REPORT TO THE MONSANTO CHEMICAL COMPANY

Cecil K. Drinker, M.D.

September 15, 1938

ADM 0034

21

TOWOLDMON0001251

C+25+

REPORT TO THE MONSANTO CHEMICAL COMPANY

by

Cecil K. Drinker, M.D.
Dean and Professor of Physiology
Harvard School of Public Health, Boston, Mass.

September 15, 1938

ACH 003430

TOWOLDMON0001252

Page

I. Experiments to determine the possible toxicity of the following substances: 2

1. Chlorcosane 2

2. Diphenyl phthalate 3

3. Chlorinated diphenyl #1268 4

4. Mixture of chlorinated diphenyl and chlorinated diphenyl benzene #5460 7

II. Some general considerations: 9

1. Evidence for the destruction of a mixture of penta and hexachlornaphthalenes (#1006) in the body 9

2. The effect of increasing the sodium chloride in the diet upon animals receiving toxic doses of #1006 10

3. The effect of high and low calcium intake on animals inhaling high concentrations of #1006 10

4. The effect of injections of xanthine on animals inhaling high concentrations of #1006 11

TOWOLDMON0001253

I. EXPERIMENTS TO DETERMINE THE POSSIBLE TOXICITY OF THE FOLLOWING SUBSTANCES

1. CHLORCOSANE

On July 14, 1937, we were informed by F. D. Smith that each 6 ounce bottle of a soft drink might contain 60 to 70 mgm. of chlorcosane. There was no pharmacological reason to believe the compound would be irritating or toxic in any way, and therefore it was decided to dose rats with it very heavily, the argument being that if they survived heavy dosage without any evidence of damage a verdict of no toxicity might be given and much time saved. If, on the other hand, the large doses proved poisonous it would be necessary to reduce dosage until a non-toxic level was reached. The first of these alternatives proved correct.

Experiments. — August 10, 1937. 20 adult white rats ranging in weight from 162 to 210 gm. were each fed 70 mgm. of chlorcosane in 1 cc. of olive oil daily by stomach tube. These animals showed no evidence of damage in any way attributable to chlorcosane. They were examined microscopically from the 35th to the 92nd day. The only constant change observed was a possible slight increase in the granularity of the cells of the liver cords, but the pathologist in charge felt this to be within normal limits. This finding is doubly reassuring since many of the animals developed a chronic suppurative lung condition very prevalent in Boston stock rats at the time. But even with this added handicap there was no evidence of damage and we thus conclude that chlorcosane even in enormous dosage is an inert material in the body and in all probability passes through the intestine unchanged.

TOWOLDMON0001254

2. DIPHENYL PHTHALATE

On August 25, 1937, we were informed by Dr. R. E. Kelly that diphenyl phthalate might be absorbed through the skin or might be inhaled and swallowed during spraying operations. After consultation it was decided to feed heavily, and if there were indications of toxicity to project other experiments.

Experiments. — September 20, 1937. 20 adult white rats ranging in weight from 242 to 301 gm. were each fed 0.5 mgm. of diphenyl phthalate suspended in 1 cc. of water by stomach tube daily. The animals were from new stock and with one exception remained clear of the lung condition which existed in the chlorcosane group. During the period of experiment, which lasted 92 days, practically all the animals gained weight. Examination of 8 animals killed at intervals during this period of test resulted as follows:

The organs were normal grossly, and on microscopic examination the liver alone was of possible interest. The changes in the liver varied from those in which the cells of the liver cords were almost normal, having only a slight swelling and granularity of their cytoplasm, to those in which these changes were pronounced. In the latter there was a moderate degree of vacuolization and a rare hyaline body. These alterations it must be understood were the result of very certain dosage, since the compound was given by stomach tube and the animals had no possible way of avoiding it. Neither the pathologist nor myself was able to consider them of enough moment to cause us to make further experiments.

I am confident that with the ordinary precautions accompanying spray lacquering no possible harm could be done, even if the concentration of diphenyl phthalate in the lacquer was far above the 5 per cent figure given us by R. E. Kelly.

