Public Health Monograph No. 57

Exposure Chambers For Research In Animal Inhalation

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U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

Arthur S. Flemming, *Secretary*

PUBLIC HEALTH SERVICE

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Exposure Chambers For Research In Animal Inhalation

Design, Construction, Operation, and Performance

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Public Health Monograph No. 57

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Foreword

The industrial physician and hygienist for many years have been aware of the importance of inhalation in industrial exposures to toxic substances. This comparatively restricted interest in inhalation toxicology has now been broadened and intensified by two developments. First, the phenomenal growth in chemicals used in industry, in the defense establishments, and in the home has accelerated toxicological research in general. Second, the recognition of the potential health effects of community air pollution has intensified the need for more inhalation studies. As a result, many new workers are entering this field.

To help these investigators avoid some common difficulties and sources of error observed in its 40-odd years of research, the Occupational Health Program has prepared this monograph on the design, construction, operation, and performance of exposure chambers for animal inhalation research. Much of the material presented here is based on original research and designs developed by the program. This research was concentrated on such factors as the relation of the size of the chamber to the type and number of animals to be exposed, dissipation of heat of the animals, and geometry of adsorbing surfaces, as well as problems of maintenance.

Scientific progress is ever dependent on new instruments and techniques. It is hoped that these tools, developed for a research activity which at first glance may seem deceptively simple, will aid in further discoveries in an increasingly complex and important area of toxicology.

Harold J. Magnuson, M.D.
Chief, Occupational Health Program

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I. Introduction

The design, construction, operation, and performance of inhalation chambers for animal exposure in toxicological research have not received the consideration commensurate with their importance in the development of inhalation toxicology. With the growing demand for inhalation toxicity data, it becomes increasingly important that information on exposure chambers be developed and made more readily available. The philosophy underlying animal inhalation exposures deserves more emphasis than it has received in the past, that it may serve as a guide for the new investigator entering the field.

This monograph describes the basic types of chambers that have been successfully employed by the Occupational Health Program, Public Health Service, to investigate problems in industrial toxicology, together with the philosophy that guided their construction and development. Considerable data are presented to demonstrate the operating performance of the chambers. It is hoped that this information will point out the characteristics of a group of serviceable chambers, stimulate interchange of ideas on design and operating techniques, and promote research and development in the field.

Included also are references and brief descriptions of exposure chambers that have been used in the past and of those being currently used by some of the most active workers in the field. In making these selections, emphasis has been placed on new or unusual features of design and construction.

Design and Construction

The studies in inhalation toxicology conducted by the Occupational Health Field Headquarters laboratory require the exposure to aerosols of large numbers and a variety of laboratory animals under closely controlled conditions. The multiplicity of conditions to be met in setting up an animal exposure program including facilities can be appreciated from the following requirements for controlled animal exposure.

The first requirement is a dynamic air system with the following properties:

1. A source of clean air supply.
2. A constant flow of air.
3. Controls to vary this flow over a suitable range.
4. Instruments for accurately metering this flow.
5. Apparatus for injecting an aerosol or gaseous contaminant into the input airstream.
6. Uniform distribution of the input airstream throughout the exposure zone.
7. A cooling system for the air supply to maintain a constant exposure chamber temperature, despite the animal heat load and variations in ambient temperature.
8. Signal devices to indicate failure of operating equipment.

Second, it is necessary to provide for the elimination of toxic products from the exposure chambers to insure a safe working environment for laboratory personnel, and to prevent the discharge of pollutants to the outside atmosphere. This necessitates:

1. Provision for exhausting air from the chambers.
2. Cleaning the exhausted air before discharge to the atmosphere.
3. Maintenance of a slight negative pressure to prevent outward leakage from the exposure units.
4. A means of cleaning the exposure chambers after each day's use.

Third, accurate determination of the concentrations of contaminants is required. This necessitates:

1. Facilities for taking representative samples of the exposure chamber atmosphere.
2. Appropriate sampling ports from which any part of the exposure chamber may be probed.

From the chamber operator's point of view, the chamber should have the following additional attributes:

1. Ease of access to exposure chamber interior.
2. Suitable observation windows.
3. Automatic control and recording of the exposure chamber variables, such as airflow, temperature, humidity, and contaminant feed.

All of the considerations enumerated apply to both total-enclosure and head-enclosure type chambers. Head-enclosure chambers should be used when skin absorption of the test contaminant would be a significant route of entry, for example, in the investigation of the inhalation toxicity of cyanide or certain mercury compounds. In other instances, operational advantages make the total-enclosure type preferable. Such chambers are easier to keep clean since they can be hosed down, and are easier to load and unload.

Ingestion of particulate matter, derived from deposition on fur during total-enclosure inhalation exposures, generally makes a relatively minor contribution to the overall toxicity. For example, inhalation studies at the University of Rochester Atomic Energy Project, as reported by Voegtlin and Hodge (*1*), showed ingestion-to-inhalation ratios for eight compounds on dogs, rats, and rabbits. For dogs exposed to uranium at 20 mg./m.^{3}, the ratio varied from 65 for UO_2F_2 to 10,000 for UF_4. Similar results were found for the rat and rabbit. When the maximal amount is used of uranium analytically determined in the gastrointestinal tract (with contents), the maximal contribution to inhalation toxicity by orally ingested dust for UF_4 would be 0.1 percent and for UO_2F_2, 15 percent.

Two main objectives to be kept in mind in the design and construction of animal inhalation chambers are performance and ease of operation. The chamber must fulfill the purpose for which it is intended, but equally important is ease of operation and maintenance. Thus, in considering alternate design features for a chamber, one can first eliminate any which would result in inferior performance. Of those features that meet the performance specifications, the choice should then be made on the basis of operating convenience.

Readymade animal inhalation exposure chambers are not commercially available and, if built to order and delivered, are usually prohibitive in cost. If funds for chamber construction are limited, best results will be obtained by building the chamber at the site, with the aid of local contractors. The job may be given to one contractor who assumes responsibility for the entire job or let out in separate contracts for sheet metal, air-conditioning, plumbing, and electrical work, depending upon local conditions.

Chambers in Occupational Health Program

The exposure chambers of this laboratory are divided into four groups determined by the type of exposure they provide. These include, (*a*) large-scale exposure chambers, (*b*) control exposure chambers, (*c*) small-scale exposure units, and (*d*) pilot exposure units. The features of each type that have proved of value in ease of operation and maintenance, in reliability of performance, and in adaptability will be subsequently discussed in detail. This laboratory has not had experience with head-exposure chambers in recent years, and such chambers are not in the laboratory at this time. Consequently, the discussions of chamber design and construction which follow will be limited to total-enclosure chambers. The four groups of exposure chambers may be briefly defined as follows.

Large-Scale Exposure Chambers

Large-scale exposure chambers are usually of permanent, heavy-duty construction, ranging in size from about 90 to 250 cubic feet. In this laboratory these chambers have permanently mounted systems for air conditioning, plumbing, air supply, and exhaust, and are expected to be used indefinitely for numerous and different studies, whose nature is unknown at present. Thus, they are airtight, rigid, and corrosion resistant; and designed to provide constant performance and uniform exposure under changing operating requirements, and to need minimal maintenance.

Control Exposure Chambers

Control exposure chambers are used in conjunction with the large-scale exposure chambers for controls and for preexposure of the

animals used in the inhalation experiments. They must meet most of the exposure chamber requirements. While they need not be capable of a uniform particulate distribution, they must provide a uniform distribution of clean inlet air.

Small-Scale Exposure Units

Small-scale exposure units are used for periods of time and contaminant levels intermediate to the short, acute exposures in the pilot units, and the long-term, chronic exposures provided by the large-scale exposure chambers. Typically, such a chamber is used for chronic exposures of 3 to 6 months, and has a volume of approximately 25 cubic feet. Two dogs, four rabbits, and twenty rats could readily be exposed in such a chamber.

Pilot Exposure Units

Pilot exposure units are of laboratory bench size, and of less than permanent type construction. They are usually made from large glass jars and flasks, adapted to permit the exposure of small numbers of small laboratory animals to accurately determined uniform concentrations of contaminants for short periods of time at acute levels.

II. Design Considerations

Large-Scale Exposure Chambers

The major problem in the design of large-scale, all-purpose exposure chambers is the difficulty of generating and maintaining a sufficiently constant and uniform dust distribution in the air. This imposes restrictions on the size and shape of such chambers that can be built at reasonable cost.

Chamber Shape and Air Movement

The experience of this laboratory has shown that good dust distribution can most easily be maintained in a chamber where the dust-laden airstream is fed in at the top, and withdrawn at or near the bottom. The inlet and exhaust must be designed so that the concentration of the air contaminant across a horizontal cross section of the chamber is uniform.

If the horizontal cross section of the chamber is a regular polygon or is circular, it is easier to obtain good distribution than if the cross section is rectangular or otherwise irregular. In addition, a chamber with an irregular cross section would have a greater surface-to-volume ratio than the corresponding regular shape which would increase surface effects. Of the regular shapes, a square is by far the most practicable. Construction is not difficult, and more important from the operating standpoint, the animal cages can be loaded and unloaded efficiently, especially if a large access door is provided.

To establish a particulate aerosol of uniform concentration in a downflow chamber having a square horizontal cross section, an effective means of distributing the aerosol must be devised. For some gases, vapors, and very fine particulates, a simple cubic chamber may be satisfactory. This laboratory obtained several cubic chambers in 1950 and used one, unmodified, for a study of cobalt metal fume (fig. 1). The air supply was at the top center, and a baffle plate just below the inlet aided uniform dispersion. Satisfactory aerosol uniformity was obtained with this arrangement.

It was realized, however, that if proper distribution was to be maintained with the generation of aerosols of larger particulates as represented by most mineral dusts, an improved chamber design would be required. The existing cubic chambers were adapted by adding a pyramidal top (fig. 2). This modification provided the conditions that led to improved distribution for aerosols of heavier particles.

The pyramidal tops added to these chambers are shaped like canopy hoods and provide the dispersion space needed for uniform flow

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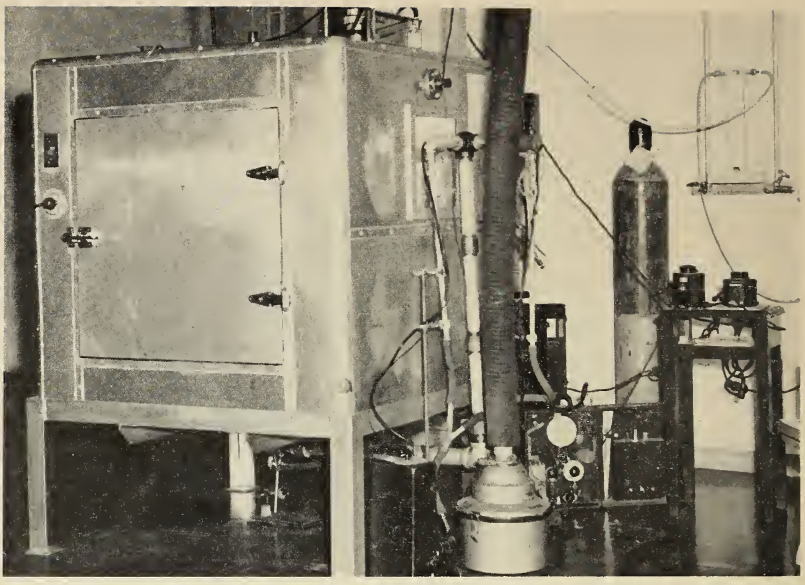


Figure 1. Unmodified 4-foot cubic chamber used in 1950 for preliminary studies of cobalt metal fume.

NOTE: Blower at top supplied air to chamber through the slender cone into which the fume was injected upstream from the metering orifice. Vacuum cleaner (lower right) exhausted chamber through electrostatic precipitators and discharged into the laboratory exhaust duct. Variacs (small table) were used to control both exhaust and supply blowers.

throughout the body of the chamber where the animals are exposed. Similar pyramidal tops were specified in the three newest large-scale exposure chambers built by the Occupational Health Program. The experience derived from the adaptation and operation of the chambers obtained in 1950 was incorporated in the design of the newest chambers (fig. 3).

In the older chambers, the air supply enters tangentially into cylinders which are extensions of the tops of the canopies. The contaminant aerosols are fed directly into these cylinders at a constant rate, appropriate to the given aerosol, and mixing is enhanced by the turbulence and aspiration imparted by the rotary motion of the supply airstream. Further mixing takes place in the canopy tops, insuring uniform air concentrations in the exposure zones. While good concentration uniformity is obtained with the mixing cylinders, the inlet air has a tendency, especially at higher airflows, to flow down the walls of the chamber rather than uniformly throughout it. This type of flow heightens temperature difference within the chamber. In the newest chambers venturi-type mixing sections were selected in place of the mixing cylinders to obtain both good mixing and more uniform airflow. For operating convenience, platforms have been built adjacent to each chamber canopy which allow the chamber operator to set up and adjust the aerosol generators.

The exhaust of each chamber is drawn through a distributing plenum below the exposure zone. These plenums consist of duct sections with holes along the bottom surfaces. Exhaust air enters through these holes, but liquid waste cannot accumulate.

In the large-scale inhalation studies of this laboratory one exposure chamber of 90 cubic

feet, four of 145 cubic feet, two of 230 cubic feet, and one large control exposure chamber of 600 cubic feet are used. These chambers all are connected to a common supply air system and a common exhaust air system of 1,300 cfm capacity each. It is desirable to install a spare supply and a spare exhaust blower to go into service automatically if the regular blowers fail. Figure 4 shows parts of the duct system. Oversized, 12-inch by 12-inch ductwork of 20-gauge galvanized sheet was used to provide a low-pressure-loss system.

The air supply and exhaust systems for a permanent multichamber require careful design, incorporating flexibility as well as ease of operation and maintenance. If a number of chambers are to be used in an inhalation program, it is desirable, from the standpoint of cost, to utilize a common air supply and a common air exhaust for all the chambers. When this is done, extra precautions must be taken to eliminate the possibility of mechanical failure, since all operating units would be affected. The initial design should accommodate foreseeable modifications such as higher air requirements, additional chambers, and additional air cleaning facilities.

The rate of airflow maintained through a given exposure chamber is dependent on a number of factors, several of which are interrelated. The airflow must be sufficient to maintain the oxygen supply and remove excess carbon dioxide. It must also maintain a nearly uniform, constant thermal environment by removing the heat generated by the animals in the chamber. The rate of airflow and the temperature of the entering air must be balanced with the rate of heat generation to maintain temperatures within an acceptable range. Mice, rats, and guinea pigs are susceptible to pneumonia when exposed to drafts of air at temperatures lower than 75° F. Exposure zone temperatures, when such animals are exposed, should be kept within the range of 76°–82° F. throughout. The amount of air required for heat removal is usually far in excess of the volume needed for O_2–CO_2 balance, and constitutes the practical lower design limit for airflow. The upper limit is usually imposed by generation or feed rate of the contaminant aerosol. If the upper and lower limits should overlap for a given animal loading it may be possible to reduce the loading, and with it the lower limit, in order to find a satisfactory operating airflow.

The airflow to each exposure chamber in this laboratory is metered by a venturi-type flowmeter in the supply duct (fig. 4). The pressure taps of each venturi-type meter are connected to a Magnehelic pressure gauge (*A*) on the panel board. The airflow calibration for each flowmeter has been drawn on the face of the corresponding gauge, so that it reads directly in cubic feet per minute. The flow through these meters can be varied from about 2.5 to 100 cfm. The rate of airflow and the static pressure in the chamber are controlled

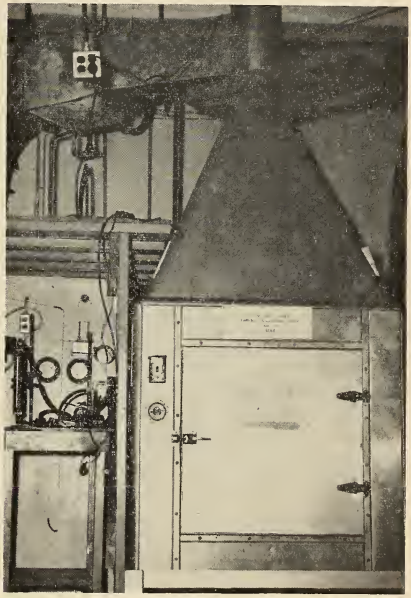


Figure 2. The same chamber shown in figure 1 modified by the addition of a pyramidal top and mixing cylinder.

NOTE: The enlarged section of air duct supplying air to mixing cylinder contains air-conditioning cooling coils. Controls for chamber air supply, exhaust, and air conditioning are on panel at rear of chamber to left. The wooden platform allows operator to service dust feed mechanism which is supported by small platform around top of pyramidal transition section.

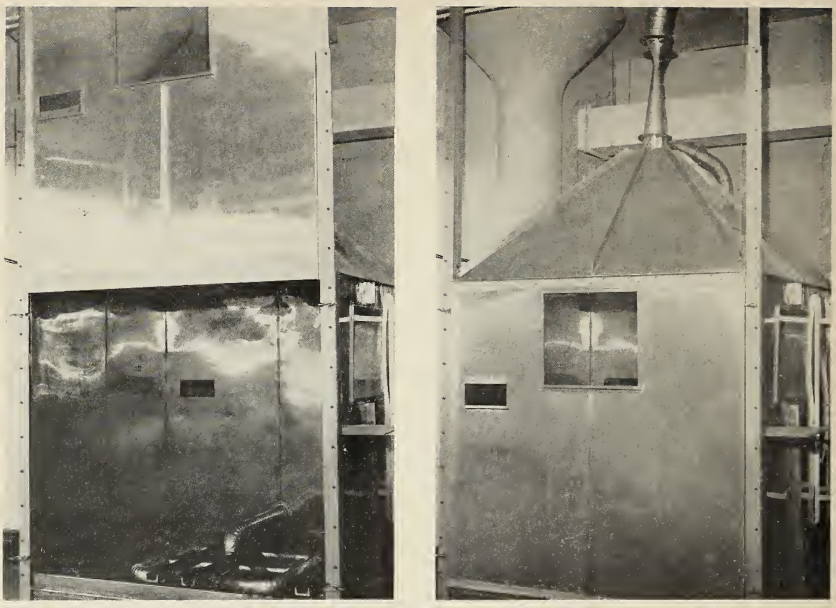


Figure 3. Newest chambers constructed by Occupational Health Program designed for 24-hour exposure of animals in long-term studies.

NOTE: The entire front wall of the chamber serves as the door and permits easy access. Standard and easily replaceable galvanized downspouting has been used for the exhaust plenum. Cage racks containing the animals are rolled into chamber on tracks shown and can therefore be exchanged quickly for clean cage racks.

by means of sliding gates mounted on orifice plates which are located within the supply and exhaust ducts. These valves are manually controlled at the control panel of each chamber through mechanical linkages. Each valve has two leaves which move toward or away from the center of the duct simultaneously, causing a linear change in airflow rate with degree of opening. A negative static pressure of 0.1 inch of water is maintained in the operating chambers.

An alternative valve design is being tested in the air supply and exhaust lines. This consists of a metal cone approximately 3.5 inches in height with a maximum diameter of 2.5 inches which is lowered by a gear arrangement into a 2-inch diameter orifice in the duct. Such a valve should not be clogged readily by animal hair or become stuck by the deposition of animal protein on the screw threads or other sliding surfaces.

Certain chambers have been equipped with commercially available (*B*) flexible pinch-type exhaust valves as shown in figure 11. Although these valves are relatively expensive, the smooth flow lines, even when the valves are almost closed, make clogging unlikely and provide positive control of the airflow over the entire range of operation.

The air supply must be clean, fresh, and at the proper temperature. A precleaner may be necessary for the air supply and the air exhausted from the chamber should be cleaned prior to discharge to the atmosphere. Three of the chambers in this laboratory have 100 cfm graded saddle-packed tower air washers

in their exhaust lines. The exhaust air from all the chambers passes through a PV–22 Westinghouse Precipitron before discharge to the atmosphere.

