Material Toxicology Evaluation by Direct Animal Exposure

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The animals used for this experiment were lawfully acquired and treated in accordance with the "Principals of Laboratory Animal Care" issued by the Animal Facilities Standards Committee on the Animal Care Panel, U.S. Department of Health, Education, and Welfare, Public Health Service, March 1963.

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Introduction

The system in use at the Civil Aeromedical Institute (CAMI) for direct animal assay of the toxicity of the combustion products of polymeric materials evolved from a 2-year program in which many concepts, designs and operating parameters were evaluated.

It is our purpose at this time, (a) to describe our system, (b) to discuss the rationale underlying certain design features, (c) to describe the tests which led to the selection of operating parameters, and (e) to present a method for "normalizing" the raw data from consecutive tests.

When we were requested by our agency to initiate a materials evaluation program based on animal exposures, a major stipulation was made which limited our options in both system design and operating conditions. The manner of burning the material sample was to correspond as closely as possible to that selected for a concurrent program at the National Aviation Facilities Experimental Center (NAFEC) in which selected major constituents of combustion mixtures were to be chemically analyzed. Under these comparable conditions, we were to observe time to physical incapacitation (ti) and time to death (td) for the animals in our exposure system, and measure the two combustion products with which we were most familiar, carbon monoxide and hydrogen cyanide, within the exposure chamber. Hopefully, the two procedures would supplement each other in that the NAFEC analyses should reveal contributing toxicants if CO and HCN obviously failed to account for the tis and tds which we observed.

After a series of preliminary tests with improvised equipment, we began to design and construct the system now in use, which is presented diagramatically in Figure I and described in detail below.

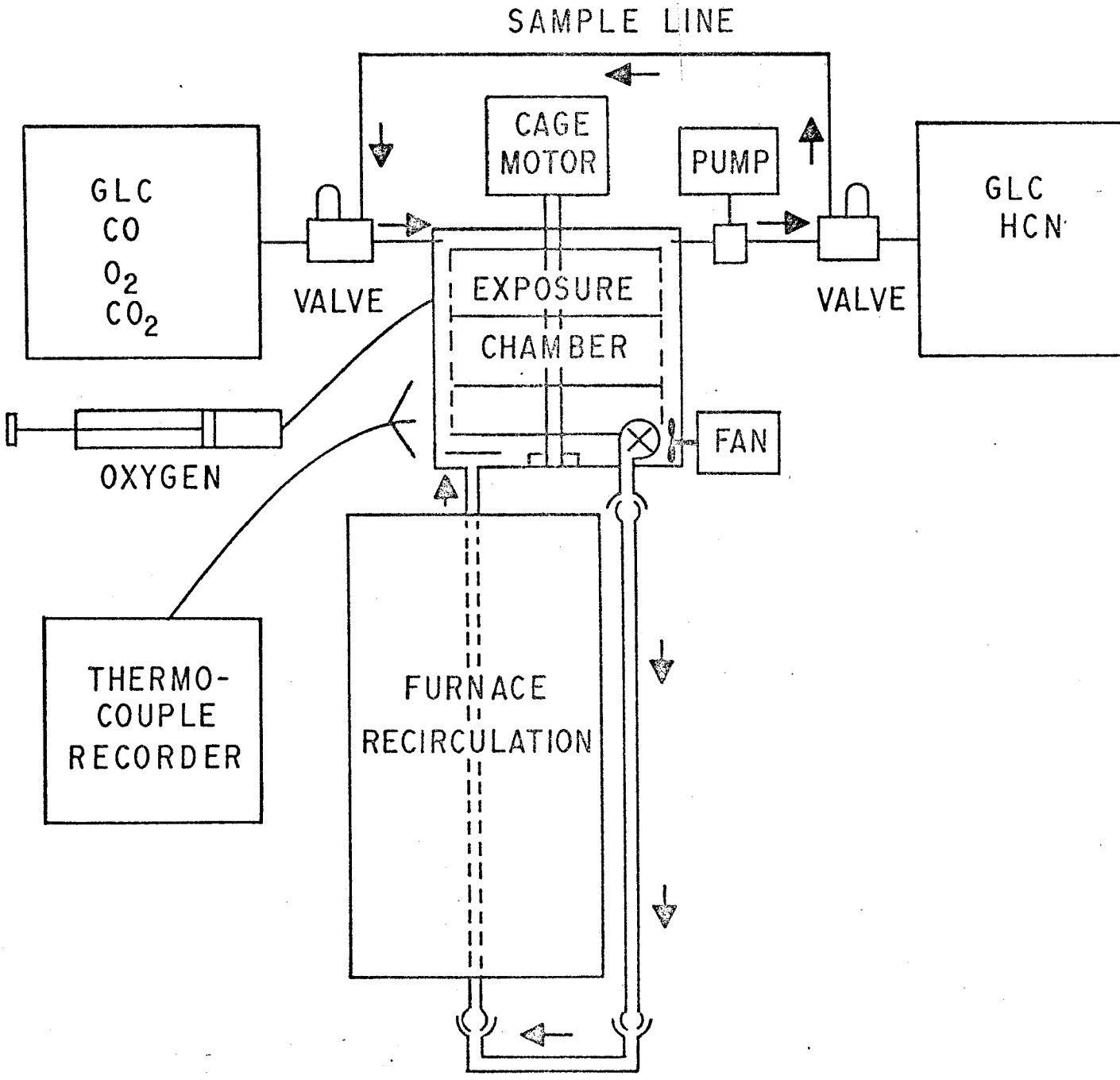
Combustion, Exposure and Analytical System

1. Combustion.

Combustion is accomplished in a tube furnace of 2-inch capacity (Lindberg Model 54231A with Model 59344 Controller). Aluminum plates attached to the furnace housing at each end position a 1-inch combustion tube precisely between the heating elements. Our use of this combustion

FIGURE I

COMBUSTION-EXPOSURE SYSTEM



device was dictated by the fact that a similar furnace was already in use in the analytical program at NAFEC. The relatively slow heating rate of such a furnace necessitates that samples be inserted into the burn zone after the selected combustion temperature has been reached. The means of accomplishing this operation at NAFEC and CAMI differs slightly. The NAFEC furnace is of 1-inch capacity. The sample is loaded into a Vycor® combustion tube which is then inserted into the closed, pre-heated furnace. In our operation, the combustion tube is in place and at burn temperature before the sample, in a quartz boat or tube of appropriate diameter, is inserted. In both systems the sample is positioned at the point of maximum temperature within the furnace. Such differences as may exist in the mode and rate of heat transfer to the sample in the two systems appear to be minor.

All samples are now burned at a temperature of 600° C. After early tests (to be reported herein) had been completed, it was found that a furnace control setting of 600° produced only 585° within the combustion tube. We now correct for this discrepancy. The combustion temperature of 600° was selected by NAFEC scientists who have had broad experience in flammability research. They believe it to be a suitable choice, particularly in the aviation context, and our own tests support this view.