ACP CC3433

TOWOLDMON0001255

3. CHLORINATED DIPHENYL — COMPOUND #1268

This material was furnished by the Monsanto Chemical Company. It was said to have a chlorine content of 68 per cent, the highest chlorine figure of any compound tested by us during the past three years. It had been our impression that toxicity and chlorine content followed one another closely, but this has not turned out to be the case for #1268. Further discussion of this point will follow the description of the experimental observations and their results.

Experiments. — August 2, 1937. The compound #1268 was administered by inhalation, the technique being that described in the paper entitled The Problem of Possible Systemic Effects from Certain Chlorinated Hydrocarbons, Journal of Industrial Hygiene, 1937, 19, 283. For the first observations 80 adult white rats were used. They were exposed as follows:

Group 1. — 80 animals. Temperature to which the compound was heated to introduce fume into air line 140-190° C. Average concentration in air breathed by rats 0.53 mgm. per cubic meter. Average daily exposure 16 hours. The experiment was continued for 119 days, animals being sacrificed at intervals for pathological examination and for the carbon tetrachloride and alcohol test (see paper previously cited).

The conditions described above caused swelling and increase of granularity of the liver cells. Hyaline inclusions were rare. These changes were quite uniform in all animals examined after the 31st day, but were not certainly progressive. The rats were very healthy throughout the period and there was an almost uniform gain in weight. The carbon tetrachloride-alcohol test was positive after 52 days, which indicates that though liver damage was apparently slight some degree of harm had been done the organ. There was no evidence of

TOWOLDMON0001256

damage in any part of the body except the liver.

At the end of 93 days 10 rats, apparently in excellent condition, were removed from the experiment and set aside for observations upon liver recovery. These recovery animals showed no clinical changes of any sort. When sacrificed 72 and 141 days after removal from exposure it was observed that the swelling of the liver cells had disappeared, but the granular and hyaline material remained in the liver cells and had apparently become permanent. There was absolutely no progression of damage after removal from exposure.

These changes may be compared with those produced in rats by inhalation of chlorinated diphenyl #4465, administered in a similar manner in concentrations of 0.57 to 0.93 mgm. per cubic meter over similar periods of time. In the case of this compound the conspicuous difference was the far greater incidence of hyalinization of the liver cells, which in our opinion was the characteristic lesion caused by #4465 and mixtures containing it. One can therefore conclude that #1268 in low concentrations is definitely less toxic than #4465.

Group 2. — After 119 days, the low concentration of #1268 having proved but slightly poisonous, it was decided to increase the concentration in the air breathed by the 34 rats then remaining. Temperature and other conditions were maintained as during the first 119 days, but by the use of 4 vaporizers instead of 1 the average concentration of #1268 in the air was brought to 6.23 mgm. per cubic meter of air. Exposure to this very high concentration was continued for 87 days.

Under these extreme conditions the animals again remained in perfect health. The carbon tetrachloride and alcohol test was positive but there was absolutely no other indication of liver damage and no evidence of disturbance to other organs.

ACM CC343

TOWOLDMON0001257

When examined at autopsy at the end of 87 days — the animals had experienced a total exposure of 206 days — there were no gross evidences of abnormality in any part. On microscopic examination no organ showed changes except the liver. The number of cells containing hyaline and the amount of hyaline in the cells involved increased during this period of exposure to the higher concentration of #1268. The hyaline was most abundant in the portal zone of the lobule with very little in the central area. After 42 days of exposure 10 rats were set aside and were sacrificed 73 days later. They were in excellent condition when removed and did not change. Grossly, at autopsy, they showed nothing abnormal, and on microscopic examination the liver cells had lost their swelling but retained increased granularity and hyaline inclusions.

