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THE PROBLEM OF POSSIBLE SYSTEMIC EFFECTS FROM CERTAIN CHLORINATED HYDROCARBONS*

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THE use of chlorinated naphthalenes and compounds of allied pharmacological possibilities is extremely wide, and with the steady growth of the use of electricity is certain to expand much farther. For years it has been known that many of these compounds cause a troublesome acne, and there is a large literature upon this phase of the subject. Our investigations have not been concerned with chloracne but with the possibility of systemic effects following ingestion or inhalation of such products. In the spring of 1936, the Halowax Corporation, a division of the Bakelite Corporation, called our attention to three fatal cases of jaundice in workmen using chlorinated naphthalenes and chlorinated diphenyl, and requested that the subject be investigated as rapidly and thoroughly as possible.† In brief these cases were as follows:

Patient 1. Male, age 21. The previous medical history of this man was in no way significant except for the fact that he had an attack of jaundice about 6 weeks prior to his fatal illness. Late in December, 1936, he became badly constipated and had much abdominal pain and distention. When admitted to the hospital he was slightly jaundiced and was evidently very ill. He was somewhat anemic and his skin, particularly upon the arms, face, chest and back, showed many pustules. He died after a brief period in the hospital, and at autopsy was found to

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† The Halowax Company makes many products besides chlorinated naphthalenes, and it has come to our knowledge that all of these products are indiscriminately called "halowaxes" by purchasers and users, and are lumped together as possible causers of acne and even of systemic disease. Since "halowax" is merely a trade designation, care should be taken to describe compounds by their chemical names and thus avoid condemnations which are both troublesome and misleading.

have a cirrhosis of the liver with acute yellow atrophy superimposed upon it. This man had been exposed to low concentrations of vapors arising from a mixture of tetra and pentachlornaphthalenes, together with approximately 10 per cent of a refined chlorinated diphenyl. While both he and others engaged in the same work had chloracne, there were no other disturbances of health in fellow workmen, nor was there any precipitating cause for the acute yellow atrophy such as treatment with arsphenamine or exposure to dangerous concentrations of carbon tetrachloride.

Patient 2. This was a young man who died in February, 1936, after an acute illness characterized by jaundice. He had been exposed to fumes arising from a mixture of penta and hexachlornaphthalenes. There is no record of chloracne. The patient worked with a large number of other people of whom but one (Patient 3), a close friend, had significant illness.

Patient 3. Another young man employed with Patient 2. He became jaundiced in March, 1936, and died after an illness of 2 weeks. A careful autopsy resulted in a diagnosis of acute yellow atrophy of the liver Here again no history could be obtained as to a precipitating cause, and there was no record of preceding attacks of jaundice.

In addition to these three very recent fatalities, we have learned of four other possible cases, none of them fatal. All of these have had jaundice and the entire group consists of isolated individuals who have been picked out of large groups having the same exposure. In but one instance, Patient 1, is there record of antecedent disturbance of health, and the general health of fellow workers has been good.

Such cases have not been reported in the medical literature and only occasionally can one find reference to systemic effects of any sort. For example, Courtois-Suffit (1934) reports on work done by Touraine and his associates (1934) who examined 60 workers who had been exposed to trichlornaphthalene. Of these 13 were found to have mild digestive complaints, anorexia, nausea and vertigo, but Courtois-Suffit remarks finally, "Absorption is certainly possible and we have for proof of it some of the digestive and general complaints which have been due to it. But they appear to be of little consequence considering the mildness of the digestive troubles and the absence of respiratory phenomena."

In Touraine's cases the exposure was to a trichlornaphthalene, whereas the American cases of acute yellow atrophy were exposed to compounds of higher chlorination. Our own experiments indicate that trichlornaphthalenes require enormous dosage, far beyond anything encountered in industry, in order to produce liver damage. Teleky (1927) reported a number of cases of chloracne in persons exposed to chlorinated naphthalenes with a chlorine content ranging from 14 to 53 per cent. He found that the lower the chlorine content the less the acne. Mittelstädt (1935) examined a number of cases of chloracne due to trichlornaphthalene and reported a number of vague general complaints but nothing in the nature of serious disease. Regarding his animal experimentation, Lehmann (1919) reported that animals fed chlorinated naphthalenes refused to eat after a time and that, whether poisoned by inhalation or by feeding, at death showed "peculiar" lesions in the liver. Flinn and Jarvik (1936) gave subcutaneous injections of enormous doses of chlorinated naphthalenes dissolved in paraffin oil to rabbits. The compounds used were as follows:

1. A mixture of tri and tetrachlornaphthalene.
2. A mixture of tetra and pentachlornaphthalene.
3. A mixture of penta and hexachlornaphthalene.

In addition, sublimates from (2) and (3) were collected in oil and injected subcutaneously.

None of the animals receiving (1) or the sublimate from (2) died, and even after 2 months were quite normal when autopsied. The first death in the animals receiving (3) occurred on the 12th day and the last died on the 26th day. Those receiving the sublimate from (3) were even more severely affected. Autopsy in these animals revealed striking changes in the liver, not, as described, entirely characteristic of acute yellow atrophy but sufficiently suggestive to cause the authors to conclude that "certain chlorinated naphthalenes or impurities contained in them are capable of producing yellow atrophy of the liver in the rabbit."

At the beginning of their paper, Flinn and Jarvik (1936) mention the fact that there have been three cases of acute yellow atrophy of the liver in men working with chlorinated naphthalenes but give no details in regard to them. These cases are undoubtedly the same as those described in the beginning of this paper.

One may summarize the meagre literature upon systemic effects from these substances as follows:

1. With the exception of the mention of acute yellow atrophy by Flinn and Jarvik (1936) there are no reports or even suggestions of serious effects upon human beings.

