TEST METHOD 5100-1A
FEBRUARY 1991
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U.S. DEPARTMENT OF AGRICULTURE
FOREST SERVICE

STANDARD TEST METHOD FOR DETERMINING ACUTE INHALATION TOXICITY OF FIRE SHELTER CLOTH LAMINATES

1. SCOPE

1.1 Scope. This test method describes apparatus and laboratory procedures to be used in acute inhalation toxicity tests of products released from thermal degradation of laminated cloth used in the manufacture of fire shelters.

2. PUBLICATIONS

2.1 Government documents

2.1.1 Publications. The following documents of the issue in effect on the date of invitation for bids or request for proposal form a part of this specification to the extent specified herein:

AMERICAN SOCIETY FOR TESTING AND MATERIALS (ASTM)

E 800 - Proposed Standard - Standard Guide for Measurement of Gases Present or Generated During Fires

(Application for copies should be addressed to the American Society for Testing and Materials, 1916 Race St., Philadelphia, PA 19103.)

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Good Laboratory Practices for Non-clinical Laboratory Studies, CFR, Title 21, Chapter 1, Part 58, pp. 209-223.

(The Code of Federal Regulations is for sale on a subscription basis from the Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402. Reprints of certain regulations may be obtained from the Federal agency responsible for issueing them.)

NATIONAL BUREAU OF STANDARDS

NBSIR 80-2177 - Development of Recommended Test Method for Toxicological Assessment of Inhaled Combustion Products

(Application for copies should be addressed to Center for Fire Research, National Engineering Laboratory, National Institute of Standards & Technology, U.S. Department of Commerce, Washington, DC 20234.)

NATIONAL INSTITUTES OF HEALTH

Guide for Care and Use of Laboratory Animals, NIH Publication 86-23.

(Application for copies should be addressed to National Center for Research Resources, National Institutes of Health, Bethesda, MD 20892.)

2.3 Order of precedence. In the event of conflict between the text of this document and the references cited herein, the text of this document takes precedence. Nothing in this document, however, supersede applicable laws and regulations unless specific exemption has been obtained.

3. REQUIREMENTS

Testing with the apparatus described herein shall be conducted by individuals with appropriate training in toxicology, chemistry, animal handling, and laboratory procedures. Specific training and experience in combustion toxicology is desirable.

The test facility shall be registered with the U.S. Department of Agriculture as an animal research facility.

Operation of the apparatus requires a laboratory with a working fume hood, appropriate bench space, analytical instrumentation, and associated equipment. Normal safeguards for working with potentially toxic chemicals, toxic gases, flammables, and electrical devices shall be observed.

3.1 Radiant Furnaces. Radiant energy shall be used to heat the sample specimens. The furnace shall be able to heat the surface-mounted sample to the prescribed decomposition temperature in less than 5 minutes. The furnace described in figure 1 is capable of consistent high temperature irradiation. The commercially available hater panel consists of a series of 14 inch metallic ribbons mounted on an inert vitrified board that measures 16L by 8.5W by 1.25D inches (Glenro Inc.). The heating panel is mounted in a custom-made stainless steel frame at a 45° angle. The frame holds the specimen a uniform distance over the exposed heating surface.

Placement of the specimen on the frame is aided by a 5.0 inch open stainless steel wire mesh support that prevents the decomposing specimens from falling on the hot ribbons.

Radiant energy control is accomplished by a heavy duty voltage regulator (Powerstat™) and monitored by thermocouple. Approximate operating conditions shall first be established by scanning the heating surface with a radiometer. A surface energy of approximately 2.5-3.0 watts/cm^2 recorded without the specimen or screen in place should provide enough irradiated for decomposition at a subsequent sample temperature of over 400°C. When the voltage setting to achieve 2.5-3.0 watts/cm^2 is obtained, the setting should be recorded. During the start of the test, this setting should will be dialed-in to initiate specimen decomposition. Final adjustments, raising or lowering the temperature to 465°C, shall be made with the regulator while monitoring thermocouple readings.

4.3 Animal Exposure Chamber. A two-part 38 liter exposure chamber (figure 2) shall be used. The two-part design significantly reduces the heat in the animal exposure chamber. The combustion chamber of 25 liters is made from temperature resistant glass (Pyroceram II™) and contains a panel mounted electric radiant heater. The 13 liter animal exposure chamber is made from 0.5 inch clear acrylic (e.g., Plexiglass™). The chambers are connected by large-diameter glass or silcone rubber tubing ending in intake and exhaust ports fitted with ACE™ ball-socket connectors. A large capacity peristaltic pump (Cole-Parmer) aids in the equilibrium of chamber atmosphere by recirculating gases.

The apparatus as described shall be assembled in a working laboratory hood for safe operation.