The second feature required to insure comparable conditions of combustion required a compromise and dictated a somewhat unique design characteristic of our system. At NAFEC, it has proved to be advantageous to draw ambient air continuously through the combustion tube, with total collection of the emerging gases in absorbers for subsequent analysis of the components of interest. Because ambient air is continuously supplied to the heated sample, combustion can best be described as oxidative pyrolysis.

Because of the different requirements of chemical analysis and toxicological testing, we could not operate in this way.

In an assay of toxicity, the sample size (concentration) must be properly matched to the total volume of the system in order to produce an observable response in the subjects (a physiological change, or death) within an appropriate time frame. Once the burn has started, this quantitative relationship cannot be disturbed for the duration of the observation period. We could not, therefore, afford to lose "overboard" any considerable quantity of the combustion mixture, as a flow-through system would demand. Moreover, in exposing animals to a combustion mixture in which the concentration of components is changing as the burn proceeds, the consequences of losing X quantity of product 1 minute into the burn would be quite different from loss of the same quantity at 2 minutes or 3 minutes.

Our system insures an oxidative burn by recirculating the gases within the closed system through the burn zone. An epoxy-bladed blower driven by a variable-speed motor draws gases from the animal chamber and drives them through an all-glass tubing system (1 inch O.D.) back into the chamber.

Since both the animals and the combustion process require oxygen, the concentration within the system is monitored and maintained within 90% of the atmospheric content by manual addition of oxygen as required. By inserting orifice plates with appropriate aperture sizes into the line and varying the speed of the blower, the recirculation rate can be controlled. The flow can be measured accurately by means of a thermistor anemometer of our own design.

Exploratory experiments had shown that variations in recirculation rate produced significant changes in the composition of the combustion mixture, and that 6 liters/min seemed optimal. Analyses of oxygen in gas samples obtained from a probe in the burn zone indicated that this flow rate maintained an adequate oxygen concentration. Higher flow rates, incidentally, transfer too much heat into the animal chamber. NAFEC, however, encountered losses from their collection system at 6 liters/min. Further tests indicated that we could tolerate a lower recirculation rate, and we therefore adopted a flow rate of 4 liters per minute to insure that their results and ours would be comparable.

2. Exposure System.

The following considerations were taken into account in constructing our present equipment, and insofar as has been possible each requirement has been met.

a. Exposure-system volume. Previous experience had shown that, for a variety of reasons, it was important to keep the volume of the exposure system as small as possible. Because of the chemical and physical properties of many of the major components of combustion mixtures, it is a practical impossibility, regardless of design, to transmit such mixtures unchanged from the combustion zone to exposed animal subjects. Some constituents are of low-to-moderate volatility and condense on cool surfaces. Many adsorb on dry surfaces; others are water soluble and collect on moist surfaces. Many are acidic or alkaline and reactive and combine chemically with each other or with system components. Smoke consists of liquid aerosol and solid particles which settle on all surfaces. Since toxicants may be present in or on these particulates, their loss alters the total toxicological potential.

The only approach to a solution to this problem, at best a partial one, is to keep surface area minimal by keeping volume low.

The minimum dimensions of our exposure chamber were set by our desire to incorporate the motor-driven rotating cages which had proved in earlier work to allow a very precise determination of the time to physical incapacitation, which is probably a more significant objective observation than death in testing aircraft materials because of its potential relationship to the time available for unassisted escape from a fire environment. The final volume of 12.6 liters, including the recirculation pathway, resulted from the inclusion of this feature. It is, of course, possible for us to record time to death in addition, as indicated by respiratory arrest after cage rotation is discontinued.

b. Design features and materials of construction, The system is fabricated almost entirely of glass and plastic; the combustion tube is Vycor®. Several types of plastic are in contact with the combustion products within the exposure chamber: Teflon®, epoxy, and polypropylene seals and fan blades, in addition to the walls themselves, which are Plexiglas®. Because of the reactive nature of many major combustion products, metal has been kept to a minimum. Only the stainless-steel shaft which carries the cages, (almost completely shielded by plastic tubing) stainless needles for gas sampling, fan-motor shafts and thermocouple tips offer reaction sites for such gases. We see no solution to the problem presented by fluorine-containing polymers, These will yield fluorinated organic fragments and hydrogen fluoride, all of which, but especially the latter, are capable of reacting with vitreous materials, We believe the animals will receive exposure to the major portion of fluorides, but cannot guarantee absolute accuracy in this regard,

The animal chamber consists of a clear plastic box made of ¼-inch thick Plexiglas®, approximately 10" x 10" x 10". It has a ½-inch thick front cover held in place with four thumbscrews.

White rats weighing from 150 to 300 gms are exposed in groups of three. They are housed in three cylindrical cages, 8" O.D. x 3" in width, mounted on a single shaft. The cages are constructed from perforated Plexiglas® discs and are enclosed by plastic mesh. The shaft extends through a Teflon® bearing at the rear of the cage and engages by a spline coupling with the shaft of a gear motor which rotates the cages at 6 rpm.

The forward end of the shaft is supported in a boss, cemented to the front cover, which engages the shaft when the cover is put in place.

The recirculating blower is mounted on top of the chamber near the right, front. The glass-Vycor® duct system is constructed in sections connected by means of standard ball and socket joints. The flow-limiting plastic orifice plate is installed at one of these joints. Special short sections containing openings which allow the insertion of thermocouples, sampling tubes or flow-monitoring devices through rubber septa can be positioned at any of three points in the system. The blower is held in place with thumbscrews for easy removal. The combustion tube enters the chamber through the front cover at the lower left. Gas-tight seals are insured at juncture of tube and blower and at the entrance of the combustion tube into the chamber by Viton® O-rings. A glass deflection plate in front of the combustion-tube mouth protects the animals from direct impact of the heated gas.

A mixing fan with plastic blades is mounted on the right side of the chamber opposite the combustion tube entrance. It can also be quickly removed for cleaning. An auxilliary mixing blower (not shown in the diagram) is mounted on the top of the chamber. Thermocouples which continuously monitor the temperature at multiple points within the chamber produce a record of the temperature to which the animals are exposed and indicate the efficiency with which incoming gases are mixed with residual gases. The average temperature within the chamber can be kept at or below 32° C, and typically averages 28° C.

Orifices in the chamber walls sealed by rubber septa permit the introduction of needles for gas sampling, introduction of oxygen, etc. In our final assembly, the preheated furnace slides forward and backward between guide rails which maintain alignment between the combustion tube and the entry port into the chamber. In its most distant position there is adequate space for removal of the front cover of the exposure chamber, insertion and removal of animal cages (which can be accomplished in 20 seconds) and other required manipulations in the vicinity of the chamber. In its forward position, the distance between the furnace and the mouth of the combustion tube within the animal chamber is 5 inches. This we consider to be an important design feature unique to our system. The length of relatively cool tubing which combustion mixtures must traverse is minimal, with lessened opportunity for condensation and deposition of aerosols, low volatility organic fragments and particulates. We would prefer an even shorter path to insure that animals are exposed to an unchanged combustion mixture, but temperature considerations preclude this possibility.

c. Time frame. Another major problem which we faced was the selection of a suitable exposure duration. It is clear that we can control this factor by properly matching the size of the material sample to the total volume of the exposure system. It appeared to us that the prolonged (1 or more hours) exposure periods used by many investigators were inappropriate in testing materials for use in aircraft, in which the catastrophic fire develops rapidly. We therefore decided to select a sample size which would produce, in the case of those polymers of major interest, an observable response within a 30-minute period. We were confident that if the proper sample size were used for all materials tested, we could obtain distribution of response times within a 30-minute time span and demonstrate differential toxicity. We have shown that this is, indeed, possible at least with the materials tested to date.

