June 1937

C+2 SAM

REPORT ON 4465

Inhalation Experiments

Animals. White rats have been employed throughout. They permit the use of a large number of animals in a relatively small inhalation installation. They thrive upon a diet very similar to man, and in the case of these chlorinated compounds it is possible that diet may be very significant. Finally, their normal characteristics have been described so well as to make the detection of abnormalities both easy and certain.

Keep the study [illegible] to C.C. Drinker Report [illegible] 7/15/38 [illegible]

Method of Exposure. The inhalation experiments were carried out in four large air-tight wooden boxes, each capable of holding ten rat cages, size 22" x 22" x 14", in two tiers of five cages each. When the experiment was not in progress the doors were opened wide and the cages kept in place (see Figure 1).

At the center of one end of each box -- the inflow end -- air was introduced through a pipe 7 inches in diameter (see Figure 2). Each box was equipped with an individual electric blower which blew the air through several feet of 7-inch pipe before entering the end of the box. An orifice was placed in the pipe line, and the flow of air in cubic feet per minute could be read off directly from calibrated flow meters. A damper placed at the entrance of the pipe into the box was adjusted to assure a uniform distribution of the stream of air to the two tiers of cages.

At the opposite end of the box -- the outflow end -- the air from each box was exhausted through a 7-inch pipe fitted with a damper and connected to a large central exhaust fan (see Figure 3).

About 4 inches from the entrance of the 7-inch pipe into the box, the fumes of the substance were introduced into the inflowing air.

MONS 054592

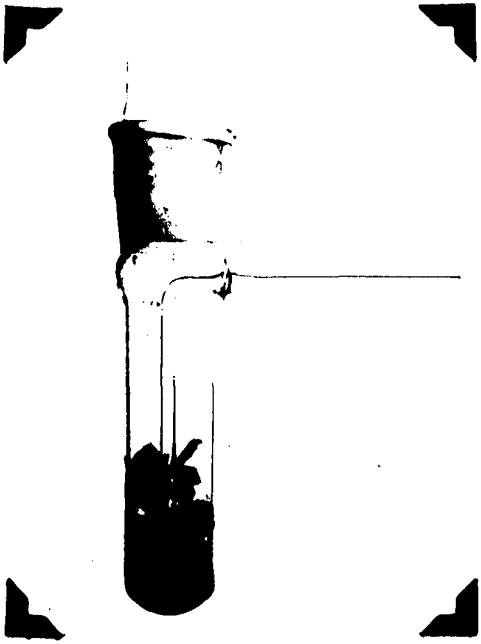

Fig. 4. Pyrex glass flasks.

MONS 054593

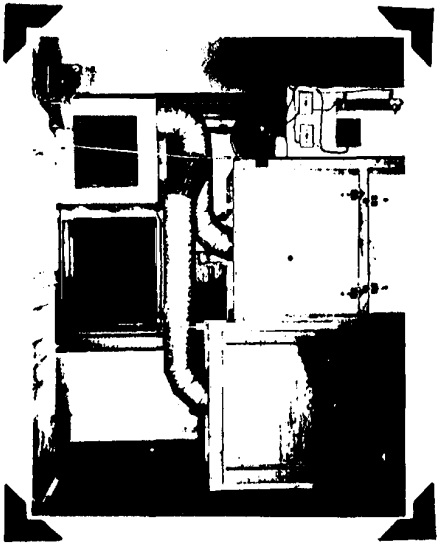

Fig. 3. Outflow end of two boxes showing connections to exhaust system.

MONS 054594

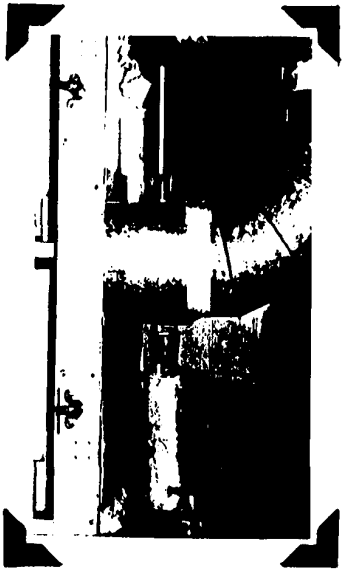

Fig. 2. Electric heater for maintaining chlorinated diphenyl at a constant temperature in place at inflow end of box.