In exhausting the air from a chamber, the uniformity of airflow established at the inlet must not be upset. A pressure distributing exhaust plenum near the floor, such as those in the chambers of this laboratory, provides the desired uniformity. The lower surfaces of such plenums should be at least 6 inches above the floor and have openings uniformly around them. Openings that are on the lower surfaces will not become filled with washings when the chambers are hosed down.

Cleaning Provisions

A major consideration, from the chamber operator's point of view, is the ease with which the chambers can be kept clean. This laboratory has found that a high-pressure water hose does a rapid and thorough job when used after each animal occupancy. Chambers that have pitched floors, center drains, and smooth interior walls are easily hosed down. The drainage from each chamber should be run into a sewerline unless it is sufficiently toxic to warrant collection in a sealed container.

Construction Materials

The materials of construction specified for an exposure chamber are determined by the nature of the contaminants introduced, the permanence of the chamber, and to a considerable degree by the preference of the chamber operator. From the standpoint of chamber performance, the important factors are the quality and the shape of the interior surfaces. The geometry of the interior is determined by the general design of the chamber, as previously discussed, while the nature of the surfaces is determined by the exposure conditions to be encountered. The interior surfaces should be smooth and nonabsorbent, or lined with a nonabsorbent material, to minimize surface effects. They must withstand the corrosive action of the materials that contact them, and must not interfere with concentration uniformity by absorbing, or reacting with, the test substance. The surfaces must also resist the corrosive effects of animal excreta.

A permanent chamber must be constructed so that it will be sturdy enough to resist battering by animal cages during loading and unloading. If highly toxic materials are used which contaminate the chamber beyond easy cleaning it may be desirable to construct it of less permanent materials for disposal upon completion of the study.

The permanent chambers most recently completed by this laboratory are constructed with walls of 14-gauge type 3S aluminum sheet and an exterior angle-iron frame. Some laboratories use heavier, self-supporting walls, and dispense with the exterior frame. Still others use wooden or concrete walls and line

Figure 1. View of air supply and exhaust ductwork at rear of chamber.

NOTE: Venturi metering air supply is in foreground.

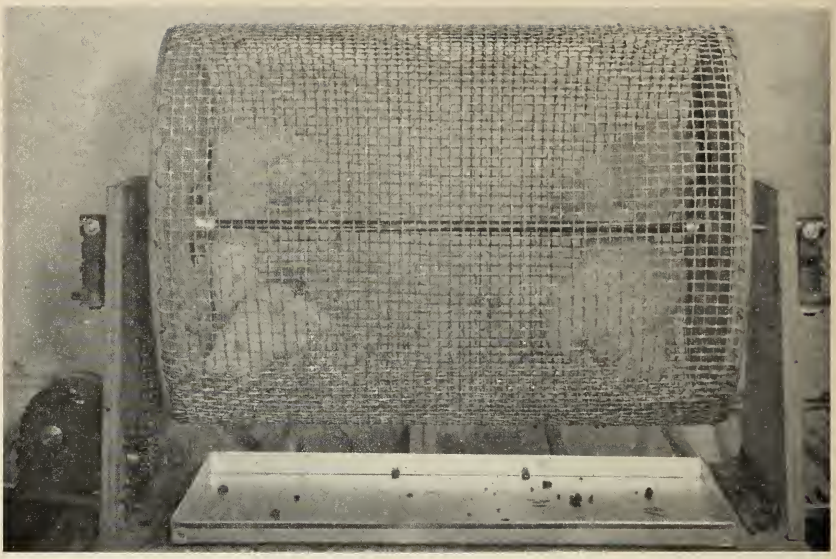


Figure 5. Motor-driven activity cage for rats and small animals accommodating 10 to 15 adult rats.

them with smooth, nonabsorbent surfaces. The performance of the resulting chamber does not depend on the type of construction, and the choice should be made on the basis of cost and personal preference. The interior surfaces could be of aluminum, stainless steel, safety glass, or a plastic, depending on the operating conditions and again on personal preference.

Space and Number of Chambers

It has been found through experience that it is much more difficult to maintain uniform dust levels in chambers with main bodies smaller than 4 feet by 4 feet by 4 feet, or larger than 6 feet by 6 feet by 6 feet, than within these limits. If a chamber volume greater than 6 feet by 6 feet by 6 feet is required, the animals should be exposed in more than one chamber.

If a group of chambers is to be constructed, it may be desirable to build them in a row, or a cluster, with common walls, rather than as completely separate units. The University of Rochester built a cluster of hexagonally shaped chambers for a study on uranium compounds (*1*). In such a manner, chamber space can be constructed economically, while retaining the advantage of optimal-sized units.

To determine the number of chambers needed for a particular study, the animal volume to be exposed must be known. This laboratory has found that best results, with regard to uniformity of exposure to sedentary animals in cages, are obtained when animal volume is limited to 3–5 percent of chamber volume. The number of chambers needed can be derived from the volume of the animals to be exposed and the volume of the chambers.

This laboratory has found that a 5-percent loading of animals fills the entire main body of the chamber with cages. To demonstrate, the 5-foot chamber, having an internal volume of 145 cubic feet (4.10 cubic meters), will hold 12 dogs and 16 rabbits in individual cages, and 20 guinea pigs, 20 rats, and 20 hamsters in individually compartmentalized cages. The

volume of this group of animals can be calculated as shown below:

Animal	Number animals	Average mass (kg.)	Average volume (l.)	Number × volume (l.)
Dog	12	11	11.6	139.2
Rabbit	16	3	3.16	50.6
Guinea pig	20	.5	.527	10.5
Rat	20	.25	.264	5.3
Hamster	20	.075	.079	1.6
				[1] 207.2

[1] 0.207 cubic meter.

NOTE. Animal volume as percentage of chamber volume for maximally loaded chamber:

$$\frac{0.207\ \text{m}^3}{4.10\ \text{m}^3} \times 100 = 5.09\ \text{percent}.$$

This loading corresponds to the 5-percent loading limit found, through experience, to be an effective limit for good air distribution, and also to the 5-percent loading limit proposed by Silver as the limit above which animal surface effects cause excessive lowering of the concentration of the test substance (*2*). If chambers of a given size are to be used, the calculated chamber volume divided by the chamber size will give the number of chambers needed.

Animal Activity Cages

More chamber volume per animal is required if any of the animals are to be exercised during exposure. Experimental evidence supplied by Stokinger suggests that toxicity is often markedly greater for animals being exercised as compared to toxicity for sedentary animals in the same atmosphere (*3*). This laboratory has used rat exercisers (fig. 5) in its large-scale exposure chambers. They are revolving cylindrical wire cages 18 inches in diameter and 24 inches long, each powered by a $\frac{1}{46}$-horsepower motor. They rotate at a speed of 6.5 rpm, and accommodate 10 to 15 adult rats.

A treadmill for four dogs or equivalent load has recently been constructed for use in a 600-cubic foot walk-in chamber (fig. 6). The tread is rubber that is waterproofed and has

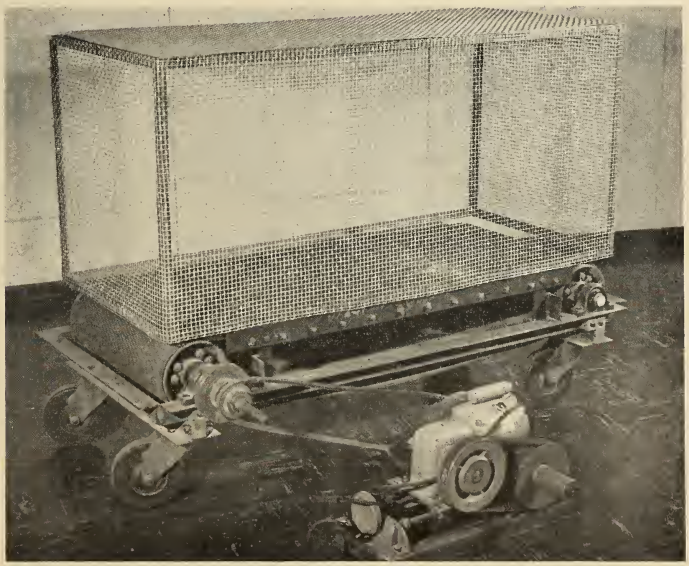


Figure 6. Treadmill exerciser for dogs accommodating four medium-sized dogs.

vulcanized edges. It has an effective area of 22 inches by 4 feet 8 inches, and rolls on 1.9-inch ball bearing rollers on 4-inch centers. It is powered by a ¼-horsepower motor with a variable drive. The speed range is from 0.9 to 7.5 miles per hour. A hydraulic jack is used to raise one end when an incline is desired.

Air Conditioning

The room in which the animal chambers of this laboratory are located is air conditioned to about 78° F. and a relative humidity of 60 percent. Approximately the same temperature is maintained in the exposure zone of the chambers, thereby reducing heat transfer through the walls of the chambers and eliminating radiant heat exchange between the chamber walls and occupants; thus the animals near the center of the chamber are in the same thermal environment as those near the walls.

The air-conditioning capacity of the room is adequate to meet the local temperature conditions, and each individual chamber has cooling coils in its air supply duct. The capacity of these individual chamber units is sufficient to cool air at Cincinnati under conditions of summertime operation to the point where it will maintain, in a chamber with a maximum animal complement, a constant exposure zone temperature of 79° ± 3°F. and an exposure zone relative humidity of less than 55 percent.

To calculate the capacity of a unit for a chamber of 145 cubic feet (4.10 cubic meters), the loading previously cited will be used. First the heat load generated by the animals must be estimated. Basal metabolic rates for laboratory animals can be found in the literature (*4*, *5*) from which the values in the table below have been taken; the table shows the calculation of the basal animal heat load.

Animal	*Number of animals*	*Average mass (kg.)*	*Average basal metabolism (kg.-cal./day)*	*Number × kg.-cal./day*
Dog	12	11	435	5,220
Rabbit	16	3	141	2,256
Guinea pig	20	.5	35	700
Rat	20	.25	31	620
Hamster	20	.075	10	200
				[1] 8,996

[1] Kg.-cal./day or basal animal heat load.

But the heat load of animals in cages is not basal. The specific dynamic action of ingested food and the effect of muscular activity cause the heat generation rate to be greater than basal. If one assumes that the average heat-producing rate is 1.25 times the basal rate, then 11,250 kg.-cal./day or 468 kg.-cal./hr. are generated. Since the animals are not fed before exposure, and since the cages limit their muscular activity, the assumption of an average metabolic rate 25 percent greater than basal gives a conservative design figure, as indicated by observations made in this laboratory.

To have both temperature and humidity control, the moisture generation rate of the animals must be known in addition to the rate of total heat generation. The rates used in the calculations to follow are values taken from experimental data in this laboratory. For the animal complement of the example, the rate of moisture generation would be approximately 4,200 grains per hour. The rate of moisture generation for any animal complement may be determined experimentally by measuring the relative humidity of the incoming and exhaust air, calculating the amount of water in grains per hour that these figures represent, and obtaining the difference between the two calculations.

If the air in the chamber is to have no temperature higher than 82° F. and no relative humidity higher than 55 percent, and at the same time carry away the animals' heat and moisture loads, the air supply in warm weather must be both cooled and dehumidified. To estimate the air-conditioning capacity required for year-round use of a chamber, the temperature and moisture content of the air entering the chamber must be specified. The air-conditioning equipment selected must be capable of supplying the desired volume of air under these conditions. The heat and moisture content of the air entering the chamber, plus the heat and moisture generated by the animals, will equal the heat and moisture content of the air at the exhaust (maximum dry bulb temperature 82° F., relative humidity 55 percent).

For the example under consideration, we have the following information:

Animal heat load	=468 kg.-cal./hr.	
Animal moisture load	=4,200 gr./hr.	
Design dry bulb temperature	=95° F.	ASHAE design cooling conditions for Cincinnati, Ohio (*6*).
Design wet bulb temperature	=78° F.	
Exhaust dry bulb temperature	=82° F.	Exhaust duct conditions: wet bulb, 68.3° F.; absolute humidity, 82 gr./lb.
Exhaust relative humidity	=50%	
Peak airflow	=50 cfm	

The maximum mass of air to be treated will be:

Mass of air=M=50 cfm=(50 ft.^{3}/min.) (60 min./hr.) (0.074 lb./ft.^{3})

M=222 lb./hr.

The 222 lb./hr. of air must pick up 468 kg.-cal./hr. of heat content, and 4,200 gr./hr. of moisture content, or:

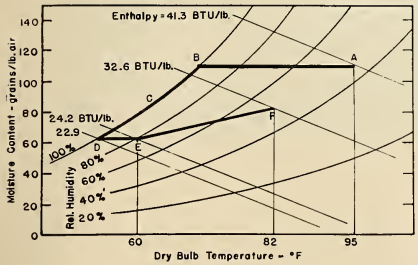
$$\frac{468 \text{ kg.-cal./hr.}}{222 \text{ lb./hr.}} = 2.11 \text{ kg.-cal./lb. or } 8.37 \text{ BTU/lb.}$$

and

$$\frac{4{,}200 \text{ gr./hr.}}{222 \text{ lb./hr.}} = 18.9 \text{ gr./lb.}$$

The air-conditioning process that takes place in the individual chamber units in this laboratory can best be explained by referring to a standard psychrometric chart, such as figure 7, with the temperature and humidity conditions plotted on it. In figure 7, point *A* is air at the summer design conditions (95° F. dry bulb, 47 percent relative humidity). Point *F* is air as it leaves the chamber (82° F., 50 percent relative humidity), and point *E* is air as it enters the chamber. The task of the air-conditioning equipment is to take air at point *A*, and condition it to point *E*.

Figure 7. Psychrometric chart showing the air-conditioning process for the design conditions: ambient temperature 95° F., relative humidity 45 percent; exhaust air temperature 82° F., relative humidity 50 percent.



The enthalpy (heat content) at point *E* must be 32.6 BTU/lb. (enthalpy at point *F*), minus the animals' heat of 8.4 BTU/lb., or 24.2 BTU/lb. The moisture content must be 82.0 gr./lb. minus the animal moisture load 18.9 gr./lb., or 63.1 gr./lb. Therefore point *E* will be 59.9° F. dry bulb, and 56.7° F. wet bulb.

Although the air supply enters the chamber at an average dry-bulb temperature of 59.9° F., the air that reaches the animals is moderated to at least 76° F. The cool air from the coils mixes in the canopy top with the warmer chamber air. Sufficient mixing takes place to insure that the animals are not exposed to cold drafts.

The air-conditioning units dehumidify air only by condensation on the cooling coils. Therefore, to get from point *A* to point *E*, the path *ABDE* must be followed. The air cools from point *A* at constant moisture content until the dewpoint, point *B*, is reached. As it cools further along the saturation curve, condensation takes place. It must be cooled until the moisture level of the air is reduced to that of point *E*, or until point *D*. The air is then reheated from point *D* to point *E*. Reheating can be performed with electrical resistance heating elements in the duct between the coils and the chamber.

The design capacity of the air conditioning must be based on cooling the air from point A to point D. The enthalpy difference between point A and point D is 41.3 minus 22.9 or 18.4 BTU/lb. Since the airflow is 50 cfm or 222 lb./hr.

$$\text{cooling requirement} = (222 \text{ lb./hr.})\ (18.4 \text{ BTU/lb.}) = 4{,}080 \text{ BTU/hr.}$$
$$= \frac{4{,}080 \text{ BTU/hr.}}{12{,}000 \text{ BTU/ton}}$$
$$= 0.34 \text{ ton of refrigeration.}$$

A ½-ton refrigeration unit will be satisfactory for a 145-cubic foot chamber with a maximum animal loading at the design conditions specified. It would be inadequate only if the air supply rate were raised over 50 cfm to 70 cfm or higher. Airflows higher than 50 cfm have never been required in the inhalation studies of this laboratory although allowance has been made for such conditions by providing two chambers with ¾-ton units. Two have ½-ton units. All have water-cooled compressors and finned evaporative condenser coils as the heat exchangers. The reheat requirement can easily be calculated. The air must be heated from point D (enthalpy=22.9) to point E (enthalpy −24.2) or 1.3 BTU/lb.

$$\text{reheat} = (222 \text{ lb./hr.})\ (1.3 \text{ BTU/lb.}) = 288 \text{ BTU/hr.}$$
$$= 84 \text{ watts}$$

Figure 8. Animal cages and racks used in newer exposure and control chambers.

Air-Conditioning Control System. The control system utilizes a thermostat-actuated evaporator pressure switch which governs a range of pressures (and corresponding temperatures) within the evaporator coils. Thus, a conventional air-conditioning unit is set up to provide heat removal at varying rates, according to demand by the thermostat. The thermostat is located for practical reasons in the exhaust airstream, although the most appropriate location would be in the midst of the group of animals. If there is no demand for

cooling, the compressor pumps down to a pressure which automatically shuts it off. However, this allows the system to be maintained in readiness, at full capacity, at all times. A manual switch actuates only the control system, and when the system is turned on the full cooling capacity is immediately available, if necessary.

Cage Arrangement and Loading

The loading of the chambers at any particular time may be dependent on the contaminant being studied, on the length of the exposure, on whether the animals are sedentary in cages or being run on exercisers, and on the number of chambers available.

There are several general rules for animal loading which are observed in all studies. First, where practicable, all animals are exposed in individual cages, or for the smaller animals, in individual sections of compartmentalized cages (fig. 8). Some larger dog cages can be used which house two dogs; dogs do not tend to nuzzle one another and may be housed in pairs. With smaller animals, intimate contact might prove to be a complicating factor. Second, larger caged animals are placed at the bottom, with successively smaller caged animals above. Third, no pans or other solid surfaces are used on cages placed in the exposure chambers. Also, when small animals are stacked in several layers, space is always provided between the layers. Thus, interference with airflow and distribution is minimized, and uniformity of concentration can more easily be maintained. To compensate for any possible nonuniformity of concentration within the chamber, the positions of the cages within the chamber are changed periodically during the study.

A highly desirable feature of a large-scale animal chamber is an arrangement by which animal cages can be rolled in and out without having to be lifted. Such a feature requires that the chamber have a large full-length access door, and an interior floor no higher than a few inches above the floor of the room so that a ramp can be used. It is desirable to install a trapped drain in the floor. However, if a hole cannot be made through the floor it is necessary to raise the chamber floor enough to accommodate a physically disconnected drain.

For convenience, the door should be as large as is structurally feasible. The most recently constructed chamber in this laboratory has a counterbalanced, vertically rising door. To use this type of door, the room height must be at least twice the door height. In the event that sufficient height for such an arrangement is not available, a full-length door sliding horizontally on a trolley, or hinged at the top or side, would serve as well.

Airlock for Short Exposures

For exposures to known concentrations for short-time intervals such as a few minutes to an hour, a new type of airlock device was developed by this laboratory (fig. 9). The airlock fits into the door openings of five of the large-scale exposure chambers, permitting its use in connection with any current inhalation study. It consists of a sheet aluminum box 30 inches wide, 24 inches high, and 15 inches deep, with the wall opposite the chamber opening as a loading door. A rotating reinforced plate at the chamber opening is supported by a vertical rod at the center and serves two purposes. It is both the door to separate the airlock from the chamber, and also the support for the animal cages. The cages, which may be used for rabbits or smaller animals, are hung from hooks on the plate while the chamber is operating at the desired conditions. Two rows of three cages each can be accommodated. The outer door can then be closed and the plate rotated through 180°, placing the animal cages within the chamber, and resealing the airlock from the chamber. The airlock can then be vented.