3. Analytical System.

a. Hydrogen Cyanide. HCN concentration within the exposure chamber is monitored at intervals throughout the entire exposure period. The first measurement is made 1 minute after the test material is inserted into the furnace, and subsequent measurements are made at 3.8-minute intervals thereafter. This interval represents the shortest time which allows separation of HCN from other components of the gas mixture and spans the retention times of all major components detected by the gas chromatograph. The instrument used for this purpose is the Shimadzu Model GC-3BF gas chromatograph which possesses an essentially all-glass transit system. A Porapak® Q (80-100 mesh) column is utilized. The detector is of the alkali flame (AFID) type, highly sensitive to HCN (picogram detection capability) when all gas flows are properly adjusted. The use of AFID for HCN measurement was pioneered in our laboratory.

b. Carbon monoxide and oxygen concentrations are similarly monitored, the first sample being taken at 1 minute, followed by sampling every 1.9 minutes thereafter. The instrument employed for these analyses is the Carle "Basic" Model 8000 equipped with the 8-10k ohm thermistor detector (TC), but with the sensitivity increased 10-fold by removing the heater from the

detector block, an expedient which is possible when gases only are to be measured. The consecutive dual column employs silica gel followed by Molecular Sieve® 5A. The detector signal is paralleled to a single recorder with dual channels set at different attenutations, so that both oxygen and CO can be recorded with maximal sensitivity.

Sampling of chamber atmosphere for analysis of these three gases is accomplished by continuous pumping through stainless-steel sample loops attached to the respective instruments. The pump (valveless) used for this purpose (Fluid Metering, Inc.) exposes the gases only to ceramic, Teflon® and stainless-steel, thus minimizing reactive losses. The gas mixture is withdrawn from the chamber through a stainless-steel needle, passed first to the HCN analyzer through Saran® tubing of minimal internal diameter and length (8-10 inches), then to the CO-oxygen gas chromatograph and from thence back into the chamber. Introduction of the samples into the analytical instruments is accomplished automatically by pneumatic valves actuated by clock-driven cams.

c. Carbon dioxide is analyzed after the burn experiment has been completed, in gas samples collected manually by syringes at suitable intervals during the burn. The "ballast" column (Porapak® Q) of the Carle instrument is utilized for this purpose.

Selection of Animal Species

For a variety of reasons, we chose the white albino rat as the test subjects for our experiments: past experience, uniformity of responses, ready availability, convenient size, and a vast pool of information on their biological characteristics. Our source of animals, of the Sprague-Dawley strain, is The Charles River Breeding Laboratories, Wilmington, Massachusetts. We have tested rats of three different strains in our system including wild Norway, and have found no differences in response attributable to strain. Animals are ordered at 100 to 120 g in weight and held in our facility for 2 weeks. They are used at weights ranging from 150 to 300 g.

Pathology

Animals are quality-checked on arrival. Approximately 1 in 10 is sacrificed and inspected for pulmonary pathology which could affect their responses to inhaled gases. All animals which die during the exposure period are subjected to autopsy. The pulmonary system is examined grossly and microscopically. Other tissues are examined in a limited number of animals.

Survivors of the 30-minute exposure are observed for 7 days or more. Those with evidence of eye damage are carefully inspected for signs of recovery or progression and are sacrificed after appropriate intervals for microscopic examination of cornea and associated structures.

Tests on Standard Polymers

The first experiments conducted with the present system were designed to test the effects of changes in certain operating parameters and to evaluate the effectiveness of the system in classifying polymers on the basis of combustion toxicity.

For these purposes we utilized a series of "standard" polymers, most of which were purchased as chemicals by generic designation (Aldrich Chemical Company, Inc., Milwaukee, Wisconsin).

1. Effect of combustion temperature.

Figures II and III demonstrate the effect of changes in furnace temperature on the production of CO and HCN, respectively. The material employed in these tests was a co-polymer of acrylonitrile (31%) and butadiene (69%) selected because of its potential capability of producing both gases at appreciable concentrations under appropriate conditions of combustion.

We believe the results indicate that 600° C. is a suitable choice for use in our test program, in which a single temperature, once selected, must be used throughout.

Temperatures of 400° and 500° seem unrealistic in relation to a fire situation in which sustained temperatures much higher than these would normally be expected to exist. In addition, many materials with superior fire resistant properties would not decompose at these temperatures and could not be appraised toxicologically. On the other hand, many materials other than that selected for this test are consumed with similar, almost explosive velocity at 800° C, and have occasionally produced explosive mixtures within the system. In addition, it proved to be difficult to protect the exposed animals from thermal stress at this temperature. It can also be seen that the process of recirculation had little effect on the concentration of CO and HCN at 600° C., but that both decreased with time in the 800° C. test.

2. Sample size and CO and HCN production.

These tests were conducted to establish the relationship between the weight of the sample and the time course of CO/HCN production. We were especially interested in this relationship for two reasons: (1) As an aid in selecting a standard sample size which would insure a range of animal response times between 1 and 30 minutes, (2) To furnish a rationale for correcting all sample weights to one exact figure. This was desirable because of the difficulty experienced in obtaining aliquots of heterogeneous materials of equal weight without sacrificing uniformity. For example, two samples weighing 0.75282 and 0.74703 g could then be corrected to an effective weight of exactly 0.75000 g, and their resultant effects compared directly. These relationships are depicted in Figures IV and V.