MONS 054595

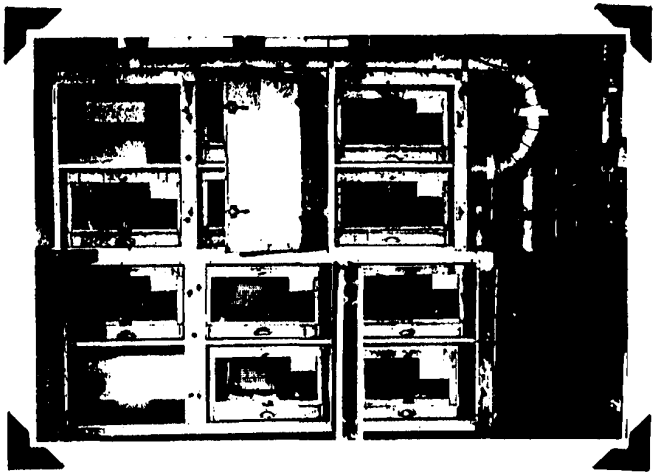

Fig. 1. Front view and inflow end of two boxes with rat cages in place and doors open.

Specially designed pyrex glass flasks (Figure 4), 7 3/4" long and with a diameter of 1 1/2", were used to hold the heated waxes. These flasks were made with a side arm and tube which extended to the bottom. The top of the flask was ground, and into this fitted a short tube 1 1/2" in length. This short tube was inserted into a large rubber stopper which fitted tightly into a hole cut out of the 7-inch pipe on the under side (see Figure 2). The flask in turn was placed in an electric heater made to cover it completely below the side arm and ground glass top. Rubber tubing connected the side arm with a compressed air reservoir and a gentle stream of air blown through the melted wax kept it in motion and assured uniform heating. Into each flask was inserted a long stem centigrade thermometer which was kept in place and could be read at any time above the 7-inch pipe through which it passed (see Figure 2).

Approximately 20 gm. of pulverized [redacted] chlorinated diphenyl were placed in the vaporizing flask and melted in the electric heater. Fresh samples were used every other day. [redacted] The collected sublimate was always removed from the upper part of the flask and a clean top used each day. No sample was ever used for more than two runs.

The flask plus the contents was carefully weighed at the beginning of the run and at the end, and the loss in weight used to calculate the average amount in a cubic meter of air per minute as determined by a series of flow meter readings. The figures obtained were not absolute because of slight variations in the air flowing through the boxes and because of deposition of material on the thermometers and on the inside surfaces of the box, but they checked well with direct determinations through air samples.

MONS 054597

Approximately one hour was allowed for the wax in the flasks to melt and come to a constant temperature. At that time the box doors were tightly closed, air bubbled through the flasks, and the blowers turned on. This was the beginning of the exposure period. By means of the theostats on the fans and the dampers in the outflow pipes, the amount of air flowing through the boxes was adjusted and an attempt was made to keep the four boxes as uniform as possible -- usually between 165 and 175 cubic feet per minute..

Experiments

Exposure to chlorinated diphenyl was begun on July 1, 1936 and ceased on November 18 of the same year. The average length of exposure was 16 hours daily for 6 days a week. Each morning at about 9:00 exposure ceased, and between this time and 4:00 p.m. the rats were cleaned, fed, weighed, etc. In order to secure uniformity of exposure the cages were shifted on a regular schedule, since wax concentrations were somewhat higher at the inflow end of the boxes. Preliminary runs showed that once properly adjusted, wax concentrations in the air remained very uniform from day to day, but to insure absolute safety readings of temperature, air-flow, etc. were made every night between 10:00 and 12:00 p.m. as well as on starting and stopping. At different times during the course of the experiment tests for free chloride were made but were uniformly negative and showed that under the temperatures used no decomposition occurred.

