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16. Abstract

In an effort to further the cause of increased safety for those who ride in commercial aircraft, this paper presents a detailed description of the genesis of a small-scale, laboratory test system that utilizes small animals to evaluate the relative toxic hazard of combustion products generated by the thermal decomposition of nonmetallic materials. It includes: a discussion of the concepts that led to the design; detailed instructions for fabricating the system; operating parameters and instructions for conducting a test; an evaluation of the system's performance as determined by the testing of 75 aircraft cabin materials; the utilization of animal response time as a basis for expressing relative toxicity; and a discussion of the derivation of an "inhalation dose" concept that promises to be more useful than "lethal concentrations."

17. Key Words Inhalation Toxicology; Combustion Toxicology; Pyrolysis Products; Time-to-incapacitation; Time-to-death; Cyanide Toxicity; Aircraft Cabin Materials; Relative Toxic Hazard; Animal Exposure System; Effective Inhalation Dose	18. Distribution Statement Document is available to the public through the National Technical Information Service, Springfield, Virginia 22151		
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It might be appropriate at this point to clarify a relationship between a test system and the "real world." In the context of an individual trapped, even though only momentarily, in a space filled with smoke and toxic gases generated by a fire, one might feel that even a 10-min exposure to such an environment represents an unrealistically lengthy dwell time. Particularly if the "space" were a burning aircraft, one might feel that the time available for escape or successful rescue would be only a minute or two, certainly not 10 min. As a consequence, it might seem only logical that, in an experimental test system designed to evaluate the toxic properties of combustion gases released from burning aircraft furnishings, the duration of exposure to the hazardous environment be limited also to only a very few minutes in order to be consistent with reality.

Let us examine, however, the real purpose of the laboratory test procedure. The objective is to measure the acute toxicity of a mixture of pyrolysis products from Material A and compare it to the toxicity of the products from Material B—and C, D, etc. If one possessed an instrument into which each of these gas mixtures could be introduced, that would produce for us a "toxicity number" such as 1000 for Material A, 910 for Material B, and 1250 for Material C, there would be no problem. We could accept that A is 10 percent better than B, and that C is 25 percent better than A, when equal weights of the three were thermally decomposed. There would be no confusion or concern over the fact that the instrument "spent" 18 seconds performing each gas mixture analysis. There would also be no concern that the significance of the stated relative toxicities might be meaningless because the quantity of toxic gas mixture utilized for the analysis would have no effect on a person if totally inhaled—or likewise even if it were sufficient to prove fatal in 30 seconds. These parameters are no more related to the significance of the analytical results than was the time required for the analysis.

In the present case, possessing no such 18-sec instrument, our instrument-of-second-choice is the albino rat. The basis for the analytical measurement is an observable effect on integrated biological activity; the analytical result is the duration of the exposure, in units of time, from initiation until the desired biological effect is produced. This interval is the time it takes our "instrument" to make an analysis of the potency of the gas mixture. It is also the quantitative analytical result, analagous to the "toxicity number" output by the first instrument. Like the 18-sec analysis time and the resultant toxicity number from the first instrument, the response time of our "mammalian instrument" has no direct relationship to either escape or survival times in a fire. It is, however, a quantity that is related to toxicity, and by which different gas mixtures can be rated relative to each other, and one whose actual magnitude can be limited more-or-less by the proper choice of other parametric values, to any range desired by the experimentalists that would satisfy the four criteria listed initially.

I. The entire system should be a closed one to minimize the quantity of sample material required for a given animal response time. In contrast to a continuous flow-through system, this approach would also allow any decomposition products that might be produced only over a short interval of time to exert their proper toxic potential relative to those products that are formed at a slower rate and over a longer period.

III. Materials.

A. *Animals.* The laboratory albino rat was selected for these studies for the following reasons:

1. Convenient size (small enough to work with, large enough to furnish adequate blood and other tissue specimens for pathological workup).

2. Readily available at reasonable cost.

3. Significant amount of preexisting toxicological and physiological data available in the literature.

4. The expertise of the authors in conducting experiments with this animal species.

All animals were males of Sprague-Dawley origin and were obtained from Charles River Breeding Laboratories, Wilmington, Massachusetts. They were ordered at a weight range of 100 to 120 g and were held in isolation in our facility for 2 wk prior to use. On arrival, approximately 1 rat in 10 was sacrificed and inspected for pulmonary pathology that might

affect its response to inhaled gases. No shipment received during this study had to be rejected for preexisting pathology; however, as a precautionary measure, they were all maintained during the first week of isolation on drinking water that contained 1 oz of sulfathiazole per each 5 gal (approximately 1.5 g/L).*

After the second week the rats were moved from the isolation quarters. Those to be used during 1 day's operation were fasted overnight so that all would be in an equivalent metabolic state. Just prior to use, each animal was weighed and coded with a color mark on the head.

B. *Test Materials.* The subject materials that were evaluated in this study were supplied by the FAA National Aviation Facilities Experimental Center (NAFEC), Atlantic City, New Jersey. All 75 materials had been previously tested by them[7,8] and were shown to possess the flammability characteristics required by the Federal Aviation Regulations (FAR)[9] for their use as transport aircraft cabin materials. Many of the materials may be found in use on current models of the wide-body commercial jet fleet.

The same materials were also the subject of a concurrent NAFEC study in which each material was to be thermally decomposed and the evolved pyrolysis products analyzed for nine toxic gases.[10] A complete description of the materials is included as Appendix B.

Following receipt of the materials from NAFEC they were kept in resealable polyethylene bags. For the 24-hr period preceding testing each material was conditioned in a constant humidity chamber maintained at 50±1 percent relative humidity at room temperature (22°–24° C). The sample specimen was then cut into small pieces; in the case of some of the composite materials and the more dense plastics, a small, powered hobby saw (model 57–2, Dremel Mfg. Co., Racine, Wisc.) was used for this purpose. A 0.75-g aliquot** of these pieces was then

weighed to a 0.1 mg accuracy and placed in the appropriate sample boat for insertion into the furnace.

C. *Combustion Furnace.* Our choice for a heat source, based on the considerations described above, was the combustion tube furnace (CTF). The CTF offers the following advantages:

1. A combination of radiant and conductive heat transfer.

2. Simple construction of total combustion system.

3. Simple and rapid introduction of test specimens.

4. Ease of sample quantitation.

5. Elimination of problems with sample melting and dripping.

6. Minimized heat transfer to the animal chamber.

The specific CTF used in this study was a Lindberg model 54231A, with a model 59344 controller, that would accommodate tubes to a maximum diameter of 5.08 cm (2 in). When 2.54-cm (1-in) tubes were used, they were centered concentrically within the heaters by means of aluminum plates mounted at each end of the furnace.

Prior to each test the furnace was preheated to 625° C at the controller's sensing element (free air temperature in the middle of the upper heating element). This resulted in a 600° C temperature at the point of contact between sample boat and test material at the required ventilation rate. The furnace controller would then maintain this temperature within ±1° C.

The decomposition temperature of 600° C was selected after considerable testing of various heating regimens that ranged from 400° C to 850° C; 600° C is above the final decomposition temperature for all materials in the study, as determined by thermogravimetric analysis (TGA),[11] and represents an environment to which any material in a real fire would be subjected. Also, any material that reaches a temperature of 600° C would have passed through all temperatures between ambient and 600° C.

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* Sul-Thi-Zol, Sulfathiazole sodium, Merck Chemical Division, Rahway, New Jersey.

** This weight of sample is a fuel-load/system-volume ratio of 60 mg/L, and was selected experimentally as the quantity that seemed most likely to yield the desired distribution among response times for most of the materials that one might want to test.

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D. *Exposure Chamber.* The animal exposure and testing chamber incorporated those considerations outlined earlier. It is shown diagrammatically in Figure 1 and described in detail in Appendix A. Basically, it is a Plexiglas box containing a three-compartment motor-driven exercise wheel and two fans that accomplish rapid mixing of the chamber atmosphere. One-inch-diameter openings at diagonal corners of one face serve as ports for connecting the exposure and combustion systems with a conduit for continuous recirculation between the two.
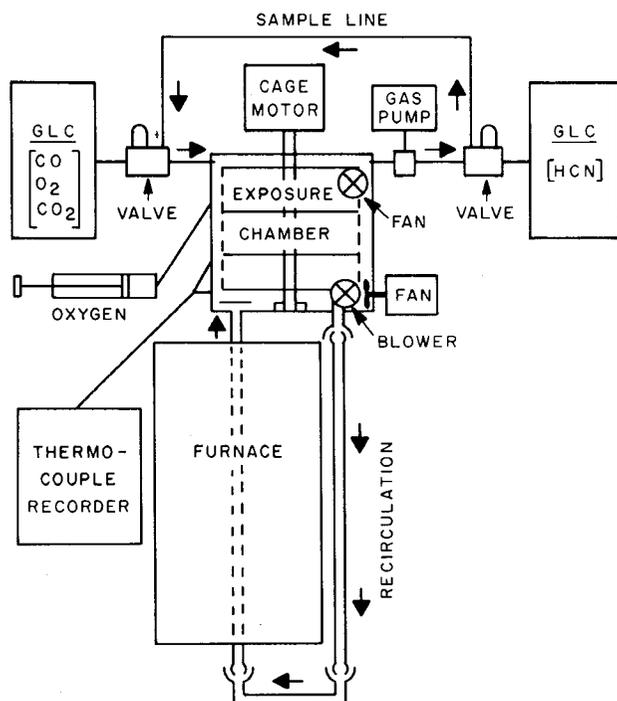

FIGURE 1.—Diagrammatic representation of overall combustion-exposure-analytical system.

Numerous small openings with silicone rubber plugs or septa provide for insertion of thermocouples, withdrawal of atmospheric samples, or introduction of gases from the exterior.

The total volume of the final system is 12.6 L; this includes the volume of the chamber, connecting 2.54-cm (1-in) conduits, and the combustion tubes minus the volumes of all interior solids. The final figure was arrived at by dilution ratios for known volumes of propane that were injected into the closed system, allowed to mix to equilibrium, and then analyzed by gas chromatography.

E. *Ventilation System.* A continuous recirculation of gases between the exposure-combustion systems served to maintain an oxygenated atmosphere in the zone of active thermal decomposition, move the decomposition products out of the furnace and into the exposure chamber, and dilute and cool these products below their flammable concentrations and flash points.

Experimental investigation of the effects of varying the ventilation rate showed the acceptable limits to be 4 and 7 L/min (Lpm). Below 4 Lpm the oxygen in the combustion zone was depleted significantly and the hot, concentrated gases tended to ignite and/or explode as they left the furnace and mixed with the oxygen diffusing back from the chamber. Above 7 Lpm the rate of heat transfer resulted in a chamber temperature of 40° C in 10 min, which was considered excessive.

We believe a ventilation rate of 6 Lpm is ideal (in our system); however, the tests reported here were accomplished with a flow of 4 Lpm. This slight compromise was desirable in order that related tests being done at NAFEC would correlate with ours, for they found it was impossible to accommodate a flow greater than 4 Lpm through their analytical samplers.*

The controlled ventilation was accomplished in our system with a variable speed blower (SCR-controlled) and a small-diameter orifice mounted inline and downstream from the blower. For details see Appendix A.

F. *Gas Analysis System.* We felt it was desirable to evaluate the concentration-time course of certain combustion products and of oxygen during each experiment. Therefore, an analytical capability was included in the test system that allowed the periodic measurement of chamber concentrations of CO, HCN, oxygen (O_2), and carbon dioxide (CO_2).

Information concerning the concentrations of CO and HCN was considered important for two reasons: (i) Many investigators feel that these two gases are the most important of the combustion products from a toxic hazard standpoint; (ii) Our previous determination of the inhalation

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* They ultimately reduced the ventilation rate even further, and their data[10] were collected at 2 Lpm.

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dose of each of these gases that would just result in incapacitation or death would allow us to calculate the resultant inhalation doses and determine whether or not these two gases were solely responsible for the observed animal response times. If they are not, then obviously at least a third component contributed significantly to the total toxicity of the combustion products.*

Gas analyses were accomplished with two gas chromatographs (GC) equipped with automated gas-sampling valves. The sample loops of these valves were continuously purged with a gas stream pumped from the exposure chamber via 3.2-mm Saran tubing, through the sample loops, and back into the chamber. The pump (Fluid Metering, Inc., Oyster Bay, NY) exposed the gases to only ceramic, Teflon, and stainless steel and thus minimized reactive losses. At fixed intervals of time the contents of these sample loops were injected into the GC's and the resultant chromatograms displayed on strip chart recorders. One GC was utilized for the determination of HCN only; the other measured the remaining gases—CO, O_2, and CO_2. Chamber HCN concentrations were measured every 3.8 min; CO and O_2 were measured at 1.9-min intervals; CO_2 samples were collected manually in syringes and analyzed after the experiment was completed.

The frequent measurement of the O_2 concentration in the chamber allowed us to make manual additions of a quantity of pure O_2 sufficient to maintain the chamber at better than 90 percent of the normal ambient partial pressure. The GC used for cyanide analyses was a Shimadzu model 3B-F utilizing a 2.1-m x 4-mm glass column of Porapak Q and was equipped with a rubidium sulfate thermionic detector of our own design. Nitrogen was the carrier gas.

The second GC was a Carle model 8000 equipped with a 3.2-mm silica gel column followed by a Molecular Sieve-5A column; helium was the carrier gas. The output signal from a thermistor detector was paralleled to a two-channel strip chart recorder. These two channels were operated at different attenuation levels; one

* A detailed account of the results of the gas analyses, and the conclusions derived therefrom, will be the subject of a subsequent Office of Aviation Medicine Report.

was selected to give a full-scale presentation for the oxygen concentration in ambient air (the nitrogen peak was allowed to go offscale); the other was adjusted for a full-scale response to the expected CO concentration in the chamber. The second column in the two-channel Carle was a 3.2-mm x 1.83-m Porapak Q column that was used for CO_2 analysis.

G. *Maximum Duration of an Exposure.* Taking into account the principles outlined in Section II,H., we selected a maximum exposure period of 30 min, at which time the experiment was terminated and any surviving animals were returned to their cages and observed for a period of 14 days.

It was determined experimentally that the CO_2 produced in 30 min by three rats in our system never exceeded a concentration of 5 percent (v/v). With 30 min as a limit, one has no difficulty in completing six experiments per day. Also, using a maximum response time of 30 min provides six response categories, each with a ± 3 min range (3 ± 3, 9 ± 3, 15 ± 3, 21 ± 3, and 27 ± 3 min); it actually provides seven categories if one calls "no response in 30 min" an additional category. Ideally once this maximum exposure time has been selected it would be achieved experimentally by selecting a fuel load to be used for all experiments that gave the best distribution of response times over the entire range of 30 min. To insure this one would have to pretest all materials, possibly at several weights of fuel load. We compromised by pretesting 12 materials that we felt would span the full toxicity range and selected a fuel load of 0.75 g for an enclosed volume of 12.6 liters.

IV. Test Procedure.

The sequence of operations for conducting an exposure experiment was as follows:

Three fasted, male rats were color coded, weighed, and placed in the rotating cage, one to a compartment. The preconditioned, weighed test material was placed in a semicylindrical glass sample boat made by cutting a 600-mm x 18-mm tube along a diameter. The material was positioned in the boat so that it would be in the middle of the hot zone when placed in the preheated furnace.

If the material to be tested were one of the foams or the bulky insulations a sample tube was substituted for the sample boat. Since these are low density materials, a 0.7-g sample would fill the combustion tube if it were positioned along a 150-mm length of an open boat. This would block the flow of air. Therefore, all foam samples were cut into 1-cm cubes and packed into a 200-mm length of glass tube (18 mm in diameter).

The insulation materials were packed in the tube around a central 6-mm glass rod. When the rod was withdrawn, just before positioning the sample tube inside the combustion tube, the stiffness of the material maintained the open channel. No channel was required with the foams because they melted down to leave an air passage as soon as they were inserted in the 600° C environment.

The cage was placed in the chamber and connected to the drive motor, then the chamber end plate was bolted in place. All thermocouples, sample lines, and the overhead pressure vent line were inserted through the proper ports. The recirculation tubes were connected together and the mixing fans turned on.

When everything was in place and the three operator-observers were ready, the combustion tube was opened, the sample boat was inserted, the system was reclosed, and a master switch was thrown. This switch turns on everything simultaneously, including a digital elapsed-time indicator and the timing device for operating the automated GC sampling valves.

The personnel activities during the run were: One observed the animals and noted the times-to-incapacitation (t_i) and times-to-death (t_d); one monitored the GC presentation of chamber oxygen concentration, added oxygen to the chamber when necessary, and took syringe samples of the chamber atmosphere for CO_2 analysis; and one monitored the sampling and GC system that measured HCN, and assisted in animal observation.

The time at which visible smoke first appeared at the furnace exit was recorded, as were the time and duration of any visible flames. A record was made of the time at which any convulsions occurred in the animals, their t_i and t_d, and the volume and time of all oxygen additions to the system.

Temperatures at the eight thermocouples were recorded automatically throughout the experiment on a calibrated multipoint strip chart recorder. This record served as an indication of any heat stress imposed on the animals and also depicted the efficiency of the air-mixing fans.

The beam from an amber-lensed, high-intensity lamp (automotive quartz-halogen fog lamp) could be directed down through the top of the chamber to improve visual observation of the animals if significant quantities of smoke were generated. Under the conditions of this test (i.e., aerobic but nonflaming combustion), most of the materials produced little smoke, and then it persisted for only a few minutes.

If there were survivors after 10 min, the recirculation blower was turned off, the conduit tubes removed from the chamber, the chamber ports stoppered, and the sample boat removed from the furnace.

Observation of the animals continued until all three had become incapacitated, at which time cage rotation was stopped to simplify the task of determining when visible respiration had ceased, which we recorded as the t_d. When the last animal had expired, the two large stoppered ports were opened to room air and a third port was connected to a vacuum source. Room air was pulled through the chamber to flush out the noxious and toxic gases.

The chamber was opened by removing the end plate, and the rotating cage was removed. The animals were immediately turned over to the Pathology Research Unit for collection of blood samples and pathological workup. Any survivors were observed until they succumbed, or for 14 days postexposure, and were then included in the pathology study.

The exposure chamber was removed from the system, all fans were dismounted from the walls, and both the chamber and rotating cage were cleaned in warm soapy water followed by an ethanol bath. All fan blades and impellers were also cleaned with ethanol. The parts were then air dried and reassembled. The system was now ready for another experiment.

