A simple system for generating low-dosage mercury vapor for animal experiments

Rune Eide and Gro R. Wesenberg
Institute of Anatomy, University of Bergen, Bergen, Norway

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A system using unlabeled, metallic mercury for generating low-dosage vapor for animal experiments is described. The system consists of four acrylic plastic chambers: one chamber containing the mercury source, one for mixing the mercury vapor with air, one exposure chamber, and one containing activated coal filters and mechanisms for regulating the airflow. The chambers are connected to each other by means of 80-mm-diameter polyvinyl chloride tubes reinforced with wire. Additional control and supporting equipment is also used. The system is easy to set up and requires minimal attendance during use. A standard deviation of 3–4% in the mercury vapor level during 6 h of exposure is typical. The conditions for the animals during exposure and the safety for the personnel during use are optimal. □ *Environment; in vivo; toxicology*

Rune Eide, Institute of Anatomy, University of Bergen, Årstadveien 19, N-5009 Bergen, Norway

Several methods exist for generating mercury vapor for animal experiments. A procedure for labeling mercury vapor with ^{203}Hg was described by Magos (1). This method utilizes the $^{200}Hg \leftrightarrow {}^{203}Hg$ exchange reaction between vapor and $^{203}HgCl_2$. It has since been modified owing to problems with decrease in the specific activity of the resulting vapor (2). However, the use of radioisotope methods demands complicated equipment and continuous sampling (typically, 4-min intervals) for scintillation counting, in addition to the expenditure and inconvenience of using radioactive materials.

Methods also exist for using unlabeled mercury to create the vapor, but they are often insufficiently described, or the mercury-monitoring systems are unsuitable for the rapid, fine adjustments needed in low-level exposures (3–5).

Several of these techniques were reviewed in connection with an investigation of the effect of low-dosage mercury vapor exposure on rats for extended periods. It was decided for the reasons mentioned above to construct new equipment, bearing the following aims in mind: 1) simple to manufacture; 2) easy to operate for a prolonged period; 3) approximately normal environmental conditions for the animals during exposure; and 4) inexpensive.

Apparatus

Design

The apparatus consists of four chambers made of acrylic plastic with additional control and measuring equipment (Fig. 1). The chambers are connected to each other by means of 80-mm-diameter polyvinyl chloride (PVC) tubes reinforced with wire.

The first chamber, the mercury chamber (Fig. 2), has a volume of 7.6 l. The chamber contains the mercury source in a acrylic plastic dish, and the top of the chamber is removable, to facilitate covering and uncovering of the mercury source. To obtain a stable temperature, the chamber is placed on a hotplate (Charles Hearson Ltd, U.K.) controlled by a thermostat. The temperature is kept just above room temperature and was initially monitored by a thermocouple connected to a plotter (Leeds & Northrup Ltd., U.K.). The chamber is also slowly shaken by an agitator (Gerhardt, FRG), to break the oxide layer constantly forming on the surface (6). Ambient air is taken in at one

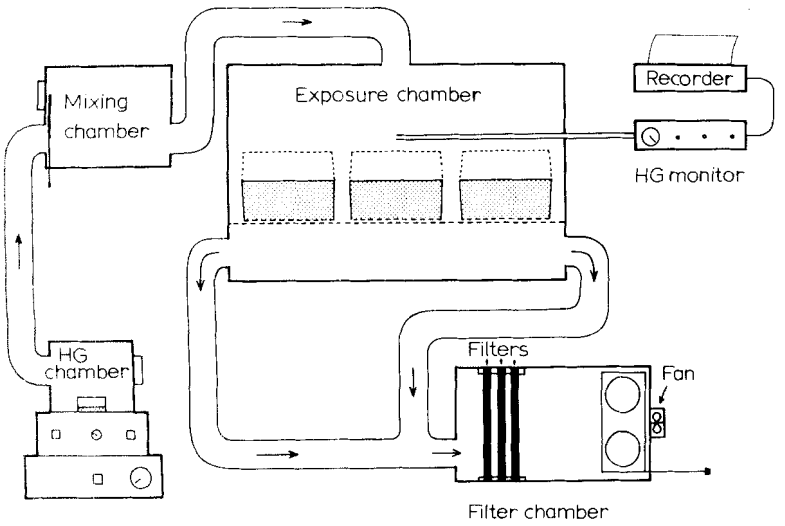

Fig. 1. The mercury-generating and exposure system.

end of the chamber and is passed on to the next chamber at the other end after mixing with mercury vapor.

