded. If no response occurs, the 1-mA footshock is initiated and, along with the CS, remains on for 20 seconds. A response during this interval also terminates the trial but is scored as an escape. The three CS consist of an increase in the intensity of the light or a 4-kHz tone or the presence of a 120-μA current on the floor. Each CS is pulsed at the rate of 2.5/second. The three CS are presented randomly 20 times each during each session. The time between

trials is also random, but averages 2 minutes. At the end of this training phase, most rats perform the CAR on 80% or more of the trials. Rats that fail to learn the escape response or the CAR are not used in tests for acute toxicity.

CAR Testing

Six animals are exposed and tested at a time. They are given several warm-up trials to ensure that the response is intact and that the equipment is functioning properly. Then the hood is secured, and an additional few trials are given. The "burn" is initiated and continued until a predetermined chamber concentration of CO or weight loss is reached, or for a predetermined time. At the end of the burn, a static condition is instituted and maintained for the remainder of a 30-minute, or longer, exposure time. The chamber is then vented, and recovery is monitored for an additional 30 minutes while fresh air is drawn through the animal chamber. During the exposure and recovery periods, trials are presented at the rate of about one per minute. The order of presentation of the three CS is random.

RESULTS

Chamber Operation

Figures 6 through 10 are representative of the data collected during a typical exposure. Figure 6 illustrates the weight loss and optical density resulting from a 4- to 5-minute pyrolysis of material #1. Once the pyrolysis is stopped, the smoke density decreases and the weight loss, of course, comes to a stop. Figures 7 and 8 show the vertical temperature profiles on each side of the chamber, from top to bottom. The thermistors on each side are spaced at 15-cm intervals, with the bottom thermistor being 15 cm from the floor of the chamber. The temperature profile reaches its highest point just at the end of the pyrolysis and then stabilizes at a lower temperature immediately. The vertical temperatures are very close to one another at each measurement period, indicating a lack of "layering" in the chamber. In other words, there is an apparent good mixing of the pyrolysis products in the chambers. Figure 9 shows the temperature at each animal cage location on the floor of the chamber. The purpose of these measurements is to ensure that the test animals are not being heat-stressed. Figure 10 shows the O_2, CO_2, and CO profiles during the 30-minute exposure to the pyrolysis products of material #1. As might be expected, there is an initial rapid loss of O_2 during the pyrolysis period (first 5 minutes) and then a much slower decrease in O_2 for the remainder of the 30-minute exposure period. The CO concentration climbed rapidly during the pyrolysis period and then stabilized and remained constant during the remainder of the exposure. The CO_2 concentration similarly increased rapidly during pyrolysis. However, it continued to increase, but at a much slower rate after the pyrolysis had stopped.

Acute Toxicity Studies

The acute toxicity of the candidate aircraft materials is shown in Table 2. The LC50 is given in terms of both weight loss of the sample