The combustion/exposure test was replicated a minimum of three times for the first 65 materials and therefore yielded response times based on a minimum of nine animals. Some of those 65 were tested a fourth or fifth time (12 and 15 animals respectively) if the variation in animal response times exceeded a standard deviation of 1 min. The final 10 materials were tested only twice for a total of six animals each.

We found there were two parameters in the experimental design that could not be quantitatively replicated in all experiments without incurring an undesirable increase in time and cost per test. These two parameters were the body weight of the test animals and the weight of material placed in the furnace. Exact replication of animal weights from test to test, especially for animals utilized during their period of rapid growth, would have entailed an almost daily receipt of animals of closely specified weight range. Weighing material specimens to within 0.5 percent of the same weight each time would have been time consuming and would have increased the risk of obtaining a nonrepresentative aliquot from the parent material, especially where the heterogeneous materials were concerned.

However, we have determined experimentally, at least for the metabolic poisons such as CO, HCN, and hydrogen sulfide (H_2S), that animal response time (t_r) is a function of body weight. If all other conditions are held constant, t_i and t_d are proportional to the fourth root of body weight and inversely proportional to the toxic gas concentration, (C):

$$t_r = k \ (wt)^{1/4} \ C^{-1}. \qquad (1)$$

We have also verified that the resulting gas concentration is proportional to the weight of material that is thermally decomposed in the furnace and similarly that animal response times are inversely proportional to this sample weight (within the limits of 0.25–1.0 g of material and 0- to 30-min response times, and under the prescribed test conditions).

These relationships were therefore utilized to circumvent the problems of animal and material sample weights that varied from experiment to experiment. We included animals at any body weight between 125 and 350 g and used material

samples at any weight near 0.75 g that would allow representative sampling. The observed response time was then normalized to that which would have resulted with a 200-g animal and a material sample weight of 1.000 g. This normalized value is referred to as a "standard" response time. (See discussion, Appendix D.)

Some of the composite materials in the test series contained one or more components that were thermally stable; e.g., glass fibers and aluminum metal. These inert materials obviously made no contribution to the final mixture of volatile combustion products; therefore, one might want to exclude their portion of the total weight of a test specimen when making comparisons of potential toxicity on an equal-sample-weight basis. For this reason, we also calculated a third set of response times, normalized for a 200-g animal and a 1-g sample weight, except that the sample weight utilized in the normalization equation is that weight *lost* during combustion, not the 0.75 g loaded into the furnace. Response times calculated in this fashion are referred to as "loss" t_i and t_d.

V. Discussion of Results.

The observed animal responses for the 75 test materials, as measured in the system we have described, yielded t_i's that ranged from 1.48 to 19.75 min for 71 of the materials; no incapacitations were observed with the other four materials within the prescribed 30-min exposure period. The observed t_d's ranged from 3.03 to 24.40 min for 56 materials; no deaths were observed in 30 min for 19 of the materials. These mean, observed response times, along with the two sets of normalized values (standard t_r and loss t_r), are presented in Appendix C, Table C–1, for each of the 75 materials and are arranged in increasing numerical order of the material identification numbers.

The 75 materials are listed by their identification numbers in Table C–2 in the order of shortest to longest response times. The observed response times are listed along with the two corresponding sets of normalized response times. The numerical value of the response time for any material can be found by reference to Table C–1.

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The statistical precision with which each of the response times was measured is depicted in Table C–3. For each test material we have listed the total number of experimental animals (N) and the relative standard deviation of the mean (coefficient of variation) calculated for each of the six response times. A majority (65 percent) of these relative standard deviations (RSD) fall between 5 and 15 percent RSD, and almost 40 percent of them are less than 10 percent RSD.*

Figure 2 is a specialized bar graph that depicts the standard t_i and standard t_d for each of the 75

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* On the basis of previous work with pure gases and of results from the burning of homogeneous polymeric specimens, we feel that those RSD above 10 to 15 percent are due primarily to lack of homogeneity in replicate specimens and/or nonreproducible thermal decomposition.

materials, arranged in the sequence of increasing t_i. It is apparent that the series of t_i's represents a near continuum of values, with no obvious or significant breaks in the pattern; therefore, any attempt to place into separate hazard categories two materials that are adjacent, or possibly even near each other, in the plot would require a most arbitrary division.

An additional observation was that materials within each "end use" category, such as foams, panels, fabrics, thermoplastics, etc., had about the same toxicity ranking. Therefore, an arbitrary division of the total group of materials into good versus bad, acceptable versus unacceptable, good versus better, etc., by drawing a line at a given t_i-value would likely place all the elements of one or more end-use categories into the unacceptable group. It seems more realistic
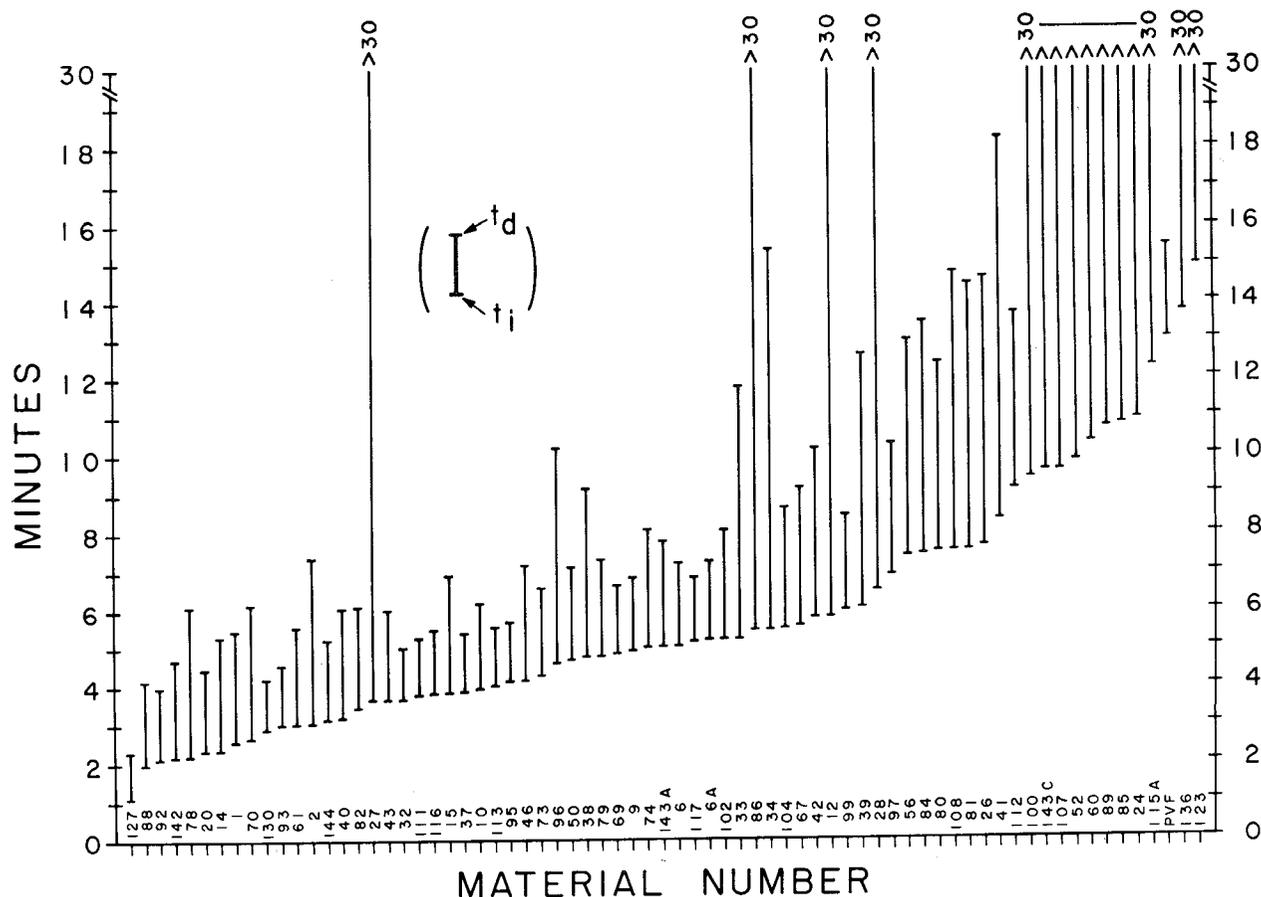

FIGURE 2.—Animal response times (std. t_i and t_d) for the 71 materials that produced incapacitation in 30 min or less. Bottom of each bar represents t_i; top of bar represents t_d. Relative toxicities decrease from left to right.

to evaluate the merit of a given material relative only to other materials that are candidates for the same functional end-use.

In the series of materials under test, there are 10 separate "functional" categories plus an 11th group composed of the individual components that make up the panel composites; e.g., honeycomb cores, adhesive, fiberglass, epoxy. The relative rank-orders of the members of each of these 11 categories are presented in Figures 3–13. In each case the materials are ranked according to their standard t_i's (bottom bar) from the most toxic on the left (shortest t_i) to the least toxic on the right (longest t_i). The materials are identified by their code numbers (see Appendix B) and by an abbreviated description of the material. These abbreviations are identified in Table 1.

A. *Panels.* Most of the 13 panel assemblies consisted of an aromatic polyamide honeycomb core covered with Fiberglas and differed from each other, mainly in the outer-most finish layer. Consequently, one would not expect them to exhibit large differences in toxicity and this was borne out experimentally. Values for standard t_i ranged from 2.36 min (material No. 20) to 5.85 min (material No. 12) and increased at a fairly constant rate (Figure 3).

Table 1. Abbreviated Titles for Materials as used in Figures 3 to 13

Material Number	Abbreviation	Material Number	Abbreviation
1	PVF/EPX-FG/AR HC/EPX-FG	78	ARAMID
2	EPX-FG/AR HC/EPX-FG	79	PLET URETH
6	PVF/AR FIBER-PHEN	80	URETHANE
6a	PVF/AR FIBER-PHEN	81	PVC
9	AL/AR HC/AL	82	WOOL:PVC 76:24
10	FG-POLYESTER	84	PVC/COTTON
12	PVF/PLST-GLASS/AR HC	85	ABS-PVC
14	PVF/AR-EPX/AR HC/EPX-FG	86	PVC
15	PVF/AR-EPX	88	WOOL
18	PVF FILM	89	PVC/NYLON
20	PVF/EPX-FG/AR HC/EPX-FG/PVF	92	ARAMID
24	EPX-FG/PVC/EPX-FG	93	COTTON
25	PVF/FG-EPX/PVF	95	RAYON
26	FG-EPX	96	WOOL:PVC 49:51
27	MELAMINE-FG	97	PVC-POLYESTER
28	ALUMINIZED PVF/NYLON	99	PVC-PMMA
32	POLYCARBONATE	100	PVC-ABS
33	WOOL/PLST/LATEX	102	POLYETHYLENE
34	WOOL/PLST/LTX/URETH	104	PLST URETH
37	PVF/PHEN-FG/AR HC	107	ABS-PVC
38	EPX COATED PHEN-FG	108	FR-PMMA
39	EPX COATED PHEN-FG	109	PMMA
40	AR HC/PHEN-FG BATT	111	POLYCARBONATE
41	EPX COATED PHEN-FG	112	SILICONE
42	PVF	113	PVF/PCARB/PVF
43	PVF/PHEN-FG/AR HC	115a	PHEN-FG
46	PVF/PVC/PHEN-FG/AR HC/EPX-FG	116	POLYCARBONATE
50	WOOL/PHEN-FG/AR HC/EPX-FG	117	POLYPHENYLENE OXIDE
52	WOOL/EPX/AL/BALSA	118a	FG-EPX/ASBESTOS
56	PVC/SS/EPX/AR-PHEN HC	123	SILICONE
60	EPX-FG	127	MODACRYLIC
61	PVF/PVC/PHEN-FG/EPX/AR HC	130	COTTON/RAYON
66	SIL TREATED PHEN-FG	136	PVC/COTTON
67	PVC/PHEN-FG/AR HC/EPX-FG	142	WOOL:NYLON 90:10
69	PVF/PVC/PHEN-FG/AR HC	143a	PLET URETH
70	WOOL:NYLON 90:10	143c	PLST URETH
73	URETHANE	144	PVF/EPX-FG/AR HC/EPX-FG
74	URETHANE		

One material, No. 12, does exhibit a property that could be worthy of consideration in some fire situation; i.e., no deaths occurred in the 30-min test period. In contrast with material No. 69, which has about the same t_i, the interval of time between incapacitation and death for No. 12 is greater than 25 min compared to less than 2 min for No. 69. The longer t_i-t_d interval would represent an increased survival potential in any fire situation that allowed rescue of an incapacitated individual by someone else, such as a building fire, or an aircraft fire at an airport or in a populated area.

B. *Panel Components*. Results obtained with these nine materials could indicate the relative contribution to the total toxicity of a composite panel that is made by each of the individual panel components. From Figure 4 it is apparent that there is very little difference between near neighbors in the first seven materials; however, the last two do differ both from the first seven materials and from each other. It would appear that the greatest toxicity is associated with the aramid component, which is an aromatic polyamide in either fiber or honeycomb form.

One must bear in mind that the relationships depicted on the bar graph and the conclusions drawn from them are all based on the "standard" t_i's. These, in turn, are based on the equivalent of a 1-g fuel load of the finished material, and no corrections are made for that residual portion of a material that does not decompose.

By substituting loss t_i's for standard t_i's, one can compare these same nine components on the basis of response time per gram of sample weight loss—essentially, this corrects for the quantity of thermally stable Fiberglas in the sample. The order of relative toxicities, from most to least toxic, then becomes: 38, 6, 6A, 40, 39, 15, 41, 42, and PVF. Three of the four Fiberglas-containing components move to positions of greater toxicity. The most dramatic shift in toxicity occurs with No. 15, which moves from second place down to sixth place; note that it does not contain any Fiberglas.

C. *Foams*. As a class, the foams decomposed rapidly under our test conditions, leaving little or no residue. Of the nine foams listed in Figure 5, seven yielded t_i values over a narrow range (4.29–5.55 min). The remaining two materials, a urethane (No. 80) and a polyester urethane (No. 143c), as well as material No. 86, a polyvinyl chloride (PVC) foam, produced no deaths during the 30-min observation period.

D. *Fabrics*. Although materials in this category represent a wide variety of chemical compositions, with few exceptions the t_i's were quite short (Figure 6). A modacrylic drapery fabric, No. 127, gave the shortest t_i (1.15 min) recorded for any of the 75 materials and the t_d was less than 3 min. Eleven of the twelve materials gave t_i values of less than 5 min and the remaining one (No. 81, an untreated PVC upholstery fabric) gave a t_i of 7.57 min.

In addition to producing relatively short t_i's, several of these materials also killed the test animals quite soon after incapacitation. The elapsed time between incapacitation and death was less than 2 min for materials No. 93, 85, 127, and 130. In our experience, a short survival time following incapacitation is usually associated with the presence of supralethal levels of CO.

E. *Coated Fabrics*. All materials in this group were fabrics that had been coated with PVC. The shortest t_i was 6.97 min for a lightweight, flame retardant-treated polyester (No. 97) used as a seat bottom diaphragm. The remaining materials were heavy upholstery fabrics and yielded t_i's of from 7.47 to 13.71 min (Figure 7).

Two of these materials appear particularly promising from a toxicological standpoint. Materials No. 89 (PVC/nylon) and No. 136 (PVC/cotton) yielded t_i's of 10.70 and 13.71 min respectively, and neither material killed the test animals in the 30-min observation period.

On a sample weight basis, both coated cotton materials (No. 84 and 136) appeared to be less toxic than their uncoated counterpart, material No. 93. However, since the weight ratio of fabric to coating in these materials is not known, it is difficult to know whether the lowered toxicity of the cotton/PVC materials is due (i) to the substitution of a significant part of their total weight with the less toxic PVC or (ii) to changes in the flammability and burning characteristics of the fabric because of the PVC coating.

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FIGURE 3.—Panels. Relative toxicity of combustion products as measured by animal response time. Bottom of each bar represents t_i; top of bar represents t_d. Relative toxicities decrease from left to right.

FIGURE 4.—Panel components. Relative toxicity of combustion products as measured by animal response time. Bottom of each bar represents t_i; top of bar represents t_d. Relative toxicities decrease from left to right.

FIGURE 9.—Thermoplastics. Relative toxicity of combustion products as measured by animal response time. Bottom of each bar represents t_i; top of bar represents t_d. Relative toxicities decrease from left to right.