The airlock was first used in a study on ozone inhalation; vinyl foam and expanded polyurethane plastic proved suitable as gasketing material. For less reactive substances, less expensive gasketing may be used. Simplicity of design and construction and a minimum of moving parts are major advantages of this device over the sliding-drawer type of airlock used by previous investigators (*7–11*). Furthermore, it has the additional feature of being readily removable and transferable to other chambers, as it is hung from the same hinges as the regular door.

Figure 9. Airlock door permitting short-term exposure of animals to established concentrations of contaminants in chamber.

Illumination, Windows, Sampling Ports

Adequate illumination in chambers may be provided by fluorescent lighting fixtures placed above windows in the canopy top. By putting the lights outside the chamber, the need for special sealed lights is avoided. For observation windows and sampling ports, Plexiglas is a convenient material. Holes are cut in the ports for the desired sampling probes. When these holes are no longer needed they are plugged with rubber stoppers. The Plexiglas is mounted so that it can be easily replaced. This practice facilitates the installation of a new sheet when a different set of holes is required.

Control Exposure Chambers

The preconditioning of all test animals and the maintenance of control animals in a control exposure chamber are fundamental requirements in animal inhalation studies. As a general rule, 25 to 50 cubic feet of control chamber space should be provided for each 100 cubic feet of exposure chamber space. When the volume of work reaches the point where it becomes desirable to devote a chamber full time for control purposes, it may be advantageous to build a large chamber especially for control. Many of the considerations applied to exposure chamber design are equally valid in designing a control chamber. These include:

1. A constant inflow of clean air, metered, maintained at proper temperature, and well distributed throughout the chamber volume.

2. An exhaust system to remove the air and maintain a slight negative pressure.

3. A means of cleaning the chambers to remove animal excreta.

4. Provision for easy loading and unloading.

However, certain limitations on design which apply to chambers for animal exposure do not apply to control chambers. Size and shape are examples. The only restriction on size and shape is that the airflow in the chamber be uniform.

Figure 10 shows a control chamber recently constructed for this laboratory. It has a height of 6 feet, 6 inches; a length of 8 feet, 4 inches; a width of 10 feet, 8 inches; and a canopy 37 inches high. An air diffuser was placed in the top of the canopy to distribute the inlet air. The chamber is easy to load; cage racks can be rolled in without having to be lifted. The entire front wall is a counterbalanced door which one man can conveniently raise and lower. Provision was made for housing the four-dog treadmill described previously. Plexiglas windows, 2 feet by 3 feet, in the front and side of the chamber were provided for observation, and sampling ports and shelves were installed in all four corners.

Small-Scale Exposure Units

For convenience of classification, exposure chambers that fall between the 1-cubic foot and smaller pilot exposure units used primarily for short-term acute toxicity studies, and the 90-cubic foot and larger large-scale exposure chambers used for long-term chronic inhalation studies, have been called small-scale exposure chambers. This laboratory has two such chambers, which have a capacity of about 25 cubic feet each, and are usually used for studies lasting from a few weeks to 6 months at subacute levels.

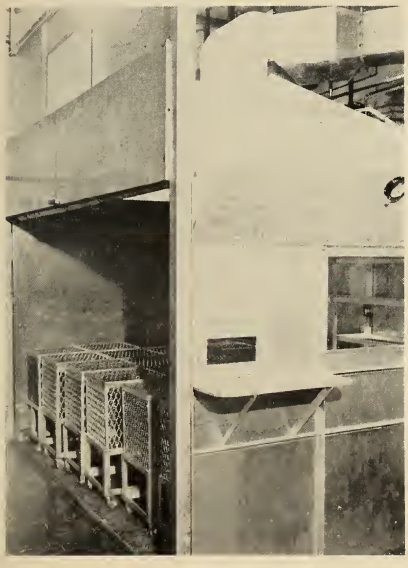


Figure 10. Chamber that houses control animals for all studies.

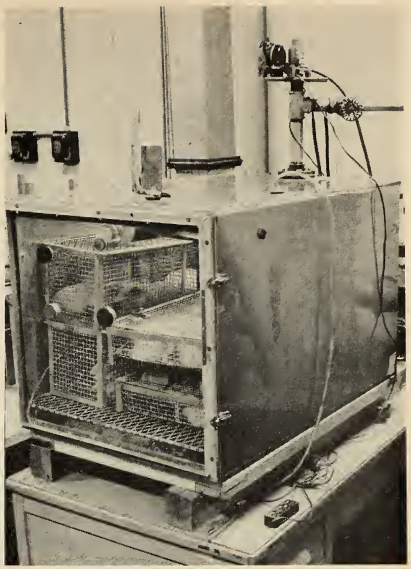


Figure 11. Small-scale stainless Steel and Plexiglas chamber designed for exposure of animals to corrosive materials.

NOTE: Air-distributing and exhaust plenums are fabricated from plastic pipe. When highly toxic materials are used, this chamber can be placed inside a large-scale chamber which serves as an exhaust canopy and protects chamber operators from exposure. Note flexible pinch-type exhaust valve at right rear of chamber.

Figure 11 shows one of the small-scale chambers. It was specifically for a study of the highly corrosive tetraalkyl thiophosphites, although it has been used for several other studies since. The walls are of stainless steel sheet with the exception of the top and front, which are of Plexiglas. The front wall is removable for loading and unloading the chamber. The inlet airflow is distributed evenly along the length of the chamber at the top by a 1½-inch nylon pipe, with $\frac{3}{16}$-inch holes at 4-inch in-

tervals, which acts as a distributing plenum. At the bottom two similar pipes serve as the exhaust. Above these lower pipes is a grate which supports the animal cages. There is a drain at the center of the chamber floor to facilitate washing. Air sampling within the chamber is done through sampling ports cut in the Plexiglas front door.

The second small-scale chamber (fig. 12) was constructed to less rigorous specifications. It was made on short notice for an inhalation study on carbon disulfide (*12*). A wooden box which had been a temporary dog cage was used as the frame of the chamber. The plywood sides and back of the box were retained and lined with aluminum foil. The open top of the box was replaced with a sheet of aluminum, and the front by a wood and Plexiglas door. The floor surface was covered by two coats of black asphaltum. The box had a metal tray which occupied the entire floor area, and it was retained and used in the chamber to catch animal excrement. Six inches above the floor, a wire screen was installed to support animal cages. Inlet and exhaust plenums similar to those in the chamber just described were installed at the top and below the screen. Supply and exhaust air connections were made to unused branches of the central supply and exhaust systems.

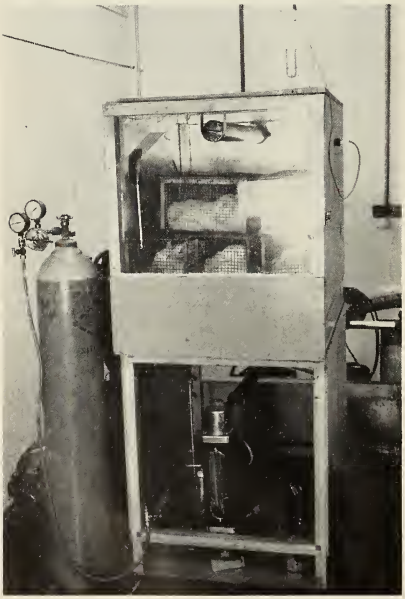


Figure 12. Inexpensive small-scale chamber constructed of wood and lined with aluminum foil.

Thus, with a minimum of effort and expense, a 25-cubic foot chamber with nonabsorbing interior surfaces was constructed from simple materials in less than 2 weeks. With very little more time the frame could also have been made, but since an appropriate rigid box was available it was used.

Pilot Exposure Units

Pilot exposure units are used by this laboratory for inhalation exposure studies on relatively small numbers of small laboratory animals. These studies are usually short-term rangefinding studies, such as tests to determine LD_{50} values which require a few hours to a day. The exposure units have been constructed around glass battery jars and belljars of about 20 liters in capacity. A representative unit is shown in figure 13. It has a volume of 18.5 liters and can hold 5 rats, 10 mice, and 10 hamsters. By using different interior frames, the same unit can be used for the exposure of various species of laboratory animals.

In the chamber shown, the airflow can be varied from 5 to 35 liters per minute. For reagents that react readily, the flow rates must be sufficiently high to prevent diminution in concentration between inlet and outlet. The air supply, drawn from the room, is cleaned and dried before entering the contaminant generator. All of the equipment is located in a laboratory fume hood. Cleaning of the air discharged from the exposure unit depends on the nature of the material being studied.

The small size of these exposure units permits easy cleaning in a laboratory sink and use in minimum sized hoods.

The elimination of leakage and accurate metering of contaminant and airflows are as important in small-scale studies in pilot units as they are in any animal inhalation work. Leakage is eliminated by tight joints. In the exposure unit illustrated, the top of the cylin-

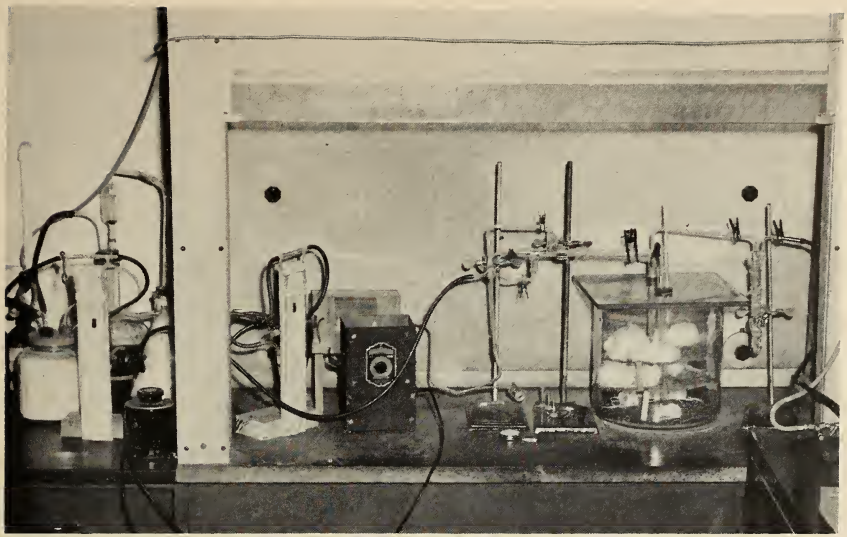


Figure 13. Twenty-liter glass pilot chambers and accessory equipment.

NOTE: These small chambers are especially useful for LD_{50} studies and experiments involving highly toxic or corrosive materials.

der is ground smooth and fits against a matching ground glass groove in the plate glass top. All of the fittings of the chamber and contaminant generators are connected with ball and socket joints which provide good connections and are easily changed. Adaptability is an important feature of equipment used for pilot exposure units. Since most studies are of short duration, and each study may require a different flow rate and a different type of contaminant, it is desirable to have contaminant generators, mixing flasks, pressure and flow measuring devices, and other equipment that can be readily fitted in any desired sequence. Ball and socket connections permit such flexibility.

Numerous pilot-sized exposure units have been described in the literature. Most of the early inhalation exposure studies, such as those of Eulenberg (*13*), von Jns (*14*), Lehmann and his associates (*15*, *17*, *18*), and Dubitski (*16*) were conducted in chambers that would be considered pilot-sized units today. Recent references to pilot-sized units are by Spiegl, et al. (*19*), and Svirbely and Saltzman (*20*) of this laboratory.

III. Operation and Performance

The purpose of operating an animal inhalation exposure chamber is to expose selected animals to known, uniform, and reproducible concentrations of aerosols or vapors having the desired chemical and physical properties. The precision with which an experienced operator can meet these objectives is the measure of performance of the chamber and its auxiliary equipment. The maintenance of the desired test atmosphere is the most important aspect of animal exposure chamber operation and the various factors which influence this atmosphere will be discussed. It should be emphasized that all factors are interdependent and should not be considered separately.

All test atmospheres fall into one of the following general categories:

1. A dispersion of solid particulates in air.
2. A mist or dispersion of liquid droplets in air.
3. A gas or vapor forming a single and homogeneous phase with air.

These general categories will be discussed in order of decreasing complexity and difficulty of producing and maintaining satisfactory performance in the animal exposure chamber.

Behavior of Solid Particulates

The characteristics of an aerosol dispersion in a chamber such as concentration and distribution throughout the chamber, as well as its physiological properties such as depth of penetration and retention in the respiratory tract of the animal, are all intimately related to the dynamic behavior of the airborne particles. The size of the particles of concern in animal inhalation studies ranges generally from the submicroscopic to the microscopic, that is, from approximately 0.01 to 5.0 microns in diameter. The chemical and physical activity of such particles are greatly increased as compared with the activity of the gross parent material. Thus the rates of oxidation and solution are greatly increased as are the vapor pressure and the phenomena of adsorption and electrostatic activity. The adsorption of a gas on a particle, however, may hinder chemical reactivity or wettability, or accelerate the same phenomena.

The term aerosol was used by Gibbs (*21*) to denote any disperse system in air, either liquid or solid. The terms dust, fume, smoke, and mist are defined to help clarify these frequently overlapping and misused terms, since the dynamic behavior of each of these may differ.

Dusts are solid particles ranging in size from the microscopic to the visible, produced by physical fragmentation. The chemical composition of the particles is usually the same as the parent material and their shape is irregular.

Fumes are solid particles usually having a particle size below 0.5 micron produced by combustion, sublimation, or condensation. The chemical composition of the fume may differ from the parent material; for example, an oxide or mixture of oxides derived from the parent metal. Fume particles are usually spherical in shape although some of the oxides, being crystalline, will have varying crystal shapes. Zinc fume, for example, is needle shaped.

Smokes are generally solid particles of carbon resulting from burning of carbonaceous material and are often coated with a film of the parent organic material or its combustion products. The carbon particles are usually extremely small (less than 0.1 micron) and an electron microscope is necessary to see them. The carbon particles are spheres but tend to agglomerate rapidly to form large aggregates and long chains.

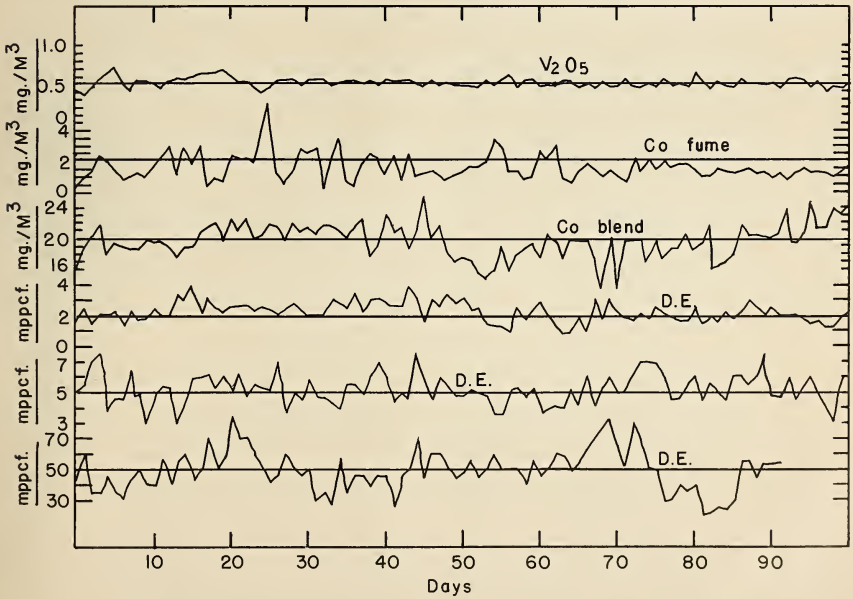
Mists or fogs are liquid droplets formed by the atomization or volatilization and subsequent condensation of water or other liquids on suitable nuclei. These nuclei may be car-

bon particles or extremely minute salt crystals. The size of these particulates is often large (5 to 20 microns) because the vapor pressure increases with increasing curvature of the surface and smaller droplets tend to vaporize rapidly. Agglomeration of smaller liquid droplets to produce larger drops is a common phenomenon.

Concentration

The chambers under discussion are termed dynamic rather than static, as the agent being studied is metered into a constantly flowing air supply. A slight negative pressure (approximately 0.1 inch of water) is maintained within the chamber by exhausting the chamber at a slightly greater flow rate than the incoming air. Thus any leak in the chamber will result in a slight flow of room air inward to retain the test agent within the chamber. The attainment of an unvarying concentration under these conditions results from a balance being reached between the metered addition of the agent under test to the diluting air supply and the loss of this material from the chamber atmosphere. The factors involved in this depletion of the established atmosphere will be discussed under a separate heading. Given a dispersing technique that can be regulated carefully to deliver the noxious agent at a uniform rate, this balanced condition should readily be reached after a period of time for the chamber to become stabilized. That this ideal condition can be approached, but never completely realized, can be seen visually in the typical plots of daily average concentrations for 100-day periods on six different inhalation studies shown in figure 14. Even if the metering of the noxious agent into the chamber were precise, the inherent error in

Figure 14. Average daily concentrations in six typical 100-day periods of chamber operation involving four dissimilar dusts.



NOTE: D.E., diatomaceous earth.

whatever analytical method was used to determine the concentration in the chamber would lead to some variation.

In all of the studies shown in figure 14, with the exception of the one labeled cobalt fume, the same dust-dispersing mechanism was used, namely, the Wright dust feed (*22*) previously described in the discussion on particle-size distribution. Varying degrees of uniformity may be attained with other dust feeds and with other compounds.

The vanadium pentoxide results (fig. 14) were obtained by a colorimetric chemical determination of the amount of vanadium collected on a filter paper from a known volume of air. Such a chemical determination can be highly accurate and therefore the variation due to the analytical method is reduced to a minimum. The results of the cobalt-blend study are based on weighing the filter paper before and after drawing a known amount of air through the filter to deposit approximately 10 mg. of the dust on the paper. Although the analytical balance used in such a determination has a sensitivity of 0.05 mg., the determination is subject to considerable error due to the differences in the degree of hydration of the paper from day to day and before and after sampling; the collection of extraneous background dust in the chamber air, including animal hair, and *nuisance dust* which would be weighed and considered as contaminant; the loss of some of the filter by insertion in the sample instrument; and other factors which are uncontrollable.

In the three diatomaceous-earth studies (fig. 14) the same dust feed was employed but the method of analysis used was a standard dust-counting technique, which has a rather high possibility for error and is influenced by such factors as dilution of the sample, great extrapolation and therefore magnification of errors, and the experience and temperament of the analyst. It would appear, therefore, that the precision of the analytical technique employed will account for an important part of the variation in concentration reported in chamber studies.

The maximal and minimal concentrations obtainable in an exposure chamber will depend on the requirements for airflow through the chamber as well as on the capacity and stability of the aerosol generator. Other factors of importance are the size of the aerosol particles being produced, the electrical charges placed on them by the dispersing apparatus, and the tendency for agglomeration of the particular particles.

Extremely small particles, such as metal fumes with diameters of the order of 0.01 to 0.02 micron which are formed by the condensation process, tend to have a high charge-to-mass ratio and therefore aggregate quickly into long chains which have a total mass sufficient to cause rapid settling out from the airborne state. At the same time their high surface area and high coefficient of friction cause them to follow the airflow patterns and thermal currents within the chamber.

Larger ground or crushed particles with diameters of 0.1 to 1.0 micron may exist as discrete particles in the air but will be subject to a differential settling of the larger particles and hence a rapid depletion of the mass concentration. Errors also can be introduced by anisokinetic sampling, the smaller particles being collected preferentially by the sampling instrument. A mass determination will indicate here also less of the contaminant than is actually present.

Another factor that will influence the concentration of dust in a chamber is the presence of the animals themselves. The breathing, motion, and metabolic heat of the animals set up air currents, thermal and otherwise, which lead to air turbulence within the chamber, increasing the collision rate and therefore the rate of agglomeration of the particles. Such an effect may change the overall size distribution of the aerosol as well as tend to decrease the concentration by encouraging settling of the particles.

