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FURTHER OBSERVATIONS ON THE POSSIBLE SYSTEMIC TOXICITY OF CERTAIN OF THE CHLORINATED HYDROCARBONS WITH SUGGESTIONS FOR PERMISSIBLE CONCENTRATIONS IN THE AIR OF WORKROOMS*

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SINCE 1937, several papers have appeared dealing with the systemic effects of certain of the chlorinated hydrocarbons. These are listed in the bibliography in the order of their appearance (1, 2, 3, 4, 5). The first of these papers included recommendations as to safe concentrations in the air of workrooms, that is, concentrations that would cause no ill effects to individuals who breathed them throughout a working day. It also mentioned certain other observations, such as the effects of diet on toxicity, which were being made at the time but which had not progressed far enough to merit description. It is now possible to report upon these matters and to list a fairly large number of chlorinated hydrocarbons together with permissible concentrations in air.

* Received for publication February 10, 1939.

THE EFFECT OF HIGH AND LOW CALCIUM INTAKE ON ANIMALS INHALING HIGH CONCENTRATIONS OF A MIXTURE OF PENTA AND HEXACHLORNAPHTHALENES (CHLORINE 62.6%).

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 (Lamson et al. (6)). To discover whether calcium in the diet would protect against liver damage 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 a mixture of penta and hexachlornaphthalenes (chlorine 62.6%), an average of 11.21 mg. per cu.m. 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.

The Effect of Injections of Xanthine on Animals Inhaling High Concentrations of a Mixture of Penta and Hexachlornaphthalenes (Chlorine 62.6%).

In 1937, R. C. Neale (7) published a brief paper in which 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 a mixture of penta and hexachlornaphthalenes (chlorine 62.6%) in concentrations averaging 15 mg. per cu.m. for 16 hours daily. One group of 20 rats was given 20 mg. of xanthine subcutaneously every other day and 40 mg. of xanthine by stomach tube on the alternate days. The second group of 18 rats had the same exposure to the compound 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.

Evidence for the Destruction of a Mixture of Penta and Hexachlornaphthalenes (Chlorine 62.6%) 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 the above compound 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 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.

The Effect of Increasing the Sodium Chloride in the Diet upon Animals Receiving Toxic Doses of a Mixture of Penta and Hexachlornaphthalenes (Chlorine 62.6%).

On the ground that chlorides might be fundamentally associated with toxicity, a group of 15 rats was fed a low toxic dose of this compound and compared with a similar group on the same dosage plus a marked increase in chloride intake secured by giving 5 cc. per kg. of body weight of 4% sodium chloride 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.

TABLE 1

A List of 14 Chlorinated Hydrocarbons, with Chlorine Contents and Permissible Limits (in mg./cu.m.) for the Air in Workrooms*

Compound	Chlorine content	Permissible limit
	%	mg./cu.m.
1. Trichlornaphthalene plus a trace of tetrachlornaphthalene. Tested upon rats by inhalation and by feeding	49.9	10.0
2. Tetra and pentachlornaphthalenes. Tested upon rats by inhalation and by feeding	56.4	1.0
3. Penta and hexachlornaphthalenes. Tested upon rats by inhalation and by feeding, and upon dogs by feeding alone	62.6	0.5
4. Tetra and pentachlornaphthalenes plus refined chlorinated diphenyl. Tested upon rats by feeding	43.5	0.5
5. 90% penta and hexachlornaphthalenes plus 10% chlorinated diphenyl benzene. Tested upon rats by inhalation and by feeding	63.0	0.5
6. Chlorinated diphenyl plus chlorinated diphenyl benzene. Tested upon rats by inhalation and by feeding	65.0	0.5
7. Chlorinated diphenyl oxide. Tested upon rats by inhalation	54.0	0.5
8. Chlorinated diphenyl oxide. Tested upon rats by inhalation	57.0	0.5
9. Chlorinated diphenyl. Tested upon rats by inhalation	50–55	0.5
10. Hexachlor diphenyl oxide plus 5% trichlornaphthalene. Tested upon rats by inhalation	50–55	0.5
11. Hexachlornaphthalene and crude chlorinated diphenyl. Tested upon rats by inhalation	Unknown	0.5
12. Special chlorinated naphthalene. Tested upon rats by inhalation	50–56	0.5
13. Chlorinated diphenyl. Tested upon rats by inhalation	68	10.0
14. Chlorinated diphenyl benzene. Tested upon rats by inhalation	60	0.5

*The analytical method and apparatus used routinely for field determinations is that described by Tebbens (This J., *19*, 204 (1937)) and by Drinker *et al.* (ibid., p. 283).

The Systemic Effects of 14 Chlorinated Hydrocarbons with Suggested Limits for Concentrations in the Air of Workrooms.

The technic for inhalation and feeding experiments upon rats has been fully described (Drinker et al. (1)). It has been uniform for all the compounds listed in table 1, and for each group of experiments 24 to 48

animals have been sacrificed and examined grossly and microscopically so as to gain very complete post-mortem reports.

The first 6 substances in table 1 have been dealt with in previous published reports (cf. 1 and 3 in bibliography). In connection with them it was recommended that in the case of trichlornaphthalene and compounds of lower chlorination a limit of 10 mg. per cu.m. be permitted, the idea being that the essential feature of the action of these compounds, namely damage to the liver, did not become prominent until a chlorine content of 50% was reached. So far as trichlornaphthalene is concerned the limit of 10 mg. should be allowed to stand, since nothing has occurred which indicates the necessity for changing it. The second compound, a mixture of tetra and pentachlornaphthalenes is in common industrial use. A tolerance of 0.5 mg. per cu.m. was formerly proposed for it, but on the basis of laboratory and field experience it is apparently entirely safe to increase this to 1.0 mg. per cu.m.

The sixth compound has been listed previously as chlorinated diphenyl. It contained 65% of chlorine and proved very destructive to the liver. Later experiments with compound 13, which contained 68% of chlorine and which was also labelled chlorinated diphenyl, were a surprise to us since this second compound was almost non-toxic. On inquiry it was found that substance 6 was in reality a mixture of chlorinated diphenyl and chlorinated diphenyl benzene and that number 13 was actual chlorinated diphenyl. We have no information as to whether this last compound lacks toxicity because it is not broken down in the body, but that would seem the probable explanation. Compound number 12 was prepared especially in order to have a substance for test which should fall in chlorination between compounds 1 and 2. It proved somewhat toxic and, in addition to changes in the liver, caused eye irritation and in one instance cataracts. The substance is not in commercial use and evidently at the temperatures used for volatilization gave off some sort of irritant, possibly hydrochloric acid.

With the exception of this isolated effect upon the eyes, we have seen no lesions from any of the 14 compounds listed except in the liver. The character of these lesions has been thoroughly described by Bennett *et al.* (3). These additional data and the tolerances recommended deal specifically with systemic effects since one can get no information as to chloracne in the skin from observations upon fur-bearing animals. But it may be pointed out that where industrial workers have been careful as to cleanliness and where air concentrations have been kept within the limits suggested, skin lesions apparently cease to occur.

Summary

Certain experiments carried out upon large groups of white rats indicate the following:

1. A high calcium diet has no protective effect against the characteristic liver lesions caused by these compounds.

2. Administration of xanthine does not prevent liver injury.

3. When a mixture of penta and hexachlornaphthalene is given to dogs by mouth, the urinary chlorides rise, indicating that in some way the body cells detach chlorine from this compound.

4. A high chlorine intake does not enhance toxicity.

5. A tabulation can now be presented covering 14 chlorinated hydrocarbons with permissible safe concentrations in the air of workrooms.

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