The experiments on inhalations of high concentrations of #1268 reenforce the conclusion that this compound is of low toxicity as compared with #4465 or with chlorinated naphthalenes above trichlornaphthalene. The question as to why #1268, the most highly chlorinated compound tested, proved but slightly harmful cannot be answered with any definiteness. It has been suggested that the toxicity of all these chlorinated compounds, even though of varied composition, may depend on the ability of the animal to decompose them after lodgment in the tissues, and that this decomposition might be shown by an increase in the chlorin in the urine in suitably conducted feeding experiments. In experiments upon this point we have shown a definite increase in the urinary chlorides when dogs and rats were fed a mixture of penta and hexachlornaphthalenes (#1006), a compound highly injurious to the liver and containing 62.6 per cent of chlorine, but simil observations have not been made with any of the chlorinated diphenyls or allied compounds. It may, however, be that when about 65 per cent chlorination is reach the substances formed are quite stable in the body and so cause a minimum of damage.

ALP CC343c

TOWOLDMON0001258

In conclusion, #1268, if handled with ordinary precautions as to ventilation should be entirely harmless to workmen. While it cannot be given an absolutely clean bill as to health, it is preferable to #4465 and #5460.

4. MIXTURE OF CHLORINATED DIPHENYL AND CHLORINATED DIPHENYL BENZENE — COMPOUND #5460

This substance was furnished by the Monsanto Chemical Company and was said to have a chlorine content of 60 per cent, being in this respect below #4465 and far below #1268.

Experiments. — The inhalation technique was used as in the case of #1268 and #4465.

August 2, 1937. 80 adult white rats were the subjects. Temperature to which the compound was heated to introduce fume into the air line 140-196° C. Average concentration in air breathed by rats 0.085 mgm. per cubic meter. Average daily exposure 16 hours. The experiment lasted 119 days. A certain number of animals were sacrificed for pathological examination, others were used for the carbon tetrachloride and alcohol test, and still others set aside for observations as to recovery from possible damage.

In spite of the fact that the concentration in the air breathed by the rats averaged about 1/6 that obtained at comparable temperatures from #1268, a number of these animals became sick and lost weight towards the end of the second month of exposure. When killed, such individuals showed gross mottling of the liver but no changes in other organs. On microscopic examination swelling of the liver cells, increased granularity and hyaline inclusions were noted in animals killed as early as the 16th day of exposure. These changes obviously occurred rapidly. Hyaline deposits were never as numerous as with #1268. Animals

TOWOLDMON0001259

removed for recovery after 51 and 119 days of exposure did not gain markedly in weight, and one died for no obvious cause. When examined at autopsy the swelling of the liver cells had subsided, but abnormal granularity and hyalinization remained.

Needless to say the carbon tetrachloride and alcohol test was positive whenever used.

In view of the fact that #5460 in such low concentration proved so definitely toxic, no higher concentrations were tested. It seems imperative that whenever this compound is used in industry, great care be taken to keep concentrations in the air at an extremely low level. No liberties can be taken with it, as with #1268.

TOWOLDMON0001260

II. SOME GENERAL CONSIDERATIONS

In addition to these tests of compounds, certain experiments were done which are of interest to those manufacturing or using chlorinated hydrocarbons. Details as to these experiments are of course available but are not included in this report since they are not of direct industrial interest.

1. EVIDENCE FOR THE DESTRUCTION OF A MIXTURE OF PENTA AND HEXACHLORONAPHTHALENES (#1006) IN THE BODY

It has always been a question as to whether the chlorinated hydrocarbons which have been examined by ourselves and by others do harm per se or whether toxicity depends on their breakdown in the body with the liberation of something harmful to the liver. A partial answer has been obtained by feeding #1006 to rats and dogs which were on a low chloride diet with uniform excretion of chlorides in the urine. When these animals received the chlorinated hydrocarbon (#1006) the urinary chlorides rose. This indicates that the body certainly has power to detach chlorine from this compound, and it is probable that the same condition is true for allied toxic compounds. It would be most interesting to see whether ingestion of #1268 results in similar findings or whether in the case of this relatively non-toxic compound there is practically no splitting off of chlorine. Neither time nor our financial resources permitted such tests.