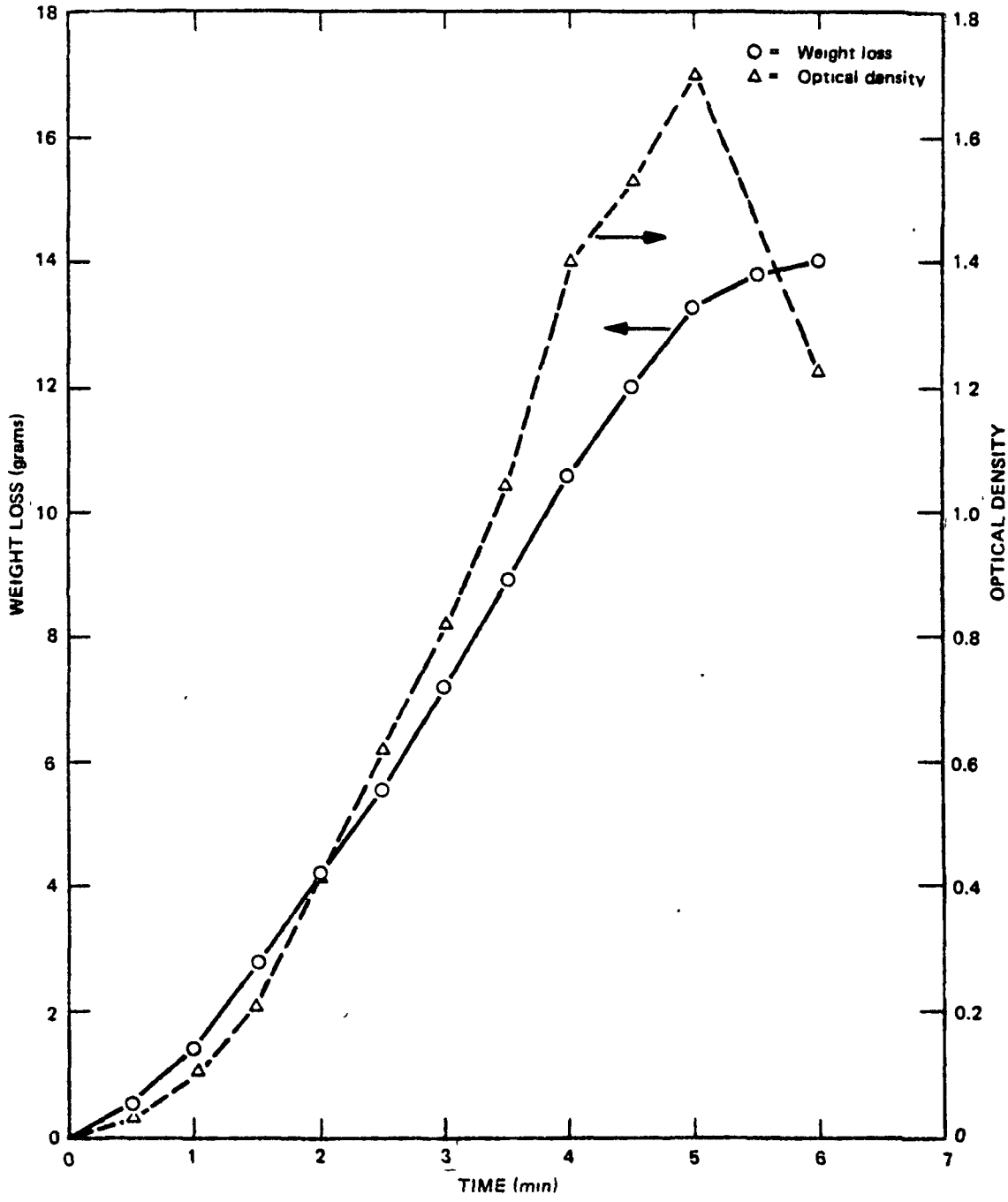

FIGURE 6 WEIGHT LOSS OF SAMPLE (MATERIAL #1) AND SMOKE DENSITY IN THE CHAMBER AS A FUNCTION OF BURN TIME

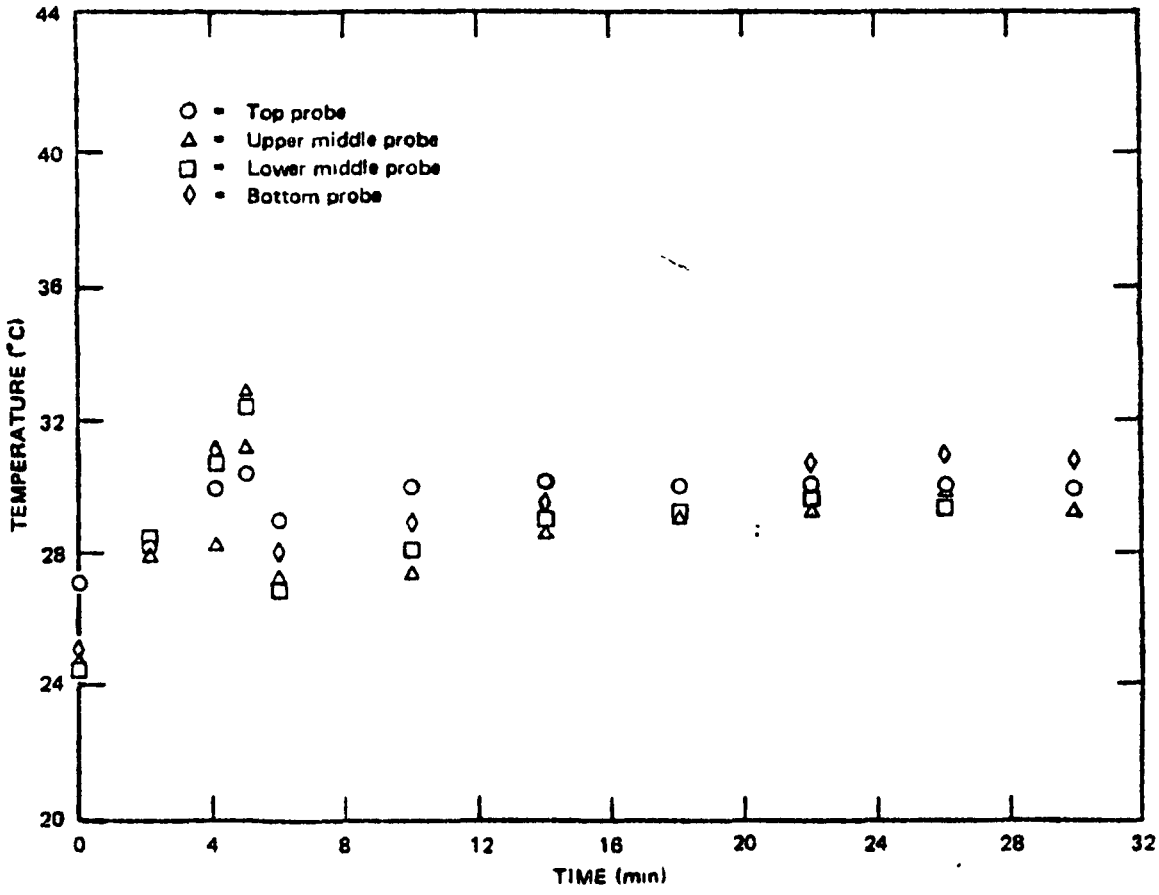