3.3 Combustion/Pyrolysis Gas Analysis. Carbon monoxide (CO), carbon dioxide (CO_2), oxygen (O_2) and hydrogen cyanide (HCN) levels shall be measured at least every 5 minutes during the 15 minute exposure test (i.e., at 0, 5, 10, and 15 minutes). Oxygen shall not fall below 16% during the animal exposure. Any of the accepted techniques described in ASTM Proposed Standard E 800 Standard Guide for Measurement of Gases Present or Generated During Fires may be used; however, automatic, continuous infrared analysis of CO and CO_2 and paramagnetic analysis of O_2 is preferred. The selective ion electrode method for HCN is recommended. A procedure for HCN analysis of chamber atmosphere is given in the appendix.

Gas analysis shall be accomplished by an instrumentation "closed-loop" in which gases are returned to the chamber. The total volume of gases taken from the chambers for destructive analysis, if any, shall not exceed 10% (3.8 liters). Oxygen levels shall not fall below 16% from a normal of approximately 21%. Anoxic conditions will affect animals' responses and confound test results; therefore, any depletion that approaches the critical 16% mark shall be corrected by replenishment as described in section 4.

3.4 Temperature Monitoring and Control. Laminated cloth sample specimen temperature shall be recorded throughout the test from at least one thermocouple that shall be placed securely on the test sample, on the surface opposite the heating elements. Radiant energy to the sample specimen shall be controlled to maintain a sample temperature of 465°C ± 15°C.

3.5 Sample Specimen Preparation. Test shall be conducted on multiple specimens cut from laminated cloth samples. A specimen shall be conveniently cut into rectangles to equal the surface area dimensions of the radiant heater (i.e., 7.5 by 12 inches--approximately 90 square inches). The individual specimens shall then be layered and sandwiched in stainless steel wire mesh for exposure to the heat.

When mounted on the heater, the inner scrim side (dull side) of the first specimen (and subsequent specimens) shall face the heat. The thermocouple wire should be placed on the shiny outer side of the last layer, in between sample and wire mesh. The mesh specimen holder is to be placed in a stationary upright position in the channel at the base of the heater frame. The 45° tilt of the furnace frame keeps specimen over the heating elements.

3.6 Test Animals. Young, healthy adult laboratory rats of uniform sex, weighing between 200 and 300 grams, shall be used. The Sprague-Dawley and Fisher Strains are satisfactory. Sources of animals shall be reported, e.g., the commercial supplier must be named if one is used. If animals from an in-house colony are used, it must be stated. In all cases, normal steps shall be taken to assure healthy animals are used in testing.

3.7 Animal Care. Animals shall be maintained on ad libitum food and water schedules and treated in accordance with Good Laboratory Practice for Non-clinical Laboratory Studies published in the Code of Federal Regulations, Title 21, Chapter 1, Part 58.90 and Guide for the Care and Use of Laboratory Animals, NIH Publication 86-23. Animals received from a supplier shall be housed at the testing laboratory for a minimum of 7 days before being used intesting.

3.8 Animal Observations and Lethality. General toxicological effects shall be measured in terms of exposure lethality and post-exposure body-weights and lethality. Individual animal body weights shall be recorded on the first day of test and at the end of 7 days observation. Weight loss, if any, shall be recorded. Necropsies shall be performed and gross pathological examination of lungs and other organs shall be made and abnormalities reported. All abnormal clinical symptoms pertaining to inhalation shall be reported.

3.9 Test System Validation. Verification of the functional integrity of the test apparatus shall precede actual animal exposure toxicity testing and is accomplished in three steps:

1. Pure Gas Trials: With the apparatus assembled and in full operational configuration, except the radiant heater, which is not switch on, introduce a known standardization gas (i.e. CO 1%) to a level approaching chamber atmosphere equilibrium. Monitor the standard gas through the equilibration phase into the steady-state phase for a time period equal to the 15 minute test period. The monitoring should reveal a "square wave" chamber exposure, that is, a rise in CO from zero to a plateau (see example 1). The maintenance of the plateau or steady-state phase indicates that there are no significant leaks in the system. If, instead, the concentration of standardization gas "decays" over time, the operators must check for and correct chamber leaks. Satisfactory chamber performance is indicated when the system retains approximately 90% or more of the standardization gas concentration during the test trials.

2. Control Sample. A control toxic sample shall be used to verify the function and reproducibility of the whole system, specifically the radiant heating system. (Toxic control samples are available from USDA Forest Service, Missoula Technology and Development Center, Building 1, Fort Missoula, Missoula, MT 59801.)

When tested in accordance with the methods herein, the control material should generate the following concentration of gases:

Specimen Size	Temperature	HCN Exposure Chamber ppm	Minimum Yield HCN
90 sq. in.	465	90 to >135	1 ppm/sq.in.
180 sq. in.		180 to >270	

Failure to meet the above measurable concentrations indicates suboptimal furnace performance. Trouble-shooting measures should investigate the radiant furnace performance, temperature controlling equipment, chamber leaks, and gas sampling and analysis procedure.

