THE INVESTIGATION OF INHALATION TOXICITY

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The techniques described in this communication are in current use in a laboratory undertaking the routine investigation of the toxicity of substances used or produced by the chemical industry. In such a laboratory it is not always possible to submit each substance to an exhaustive study, but enough work must be done on each to ensure that no important toxic action is likely to be missed. The " range-finding tests " described by Smyth and Carpenter, which concentrate solely on the acute effects produced by single exposures, are not likely to be adequate for this purpose, for the most serious hazard in industry from the inhalation of gases, vapours, mists and dusts usually derives from prolonged exposure to lower concentrations.

The majority of substances received for investigation are volatile liquids or solids. With these, the first step is to ascertain whether, under the most adverse circumstances, the vapour is likely to present a hazard. If the sample is a liquid, it is introduced into a bubbler maintained at constant temperature and an air stream is passed through it. A concentration approaching saturation can be achieved if a sintered glass disc is used to break up the air stream into fine bubbles. The atmosphere then enters an exposure chamber in which a group of experimental animals is exposed daily for a period of at least three weeks, though the experiment will be terminated earlier if severe toxic signs or death occur. The vapour is regarded as presenting no special hazard if a careful examination of the animals during the exposure period reveals no signs of any toxic action, and nothing abnormal is seen at autopsy or at histopathological examination of the major organs. If the sample is supplied in the form of a granular solid, then the air supply for the exposure chamber is passed through a column of the granules. If the particles are very fine it may be necessary to disperse them on a granular material such as pumice.

If a potential toxic action of the vapour is revealed in these experiments, it is necessary to expose groups of animals to progressively decreasing concentrations for periods of at least three weeks, until the threshold concentration for toxic action is reached. For this purpose, the vapour concentrations used must be known with a reasonable accuracy. A dynamic procedure, that is, one in which the test concentrations in air are continuously generated and led into the exposure chamber, is to be preferred to a static method, as the former permits adequate ventilation with removal of carbon dioxide, water vapour and other waste products. The dynamic procedure also

permits a reasonably compact apparatus, and it eliminates the progressive decrease in the test concentrations due to adsorption and absorption which are encountered with the static procedure. In many earlier publications, dynamic air concentrations were prepared by saturating air with the vapour and then diluting it with clean air as required. This procedure has many disadvantages; it assumes that the sample is pure and its vapour pressure is known. It cannot be used if the sample undergoes a chemical change when continously aerated due to oxidation or polymerization, and a two-stage dilution must be used for low concentrations of volatile materials. In more recent years there has been a tendency to abandon this procedure in favour of a controlled introduction of the sample into the air stream; usually heat is applied to expedite the evaporation of the liquid as it enters the air stream. This procedure can be used efficiently for some materials, but it is unsuitable if the sample is heat-labile.

In my own laboratory, dynamic test atmospheric concentrations have been, for a number of years, prepared by means of a controlled fluid-feed atomizer. In this, a volatile liquid is contained in a glass syringe, from which it is expelled by a ram at a known constant rate into a concentric air jet atomizer. The liquid is sub-divided by the atomizer into fine particles which rapidly and completely evaporate to give a uniformly mixed atmospheric concentration. From a knowledge of the diameter of the syringe, the rate of air flow, and the rate of movement of the ram, the atmospheric concentration can be calculated, and the concentration can readily be changed by altering one or more of these variables. The ram driving the syringe piston operates by means of a synchronous motor through a set of gears, which enables its speed to be varied between 0·25 and 0·008 cm/min. For atmospheric concentrations lower than can conveniently be achieved with the smallest syringe available, a solution of the sample in an inert volatile solvent may be used.

Low atmospheric concentrations of gases may be prepared in a similar manner by filling the gas into a large, well-lubricated glass syringe. An atomizer cannot here be used, as the Venturi effect at the jet would cause a reduction of pressure of the gas within the syringe. The gas is injected through a capillary jet into a slowly moving transverse current of air, and care is taken that no changes in the pressure drop in the system occur during the experiment as this might cause a loss of gas from, or an introduction of air into, the syringe. Atmospheric concentrations of a gas may also be prepared in the manner used for volatile liquids, if it is soluble in a suitable inert volatile solvent.