FIGURE 7 VERTICAL TEMPERATURE PROFILES NEAR ONE SIDE OF THE CHAMBER (MATERIAL #1)

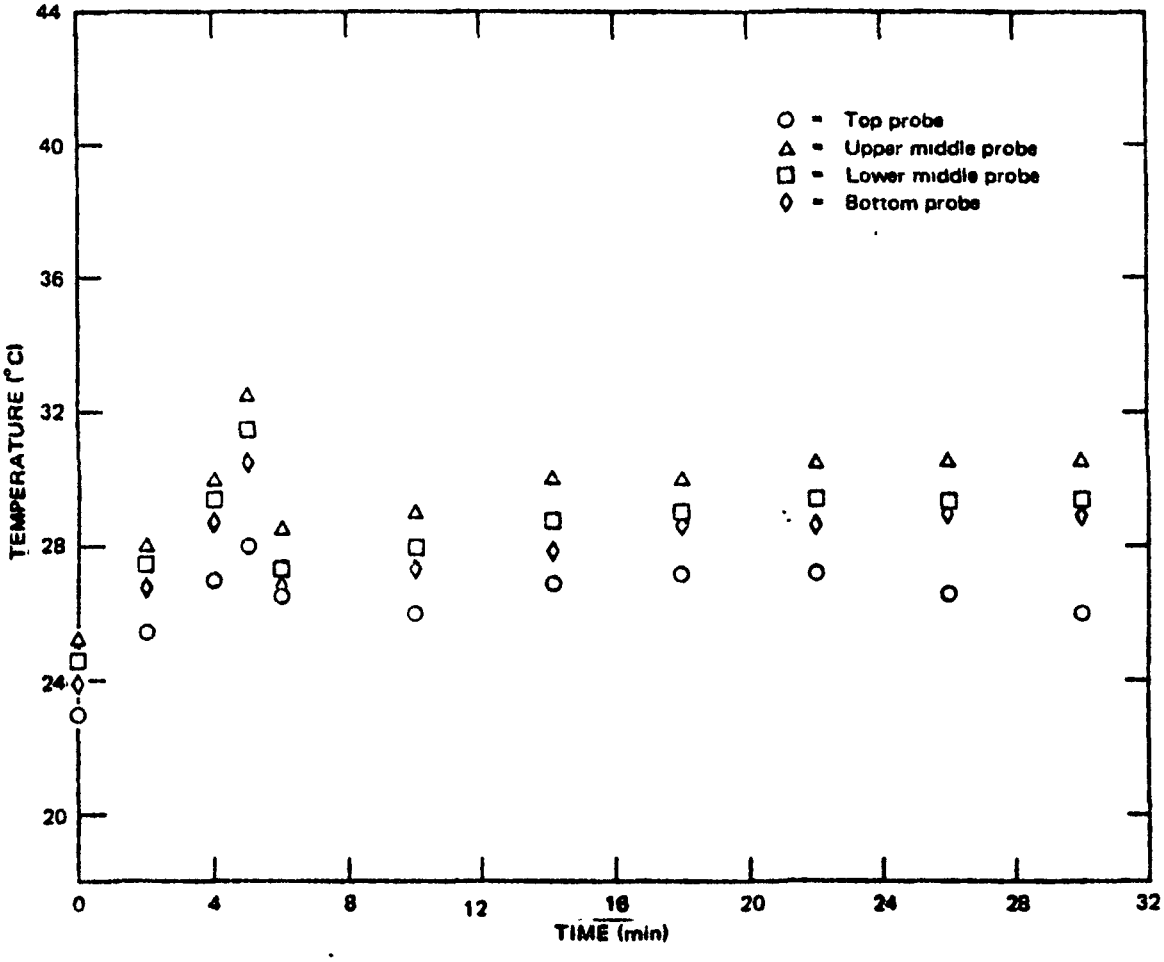

FIGURE 8 VERTICAL TEMPERATURE PROFILES AT OPPOSITE SIDE OF THE CHAMBER (MATERIAL #1)

