Effect of Hexachlorobenzene (HCB) on Growth of *Tetrahymena pyriformis*

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Introduction

In recent years many papers have been published showing the extensive distribution of hexachlorobenzene (HCB) in the environment. High residues of this pollutant have been found in raptorial birds (VOS et al. 1968, KOEMAN et al. 1969a), fish (HOLDEN 1970, ZITKO 1971, KOEMAN et al. 1969b), sea birds, and mussels (KOEMAN et al. 1969b). HCB residues were also reported in cereals and other plant material (STIJVE 1971), and recently relatively high concentrations of HCB were also found in human milk and adipose tissue (ACKER and SCHULTE 1970) where this pollutant was accumulated to considerable higher concentrations than DDT plus DDE.

The origin of these relatively high concentrations of HCB in the environment is not yet well understood, as on one hand HCB is used in industry as a plasticiser for PVC as well as a flame retardant and on the other hand this compound has been used since 1945 in agriculture for seed dressing against seed born and soil born diseases (SCHLÖR 1970). So the relatively high concentrations of this pollutant in the environment may be the result of agricultural application as well as of industrial activity. But only little is known about the persistence of HCB.

Only recently FREITAG et al. (1974) were able to show that it was not possible to find degradation products of HCB in plants and soil. From these results they concluded that HCB might even be more stable in the environment than dieldrin and DDT. On the other hand studies with rats did show a degradation of HCB to pentachloro-

Bulletin of Environmental Contamination & Toxicology,
Vol. 16, No. 3 © 1976 by Springer-Verlag New York Inc.

phenol and trichlorophenol and other unknown metabolites (MEHENDALE et al. 1975).

From recent experiments with rats (MEHENDALE et al. 1975, ELDER 1972, TALJAARD et al. 1972, RAJAMANICKAM et al. 1972, IPPEN et al. 1972) it is known that HCB can induce porphyria which might be a result of the induction of δ-aminolevulinic acid synthetase by HCB which became evident from studies with liver cells of chick embryo (GRANICK 1967). Apart from these data only little information about the effect of HCB on metabolic pathways in animals is available. Therefore, these preliminary studies were undertaken with Tetrahymena pyriformis which is known to be a good tool for pharmacological research (HUTNER et al. 1973).

Materials and Methods

Tetrahymena pyriformis Wh 14 from the Pringsheim algae collection, University of Göttingen, Germany, was grown in axenic cultures in normal test tubes with about 10 ml of nutrient broth in an incubator at 30°C as described by GEIKE (1969). From these stock cultures equal amounts (1.0 ml) were transferred to 500-ml-Erlenmeyer flasks containing 200 ml of nutrient solution after adding the appropriate amount of HCB in acetone solution (1.0 ml); the controls received the same amount of acetone. The cultures were grown under continuous stirring with a magnet stirrer arrangement in an incubator for 10 days at 30°C. After incubation the cells were harvested by centrifugation for 10 min at 3000 x g, washed three times with distilled water and were made up to 25 ml of suspension from which aliquots were taken for the determination of dry matter, carbohydrates, and total nitrogen. For measurements of the "porphobilinogen" in the nutrient solution an aliquot of the cell-free supernatant of the nutrient broth was estimated by the

method of MAUZERALL and GRANICK (1956). Carbohydrates were determined by the anthrone method and for the determination of total nitrogen the Kjeldahl method was used.

Results and Discussion

The effect of HCB on three growth parameters of Tetrahymena pyriformis and on the excretion of compounds into the medium giving with modified Ehrlich reagent the porphobilinogen reaction was studied in experiments with 0, 0.001, 0.01, 0.1, 0.25 and 0.5 ppm HCB in the nutrient solution which contained 0.5 % acetone after addition of this compound. The data presented here are drawn from one experiment and are representative of a further eight replicates. As can be seen from Table 1 the effect of HCB on the growth of this organism is very striking. Incubation of Tetrahymena pyriformis with HCB over a period of 10 days led to a decrease of growth as is evident from all growth parameters studied and to an increase in the excretion of "porphobilinogen" into the nutrient medium. The effect of HCB on the growth parameters and excretion of the "porphobilinogen" is well correlated with the concentration of this compound applied. As can be seen from Table 1 total nitrogen of the cells is most strongly affected by HCB as it is decreased by a factor of 10 at the lowest concentration applied. These results are in good agreement with studies of YAMAGUCHI et al. (1973) about the toxicity of heavy metals to Tetrahymena pyriformis. They found that the degree of toxicity was negatively correlated with the protein concentration of the organism. The carbohydrate content is also affected very strongly while the dry matter at the highest HCB concentration applied is decreased only by a factor of two compared to the control. This relative-