2. There is evidence (Teleky, 1927) that the degree of chlorination is significant in relation to the production of acne. In the work of Flinn and Jarvik (1936) the compounds producing serious liver injury were the most highly chlorinated of those tested, though the chlorine contents as given by analysis vary surprisingly little.

3. There are no published figures upon the amounts of various chlorinated naphthalenes in the air which will produce injury of any sort, and while the work of Lehmann (1919) and of Flinn and Jarvik (1936) point to the liver as a possible site of injury this indication rests upon such extreme dosage as to fail to apply directly to human exposure.

Experimental Work

In appraising the possible toxicity of any substance met in industry it is first necessary to determine the principal route of absorption. In the case of the compounds under consideration there can be no doubt that inhalation is their chief means of entering the body. They are used hot in a great variety of operations and volatilize in varied degree. They are often applied in solution in such volatile solvents as carbon tetrachloride and toluene. The amounts reaching the air under such circumstances are hardly detectable. It will however be shown that carbon tetrachloride adds to the toxicity of the chlorinated naphthalenes and allied compounds, and if there is possibility of inhaling these compounds in other parts of the factory then inhalation of carbon tetrachloride adds a decided hazard. Under such circumstances solvents such as toluene should be used.

Observation in a number of plants causes us to feel that even though

workmen may be extremely dirty and careless, comparatively little of these waxes is eaten. They are tenacious substances, insoluble in water, and if they get on the hands they stick there and are not transferred to the food.

Skin absorption is the third possible means of entering the body. It may occur but at best must be slight when compared to the steady inhalation of finely divided or gaseous material in the air.

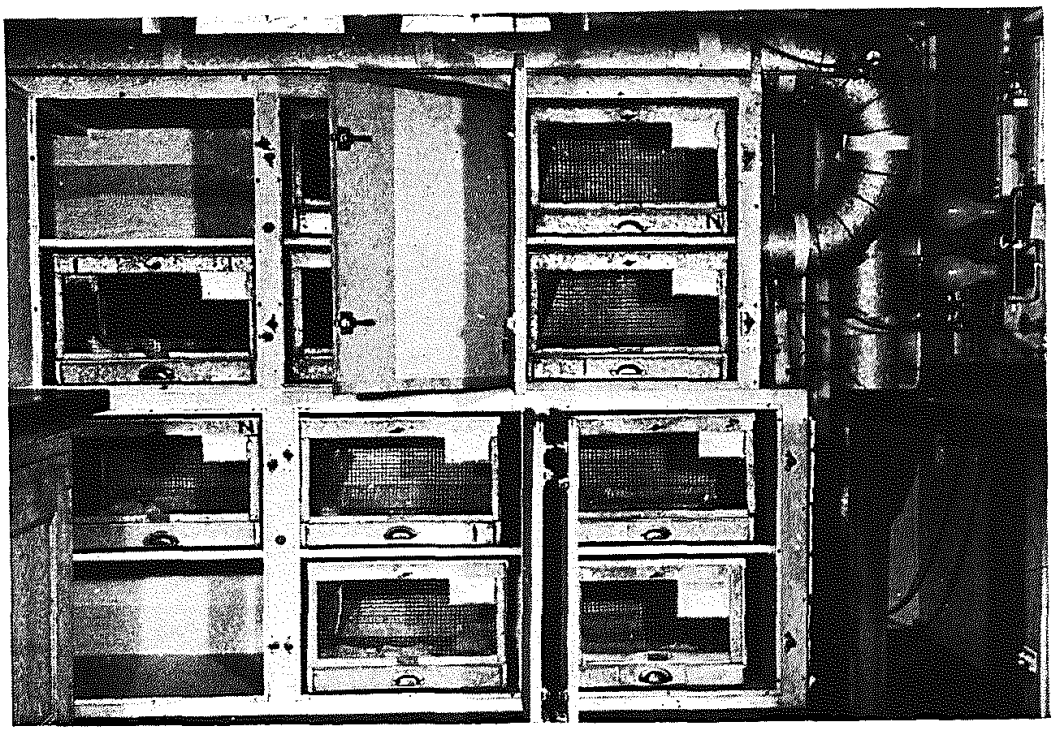

FIG. 1. Front view and inflow end of two boxes with rat cages in place and doors open.

Inhalation experiments are then the most important sources of information, but to them we have added a certain number of observations upon ingestion and subcutaneous injection of various compounds.

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 (figure 1).

At the center of one end of each box —the inflow end—air was introduced through a pipe 7 inches in diameter (figure 2). Each box was equipped with an individual variable-speed electric blower which blew the air through several feet of 7-inch pipe before enter-

ing the end of the box. An orifice meter was placed in the pipe line, and the flow of air in cubic feet per minute could be adjusted and read off directly from a calibration curve. A vane deflector 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 (figure 3).

About 4 inches from the entrance of the 7-inch pipe into the box, the fumes of the substances listed were introduced into the inflowing air. Specially designed pyrex glass flasks (figure 4), $7\frac{3}{4}$ inches long and with a diameter of $1\frac{1}{2}$ inches, were used to hold the heated waxes. These flasks were made with a side arm and tube that extended to the bottom. The top of the flask was ground, and into this fitted a short tube $1\frac{1}{2}$ inches in length. This short tube was inserted into a large rubber stopper that fitted tightly into a hole cut out of the 7-inch pipe on the under side (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 (figure 2).

Approximately 30 gm. of pulverized chlorinated naphthalenes or 20 gm. of chlorinated diphenyl were placed in the bottom of each flask and melted in the electric heater. Fresh samples were used every other day, but it was often found necessary to add 20 gm. of new material even after one run since so much had sublimed. Whatever the case, 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.