TOWOLDMON0001261

2. THE EFFECT OF INCREASING THE SODIUM CHLORIDE IN THE DIET UPON ANIMALS RECEIVING TOXIC DOSES OF #1006

On the ground that chlorides might be fundamentally associated with toxicity, a group of 15 rats was fed a low toxic dose of #1006 and compared with a similar group on the same dosage of #1006 plus a marked increase in chloride intake secured by giving 5 cc. per kilogram of body weight of 4 per cent NaCl solution daily.

No differences were found between the two groups, and it may be concluded that chlorine increase secured through the diet does not enhance toxicity. This experiment was done in order to find out whether increase in chloride intake during hot weather might be harmful.

A particular phase of the problem, the possible enhancement of typical skin lesions by increasing chloride intake, cannot be decided by experiments on fur-bearing animals with no sebaceous glands. All that can be said at the moment is that increased chloride intake does not increase systemic toxicity.

3. THE EFFECT OF HIGH AND LOW CALCIUM INTAKE ON ANIMALS INHALING HIGH CONCENTRATIONS OF #1006

It is well known that a diet rich in calcium is markedly effective in preventing the acute yellow atrophy of the liver produced by carbon tetrachloride. (The Prevention and Treatment of Carbon Tetrachloride Intoxication. By P. D. Lamson, M.D., A. S. Minot, Ph.D., and B. H. Robbins, M.S., Journal of the American Medical Association, 1928, volume 90, page 345).

To discover whether calcium in the diet would protect against liver damage

TOWOLDMON0001262

from a toxic chlorinated hydrocarbon, 30 adult white rats were placed upon a diet of lean horsemeat, starch and lard, a combination adequate for maintenance but very low in calcium. Another group was given a diet consisting of dog chow, milk, lettuce and eggs, with added calcium lactate — a ration very high in calcium.

Both groups were exposed simultaneously to inhalation of high concentrations of ƒ1006, an average of 11.21 mgm. per cubic meter for 16 hours a day.

After 16 days, 12 high calcium diet rats were alive and 7 of the low calcium group. At intervals animals were killed for examination. In both groups the liver was abnormal grossly and microscopically, and in both groups animals died from liver damage. It was impossible to consider that the high calcium diet was in the least degree protective. It may, therefore, be concluded that adding calcium to the diet of workers either in the form of extra milk or of calcium lactate will not prevent liver damage.

4. THE EFFECT OF INJECTIONS OF XANTHINE ON ANIMALS INHALING HIGH CONCENTRATIONS OF ƒ1006

In 1937, R. C. Neale published a brief paper (The Protective Action of Certain Purines against Liver Necrosis Produced by Carbon Tetrachloride and Chloroform. Science, 1937, volume 86, page 83). He claimed that rats injected with sodium xanthine became markedly resistant to carbon tetrachloride. This suggested that xanthine might have similar protective power over liver damage from the chlorinated hydrocarbons on examination in this laboratory.

Accordingly 38 adult white rats were caused to inhale ƒ1006 in concentrations averaging 15 mgm. per cubic meter for 16 hours daily. One group of 20 rats was given 20 mgm. of xanthine subcutaneously every other day and 40 mgm.

TOWOLDMON0001263

of xanthine by stomach tube on the alternate days. The second group of 18 rats had the same exposure to #1006 without xanthine treatment. No differences were noted and one cannot expect any efficacy from xanthine either in the prevention or treatment of liver disease due to this chlorinated hydrocarbon, and in all probability the same negative result would be encountered in connection with allied toxic compounds.

TOWOLDMON0001264

REPORT TO THE MONSANTO CHEMICAL COMPANY

Cecil K. Drinker, M.D.

September 15, 1938

ADM 003416

35

TOWOLDMON0001265

C+25MM

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.

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.