A typical combustion trial using the system will produce a CO chart fingerprint revealing the accelerated thermal decomposition phase ,peak concentration, equilibrium phase of gases between chambers, and finally equilibrium plateau (see example 2). The final plateau state indicates a tight system even though minor leakage may have occurred during decomposition when gas pressure and heat cause volume expansion. In control sample tests the investigators should prolong the test time to assure that gas concentrations are not decaying. Monitoring chamber performance for a longer time is important because the equilibrium phase may not be revealed in 15 minutes with some samples.

3. Comparative Response Trial(s): The toxic control material has been shown through tests at independent laboratories to reproduce a toxic response in animals during the test period measured as incapacitation of death within 15 minutes when 90 square inches or more have been heated.

The testing laboratory shall report the results of tests conducted on the control material along with its results from test samples. Historical dose-response data shall be permitted but, with any new test, at least one current toxic control trial shall be included to reverify comparative data.

4. TESTING

4.1 Screen Test. To quickly eliminate certain laminates with toxic potencies that might compare to those previously found unacceptable, and to limit the unnecessary use of animals in a complete test, a combustion gas-analysis test without animals optionally may be conducted.

When tested under the same chamber conditions of temperature and time, a sample laminate shall fail the toxicity test if it produces or exceeds measurable HCN concentrations of 1 ppm/square inch of specimen, with specimen sizes from 90-180 square inches (90 to 180 ppm chamber concentrations respectively).

4.2 Complete Test. The apparatus shall be assembled and it's function validated with calibration gas and controls as in section 3. Three test animals, preferably fasted overnight, shall be identified and placed in separate compartments within the activity wheel of the exposure chamber. The wheel is then secured and should be set in motion shortly before the test time to adapt the animals to walking. The lid of the exposure chamber is kept off to provide air and circulation until moments before the sample is combusted when it is then sealed firmly. After positioning the specimen on the heater, the furnace chamber is sealed and all of the apparatus and analytical systems are also checked. The test is initiated by switching on the heater and recirculating pump. A 30 second "lag" time is incorporated when timing the experiment in order to make final apparatus checks and heating adjustments.

The specimens shall be exposed to radiant heat for 15 minutes and attain a surface temperature of 465° ± 15°C. During the test, animal observations shall be made, recording incapacitation and lethality. Exposure chamber conditions shall be measured initially and at least every 5 minutes to include temperature and O_2, CO, CO_2, and HCN. Gas analysis for other smoke constituents is Optional. In the event that O_2 levels drop from 21% to the critical low of 16%, a cylinder of oxygen and a flow meter shall be available to meter in the exact amount of oxygen needed to replenish the chamber atmosphere within 2 minutes.

A minimum of three experiments shall be conducted spanning a range of test laminate surface areas that produce both a non-toxic response with the smaller areas tested and a toxic response with greater areas. His "dose-response" determination may be achieved by basing specimen increments on logical progressions of the previous "dose" (e.g., 45..90..135 square inches).

In each experiment the specimen shall be weighed and mounted in the holder and positioned on the radiant furnace with the thermocouple in place. After Exposure, animals shall be immediately removed from the activity wheel. The specimen, when cool, shall be reweighed to determine the percent combusted. Surviving animals shall be observed for 7 days for post-exposure lethality, if any, and general toxicological effects.

4.3 Records. Raw data from each test series shall be recorded and submitted on the TM 5100-1 data sheet and submitted with the final report. Chart recordings, where appropriate, should be submitted to support test validation and gas analysis.

4.4 Interpretation. Toxicity of the fire shelter laminate shall be based on animal lethality in the complete test.

Independent laboratory reports have shown acceptable laminate materials to have toxic potencies significantly less than the control material.

Actual results, for example, have produced the following data:

Surface Area Sq. in.	Toxic Response as Lethality (15-Minute Exposure) Toxic Control	Sample Laminate
360	Toxicity	Toxicity
270	Toxicity	Toxicity
180	Toxicity	None
135	Toxicity	None
90	Toxicity	None
45	None	None

The control sample has demonstrated toxicity from the heating of 90 square inches in the test apparatus. For laminates to be considered acceptable, data shall reveal, at a minimum, no toxicity at one dose level greater than the toxic control (incremental dose factor of 45 square inches) starting with 90 square inch specimen.

Additionally, using the average of individual trials, the test laminate shall not produce measurable HCN concentrations exceeding 0.5 ppm/square inch.

APPENDIX--HCN DETECTION PROCEDURE

Procedure: Detection of HCN in an atmosphere by Specific Ion Electrode (1)

Equipment: Orion Digital Analyzer model 701A or equivalent

Calibration: 1.0) Prepare five CN standard solutions from 10^{-2} to 10^{-6}.