H. *Cargo Liners.* Materials in this category consisted of Fiberglas-reinforced plastic sheets containing variable quantities of nonflammable material (Figure 10). In general, the observed t_i's were related to the quantity of noncombus-
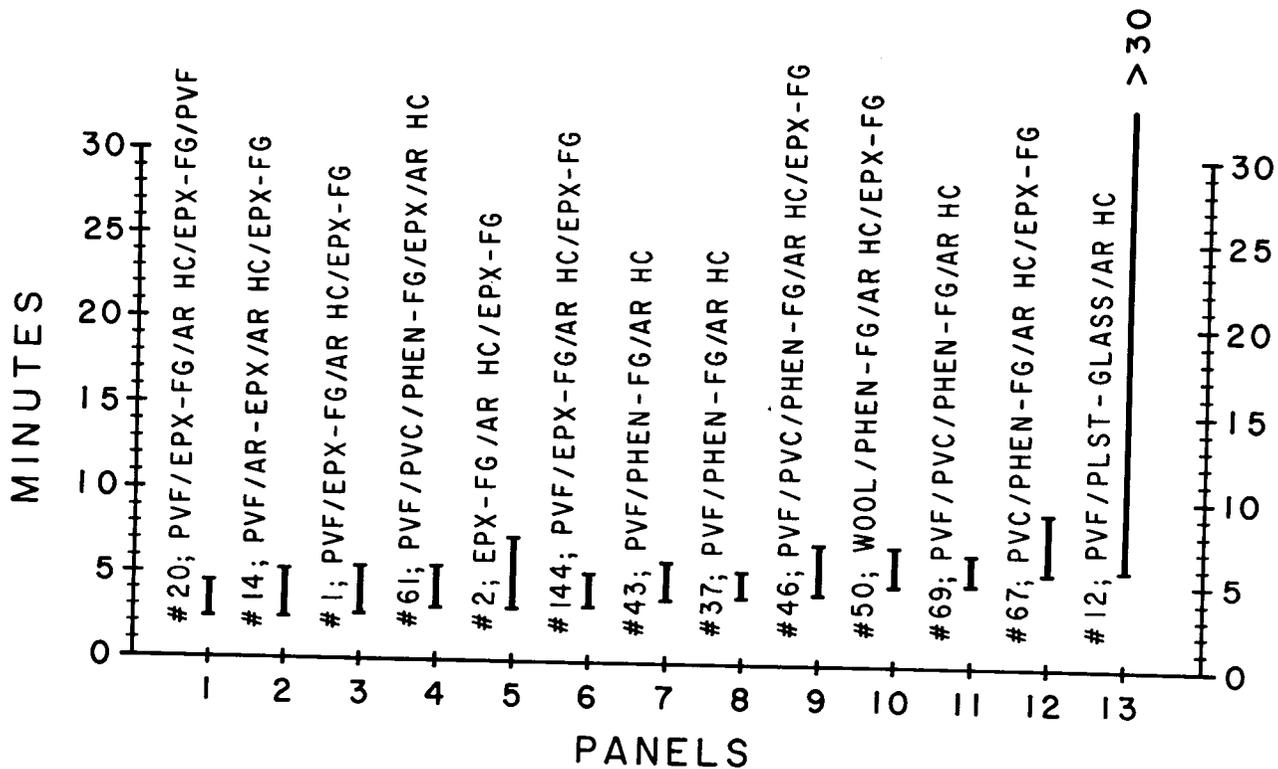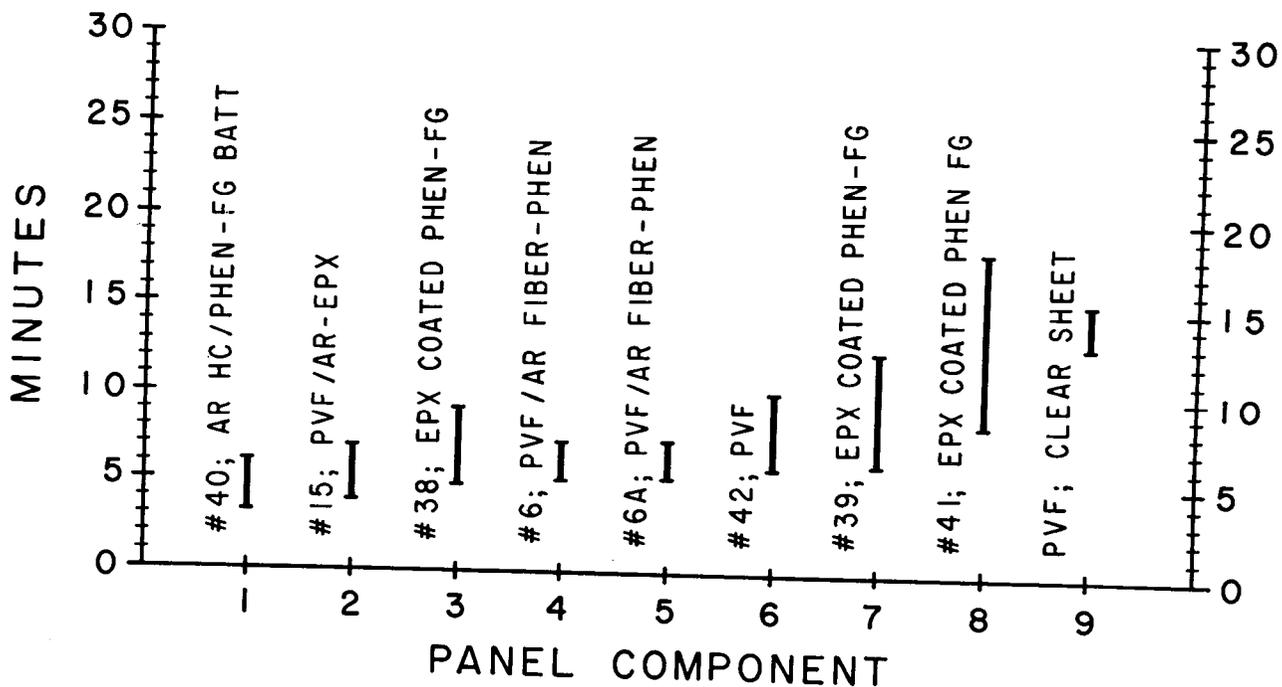

FIGURE 10.—Cargo liners. Relative toxicity of combustion products as measured by animal response time. Bottom of each bar represents t_i; top of bar represents t_d. Relative toxicities decrease from left to right.

tible material in each sample; i.e., the larger the fraction of thermally stable component, the longer the t_i.

Materials that produced animal incapacitation within the 30-min observation period were a Fiberglas/polyester (No. 10) and two Fiberglas/epoxy sheets (No. 26, 60). Their respective t_i's were 3.99, 7.68, and 10.33 min. Two materials neither incapacitated nor killed the animals: No. 25, a PVF-coated Fiberglas/epoxy and an asbestos-impregnated Fiberglas/epoxy (No. 118A). The animal survivors from tests of No. 118A were observed for 8 days posttest and were then sacrificed. No obvious ill effects of the potential asbestos exposure were noted during the 8 days, and no gross or microscopic pathology attributable to asbestos was found on postmortem examination.*

I. *Transparencies.* The three materials in this category yielded a wide range of toxicity values. One polycarbonate (No. 111) gave a t_i of 3.80 min; the second acrylic (No. 109) did not incapacitate in 30 min (Figure 11). Material No. 111 gave the rapid production of a very high CO level, which led to the usual short t_i-t_d interval. Material No. 109 provided a surprising, although not completely unexpected, incident in the latter phases of the test series. After the initial tests had confirmed that we were not going to observe a $t_i < 30$ min for some materials, larger samples were loaded to determine what weight would be necessary to obtain an observable incapacitation. The required weight of the larger sample was estimated on the basis of the quantity of CO and HCN resulting from the original decomposition of 0.75 g of material and the knowledge of the incapacitating doses of these gases for the rat. When a calculated incapacitating load of No. 109, 2.25 g, was tested, the mixture of pyrolysis gases generated was sufficiently concentrated, at the ventilation rate of 4 L/min, to produce an explosive mixture in the chamber. These gases were ignited by a flame that originated in the furnace tube, and the resultant explosion severely damaged the exposure chamber. The experience was an excellent demonstration of one of the advantages of a chamber constructed from plexiglas rather than glass, and it prompted us to add a large blowout panel to the design of future chambers.

J. *Insulations.* Materials from this end-use category that were included in the test series were limited to three Fiberglas insulation batts containing various binders (No. 27, 66, 115A) and a single insulation batt cover material (No. 28) composed of aluminized PVF and nylon (Figure 12). The range of t_i's for the batts was from 3.70 min for a melamine batt (No. 27) to greater than 30 min for a silicone-treated phenolic-Fiberglas batt (No. 66). A nonsiliconed phenolic batt (No. 115A), on the other hand, gave a t_i of 12.26 min. No deaths were observed with any of the four materials.

The Fiberglas content of these materials is about 80 percent (by weight); therefore, the amount of material undergoing decomposition in

FIGURE 11.—Transparencies. Relative toxicity of combustion products as measured by animal response time. Bottom of each bar represents t_i; top of bar represents t_d. Relative toxicities decrease from left to right.
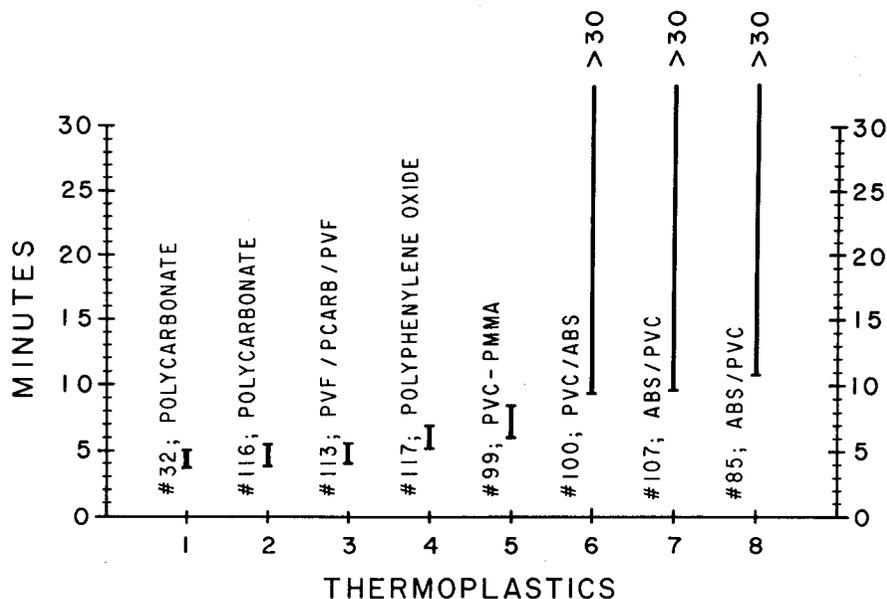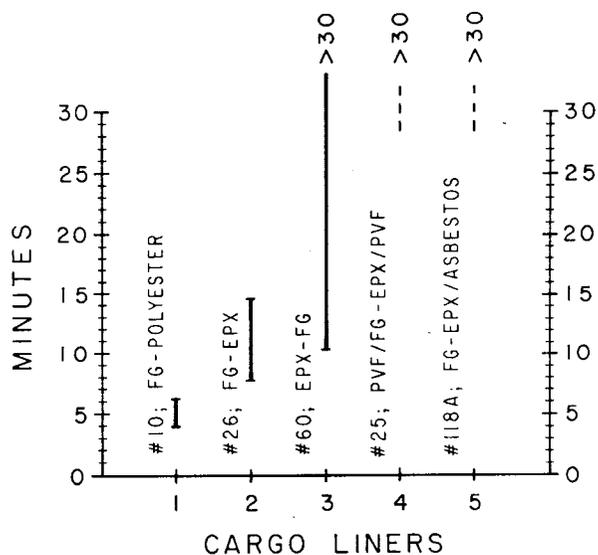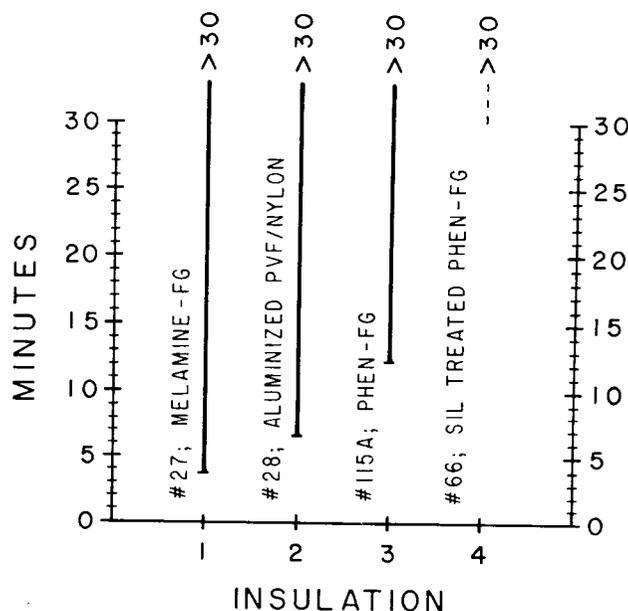

FIGURE 12.—Insulations. Relative toxicity of combustion products as measured by animal response time. Bottom of each bar represents t_i; top of bar represents t_d. Relative toxicities decrease from left to right.

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the furnace is only about 20 percent of the loaded weight. It is this small amount of binding material that is responsible for the animal responses. For material No. 27, for instance, if the melamine alone accounts for the t_i of 3.7 min and is only 20 percent of the total weight, then on an equal weight basis, pure melamine would be about the most toxic polymeric material we have tested. Careful consideration should be given to this fact when such materials are used, for although they are light in weight they can still comprise a significant fraction of the total weight of polymeric material used in aircraft.

K. *Elastomers.* Two door-sealing materials, both of which were silicone elastomers, were tested in the series (Figure 13). From the toxicological standpoint, material No. 123 proved to be the better of the two, producing a t_i of 14.96 min and no deaths. Material No. 112 incapacitated the animals in 9.16 and killed them about 4 min later.
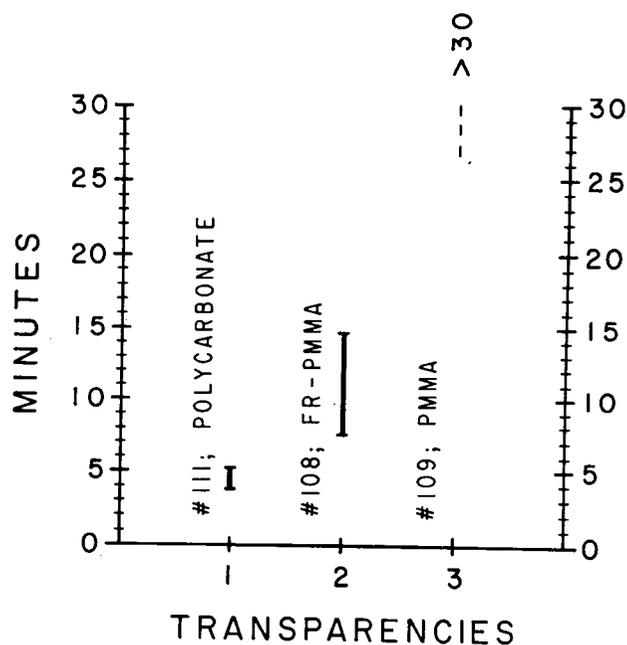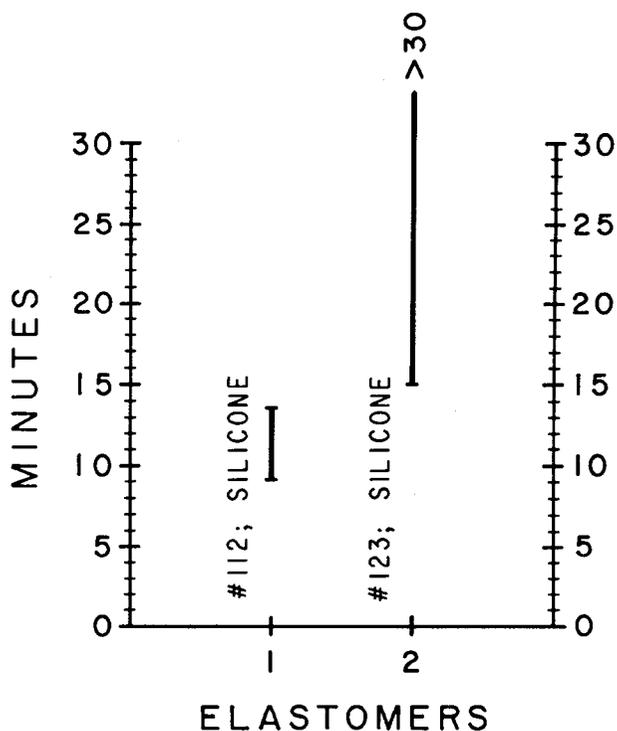

FIGURE 13.—Elastomers. Relative toxicity of combustion products as measured by animal response time. Bottom of each bar represents t_i; top of bar represents t_d. Relative toxicities decrease from left to right.

VI. Summary and Conclusions.

We have described in some detail the concepts, design features, construction, and testing of a small-scale system that utilizes small laboratory animals for evaluating the relative toxicities of combustion products from polymers. Overall system performance was demonstrated by testing 75 materials used in aircraft cabin interiors and ranking them according to their relative inhalation toxicities to the albino rat.

Relative rank-orders for these 75 materials have been presented in several ways:

A. On the basis of two animal end points; i.e., relative times-to-incapacitation and times-to-death.

B. On the basis of equal material weights loaded in the furnace.

C. On the basis of the loss of equal weight of material during pyrolysis.

D. On the basis of animal response times corrected for differences in animal weights and sample weights.

The significance of the various bases for these multiple rankings has been discussed, as have the relative merits for selecting one base over another for a specific application. We feel that use of either "loss" or "standard" response times is equally valid provided the choice is based on the proper recognition of what each measurement signifies and its relationship to the weight of the material involved. If one were primarily interested in evaluating relative performance of materials as they would be used in an installation, the use of "standard" response times would probably be preferable. However, if one were interested in the potency of the decomposition products, per se, one might choose to look at "loss" response times. For most comparisons, the use of "standard t_i" is the one preferred by the authors.

Judging from the distribution of t_i's throughout the 0 to 30-min exposure period, it appears that a more uniform distribution might have resulted with a fuel load slightly less than the 0.75g/12.6 L that was selected.

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The system is small (12.6 L), easily constructed by laboratory personnel, and relatively inexpensive to assemble. It will accommodate from 8 to 12 tests per day per unit, utilizing three rats per test, with cleaning between successive tests. For such biological end points, reproducibility of animal response times from test to test is surprisingly good, as is the precision of the measurement from animal to animal within a given test.

The simplicity of design, low cost, ease of operation, and reproducibility of results suggest that the significant features of this system and its operating parameters are worthy of consideration in the current search for a standard system with which to measure the relative inhalation toxic hazards associated with nonmetallic polymeric material.

One must, however, bear in mind that this test system, reproducible as it is and useful as it may be, shares a fault common to all such systems in use or proposed. The parameters of the thermal decomposition process do not necessarily represent, and are not presented as a representation of, the processes that exist in a "real" fire. Therefore, the resultant relative toxicity ranking may not reflect the relative hazards of these or other such materials under actual fire conditions. We consider this to be a preliminary study with the primary objective that of designing a suitable system for making toxicity measurements of gaseous environments, not reproducing actual fire atmospheres. The described system accomplishes that objective surprisingly well. We recommend that additional studies be conducted to refine the parametric values in order that they may more adequately reflect those of significance in a fire.