TABLE 1

Effect of HCB on some growth parameters of Tetrahymena pyriformis and on the excretion of "porphobilinogen"

HCB in nutrient broth (ppm)	Total yield of culture (in mg)			"Porphobilinogen" * (pMoles in nutrient solution)
	Dry Matter	Carbohydrates*	Total Nitrogen*	
0.000	175.0	36.5	12.3	19.4
0.001	115.0	17.5	1.16	387.0
0.010	107.5	11.5	0.93	657.9
0.100	103.8	8.5	0.82	677.3
0.250	97.5	7.5	0.5	735.3
0.500	88.8	3.5	0.36	774.0

* corrected for blank

ly small decrease of dry matter compared to the decrease of the other growth parameters studied is of special interest and indicates an interaction of HCB with several metabolic pathways. An accumulation in the cells or precipitation of HCB accounting for the relatively high values of dry matter can be excluded as the total amount of HCB in the culture which receives 0.5 ppm was only 0.1 mg.

The reasons for the large decreases in carbohydrates and total nitrogen content in treated cells is not yet understood. From preliminary studies in this laboratory, however, it is known that respiration of Tetrahymena pyriformis is depressed by HCB the result of which might be an energy depletion which in turn might influence the uptake of substances from the nutrient solution resulting in a depletion of storage products within the organism. On the other hand HCB might also directly inhibit food uptake and / or several other factors involved in the growth of this organism.

Very striking is the increase of "porphobilinogen" in the nutrient solution induced by HCB. The data (Tab. 1) show a high increase in the excretion of "porphobilinogen" into the medium even at the lowest concentration studied (0.001 ppm) so that the threshold concentration for the induction of the excretion of these compounds may be far below this value. The nature of the compounds in the medium giving the colour reaction with modified Ehrlich reagent according to the method of MAUZERALL and GRANICK (1956) and termed here "porphobilinogen" is not yet clear. As an incubation of the nutrient solution with 0.25 ppm HCB does not bring any colour reaction the excreted substances are formed by Tetrahymena pyriformis as an effect of HCB treatment. We are fully aware that other compounds than porphobilinogen or porphyrins may be determined by the method of MAUZERALL and GRANICK (1956) but from the literature it seems likely that they may be compounds from the

pathway of porphyrin biosynthesis. The effect might be due to an induction of δ-aminolevulinic acid synthetase by HCB as has been shown with liver cells of chick embryo (GRANICK 1967) and rats (RAJAMANICKAM et al. 1972). As porphyria can be induced by HCB application in rats (MEHENDALE et al. 1975, ELDER 1972, TALJAARD et al. 1972, RAJAMANICKAM et al. 1972, IPPEN et al. 1972) the results presented here could indicate a striking similarity in this respect between Tetrahymena pyriformis and other animals.
As it is known from studies with Japanese quails (VOS et al. 1971) that HCB can induce liver damage and reproductive failure in birds, studies with Tetrahymena pyriformis might reveal possible harmful effects not only on man but also on mammals and other animals. Further studies on this aspect, however, are necessary.

Summary

Tetrahymena pyriformis Wh 14 was incubated with HCB (0.001 - 0.5 ppm) in Erlenmeyer flasks under continuous stirring at 30°C for 10 days in an incubator. HCB decreased growth as deduced from measurements of dry weight, carbohydrates, and total nitrogen. Total nitrogen and carbohydrates are decreased drastically while dry matter is reduced only by a factor of two. The excretion of a compound into the nutrient solution which yields a colour with modified Ehrlich reagent like porphobilinogen is very strongly increased by HCB. All effects observed were very well correlated with the concentration of this compound applied.

Acknowledgment

The authors wish to thank the Deutsche Forschungsgemeinschaft for the financial support of this work.

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