1.1) Use .490g of NaCN in 1 liter distilled water containing 10 mls of 10M NaOH (ISA).

1.2) Make serial dilutions from 10^{-3} to 10^{-6} so that each dilution contains 100 ml.

2.0) To each dilution add 1 ml of ISA; the pH should be approximately 13.0.

3.0) Set up Orion 701A meter with CN electrode 940600 and Single junction reference electrode no. 900100.

3.1) Set temperature dial and then set function switch to REL. MV.

3.2) Using 10^{-4} std. Calibrate meter to 0.00.

3.3) Plot concentration curve on 4-stage semilog paper for all dilutions.

Note A) Each fold change in dilution should approximate ± 56MV; if not, this indicates electrode disfunction. Clean, soak, or lightly polish electrode. Check manual for maintenance.

Note B) Electrode will not tolerate high concentrations of CN without some damage; 10^{-2} dilution should be recorded rapidly.

(1) Based on method S-250, NIOSH Analytical Manual, 2nd ed. U.S. HEW Publication and Orion electrode methodology, Orion Instruments.

Air Sampling: 4.0) Prepare a 0.2 M NaOH scrub solution: i.e., 2 mls of 10 M NaOH in 98 mls of distilled water.

4.1) Add exactly 6 mls of scrub to a clean 50 ml beaker and cover with a watch glass.

4.2) With a dry 100 cc plastic or glass syringe and needle capture the atmosphere using the total 100 cc volume.

4.3) Using the same gas filled syringe, withdraw the 6 mls of scrub solution from the beaker; plug the syringe and swirl/shake for not less than 1 minute.

4.4) Eject the scrub solution back into the same beaker and measure its relative potential.

Calculation: Given the above sampling procedure, the following is the conversion Factor used in determining HCN in air.

	HCN ppm Using 6cc scrub	Multiply by	
At 25°C	value from std. curve	54.4	= HCN in atmosphere

Interference with CN; Ions

S	Must be absent
Br	$5x10^{3}$ moles/1 max.
I	0.1 moles/1 max.
Cl	CI 10^{6} moles/1 max.

TM 5100-1A Data Sheet

USDA FOREST SERVICE ACUTE INHALATION TOXICITY REPORT
ON FIRE SHELTER CLOTH LAMINATES

TEST RUN DATA

Testing Laboratory _____ Run No. _____

Sample Indetification _____ Sample Weight Initial (g) _____

Set Point Temperature 465 ±15°C yes ___ no ___ Sample Weight Final (g)_____

Sample Size (sq. in.) _____ % Loss On Ignition (LOI)_____

EXPOSURE EFFECTS

Animal No.	Incapacitation Time	Lethality Time (est)	Incapacitation Ratio	Mortality Ratio
____	_____	_____	_____	_____
____	_____	_____		
____	_____	_____	Note:	

POST EXPOSURE EFFECT AND OBSERVATIONS
BODY WEIGHT AND LETHALITY

Animal No.	Weight(g) Initial	Weight(g) Day 7	Final Mortality Ratio
____	____	_____	_____
____	____	_____	_____
____	____	_____	_____

- -

EXPOSURE CONDITIONS
MINUTES

	(0)	(5)	(10)	(15)	HCN Yield ppm/sq.in.
Furnace Temp (°C)					
Exposure Chamber Temp (°C)					
O_2 (%)					
HCN (ppm)					
CO (ppm)					
CO_2 (ppm)					

Suggested List of Suppliers

Description	Supplier	Technical Note
Radiant Heater		
Panel: (8) 14 x .5 inch heating ribbons mounted on double vitrious board	Glenro Inc. 29 McBride Ave. Ext Paterson, NJ 04501	Recess panel in frame .63 from face with vitrious board spacer
Specify Series 80 Heater Module No Sheet Metal, No Grill, 80 V, 1.6 KW		
Frame: Stainless Steel Sheet	Custom Fabrication	Provide channel to hold Sample support at base
Sample Support: Stainless Wire Mesh 0.5 inch open	Commercial Suppliers	
Power supply: Powerstat®, Transformer 120V 1156C or equivalent	Superior Electric Co. available from Newark Electronic, Chicago, IL 60624	
Temperature Recorder: Omega Model 99 Type K Recorder	Omega Engineering Stamford, CT 06907	
Thermocouple: Type K 12 inch probe, miniature, K116 G-1600-0-12-24.1H x/Kx 41102; Kx 41202 connectors or equivelent	Thermo Electric Saddle Brook, NJ 07662	
Radiometer: 1 inch water cooled radiometer	Medtherm Corporation Huntsville, AL 35804	Requires high quality MV Multimeter to operate
Chamber:		
Furnace Chamber Pyroceram II® (2) plates 17.4 x 10 x .2 inch (2) plates 17.4 x 9.4 x .2 inch (2) plates 9 x 10 x .2 inch assembled volume 25L	F.J. Gray & Co. Queens Boulevard Jamaica, NY 11435	Specify no irregularities to assemble and seal with G.E. Silicone® at seams
ports drilled .75 inch diameter w/diamond bit	Diamond bits available from New Jersey Diamond Tool Kentucky Avenue Paterson, NJ	Drill press required
Porting: Drill glass with diamond drill bit		
Fittings: Ace® ball/socket 28/15 connectors, stock and custom sizes for smoke pathways	Ace Scientific Supply East Brunswick, NJ 08816	Mate with rubber stopper or cut silicone tubing at bulkhead of chamber
Top Gasket: cut from silicon tubing Masterflex® 6411-16	Cole-Parmer 7425 N. Oak Park Ave. Chicago, IL 60648	Tack in place with G.E. Silicone II, seal before each test with silicone lab stop- cock grease
Exposure Chamber		
Fabricate from 0.5 inch thick Plexiglass® or equivalent to volume of 13 liters	Commercial Plastics 98-31 Jamaica Avenue Richmond Hill, NY 11481	Order pre-cut sections or fabricate/machine in-house