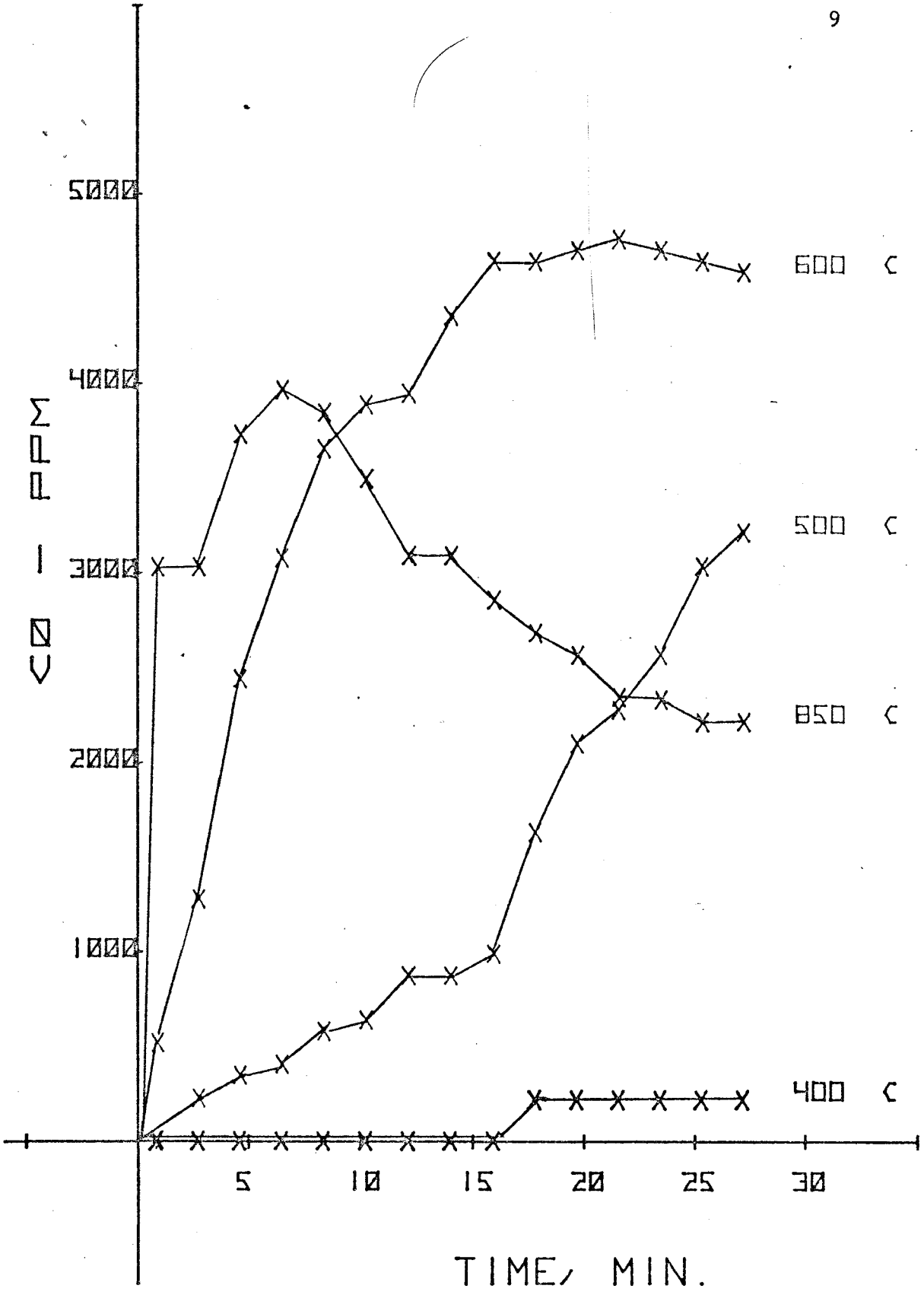


FIGURE II. Effect of temperature on evolution of carbon monoxide

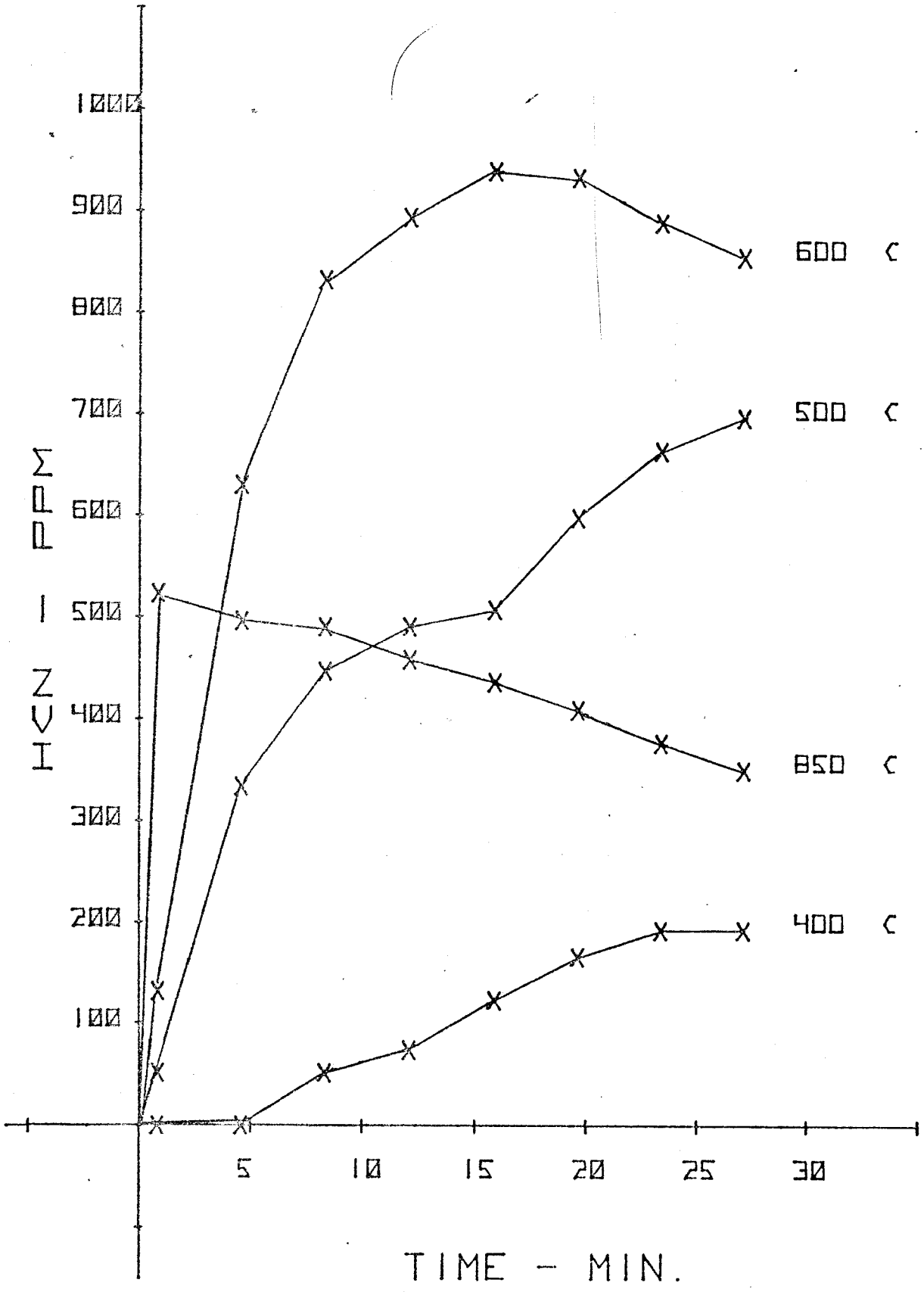


FIGURE III. Effect of temperature on evolution of hydrogen cyanide

3. Animal exposures.

Figure VI presents, in graphic form, the results of our first animal exposures in the present system. It should be emphasized that none of the 15 materials, with the exception of Douglas fir, represents a product in end-use form. To the best of our knowledge, none contains an additive which would affect inherent properties or introduce unusual toxic constituents into the combustion mixture.

The bars are arranged in the order of apparent toxicity of combustion products as indicated by animal responses (ti) under the test conditions described. Each ti (lower end of bar) and each td (upper end of bar) represents the mean for at least six animals (two replicate exposures); in some instances more than six.