APPENDIX A

DESCRIPTION OF THE ANIMAL EXPOSURE CHAMBER AND ITS OPERATION
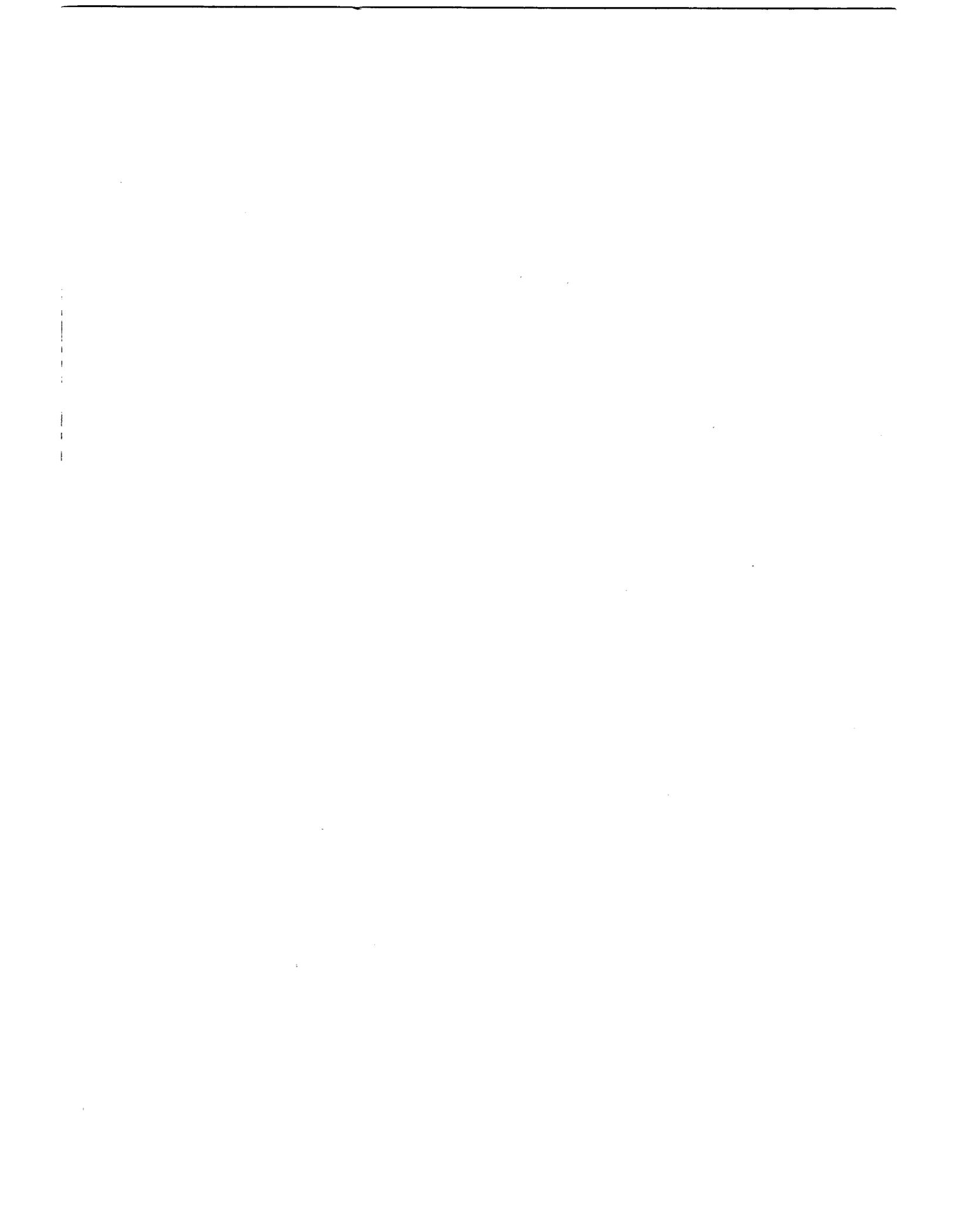
APPENDIX B

DESCRIPTION OF THE 75 AIRCRAFT INTERIOR MATERIALS
AND THEIR PHYSICAL PROPERTIES
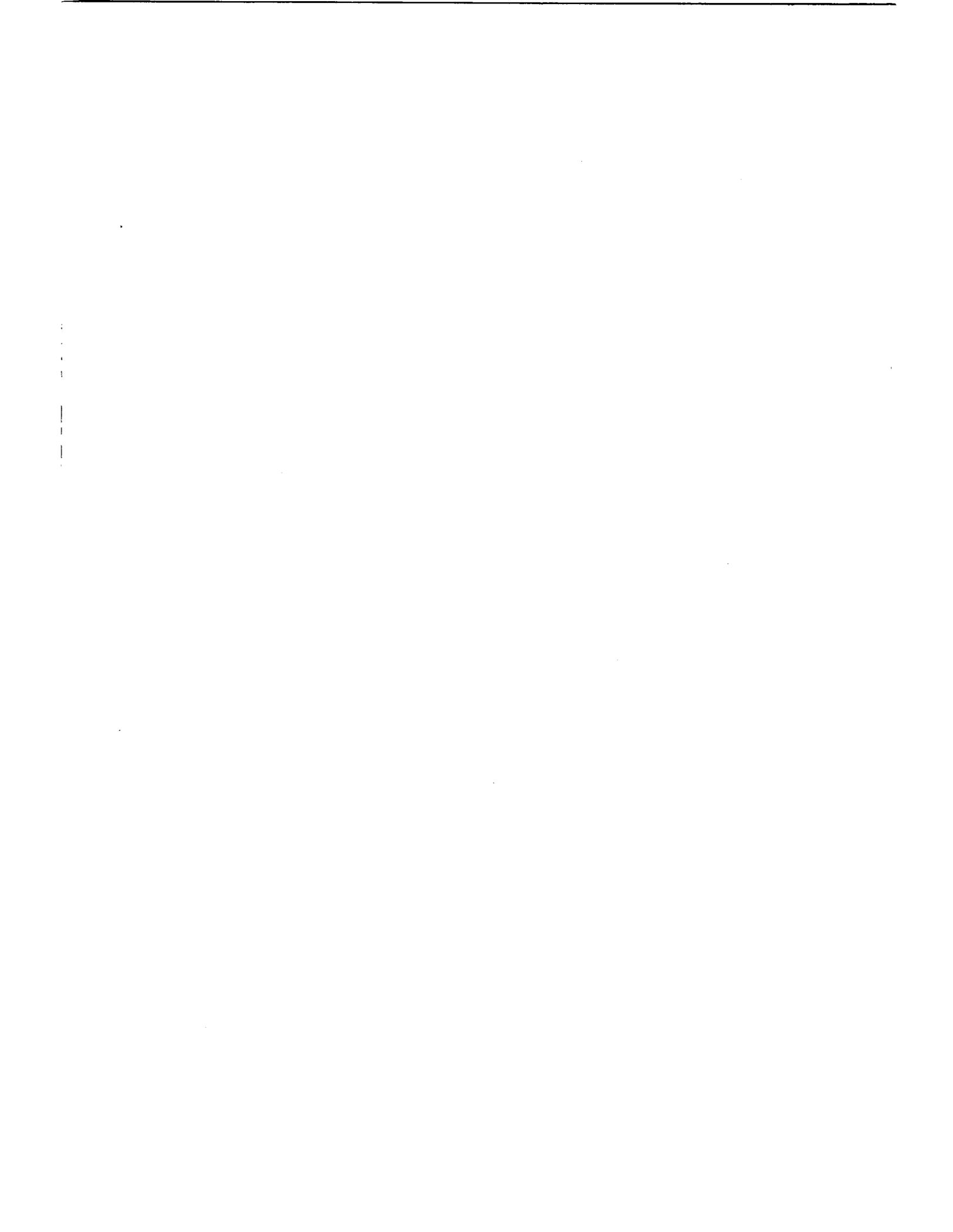
No.	Chemical Composition	Thickness (in)	Unit Weight (oz/yd²)	Designation	Cabin Use
84	PVC/Cotton (untreated)	0.058	26.9	Coated fabric	Arm rest cover
85	ABS-PVC (untreated)	0.060	56.4	Thermoplastic	Seat side panels and trays
86	PVC (untreated)	0.500	28.8	Foam	Flotation cushion and padding for seat back and arm rest
88	FR Wool	0.055	17.2	Fabric	Upholstery
89	FR PVC/Nylon	0.059	26.3	Coated fabric	Seat arm cap
92	Aramid	0.036	11.8	Fabric	Upholstery
93	FR Cotton	0.012	3.06	Fabric	Upholstery
95	FR Rayon	0.041	15.4	Fabric	Upholstery
96	Wool (49 percent)/PVC (51 percent)	0.044	13.8	Fabric	Upholstery
97	FR PVC-Polyester	0.018	11.4	Coated fabric	Seat bottom diaphragm
99	FR PVC-Polymethyl Methacrylate	0.044	39.6	Thermoplastic	Seat shroud
100	FR PVC/ABS	0.092	86.9	Thermoplastic	Seat shroud
102	FR Polyethylene (rigid)	0.500	13.7	Foam	Flotation cushion
104	FR Polyester Urethane	0.500	40.1	Foam	Seat cushion
107	ABS-PVC	0.127	122	Thermoplastic	Molded part
108	FR Polymethyl Methacrylate	0.054	46.6	Transparency	Scratch shield
109	Polymethyl Methacrylate	0.260	228	Transparency	Window pane
111	Polycarbonate	0.052	46.2	Transparency	Windscreen
112	Silicone	0.094	86.3	Elastomer	Door seals
113	PVF/Polycarbonate/PVF	0.431	151	Thermoplastic	
115a	Phenolic-Fiberglas	1.09	6.40	Insulation	Fuselage insulation
116	Polycarbonate	0.043	36.8	Thermoplastic	Passenger service units and luminaires
117	Polyphenylene Oxide	0.041	31.4	Thermoplastic	Flight station and lavatory parts
118a	Fiberglas-Epoxy/Asbestos	0.020	28.9	Cargo liner	Cargo liner
123	Silicone	0.124	116	Elastomer	Door seals
127	Modacrylic	0.032	8.63	Fabric	Drapery
130	Cotton/Rayon	0.040	15.0	Fabric	Upholstery
136	PVC/Cotton	0.057	28.3	Coated fabric	Upholstery
142	FR Wool (90 percent)/Nylon (10 percent)	0.035	10.3	Fabric	Upholstery

No.	Chemical Composition	Thickness (in)	Unit Weight (oz/yd^2)	Designation	Cabin Use
143a	FR Polyether Urethane	0.500	13.9	Foam	Seat cushion
143c	FR Polyester Urethane	0.500	38.8	Foam	Seat cushion
144	PVF/Epoxy-Fiberglas/Aramid Honeycomb/Epoxy-Fiberglas	0.276	43.3	Panel	Wall panel

ABBREVIATIONS

ABS – Acrylonitrile/Butadiene/Styrene
FR – Flame-retardant treated
PVC – Polyvinyl chloride
PVF – Polyvinyl fluoride

Table C-1. Mean Response Times for Each of the 75 Test Materials

Mean Response Time (Minutes)

Material No.	Observed ①		Standard ②		Loss ③	
	t_i	t_d	t_i	t_d	t_i	t_d
1	3.34	6.99	2.61	5.47	1.64	3.44
2	4.04	9.69	3.07	7.38	1.76	4.23
6	6.51	9.29	5.07	7.23	1.97	2.80
6a	6.68	9.37	5.22	7.31	2.20	3.08
9	6.61	9.20	4.94	6.88	2.15	2.99
10	5.09	7.89 ④	3.99	6.20	3.60	5.59
12	7.57	----	5.85	----	3.60	----
14	3.09	6.89	2.38	5.31	1.80	4.02
15	5.09	9.05	3.89	6.94	3.74	6.66
20	3.04	5.77	2.36	4.48	1.66	3.15
24	14.68	----	10.90	----	5.37	----
25	----	----	----	----	----	----
26	9.95	18.92	7.68	14.61	3.64	6.91
27	4.91	----	3.70	----	0.69	----
28	8.71	----	6.56	----	6.24	----
32	4.94	6.71	3.70	5.02	3.61	4.90
33	7.07	15.80	5.26	11.73	4.95	11.07
34	7.35	20.43	5.53	15.35	5.13	14.26
37	5.06	7.06	3.90	5.43	2.06	2.87
38	6.27	11.96	4.79	9.15	1.44	2.75
39	7.88	16.15	6.09	12.56	2.76	5.69
40	4.31	8.14	3.22	6.08	2.51	4.74
41	11.19	24.40	8.36	18.22	4.69	10.24
42	11.40	19.91	5.82	10.16	4.71	8.24
43	4.85	7.90	3.70	6.02	2.31	3.76
46	5.59	9.61	4.18	7.17	2.37	4.04
50	6.17	9.32	4.70	7.10	3.05	4.62
52	13.11	----	9.84	----	5.45	----
56	10.12	17.60	7.46	12.97	4.75	8.26
60	13.63	----	10.33	----	4.37	----
61	4.07	7.39	3.07	5.57	2.09	3.79
66	----	----	----	----	----	----
67	7.33	11.97	5.58	9.15	3.34	5.48
69	6.49	8.92	4.86	6.68	2.90	3.98
70	3.66	8.27	2.72	6.16	2.36	5.36
73	5.59	8.57	4.29	6.59	4.46	6.80
74	6.61	10.61	5.04	8.08	5.10	8.15
78	3.04	8.36	2.23	6.13	2.17	5.97
79	6.35	9.70	4.80	7.34	4.74	7.25
80	9.91	16.37	7.55	12.40	7.21	11.82
81	9.99	19.09	7.57	14.45	7.44	14.18
82	4.72	8.32	3.47	6.12	3.43	6.04

Table C-1 (continued)

Material No.	Observed		Standard		Loss	
	t_i	t_d	t_i	t_d	t_i	t_d
84	9.73	17.52	7.47	13.43	6.91	12.46
85	14.22	----	10.79	----	9.97	----
86	7.10	----	5.50	----	4.03	----
88	2.70	5.62	2.00	4.17	1.75	3.63
89	14.29	----	10.70	----	9.89	----
92	2.83	5.55	2.15	4.22	2.00	3.94
93	3.95	5.88	3.07	4.58	2.74	4.10
95	5.43	7.43	4.18	5.72	3.51	4.81
96	5.98	13.19	4.64	10.18	4.53	9.96
97	9.23	13.59	6.97	10.28	6.51	9.60
99	7.91	11.10	6.01	8.44	5.88	8.25
100	12.30	----	9.41	----	8.49	----
102	6.66	10.26	5.25	8.08	5.55	8.60
104	7.09	11.05	5.55	8.65	5.50	8.57
107	12.60	----	9.59	----	8.89	----
108	10.06	19.75	7.56	14.73	7.50	14.60
109	----	----	----	----	----	----
111	4.98	6.94	3.80	5.28	3.75	5.21
112	12.02	17.95	9.16	13.66	2.26	3.38
113	5.13	7.06	4.04	5.56	3.99	5.49
115a	13.08	----	12.26	----	2.98	----
116	4.85	6.97	3.83	5.50	3.76	5.40
117	6.87	9.12	5.19	6.89	5.11	6.78
118a	----	----	----	----	----	----
123	19.75	----	14.96	----	2.99	----
127	1.48	3.03	1.15	2.34	1.10	2.24
130	3.76	5.46	2.93	4.24	2.72	3.94
136	18.12	----	13.71	----	11.87	----
142	2.85	6.04	2.22	4.70	2.01	4.26
143a	6.34	9.77	5.06	7.80	5.06	7.80
143c	12.20	----	9.58	----	8.43	----
144	4.28	7.02	3.19	5.26	1.77	2.92
PVF	17.17	20.33	13.02	15.42	13.19	15.61

1. Observed response times are those determined experimentally; they have not been transformed in any way except for calculation of the mean value of all observed times.

2. Standard response times are the observed times normalized to those for a "standard" 200-g animal and a "standard" fuel load of 1.000 g rather than the weight actually used.

3. Loss response times are the observed times normalized to those for a "standard" 200-g rat and for a loss of fuel weight of 1.000 g rather than the actual weight loss.

4. A (----)entry indicates no response during the 30-min observation period.

Table C-2. Materials Ranked in Order of Decreasing Toxic Hazard

Material Numbers Listed by Ranking Criteria

Rank Order	Observed t_i	Standard t_i	Loss t_i	Observed t_d	Standard t_d	Loss t_d
1	127	127	27	127	127	127
2	88	88	127	130	88	38
3	92	92	38	92	92	6
4	142	142	1	88	130	37
5	78	78	20	20	20	144
6	20	20	88	93	93	9
7	14	14	2	142	142	6a
8	1	1	144	32	32	20
9	70	70	14	14	144	112
10	130	130	6	111	111	1
11	93	93	92	116	14	88
12	2	61	142	1	37	43
13	61	2	37	144	1	61
14	144	144	61	37	116	92
15	40	40	9	113	113	130
16	82	82	78	61	61	69
17	116	27	6a	95	95	14
18	43	43	112	10	43	46
19	27	32	43	43	40	93
20	32	111	70	40	82	2
21	111	116	46	70	78	142
22	37	15	40	82	70	50
23	15	37	130	78	10	40
24	10	10	93	73	73	95
25	113	113	39	69	69	32
26	95	95	69	15	9	111
27	73	46	115a	117	117	70
28	46	73	123	9	15	116
29	96	96	50	6	50	67
30	50	50	67	50	46	113
31	38	38	82	6a	6	10
32	143a	79	95	46	6a	39
33	79	69	10	2	79	78
34	69	9	12	79	2	82
35	6	74	32	143a	143a	15
36	9	143a	26	102	74	117
37	74	6	15	74	102	73
38	102	117	111	104	99	26
39	6a	6a	116	99	104	79
40	117	102	113	38	67	143a

Table C-2 (Continued)

Rank Order	Observed t_i	Standard t_i	Loss t_i	Observed t_d	Standard t_d	Loss t_d
41	33	33	86	67	38	74
42	104	86	60	96	42	42
43	86	34	73	97	96	99
44	67	104	96	33	97	56
45	34	67	41	39	33	104
46	12	42	42	80	80	102
47	39	12	79	84	39	97
48	99	99	56	56	56	96
49	28	39	33	112	84	41
50	97	28	143a	26	112	33
51	84	97	74	81	81	80
52	80	56	117	108	26	84
53	26	84	34	42	108	81
54	81	80	24	PVF	34	34
55	108	108	52	34	PVF	108
56	56	81	104	41	41	PVF
57	41	26	102			
58	42	41	99			
59	112	112	28			
60	143c	100	97			
61	100	143c	84			
62	107	107	80			
63	115a	52	81			
64	52	60	108			
65	60	89	143c			
66	85	85	100			
67	89	24	107			
68	24	115a	89			
69	PVF*	PVF	85			
70	136	136	136			
71	123	123	PVF			

No animal deaths were noted during the 30-min observation period for materials No. 12, 24, 25, 27, 28, 52, 60, 66, 85, 86, 89, 100, 107, 109, 115a, 118a, 123, 136, and 143c.

No animal incapacitation was noted during the 30-min observation period for materials No. 25, 66, 109, and 118a.