TOWOLDMON0001266

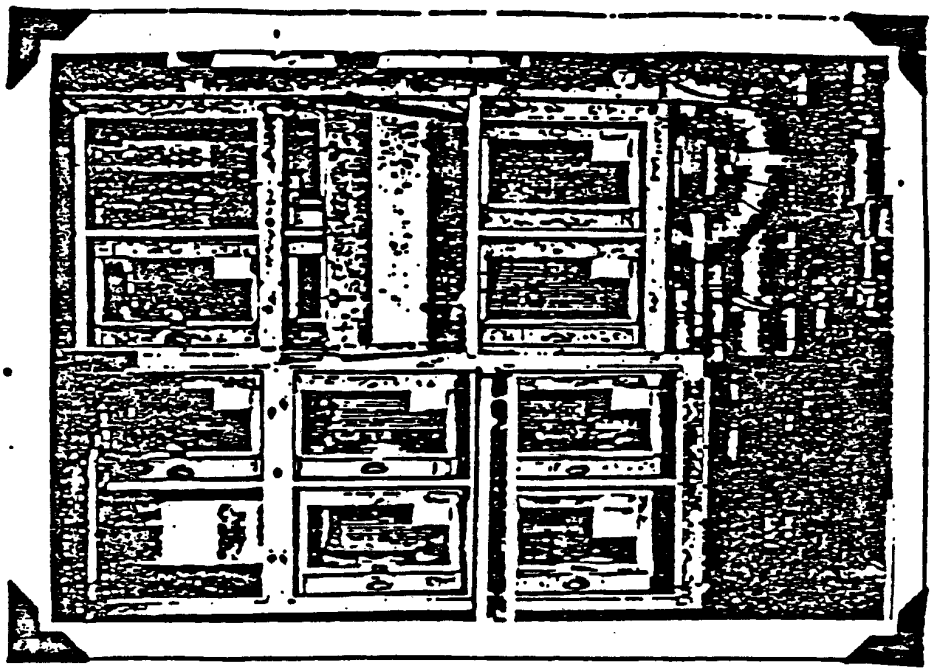


Fig. 1. Front view and inflow end of two boxes with rat cages in place and doors open.

TOWOLDMON0001267

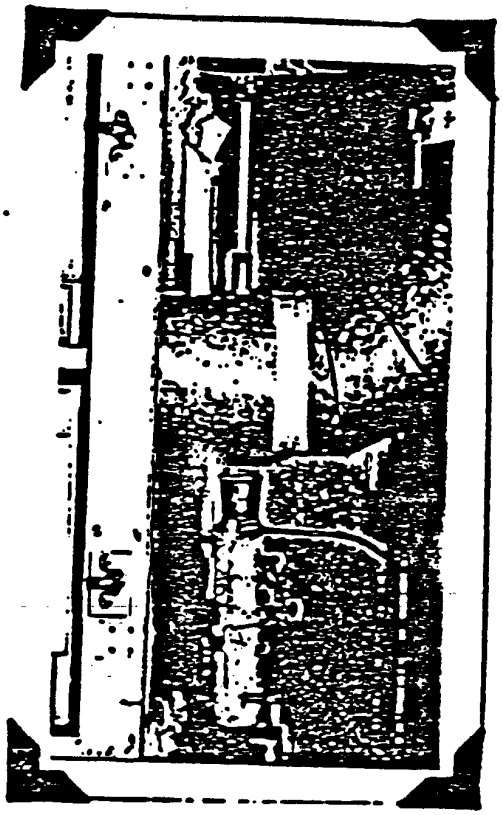


Fig. 2. Electric heater for maintaining chlorinated diphenyl at a constant temperature in place at inflow end of box.