Table 1 presents the same data in numerical form, accompanied by CO and HCN concentrations at incapacitation and death, obtained by interpolation from curves representing the concentration-time profile for the two gases. When a zero appears in the HCN columns, it indicates that none was detected. A dash means that an interfering peak prevented a precise HCN analysis, a consequence of the short time allowed for the gas-chromatographic cycle.

From our earlier studies with pure gases, we have been able to calculate the approximate 5-minute incapacitating concentrations for HCN and CO. These values are, respectively, 107 and 5000 ppm for a 200 gm rat.

When these values are considered in conjunction with both the bar graph and the table, several points of considerable interest are apparent.

In the case of polymer number 1, for instance, the very rapid evolution of HCN would almost certainly insure rapid incapacitation even if no other constituents were present. In the case of material number 2, both gases were certainly involved in producing a short ti, because neither one alone could be expected to incapacitate in so short a time.

Of course, we are not unaware of the certain presence of other toxic products in the combustion mixture, but it is of interest to note that in this series of tests on pure polymers, CO and HCN concentrations alone or in combination are in rough agreement with the animal responses.

Another interesting feature of these tests is that they incorporate, incidentally, the heat-resistance properties of the polymer. Certain polymers which eventually evolve sufficient concentrations of CO or HCN to incapacitate at or within 5 minutes obviously do so slowly, thus permitting prolonged activity and/or survival (material 6 is an outstanding example).

It will be noted that the bars representing certain materials (3 and 6 for instance) are short. We believe this means simply that when CO (and perhaps other toxicants) reach overwhelming concentrations, regardless of the time required, survival past the point of incapacitation will be brief. This is especially apparent in the case of CO.

Polymer number 15 failed to incapacitate within 30 minutes and the animals were removed, alive, from the exposure chamber at that point.