Description	Supplier	Technical Note
Whole-body chamber dimension 11L x 10W x 10H inches		
Activity Wheel: Acrylic & polypropylene mesh 7.25D x 9.63L inch cylinder. Wheel separators are .25 x 7.25 inch acrylic drilled on center for .25 inch s.s. rod. Polypropylene mesh is .25 opening size.	McMaster-Carr Chicago, IL 60648	Fabricate wheels and chuck support to drive. Seal bearings in chamber To prevent leaks
Activity Wheel Motor 6rpm: Dayton Model No. 3M104	McMaster-Carr	Chuck to center rod, build support
Exposure chamber seals: .38 inch neoprene gasket	Commercial Hardware Supplier	
Lid Latches: 2 inch "South Co" (Lancaster, PA) rubber latches or equivalent	McMaster-Carr	
gas fittings may be .25 MPT nipples drilled into chamber		

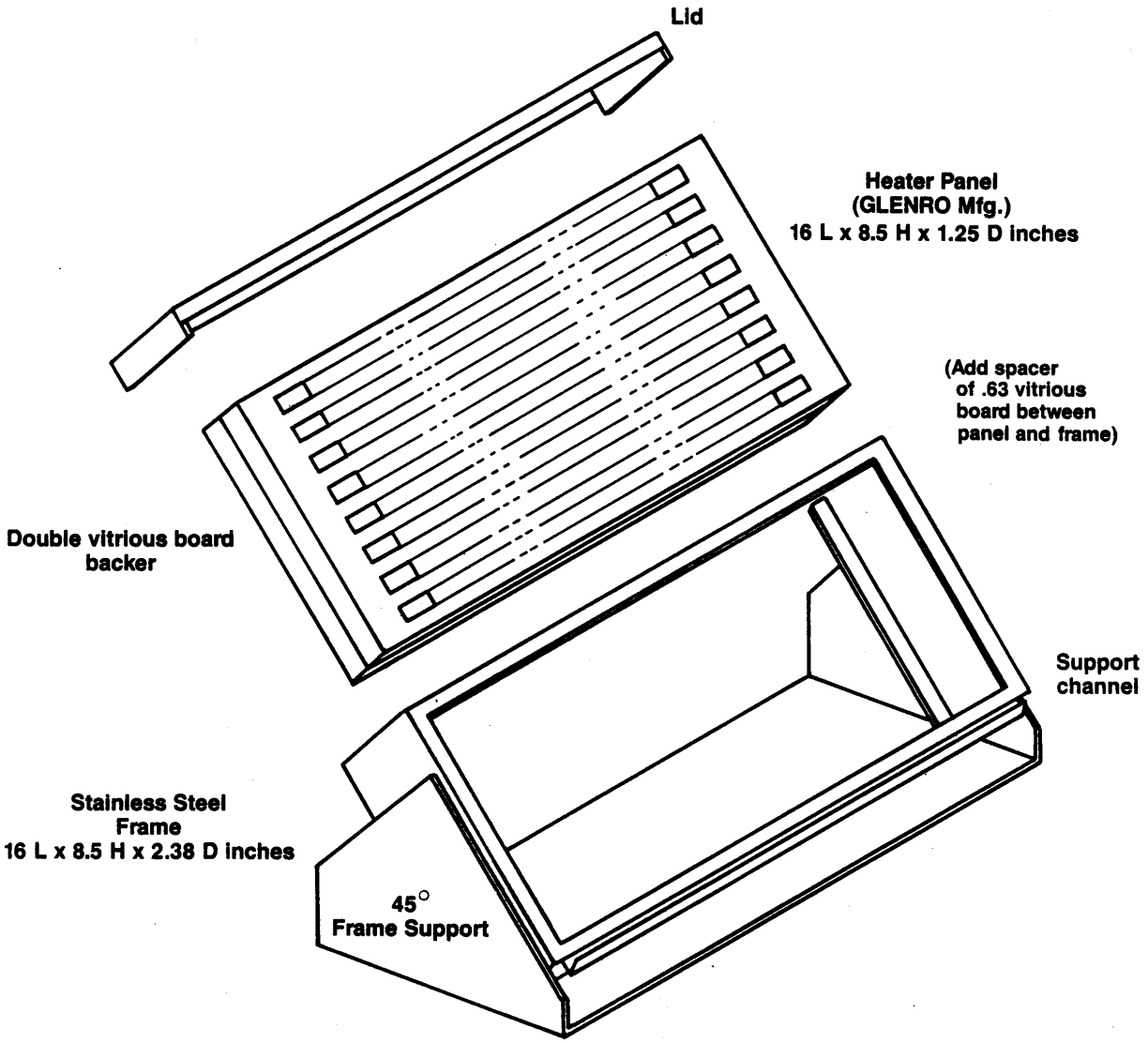
Recirculating Pump:

Model 7019 large capacity Peristaltic pump with Dayton® variable speed motor and controller	Cole-Parmer 7425 N. Oak Park Ave. Chicago, IL 60648	

Analytical Equipment

(2) Beckman 864 Infrared Analyzers or equivalent	Beckman Instruments Fullerton, CA 92634	One analyzer each for CO and CO_2 (Peristaltic Sampling Pump required, strip chart recorder recommended)
Beckman D-2 Automatic Oxygen Analyser		
ORION 701A Digital Ion analyzer w/ 940600 HCN electrode and 900100 reference electrode	Orion Research 380 Putnam Avenue	

The above list is not a complete list of sources nor does it imply endorsement of the companies listed or their products.



Radiant Heater

Figure 1

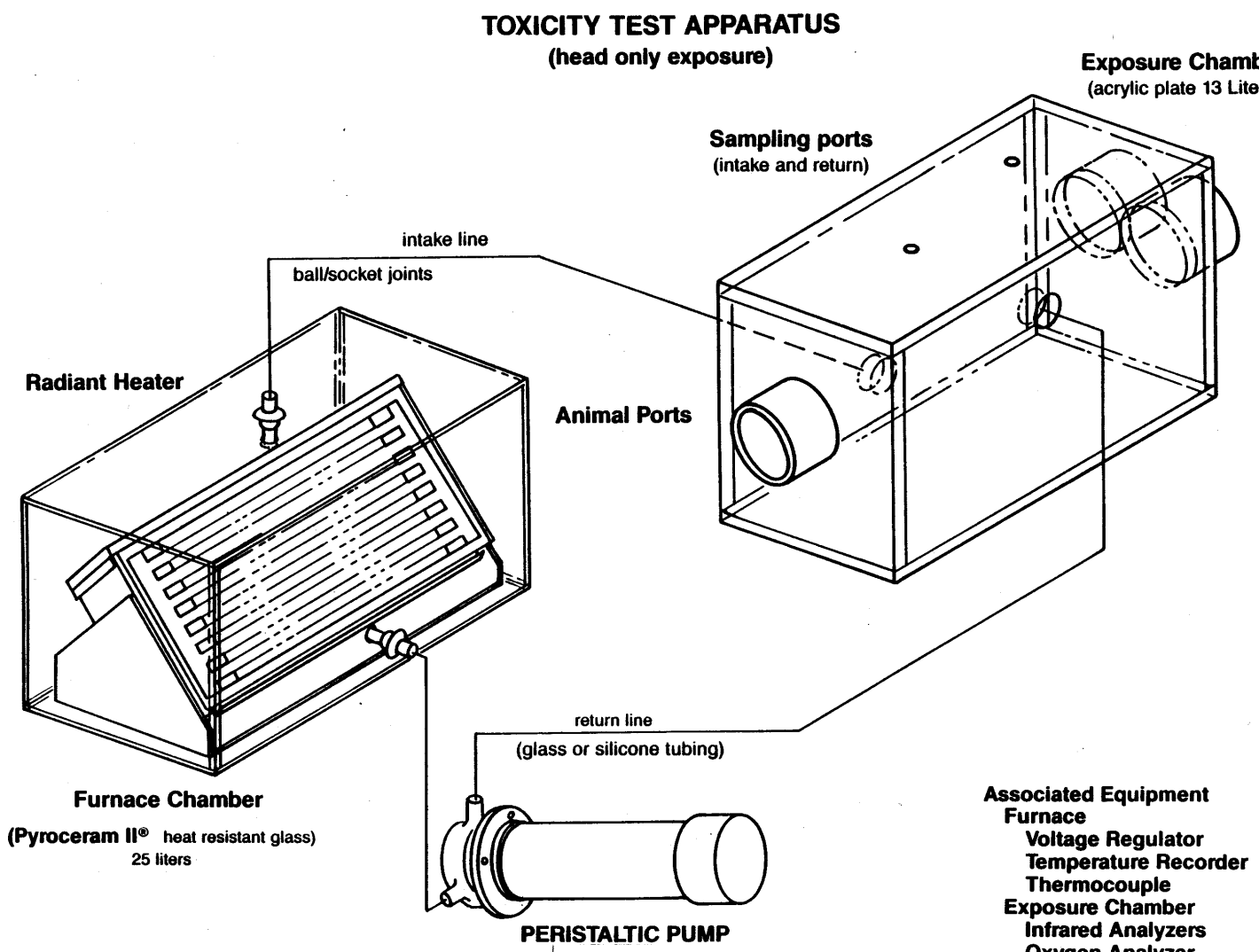
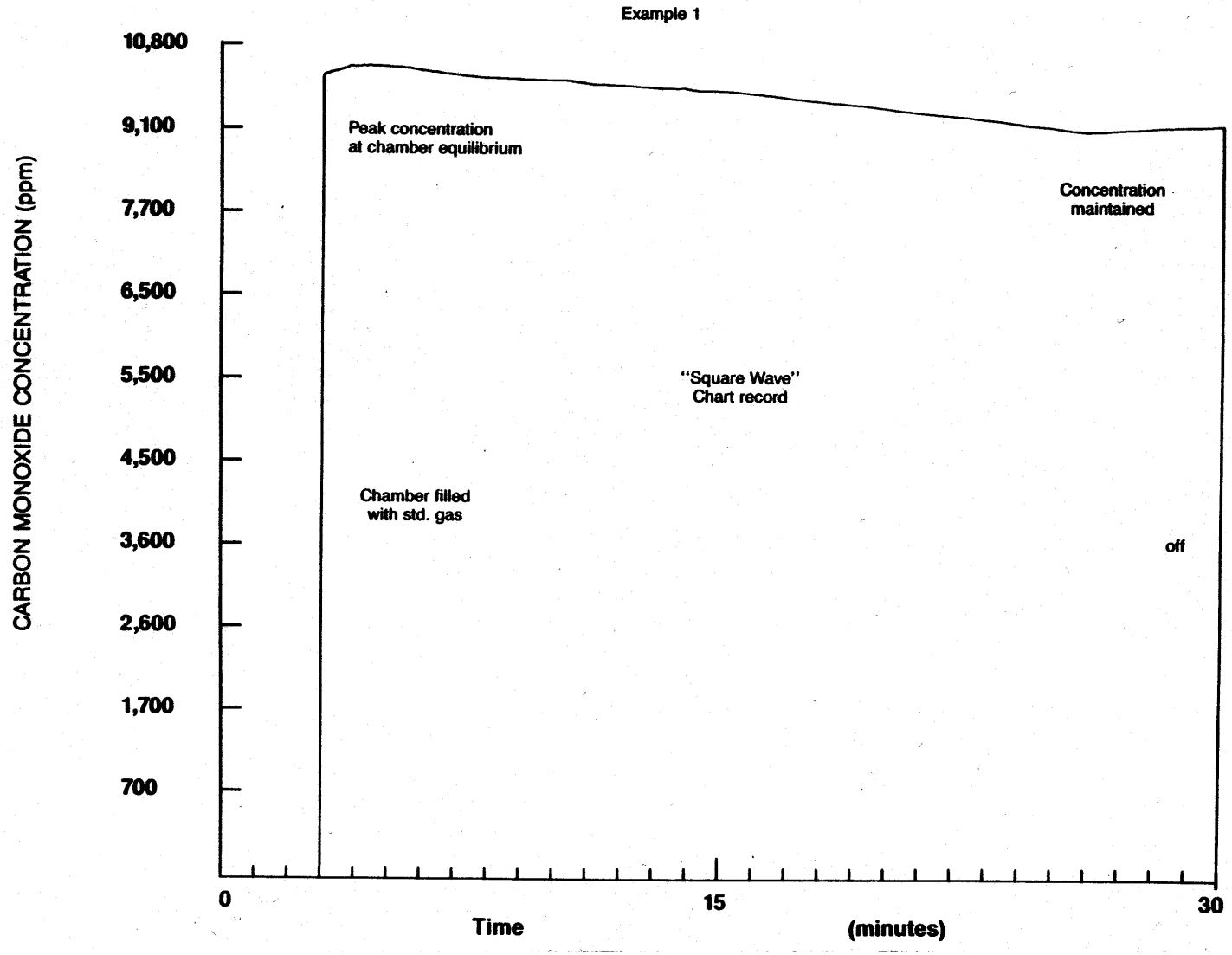