The fur of the animals tends to collect an inordinately large amount of dust from the chamber atmosphere. This may be due partly to an electrical attraction between the animal fur and the particulates but it is also an extension of the *wall effect*, a diffusional phenomenon in which particles close to a surface collide with it and are held to it by electrical forces, van der Waals forces, gravity, or other mechanisms. As these particles are removed from the layer of atmosphere in close proximity to the surface the concentration of par-

ticles in this layer is reduced. There will be a diffusion of particles from the higher concentration farther away from the surface toward the area of lower concentration, in an attempt to eliminate the concentration gradient. As this condition of equilibrium is achieved, the process of collision with the surface continues so that there is, in effect, a tendency of particles to move toward the surface and be collected there.

Distribution

A factor of importance equal to the aerosol concentration is its distribution throughout the chamber. Considerable thought must be given to this in the design of the chamber so that there is not a direct flow of the test atmosphere from the entrance to the exit of the chamber but rather a complete circulation through the entire chamber volume.

If care is not exercised it is possible to have dead spaces in the chamber where there is little or no circulation of the *fresh* atmosphere. Animals placed in these dead spaces would receive considerably less than the calculated concentration of contaminant. The elimination of these spaces is also important from the point of equalizing the temperature throughout the chamber. This is especially true when the incoming air is cooled to counteract the metabolic heat of the animals. When this condition obtains it is possible for some of the animals to be subjected to a blast of cold incoming air while others are in a pool of stagnant and relatively warm air. In addition to the possible increase in incidence of pneumonia among the animals in such a situation, there is the possibility of differing response of the same animal at different temperatures and a change in the actual response of warm animals because of such factors as altered breathing patterns and circulation rates.

As standard operating practice during a long-term study, the locations of all animals in the chamber should be rotated from day to day to equalize any differences in exposure or temperature that may otherwise go undetected.

In practice, the distribution of the aerosol in the chamber should be tested under operating conditions before the animal exposure is begun. This test, repeated with the animals in the chamber and periodically throughout the entire study, is accomplished by taking a series of simultaneous samples comparing the concentration in each of the eight corners of the chamber with a standard or reference sample. The standard sample is ordinarily taken at a convenient reference location used throughout the study for routine sampling of the chamber atmosphere. It should be located centrally within the chamber and be in or near the breathing zone of the animals.

Figure 15 shows the eight corner sampling positions and the reference sample position. Table 1 gives the results of a series of samples taken to show the uniformity of distribution of a dust in this chamber. All samples were taken by drawing a known volume of air through a filter paper held in a brass sampling head at the end of a probe of copper tubing. The reference probe extended about 18 inches into the chamber from a sampling port cut in a Plexiglas window. The corner probe was a length of copper tubing sufficiently long to reach all corners and flexible enough to be bent around animal cages if necessary. The corner sample and reference sample were taken simultaneously in all cases.

The average percent deviation of the corner position concentrations was almost twice that of the reference positions (5.6 and 3.0 percent, respectively). The percentage difference between the reference sample mean and the corner sample mean was 2.5 percent which is considered to be excellent performance in attaining uniform distribution. That this is not statistically significant is shown by Student's t test shown in table 1. In a large chamber (90 cubic feet or larger) an average difference of less than 10 percent would be acceptable.

Losses

The actual concentration of airborne particles attained in a dynamic chamber bears little relation to the weight of contaminant dispersed into the supply airstream. This is shown in figure 16 where the weight of dust dispersed and the calculated chamber concentration are compared with the actual concentration found by sampling the chamber air. In the case reported, approximately 60 percent of the dust blown into the chamber remained suspended in the air. Additional information

is given in table 2 which compares the results of simultaneous samples taken in the body (reference point) of the chamber and in the exhaust plenum immediately outside the chamber. Although the distance through large ducts and openings may be short, a significant decrease in concentration can be observed.

It is apparent that any method of determining chamber concentration based on the amount of contaminant dispersed into the chamber will be unreliable. The loss of contaminant from the chamber air is a composite result of a number of factors such as particle-size distribution, particle density, temperature, and charge on the particles; contaminant loss will, therefore, vary considerably from one contaminant to another.

Although the theories concerning the dynamics of small particles have been discussed by DallaValle (*23*), Drinker and Hatch (*24*), and more recently in the excellent treatise by Green and Lane (*25*), the more important concepts will be reviewed here.

Settling and Stokes' Law. Although all airborne particles are accelerated toward the

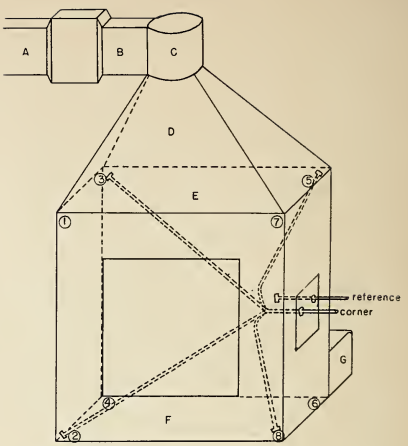


Figure 15. Sketch of chamber indicating the eight corner and one reference sampling positions.

NOTE: Letters indicate positions of thermocouples used for measurement of temperature distribution within chamber.

Table 1. Comparison of cobalt dust concentration at eight corner sampling points of a chamber with simultaneous samples at the reference point

Corner position	Corner concentration	Reference concentration	Difference (δ)	Difference squared (δ^2)
1	21. 8	24. 0	2. 2	4. 84
2	22. 4	19. 8	−2. 6	6. 76
3	23. 1	22. 6	−. 5	. 25
4	21. 3	21. 3	0	0
5	20. 8	23. 0	2. 2	4. 84
6	21. 8	25. 0	3. 2	10. 24
7	21. 7	23. 2	1. 5	2. 25
8	23. 2	21. 5	−1. 7	2. 89
$N=8$	Mean=22.01 or μ_1	Mean=22.55 or μ_2	$\Sigma\delta=4.3$ Mean$=\bar{\delta}=0.54$	$\Sigma\delta^2=32.07$

$H_o: \mu_1-\mu_2=0$

$$s^2_d=\frac{\Sigma\delta^2-\frac{(\Sigma\delta)^2}{N}}{N-1}=\frac{32.07-2.31}{7}=4.25$$

$s_d=2.0615$

$$\bar{s}_d=\frac{s_d}{\sqrt{N}}=\frac{2.06}{2.83}=0.728$$

$$t_{calc.}=\frac{\bar{\delta}-0}{\bar{s}_d}=\frac{0.54}{0.728}=0.7418$$

$t_{tabular}=1.895$, $t_{tabular}>t_{calc.}$

This t-test shows no significant difference between μ_1 and μ_2 at a risk level of 1 percent.

earth by the force of gravity, the small particles have a great surface area per unit of mass. Therefore, the resistance of the air quickly balances the force of acceleration and a constant or terminal velocity is obtained. The force (F) attracting the particles to the earth can be written

$$F=\frac{4}{3}\pi r^3(\rho-\rho')g$$

where

r=radius of the particle
ρ=density of the particle
ρ'=density of air (negligible compared to ρ)
g=acceleration of gravity

The resistance (R) of the air to the motion of the particle can be written

$$R=6\pi\eta rV$$

where

η=viscosity of air
r=radius of the particle
V=velocity of the particle

At constant velocity, acceleration is zero then $R=F$ and

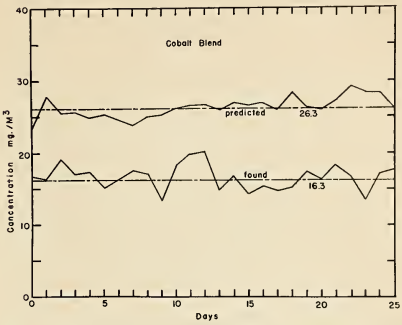
$$6\eta rV=\frac{4}{3}r^3(\rho-\rho')g$$

or

$$V=\frac{2}{9}\frac{r^2(\rho-\rho')g}{\eta}$$

This is Stokes' equation for the terminal velocity of a falling spherical particle, assuming streamlined flow. Streamlined motion can be assumed for spherical particles up to a diameter of 115μ or for irregular particles up to 85μ and therefore Stokes' equation is valid for the particle sizes of interest in exposure chamber work. For extremely small particles approaching the size of the mean-free path of air molecules the frictional resistance decreases

Figure 16. Actual concentrations of a solid particulate aerosol found in a chamber compared to concentration predicted from amount of material actually dispersed by the dust feed during operating day.



and it is necessary to apply a correction developed by Cunningham (*26*):

$$V'=V\left(1+K\frac{\lambda}{r}\right)$$

where

V'=corrected velocity of particle
V=uncorrected velocity
K=0.86, found empirically for airborne mineral particles
λ=mean-free path of air molecules=10^{-5} cm., standard temperature and pressure
r=radius of the particle

Although this correction is negligible for particles larger than 0.5μ it amounts to a fivefold increase in settling velocity for particles of 0.05μ in diameter.

Table 3 shows the relative settling velocities for spheres of different sizes and unit density. It is apparent that the loss of particles from the chamber air due to settling will affect only

Table 2. Comparison of aerosol concentration found simultaneously in chamber body and exhaust plenums

Contaminant	Chamber	Exhaust	Loss (percent)
25 percent $CoCl_2$ (liquid droplets)	4.66 mg./m.^{3} [1]	3.30 mg./m.^{3} [1]	29. 2
Co fume	0.94 mg./m.^{3} [1]	0.70 mg./m.^{3} [1]	25. 5
Diatomaceous earth	5.17 mppcf [2]	3.59 mppcf [2]	30. 6

[1] mg./m.^{3}=milligrams of aerosol per cubic meter of air.
[2] mppcf=millions of particles per cubic foot of air.

that part of the distribution greater than approximately 5μ. Although this will be a relatively small percentage of the particles the loss would be appreciable if the determination were made on the basis of mass.

Diffusion and Brownian Motion. Particles with diameters of the order of 1 micron and less behave increasingly like gas molecules as their particle size decreases. Their movement is more subject to thermal or turbulent air currents in the chamber than to settling due to gravity. Diffusion becomes more important in the removal of these smaller particles onto collecting surfaces. Particles of 0.1 micron and less in diameter exhibit Brownian motion which can be described mathematically by the equation obtained by Einstein (*27*).

$$\frac{\lambda^2}{2t}=\frac{RT}{KN}$$

where

λ=displacement of the particle in time, t
R=gas constant
T=absolute temperature
N=Avogadro's number
$K=6\pi\eta r$, Stokes' resistance for a sphere in streamlined motion

Diffusion in the chamber, however, involves not only Brownian motion but also the effect of gravity and the motion of the air in the chamber. Corrections for these effects which become more complex and less definitive will not be discussed here; additional material will be found in the reference by DallaValle (*23*).

The equation of Brownian motion expresses qualitatively that the movement of the particles becomes greater as the size decreases. This is the opposite to the effect of gravity and there is a point at which the combined effects cause motion to be at a minimum. Diffusion is primarily responsible for the deposition of airborne particles on the chamber walls, roof, and animal fur by the process which has been described in the discussion on distribution.

Centrifugation. In some of the chambers previously described, the contaminant is injected into a mixing cylinder, which the supplying airstream enters tangentially, and follows a spiral path before reaching the body of the chamber. The air in the mixing chamber is subjected to centrifugal forces which tend to remove larger particles from the airstream. Davies (*28*) has developed a formula giving the minimum size of particle which will be removed from a spiraling airstream by centrifugal force. The formula follows:

$$d=\left[\frac{9\eta R_2}{8(\rho-\rho')v_o H}\left\{1-\left(\frac{R_1}{R_2}\right)^4\right\}\right]^{\frac{1}{2}}$$

where

d=minimum diameter of particle
η=viscosity of air
R_2=outer radius of airstream
ρ=density of the particle
ρ'=density of air
v_o=linear velocity of rotating airstream
H=height of cylinder
R_1=inner radius of airstream

Under the conditions of the chambers previously described, it can be shown that particles less than 20 microns in diameter will not be lost in this type of mixing cylinder.

Flocculation. Flocculation or agglomeration of particles in air comes about as a result of Brownian motion and therefore is directly related to the size of the particles. In general, the larger dust particles show no tendency to agglomerate whereas the small fume or mist particles flocculate rapidly. Aggregates of fine particles behave dynamically as equivalent large particles, and will be removed from the chamber air by settling or centrifugation, or if inhaled by the animals, will be retained in the upper respiratory passages, where they are relatively innocuous. An equation relating the concentration change of a cloud of particles with time has been given by Whitlaw-Gray and Patterson (*29*), as follows:

$$\frac{1}{n}-\frac{1}{n_o}=kt$$

where

n=number concentration of particles at time, t
n_o=number concentration of particles at $t=0$
k=empirical constant, 3×10^{-10} cc./sec.

This equation shows that the rate of flocculation of an aerosol depends only on the product of the number concentration of particles and the constant k. Experimentally different values of k have been reported for aerosols of

Table 3. Terminal settling velocities in centimeters per second for spheres of unit density as calculated from Stokes' equation and corrected with application of Cunningham's correction

Sphere diameter (μ)	Uncorrected velocity	Correction	Corrected velocity
50	7.5	0.0255	7.5255
10	0.3	0.00516	0.30516
5	0.75×10^{-1}	0.0255×10^{-1}	0.775×10^{-1}
1	0.3×10^{-2}	0.0516×10^{-2}	0.35×10^{-2}
0.5	0.75×10^{-3}	0.255×10^{-3}	1.01×10^{-3}
0.1	0.3×10^{-4}	0.516×10^{-4}	0.816×10^{-4}
0.05	0.75×10^{-5}	2.55×10^{-5}	3.20×10^{-5}
0.01	0.3×10^{-6}	5.16×10^{-6}	5.46×10^{-6}

different substances and different particle-size distributions (*25*) but these variations are probably due to the effect of electric charges on the particles. Table 4 shows the degree of flocculation that can be predicted for various number concentrations of particles in a 10-minute period. It is apparent that a higher chamber airflow is required when the aerosol concentration is high.

Improper dispersing of the dust from the dust generator is often mistaken for flocculation. This may occur when finely divided particulates have been collected and packed into a dust-feed cylinder. A great deal of energy is required to disaggregate this dust and to disperse it in air as discrete particles. Such energy is often supplied by forcing the dust-laden airstream through a jet of near critical dimensions and against an impaction plate placed close to the orifice. Impaction against the plate shatters the larger aggregates and the shear forces of the high velocity airstream tend to tear apart the smaller flocks. In this treatment of airborne dusts there is the possibility that an electrostatic charge could be placed on the particles which would cause reagglomeration. However, the rapid dilution of the relatively concentrated dust stream issuing from the generator with large quantities of clean air and the humidity conditions realized in the animal exposure chambers quickly dissipate any such charges on the particles and this reagglomeration has not been observed in this laboratory.

Turbulent airflow in the chamber has been shown (*30*) to increase greatly the rate of flocculation for fumes and smokes, but only in those particulates which exhibit Brownian motion. Larger aerosols would not be expected to be affected by turbulence.

Although it has been suspected that humidity would affect the rate of agglomeration, this has not been demonstrated (*24*) until a condition of saturation is reached. At saturation, the airborne particles act as condensation nuclei for the water vapor and will be lost rapidly by settling when coated with water.

Chamber Response

The speed with which the concentration in a dynamic chamber can be changed is a function of the volume of the chamber and the total airflow through it. This is commonly expressed as the time required for a complete *air-change*. Such terminology, however, is inaccurate and can be misleading for it can be shown that one air-change does not completely renew the chamber air but will change the concentration by a maximum of only 63.2 percent.

If perfect mixing within the chamber is assumed and the contaminant is introduced uniformly into the chamber air supply, both rates being held constant, the normal concentration can be calculated for any time, t, by the following equation:

$$c=\frac{w}{b}\left(1-e^{-\frac{bt}{a}}\right)$$

Table 4. Degree of flocculation of three sizes of particles (density 2.38) in 10 minutes

Diameter of particles (μ)	Mass concentration (mg./m.^{3})	Number concentration at $t=0$ (particles/cm.^{3})	Number concentration at $t=10$ min. (particles/cm.^{3})	Particles flocculated (percent)
2.0	10	1×10^3	0.9998×10^3	0.02
0.2	10	1×10^6	0.85×10^6	15.0
0.02	10	1×10^9	0.0055×10^9	99.45

where

c=concentration in the chamber
w=amount of contaminant introduced per minute
b=volume of air passing through chamber each minute
a=volume of chamber

It is apparent that the chamber concentration rises rapidly at first and then approaches a constant value at infinite time. The desired concentration is w/b and the percent of this actually attained, $\frac{c}{w/b}100$, will be

$$\left(\frac{c}{w/b}\right)100=100\left(1-e^{-\frac{bt}{a}}\right)$$

let

$$100\left(1-e^{-\frac{bt}{a}}\right)=X$$

then

$$e^{-\frac{bt}{a}}=\frac{100-X}{100}$$

$$-\frac{bt}{a}=ln\frac{100-X}{100}$$

hence

$$t=K\frac{a}{b}$$

It will be noted that the ratio a/b is the time required for one air change in the chamber. Therefore, K is the number of air changes required to change the chamber concentration to the desired percentage of the input concentration. The value of K varies with the percentage of the input concentration that it is desired to obtain. For $X = 99$ percent, $K = 4.605$, and for $X = 95$ percent, $K = 2.996$. These values have been verified experimentally for mixtures of gases by Silver (*2*).

It is seen that t is independent of any previous concentration level in the chamber and that at constant airflow the time required to change from one level to another must always be the same. Thus, the time required to clear the chamber after turning off the contaminant feed, is the same as that required to establish the concentration. Figure 17 is an actual record of chamber buildup and depletion for a dust aerosol dispersed from a Wright dust feed. It is seen that the buildup and depletion times, although similar, do not duplicate each other exactly. This can be explained by assuming less than perfect mixing and recognizing that the above equations were developed for mixtures of two gases rather than for a mixture of a gas and particulates. Inhalation toxicology, however, is usually concerned with small particles which approach the behavior of gas molecules and therefore the theoretical equations may be used to give a reasonable prediction of the behavior of an aerosol in a chamber.

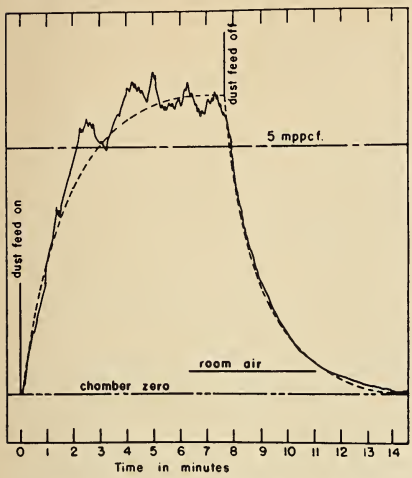
Particle-Size Distribution and Dispersal

The size distribution of the particles in a dust cloud is of interest because particle size governs not only pulmonary alveolar deposition, but also governs losses from settling and diffusion which affect the stability of the dispersion. (See discussion on losses.)

The production of a dust can be accomplished in either of two ways. In one, the parent material can be ground to the desired size, sieved or elutriated to eliminate unwanted sizes, and the product dispersed into the airstream; in the second, the grinding or atomizing process itself can be used to meter the aerosol directly into the air supply. The first way permits the selection of the desired particle size and the major problem becomes that of breaking up the aggregates of particles formed when the dust is collected and packed into the dust-feed mechanism. This is effected in the Wright dust feed (*22*) by impacting the dust particles on a tungsten-carbide plate, thereby fracturing the large aggregates. The device involved is a gear-train assembly powered by a synchronous motor which drives a brass cup packed with the dust down a spindle shaft and against a scraper blade which continuously removes a uniform amount of the caked dust and disperses it into the airstream to the chamber.