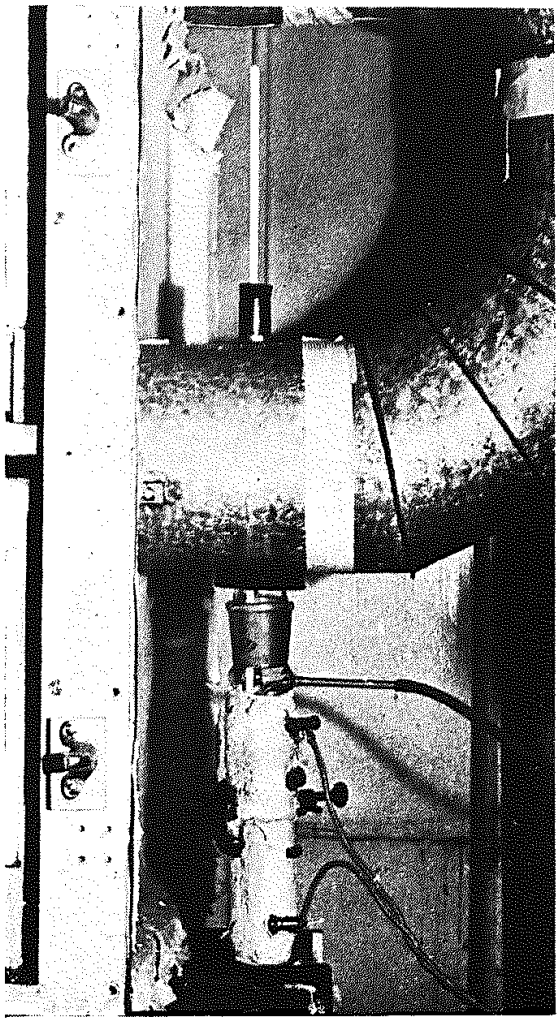

FIG. 2. Electric heater for maintaining chlorinated compounds at a constant temperature in place at inflow end of box.

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

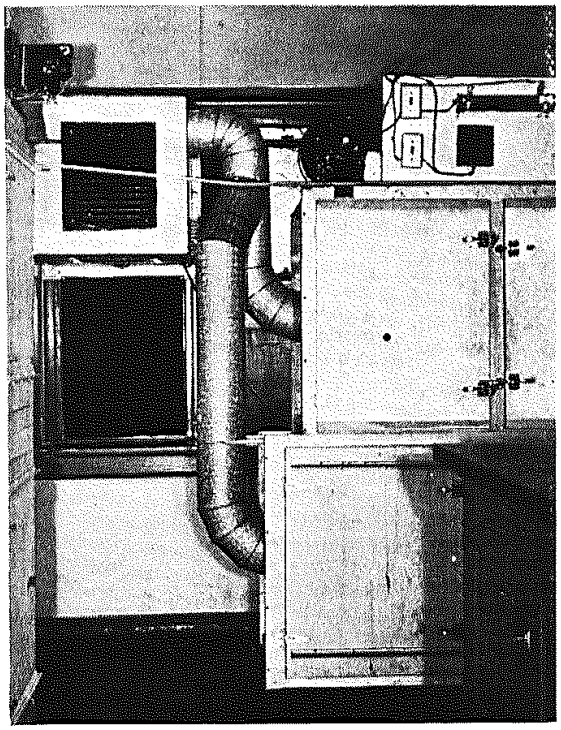

FIG. 3. Outflow end of two boxes showing connections to exhaust system.

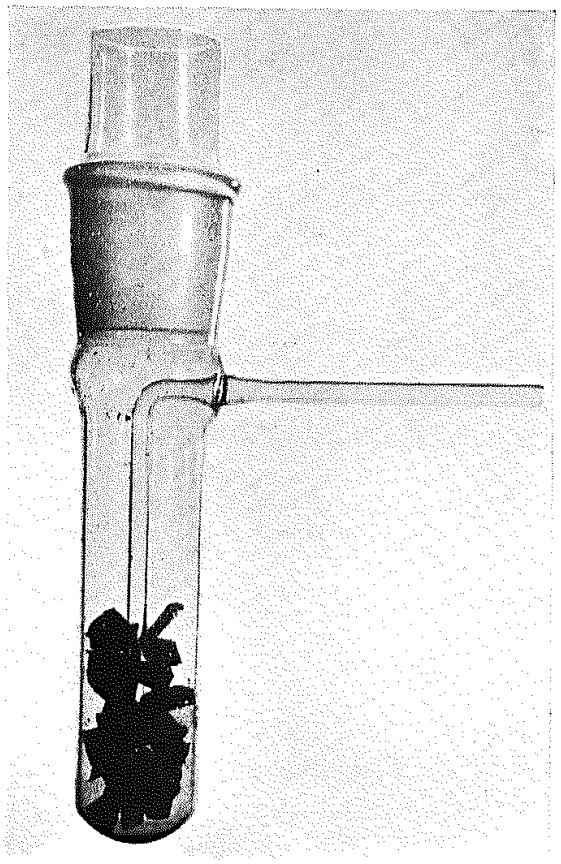

FIG. 4. Pyrex glass flask

flowmeter 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.

Approximately 1 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 rheostats on the fans and 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 c.f.m.

In the first group of experiments the following substances were tested:

1. A mixture of trichlornaphthalenes plus a trace of tetrachlornapthalene. Chlorine content 49.9 per cent.
2. A mixture of penta and hexachlornaphthalenes. Chlorine content 62.6 per cent.
3. A mixture of 90 per cent penta and hexachlornaphthalenes plus 10 per cent refined chlorinated diphenyl. Chlorine content 63.0 per cent.
4. Chlorinated diphenyl. Chlorine content 65.0 per cent.

The compounds were selected as representing a certain range in chlorination and also because of their industrial importance. In each instance 80 animals were exposed, 10 rats being placed in each cage. They were fed Purina

Dog Chow supplemented by lettuce, eggs, milk and cod liver oil.