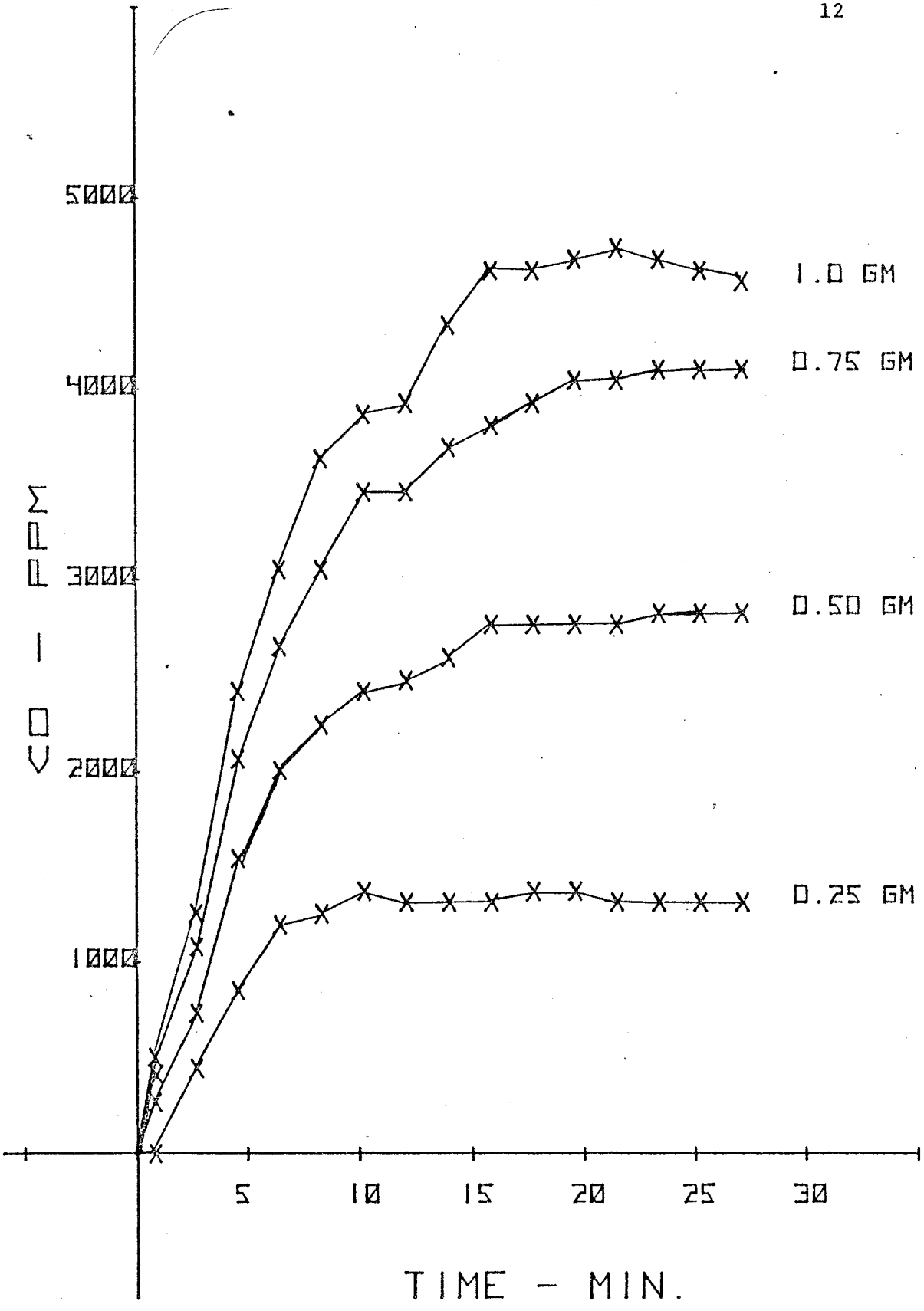


FIGURE IV. Effect of sample size on evolution of CO

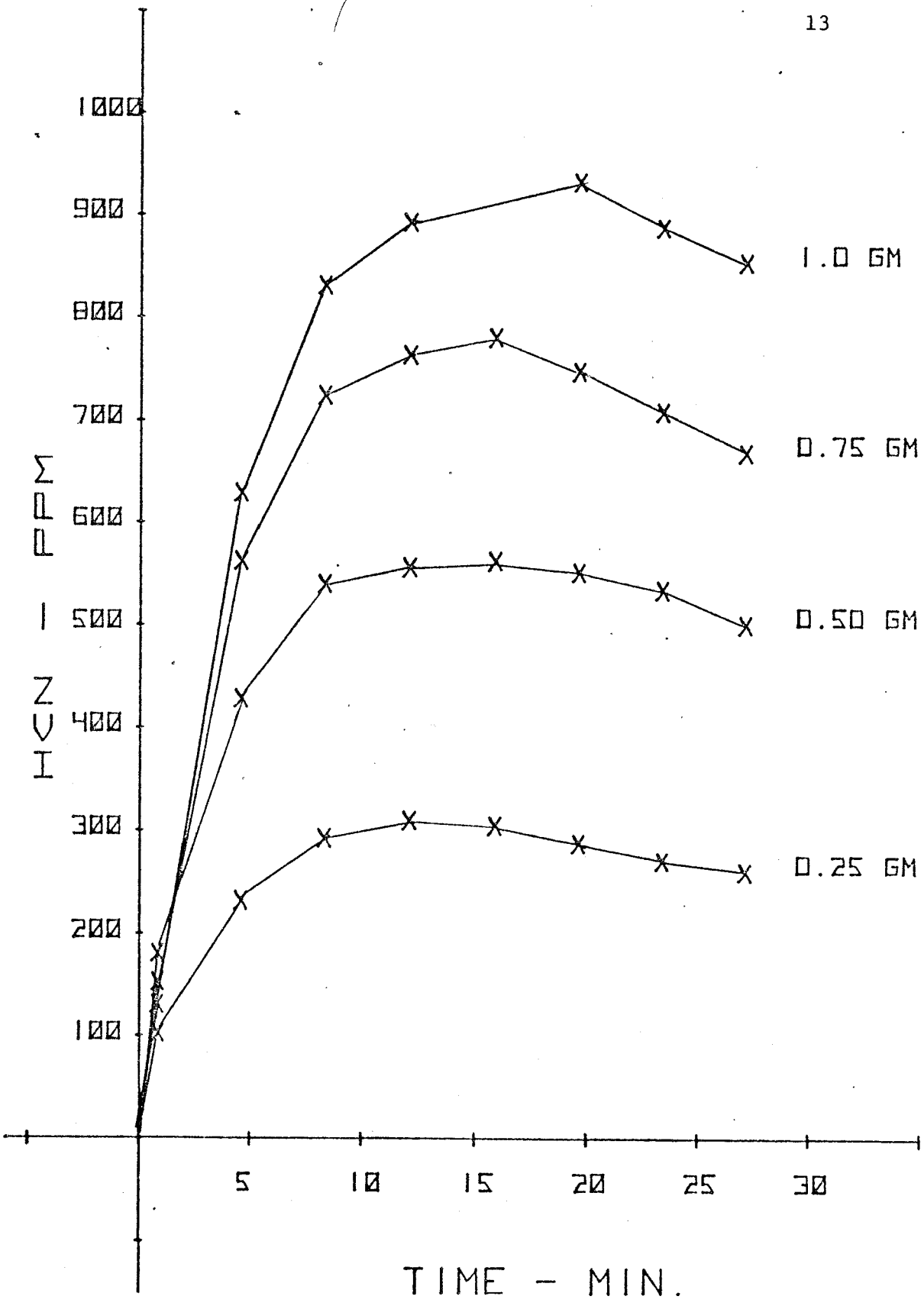
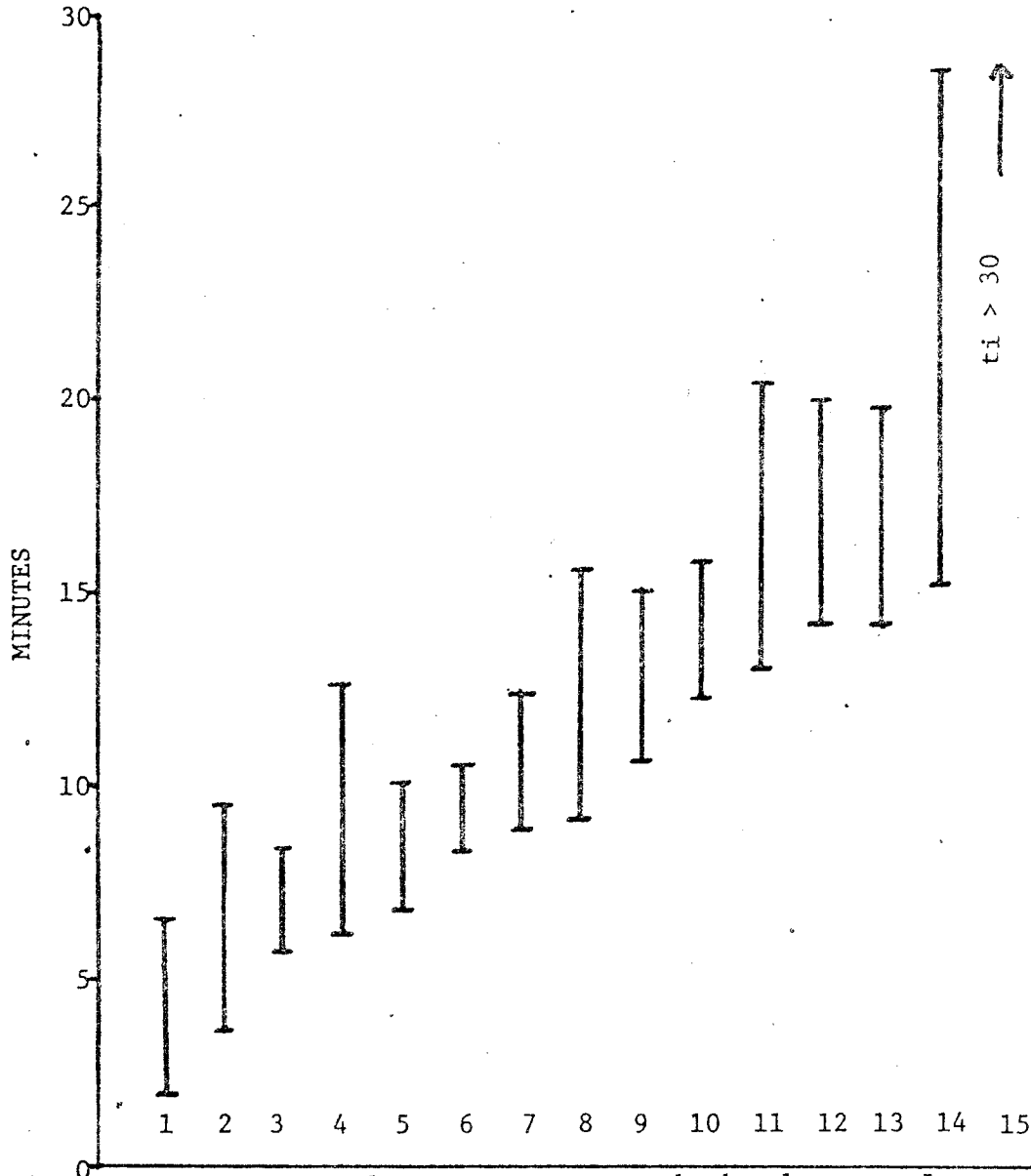


FIGURE V. Effect of sample size on the evolution of HCN



MATERIALS

1. Acrylonitrile:butadiene 44:56
2. Acrylonitrile:butadiene 31:69
3. Douglas Fir
4. Acrylonitrile:butadiene 20:80
5. Polyethylene, chlorinated 25%
6. Polysulfone Resin
7. Polyurethane Foam
8. Polyethylene, chlorinated 36%
9. Polychloroprene
10. Polyvinylchloride
11. Nylon 6/6 (Adipamide)
12. Acrylonitrile:butadiene:styrene
13. Nylon 6/12 (Dodecane diamide)
14. Nylon 6/10
15. Polyphenylene sulfide

FIGURE VI. Animal responses to standard polymers. Lower end of bar represents mean ti, upper end represents mean td for a 200-gram rat, 1-gram fuel load, 585° C furnace temperature and a 6 1/min airflow.