When the dust is dispersed from a liquid suspension by atomization or nebulization, the liquid droplet formed may contain several solid particles which will be drawn together upon the evaporation of the liquid to form a firmly bound aggregate. The remedy in this situation is to use a sufficiently dilute suspension so that the liquid droplet can contain only one solid particle. This may limit the maximal concentration that can be obtained since

Figure 17. Buildup and depletion times for a 145-cubic foot chamber with an airflow of 45 cfm as recorded by an automatic light-scattering photometer.



NOTE: The dust was diatomaceous earth dispersed by a Wright dust feed set to deliver 5.5 mppcf. Vertical scale, approximately arithmetic.

the evaporation of the liquid tends to saturate the atmosphere and retard further evaporation.

Two devices for this purpose have been used successfully in this laboratory. For the small or pilot chambers, the commercially available all-glass Vaponephrin nebulizer modified by the addition of a side arm to supply a constant level of liquid in the chamber adequately supplies fairly high concentrations of liquid droplets, 5–15μ in diameter. The other device is known as the Laskin atomizer and was designed for the Atomic Energy Project of the University of Rochester. (These instruments are described more fully in the discussion on aerosol generators and concentration.)

A great number of dust dispensers have been described in the literature (*31*) and the references given here are not exhaustive but merely indicate several types that have been suggested. Most of these have been developed to solve a specific problem and where feasible it is usually more economical to purchase a commercially available instrument rather than invest the great amount of research time required for the development and testing of an instrument. Table 5 lists a number of aerosol generators or feeders which have been used by various institutions. Only the last one on the list, the Wright dust feed, is known to be commercially available (*C*).

An example of the second technique of solid particulate generation is the production of a fume by an electric arc composed of two rods of the parent material. Another example is the burning of an organic compound such as tetraethyl lead to produce a lead fume. The La Mer-Sinclair aerosol generator is also in this category. With precise regulation and control, uniformly sized particles can be obtained by these methods but the development of the proper technique to be applied to each substance must be considered a separate research problem.

Regardless of which dispersing technique is selected, certain facilities will be required and can be incorporated conveniently in the control panel of the chamber. A regulated air supply of reduced pressure will be necessary. This laboratory has found it convenient to mount a variable pressure control valve (0–60 psi) with indicating pressure gauge and built-in filter on the control panel (*D*). Compressed air is taken directly from the regular laboratory air line which supplies air at a pressure of 120 psi. Most dispensing instruments will require an air pressure of from 1 to 20 psi, but it is advantageous to have a reserve of higher pressure available for exceptional situations. A flowmeter of the manometer type or the floating-ball type can be connected downstream from the regulator and upstream from the dispersing device. A needle valve between the pressure regulator and the flowmeter can be used to control the airflow.

Many dust-feed devices require dry and filtered air to avoid clogging of the mechanism. The above arrangement has been found to be generally satisfactory in providing dry air, as well as filtered air, since the expansion of the air in the pressure-reducing valve condenses excess moisture from the air and a drain in the valve itself allows removal of this condensed water. Exceptional conditions may re-

Name	Feeder principal
Air Filter Institute	Moving horizontal tray with leveling gear
Aircraft Nuclear Propulsion	Tube elevated by hydraulic piston into 90-psi air line
Atkins	Screw feeder to recirculating unit
Bureau of Mines	Fluidized bed from solids feeder
Bureau of Mines	Tube elevated by winch into spiraling airstream
Bureau of Standards	Rotating gear with tooth reduced to pitch circle, grooves fed by gravity from hopper.
Colorado	Tube elevated by screw, dust scraped into spiraling airstream
Dustshaker	Oscillating vibratory screen, 40 to 60 mesh
Farr	4-grooved plate moves horizontally with leveling gear
Geyser	Powder in small chamber, dispersed by air jets
Harvard No. 1	Air dispersed from vibrating tube
Harvard No. 2	Revolving disk screen from vibration feeder
Harvard No. 3	Rotating 300-mesh screen cylinder, vibrated, inside lucite container.
Harvard No. 4	Rotating turntable with scraper, fed from vibration feeder
Harvard No. 5	Small screw feeder, variable discharge openings, to moving belt with rotating scraper to adjust height, width fixed by belt or notches in scraper.
Haskell Laboratory	Pulsating airflow through material in tube
Knolls Atomic Power Laboratory	Rotating steel wire brush held against aluminum metal
Minnesota No. 1	Rotating disk with dust ribbon
Minnesota No. 2	Moving horizontal trough with level dust layer
New York State No. 1	Hopper feed to moving belt, dust discharged from end into hopper with airflow through.
New York State No. 2	Hopper feed to vibrating chute
Research Cottrell	Funnel feed to plate in horizontal fan impeller
Research Products	Compressed air jet in flask with dust on bottom
Rotating drum	Rotating drum with friable material, airstream passes through
Stanford Research Institute	Moving horizontal vee trough
Screw	Variable speed stoker screw, or wood auger 0.5–2 inches
Sonic	High frequency vibration from speaker cone directed at dust
Texas	Modified Bureau of Mines respiratory test dust feeder
University of Chicago No. 1	Vibrated sintered 40-mesh glass filter, air from top to bottom
University of Chicago No. 2	Multiple impinging nozzles fed from dust in container
Vibratory	Trough or tray pulsed by electromagnetic vibrator
Wright	Rotating packed drum moved against scraper, dust entrained in low-pressure airstream.

[1] Reference No. 31.

quire the insertion of a drying column placed directly after the pressure reducing valve. In instances in which hygroscopic dusts are used, oil-pumped (dry) nitrogen may be required.

Background Dust Counts

When low concentrations of contaminants are used in the chamber a determination of the background (nuisance) dust contamination of the chamber air is important. This is a requisite when the method of analysis is not specific, such as gravimetric analysis of filter paper samples or analysis by light reflectance from the filter paper. In this instance a sample of a large air volume is required and equally important the collected material should be analyzed by the same method used for the test agent.

If the method of analysis is a standard dust-counting technique, it is well to remember that the background dust can range between 0.5 and 1.0 million particles per cubic foot of air (0.2–0.3 mg./m.^{3}). Thus, if a toxic dust level of 2–3 mppcf is desired, the background dust will constitute an appreciable part unless it is taken into consideration and a correction is made for it.

It is usually good practice to insert a filter in the main air supply line to the chamber. However, the efficiency of such a filter will

used by different laboratories [1]

Aeration method	Classification method	Feed rate per minute	Remarks
Venturi throat	None	1 gram	Fly ash.
Sonic jet	Settling chamber	1–75 grams	Copper oxide.
Ejector	Recirculation by fan	8 grams	Pulverized coal.
Fan	Cyclone	26 lb./ft.^{3} (maximum)	Pulverized coal.
Ejector	Cyclone or settling chamber.	Milligrams	Respirator test dusts.
Ejector	Baffle	1 gram	Fly ash.
Ejector	Plenum and filter	Milligrams	Aluminum hydroxide therapy.
Airstream	None	Low	Toxicity studies.
4 ejectors	None	5 grams	Fly ash.
Aspirator	None	4 grams	Lithopone, egg albumin, Kadox.
Airstream	Settling chamber	Milligrams	Silica.
Air jet under disk, blower inlet above.	Elutriating column	Grams	Talc, glass spheres.
Aspirator from bottom tube	None	1,000 particles	Pollen.
Ejector	None	Pound	Most dry dusts.
Ejector	None	300 milligrams	Flake iron powder.
Airstream	Three elutriating columns.		
Airstream	Impingement plate	Micrograms	Aluminum or other solids.
Venturi throat	None	0.1–2 grams	Fly ash.
Venturi throat	None	Same	Fly ash.
Airstream	Two impingement nozzles, plus four elutriating columns.	0.1–2 grams	Mineral and organic test dusts.
Ejector	Impingement plate	3 pounds	Most dry dusts.
Airstream and fan blades	None		Fly ash.
Airstream	None	0.1–2 grams	Fly ash.
Airstream	None	Milligrams	Charcoal.
Glass aspirator	Settling chamber	Milligrams	Carbon black, powders.
Ejector			Most dry powders.
Airstream		Low	Many dry powders.
Ejector	Cyclone		Antimony, sulfur.
Ejector	None	Milligrams	Similar to dustshaker.
Airstream	Series impinging nozzles	Micrograms	
Ejector		Grams-pounds	Many dry powders.
Ejector	None	Micro-Milligrams	Pulverized coal, silica, and lead dusts.

change with various conditions of loading and therefore, unless checked, cannot be relied upon to give a low background dust level.

Typical of the difficulties that can arise was a situation involving the use of reflectance measurements from a filter paper sample for determining an index of chamber concentration. Erratic changes in concentration were explained only after it was discovered that on certain days trash was burned some distance from the building housing the laboratory and exposure chambers. The fine smoke particles easily penetrated the filter in the chamber air supply system and being black added to the absorbance of the filter paper sample out of proportion to the mass of smoke present.

Behavior of Liquid Aerosols

The characteristics of liquid aerosols required for animal inhalation research are almost identical to those of solid dispersions. The median diameter of the droplets must also be in the size range of from 0.01 to 5.0μ to insure penetration past the upper respiratory tree. However, the fact that liquids usually have an appreciable vapor pressure means that in a mist or spray the test agent will be pres-

ent in the vapor as well as in the liquid phase. To interpret meaningfully the physiological response of the animal, it may become important to know the relative amounts of the test substance present in both the vapor and liquid phases. This subject will be discussed further under sampling.

Aerosol Generators and Concentration

The problem of attaining a desired concentration of a mist or fog becomes one of choosing a generator of sufficient capacity. The most common devices for reducing a body of liquid to a mist of minute droplets fall into the category of nebulizers or atomizers. Two such devices which have been used successfully in this laboratory are the Vaponephrin nebulizer tested by Palmer and Kingsbury (*32*), and the Laskin atomizer described by Voegtlin and Hodge (*1*).

The Vaponephrin nebulizer (fig. 18) is commercially available and is used as a therapeutic device by the medical profession. It is constructed entirely of Pyrex glass and consists of a small nozzle which directs a stream of air at near sonic velocity across the top of a vertical capillary tube. The bottom of this capillary tube is submerged in the liquid to be dispersed. Directly behind the capillary tube is a relatively large, fixed sphere of solid glass which causes an abrupt change in the direction of the airflow from the nozzle.

In practice, the flow of air over the top of the capillary tube causes a reduction of pressure which sucks the liquid up to the top of the capillary tube where it is sheared off in the form of small drops by the blast of air. The inertia prevents the larger drops from following the airstream around the sphere and they either impinge on the sphere itself or are hurled away to impinge on the surface of the glass enclosing the nebulizer and are thus eliminated from the airstream. The smaller particles can follow the airflow around the sphere into the turbulent area behind it and then move more slowly out of the mouth of the nebulizer.

In a device of this type it is apparent that there are many critical dimensions. First, the diameter of the orifice of the nozzle limits the volume of air which can be passed through the

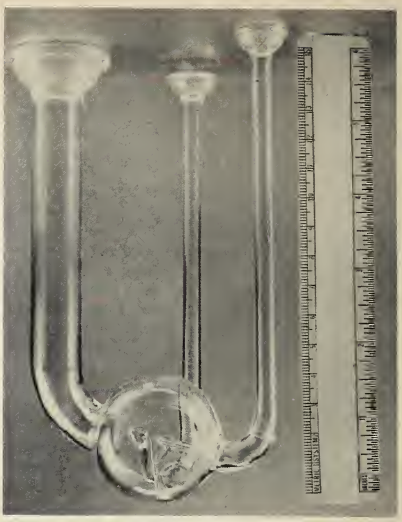
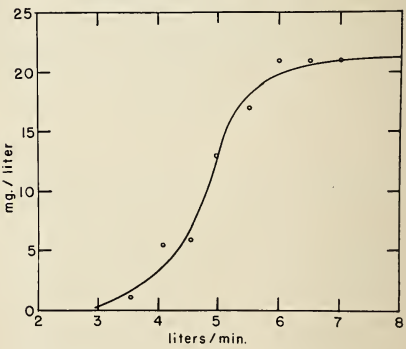


Figure 18. Vaponephrin nebulizer modified with ground glass ball and socket connections and a side arm to supply a constant level of liquid.

Figure 19. Change of output of Vaponephrin nebulizer with increasing airflow.



device and determines the air pressure necessary for optimal operation. The distances from the jet to the capillary and from the capillary to the sphere are critical as is the vertical positioning of the top of the capillary tube. All of these factors indicate the com-

plexity of attempting to make such an instrument in the laboratory.

Experience in this laboratory with the device showed that a pressure of approximately 10 psi and an airflow of 5 liters per minute are required for optimal performance. The data in figure 19 were obtained with the use of a light-scattering instrument to record changes in nebulizer output, on a mass basis, as the airflow through the nozzle was varied. It is seen that increasing the airflow above 6.5 liters per minute does not increase the output of the nebulizer. An undiluted aerosol of a light mineral oil produced at an optimal airflow of 6 liters per minute had a concentration of 20 mg. per liter. This aerosol could be then diluted to any desired lesser concentration for inhalation experiments. The physical properties of the liquid such as surface tension, viscosity, and vapor pressure will greatly affect the amount of aerosol produced as well as the particle size.

The Laskin atomizer is shown in figure 20. Air under pressure is first pumped through the hollow-center tube and passes through the four radially drilled holes at the top. The jets of air then pass over the top end of four capillary holes drilled vertically in the collar and alined with the radial holes. Liquid is thus drawn up the capillary tubes and sheared off from the edge of the collar as small droplets. Aqueous solutions have produced aerosols with a median size of from 8 to 10μ when the atomizer is operated at a pressure of 10 psi and an airflow of about 30 liters per minute. Under these conditions and with the use of the same light mineral oil that was used to test the Vaponephrin nebulizer it was found that the undiluted aerosol concentration produced by this device was approximately the same as found in the previous example. Thus, with the higher airflow the Laskin atomizer could be used to supply a chamber approximately six times larger than could be supplied with the Vaponephrin nebulizer.

In fact, the Laskin atomizer has been used to supply aerosols to the large 125-cubic foot chambers previously described. For this application it was built into the apparatus as shown in figure 21. In this assembly the larger flask at the bottom acts as a reservoir for the solution. The wide-bore capillary tubing connecting this flask with the upper flask performs as an airlift pump, constantly raising a small amount of liquid into the top flask which contains the atomizer.

The two flasks are also connected by a center tube which allows the liquid to return to the reservoir when the liquid level in the reservoir falls and thus reduces the air pressure in the space above it. This airspace in the top of the reservoir flask, therefore, acts as a pressure chamber controlling the level of liquid that is maintained in the atomizer chamber. The tubing connections on the outlet of the aerosol generator serve to deliver the aerosol to the exposure chamber, and also provide a degree of elutriation to eliminate any large droplets that might be carried over from the atomizer.

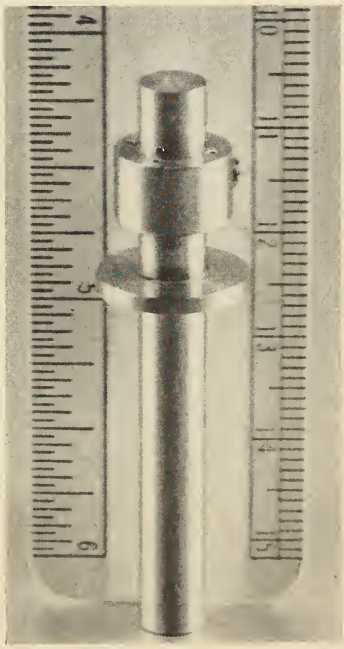


Figure 20. Laskin atomizer showing radially drilled hole in center shaft and capillary holes in metal collar around shaft.

A device which has been developed by the military for atomizing large quantities of liquids is a modification of the so-called Hartmann whistle. This is an ultrasonic device in which a jet of compressed air at a pressure of about 40 psi is directed into a cavity of resonant dimensions producing a sound wave of high frequency, greater than 25 kilocycles. The liquid is introduced into the air jet at a rate of 50 ml. per minute and is instantly atomized into a dense mist of fine droplets. When extremely high mist concentrations are desired this device may be useful; aerosol production is sufficiently great so that unwanted size fractions can be rejected and enough particles of the desired size retained to disperse into the exposure chamber.

Distribution

The difficulties encountered in obtaining uniformity of concentration of a liquid aerosol throughout the entire volume of the exposure chamber are similar to those previously described for dusts. The problems are accentuated inasmuch as the liquid droplets are often produced having larger, though perhaps more uniform, diameters. The problem is further complicated by the presence of the gaseous phase of the contaminant although the greatest mass of contaminant is concentrated in the condensed phase; if this is not the case, the exposure may be considered to be an exposure to a vapor contaminant and treated as indicated in a later discussion.

Losses

All of the factors discussed in the section on dust dispersions which contribute to the loss of aerosol from the chamber atmosphere apply equally to dispersions of liquid droplets. Because dispersed liquid droplets are usually larger than similarly dispersed dust particles, and are of spherical shape, the effect of settling can be calculated more accurately by Stokes' Law, and the Cunningham correction becomes of less importance.

Diffusion and Brownian motion will be less important in considering a dispersion of liquid droplets than in the case of dusts. This is due, again, to the relatively larger median size of the liquid droplets. However, centrifugal forces developed in sharp bends or curves in

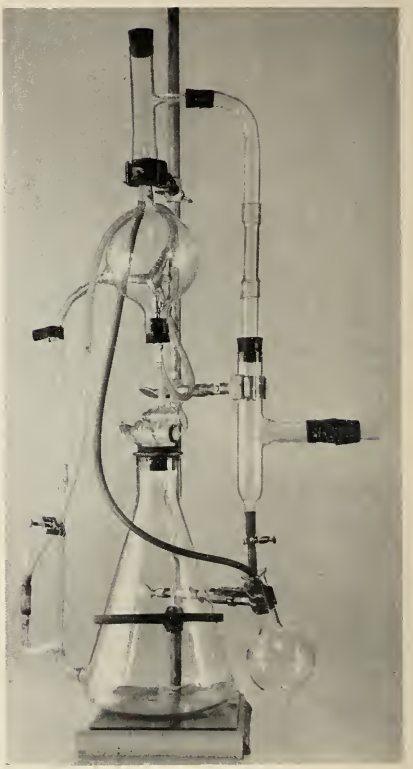


Figure 21. Glass aerosol generator incorporating Laskin atomizer, a large reservoir flask, an air-lift pump, and wide-bore tubing to connect generator to the chamber.

the ductwork leading to the exposure chamber may cause significant losses of droplets from the liquid aerosol dispersion. It was seen in table 2 that the loss of aerosol between the chamber body and the exhaust plenum was greatest in the case of the liquid $CoCl_2$ particles.

Because of the decreased Brownian motion of the liquid drops, flocculation will play a less important part in reducing the concentration of liquid particulates. The effects of flocculation can easily be overlooked, however, in liquid dispersions because collision of the drop-

lets forms a larger drop rather than chains or agglomerates of particles. Thus, in sampling a liquid aerosol it is possible to obtain a false impression of the particle-size distribution unless care is taken to prevent coalesence in the sampling instrument.

Chamber Response

The time required for the chamber to respond to changes in concentration of a liquid aerosol will be similar to the time required for solid particulates. The same formula can be used to calculate the time required to attain a desired concentration or to clear the chamber. In using this formula it should be noted that it was developed to describe the mixing of gases rather than particulates. Because the size of liquid droplets is generally larger than solid particulates the inaccuracy of applying this formula to liquid dispersions will be even greater than its application to solid dispersions.