This first group of experiments was begun on July 1, 1936 and the last exposure to number 2 (penta and hexachlornaphthalenes) was on November 16th. The exposure to the other three compounds ceased upon November 18th. On October 15th representative animals were taken out of exposure from groups 1, 2, and 4 and were killed after 2 months on December 15th, in order to see whether this clear period would bring about recovery in the affected livers. Animals from group 3 were similarly removed from exposure on October 4th and killed for examination on December 4th.

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, airflow, 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 NOVEMBER 16 AND 18

MATERIAL	CHLORINE CONTENT	TEMP.	AV. CONCENTRATION OF AIR IN BOX		TOTAL EXPOSURE	AV. DAILY EXPOSURE
	%	°C.	mg./cu. m.	range	hours	hours
Trichlornaphthalenes plus traces of tetrachlornaphthalene	49.9	150–160	1.31	High 2.60 Low 0.10	1896	16
Penta and hexachlornaphthalenes	62.6	160–170	1.16	High 2.19 Low 0.51	1864	16
90% penta and hexachlornaphthalenes plus 10% chlorinated diphenyl	63.0	165–175	1.37	High 3.17 Low 0.64	1896	16
Chlorinated diphenyl	65.0	165–175	0.57	High 1.19 Low 0.23	1896	16

Table 1 shows the temperatures at which the various substances were held, the average concentrations per cubic meter of air, and the average exposures from July 1 to November 16 and 18.

The concentration of chlorinated naphthalenes and chlorinated diphenyl in the air of workrooms.—Table 1 shows that animals have been exposed to varied concentrations of the substances under

test. The concentrations employed may be regarded as fairly representative of industrial experience. Prior to the initiation of inhalation experiments a number of estimates of chlorinated hydrocarbons in the air of different factories were made and the concentrations chosen for inhalation experiments depended on these examinations. The technic of analysis and the apparatus employed were the work of Frederick R. Millhiser and William F. Hemperly.

The method and apparatus used for determining concentrations of chlorinated hydrocarbons in air were adapted from well-known procedures. The air in question is passed over heated platinum in an electrically heated quartz tube and the effluent gas scrubbed in a column of glass beads moistened with sodium carbonate containing a trace of sodium sulfite. The beads are then washed down and the chloride determined nephelometrically as silver chloride.

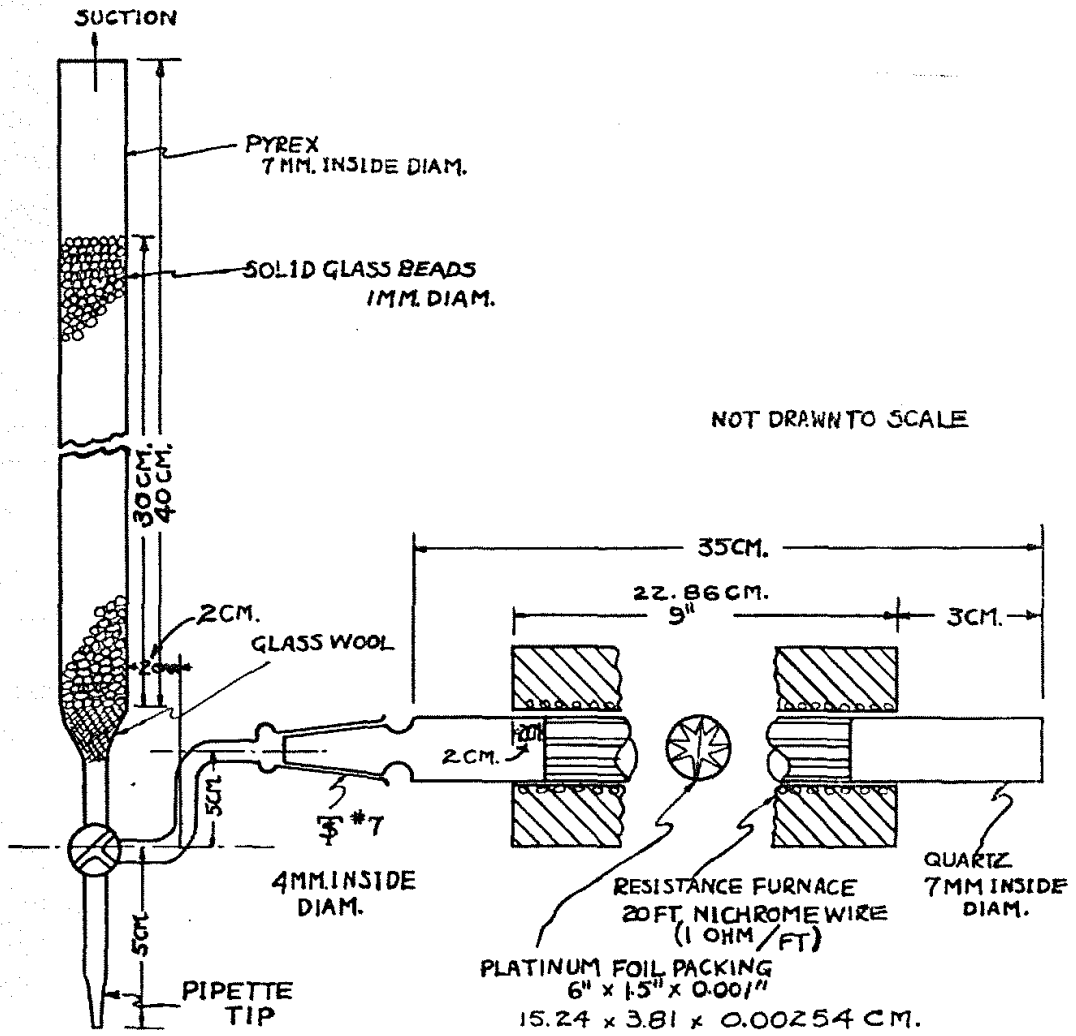

FIG. 5. Dimensioned sketch of combustion tube and absorption apparatus.