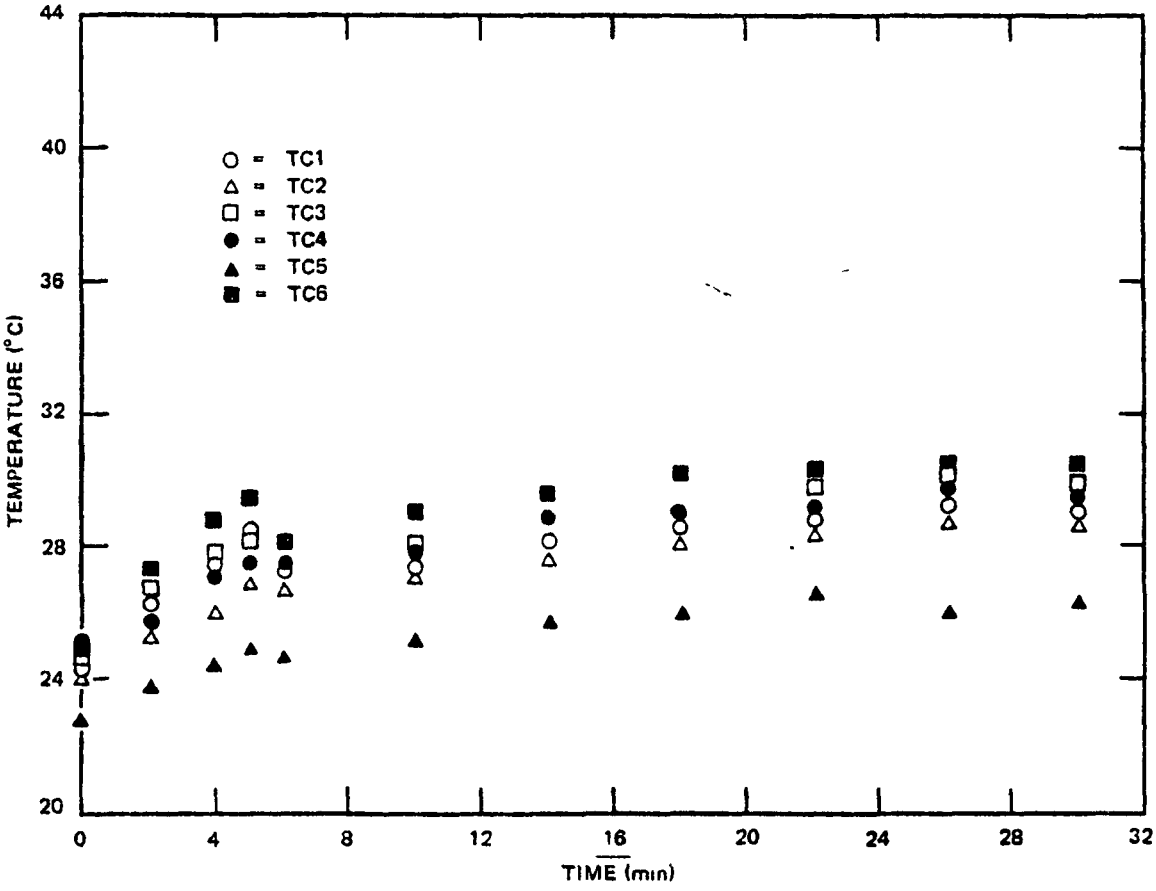

FIGURE 9 TEMPERATURE AT THE SIX CAGE POSITIONS DURING 30-MINUTE EXPOSURE TO MATERIAL # 1

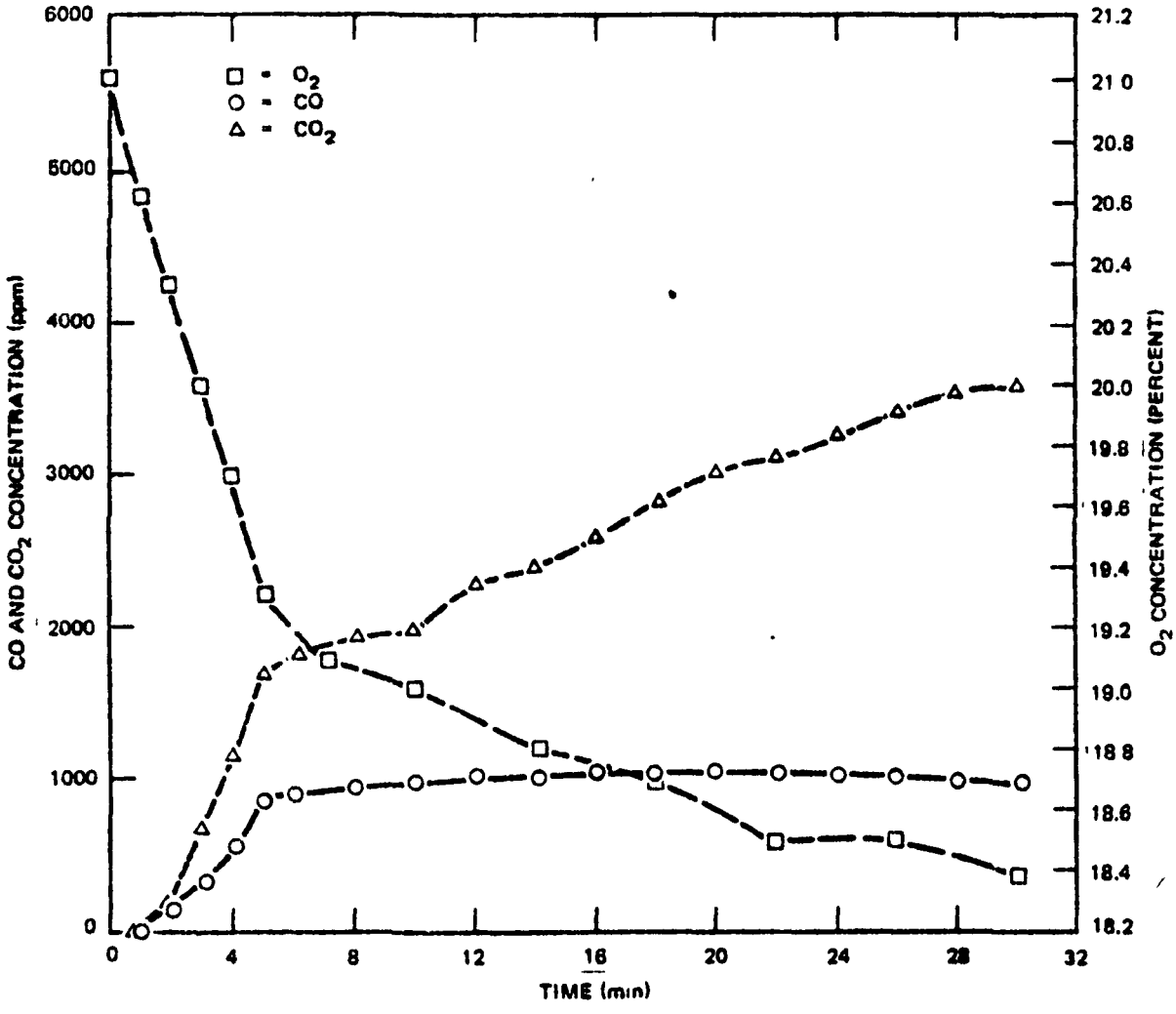

FIGURE 10 O_2, CO, AND CO_2 PROFILES DURING 30-MINUTE EXPOSURE TO PYROLYSIS PRODUCTS OF MATERIAL # 1

and CO concentration. The $LC_{t_{50}}$, the concentration of CO (in ppm) multiplied by the minutes of exposure, is also shown. The $LC_{t_{50}}$ permits a comparison of values when the exposure time varies.

The sample of material #1 tested consisted of a combination of two dissimilar samples of that material received in two different shipments. It is a foam laminated between two layers of fiberglass. The variables included not only the foam-to-fiberglass surface ratio, but also the amount of adhesive material. In addition, the color intensity and shade varied within samples so that energy absorption rates (fluxes) were different. We were unable to produce mortality with a 30-minute exposure to the pyrolysis products of material #1, so we exposed the animals for 60 minutes. All other exposures were for 30 minutes. We could not produce mortality with material #5 since it would not pyrolyze.

Table 2

ACUTE TOXICITY OF THE PYROLYSIS PRODUCTS FROM CANDIDATE AIRCRAFT MATERIALS AFTER A 30-MINUTE EXPOSURE*

Material Number	LC50 By Weight Loss (g/m^3)	LC50 By CO Concentration (ppm)	$LC_{t_{50}}$†
1	28.00	2280	135,800
2	9.43 (9.04-9.98)	3157 (2986-3310)	94,710
3	35.43 (34.11-36.16)	3683 (3625-3715)	110,490
4	7.95 (6.42-12.12)	520 (459-571)	15,600
5	Much greater than 60 grams	--	--
6	24.00 (21.00-28.00)	1525 (1381-1683)	45,750
CO alone	6.99 mg	6112 (5799-6347)	183,510

* Exposure for material #1 was 60 minutes.