If the syringe of the atomizer contains a solution of a non-volatile liquid or solid in a volatile inert solvent, the atmosphere produced will contain a mist or a dust. If the solution used is dilute a very fine particle size may be obtained by this method; for example, a potassium permanganate dust cloud with a maximal particle diameter of 5μ has been prepared by this method. The latter method cannot, however, be used for insoluble dusts, which present greater problems. With some dusts, the Wright Dust Feed Mechanism is very convenient, but it often gives an intermittent flow with organic solids. In my own laboratory, a modification of the U.S. Bureau of Mines apparatus has proved satisfactory for this purpose. The powder,

which should have been passed through a 300-mesh sieve, is placed in a vertical glass tube which is slowly raised at a constant speed to bring the surface of the powder into contact with a static head which picks up the powder and introduces it into an air stream. Intermittent flow can be avoided by applying an oscillating rotary motion to the vertical glass tube.

A variety of chambers may be used to expose experimental animals to the test atmosphere, but the preferred design for small animals is in the form of a box with the dimensions (50 × 50 × 30) cm high, with an open bottom and a detachable lid. The open bottom rests on an aluminium tray and the animals are placed on a grid of stainless steel wire. The test atmosphere enters the base up through a central glass column, then passes over the animals and out through a series of holes near the base. The chamber is surrounded by an outer shell of glass which is maintained at a slightly reduced pressure by means of an exhaust fan, to avoid contamination of the laboratory atmosphere. The inner chamber may be sub-divided by radial partitions.

The instruments for controlling the air flow rate are mounted on a panel in front of the chamber. Air is supplied to the chambers at a line pressure of one atmosphere, kept constant by means of a Nullmatic pressure regulator and the flow to each atomizer is controlled by means of a needle valve and a pressure gauge. At least once a week the calibration of the pressure gauge in terms of air flow is checked by attaching a Rotameter to the outlet of the atomizer. This system of flow control by needle valve and pressure gauge with a constant air line of pressure has been found to be simpler and at least as satisfactory as an individual pressure-reducing valve and flowmeter on each exposure chamber.

Although the apparatus as described is capable of providing an accurate and reproducible atmospheric concentration, a frequent analytical check is desirable in order to avoid human error and to ensure the proper functioning of the apparatus. For this purpose a sampling port is provided in the side of the exposure chamber, kept closed when not in use by means of a rubber stopper. The sample of the test atmosphere is extracted by means of a vacuum line controlled by a critical orifice plate; this provides a sampling rate independent of variations in the vacuum line and determined solely by the pressure between the orifice and the absorber used. For sampling speeds of 0·5–1 l·min an orifice with a diameter of 0·45 mm, flared on the upstream side to prevent turbulence, is suitable. A vacuum line is connected to the orifice through a valve and a vacuum of at least 50 mm Hg is applied to the orifice. Between the sampling equipment and the orifice plate is a needle valve and a vacuum gauge, the latter being calibrated in terms of flow rate by replacing the sampling equipment with a suitable Rotameter and taking readings of the Rotameter and the vacuum gauge over a range of settings of the needle valve. A check on this calibration is made at least once a week. The orifice plate in its holder, the valves and the vacuum gauge are mounted on the instrument panel.

The exposure chamber, as described, is suitable for daily exposures of up to 8 hours, after which the animals are removed to overnight cages in which they receive food and water. In some experiments, particularly when the metabolism of an inhaled vapour is under investigation, it is required to maintain the animals in the test atmosphere continuously, with a collection

of all the urine and faeces excreted. For this purpose, a chamber for a single rat may be constructed from a polyethylene funnel containing a stainless steel grid on which the animal sits. A lid is provided through which passes the entry and exit tube for the test atmosphere, and it also carries a water bottle and a container for moistened powdered diet. The urine and faeces falling through the bottom of the funnel are separated in a glass collector constructed from two concentric tubes. A series of 18 such individual chambers have been arranged in a single outer glass shell under exhaust ventilation.