Tebbens (1937) has recently described a method and apparatus which should be equally satisfactory for this work. In figure 5 we show a dimensioned sketch of the combustion tube and absorption apparatus as used by us in both our laboratory and field problems. The absorption tube is

somewhat easier to wash down than is Tebbens's but the essentials of the two devices are the same.*

In both cases conversion of the chlorinated hydrocarbons to hydrochloric acid and subsequent absorption as sodium carbonate should be complete. In our case concentrations in air are apt to be very low—the objective is less than 1 mgm. per cu. m. Consequently the amount of silver chloride actually formed in the final reaction is so small that it can not be determined either gravimetrically or by titration. For this reason we have been forced to use the nephelometric procedure which is sensitive to concentrations as low as 0.1 mgm. per cu. m. of air.

It is doubtful if the sampling rate should exceed 1 liter per minute as conversion of the chlorinated compound to hydrochloric acid and its subsequent absorption are not efficient unless the velocity of the gas stream is low. Another precaution to be noted is that the method is not selective—any chlorinated substance will be determined. Furthermore it does not distinguish between solid particles and gases but determines them all alike and the results must, of course, be computed in terms of total chlorine.

At the present time we possess information as to the amounts of chlorinated naphthalenes in the air of 30 different plants, and in a number of instances the measurements have been repeated several times. Frequently the amounts have been greater than those used in our experiments, but it must be remembered that the rats have been exposed for 16 hours to an atmosphere constantly impregnated with the substance under test, whereas human exposure is usually a variable quantity, intense for a short time and then negligible. It is our opinion at the present time that the concentrations of chlorinated hydrocarbons used in our experiments would be dangerous for workers in the case of compounds above trichlornaphthalene in chlorination. Fortunately it is easy to ventilate processes of manufacture which require these substances and to reduce air contamination practically to the vanishing point. Such treatment of the problem at once removes both the possibility of systemic poisoning and the annoyances that arise from cases of acne.

Results of Inhalation Experiments.

1. Animals exposed to a mixture of trichlornaphthalenes plus small amounts of tetrachlornapthalene. Living animals were apparently entirely normal. Autopsies performed near the end of exposure seemed to show slight swelling of the liver, and microscopic examination occasionally showed swollen and hypergranular liver cells. The changes were, however, never more than slight.

2. Animals exposed to a mixture of penta and hexachlornaphthalenes. No abnormalities were observed in the living animals. Rats were killed and autopsied every 6 weeks. In the first animals sacrificed liver changes were observed. These were swelling of cells, slight granulation and hyalinization. In September and October these conditions were somewhat more advanced, and in November the process became stationary. There were highly

* The combustion and absorption apparatus suited to field sampling is now made by Willson Products, Inc., Reading, Pa. The equipment includes flowmeter and suction pump with a carrying case.

granular cells, hyaline inclusions and mitotic figures, but no more than 2 months previously.

3. Animals exposed to a mixture of 90 per cent penta and hexachlornaphthalenes plus 10 per cent refined chlorinated diphenyl. No abnormalities were seen in the living animals. After 6 weeks the livers showed changes similar to those in the animals exposed to penta and hexachlornaphthalenes. These advanced in grade during August and September and then became stationary.

4. Animals exposed to chlorinated diphenyl. No abnormalities were seen in the living rats. After 6 weeks' exposure there was slight liver damage which advanced during the next 2 months. The changes consisted in slight to moderate swelling of the liver cells, an increased granularity and many mitotic figures. Hyalinization was always present as a result of inhalation of chlorinated diphenyl.

Summary of the first inhalation experiment.—In these experiments careful observation of appearance, body weight, activity, blood, and urine showed no abnormalities of any sort. Yet after 6 weeks' exposure all the compounds with chlorination above trichlornaphthalene caused minor degrees of liver damage, and no changes whatsoever in other organs. There was no acute yellow atrophy or anything suggesting it except that a slight degree of liver damage was always present and was quite clear in the liver sections examined microscopically. This damage had no detectable effect on the health of the animals. They held their weight, ate and behaved normally, being in every respect similar to the many people who have been exposed to these compounds without illness of any sort.

The functional appraisal of the liver damage caused by certain chlorinated naphthalenes and by chlorinated diphenyl.—There are no tests of liver function useful in such minor degrees of liver damage as were produced in these experiments. Indeed the animals resisted the injury so perfectly as to display no abnormalities except upon histological examination of the liver. The situation was perhaps similar to that met in industry, where, barring acne, the health of workers in these compounds has been good with the exception of the fact that in isolated instances jaundice has occurred which upon at least three occasions has gone on to acute yellow atrophy.

During recent years this disease has been seen following administration of carbon tetrachloride, arsphenamine and cincophen. In the case of carbon tetrachloride it is known that a low calcium diet and alcohol favor the production of liver damage. For the acute yellow atrophy that occasionally complicates use of the other two drugs no cause can be assigned. One cannot produce acute yellow atrophy with arsphenamine, but somehow or other this now and then does happen to patients under antisyphilitic treatment.

It occurred to us that something of the same sort might be involved in this problem. The human cases have been scattered and few. They have been isolated instances out of large groups of healthy employees who have had equal degrees of exposure. It was our idea that perhaps many of these people got liver changes such as existed in our rats, changes not recognizable through

any means other than autopsy examination. If, upon the substratum of such changes, they got an acute liver disturbance, acute catarrhal jaundice, not a very common disease but one which any of us may experience, would this relatively innocuous disease go over to acute yellow atrophy?