† Expressed as the CO concentration in ppm multiplied by the minutes of exposure.

Ranking of the materials from the most toxic to the least toxic on either a weight-loss or a CO basis was practically the same except for material #2. On a weight-loss basis, the ranking is 4, 2, 6, 1, 3 (and 5); ranking on the basis of CO is 4, 6, 1, 2, and 3.

During the expsoure periods, the animals usually displayed an initial period (2-3 minutes) of varying degrees of excitement, followed by lying very quietly for the remainder of the exposure period. The 2-week recovery period indicated some residual toxicity in those animals that survived the exposures. Table 3 lists the body weights of survivors from exposures in the lethal range of concentrations of pyrolysis products of each material. Whereas rats exposed to materials #1, #2, and #3 generally gained weight in a normal fashion, those exposed to materials #4 and #6 not only had a decrease in weight gain but a moderate to severe weight loss during the recovery period. Mortality usually occurred in the chamber during exposure or within a few hours after exposure. Material #6 was an exception in that mortality occurred over a period of days after exposure.

Gross pathology of those animals that died or were sacrificed at 2 weeks post-exposure was confined to the lungs and spleen. The changes seen in the spleen were rough surfaces, which may be explained by the stress of the exposure. The lungs were heavy and edematous. Total areas of atalectasis and congestion were a frequent observation. Petecheal hemorrhages were often observed. Based on the gross pathology, there was little doubt that the lung was the primary target organ in all cases of toxicity.

Blood-gas analysis was performed on rats exposed to the pyrolysis products from each material at 5 and 30 minutes after exposure to the material. These data are summarized in Tables 4 through 8. In all cases, except for material #4 (Table 7), there was an initial elevated carboxy-hemoglobin level, which was readily reversible, as evidenced by the 30-minute post-exposure measurements. (It should be noted that the rat has a much more efficient carboxyhemoglobin-reducing system than

Table 3

INITIAL AND FINAL BODY WEIGHTS OF RATS
SURVIVING 14 DAYS AFTER EXPOSURE TO THE
PYROLYSIS PRODUCTS OF CANDIDATE AIRCRAFT MATERIALS

Material Number	Initial Body Wt* (grams)	Final Body Wt* (grams)	2-Week Gain
1	(10) 197 ± 11.8	(5) 259 ± 13	62
	(10) 220 ± 10.0	(10) 260 ± 11	40
	(10) 223 ± 12.0	(5) 249 ± 12	26
2	(10) 243 ± 4.7	(10) 303 ± 26.4	60
	(10) 249 ± 10.1	(8) 307 ± 13.9	58
	(10) 225 ± 27.2	(2) 307 ± 27.6	82
	(10) 227 ± 12.4	(1) 341	114
3	(10) 214 ± 13.2	(10) 249 ± 12.5	35
	(10) 203 ± 22.0	(6) 252 ± 13.2	49
	(10) 189 ± 16.4	(4) 211 ± 9.9	22
4	(10) 248 ± 17.3	(10) 265 ± 17.5	17
	(10) 259 ± 16.1	(8) 264 ± 23.2	5
	(10) 237 ± 12.3	(4) 214 ± 39.8	-23
6	(10) 269 ± 12.2	(10) 271 ± 14.1	2
	(10) 322 ± 18.7	(7) 273 ± 49.7	-49
	(10) 227 ± 19.0	(1) 189 ± --	-38
	(10) 328 ± 14.2	(1) 220 ± --	-108

* Body weights were taken just before exposure and just before sacrifice, 14 days later. Numbers in parentheses are the number of animals per group.

Table 4

BLOOD-GAS ANALYSIS OF MALE RATS*
5 AND 30 MINUTES AFTER A 30-MINUTE EXPOSURE
TO THE PYROLYSIS PRODUCTS OF MATERIAL #1

(CO concentration, 1100 ppm)

Measurement	Time After Exposure	
	5 Minutes	30 Minutes
Hemoglobin (g)	10.4-11.8	10.1-10.4
Carboxyhemoglobin (%)	27.6-28.3	18.2-18.6
pH	7.371-7.500	7.381-7.445
P_{CO_2} (mm Hg)	28.3-41.1	41.4-41.9
P_{O_2} (mm Hg)	82-130	30-44
HCO_3^- (mole %)	21.8-23.5	24.5-28.0
Total CO_2 (mole %)	22.6-24.8	25.8-29.3

* Two rats per group.