Table 1

Conditions Maintained in Inhalation Experiment from July 1 to Nov. 18

Material	Temperature	Average concentration of air in box		Total length of exposure	Average daily exposure
	°C	Mg./cu.M.	Range	hours	hours
Chlorinated diphenyl	165-175	0.57	High 1.19 Low 0.23	1896	16

MONS 054598

Results of Inhalation Experiments

No abnormalities were seen at any time in the living rats. Blood and urine examinations were made; there were no losses in weight, and one would have considered that the animals were perfectly sound. However, rats sacrificed after 6 weeks exposure showed slight liver damage, which advanced during the next two months. The changes consisted in slight to moderate swelling of the liver cells, increased granularity, and many mitotic figures. Hyaline inclusions were present and were invariably plentiful in animals receiving chlorinated diphenyl by inhalation or by mouth.

One is forced to conclude from the experiments that an average concentration of 0.57 mg. per cubic meter inhaled 16 hours daily produces definite slight changes in the liver and in this organ alone. These changes are resisted efficiently by the animals and cause no depreciation of health. The situation is not unlike that seen in factories where acute yellow atrophy of the liver has occurred. In the case of such instances the patients have been singled out in some way or other from large groups of fellow workmen who have been perfectly healthy.

In considering the entire matter it seemed to us that the chlorinated hydro-carbons,/if inhaled in sufficient concentration, might cause a slight degree of damage to the liver. This damage is resisted efficiently and causes no depreciation of health, but if the individual in question happens to suffer some ordinary disease of the liver the condition is superimposed upon a substratum of injury. In accordance with this hypothesis we determined that a dose of 0.75 cc. of carbon tetrachloride plus 0.75 cc. of ethyl alcohol per kilogram of rat was entirely non-toxic to normal animals. When, however, this dose was given to animals which had inhaled chlorinated diphenyl as has been described in this experiment, the result was acute yellow atrophy

MONS 054599

of the liver. It would seem therefore that inhalation of a concentration of chlorinated diphenyl in the manner I have described is capable of producing a condition which may be dangerous to the individual inhaling it, though of itself no visible harm will be done.

Animals removed from exposure to chlorinated diphenyl after two months inhalation and given two months for recovery still showed liver changes when killed and examined, so that it is evident the alterations produced by this hydro-carbon are persistent and individuals who have been caused trouble by it should be removed completely and for a long time.

The Inhalation of a somewhat higher concentration of chlorinated diphenyl over an 8 hour day

The circumstances attending this experiment are given in Table 2.

Table 2

Material	Temperature	Average concentration of air in box		Total length of exposure	Average daily exposure
	°C	Mg./cu.m.	Range	Hours	Hours
Chlorinated diphenyl	153-174	0.93	High 3.23 Low 0.03	920	8

Again 80 rats were used in this experiment. None at any time showed the slightest evidence of illness. Microscopic examinations, however, showed a degree of change very comparable to that found in the 16 hour experiments which have been described and the carbon tetrachloride test was uniformly fatal.

General Summary

From these experiments we are forced to conclude that concentrations of chlorinated diphenyl in workrooms should not be allowed to rise above 0.5 mg. per cubic meter of air. This concentration is readily obtained by careful

MONS 054600

hooding. It is in our opinion safe for workmen who would not be exposed with the absolute steadiness characteristic of our experiments on rats and such a concentration limit would probably do away with the troublesome skin lesions which have been the particular cause for worry in regard to these compounds in the past.

In addition to the inhalation experiments many feeding experiments were done with this chlorinated diphenyl. The general changes in the liver were the same as those already described. No diarrhea or gastro-intestinal aspects seemed to be present.