Knowing that our inhalation rats had liver changes, but changes too slight to cause recognizable symptoms, we decided to test their resistance to substances known to cause liver destruction. Carbon tetrachloride and alcohol were selected. Having information that 1.0 cc. of carbon tetrachloride plus 1.0 cc. of ethyl alcohol per kilogram would kill 14 per cent of normal white rats, we reduced the dose to 0.75 cc. carbon tetrachloride and 0.75 cc. of ethyl alcohol.

This mixture was given by stomach tube to the following groups of animals and with the results found in the following tabulation.

Rats fed 0.75 cc. per kgm. each of carbon tetrachloride and ethyl alcohol by stomach tube

1. *Trichlornaphthalene plus trace of tetrachlornaphthalene.* Fed at 9:00 a.m., Nov. 10, 1936. 10 rats. No deaths.

2. *Penta and hexachlornaphthalenes.* Fed at 9:30 a.m., Nov. 10, 1936. 10 rats.
   - 3 died Nov. 11th between 4:30 and 10:30 p.m.
   - 1 died Nov. 12th at 3:20 p.m.
   - 1 died Nov. 12th at 4:30 p.m.
   - 1 died Nov. 13th between 9:30 and 11:30 a.m.
   - 1 died Nov. 15th between night of Nov. 14th and 12:30 p.m. Nov. 15th. 2 died Nov. 16th between 10:15 p.m. Nov. 15th and 9:00 a.m. Nov. 16th (stiff).

3. *90 per cent penta and hexachlornaphthalenes and 10 per cent chlorinated diphenyl.* Fed at 10:00 a.m., Nov. 10, 1936. 10 rats.
   - 1 died Nov. 10th between 5:00 and 10:30 p.m.
   - 5 died Nov. 11th between 4:30 and 10:30 p.m.
   - 1 died Nov. 11th between 4:30 p.m. and 9:00 a.m. Nov. 12th.
   - 2 died Nov. 12th about 1:00 a.m.
   - 1 died Nov. 13th between 5:00 and 10:00 p.m.

4. *Chlorinated diphenyl.* Fed at 10:00 a.m., Nov. 10, 1936. 10 rats.
   - 1 killed Nov. 10th at 10:30 p.m. (almost dead).
   - 2 died Nov. 11th between 4:30 and 10:30 p.m.
   - 1 died Nov. 12th before 8:30 a.m. (stiff).
   - 1 died Nov. 13th between 9:30 and 11:00 a.m.
   - 1 died Nov. 16th between 10:15 p.m. Nov. 15th and 9:00 a.m. Nov. 16th (stiff).

Controls: Fed at 9:30 a.m., Nov. 12, 1936. 10 rats. 1 killed Nov. 13th for normal liver.

This tabulation summarizes into the facts that:

1. No normal rats were killed by carbon tetrachloride and ethyl alcohol.

2. No trichlornaphthalene exposed rats were killed, and this finding agrees with our inability to find lesions of moment in the livers of the animals that inhaled this substance.

3. The penta and hexachlorinated naphthalene, the mixture of these with 10 per cent chlorinated diphenyl, and finally the latter substance alone seem to have produced conditions lowering the resistance to an agent capable of producing serious liver disease.

4. The sole lesion produced by this test of liver function was acute yellow atrophy usually accompanied by jaundice.

This test of liver function was accomplished with a substance which itself is an organic chloride and curiously enough it is the only substance we were able to find that was effective.

In animals with the minor degree of liver injury which has been described we were unable to produce acute yellow atrophy or any variety of liver effect with arsphenamine, cincophen, butyl chloride, ethylene chloride and tetrachlorethylene, but carbon tetrachloride was uniformly effective in disclosing the existence of liver damage.

Recovery from liver damage following removal from exposure.—Animals exposed to trichlornaphthalenes plus traces of tetrachlornaphthalene being practically normal on removal from exposure need no consideration. In the case of the penta and hexachlornaphthalene, rats after 2 months' removal from exposure still showed swollen liver cells, increased granularity, hyalinization and mitotic figures. The condition was not advanced as compared with rats killed at the time of removal from exposure but on the other hand 2 months were insufficient for recovery.

The same findings were true of the mixture of penta and hexachlorinated naphthalenes and chlorinated diphenyl and for chlorinated diphenyl alone.

Apparently the changes induced in the liver cells by these substances are exceedingly persistent and one must expect that an individual showing any signs of liver injury will be many months in returning to normal.

The effect of high concentrations of trichlornaphthalenes with traces of tetrachlornaphthalene.—The first group of inhalation experiments showed that this material in concentrations averaging 1.31 mgm. per cu. m. of air was relatively innocuous, judged both by direct observation and by the carbon tetrachloride test. This is an interesting fact since such compounds cause acne, though less potently than substances of higher chlorination. In order to explore the matter further one of the inhalation boxes was arranged so that fumes from four glass containers were delivered to the air line instead of one. This resulted in the conditions shown in table 2. The animals subjected to these conditions showed no clinical effects of any sort. After 1 month the liver cells were slightly swollen and over-granular and there were occasional mitotic figures. These changes were similar to the early effects of more highly chlorinated compounds, and progressed only slightly during the third and fourth months. When rats in this condition were given carbon tetrachloride and alcohol, in some instances their livers showed massive central necrosis and

TABLE 2

CONDITIONS MAINTAINED DURING INHALATION OF HIGH CONCENTRATIONS OF TRICHLORNAPHTHALENES PLUS TETRACHLORNAPHTHALENE

MATERIAL	TEMP.	AV. CONCENTRATION OF AIR IN BOX		TOTAL EXPOSURE	AV. DAILY EXPOSURE
	°C.	mg./cu. m.	range	hours	hours
Trichlornaphthalenes plus traces of tetrachlornaphthalene	137–200	10.97	High 16.49 Low 5.78	1232	16

in others this did not develop. Fifty rats were used in this experiment and one may conclude that while the trichlornaphthalenes are in no degree as toxic as those of higher chlorination they are not entirely free from effects upon the liver if high enough concentrations are inhaled over long periods.