Table C-3. Statistical Data. The Relative Standard Deviation of the Means of the Various Measured or Calculated Response Times

| Material Number | N* | Relative Standard Deviations (%) | | | | | |
| | | Observed Response | | Standard Response | | Loss Response | |
		ti	td	ti	td	ti	td
1	9	17.1	14.9	16.0	14.2	17.1	15.1
2	6	10.6	16.7	8.4	15.2	8.5	15.2
6	9	7.0	7.9	7.5	8.4	7.2	6.8
6a	9	8.5	7.2	8.6	6.7	5.3	4.7
9	9	9.4	5.7	8.8	6.0	10.1	6.4
10	9	18.2	15.2	15.7	12.6	12.0	8.9
12	9	11.2	---	11.1	---	10.6	---
14	9	7.5	10.4	7.2	11.1	7.3	11.7
15	6	3.9	12.0	5.4	14.7	5.4	14.7
20	9	9.2	18.0	8.7	14.7	7.8	17.1
24	9	14.5	---	13.2	---	11.8	---
25	9	---	---	---	---	---	---
26	9	8.9	23.2	9.3	23.3	14.8	25.8
27	9	16.0	---	13.2	---	17.5	---
28	12	26.1	---	23.2	---	25.2	---
32	12	6.8	7.2	6.1	6.4	6.0	6.7
33	9	6.9	25.5	7.2	24.8	6.5	25.9
34	9	7.1	32.3	8.3	32.0	7.8	31.8
37	9	10.5	8.2	11.3	8.7	9.8	6.2
38	6	5.0	13.1	6.7	15.3	4.5	13.6
39	6	10.6	39.3	12.7	41.5	12.5	41.3
40	6	13.6	12.2	13.9	11.7	13.1	11.8
41	6	2.9	9.9	3.8	9.6	8.2	13.7
42	6	6.4	27.4	6.6	27.6	6.0	28.0
43	9	7.9	9.6	8.1	9.7	9.4	9.8
46	12	14.3	11.9	14.6	12.2	20.2	12.9
50	9	9.9	3.5	9.0	3.7	10.9	10.3
52	12	25.2	---	23.6	---	23.7	---
56	9	9.6	22.2	9.1	21.6	9.6	22.6
60	9	11.4	---	12.3	---	13.6	---
61	9	8.8	6.8	8.5	6.8	9.3	6.3
66	15	---	---	---	---	---	---
67	9	12.1	11.3	11.3	14.8	11.3	14.7
69	9	7.1	10.1	6.6	9.5	6.7	8.2
70	12	25.2	27.7	25.1	27.7	24.8	27.4
73	12	29.8	32.3	27.7	31.3	19.0	22.4
74	12	22.0	33.1	18.7	29.3	14.5	25.7
78	9	8.3	9.6	9.4	11.6	8.8	11.0
79	9	16.9	15.1	12.2	10.6	12.2	10.6
80	9	13.9	27.3	8.2	22.2	7.0	20.6

* N = number of animals tested

--- = no response during 30-min observation period

Table C-3 (Continued)

| Material Number | N | Relative Standard Deviations (%) | | | | | |
| | | Observed Response | | Standard Response | | Loss Response | |
		ti	td	ti	td	ti	td
81	9	7.7	23.3	7.7	22.7	7.6	22.3
82	9	12.4	12.6	12.8	13.2	12.8	13.2
84	9	9.1	24.5	9.2	23.0	10.1	25.1
85	9	16.7	---	15.1	---	14.1	---
86	12	40.7	---	38.2	---	39.9	---
88	9	12.7	13.2	12.3	13.6	13.1	14.4
89	15	19.0	---	17.4	---	16.7	---
92	12	6.3	27.8	8.1	27.7	7.9	28.3
93	9	15.1	9.6	13.1	9.0	10.9	7.4
95	9	21.3	19.4	14.8	12.0	14.4	11.8
96	9	4.7	22.8	7.8	20.1	6.4	20.9
97	9	8.7	11.2	7.6	11.2	7.1	11.2
99	9	10.5	8.8	8.8	8.0	9.2	8.1
100	9	18.3	---	16.7	---	16.1	---
102	9	12.7	6.0	13.9	6.9	13.9	14.0
104	9	13.0	18.5	13.8	19.5	13.9	19.5
107	15	25.5	---	23.4	---	22.9	---
108	9	20.0	32.0	17.2	27.8	17.6	27.8
109	12	---	---	---	---	---	---
111	12	7.5	4.7	8.5	5.6	8.5	5.6
112	15	11.6	18.2	8.4	15.8	34.4	37.3
113	9	9.4	10.0	10.1	10.4	10.1	10.4
115a	9	69.2	---	59.0	---	57.3	---
116	9	9.6	6.4	7.5	3.7	7.0	3.2
117	18	13.5	12.4	10.5	9.7	10.0	9.2
118a	9	---	---	---	---	---	---
123	6	27.4	---	28.2	---	24.4	---
127	9	7.4	9.4	4.7	8.1	6.5	10.3
130	9	8.5	9.0	7.0	6.7	7.0	6.5
136	12	21.0	---	22.6	---	23.9	---
142	9	20.4	7.7	21.7	8.2	21.4	9.1
143a	9	9.5	14.1	9.6	14.1	10.3	14.6
143c	9	13.2	---	13.7	---	13.3	---
144	9	12.0	11.2	10.6	12.7	10.2	14.8
PVF	6	18.3	15.6	18.6	16.1	18.4	15.5
Mean RSD:		14.0	15.6	13.2	15.1	13.4	15.5

APPENDIX D

DISCUSSION OF THE BIOLOGICAL PRINCIPLES AND MATHE-MATICAL RELATIONSHIPS PERTINENT TO THE CONCEPT OF A DOSE-RESPONSE RELATIONSHIP IN INHALATION TOXICOL-OGY.

APPENDIX D

DOSE-RESPONSE RELATIONSHIPS IN INHALATION TOXICOLOGY

In earlier CAMI studies of the toxicology of atmospheres containing CO, HCN, or mixtures of the two, we were faced with an experimental design problem. To properly communicate the nature of this problem and our efforts to solve it, a short digression will be necessary.

In the classical approach to an evaluation of a toxicological dose-response relationship, it is axiomatic that a constant, and known, quantity of the substance under investigation be administered reproducibly to a large number of test animals. These animals must all be as nearly identical as is practical. Repeated administrations over a selected dosage range are then made to additional animals. Results of such investigations may then be expressed as the dosage required to elicit a particular animal response. This dosage is commonly expressed as the quantity of test substance administered per unit of animal body weight. In toxicological terms this effective dose may be referred to as a lethal dose, an hypnotic dose, an incapacitating dose, etc.

All animal experimentation involves one source of variability with which most physical scientists never have to contend, and that is the variability in response from one biological individual to the next, even when presented with identical stimuli. The existence of such a variability therefore requires that each experimental dosage be administered to a sufficiently large population of otherwise identical subjects to attain a statistically reliable response. In classical toxicology this led to the expression of "effective dose" in terms such as ED_{50}, which signifies a dose sufficient to elicit the measured response in exactly 50 percent of the test animals. Obviously other homologous terms are in common usage; examples are: LD_{25} (lethal dose for 25 percent of the test population), ED_{99}, LD_1, etc.

In addition, the biological response may vary both qualitatively and quantitatively according to the route of administration of a given dose. Therefore, if one is to adequately describe a toxicological dose-response relationship, the route of administration must also be specified; e.g., oral LD_{50}, dermal ED_{75}. These dose-response relationships are quite straightforward and logical and present little difficulty for the experimentalist in properly designing and executing most toxicological investigations; that is, unless the route of administration is to be by way of inhalation.

In the case of a gaseous agent, how does one quantitate the dose received by each animal? In these instances, the common approach (historically) has been to express the *concentration* of the substance under investigation as it exists in the atmosphere to which the animals are exposed, and specify one additional experimental parameter—the time interval over which the exposure took place. The terms corresponding to effective *doses* thus become effective *concentrations*, administered for specified times. Examples would be: lethal concentration for 50-percent mortality in a 5-min exposure (5-min LC_{50}) or, similarly, 10-min EC_{25}, etc.

Now, after such a digression, we return to our experimental problem. We were interested in defining the dose-response relationships for the effects of two toxic gases, CO and HCN, on two responses of the albino rat. The responses we chose to measure were physical incapacitation (that is, loss of coordinated psychomotor control) and death (operatively defined as cessation of visible respiration for 20 seconds). We felt that these two responses were the most appropriate for an investigation concerned with the effect of combustion gases on an individual's ability to escape from a fire environment.

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The animal exposure chamber designed by the authors for conducting these experiments included four wheel-type cages similar to free-running exercise wheels except that they were driven externally at a constant speed. This feature of the chamber was specifically designed to allow the determination of physical incapacitation. It would also control each animal's physical activity at a constant and reproducible level during an experiment as well as from one experiment to the next.

There were two aspects to the problem, and they became obvious when we tried to expose multiple sets of four animals to identical gas concentrations. We found it extremely difficult to quantitatively reproduce the HCN atmosphere from test to test and also discovered that it was going to be a logistic nightmare (as well as uneconomical) to attempt through a long series of tests to have each rat at the same weight at the time of his exposure.

The inability to control these two factors to the desirable degree meant that both of the elements of dosage (namely, quantity of toxicant inhaled and animal body weight) would have an undesirable amount of statistical variation and thus compromise the utility of these derived values. We therefore decided to evaluate a different approach, one that would allow us to calculate the dose administered to each animal and also circumvent the requirement for using animals at only one weight.

The magnitude of a "dose" acquired by inhalation would be dependent on the following factors: (i) the respired alveolar volume, (ii) the concentration of the gas in the inspired air, (iii) the efficiency of extraction of that gas from the alveolar space into the blood, and (iv) animal body weight. It seemed to us that each of these parameters could be quantitated, either with certain assumptions or under specified conditions.

The volume of the respired alveolar air could be approximated to the extent that Guyton's relationship[12] held for our experimental conditions. He reported that the correspondence between minute-respiratory-volume and body weight of an albino rat was expressed by the equation:

$$RV(minute) = 2.1 \ (body \ Wt)^{0.75}. \quad (D1)$$

Body weight is in grams, the minute-respiratory-volume (RV) is in milliliters per minute, and the units of 2.1 are ml min^{-1} g$^{-0.75}$. Thus, RV could be easily calculated. Since the gas concentration in the enclosed atmosphere could be measured, and the extraction efficiency was assumed to be unity, the dose could be calculated.

If the toxicological principle of equal doses eliciting equal responses (provided all other pertinent parameters are held constant) were to apply here, then animals of equal weight would all inspire the same total quantity of the toxic constituent before exhibiting the same response—in our case, before becoming incapacitated or by the time of death. Therefore the incapacitating dose for all animals of equal weight should be identical, as should be the lethal dose.

If the effective dose for an animal were divided by that animal's body weight, we should have an "effective dose per gram of body weight." Furthermore, we hypothesized that at least for poisons such as CO and HCN, the ED per gram of body weight should be constant for animals of any weight—with the possible exception of the very young and the very old. We therefore had a relationship that would allow us to make exposures at any concentration to animals of differing body weights and still collect useful data.

The dose equation is derived in the following manner: Quantity of air respired would be the product of the minute-respiratory volume (RV) and the time, in minutes, t:

Total Volume Respired Air $= (RV)(t)$. $\quad (D2)$

The total volume of toxic gas respired in time, t, would be the product of the total volume of respired air and the concentration (V/V) of the gas in the air, (C); this would be a dose, d, for the whole animal:

$$d = C \ (RV) \ t \quad (D3)$$

Dose on a per gram body weight basis would be:

$$D = d(Wt)^{-1} = C(RV) \ t(Wt)^{-1}. \quad (D4)$$

If this time, t, is the exposure interval required to bring about the specified response, incapacitation or death, then the dose, D, is respectively the incapacitating dose, D_i, or the lethal dose, D_d. The corresponding times would be time-to-incapacitation, t_i, and time-to-death, t_d.

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Substituting Guyton's formula for RV (for the albino rat):

$$D = [C \ t \ 2.1 \ (Wt)^{0.75}](Wt)^{-1}. \quad \text{(D5)}$$

By simplying this equation, we can see how the effective dose is a function of body weight:

$$D = 2.1C \ t \ (Wt)^{-0.25}, \quad \text{(D6)}$$

where Wt is in grams. Thus dose is inversely related to the fourth root of body weight.

It is obvious that the equations just derived are based on an exposure to a constant concentration of the active material. The relationship is equally valid for a changing concentration, provided one has adequate knowledge of the time-concentration profile over the time interval from $t=0$ to $t=t_i$ or t_d. The value for the integal (or area under the curve) of the concentration versus time curve, from zero time to the response time, can be entered in the equation as the $(C)(t)$ product. One could also think of this as finding the Ct product, dividing by t to give an average (or effective) concentration (C) over the exposure interval, and entering this as (C) in the original equation.

Exposure of a large number of rats, whose body weights ranged from 100 to 500 g, to various concentrations of CO and HCN has verified that the derived relationship is a useful one. For the level of activity represented by the driven wheel in our exposure chamber, we found the following effective doses for CO and HCN for the albino rat.

For HCN: Incapacitating dose = 0.32 mg/kg
         Lethal dose   = 1.95 mg/kg
For CO: Incapacitating dose = 15.35 mg/kg
         Lethal dose   = 50.53 mg/kg

These doses are expressed as the weight of toxic material that must be inhaled per kg body weight to produce a given effect. They may be converted to an equivalent concentration value (concentration of the toxic gas in the atmosphere), and if expressed in (volume/volume) parts per million (ppm), they now represent the "dose" for a given response when the (C) in the equation is expressed in ppm (V/V). These values for D would then be:

For HCN: D (incap) =   299 ppm (ml/g)
         D (death) = 1,834 ppm (ml/g)
For CO : D (incap) = 13,907 ppm (ml/g)
         D (death) = 45,790 ppm (ml/g)

Now that the effective doses for either response are known, we can demonstrate one of the very useful features of this relationship that is lacking in the usual expressions of "X-minute ED_y."

If one had a literature value of a 30-min LC_{50} (or LC_{99}) for a particular gas and for an animal weight of 350 g but was interested in a 5-min lethal concentration for 200-g animals, the literature value is of little use beyond affording one an approximate value from which he might speculate. Using equation (D6), however, the response time for a rat of any weight exposed to any concentration (within the limiting values) can be calculated once D has been determined.

For example, the HCN concentration, maintained at a constant value, that would incapacitate a 200-g rat in 5 min would be (from equation (D4)):

$$C_i \ (5 \ \text{min}) = D \ (WT)/t \ (RV)$$
$$= (299)(200)/[5(2.1)(200)^{0.75}]$$
$$= 107.1 \ \text{ppm.}$$

The 5-min lethal concentration would be:

$$C_d \ (5 \ \text{min}) = (1834)(200)/[5(2.1)(200)^{0.75}]$$
$$= 657 \ \text{ppm.}$$

The time to incapacitation for a 300-g rat exposed to a CO concentration of 15,000 ppm would be:

$$t_i = D(Wt)/[2.1 \ (C)(Wt)^{0.75}]$$
$$= (13,907)(300)/[(2.1)(15,000)(300)^{0.75}]$$
$$= 1.84 \ \text{min.}$$

The t_d for a 500-g rat, exposed to 10,000 ppm CO, would be 10.3 min.

These four calculations indicate the increased utility of such a relationship.

We have independently evaluated the accuracy of the lethal cyanide dose as calculated from our inhalation experiments. At the same time we have confirmed our belief that the extraction of HCN from alveolar air into blood would be essentially 100 percent efficient. (This belief was based on the known physical and chemical properties of HCN and the conditions under which alveolar exchange takes place.)

Since the results of our inhalation experiments yielded a calculated lethal cyanide dose for rats of 1.95 mg/kg, we decided to give a series of rats a graded dosage schedule of CN⁻ by direct intra-

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venous (i.v.) injection and compare lethal doses. A solution of buffered NaCN was injected into the exposed femoral vein of an ether-anesthetized rat; his heart action was monitored by palpation and his respiration was observed visually. Animals receiving doses of CN^-, based on individual body weights, that were from 50 percent to 90 percent of the lethal *inhalation* dose (1.95 mg/kg) survived the administration, while those receiving 100 percent to 200 percent of the lethal inhalation dose succumbed.

In most of the animals, we observed rather dramatic responses immediately following the injection. In particular, respiration was erratic with varying periods of apnea. Cardiac activity also became erratic at the higher doses and, in the case of the nonsurvivors, continued long after respiration had terminated.

(An interesting sidelight to this experiment that illustrates the reversibility of the observed, intermittent periods of apnea occurred with the animal that received 90 percent of the lethal dose. Because this animal had experienced a prolonged period of apnea, our attending pathologist pronounced it dead when cardiac activity could no longer be felt; so it was placed in a plastic bag on top of the other carcasses—the 90-percent dose was the last experiment of the day. The plastic bag was placed in a freezer overnight, as the animal caretaker who handled disposal had left for the day. In the morning, on retrieving the bag for disposal, there sat the "90-percent animal" on top of its deceased and frozen litter mates. Except for frostbitten (or frozen) nose, ears, toes and tail, it seemed none the worse for its "cyanotic" ordeal. We mention this primarily because it may suggest that the use of some degree of hypothermia could have value in the treatment of near-fatal cyanide poisonings.)

This correspondence between the values for lethal dose, as determined from inhalation experiments and by direct i.v. injection, supports the validity of the inhalation data and verifies that the efficiency of HCN extraction from alveolar space into blood approaches 100 percent.

Alveolar extraction efficiencies for CO are approximately 50 percent until the blood carboxyhemoglobin (COHb) level reaches 40-percent to 50-percent saturation. Above this COHb level, the extraction efficiency for CO will decline. However, in our treatment of the dose-response relationship, we chose to define dose as the quantity of the toxic agent that is *inspired* and to ignore any fractional portion that may not be absorbed and would therefore be exhaled. In other words, for the purpose of calculating what we define as dose, we arbitrarily assume that the extraction efficiency is 100 percent.

We have also found that dose-response relationships measured in the above fashion may be of use in extrapolating data from one animal species to another, provided the minute-respiratory volumes are available or can be suitably estimated. As an example, using a literature value for the 5-min LC_{99} for mice exposed to CO, we have converted this concentration figure into its corresponding 5-min lethal inhalation dose per gram of body weight by utilizing the Guyton equation for RV and the reported value for animal weight. This lethal dose (per gram of mouse), calculated from mouse LC_{99} data, agreed within 1 percent with the lethal dose (per gram of rat) calculated from our rat experiments. Obviously, the effective CO dose for 1 g of rat tissue equals the effective CO dose for 1 g of mouse tissue. We have found the same agreement between the rat data from our experiments and 5-min LC_{99} data from the literature for rat exposures to CO.

An additional observation is even more pertinent to the task of attempting to solve human problems by doing research on nonhuman species. We found that if one converted the incapacitating CO dose for rats to the equivalent dose for a "70-kg rat," the resultant dose agreed within 0.1 percent with the dose predicted by the Peterson and Stewart equation[13] for human acquisition of a 46.5-percent COHb saturation.*

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* Assuming that human incapacitation occurs when the blood COHb level reaches 46.5-percent saturation and using Guyton's constant for adult human (resting) RV of 2.06 $(Wt)^{0.75}$.