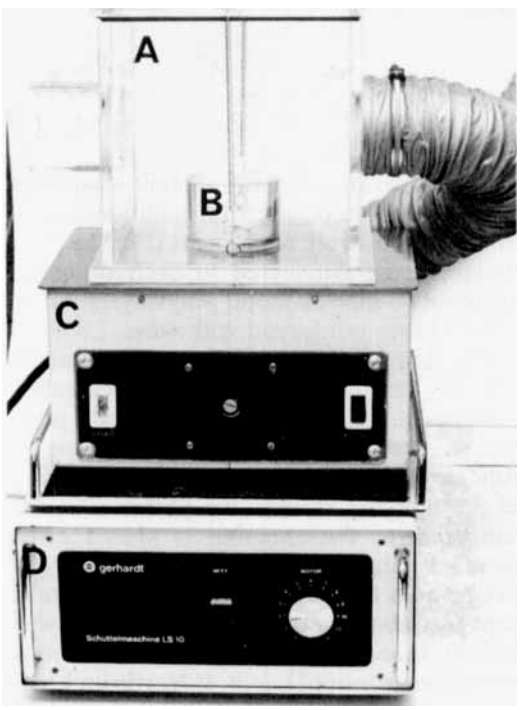

Fig. 2. The mercury-generating equipment, consisting of the mercury chamber (A), the mercury source (B), the hotplate (C), and the agitator (D).

The next chamber, the mixing chamber (Fig. 3), has a volume of 12.0 l. Air is taken into the chamber in parallel with the air/mercury mixture from the mercury chamber through equal-sized openings (diameter, 70 mm). On the inside is a movable plate connected to the outside by a thin metal rod. The size of this plate is such that it always covers 50% of the total area of the two openings regardless of its position. This makes it possible to blend the two inputs in any ratio by moving the plate. After the correct proportions between mercury vapor and air are obtained, the mixture is transported to the next chamber.

The third chamber, the exposure chamber (Fig. 4), has a volume of 325.9 l. The air/mercury mixture is taken in at the top and leaves through two holes near the bottom, one at each end. These are covered on the inside with a fine-meshed, plastic grid, to prevent escaped rats from entering the connecting tubes. The top of the chamber is removable to enable placement of the rats. The lower part of one of the sides is hinged, to provide access for cleaning purposes. A metal frame covered with chicken wire is placed on four legs on the floor of the chamber, one leg in each corner. The structure represents a minimal hindrance to the air current. Three 28- by 45-cm polypropanol animal cages can be placed on the frame.