Particle-Size Distribution

Probably the most important physical characteristic that distinguishes a dispersion of liquid droplets from an aerosol composed of solid particles is the fact that the former is in a state of dynamic equilibrium whereas usually the latter is in a state of static equilibrium. The greater vapor pressure of the liquid droplets means that a process of evaporation is constantly taking place. Thus the diameter of any single liquid droplet is not fixed. That the vapor pressure of the liquid itself is dependent on the diameter of the drop is shown in the following equation.

$$\ln \frac{P}{P_o} = \frac{2\gamma M}{r\rho RT}$$

where

P = vapor pressure over a convex surface
P_o = vapor pressure over a flat surface
γ = surface tension of the liquid
M = molecular weight of the vapor
r = radius of the drop
ρ = density of the liquid
R = gas constant
T = absolute temperature

From this equation it is seen that small drops may have considerably higher vapor pressures than larger droplets. As a consequence, the smaller droplets are evaporating more rapidly than the larger ones. If a condition of equilibrium exists and the air is saturated with the vapor of the liquid, it may be said that the larger droplets will grow in diameter at the expense of the smaller ones. Because of the small size of the liquid droplets, this condition of equilibrium is rapidly attained.

The choice of sampling instrument becomes a matter of utmost importance and must be considered with extreme care inasmuch as the conditions which exist during the course of sampling, and after collection, are seldom the same as those in the exposure chamber. In particular the time lapse between the collection of the sample and the examination or analysis must be kept to a minimum.

In the examination of a sample collected from a liquid aerosol many common analytical techniques must be excluded. For instance, the electron microscope is not applicable to this type of sample. If the sample has been collected on a glass microscope slide for optical examination, care must be taken that no film exists on the surface of the glass slide which would alter the contact angle of the droplets on the clean glass. Because of the extreme difficulties involved in optical examination of the liquid droplets other analytical techniques are often preferable.

The cascade impactor (*33*) may be useful in this situation in conjunction with a rapid colorimetric method for the determination of the mass of liquid collected on each of the impactor stages. The newer light-scattering techniques may also be useful for determining concentrations of liquid aerosols once the instrument has been calibrated. When the cascade impactor is used along with a chemical analytical technique, the background or nuisance dust does not interfere with the determination. However, with the light-scattering techniques the background dust contributes directly to the amount of light scattered by the liquid aerosol and thus must be discounted.

Characteristics of Vapors and Gases

Of the three states of matter the gaseous state presents fewest difficulties in the maintenance of animal inhalation exposure levels.

The problems which arise are usually associated with the chemical characteristics of the noxious agent rather than with its physical state.

After the contaminant has been thoroughly mixed with the diluting air to form the chamber atmosphere, it may be assumed that a stable condition exists. The contaminant is molecularly dispersed in the air of the chamber atmosphere and the problem of uniformity of distribution throughout the volume of the chamber is greatly simplified.

If the distribution of the air itself throughout the chamber can be assumed to be satisfactory, which can readily be determined by the introduction of phosphorus trichloride fumes, the gaseous contaminant may also be assumed to be uniformly distributed.

None of the factors which contribute to the loss of solid or liquid particulates from the atmosphere affects the gaseous contaminant. The only mechanisms causing the depletion of the gaseous contaminant are chemiadsorption reactions characteristic of the particular contaminant. Such reactions may constitute a significant reduction in the ambient concentration of the test agent. To this end, it may be necessary to analyze carefully the gaseous contaminant before and after its residence in the chamber. It may also be necessary to determine whether the agent has been changed chemically by air oxidation or hydrolysis, and to establish the exact nature of the material to which the animals are being exposed. This may often be done conveniently with infrared spectrophotometry by making a direct comparison of the absorption spectra of the parent material and the diluted material. If the interest of the experiment lies in a particular functional group a simple chemical test for this functional group may suffice. Should it be found that appreciable decrease in concentration of the agent has occurred for any of the above reasons, increasing the flow rate to compensate for the loss is the remedy.

If the exposure is to be to one of the more common gases, it may be possible to purchase the gas in the liquefied state in a pressure tank. If this is possible a pressure reducing valve, a needle valve, and a flowmeter are all that is necessary to inject a measured amount of the gas into the chamber air supply. It is still necessary, however, to sample the chamber atmosphere in the breathing zone of the animals to establish the actual concentration of the contaminant to which they are exposed.

Physical Environment

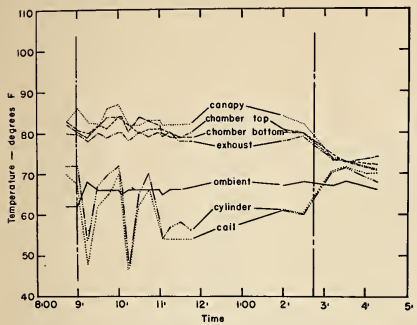
In carefully performed inhalation toxicity studies the physical environment of the animals assumes a position of importance equal to the careful control of the concentration and size-distribution of the test agent.

At high temperatures and humidities the respiratory pattern of the animals will be changed from the normal slow rate of breathing and deep inhalation to the rapid panting and shallow breathing often seen in dogs at elevated temperatures and humidities. This will alter the tidal volume of the animals and thus affect the amount of air contaminant inhaled and retained in the lungs. It will also seriously affect the pattern of deposition of the contaminant in the lung by preventing penetration into the lower lobes, resulting in a considerable portion of lung unexposed to the contaminant. The result of this altered respiratory pattern will vary from species to species and will depend on the characteristics of the inhaled substance.

Unless the effects of the physical environment on the exposed animal are being studied specifically, the temperature and humidity conditions in the inhalation chamber should duplicate as nearly as possible the conditions for normal animal comfort. Rats and mice thrive best at temperatures somewhat greater than that for dogs and rabbits. A range of temperature from 75° to 80° F. (21° to 23° C.) and a relative humidity of about 50 percent are a reasonable compromise. The metabolic heat generated by the animals and the moisture added to the air are both appreciable, even when the metabolic state of the animals approaches the basal condition.

In a previous discussion, under design considerations, it was pointed out that the normal animal complement in a 145-cubic foot chamber develops a metabolic heat load of almost 500 kg. cal/hr. and approximately 4,000 grains of water per hour are added to the chamber atmosphere. It was shown that a ½-ton refrigeration unit would be required to

Figure 22. Fluctuations in temperature at locations in chamber system shown in figure 15 (letters A–G) during a typical operating day.



cool and dehumidify such a chamber atmosphere to maintain reasonable control of the temperature and humidity. Figure 22 shows the temperatures found at various positions in the chamber system during a typical operating day.

From the viewpoint of the toxicologist it is not enough that he be assured that a given chamber is operating normally. He must assure himself that the conditions are satisfactory by actual measurement and recording of these observations. The temperature within the chamber can be measured simply and accurately by hanging a thermometer inside so that it can be read from the outside through a window or sampling port. Dial-reading remote thermometers may conveniently be installed, but as with all more complicated indicators they should be checked periodically against the primary standard, the mercury thermometer.

The wet-and-dry-bulb psychrometer is the primary standard for measuring relative humidity. Many more convenient secondary instruments are available but all of them depend on some phenomenon such as the expansion and contraction of human hair, or the electrical conductivity of a hygroscopic ceramic surface. The sensing elements of these instruments soon adsorb films of the agent under test and animal byproducts, and thus become unreliable.

The only reliable instrument, therefore, is the wet-and-dry-bulb psychrometer which can be inserted through a sampling port and read several times a day. If the psychrometer is mounted inside the chamber it must be checked frequently to determine that the wet bulb is actually saturated with water. The knitted boot of the wet bulb should be cleaned at least once a week and the psychrometer should be checked against a standard, kept clean, and in another room separated from the animal quarters.

Recording thermometers and hydrometers are available and serve well if their accuracy is checked frequently against the primary standards. They are usually equipped with a 24-hour clock motor and have the additional advantage that the chart serves as a permanent record of chamber conditions to which can be added other daily chamber data. Usually only a portion of the 24 hours is used; accordingly a strip chart that can be stopped when the chamber is not in use is more economical than the more common circular chart.

Another aspect of the physical environment of the animals that should be considered is the air pressure maintained in the chamber. It is customary to operate exposure chambers at a slightly negative pressure. If there is a small leak in the chamber, it is thus assured that room air will flow into the chamber rather than the noxious agent flowing outward into the room.

The negative pressure required is obtained by exhausting air from the chamber at a slightly greater rate than it is supplied. The chambers are ordinarily almost airtight so that this differential adjustment of pressure is rather critical. In practice, a tenth of an inch of water has been found to be an ample negative pressure to maintain and, with the chamber air supply adjusted to the desired airflow, the exhaust valve is merely adjusted to give the desired 0.1-inch pressure within the chamber.

The construction of a satisfactory gauge sensitive to such a small pressure differential posed several problems. The ordinary water manometer, even when inclined to an angle of 20° can be read only with difficulty and as the water becomes contaminated it will often not reproduce a reading within the limits of a tenth of an inch. The most successful gauge has been found to be a diaphragm, mechani-

cal-linkage, type which is sensitive to 0.01 inch of water pressure changes and can be read easily to this same pressure (*A*).

Some variation in chamber pressure is unavoidable while the airflow is being adjusted but continued operation at either high negative or positive pressures should not be permitted; the latter, because of the obvious hazard of exposing personnel, and the former, to avoid animal damage, and finally, in either instance, to insure that conditions simulate those of normal human working conditions.

Chemical Environment

Factors of concern in the chemical environment are, in general, and in order of decreasing importance, oxygen, carbon dioxide, and ammonia. Oxygen content of the exposure atmosphere should be maintained at the normal 20-percent value. If, for economical use of the test agent, flow rates through the chamber must be reduced, this reduction should not result in an atmospheric content of oxygen below 15 percent.

Similarly, airflow rates should not be such as to permit increases in carbon dioxide to reach levels that alter the respiratory pattern of the animals with its resultant effect on intake of the test agent.

With sluggish air change, ammonia liberated from animal excreta can similarly accumulate to undesirable levels. Ammonia, even more than carbon dioxide, may be undesirable for two reasons. Respiratory patterns can be altered appreciably by ammonia. And like carbon dioxide, ammonia through chemical combination with an appropriate test agent may alter the response of the reagent under test. The degree of the effects is dependent on the level at which the test agent is used; at air pollution levels, for example, inadequate control of carbon dioxide and ammonia concentrations could vitiate the results of an entire study.

Sampling

In the discussion concerning factors involved in the loss of the contaminant from the chamber atmosphere it was shown that the only reliable estimate of the concentration of test agent in the chamber atmosphere is obtained by sampling near the breathing zone of the animals. This sampling, however, must be done with a suitably selected instrument and a technique capable of furnishing the information with the desired accuracy.

Not all of the available air-sampling instruments will be described; these instruments and instructions for their calibration and use are given in a number of standard texts on industrial hygiene and air pollution (*24*, *25*, *34*). Most of these instruments have been used in this laboratory at one time or another but two of them, the midget impinger and the filter paper dust sampler, have been generally found useful and are adaptable to most sampling needs.

The midget impinger (*35*) is an all-glass apparatus through which air is sampled at high velocity and impinged from a jet onto the flat glass bottom of the flask which is covered by the absorption medium, usually water. The dust particles are momentarily arrested by the impingement process, wetted by the liquid, and thus trapped. The flow rate of 0.1 cubic foot per minute is critical since the air must pass through the jet at near sonic velocity. The dimensions of the jet and its distance from the impingement plate are likewise critical.

The collecting efficiency of the impinger has been investigated both theoretically and experimentally by Davies, Aylward, and Leacey (*33*) and found to decrease rapidly for particles smaller than 0.7μ in diameter. Many gases that are soluble in the absorbent are collected with high efficiency so that, in some instances, this instrument will often outperform the classical fritted bubblers.

Probably the most useful sampling instrument for particulates is the brass filter paper holder shown in figure 23. The original drawing for this sampler was made by Laskin at the University of Rochester Atomic Energy Project, but similar models were used for many years previously by Fairhall and Sayers (*36*), and others. The addition of the 100-mesh supporting screen which permits the use of the Millipore filter was made by Marsh of this laboratory.

When the Millipore filter is used, a sampling rate of 0.1 cubic foot (2.7 liters) per minute is convenient. This permits the same pump to

Figure 23. Brass filter-paper sampling head.

NOTE: Filter paper or Millipore filter is placed between the brass ring and the supporting screen.

be used as a source of suction for either the impinger or the filter sampler. When filter paper is used, a flow rate of approximately 18 liters per minute is required to produce a face velocity of 100 linear feet per minute through the 0.786-inch^2 filter (1.0 in. diameter). This face velocity has been shown to be most efficient for the retention of small particles by filter-paper media (*37*).

Whatman paper No. 41 has been found to be a surprisingly efficient medium for air sampling even for small particles (0.1μ). Comparison with the Millipore filter, which can be accepted to approach 100 percent efficiency, indicated an efficiency of 97 percent for this analytical grade paper (*38*).

This type of paper has been used to determine the mass concentration of an aerosol in a chamber by merely weighing the filter before and after sampling. In most cases, however, the filter is extracted or digested and a more specific, colorimetric chemical determination is made.

The Millipore filter lends itself readily to chemical procedures because of the high degree of purity of the filter. Being less hygroscopic than paper, it is better suited to direct weight determination of mass.

If particle-size analyses are desired, techniques have been described using the Millipore filter sample for both optical (*39*) and electron microscopy (*40–42*). It is also useful for X-ray diffraction analysis of samples, providing less background than the usual technique which involves spreading the sample on glass slides (*43*).

It is convenient to have a source of suction, such as an extension of the laboratory vacuum line, available at the chamber control panel along with a pressure gauge and a flowmeter having a range up to about 20 liters per minute.

An equally convenient alternative is a portable unit consisting of a vacuum pump, flowmeter, pressure indicator, and a timer which can be set to turn off the pump after the desired sampling time. This unit when built into a box about the size of an 8-inch cube can be easily carried from chamber to chamber. A small diaphragm-type pump which delivers 0.1 cfm through both the impinger and Millipore filter has been found to be satisfactory (*E*).

Standard Sampling Procedures

A knowledge of the technical aspects of sampling is of the utmost importance for determining the actual chamber concentration at any given time. Thus a thorough understanding of the instrument used, and the technique to be followed, must be assumed.

The collected data will yield a valid picture of the performance of the chamber over a long period of time only if a well organized and integrated program of sampling has been followed. To encourage uniformity, general suggestions have been issued to guide the project leaders as well as the chamber operators. It is felt that the following instructions are self-explanatory and may emphasize some important points that deserve consideration.

Need for Standardization

Standard procedures of sampling the chambers are necessary for the following reasons:

1. To assure proper weighting of the concentration values in calculating the average concentration daily, weekly, and for the entire exposure.

2. To assure uniformity so that the same techniques are used in all experiments for any substance at different exposure levels.

3. To afford a set procedure which will serve in

emergencies when specific instructions from the project leader cannot be obtained.

4. To enable the project leader and section chief to be informed completely on the procedures in use.

Specific Instructions

1. Samples should be taken at specified and uniform intervals throughout the exposure day as determined by the project leader. The time of sampling should be noted on the daily worksheet (fig. 24) along with the data on flow rate, length of time of sample, comments on general appearance of sample, and an estimation of the chamber concentration.

2. If a sample deviates appreciably from the normal, a repeat sample should be taken immediately and the record clearly marked Repeat Sample. Such repeat samples will not be included when computing the average concentration unless they are obviously more representative of the overall concentration than the normal sample.

3. If the repeat sample also deviates from the normal, allow time for the chamber to return to equilibrium and sample again at next specified time. If sample is low, check feed mechanism to see if it is operative.

4. If the chamber concentration continues to deviate from the normal over a 2-hour period ask the advice of the project leader. If this is not possible, make minor adjustments to the chamber airflow to attempt to return the concentration to the desired level. *Note in detail* on the worksheet any such adjustments made *and the results.*

5. Take as many repeat samples as necessary, but be sure to mark them as such on the worksheet.

6. If the chamber concentration deviates more than 50 percent from the desired level, and advice is not available, stop the exposure and remove the animals from the chamber on the basis that it is better to lose an exposure day than to ruin the entire experiment. An uncontrollable variation of more than four or five times the normal daily deviation should be suspect.

7. Filter paper samples are normally taken at a sampling rate of 18 liters per minute (14.5 on flowmeter). Millipore filter samples and midget impinger samples are taken at 2.7 liters per minute (8.0 on flowmeter). These sampling rates should not be changed except on specific instructions from the project leader and then the changes should be noted clearly on the worksheet.

8. Never take a sample less than 30 minutes after the chamber feed has been started.

9. At each sampling time check all other chamber controls and readings, and record at least once a day. The chambers should be maintained at approximately 0.1 inch of water, negative pressure. Under no circumstances allow the chambers to operate under a positive pressure.

10. Samples are taken routinely at the breathing zone of the animals with the sampling head attached to a short copper tube and extending 12 to 18 inches inside the chamber wall.

11. Occasionally simultaneous samples should be taken in the exhaust duct and at the normal sampling position to serve as a check on excessive losses and nonuniform distribution in the chamber. Once or twice weekly should be sufficient.

12. Once a week an all-day sample should be taken in the control chamber and on another day an all-day sample of the room air in the exposure room. These will serve as a check on leakage from the chamber, and establish the background of exposure for the control animals and of personnel. All of these samples should be clearly marked and filed with the permanent chamber record.

13. When the chambers are started in the morning make sure that the water in the scrubbing column behind each chamber is turned on as well as the Precipitron unit in the central exhaust system. Turn these off when the chambers are shut down.

The above instructions should serve as a guide to be followed when individual samples are taken by the chamber operator. It has been the custom in this laboratory to take hourly samples, or five samples during a 6-hour exposure. These samples may be analyzed by the chamber operator following a simplified routine procedure or turned over to the analytical laboratory for a more detailed evaluation involving X-ray diffraction analysis, infrared determinations, or other analytical procedures.

In any event there may be a considerable time lapse between the taking of the sample and delivery of the results. Thus the samples may serve rather as a record of the conditions that prevailed in the chamber than as an effective means of control. Automatic sampling devices may serve more effectively as a means of controlling chamber concentration.

Automatic Sampling Devices

There are a number of instruments available that can measure continuously and directly the concentration of a contaminant in the atmosphere. If this cannot be done directly in a particular case an instrument can usually be modified to accomplish the purpose by indirect means. One such instrument is the Thomas Autometer (*44*) which continuously analyzes and records the atmospheric concentration of sulfur dioxide. By merely changing the reagents this instrument could be made to measure at least empirically a number of other chemical vapors.

There is a continuously operating residual chlorine recorder which is used by water departments to determine trace amounts of chlorine at various distances from the chlorination plant. The chamber atmosphere could be bubbled through a constantly flowing stream of water and the resulting solution analyzed by a modification of this instrument.

The great interest in air pollution has led to the development of automatic and recording analyzers for oxidants and several other atmospheric contaminants (*45–47*) which may be useful to record the concentration of ozone or other substance to which the animals are being exposed.

The adaptation of these instruments to chamber work is greatly simplified by the fact that the only impurity ordinarily found in the chamber atmosphere is the contaminant which has been added. Also the instrument need not give direct readings but can be calibrated empirically by exact chemical methods and a reading that is only proportional to the contaminant concentration may be wholly satisfactory.

For particulate material there is available commercially a light-scattering photometer which measures the light scattered by the particles under dark field illumination by means of a photoelectric cell and a logarithmic amplifier (*F*). This instrument has been used successfully to monitor the concentration of airborne silica particles over a 2-year period, the chemical analysis of which would have involved a long and tedious procedure.

Newer electronic instruments based on similar principles have recently been developed (*48*), and have much greater stability and sensitivity so that instrumental error is negligible and erroneous response practically impossible.

Instruments of the type mentioned above may be considered expensive, ranging from $500 to $2,500 in cost, but the cost can be amortized rapidly when account is taken of the time spent by a skilled chemist analyzing the five routine samples daily and the equipment and reagents that are used.