TOXICITY TEST APPARATUS
(head only exposure)
Exposure Chamber
(acrylic plate 13 Liters)
Sampling ports
(intake and return)
intake line
ball/socket joints
Radiant Heater
Animal Ports
return line
(glass or silicone tubing)
Furnace Chamber
(Pyroceram II® heat resistant glass)
25 liters
PERISTALTIC PUMP
Associated Equipment
Furnace
Voltage Regulator
Temperature Recorder
Thermocouple
Exposure Chamber
Infrared Analyzers
Oxygen Analyzer
Specific-Ion Analyzer

Figure 2

EXPOSURE CHAMBER GAS CONCENTRATION

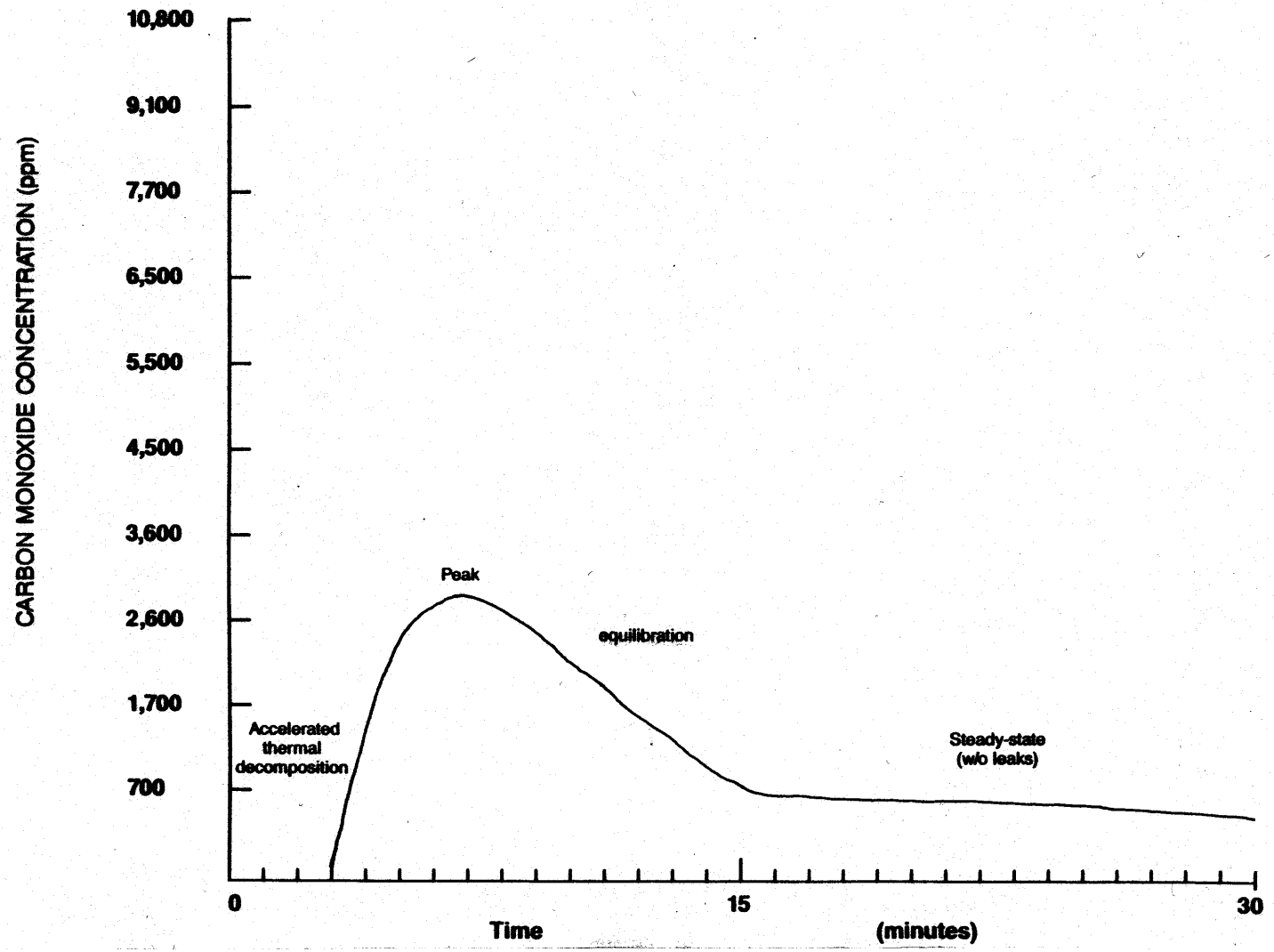
(non-integrated data)



EXPOSURE CHAMBER GAS CONCENTRATION

(non-integrated data)

Example 2



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