The effects of high concentrations of penta and hexachlornaphthalenes.—There can be no doubt as to the damage done the liver by these compounds but in the concentrations so far reported no symptoms were caused that could be recognized clinically and nothing approaching acute yellow atrophy occurred except through the use of the carbon tetrachloride test. On December 1, 1936, 80 rats were subjected to the conditions summarized in table 3. This experment was terminated on January 21. All of the animals lost weight and appetite and deaths began after 8 days of exposure. Fifty-five rats died, most of them heavily jaundiced, 8 were killed for microscopic examination, 9 were killed by the carbon tetrachloride test and 8 lived through the period of exposure. The livers of the animals killed for examination showed marked fatty degeneration, central in type with necrosis of liver cells. Surviving animals showed pronounced changes in the liver after 35 days of freedom from inhalation. It is thus evident that penta and hexachlornaphthalenes are markedly toxic compounds and that recovery from their effects is extremely slow.

The effects of penta and hexachlornaphthalenes, the mixture of these with 10 per cent chlorinated diphenyl, and of chlorinated diphenyl alone, when inhaled in low concentrations through an 8-hour day instead of a 16-hour day as in the first experiment.—Since steady human exposure to any of the compounds tested would invariably be for 8 hours rather than 16-hour periods, a further inhalation experiment was arranged under the conditions shown in table 4 which are quite comparable to those in table 1 except for the shorter inhalation periods. Eighty rats were used for each of the three compounds tested. None at any time showed the slightest evidence of illness. Microscopic examination beginning after 6 weeks' exposure showed swollen liver cells, excess granulation, hyaline inclusions and occasional mitotic figures. These changes did not progress and were very similar to those in the animals exposed for 16 hours under the conditions set forth in table 1. The carbon tetrachloride test was uniformly fatal to them and one must conclude that con-

TABLE 3

CONDITIONS MAINTAINED DURING INHALATION OF HIGH CONCENTRATIONS OF PENTA AND HEXACHLORNAPHTHALENES

MATERIAL	TEMP.	AV. CONCENTRATION OF AIR IN BOX		TOTAL EXPOSURE	AV. DAILY EXPOSURE
	°C.	mg./cu. m.	range	hours	hours
Penta and hexachlornaphthalenes.....	137–195	8.88	High 14.0 Low 5.75	608	16

centrations of these compounds such as were employed cause a certain degree of liver damage even if inhaled for but 8 hours daily. This injury is resisted successfully by the rats just as was the 16-hour injury but it is none the less there and destroys the ability of the animal to resist the carbon tetrachloride test.

Summary of inhalation experiments. The findings that have been described briefly will be amplified upon the pathological side in a further paper by Dr. G. A. Bennett. What has been given is, however, sufficient to indicate that compounds more highly chlorinated than trichlornaphthalene are capable of causing liver injury when inhaled steadily in quite low concentrations. It is an extraordinary thing that even the most searching examination fails to show injury in any other region. It is not easy to grade the toxicity of the different compounds tested, but the chlorinated diphenyl is certainly capable of doing harm in very low concentrations and is probably the most dangerous. Industrial experience combined with these experiments make it appear probable that workmen never inhale enough of any of these substances to get acute yellow atrophy. They may, however, acquire a substratum of liver damage upon which acute yellow atrophy may develop. Experience in a number of plants has shown how easy it is to reduce concentrations of these compounds practically to the vanishing point, and every effort should be made to attain such conditions.

TABLE 4

CONDITIONS MAINTAINED DURING INHALATION OF LOW CONCENTRATIONS OF THREE COMPOUNDS DURING 8-HOUR INSTEAD OF 16-HOUR PERIODS

MATERIAL	TEMP.	AV. CONCENTRATION OF AIR IN BOX		TOTAL EXPOSURE	AV. DAILY EXPOSURE
	°C.	mg./cu. m.	range	hours	hours
Penta and hexachlornaphthalenes.....	149–193	1.44	High 2.58 Low 0.42	920	8
90% penta and hexachlornaphthalenes plus 10% chlorinated diphenyl.....	150–197	1.66	High 3.30 Low 0.56	912	8
Chlorinated diphenyl.....	153–174	0.93	High 3.23 Low 0.03	920	8

Gross Feeding Experiments

The various compounds used were ground as finely as possible and mixed with a standard balanced ration for the white rat. The food was placed in a single container, at which the 10 animals in a cage had an equal chance, the amount of the compounds added being reduced as the number of rats lessened during the feeding period. The experiments were designed to give an idea as to the possible toxicity of the compounds selected and, if toxicity was observed, some idea as to the site or sites of damage.

The experiments were successful in both respects, and indeed one may anticipate that future appraisals of the possible toxicity of chlorinated hydro-

carbons may often be made by simple feeding and do not require the elaborate apparatus and the expense necessary for inhalation experiments. The essential reason for this opinion resides in the identity of the lesions produced by both methods, and the fact that the different compounds so far studied seem to arrange themselves identically so far as toxicity is concerned whichever way they are administered.

Trichlornaphthalene plus traces of tetrachlornaphthalene.—Feeding began on May 4, 1936 and continued until November 2, 1936. In the beginning 3 gm. of this mixture were added to the food for 10 rats each day, and this concentration was maintained throughout the experiment. All the animals were killed except the last which died on Nov. 2. There was no loss of weight and no appearance of wasting illness of any sort. The single death was due to some variety of respiratory infection which had no relation to the material inhaled.

Histological examination showed slight changes in the liver but nothing of great significance.