Table 1

Animal Responses to Standard Polymers with Associated CO and HCN Concentrations, Response times in min; Concentrations in ppm v/v.

Polymer	Times to		HCN Concentration At			CO Concentration At		
	Incapacitation	Death	ti	td	Peak	ti	td	Peak
A/B (44/56)	2.06	6.51	500	750	800	1200	3500	4730
A/B (31/69)	3.38	9.49	200	325	350	2000	5000	5400
Douglas Fir	5.64	8.46	100	90	110	17000	17000	17350
A/B (20/80)	6.02	12.83	325	245	350	4500	5000	5000
Chlorinated Polyethylene (25% Cl)	6.78	9.89	0	0	0	10500	10750	10900
Polysulfone Resin	8.10	10.27	0	0	0	19500	23000	26500
Polyurethane Foam	8.86	12.18	0	0	0	8650	8700	8800
Chlorinated Polyethylene (36% Cl)	8.89	14.51	0	0	0	6850	7000	7000
Polychloroprene	10.63	14.93	0	0	0	6300	7000	7150
PVC, pure	11.91	15.36	0	0	0	7250	8800	8800
Nylon 6/6	13.00	20.17	-	-	-	2500	2550	2550
A/B/S	13.81	19.77	150	140	275	4600	4900	4900
Nylon 6/12	13.94	19.59	-	-	-	4600	5000	5000
Nylon 6/10	15.11	28.59	-	-	-	1675	1600	1700
Polyphenylene Sulfide	>30	>30	-	-	-	-	-	<100

Concept and Evaluation of Dose-Response Relationships in Inhalation Toxicology

In our earlier studies of the toxicology of atmospheres containing CO, HCN, or mixtures of the two, satisfactory solutions to our design and analytical problems left us with a final pair of problem areas.

The investigation of any chemical dose/biological response relationships must include a consideration of the magnitude of the receptor biological system in relation to the magnitude of the administered dose. In our system this meant that the most meaningful and precise data would result from administration of replicate doses of exactly the same quantity of toxicant to replicate animals of exactly the same body weight. This is the usual procedure for determining such toxicological properties as LC-50, LD-90, MLD, etc.

We initiated our studies using exactly this standard protocol. We soon found, however, that the use of animals within a weight range of only a few grams created an unrealistic economic and logistic situation. At the same time we discovered that, in our system at that time, we could not repeatedly generate identical toxic gas time-concentration curves in the chamber atmosphere. The inability to control these two parameters would introduce an unnecessary and undesirable lack of precision in any calculation of toxicological properties by the usual techniques.

We decided to take advantage of this otherwise undesirable situation, and develop a more useful and more versatile relationship between toxicant dose (administered by inhalation) and biological system response.

A relationship was developed from the following assumptions:

(1) The animal's minute-respiratory volume remains constant over the exposure period and is equal to (or if variable, it averages out to) that given by the Guyton formula: $RV = 2.1\ (\text{Body Wt, g})^{0.75}$; RV is in ml.

(2) The total volume of chamber atmosphere which is inspired over time, t, is: (RV) (t).

(3) The total quantity of toxicant which is inspired over time, t, is equal to the area under the concentration-time profile over the time interval of interest:

Q, quantity $= \overline{CT}$, integrated area
C, concentration $= \overline{CT}/t_r$, tr = response time (ti or td)

(4) The total inhalation dose required to produce a given response (incapacitation or death) is d_r:

$$d_r = Ct_r(RV)$$

The dose per gram of body weight for a given response is D_r:

$$D_r = \frac{Ct_r(RV)}{Wt}$$

All the data from our CO and HCN studies were evaluated according to this relationship. The calculated "Doses" for incapacitation and for death proved to be surprisingly constant,

In the case of CO, with animal weights varying over a 3-fold range and CO concentrations over a 2.5-fold range, the incapacitating inhalation dose was 15.4 mg/kg and the inhalation lethal dose was 50.5 mg/kg,

The increased versatility of this relationship over that afforded by LC or LD figures is indicated by the fact that once D_r is known, t_rs can be predicted for selected concentrations and conversely C_rs can be predicted for selected t_rs.

An unexpected example of the equation's utility is evidenced by the fact that inserting the appropriate RV for mice or for man (and the appropriate body weight) yields incapacitating CO inhalation doses for those two species which agree within 1 to 2% of experimentally-derived values found in the scientific literature,

The correspondence between our equation and human experimental data is shown by Figure VII which is a regression of our equation for the incapacitating dose of CO in the rat on data for CO incapacitation in man derived by Peterson and Stewart,

The precision for cyanide values is not so good as that for CO, but, for the rat, they are:

Incapacitating Dose (inhalation) = 320 µg/kg
Lethal Dose (inhalation) = 1.95 mg/kg

We have further verified the calculated value for a lethal dose in the rat by administering intravenous injections of cyanide solutions. An iv dose of 2.0 mg/kg proved lethal while one of 1.80 mg/kg did not. This experiment also verified our supposition that the efficiency with which HCN is extracted from inspired air is essentially 100%, while the extraction efficiency for inspired CO is about 50% up to a COHb saturation of 40-50% and then it decreases.

If we attempt to extrapolate our HCN toxicity data on rats to its significance for man, we can only say that our equation predicts the lethal blood level in man would be 3.4 µg/ml. Forensic data for human fatalities indicate the lethal blood level to be from 2 to 5 µg/ml.

So far we have been discussing the utilization of our theoretical equation in situations dealing with known toxic gases. In combustion toxicology, both the nature and quantity of toxic gases in the combustion mixture are unknown. The equation should be useful, however, in estimating the relative contribution to the total toxicity of any component which can be quantitated and studied alone.

We have made one further use of the relationship in our studies of the toxicity of polymer pyrolysis products. Since we cannot measure the concentration of the total toxic gases, we assign this quantity to the weight of sample loaded in the furnace tube. The relationship between this quantity (fuel load) and animal response (incapacitation) follows the relationship that would be predicted by the equation; i.e., $C = K (1/ti)$. This is indicated graphically in Figure VIII.