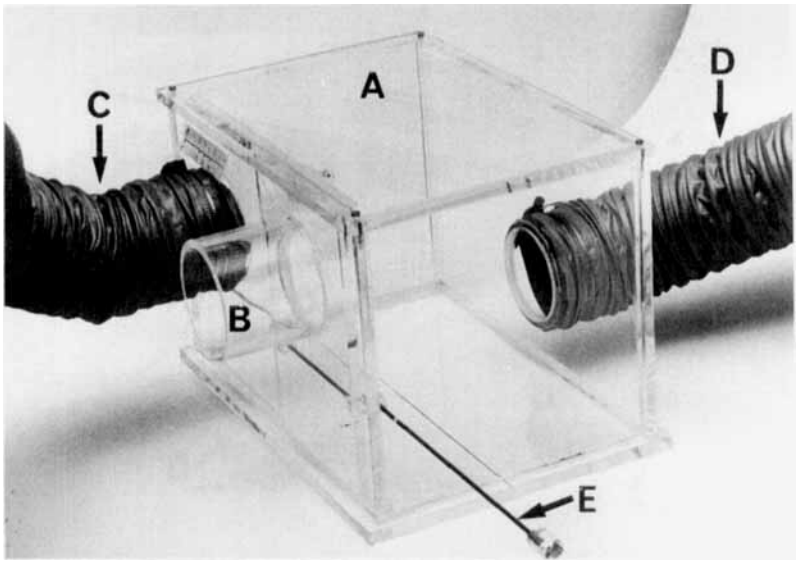

Fig. 3. The mixing chamber (A) with apertures for air (B) and mercury vapor (C), and an outlet for the resultant mixture (D). A movable plate with a metal rod (E) determines the resultant mixture.

These cages have stainless steel grids both on the top and at the bottom to ensure an uninterrupted air flow.

A mercury-monitoring device (Mercury vapor monitor 791, Kipp Analytica, The Netherlands) continuously measures the mercury concentration in the exposure chamber via a Teflon tube with a diameter of 4 mm. The monitor extracts 1 l of air per minute from the chamber for this purpose. The results are recorded on a plotter (Linseis GmbH, FRG).

Any mercury adhering to or chemically combining with structures inside the chamber may conceivably later act as sources of mercury. Owing to the constant supervision of the mercury concentration in the chamber, however, the input of 'fresh' mercury will be reduced accordingly.

The air/mercury mixture passes from the

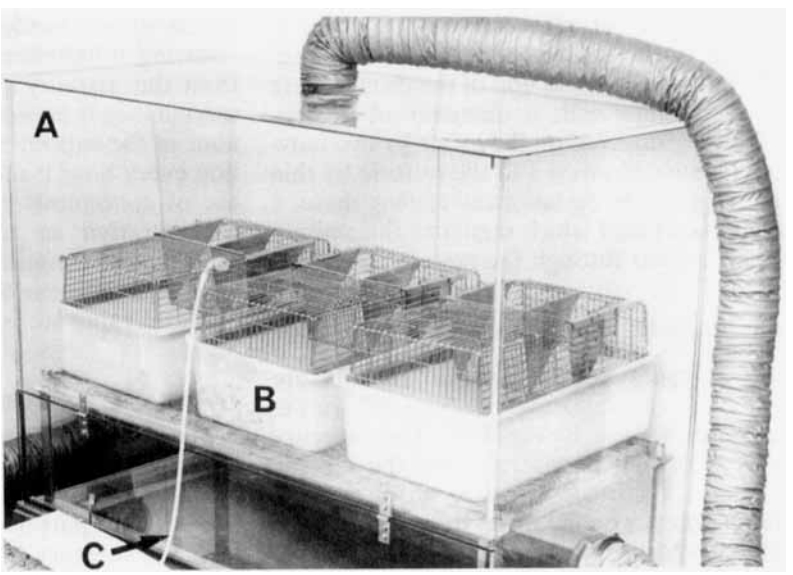

Fig. 4. The exposure chamber (A) containing three animal cages (B). The sampling tube to the mercury monitor is also shown (C).