Tetra and pentachlornaphthalenes.—Feeding began on June 29, 1936. A dosage of 0.5 gm. daily was employed. All the animals sickened gradually and were either killed or died by August 29, 1936.

At autopsy both grossly and histologically the liver was the single organ affected. The liver cells showed swelling, hypergranulation, hyaline inclusions and vacuolation. Here and there necrotic cells were found. There was a tremendous accumulation of fat.

Penta and hexachlornaphthalenes.—Feeding began May 4, 1936, a 3 gm. dosage being used. By June 6, nine rats had died. The last one was obviously ill and was killed for autopsy examination. All animals lost weight from the beginning and were ill. At autopsy the liver alone was affected, the lesions observed being similar to those that have been described for tetra and pentachlornaphthalenes but worse.

90 per cent penta and hexachlornaphthalenes and 10 per cent chlorinated diphenyl.—Feeding began May 4, 1936 on a 3 gm. dosage. On May 16th feeding was stopped but all the animals went on to death, the last dying on June 8th. An autopsy the liver lesions were extremely severe and of the usual type.

On account of the high toxicity of the 3 gm. dosage, four rats were given a 0.5 gm. dose every other day. Feeding began June 24, 1936 and the last animal was killed Sept. 11th. There were no deaths but all the animals lost weight. At autopsy the liver as usual was the single organ affected, the lesions being characteristic and extensive.

Chlorinated diphenyl.—Feeding began May 4, 1936 on a 3 gm. dosage and was discontinued on May 10th. Seven of the 10 rats were dead by May 12th. The three remaining rats gained in weight but were sacrificed for autopsy purposes on July 8th. The liver changes began at once. There were no changes in other organs.

A second group of 10 rats was fed a much smaller dose—0.5 gm. every other day. Feeding began May 20, 1936. The first rat died on May 29th and four more before June 24th. The remaining were sacrificed. Those rats that died showed losses in weight, while those sacrificed had recovered

their initial loss in weight and were gaining. The liver lesions were similar to those found in rats fed penta and hexachlornaphthalenes plus 10 per cent chlorinated diphenyl but not so marked.

Summary of gross feeding experiments.—Of the various materials fed rats in large doses trichlornaphthalene plus traces of tetrachlornaphthalene was quite innocuous. Tetra and pentachlornaphthalene showed definite liver damage. Penta and hexachlornaphthalenes caused a similar grade of injury. The addition of chlorinated diphenyl to penta and hexachlornaphthalenes increased the toxicity. Chlorinated diphenyl alone produced liver lesions but in the dosage used was less effective than when mixed with highly chlorinated naphthalenes. In no case did the compounds used produce acute yellow atrophy but the lesions observed indicate this might be possible if one found a dosage which could act for the proper period of time.

Feeding Precise Doses by Stomach Tube

The compounds employed were suspended in gum acacia. In figuring the dosage the total amount a man of 50 kg. would inhale in an 8-hour day assuming an air concentration of 20 mgm. per cu. m. was first calculated and reduced to milligrams per kilogram. The rats and rabbits received this dose each day. The compounds used were those employed in the gross feeding experiments and the results were essentially similar though the lesions were less severe.

Subcutaneous Injections

The same gum acacia suspensions were injected subcutaneously into rats and rabbits, the dosage being calculated on the basis of 4 mgm. per cu. m. of air. Again similar results were obtained. In all such experiments there must of necessity be differences in the degree of effect but invariably the liver was the sole organ affected and the lesions were those already described many times.

Discussion

These experiments leave no doubt as to the possibility of systemic effects from the chlorinated naphthalenes and chlorinated diphenyl. As in the case of the effects upon the skin, the degree of chlorination seems to determine the systemic toxicity, and it is a striking thing that when trichlornaphthalene is reached systemic effects are never marked and are produced with the greatest difficulty. It is most remarkable, too, that all the compounds tested attack the liver and the liver alone. During the past few months we have determined the organically combined chloride in the livers of animals very severely poisoned by penta and hexachlornaphthalenes but have found no increase over normal figures, though the livers, as determined histologically, were very severely affected. At the present time we are conducting inhalation experiments on a chlorinated diphenyl containing 55 per cent of chlorine instead of 64 per cent as in the case of the experiments reported in this paper and on a compound with a chlorine content between tri and tetrachlornaphthalene. We are also determining the degree to which the diet may increase or decrease toxicity, this being suggested by similar work upon carbon tetrachloride.

In the basis of these experiments

and on many field determinations of different compounds in the air of workrooms, it appears safe and it is certainly easily attained, to ventilate so that the air breathed does not contain more than 0.5 mgm. per cu. m. of any of these compounds above trichlornaphthalene. In the case of the latter compound concentrations of 10.0 mgm. are permissible. We know from many examinations in many different plants that such concentrations have been greatly exceeded during the past 20 years, and we are conscious of the fact that our rat exposures have been inexorably constant whereas human exposure is never so ordered. Time and careful observation may change these opinions as to standards, but today we are convinced they are safe. Impregnating tanks and other arrangements utilizing the chlorinated hydrocarbons are easy to hood and to safeguard. Compared with benzene, lead tetraethyl and many other compounds, these substances are very little toxic and operations employing them can easily be safeguarded. It may be argued that where possible trichlornaphthalene should be used, but this compound will cause acne and if employed very carelessly might do more. Furthermore, higher chlorination is often essential for highly practical reasons. The solution consists in thoroughly adequate ventilation plus good housekeeping around all wax containers.

A final word of caution bears upon the use of carbon tetrachloride as a wax solvent. Obviously this compound adds readily to the toxicity of the highly chlorinated waxes. If carbon tetrachloride is used, ventilation should be excellent, but in our opinion it would be better to dispense with carbon tetrachloride and depend on other solvents, especially upon those containing no chlorine.

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