Evaluation of similar extrapolations for HCN, from our rat data to humans, is made difficult by the paucity of quantitative data for HCN dose-response relationships in humans. However, the lethal inhalation dose that we measure for the rat does convert to a blood cyanide concentration of 3.4 μg/mL (assuming the HCN is distributed uniformly throughout the total body water). Forensic data for human fatalities indicate blood cyanide concentrations from 2 to 5 μg/mL.

The dose-response relationships just discussed for CO and HCN exposures have been applied to the combustion experiments in the following manner:

If equation (D6) is rearranged to

$$C^{-1} = 2.1(D)^{-1} t(Wt)^{-0.25}, \qquad (D7)$$

and it is recognized that the effective dose, D, is a constant (the quantity of toxic gas(es) required to produce the observed response) with the effective concentration, C, proportional to the quantity of fuel, F, that decomposed, C=kF, we can see that for all experiments in which F is kept constant:

$$t = K(Wt)^{0.25} \qquad (D8)$$

where $K = D(2.1 \ kF)^{-1}$. Therefore, for such experiments, t can be corrected from that obtained with an experimental animal of any weight to that t which would have been obtained with a standard animal weighing 200 g.

The variation in t due to different fuel quantities, F, can be similarly corrected to that value which would have resulted from a quantity of fuel equal to one gram.

Transformations, or normalizations, based on these concepts and assumptions were utilized in converting the observed data to "standard" and "loss" response times.

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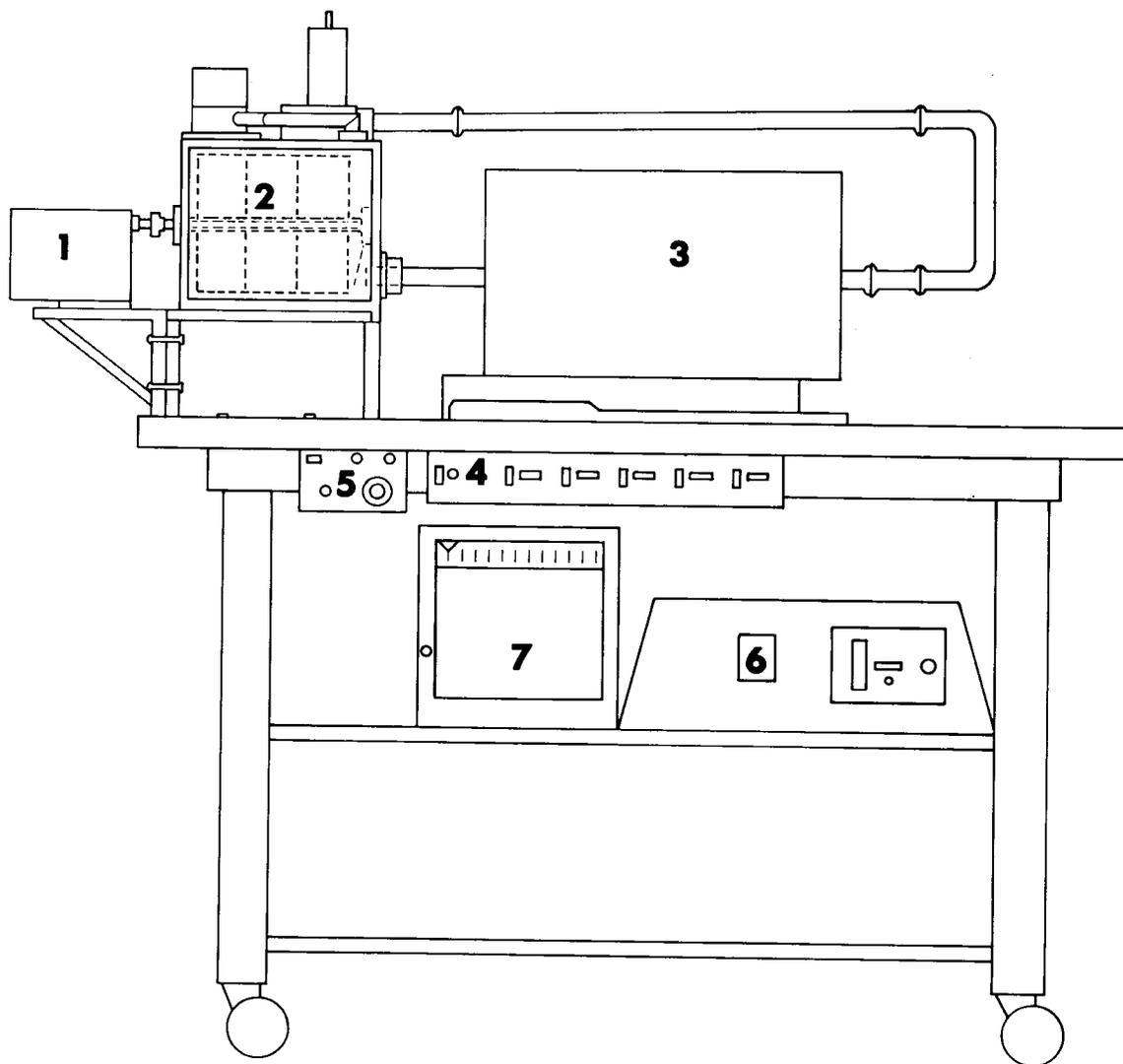
1. Gearmotor, model 3M126, 6 rpm, 1/20 hp, Dayton Electric Mfg. Co., Chicago, Ill.

2. Animal Exposure Chamber.

3. Furnace, Type 54231A, 1300 W, Lindberg, Div. of Sola Basic Industries, Watertown, Wisc.

4. Electrical Outlet Strip, model 1577, Cole-Parmer Inst. Co., Chicago, Ill.

5. Speed Controller for Recirculating Blower, Stir-Pak solid state model #4556-03, Cole-Parmer Inst. Co., Chicago, Ill.

6. Furnace Temperature Controller, Type 59344, Lindberg, Div. of Sola Basic Industries, Watertown, Wisc.

7. Chamber Temperature Recorder, Speedomax model H, Temperature range 25-50° C, Leeds and Northrup Co., Philadelphia, Pa.

FIGURE A–1.—Animal exposure assembly.

ANIMAL EXPOSURE CHAMBER — End View
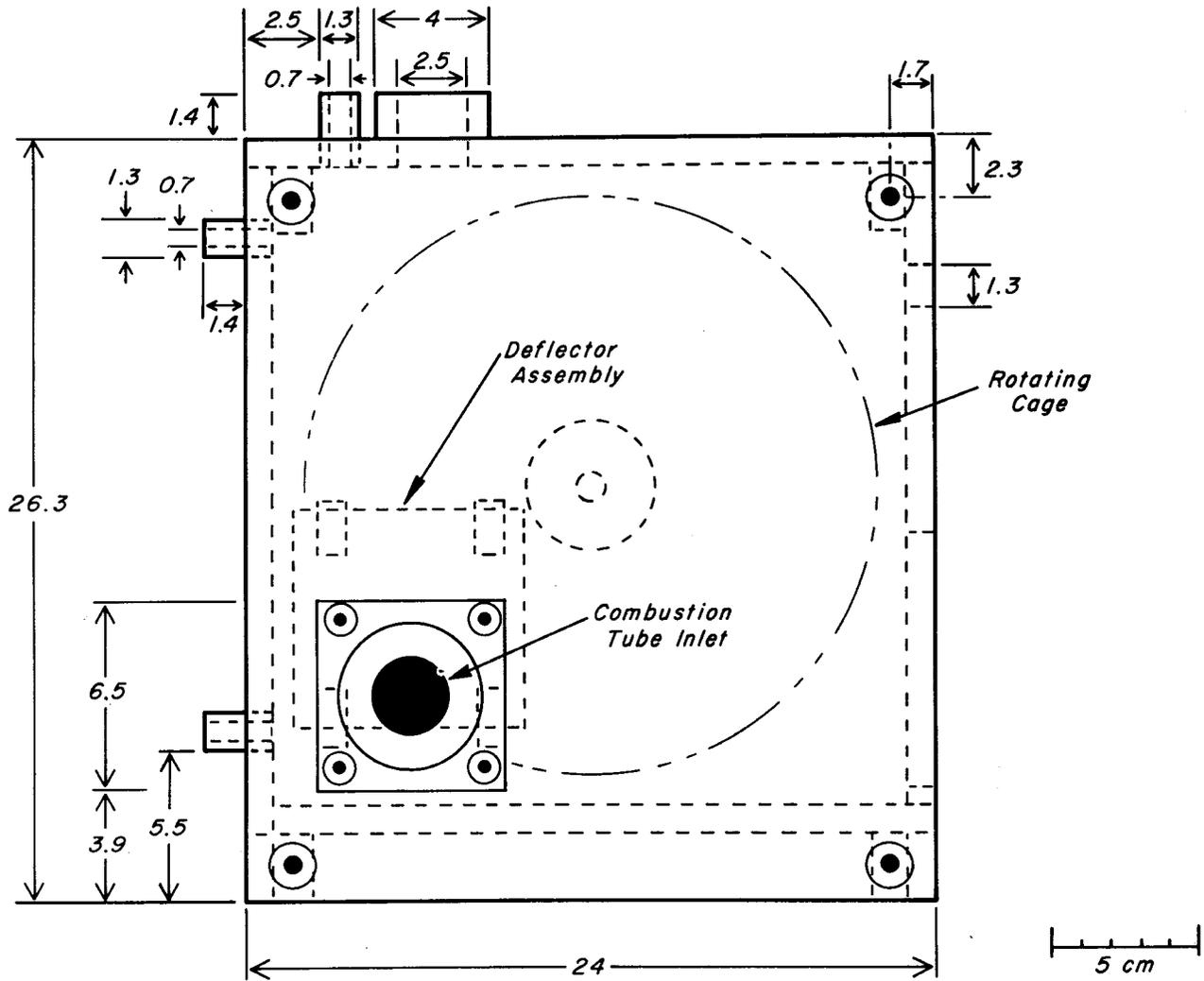
FIGURE A–2.—Animal exposure chamber, end view.

ANIMAL EXPOSURE CHAMBER — Left Side View

All dimensions in centimeters

5 cm
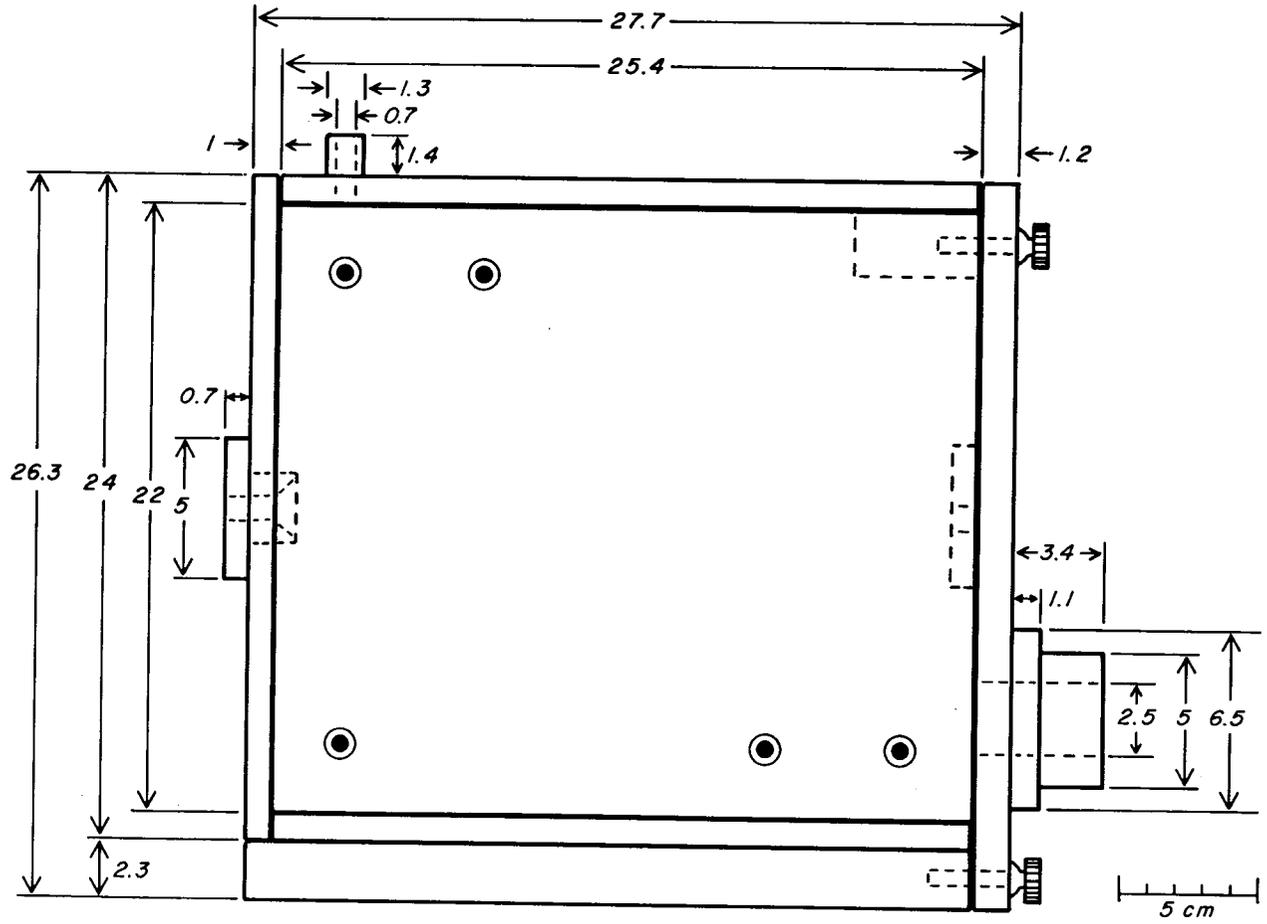
FIGURE A–3.—Animal exposure chamber, left side.

ANIMAL EXPOSURE CHAMBER — Right Side View
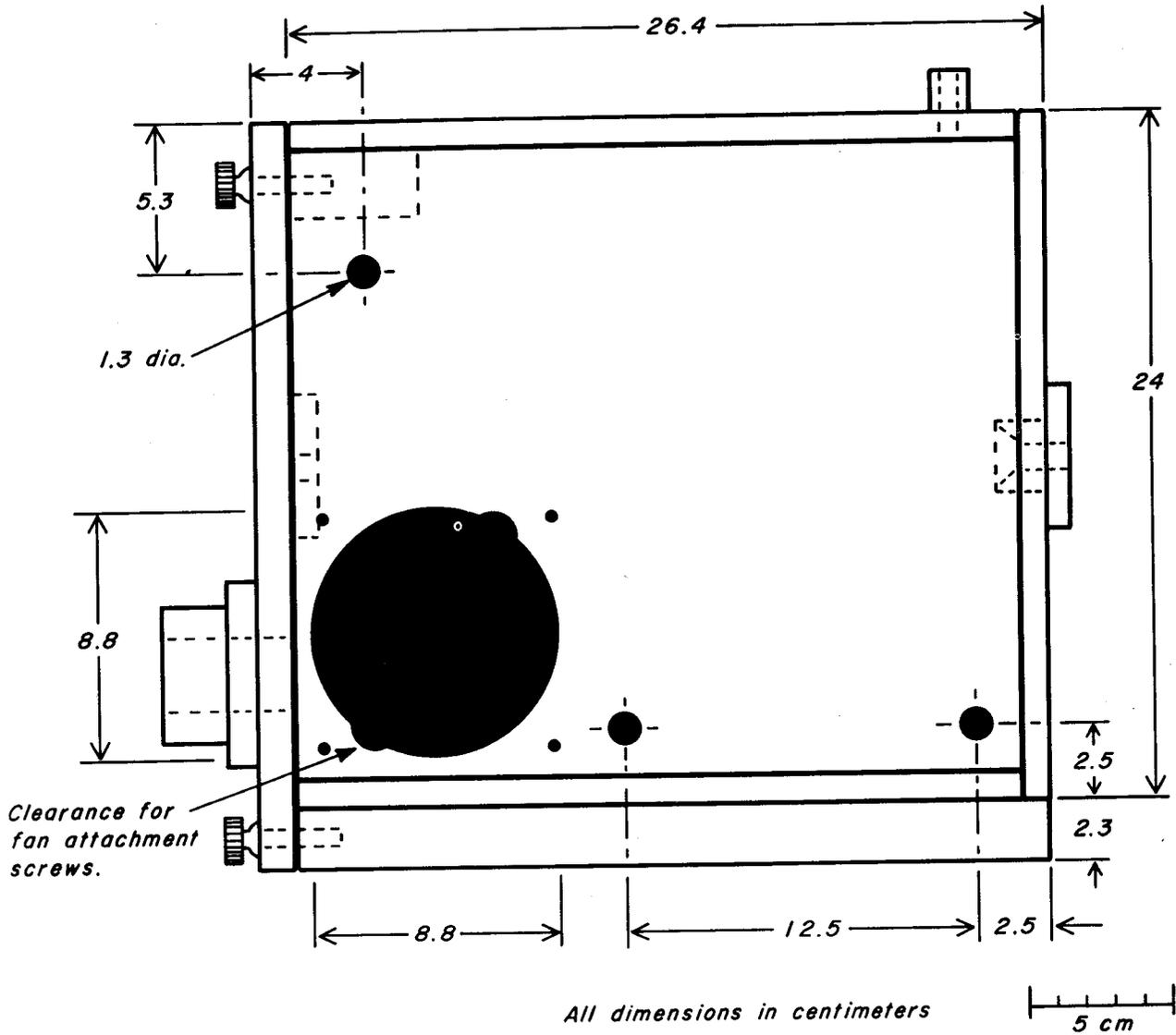

All dimensions in centimeters

5 cm

FIGURE A-4.—Animal exposure chamber, right side.