In addition to the economic considerations, there are certain other advantages in the use of physical instruments that inevitably point to their assuming a prominent position as the science of inhalation toxicology continues to grow. The immediate response of these instruments enables the operator to know at all times the concentration in the chamber and therefore make the adjustments necessary to maintain the desired concentration. The instrument chart showing the chamber concentration becomes a part of the permanent record of the experiment.

But probably the most important offering of the direct-reading instruments is that their instantaneous response provides a means for developing automatic control of the chamber feeds. This is the natural result of instrumentation and will be the next important development in the design of animal inhalation exposure chambers.

Warning Devices

The importance of maintaining the desired conditions in the chamber within known limits requires the use of warning devices to indicate to the operator if the specified limits have been exceeded. With the increasing dependence on automatic sampling devices and the decreased attention paid to the chamber by the operator, these warning devices become more and more necessary.

To insure the maintenance of a negative pressure in the chamber, a differential pressure switch may be mounted on the chamber and used to control two lights (*G*). If the pressure rises to less than 0.1 inch a red light comes on. If the negative pressure is greater than 0.1 inch a green light indicates that the chamber is operating satisfactorily. This switch is sufficiently sensitive that opening the chamber door and consequent attainment of atmospheric pressure will cause the red light to come on and remain on. Thus the operator is also informed of past deviations from normal operation.

The same type of switch connected between the central exhaust and central air-supply ducts will immediately indicate failure in either of these systems. This indicator should control a bell-alarm system rather than lights because of the seriousness of affecting all the chambers on the line. The device also protects against inadvertent turning off of one or

both main blowers by a contractor or temporary workman who would not be aware of the gravity of such an action.

Similar devices might be used to indicate excessive temperatures or malfunctioning of the air-conditioning system. Whatever device is used to give warning of a detrimental condition, the device should be a measure of the actual environmental factor under consideration, such as temperature, pressure, or humidity, and not a related quantity such as electrical power consumption, coil pressure, or airflow.

Loading of Chambers

Early experimenters placed their animals in inhalation chambers without restraint, allowing them to roam freely about the floor of the chamber. It is now considered better practice to place the animals in separate cages no larger than necessary to contain the animal comfortably. For example, expanded steel mesh cages about 18 inches in each dimension have been found to be satisfactory for most dogs and monkeys. Rabbits require cages made of ½-inch hardware cloth about 9 inches by 9 inches by 15 inches. Individual rat cages are made of ¼-inch hardware cloth and are about 3 inches by 3 inches by 6 inches. But for convenience in handling, a single tray containing up to 20 such enclosures can be fabricated from the four-mesh screening reinforced with angles of galvanized sheet iron. With these three cage sizes most species of animals can be accommodated.

Individual cages are advisable to overcome the tendency of certain species to nuzzle into each other's fur and thus effectively filter particulates out of the air that they breathe, also to prevent fighting and, among some species, cannibalism.

Normally the cages containing the larger animals are placed in the bottom layer with the smaller animals tiered above, separated by board slats, and without drainage pans which would alter the dust distribution in the chamber atmosphere.

From day-to-day the positions of the individual animals in the chamber are rotated as much as possible to compensate for any undetected variation in air distribution.

As discussed earlier, the body volume of the animals placed in the chamber should not exceed 5 percent of the total volume of the chamber. If cages of the dimensions given above are used to contain the animals it is found to be nearly impossible to exceed this 5 percent.

In designing the animal cages, and in fact the chamber itself and all of its associated equipment, consideration must be given to the safety of personnel and the animals. Sharp edges and unfinished sheet-metal work should be avoided since cuts and scratches from metal contaminated with toxic agents and animal excreta may be hazardous. The chamber door should be large enough to provide easy access and should be padded, if necessary, to prevent injury to the head, fingers, arms, or back of the person loading it. Carts and dollies used to move the animals from their living quarters to the chamber should be of sufficient height so that the workers will find bending reduced to a minimum, thus decreasing the incidence of fatigue and low back injuries.

Before beginning an exposure, the time required to clear the chamber of the toxic material should be calculated or determined by actual measurement. The operator should not open the door to remove the animals until the calculated time has elapsed after stopping the dust feed. Thus, unwarranted exposure of the operator can be eliminated.

Records

The methods used for recording data will vary with each laboratory according to the custom and experience of the personnel. Some of the forms and methods used in this laboratory will be discussed and may serve as a guide to newcomers in the field.

For short-term exposures (30 days or less) the daily worksheet shown in figure 24 is kept on a shelf beside the chamber and the entries are made as the sample is taken. These sheets are punched for insertion in a looseleaf notebook. When an occasion arises to refer to a study after it has been completed there is no substitute for having the original worksheets on file. For long-term studies (more than 30 days) a mimeographed stencil may be cut and this, or a more appropriate form imprinted on

Figure 24. Daily worksheet for recording sampling data and operating conditions.

DAILY CHAMBER RECORD

10-5-54

STUDY W-25-05-54-2489-000

CONCENTRATION 0.50 mg./M³

WEEK 1 DAY 2

EXPOSURE - ON: 0915

OFF: 1515

TOTAL TIME: 6 hrs.

CHAMBER NO. C-1

CHAMBER AIR INTAKE SETTING 42.5 CFM

AEROSOL GENERATOR: AIR FLOW 10 L/min. PRESSURE 20 lb./in.² TEMP. 78°

ANALYSIS PERFORMED BY: P. W.

METHOD: Phosphotungstic acid

Item	Sample Number						Average
	1	2	3	4	5	6	
TIME SAMPLE TAKEN	0955	1055	1230	1330	1430	1455	—
TEMPERATURE (°F)	80	79	79	78	78	79	79
RELATIVE HUMIDITY (%)	78	75	74	75	74	74	75
SAMPLING TIME (min.)	20	20	20	20	20	20	—
RATE OF SAMPLING (L/min.)	18	18	18	18	18	18	—
OPTICAL DENSITY	18	18	17	17	17	17	—
mg. CONSTITUENT	0.13	.13	.12	.12	.12	.12	—
CHAMBER CONCENTRATION (mg./M³)	0.36	.36	.33	.33	.33	.33	.34

AV. CONCENTRATION 0.34 mg./M³ DEVIATION .3

REMARKS & COMPUTATIONS: Control chamber — av. temp. 76° F

the pages of a bound record book, may be used to record the samples sent from the chamber to the analyst's bench and back to the chamber.

The chamber-data sheets, one of which is shown in figure 25, constitute a permanent record of the entire experiment. Each day the results from the daily worksheets are entered, giving the average concentration and the range, as well as the species-by-species record of the number of animals placed in the chamber and the number of deaths occurring during the exposure that day. For convenience the animal species are coded numerically. A column

Figure 25. Chamber data sheet.

Sheet No. 3

EXPOSURE CHAMBER CALENDAR

Expt. Title: Cobalt by inhalation

Expt. Code: F-002-10-I-002Y-9

Special Data: -8, -9, -4

Date M D Y	Calendar Day	Exposure Day	Hrs. of Exposure	Exposure Concentration Range	Exposure Concentration Mean	Cumulative Hours of Exposure	Av. Temperature	Rel. Humidity, %	Number of Animals Exposed by Species 1	2	3	4	5	6	7	8	9	Mortality 1	2	3	4	5	6	7	8	9	Weight	Hematology	Clinical Chemistry	Pathology	Remarks
3-19-52	51	36	6	.22-.40	3.27	217	81	42				6			12	4	15				0			0	0	0					Continuous rabbit 109 died
3-20-52	52	37	6	.11-1.7	.60	223	80	41				6			12	4	15				0			0	0	0					
3-21	53	38	6	.37-.88	.48	229	82	42				6			12	4	15				0			0	0	0					
3-22																															
3-23																															
3-24	56	39	6	1.0-2.4	1.80	235	84	46				12			26	10	30				0			0	0	0					Int. and cont. groups
3-25	57	40	6	2.0-2.6	2.52	241	83	49				12			26	10	29				0			0	0	2					cont. rabbit 9 died - Rat 2 killed
3-26	58	41	6	1.7-2.2	2.11	247	84	44				12			26	10	28				0			0	0	0					
3-27	59	42	6	.72-1.3	1.16	253	85	48				16			29	10	33				0			0	0	0					cont., int. and prelim. expts.
3-28	60	43	6	1.0-4.0	2.29	259	85	50				16			29	10	33				0			0	0	0					End exposure 9 w. rats until 4-28-52 1 cont. mouse died
3-29																															
3-30																															
3-31	63	44	6	.28-1.0	.57	265	79	47				6			11	4	13				0			0	0	0					Continuous groups
4-1-52	64	45	6	2.0-2.8	2.47	271	80	45				6			11	4	13				0			0	0	0					
4-2	65	46	6	.33-3.0	1.08	277	81	38				6			11	4	13				0			0	0	0					
4-3	66	47	6	.85-1.7	1.17	283	80	38				6			10	4	13				0			1	0	0					1 cont. mouse died
4-4	67	48	5	.22-2.2	1.21	288	83	38				6			10	4	13				0			0	0	0					
4-5																															
4-6																															
4-7	70	49	6	.33-1.5	1.04	294	79	38				6			10	4	13				0			0	0	0					Cont. groups
4-8	71	50	6	.27-1.5	.74	300	79	38				6			10	4	13				0			0	0	0					
4-9	72	51	6	.44-1.7	1.02	306	79	43				6			10	4	13				0			0	0	0					
4-10	73	52	6	.23-1.9	1.23	312	79	42				6			10	4	13				0			0	0	0					

NOTE: All data required by the toxicologist are recorded on this form to serve as a permanent record of exposure of animals.

is also provided for recording information on items such as chamber breakdowns, accidents, special treatment of animals, and unusual animal behavior.

From this sheet the toxicologist and pathologist make their evaluation, and therefore the need for accuracy, completeness, and neatness cannot be overemphasized.

It has been the custom in this laboratory to make photostatic copies of these sheets and to distribute them among the several interested workers, project leader, and section chief for each to study and file. When automatic or recording instruments are used to monitor the chambers, the charts yielded by the instruments are evaluated and the results entered on the chamber data sheet as soon as possible. The charts are then filed by the project leader along with the daily worksheets and other information or procedures pertinent to the study.

IV. Review of Literature

Controlled animal inhalation exposure studies were described as early as 1865 by Eulenberg (*13*), who used a cubical wooden chamber, 12¾ inches on a side with two glass walls, for the exposure of small laboratory animals to high concentrations of numerous toxic and asphyxiant gases. The inner walls of the chamber were coated with varnish which contained rubber. Auxiliary equipment used included a gasometer for measuring flow rates and a manometer for measuring chamber pressure. The airflow was driven by water displacement.

In 1875–76 von Jns (*14*) reported the use of a carefully designed chamber for dust exposure of small laboratory animals. The chamber was a total-enclosure type 20 cm. by 20 cm. by 10 cm. The dust feed was an ingenious arrangement of a mechanical shaker on a dust-filled funnel, from which the dust was dispersed by a motor-driven bellows into the exposure chamber. The chamber was used to study the effects of the inhalation of diatomaceous earth.

Lehmann and his associates published numerous articles on inhalation toxicity. The first work was done at the Hygienic Institute in Munich, and the bulk of the work at the Hygienic Institute of Würzburg. The first paper in the series, by Lehmann (*15*) in 1886, presented an illustration and description of an exposure apparatus. The chamber was rectangular with glass walls, and was used for the dynamic exposure of cats, rabbits, guinea pigs, and frogs. Although the exact size of the chamber was not given, its volume was apparently about 200 liters. A metered airflow of 5 to 50 liters per minute was used, the air driven by water displacement.

Reports on inhalation experiments by Lehmann's group in which exposure chambers were described and illustrated include papers by Dubitzki (*16*) in 1911, describing an inhalation chamber for gases; by Saito (*49*) in 1912, describing a dust exposure unit; by Lehmann, Saito, and Majima (*17*) in 1912, describing a mist exposure unit; and by Lehmann and Hasegawa (*18*) in 1913, describing an additional gas and vapor exposure unit.

Dubitzki (*16*) used a glass chamber formed by placing two glass cylinders end-to-end, for a study on arsenic hydride. Innovations included a circulating fan in the chamber, an absorption train for the chamber exhaust, and an exhaust pump as the air mover.

Saito showed a thorough appreciation of the problems of dust-chamber requirements (*49*). His apparatus was a dynamic flow, head-exposure unit for dogs and rabbits. The airflow was downward through a vertical cylinder through which the animal heads protruded. The dusty discharge air was passed through a scrubber before discharge. In experiments with white lead, concentration profiles in the exposure zone showed good uniformity and consistency. Considerable attention was paid to the complete elimination of leakage, to the necessity of preexposure, and to the importance of constant known conditions.

A total-enclosure exposure unit for exposure

to mists, described by Lehmann, Saito, and Majima (*17*), was constructed from two 12-liter glass cylinders joined at their open ends and placed horizontally. The airflow was metered at the inlet, and the chamber concentration was sampled at two points, near the inlet and at the exhaust.

Lehmann and Hasegawa (*18*) used the same type of glass chamber in work with nitrogen oxides. The gas under test was forced into the exposure unit by hydrostatic pressure, and the exiting gases drawn through analytical scrubbers by means of a pump. Metered air of known contaminant concentration was passed through the unit. Attention was paid to the importance of chamber design and accessories.

The crudity of some of the subsequent work was in sharp contrast to that of these early investigators. In the early work, and in fact in much of the contemporary work on animal inhalation, a definite distinction was made between exposures of animals to particulates and to gases because the chambers required for dust exposures posed the more difficult problems of dust generation, distribution, and control.

The chamber used by Mavrogordato for dust exposure in 1918 (*50*) and the one used by Gardner in 1920 (*51*) were surprisingly crude in comparison with the previously described dusting chambers of von Jns (*14*) and Saito (*49*). The later chambers contributed nothing of note to animal inhalation chamber design or construction. A simple wooden box was used by Mavrogordato to expose guinea pigs to coal, shale, flue, and flint dust. A two-bladed electric fan dispersed the material contained in a wooden trough. Dust concentrations, varying from 27,000 to 45,000 mg./m.^{3}, were determined by inserting a cotton-plugged tube in the side of the chamber and withdrawing a volume of dusty air. The chamber used by Gardner was a box containing animals on trays in the upper portion, and a barrel of finely divided granite in the bottom which was agitated by a paddle wheel. To compensate for the variation of dust concentration with location, the animals were placed in different positions each day.

Marshall and Kolls in 1919 (*52*) described a total-enclosure chamber for gaseous exposure which was claimed to be an improved version of Lehmann's apparatus. It was a plasticene sealed glass, 130-liter box with a sliding door for easy access.

Lehmann's textbook on industrial hygiene published in 1919, Kurzes Lehrbuch der Arbeits- und Gewerbehygiene (*53*), illustrated on page 108 the dust inhalation chamber of Saito (*49*), on page 125 the droplet inhalation apparatus of Lehmann, Saito, and Majima (*17*), and two gas and vapor inhalation chambers. One apparatus shown on page 134 was a modification of Lehmann's (*15*) for use with the vapors of volatile liquids, and incorporated the improvements of Dubitzki (*16*). Another apparatus, on page 136, was a modification of Dubitzki's (*16*) for use with mixtures of very toxic gases.

A total-enclosure gassing chamber of 512 liters' capacity was described by Walton and Jones in 1926 (*54*). With a chamber larger than most previous ones, it was hoped to lessen wall effects. Provision was made for rapid air change in the chamber to reduce the time for attainment and for purging of chamber concentrations.

A novel group of three dust exposure chambers was described by Jötten and Arnoldi (*55*) and Jötten and Kortmann (*56*) in Gewerbestaub und Lungentuberkulose, 1927–29. The chambers consisted of a main cylinder of sheet metal about 90 cm. high and 27 cm. in diameter, with flat glass tops and conical bottoms and with 12 removable animal compartments attached to the cylinder wall in 2 radial planes, the cylinder acting as a distributing plenum for the exposure compartments. The compartments were truncated four-sided pyramids with the bases away from the cylinder. They were separated from the cylinder at the junction by metal screens. Each compartment was large enough to accommodate a single rabbit, which was placed with its head toward the center. The cages were removed for cleaning and disinfection, and were interchangeable. There was an opening on the top of each compartment which served as an air exhaust. These were plugged with cotton filters to prevent the escape of dust into the room.

The dust generating apparatus consisted of an electric compressor and three vertical glass tubes 40 mm. in diameter and 80 cm. long,

connected in series. The first tube contained a measured amount of dust. The air from the compressor entered the tube at the bottom and was discharged from the top, passing through the two following tubes to become more uniform in concentration before entering the chamber at the bottom of the cylinder. Dust concentrations were determined from samples taken through ports in the glass tops.

Froboese and Brückner (*57*) in 1929 described a procedure for exposing small animals to metal fumes. The exposure chamber was simply a glass cylinder 27 cm. in diameter and 24 cm. high. The electric arc fume generator and the mixing and metering apparatus were described in detail.

In 1929, Wigdortschik and Petroff (*58*) gave a thorough description and performance analysis of a cubic dust exposure chamber having a volume of 2.2 $m.^3$. It was constructed of oak wood. Most of the interior surfaces were glazed, and the remaining surfaces were lead lined. The chamber stood on four legs. In the space below the exposure zone an electric fan was set up to insure good air distribution. There were inlet and exhaust slits for the dusty air along opposite edges of the chamber, and an exhaust duct opening for purging the chamber.

Considerable discussion was devoted to the dust generating apparatus. The air which carried the dust was metered, cleaned, and dried before entering the dust generator, which was a mechanical shaking flask. The dusty air was mixed further in a mixing device consisting of a perforated steel ball in a double conical section. The dusty air entered the ball and discharged through the perforations while clean air flowed around the ball. The dusty air passed through two settling traps before entering the chamber.

Considerable data and a number of graphs were given for test runs made with the chamber, showing among others, the relation between dust feed rate and concentration; dust concentration and time; dust concentration and circulating fan speed; dust concentration and location within the chamber; and temperature and humidity and airflow rate.

Sayers, et al. (*59*) described in 1929 a 250-cubic foot chamber used at the Pittsburgh Experiment Station of the U.S. Bureau of Mines to expose guinea pigs to static concentrations of halogenated hydrocarbons. The concentrations were established by pouring the desired amount of liquid onto a large, flat surface in the chamber. Distribution was aided by a fan. Air samples were taken at regular intervals throughout the exposure.

In a later report from the same station, Sayers, et al. (*60*) described in 1930 a 5.5-cubic foot horizontal steel cylindrical dynamic flow chamber, made from a 20-inch diameter pipe section which was designed specifically for use with explosive vapors. A large chamber of 252 cubic feet was also described which resembles the chamber presented by Lehmann and Hasegawa (*18*). Fans were used in both chambers to improve mixing.

In 1931, Yant, Schrenk, and Sayers (*61*) used four 288-cubic foot total-enclosure box-type chambers to study methanol inhalation by laboratory animals. An innovation was a pressure-equalizing section (plenum) along a lower edge of the chamber through which air supply entered. The air was exhausted through three perforated pipes situated in each of three corners and extending vertically from the bottom to the top of the chamber. There was a fan opposite to the supply plenum for circulation. These supply and exhaust systems represented a distinct improvement over previous air-exchange methods.

In 1931, Haynes (*62*) used the dust chamber of Mavrogordato. Gross and Kuss (*63*) described in 1931 a procedure and apparatus for the regulation of airflow and vapor generation rates used for inhalation studies on mixtures of volatile compounds. The animals were exposed in a hemicylindrical glass chamber of about 50 liters.

A handbook section by Gross and Hebestreit (*64*) on animal experimentation methods in occupational medicine appeared in 1932. The authors reviewed the techniques of animal exposure in use at that time. Areas discussed included the inhalation of dusts, fumes, droplets and fogs, vapors, and gases; and also feeding, skin absorption, and injection.