TOWOLDMON0001268

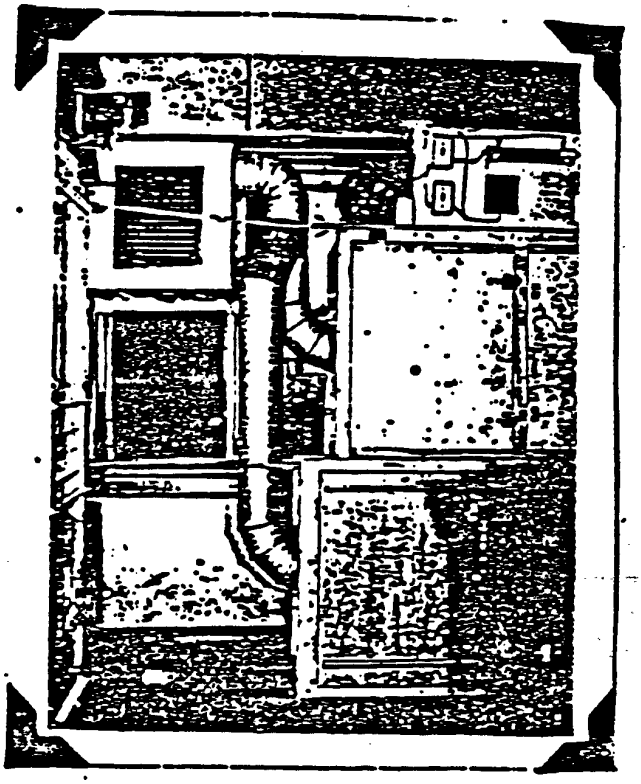


Fig. 3. Outflow end of two boxes showing connections to exhaust system.

TOWOLDMON0001269

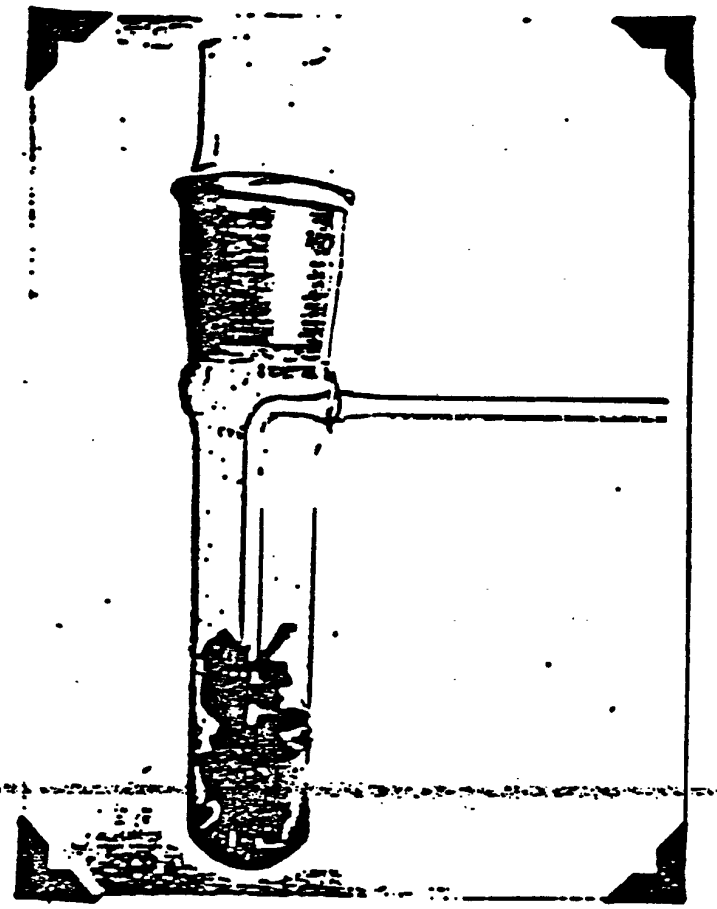


Fig. 4. Pyrex glass flasks.

TOWOLDMON0001270

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.

TOWOLDMON0001271

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 ACM CC34.

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

Material	Temperature	Average concentration of air in box		Total length of exposure	Average da: exposure
	°C	Mg./cu.M.	Range	hours	hours
Chlorinated diphenyl	165-175	0.37	High 1.19 Low 0.23	1896	16

TOWOLDMON0001272

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

TOWOLDMON0001273

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 exp
	°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

TOWOLDMON0001274

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.

ACP C03426

TOWOLDMON0001275