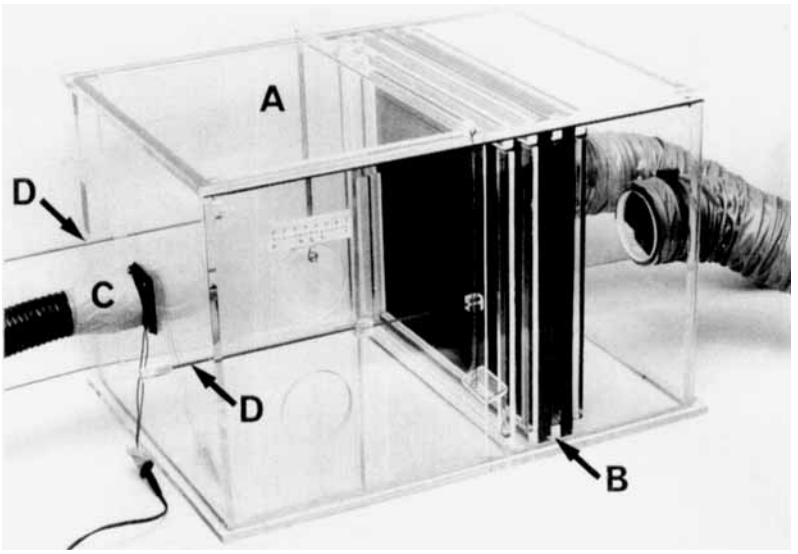

Fig. 5. The filter chamber (A) containing two of the three possible activated coal filters (B), a fan (C) and two movable plates, which regulate the airflow (D).

exposure chamber to the fourth chamber, the filter chamber (Fig. 5). This chamber has a volume of 53.17 l. The tubes from the exposure chamber are connected at one end of the chamber. In the middle of the chamber are three attachments for activated coal filters (Electrolux, Sweden), used to remove the mercury from the air. At the far end is a small direct-current fan (Micronel, Switzerland) with varying voltage. It has a maximum capacity of 320 l/min. From this fan the residual air after mercury removal is passed to the environment via a PVC hose. On both sides of this end of the chamber are two openings with a diameter of 70 mm. These are covered on the inside by two movable plates connected to the outside by thin metal rods. By opening and closing these, a shunt is created which regulates the amount of air passed through the system.

Operation

The cages containing the animals are placed in the exposure chamber before the mercury source is exposed. The mercury source is then uncovered, and the electric appliances started. The size of the source, the frequency of agitation, the proportion of mercury to air, the size of the shunt, and the voltage of the fan are adjusted in accordance with the intended mercury level.

The intended mercury level is usually achieved and stabilized within approximately 30 min, and adjustments hereafter are seldom needed. A pilot study showed that the mercury concentration fell inside the chamber during the placement of the animals and needed about 30 min to regain the initial proportion even when the mercury concentration had reached working level. The modifications needed are mainly done by reducing or increasing the supply of mercury from the mercury chamber. A continuous surveillance is necessary during the first half hour of the exposure. Thereafter an inspection every hour is more than sufficient. The use of continuous plotting of the mercury concentration in the exposure chamber shows any deviations from the intended level, and these can then be considered when the total exposure is calculated.

Testing of the system

The system has been tested at several concentration levels. Typical graphs from the recording plotter are shown in Figs. 6 and 7. Fig. 6 shows part of a 6-h exposure with an intended mercury level of 20 $\mu g/m^3$, which

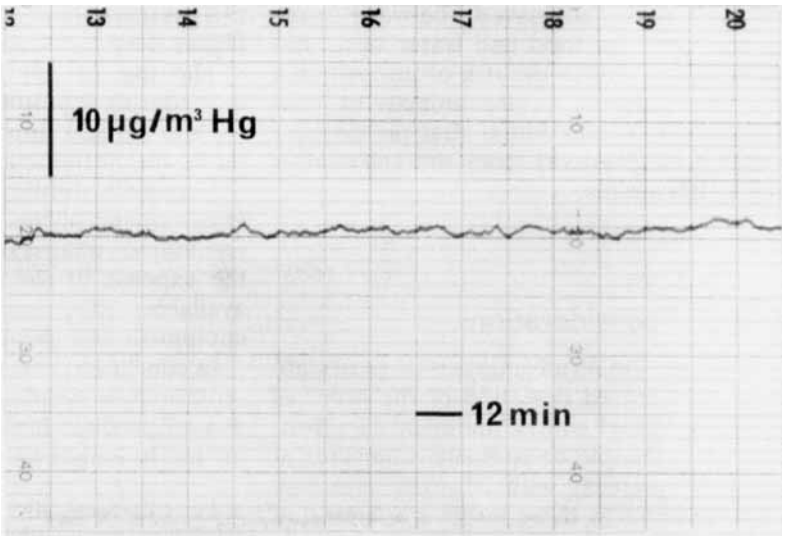

Fig. 6. Part of a 6-h exposure with an intended mercury level of 20 µg/m³.

gave a mean mercury value of 19.09 µg/m³ and an SD of 1.37. If only the stabilized period is analyzed, however, the mean value increases to 19.67 µg/m³ mercury, and the SD decreases to 0.84.