In the section on dust inhalation, the methods and apparatus of Saito (*49*), Wigdortschik and Petroff (*58*), and Jötten, Arnoldi, and Kortmann (*55*, *56*) were discussed in detail. For acute tests at high dust levels, a

simplified version of the Jötten, Arnoldi, and Kortmann apparatus was described.

In the section on fume inhalation, the method and apparatus of Froboese and Brückner (*57*) were described and illustrated in detail.

The section on droplets and fogs contained a description of the apparatus and methods of Lehmann, Saito, and Majima (*17*).

For the inhalation of vapors of volatile liquids, the apparatus and methods of Lehmann (*53*), and Gross and Kuss (*63*) were described.

For gases, the apparatus and methods described were those of Lehmann (*53*), Dubitzki (*16*), and Gross and Kuss (*63*). The method of Gross and Kuss was designed for the continuous inhalation of very dilute gas-air mixtures, and had not previously been described in the literature. The Gross and Kuss chamber was a simple enclosure formed by joining two cylinders end-to-end. The complicated part of the apparatus was the system of gas burettes for the continuous and exact addition of small quantities of toxic gases to the main air current.

A large inhalation unit for gas and vapor exposure was described in 1935 by Heubner and Schellberg (*7*). It consisted of a chamber 9 feet by 5¼ feet by 6½ feet (310 cubic feet) and an anteroom 5¼ feet by 5¼ feet by 6½ feet (180 cubic feet), both lined with glazed tile. The anteroom contained the control equipment and utility connections. A sliding drawer air lock between the two rooms permitted the introduction of the test animals to a preestablished concentration. The animals were not confined by cages within the exposure chamber. The airflow could be regulated and the chamber could be purged within 30 seconds. A large fan was mounted in the ceiling for circulation purposes, and windows were provided in one wall.

In 1936 von Oettingen, et al. (*65*), of the DuPont Haskell Laboratory, exposed mice, rats, cats, and rabbits to chloroprene in an 8-liter bell jar. The air supply bubbled through a cylinder containing a weighed amount of chloroprene, and the chamber concentration was estimated by weight difference. For cats and rabbits in such a small chamber the absorption of the chloroprene on the fur must have been high.

In 1936, von Oettingen (*66*) reviewed the equipment needed in an industrial toxicology laboratory. Included in the discussion were diagrams and descriptions of the exposure chambers of von Oettingen, et al. (*65*), Gross and Kuss (*63*), Gross and Hebestreit (*64*), Lehmann, Saito, and Majima (*17*), Froboese and Brückner (*57*), Jötten and Arnoldi (*55*), and Jötten and Kortmann (*56*).

Dudley and Miller (*8*) of the former Division of Industrial Hygiene, Public Health Service, described in 1937 a 1-cubic meter exposure chamber of masonite fiberboard and glass, used at the Public Health Service's National Institutes of Health. A feature of this chamber was a sliding cage-carrier which permitted exposure of animals to previously established concentrations of toxic agents. By such an arrangement, the dilution was estimated to be less than 5 percent for the first minute, and the desired concentration could be reached in 3 minutes.

Another type of chamber used by the Division of Industrial Hygiene of the Public Health Service was described in 1940 by Fairhall and Sayers (*67*). This box-type chamber had heavy glass fronts fitted against soft rubber gaskets, which were removable for purposes of cleaning and transferring animals. The dust feed passed through an elutriator to provide a uniform dust dispersion. Air samples of the chamber atmosphere were taken at a rate of 1 cfm through filter paper disks mounted in a side wall. These samples could be analyzed chemically and microscopically for concentration, particle size, and composition.

Irish and Adams (*68*) of the Dow Chemical Co. presented in 1940 a discussion on apparatus and methods for testing the toxicity of vapors. Topics discussed included air supply, metering, mixing, the exposure chamber, the exhaust system, sampling in the chamber, and analytical methods. Apparatus described included two gas-feeding devices and two types of exposure chambers. It was stated that the essential factors for the design of exposure chambers are: "an airtight space of known volume; a surface readily cleaned, impervious and resistant to the vapors being tested; ready visibility for viewing the activity of test animals; and a design and cost which is practical."

The first type of chamber referred to was a

small box with glass walls set in a Monel frame. A battery of such chambers and their associated control equipment was shown. The air supply, at room conditions, entered a Monel pipe into which the vapor was pumped, and the mixture discharged into a baffle chamber before entering the exposure chamber. The gases entered through a narrow slit running the length of the front of the chamber and were exhausted through a similar slit along the lower back edge of the chamber. An orifice meter was used to measure the flow.

The second type of chamber described was much larger, about 66 cubic feet, and was a wooden box lined with a thin sheet of corrosion-resistant metal. Instead of a mixing chamber, as in the smaller units, a large slow-revolving fan was provided to mix the gases. The animals were not confined by cages in these chambers.

In 1943 Werner, et al. (*69*) described a bell-jar exposure unit used to test the toxicity of organic solvent vapors. The vapors were generated by passing a metered volume of air through two bubblers immersed in a constant temperature bath and mixed with a metered stream of clean air.

Stead, Dernehl, and Nau (*70*) of the University of Texas described in 1944 an animal chamber for dust exposure made from a 50-gallon steel drum. It was mounted on its side, provided with a removable glass front and electric illumination, and equipped with a rack to hold three animal cages. The dust feed consisted of a rotating dust tube which was raised at a constant rate, feeding dust to an air ejector arrangement. The rate could be varied to give the desired dust concentration. The dusty air passed through a cyclone separator which was made from a glass desiccator jar. It removed the particles larger than 4 microns in diameter. Air was exhausted from the chamber through an electrostatic precipitator. The entire exposure unit was set up in a constant temperature, constant humidity room, and it was claimed that reproducible dust concentrations could be established.

Principles influencing design and operation of constant-flow chambers for gas and vapor inhalation exposures were discussed in 1946 by Silver (*2*) of the Army Chemical Center, Edgewood, Md. Data were presented and formulas derived for predicting equilibration time for chamber concentrations. Also discussed were the effects of airflow, chamber size, the character and quantity of the interior surface, the shape of the chamber, the relative areas of air inlet and door opening, and the number and size of the animals, on chamber concentrations, equilibration times, surface effects, and animal loadings. The data presented were obtained on a group of chambers, including several designed for human exposure.

Of the animal exposure chambers referred to by Silver (*2*), one of 629 liters' capacity had been described in 1940 by the same author (*9*). It was constructed of steel plate and was approximately cubical in shape, with large, plate glass windows in each of two opposite sides. A feature was a sliding cage carrier supported in the door. Small, gasketed circular steel plates covered openings near the corners of the chamber. These plates could be removed for the insertion of various sampling devices and feed mechanisms. A calibrated orifice meter in the exhaust line was used to measure the airflow through the chamber. The exhaust pump maintained the chamber under negative pressure, drawing the air supply from the room through a mixing flask into which the contaminant aerosol was fed.

In 1946, Boyland, McDonald, and Rumens (*71*) described a 20-liter gas chamber in which small animals were exposed to phosgene gas.

Princi, Church, and McGilvray (*72*) in 1949 described a head-exposure chamber of effective design. It was made from two 55-gallon drums welded together to make one vertical cylinder, had inlet and exhaust cones at the top and bottom and, in principle, was similar to the chamber of Saito (*49*). The top cone had an anemostat air diffuser and distributing plate to provide uniform inlet dusty air.

A group of small exposure units used at the University of Rochester was described in 1953 by Spiegl, et al. (*19*). The units were made from 5-gallon battery jars placed horizontally on wooden stands, and bolted against sponge rubber gaskets recessed into upright front boards, serving also as an instrument panel. These chambers were used for rapid range-finding toxicity tests with rats, mice, guinea pigs, and hamsters.

A variety of large exposure units for inhalation toxicity studies of atomic energy materials for the Manhattan Project at Rochester, N.Y., has been described by Stokinger in a publication edited by Voegtlin and Hodge (*1*). The production of the chambers representing the combined effort of a group of project engineers, ranged in size from 4-foot cubes to approximately 9-foot cubes. Construction materials varied from wood and glass, for unreactive substances, to stainless steel for corrosive uranium halides, and copper-lined units for fluorine and hydrogen fluoride gases. Convertible head-exposure units were described that were suitable for the exposure of large numbers of dogs, rabbits, and guinea pigs. Either one or two entire sides of these chambers were occupied with head-exposure ports and cages, those for larger animals being near the floor, with successively smaller fittings above. A description of an airtight method of gasketing the head-exposed animals in the chamber was given. Pictorial and written descriptions were also given of the methods of providing ventilation and distribution of the test agents in the chambers. All units were connected to a single air intake and exhaust.

Designs were given for a variety of dust-feed mechanisms for materials difficult to disperse, such as the highly hygroscopic uranium tetrachloride, or hard-packing materials such as uranyl nitrate hexahydrate.

Because fans were used to provide distribution of the test agents within the chambers, certain of the extremely dense uranium compounds caused considerable difficulty in maintaining uniform distribution.

A chamber was designed and reported on in 1950, by Wilson and Laskin (*73*), that avoided this difficulty. The chamber, although of smaller capacity, was in the form of a hexagonal prism with sides measuring 4 feet in width and slightly less than 4 feet in height, and top and bottom sections in the form of hexagonal pyramids approximately 2 feet high. The chamber appeared pictorially in the report (*73*). By introducing the agent at the top with the incoming air, this design provided uniform distribution both of concentration and particle size of dust and ease of maintenance. The chief drawback of the chamber was its height requirement, which is greater than 9 feet, for a comparatively small animal capacity. The stainless steel-plastic construction, however, provided complete visualization of the animals' response to exposure at all times.

To obtain increased animal capacity for a 5-year chronic uranium dioxide study using primarily dogs and monkeys, the Rochester group combined four separate chambers of the hexagonal type to operate from a common air intake and dust-feed system. The exposure chambers were installed in a semicircular arrangement in front of a master control panel. A report (*74*) describing this installation in detail was prepared in 1957. Each chamber has an internal volume of about 80 cubic feet with an animal capacity of 4 monkeys, 8 dogs (beagles), and 40 rats. The total animal capacity of the complete unit is 16 monkeys, 32 dogs, and 160 rats.

During normal operation the airflow pattern through the exposure unit is as follows. Room air that has been filtered and conditioned previously is supplied to the inlet air duct by a centrifugal fan. The test material, UO_2 dust, is introduced under a slight positive pressure into the throat of a Venturi section located in this duct, and the dust-laden air is transported through the duct into a plenum chamber. The individual chambers which operate at a slight negative pressure draw aliquots of the dusty air from the plenum through their respective chamber inlet air ducts.

The dust and air mixture is admitted tangentially to the short cylindrical section at the top of each chamber. In this way, the air and test material are mixed and distributed uniformly throughout each chamber as a result of a mild swirling motion. The exhaust air is drawn axially from the bottom of each chamber through individual exhaust ducts, through a header and into a main exhaust system which is connected to a rotoclone. After several stages of filtration the exhaust air is discharged through an outside stack.

While the exposure unit described above was primarily designed for a long-term chronic study with large numbers of animals, it may easily be modified to handle other types of inhalation experiments. By separate con-

trol of individual dust inlets as many as four different concentrations of the same compound, or four different compounds, can be tested simultaneously.

A chamber designed for inhalation exposure of animals to highly hazardous materials or for continuous exposure has been described by Leach, Laskin, and Lauterbach (*75*). The chamber was basically cubic in shape, with pyramidal top and bottom, and with all internal corners filleted and smooth.

Constructed of plywood and glass, the chamber had an internal volume of approximately 700 liters. On one side there was a *dry* box, used for the isolation of the animals during washing and maintenance of the chamber, which was provided for by appropriately placed nozzles. Standard arm-length veterinarian gloves attached to ports in the chamber door permitted shuttling the animals from the chamber to the dry box. The shape of the chamber was similar to the hexagonal unit described above, and it had all the characteristics of uniformity of dust concentration control desired.

The Rochester group feeling the need for a small, highly flexible animal exposure unit for use in screening a wide variety of materials for toxicity, developed such a unit which was described by Leach and Spiegl (*76*) in 1956.

Basically, the chamber itself is a miniature replica of the larger hexagonal units described by Wilson and Laskin (*73*). All dimensions of the larger chamber were scaled down to two-thirds size, resulting in a chamber with a capacity of about one-half that of the full-size models. The entire unit is self-contained, with its own exhauster and provision for various exhaust air cleaners (cleaner depending on the material being used).

This unit was used with a number of materials in a variety of forms, including dusts, mists, and vapors, and proved most useful for quick, small-scale investigations of a pilot nature.

Baurmash, et al. (*77*) of the University of California at Los Angeles described an attempt to develop an exposure unit in which a quantity of animals could be exposed to the same concentration regardless of the location of the animal. The authors made the assumption that the concentration in a unit that was square or rectangular in shape would be inherently nonuniform, and they stated that the solution is a circular chamber with the contaminated air entering the chamber somewhere along the central axis. Going one step further, the investigators specified that the position of the animals be changed during the exposure time to eliminate the possibility of variation in the concentration along radial lines.

The chamber was described as circular, about 6½ feet in diameter at the center, and about 3 feet high, with an elliptical vertical cross section. It resembled the common conception of a flying saucer. The chamber was constructed of Fiberglas and plastic laminations in two halves, with a rubber gasket sealer. In the upper half was a lucite door and window. The upper half could be raised with block and tackle for loading and cleaning. A horizontal sheet metal platform for supporting the animal cages was installed near the center. This platform was designed to revolve slowly, about one-sixtieth rpm, about the vertical axis to minimize variation in radial air concentration.

Dust and air were fed in at the top where a deflector directed the air mixture downward and outward. The exhaust was at the bottom center. The sheet metal platform at the center restricted the flow to a narrow annular space at the edge of the chamber. It was planned to locate the heads of the animals at the rim of the platform.

It was stated that the concentration in the chamber had a maximum variation of 4.3 percent. However, no data were given for the variation in concentration with radial distance from the center. With the solid platform restricting the flow, such a variation is likely. Therefore, it would appear necessary to build the animal cages so that the animals could not turn around within them.

The dust feed (*H*) used was reported by the authors as giving a uniform feed for periods up to 8 hours in duration. Other features of the exposure unit were an automatic solenoid shutoff valve for the dust feed in the event the exhaust blower failed, and a drain at the bottom of the chamber to facilitate cleaning.

Urban (*78*) in 1954 described two types of

chambers in use at the Saranac Laboratory. One type was a box 4 feet 7 inches by 3 feet 2 inches by 4 feet 7 inches high. Features included a plenum at the bottom, below a perforated plate from which the air was exhausted, and a drain at the center of the floor for easy cleaning. The second type of chamber patterned after chambers at the University of Rochester was octagonal in cross section, 5 feet across, and 30 inches high with 22-inch canopies at the top and bottom. Good air distribution and easy cleaning were notable features.

A stainless steel animal exposure chamber, designed for inhalation studies of toxic gases and vapors, and constructed as a cube, 30 inches along each side, was described in 1956 by Sunderman, et al. (*10*), of Jefferson Medical College, and Rohm & Hass Co., Philadelphia.

An exhaust fan outside the building provided the air movement and maintained the chamber under negative pressure. The airflow was measured with an orifice meter and inclined manometer. Uniform airflow through the chamber was aided by an inlet and an outlet baffle, which were located along diametrically opposite edges. Each baffle plate had five 5-inch by ⅜-inch slots with adjustable slides.

The two sides and top of the chamber had 17-inch by 17-inch by ¼-inch safety glass windows; the top window, having a circular-type fluorescent light resting on it, acted as a light port. The front wall of the chamber was hinged and had a sliding drawer located in its center. Four rabbits or equivalent animal complement could be placed in the chamber through the drawer, or three times as many animals could be accommodated if the front wall were opened. A mechanically actuated hypodermic-syringe feed for volatile liquids was described, which was used for introducing nickel carbonyl into the chamber. Four ports for sampling and insertion of special devices were located on each side of the chamber near the corners.

MacFarland in 1956 (*11*) described a chamber built by the Canadian Department of National Health and Welfare, Ottawa. The basic design was patterned after Silver's design (*2*). The chamber was a cube, 1 meter on a side. The sides and bottom were fabricated of ¼-inch boiler plate and the top of glass. The author suggested that steel plate is a convenient construction material since it gives a rigid chamber, strong enough to bear the weight of piping for airflow circuits and thick enough to permit tapping drilled holes for the entry of sensing elements and sampling devices.

Features of the exposure equipment included an elaborate automatically controlled air system, and a compressed air-driven sliding cage carrier. The airflow circuits permitted scavenging, bypassing, and circulation of the test atmosphere. In addition, three extra flow loops permitted the introduction of a flowmeter, an absorbing canister for air cleaning, an arc box for arc-produced fumes, or other attachments.

Two pumps were used, which could be operated singly or in parallel. A total of sixteen 2-inch gate valves was used to control the system. The valves were air operated, and the air control was solenoid actuated. Each airflow pattern used was programed at the control board, and upon demand, the 16 valves assumed the requisite pattern in 6 seconds or less.

Since short-term acute exposures of 10 to 30 minutes were planned, an airlock mechanism with a basket for 12 rats was built into the front plate. The time of travel of the carriage was 3 seconds, and it could be moved out either manually or automatically by a preset program.

Four large-scale inhalation chambers are being used (1958) at the National Cancer Institute, Public Health Service, in Bethesda, Md. They have octagonal cross sections, with the octagons measuring 54 inches on a side, and are derived from the designs used at the University of Rochester (*73*, *74*, *76*), and the former Saranac Laboratory (*78*). The sides are lucite windows, and the frame and top and bottom canopies are stainless steel. Pie-shaped animal cages are used which are supported on a rotating platform. One face of the chamber opens for loading and unloading the animals.

Wright (*79*) published in 1957 a description of exposure chambers and techniques

used by the Pneumoconiosis Research Unit, Llandough Hospital, Cardiff, South Wales. These techniques evolved over a 10-year period of experience in this field.

The chambers described are cubic, 2 feet on a side, with truncated pyramidal tops. The dust is blown directly into the chamber by the Wright dust feed (*22*) through a hole in the flat top of the pyramid. A unique feature of the chambers is a safety door which opens automatically if the dust feed should fail; this is particularly important when the exposures are carried out unattended through the night as was done in this case.

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(*C*) Wright dust feed. L. A. Adams, Ltd., Minewa Road, Chase Estate, London, N.W. 10.

(*D*) Variable pressure control valve. Conoflow Regulator Corp., 2100 Arch Street, Philadelphia 3, Pa.

(*E*) Dynapump. Standard Scientific Co., 34 West 4th Street, New York 12, N.Y.

(*F*) Forward scattering smoke photometer. Phoenix Precision Instrument Co., 3803–05 North 5th Street, Philadelphia 40, Pa.

(*G*) Differential pressure switch, No. 1626. F. W. Dwyer (reference *A*).

(*H*) Far-Air model 4 dust feeder. Farr Co., Airport Station, Los Angeles 45, Calif.

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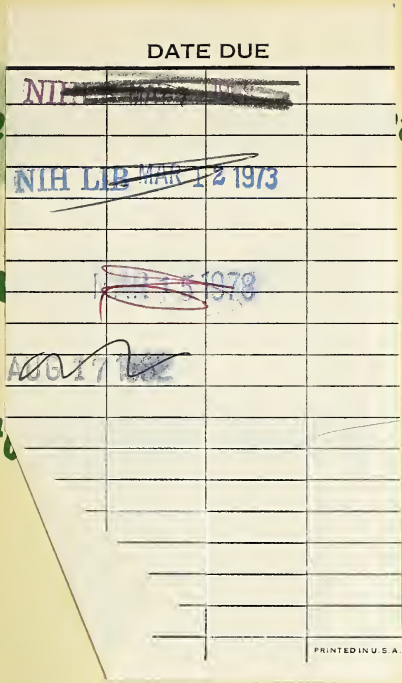

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