ANIMAL EXPOSURE CHAMBER — Top View
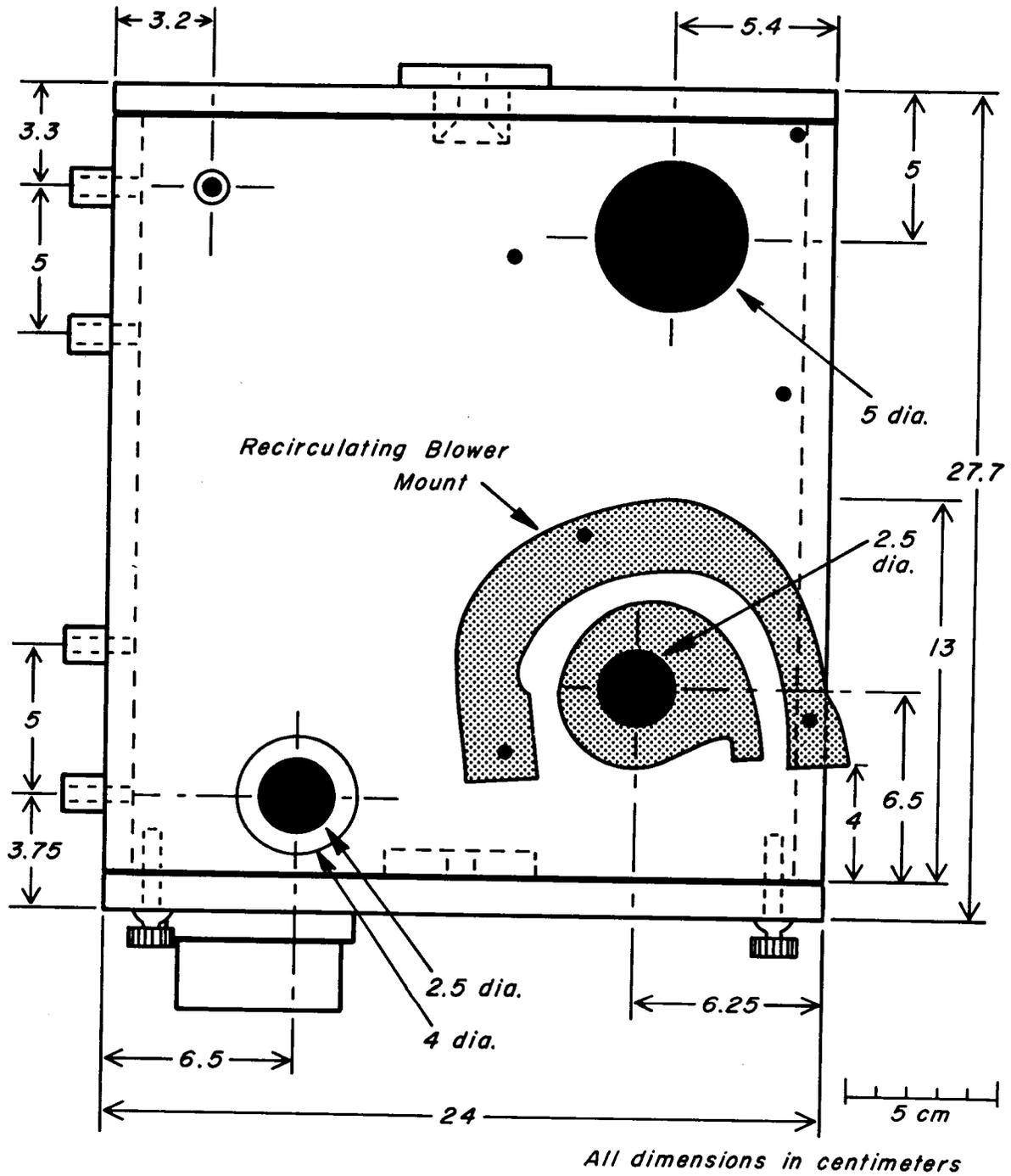
FIGURE A-5.—Animal exposure chamber, top view.

ANIMAL EXPOSURE CHAMBER — Top View
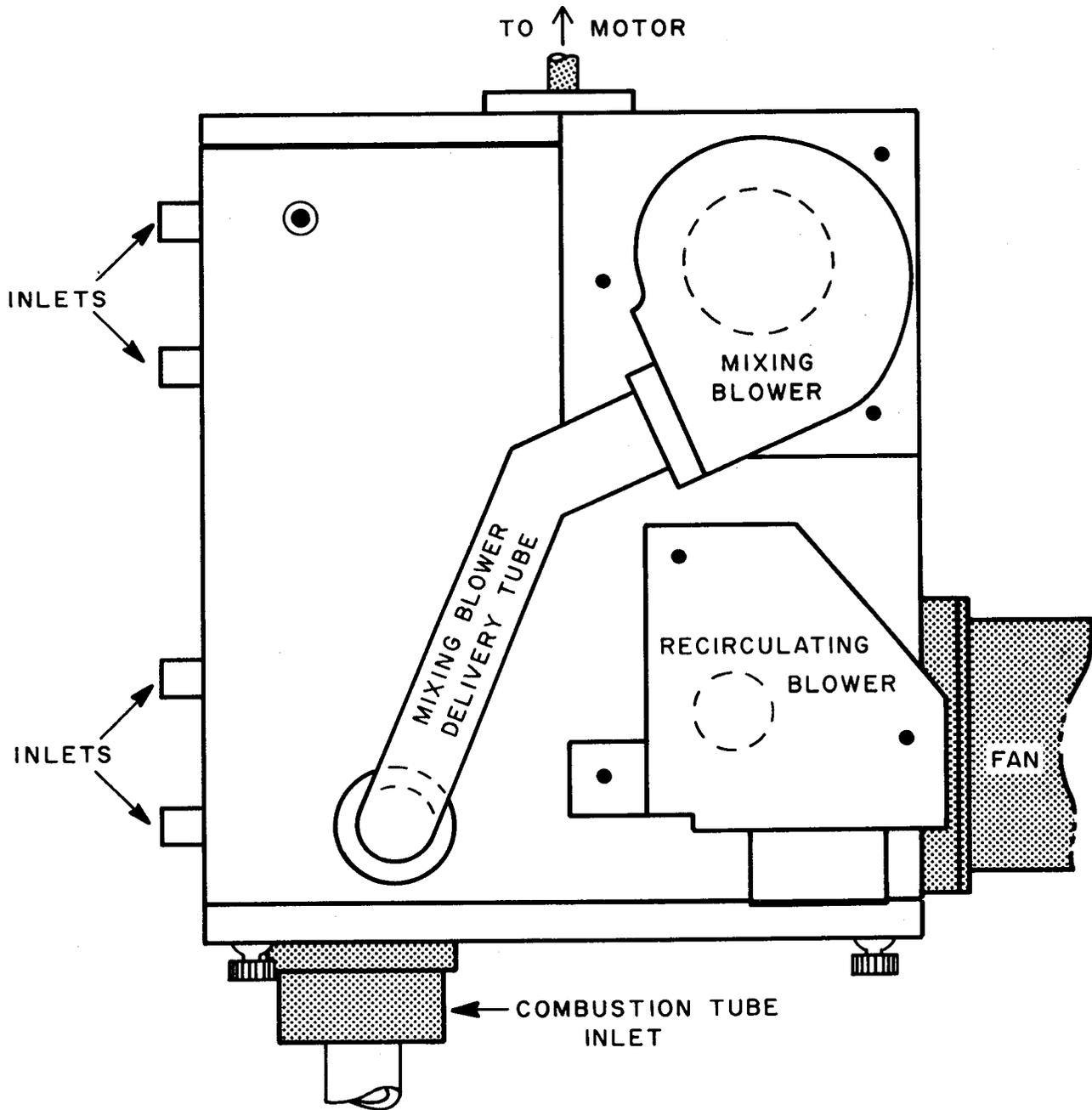## Showing Blowers and Fan Installed

TO ↑ MOTOR

INLETS

INLETS

MIXING BLOWER

MIXING BLOWER DELIVERY TUBE

RECIRCULATING BLOWER

FAN

← COMBUSTION TUBE INLET

FIGURE A–6.—Animal exposure chamber, top view, with blower and fan installed.
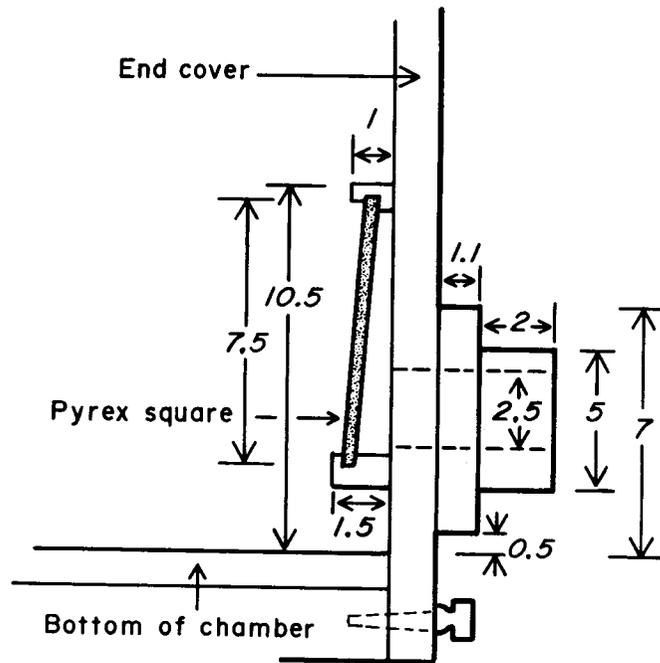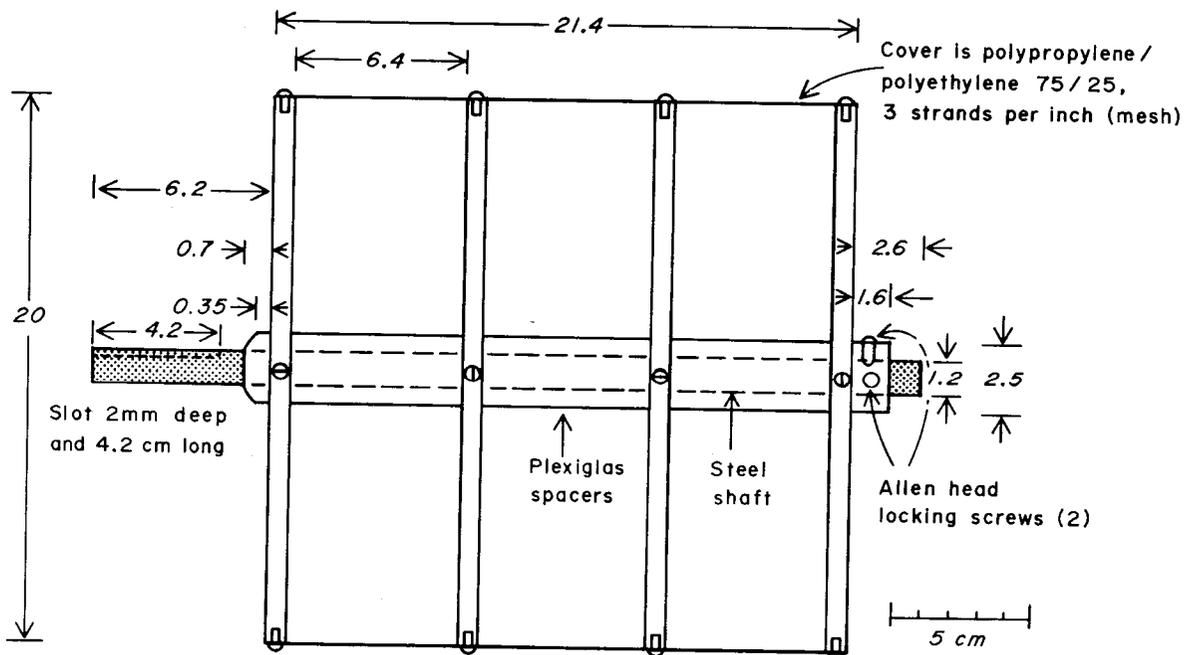
DETAIL OF DEFLECTOR ASSEMBLY

End cover

/

1.1

10.5

7.5

2

2.5

5

7

Pyrex square

1.5

0.5

Bottom of chamber

FIGURE A–7.—Detail section, gas deflector assembly, side view.

ROTATING CAGE (SIDE)

21.4

6.4

Cover is polypropylene/
polyethylene 75/25,
3 strands per inch (mesh)

6.2

0.7

2.6

0.35

1.6

4.2

1.2   2.5

20

Slot 2mm deep
and 4.2 cm long

Plexiglas
spacers

Steel
shaft

Allen head
locking screws (2)

5 cm

All dimensions in centimeters

FIGURE A–8.—Rotating cage assembly, side view.

ROTATING CAGE (END)

Heavy rubber band (3)

Steel wire hook

7 cm overlap in plastic mesh

0.6 cm thick Plexiglas disc. 20 cm diameter

1.3 cm holes cut in all 4 plastic ends and dividers

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Polypropylene/polyethylene 75/25 plastic mesh 3 strands per inch

5 cm

All dimensions in centimeters
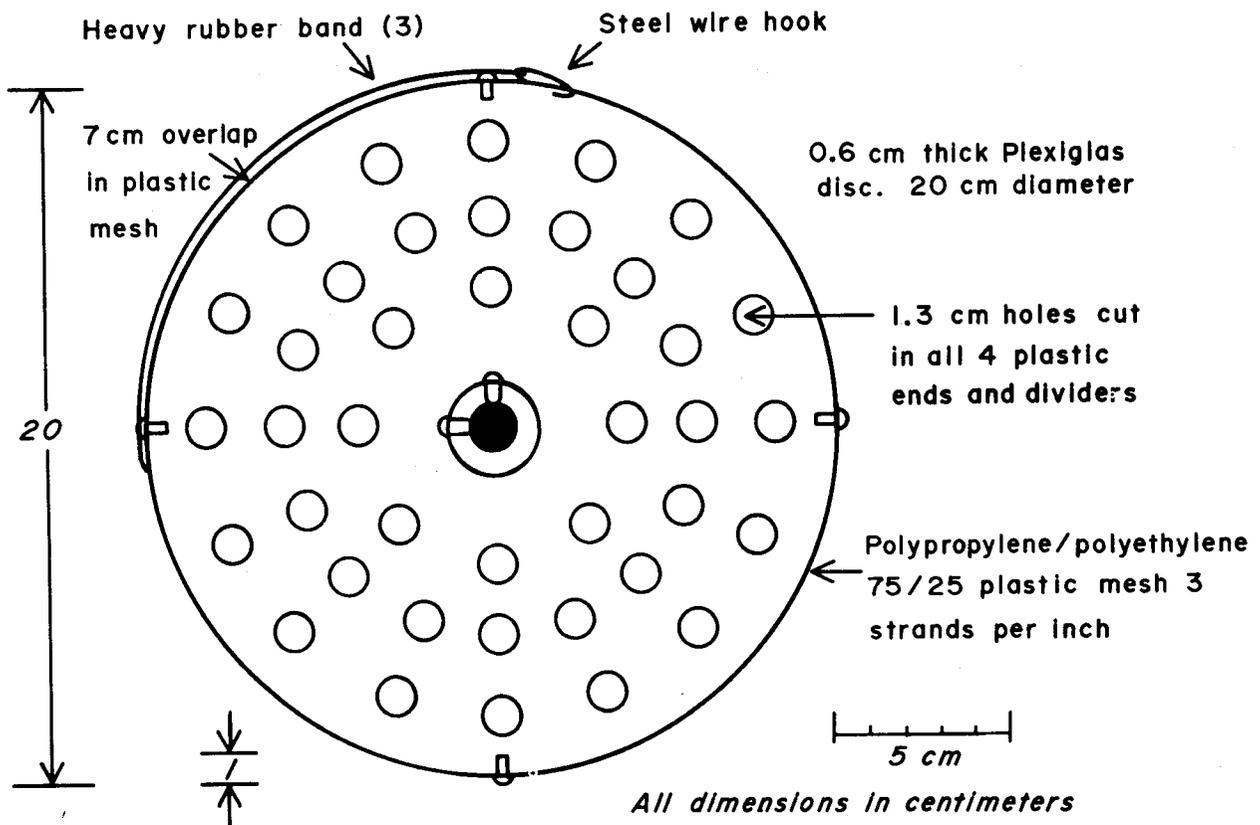
FIGURE A–9.—Rotating cage assembly, end view.

RECIRCULATING BLOWER ASSEMBLY

Cole Parmer
Stir-pak motor
4556

Teflon washer

Rubber washer

Rotor molded
from epoxy resin

Viton O-rings
2.8 i.d.