Table 5

BLOOD-GAS ANALYSIS OF MALE RATS*
5 AND 30 MINUTES AFTER 30-MINUTE EXPOSURES
TO THE PYROLYSIS PRODUCTS OF MATERIAL #2

	Time After Exposure			
	5 Min	30 Min	5 Min	30 Min
CO concentration (ppm)	2448	2448	1896	1896
Oxyhemoglobin (%)	37.2-40.6	59-75	23-34	38-49
Hemoglobin (g)	9.7-11.3	9.6-13.1	7.6-9.4	9.4-11.8
Carboxyhemoglobin (%)	49-50	24-27	33-34	13-19
pH	6.952-7.030	7.098-7.324	7.106-7.413	7.324-7.413
P_{CO_2} (mm Hg)	28-50	33-36	14-29	38-40
P_{O_2} (mm Hg)	22-26	55-57	42-143	33-38
Base excess	-19 to 21	-6 to 8	-23 to 17	-4 to 1
HCO_3^- (mole %)	7.2-10.8	10-18	4-9	20-24
Total CO_2 (mole %)	8.1-12.4	11-20	5-10	22-26

* Two rats per group.

Table 6

BLOOD-GAS ANALYSIS OF MALE RATS*
5, 15, AND 30 MINUTES AFTER A 30-MINUTE EXPOSURE
TO THE PYROLYSIS PRODUCTS OF MATERIAL #3

(CO concentration, 3678 ppm)

Measurement	5 Minutes	15 Minutes	30 Minutes
Hemoglobin (g)	8.2-12.4	9.2-11.7	8.9-11.8
Carboxyhemoglobin (%)	43.6-59.2	36.3-42.3	30.0-35.2
pH	6.786-6.934	6.957-7.117	7.075-7.204
P_{CO_2} (mm Hg)	43.9-81.4	39.8-60.1	44.6-65.2
P_{O_2} (mm Hg)	6-88	6-13	4-32
HCO_3^- (mole %)	8.3-12.0	11.2-18.3	14.9-22.3
Total CO_2 (mole %)	9.8-14.5	12.4-20.1	15.6-24.1

* Five rats per group. Rats anesthetized with pentobarbital before bleeding.

Table 7

BLOOD-GAS ANALYSIS OF MALE RATS
5 AND 30 MINUTES AFTER A 30-MINUTE EXPOSURE
TO THE PYROLYSIS PRODUCTS OF MATERIAL #4

(CO concentration, 310 ppm)

Measurement	Time After Exposure	
	5 Minutes*	30 Minutes†
Hemoglobin (g)	11.9-14.3	12.3-14.2
Carboxyhemoglobin (%)	0	0
pH	7.375-7.517	7.245-7.428
P_{CO_2} (mm Hg)	21-46	27-39
P_{O_2} (mm Hg)	73-111	64-98
Base Excess	-2.8 to 1.3	-12.1 to -3.0
HCO_3^- (mole %)	17.1-26.2	13.5-21.3
Total CO_2 (mole %)	17.7-27.7	14.5-22.5

* Four rats.

† Five rats.

Table 8

BLOOD-GAS ANALYSIS OF MALE RATS
5 AND 30 MINUTES AFTER A 30-MINUTE EXPOSURE
TO THE PYROLYSIS PRODUCTS OF MATERIAL #6

(CO concentration, 1440 ppm)

Measurement	Time After Exposure	
	5 Minutes*	30 Minutes†
Hemoglobin (g)	13.3-14.6	13.8
Carboxyhemoglobin (%)	25.6-37.8	22.5
pH	6.631-7.383	7.390
P_{CO_2} (mm Hg)	29-65	32
P_{O_2} (mm Hg)	15-84	46
Base Excess	-29 to 5.2	-3.8
HCO_3^- (mole %)	6.6-17.4	18.9
Total CO_2 (mole %)	8.5-18.0	19.9

* Five rats.

† One rat.

Table 9

SUMMARY OF THE BEHAVIORAL PERFORMANCE DATA FROM RATS EXPOSED TO THE PYROLYSIS PRODUCTS OF CANDIDATE AIRCRAFT MATERIALS*

Material Number	CC50	IC50	LC50
1	1229	1767	1787
2	1387	1964	1996
3	1615	2715	2257
4	121	176	124
6	1492	3043 (approx)	1430
CO alone	1600	3125	3650

* Values expressed as ppm of CO. Each value was determined from several trials by regression analysis. Each exposure was done with six animals.

man has.) Materials #2, #3, and #6 produced a moderate to severe acidosis, with partial depletion of the bicarbonate reserve, but this was also reversible in surviving animals at 30 minutes after exposure even though recovery may not have been complete. The partial pressures of O_2 and CO_2 (from venous blood) probably reflect a normal condition to slight hyperventilation. However, these samples were taken 5 minutes after the rats were removed to room air. Had the blood been drawn in the chamber at the end of the exposure period, there probably would have been much high P_{CO_2} values (evidence of breath-holding, or hypoventilation).