Fig. 7 shows a section of a 6-h exposure with an intended mercury level of 500 µg/m³ mercury. This gave a mean of 454.41 µg/m³ mercury with an SD of 73.63. Analysis of the stabilized period gave values of 472.03 and 14.63, respectively.

The temperature-monitoring system showed that the temperature inside the mercury chamber was stable shortly after start, and the equipment has since been removed.

The animals were transferred to ordinary cages immediately after each session in the exposure chamber. The exposure cages were

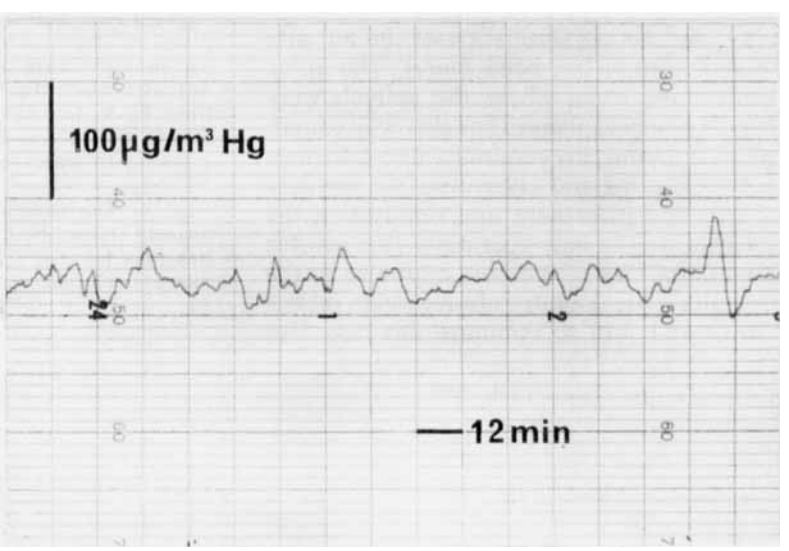

Fig. 7. Section of a 6-h exposure with an intended mercury level of 500 µg/m³.

washed and autoclaved between each period, and the food and water were also changed, to reduce the risk of uncontrolled mercury exposure of the animals by contamination. It was found that no mercury vapor was given off from the cages after these procedures.

General considerations

The system's main advantage is its great stability. A standard deviation of the order of 3–4%, attained with a minimum of effort, makes it possible to compare exposures at 10 $\mu g/m^3$ mercury with 20 $\mu g/m^3$ mercury. Investigations at these levels are important in relation to the effect of mercury vapor in environmental situations and also when considering the potential long-term vapor exposure from amalgam fillings.

The stability of the system makes it possible to leave the setup unattended for at least an hour. This is important because it simplifies the running of the experiment considerably. It is also unnecessary to use radioactive materials as concentration markers for a prolonged time period during investigations, thus avoiding the costs and disadvantages of these materials. This procedure reduces the running costs.

The ambient air in the room where the system was situated has been tested repeatedly, and no contamination of the air with mercury vapor has been found. The air in the storage room where the animals were kept between exposures has also been tested, without finding any contamination with mercury. Covering and uncovering the mercury source and placement and removal of the animals are the only operations that involve a potential risk of mercury exposure to the operator. The operator always wears rubber gloves and cuffs to minimize this risk. Use of a gas mask should also be considered when higher doses are used.

The use of modified, standard animal cages during exposures makes the conditions for the animals approximately normal and limits the introduction of stress factors for the animals during exposures. The main disadvantage of the system is the need for continuous mercury monitoring, including the expense of the monitor, but if this is available, then only ordinary laboratory equipment and cheap parts are used.

In conclusion, we believe that this system represents a simple, stable, and economic way to produce low-dosage mercury vapor for use in long-term animal experiments.

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