Bottom

Side

5 cm

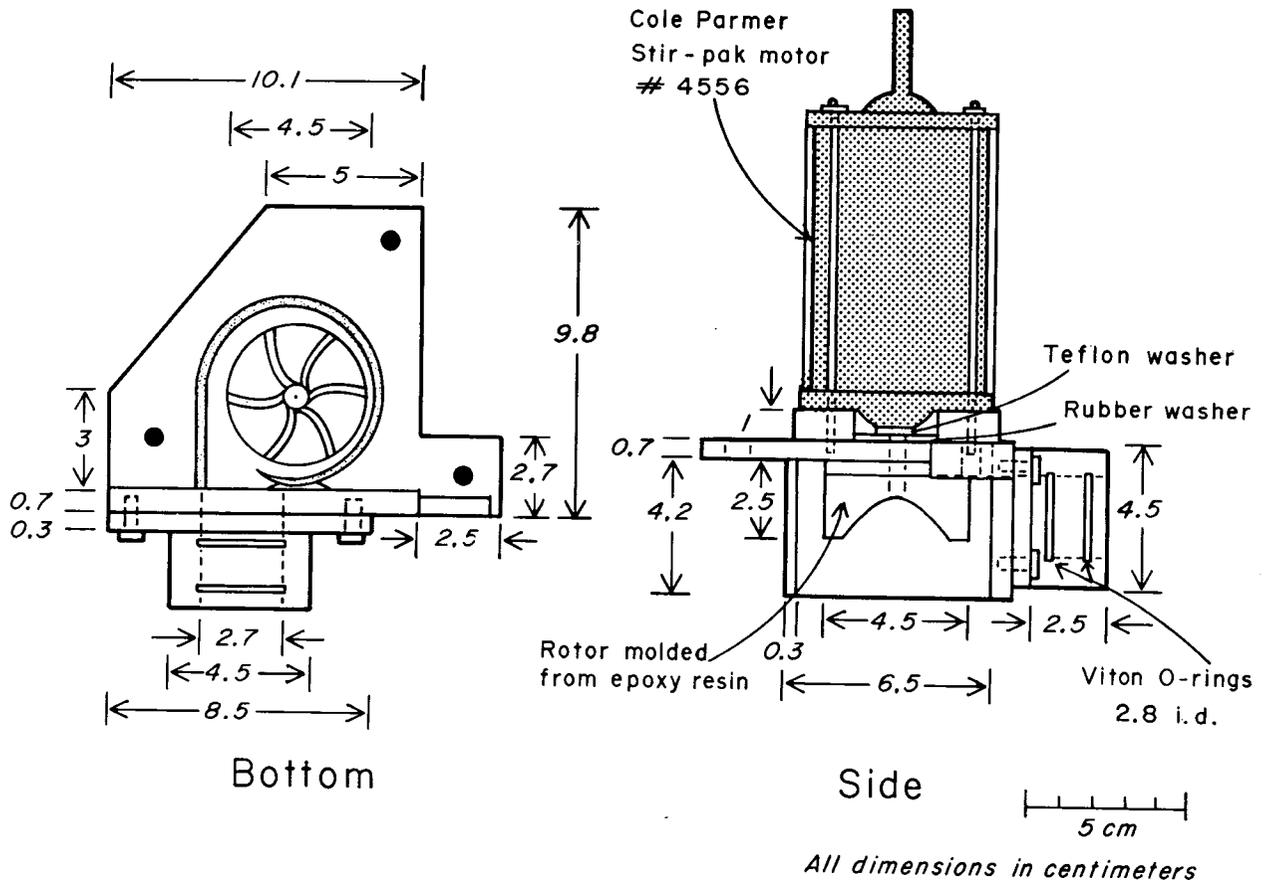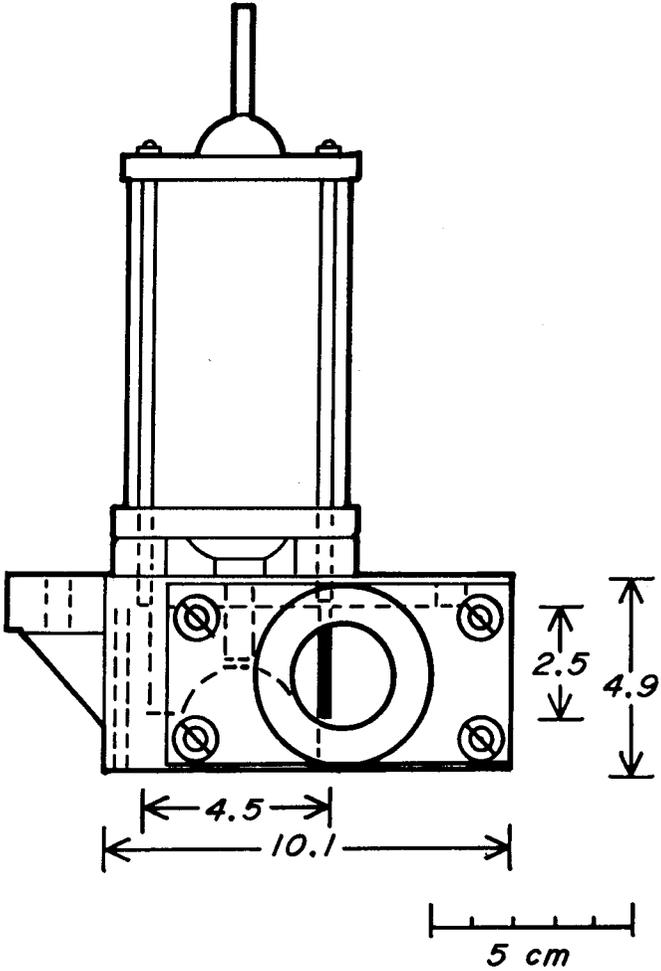*All dimensions in centimeters*

FIGURE A–10.—Recirculating blower assembly.

RECIRCULATING BLOWER
ASSEMBLY

All dimensions in centimeters

Front
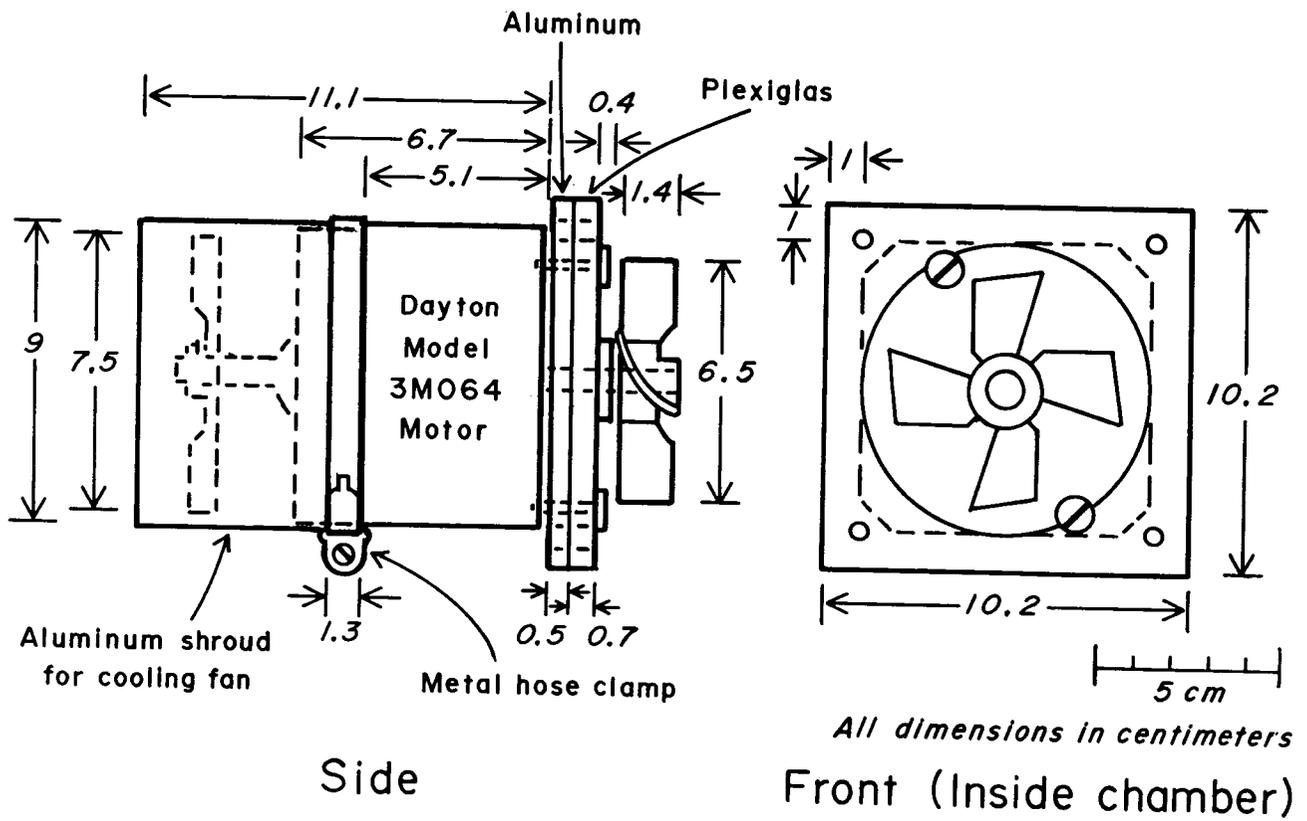
FIGURE A–11.—Recirculating blower assembly, front view.

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FAN ASSEMBLY

Side

Front (Inside chamber)

All dimensions in centimeters

FIGURE A–12.—Fan Assembly.

MIXING BLOWER

Viton O-ring

Bottom

Side

ROTOR DETAIL

5 cm

All dimensions in centimeters
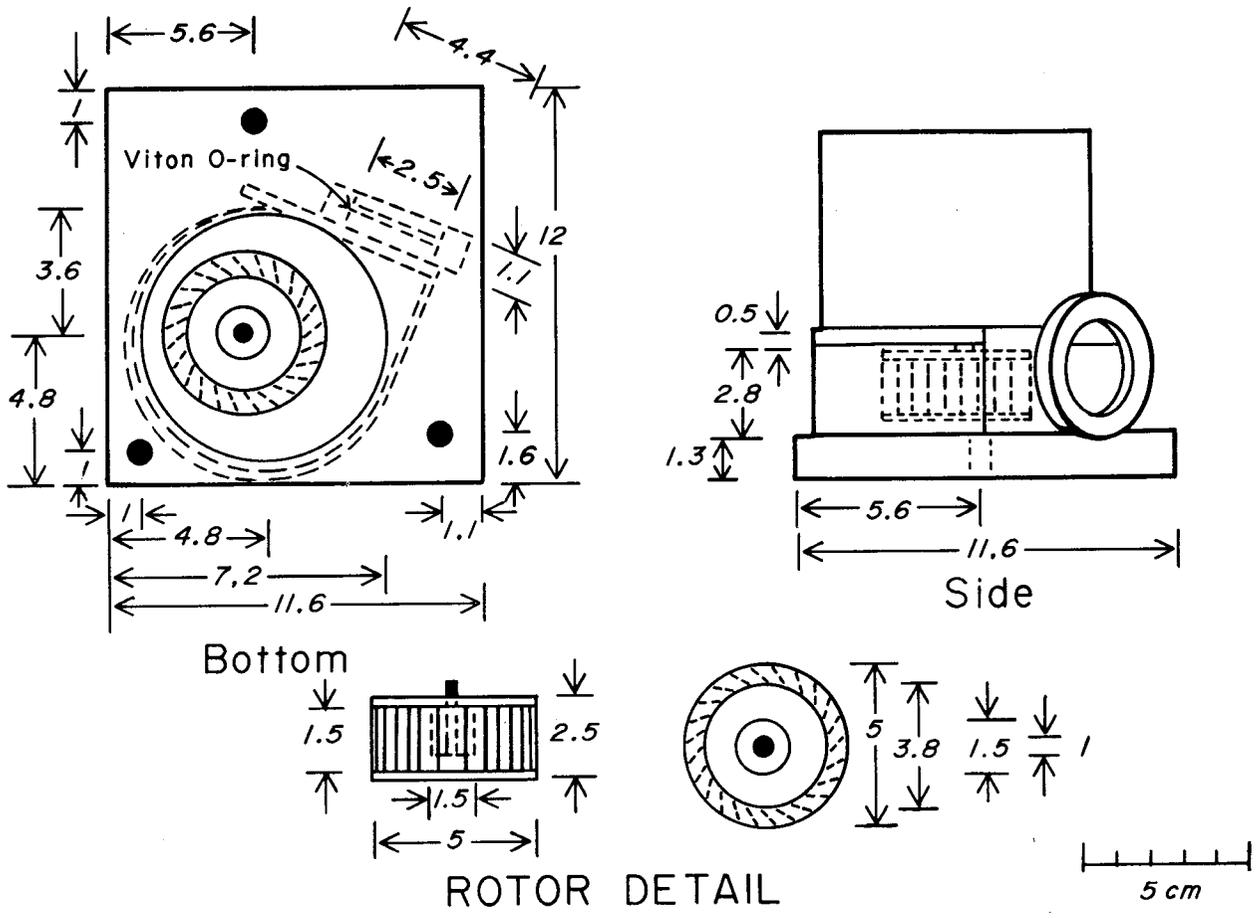
FIGURE A–13.—Mixing blower with rotor.

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MIXING BLOWER DELIVERY TUBE
(Plexiglas)

Top View

Left Side View

5 cm

All dimensions in centimeters
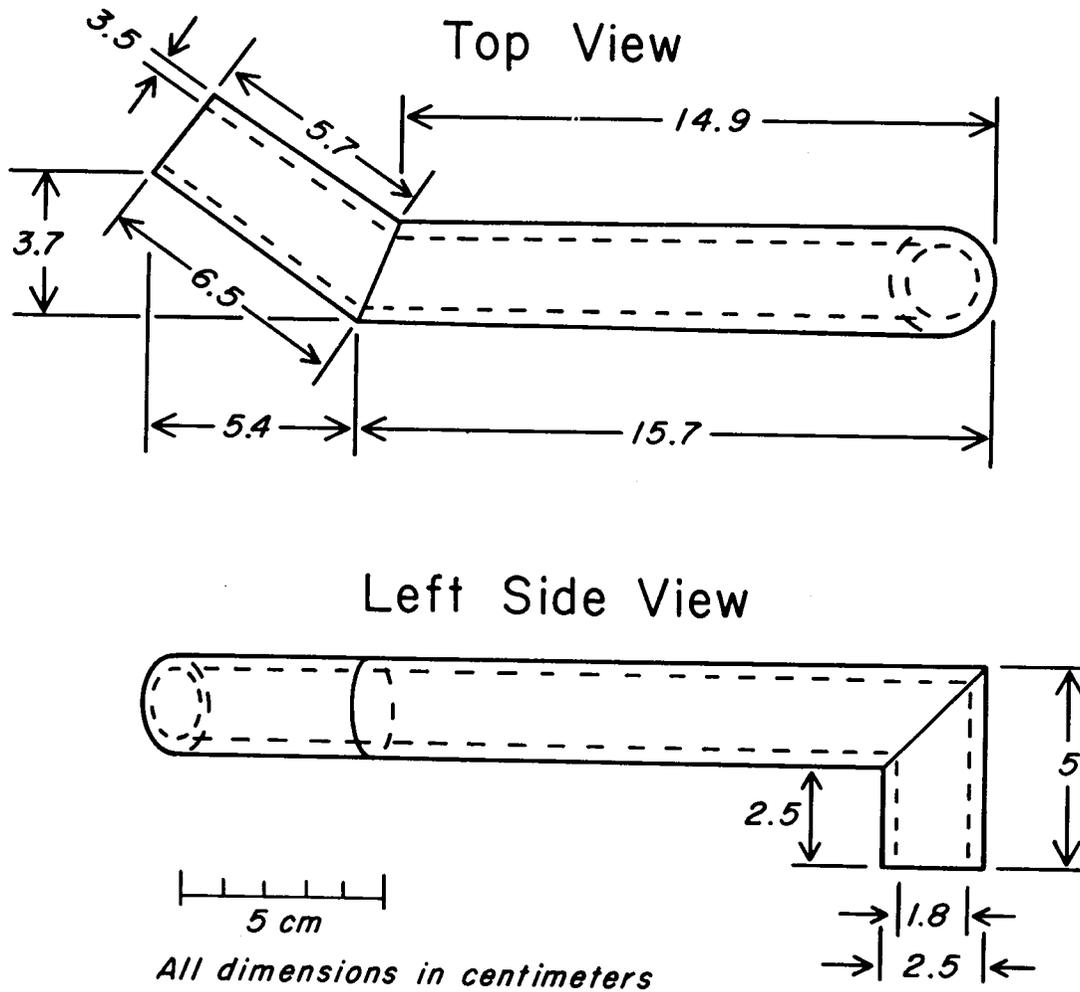
FIGURE A–14.—Detail, mixing blower delivery tube.

RECIRCULATION ASSEMBLY

Pyrex, 2.5 O.D., 2.2 I.D.

Pyrex ground joint socket 2.8 O.D., 2.4 I.D.

All ball joints are 35/25 Pyrex

Vycor combustion tube 2.5 O.D., 2.2 I.D.

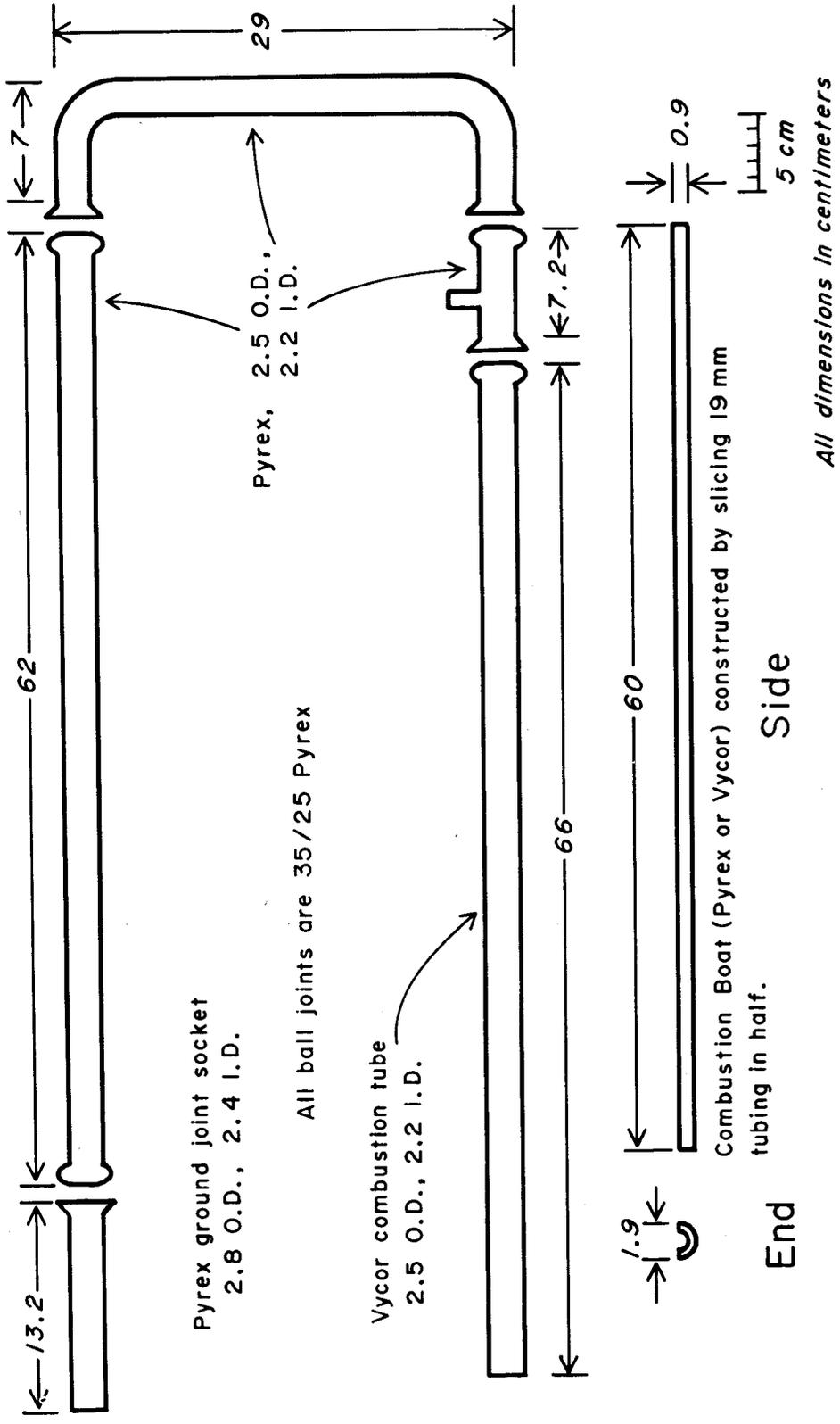Combustion Boat (Pyrex or Vycor) constructed by slicing 19 mm tubing in half.

Side

End

All dimensions in centimeters

5 cm

FIGURE A–15.—Recirculation assembly.

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