No blood gases were done on Material #5 since nothing could be pyrolyzed from this material.

Behavioral Studies

The results of the behavioral studies are summarized in Table 9. The loss of the Conditioned Avoidance Response (CC50), incapacitation (IC50), and lethality (LC50) are expressed in terms of the CO concentration. First, note that the LC50 values are lower for the animals in the behavioral chambers. This is probably because these animals are required to expend more energy in task performance and therefore have a higher respiratory minute volume than those allowed to rest in the exposure chamber. Consider, for example, the LC50 of CO alone. In the acute toxicity studies this was 6112 ppm, whereas in the behavioral chamber this was reduced to 3650 ppm, or nearly half the "resting" LC50 that was obtained in the wire cages.

Next, note that the incapacitating concentration of each material is the same (#1 and #2) or greater (#3, #4, and #6) than the LC50, in contrast to CO, for which the IC50 is about 85% of the LC50. (The IC50 for Material #6 is an approximation since CO concentrations that high could not be reached.) Materials #3 and #6 present an interesting phenomenon since the pyrolysis products apparently contain some substance that is antagonistic to CO incapacitation.

Inhibition of the conditioned avoidance response (CC50) was the most sensitive measure with Materials #1, #2, and #3, but was approximately the same as the LC50 for Materials #4 and #6.

Recovery of behavioral activity was complete within 24 hours in all animals except those exposed to the pyrolysis products of Material #6. These animals took up to 7 days to regain their pre-exposure level of performance.

DISCUSSION

This study was initiated to evaluate the toxicity (i.e., safety) of candidate aircraft materials since they may become involved in situations of thermal decomposition. This requires test methodology for evaluating not only the toxicity of the thermal decomposition products, but also the incapacitating effects of the decomposition products and the thermal stability of the initial product. First, an exposure chamber was built that allowed the controlled pyrolysis of material by external heat fluxes. The flux rates are adjustable over a wide range so that pyrolysis or flaming mode is easily achieved. This capability also allows us to complete the pyrolysis of a sample in a short time relative to the animal exposure time.

The exposure chamber is designed so that the pyrolysis area and animal exposure area are essentially one chamber. This design avoids large losses of combustion products on the walls of any transfer apparatus. At the same time, the animals are protected from direct exposure to the burning material. Thus, even a relatively long pyrolysis time does not cause a temperature rise of more than a few degrees at the animal locations in the chamber. Continuous monitoring of sample weight loss and the chamber concentrations of CO, CO_2, and O_2 gave us good control of the pyrolysis and permitted us to reproduce any desired exposure. We found that using the CO concentration produced by the pyrolysis of each material provided us with a satisfactory "internal standard" to determine our median effective doses.

In summary, the chamber and methodologies used in these studies generally meet or exceed those recommended by the National Academy of Sciences (Fire Toxicology: Methods for Evaluation of Toxicity of Pyrolysis and Combustion Products, Report No. 2, NAS Committee on Fire Toxicology, August 1977). Specifically, (1) we cannot do testing in both the flaming and the pyrolysis mode; (2) the pyrolysis time is short (1 to 4

minutes) with respect to the animal exposure time; (3) the animal chamber and pyrolysis unit are essentially one chamber and the sample is pyrolyzed in that chamber, whereas the energy (heat) source is located outside the chamber; (4) we use small animals and expose six to twelve at one time; (5) we use 30-minute exposures but can expose for longer or shorter times, as necessary; (6) the temperature in the exposure chamber never exceeds 35° C; (7) we measure incapacitation and avoidance as well as mortality; (8) we monitor CO, CO_2, and O_2 continuously during exposure, and the O_2 concentration is never below 17%.

The toxicity studies have been expressed in terms of CO concentrations because that has been a convenient and consistent measurement. However, we do not mean to imply that CO is the only--or even the main--factor contributing to the toxicity of the pyrolysis products of the various materials. This is evident from both the blood-gas data and the variable rate of body weight recovery seen after exposure. For example, the survivors after exposure to materials #4 and #6 lost weight during the 2-week postexposure period. After exposure to material #3, weight gain was reduced somewhat.

Gross pathology was confined to the lungs and, to a lesser degree, the spleen. The lungs were generally edematous and atalectatic, and occasionally petechial hemorrhages were seen. This is not characteristic of CO but, rather, was probably induced by the myriad of other compounds in the pyrolysate. For example, materials #3, #4, and #6 contained a great deal of SO_2.

The behavioral performance of the animals was somewhat surprising in that the decrement of CAR performance and/or incapacitation often occurred at concentrations that were about the same as or higher than the LC50. This seems to point out the need for doing both tests for incapacitation and those based on mortality data when evaluating these compounds.