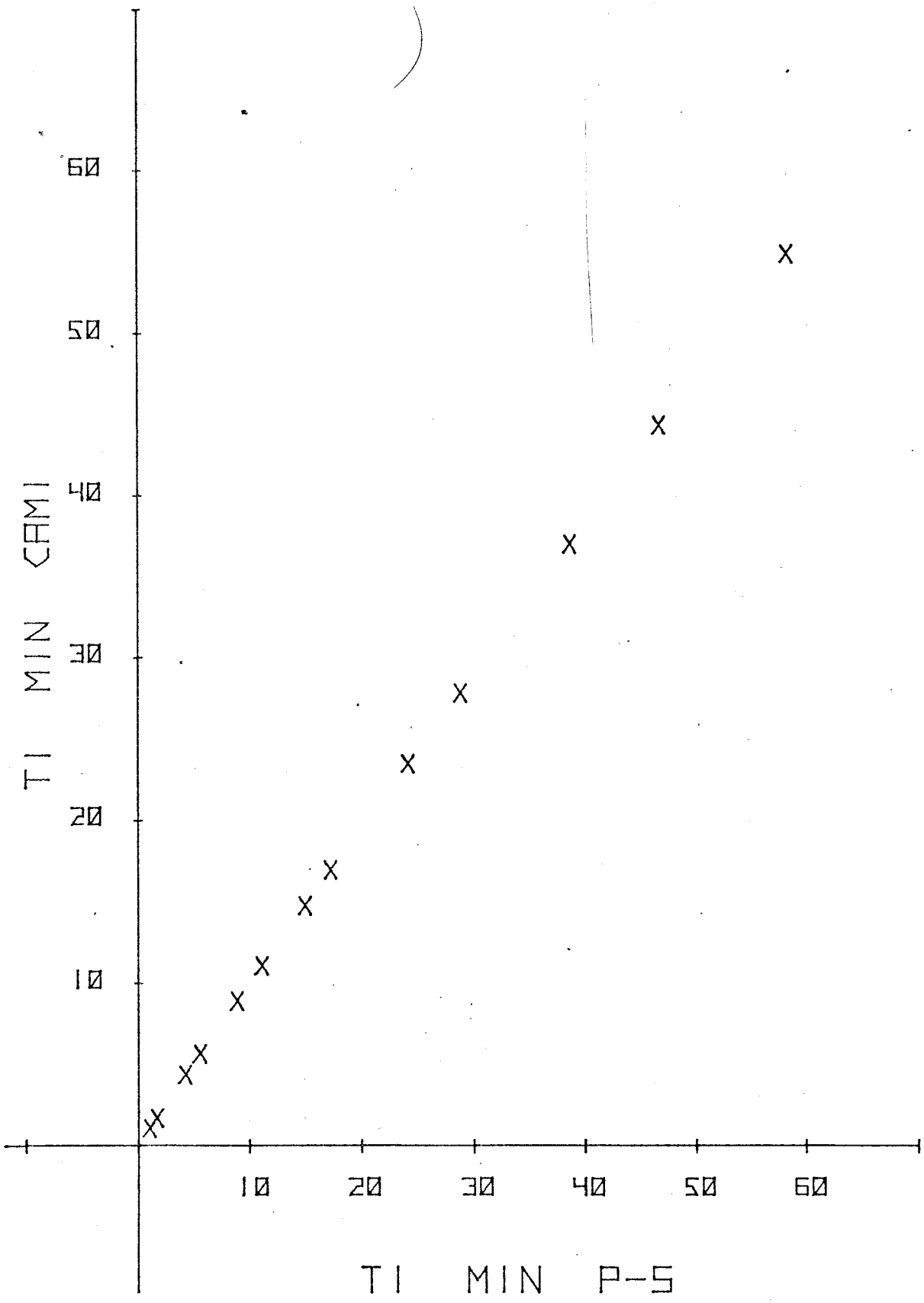


FIGURE VII. Correspondence between times-to-incapacitation for equal inhalation doses of CO in man and rat, Based on CAMI equation derived from rat data and Peterson-Stewart equation derived from human data.

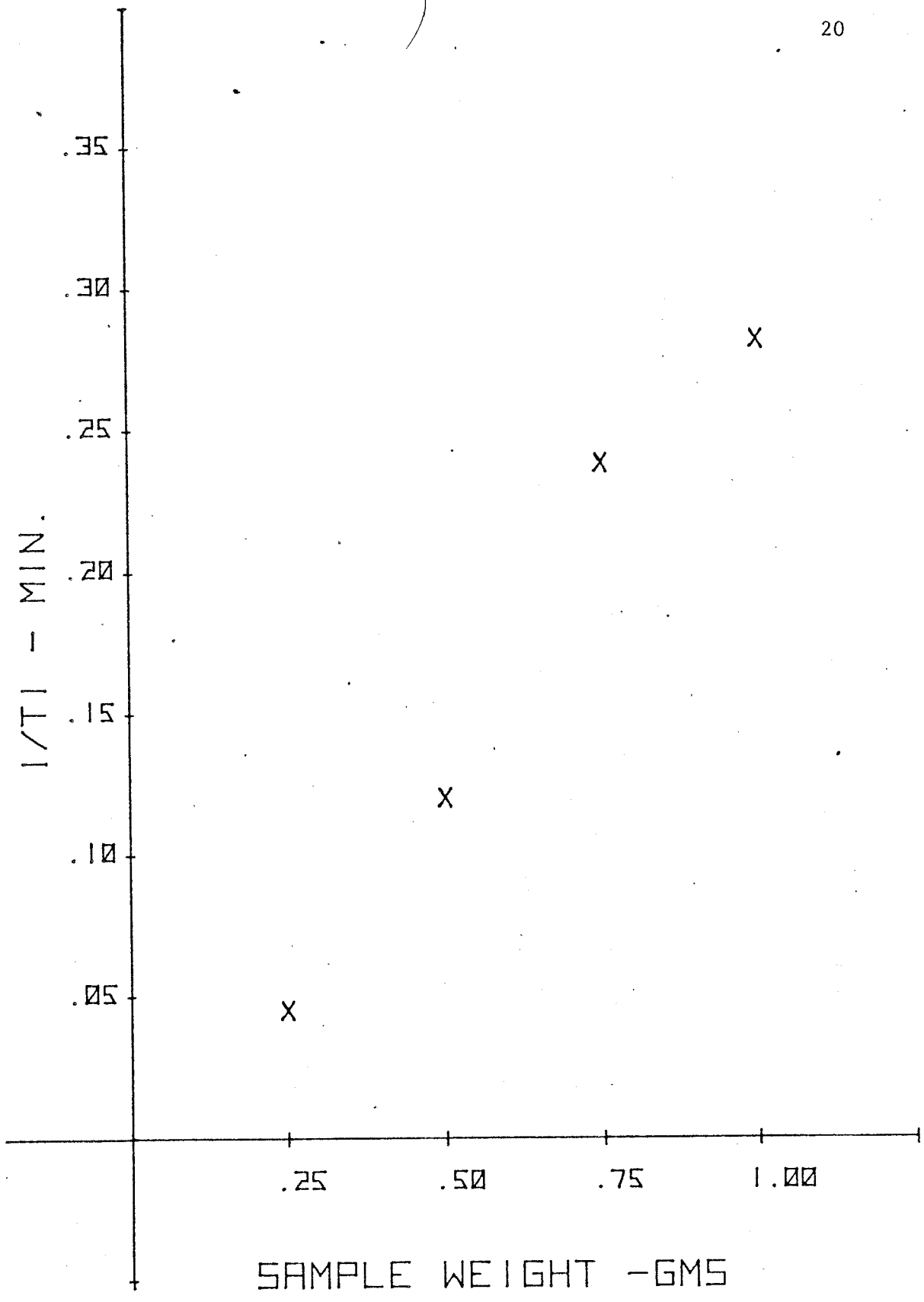


FIGURE VIII, Animal responses in relation to sample size