Vol. 80, No. 4, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 28, 1978 Pages 781-788

HEXACHLOROBENZENE PORPHYRIA AND HEXACHLOROBENZENE CATABOLISM IN RATS

F. Kószó, Cs. Siklósi and N. Simon

Department of Dermatology and Venerology, University School of Medicine
Szeged - Hungary

Received December 9, 1977

Summary: Gas-chromatographic examinations were made on the amounts of hexachlorobenzene accumulating in the liver and fatty tissue of rats chronically poisoned with a diet containing 0.2 % hexachlorobenzene, and on the amounts of hexachlorobenzene and pentachlorophenol excreted with the urine and the faeces in the course of the poisoning. The results indicated a constant rise in the hexachlorobenzene levels in these tissues. Pentachlorophenol formed in the catabolism of hexachlorobenzene appeared in increasing concentration in both the urine and the faeces from the commencement of the poisoning. After the 5th-6th week of poisoning, the presence of other apolar and polar products in the excretions was also markedly enhanced. After a single dose of hexachlorobenzene /0.2 g/animal/, of all the decomposition products only pentachlorophenol was produced in high concentration, showing that this is a primary catabolite. A hypothesis is put forward as to the possibility of a role being played in the mechanism of action of hexachlorobenzene by a membrane permeability change.

The porphyrinogenic effect of HCB has been known since the description of "Turkish porphyria" (1). Of the many subsequent authors, Ockner and Schmid were the first to demonstrate on rats that HCB-induced experimental porphyria serves as a useful model for the investigation of chronic hepatic porphyrias (2). However, the factors inducing human chronic hepatic porphyria have not been elucidated. The study of the mechanism of action of HCB in HCB-induced experimental porphyria in animals offers some hope that more may be learnt on the mode of occurence of the human disease.

Since HCB is a compound with fairly low reactivity, it was earlier assumed that it also behaves as an inert substance in the living organism (3); only recently has its catabolism in the organism been suggested. Mehendale et al. considered the primary catabolites to be chlorobenzenes, formed as a result of reductive dehalogenation processes (4). Their experiments were made on rats

Abbreviations: HCB = hexachlorobenzene
PCP = pentachlorophenol

0006-291X/78/0804-0781$01.00/0

which had been preliminarily poisoned with a single dose of ca. 5 mg HCB/kg. After such a brief poisoning period, at most transient coproporphyrinuria is observed in the animals, and in effect, therefore, the aim could only be the confirmation of the actual catabolism of the HCB. Lui and Sweeney put forward two alternative routes for the mechanism of the catabolism (5, 6). In the rat experiments of Koss with [^{14}C]HCB, the radioactivity appeared in every tissue a certain time following the poisoning. Approximately half of the radioactivity measured in the faeces and urine was due to HCB catabolites (7).

The more distant aim of our own experiments is to elucidate the catabolism of HCB in rats during chronic poisoning, from the beginning of the diet until the complete development of the porphyric state, with regard to the aetiologic factors of porphyria cutanea tarda and tarda-like acquired toxic porphyrias. In the present paper we support the possibility of the catabolism of HCB, and present data on the amount of HCB accumulating in the organism in the course of chronic HCB poisoning, and on the excretion of HCB and PCP.

MATERIALS AND METHODS

The HCB used /Fluka 52100/ was first recrystallized from benzene. The melting point of the resulting material was 225-226 °C. Gas-chromatographically, only trace amounts of tetrachlorobenzene could be detected as impurity. The other chemicals employed were REANAL products of analytical purity.

Treatment of animals; extraction.

A total of 80 young laboratory albino rats /Wistar strain, 40 males, 40 females, initial body weight ca. 120 g/ were chronically poisoned with a diet containing 0.2 % HCB. The animals received food and water ad libitum. At appropriate times after the commencement of the poisoning, the amounts of porphyrins excreted with the urine and faeces were determined by the method of Doss (8), and the HCB and PCP contents of the excretions were also measured. On the days following the group urine and faeces collections, one male and one female animal were sacrificed and the HCB contents of the liver and fatty tissue were examined. The HCB and PCP contents of the excretions and the tissues were extracted with n-hexane containing 5 % ether. The combined extract portions were purified on columns containing a suitable amount /2-5 g/ of partially deactivated 60/100 mesh Florisil (9).

Gas-chromatography.

Analyses were performed with Selectograph /Hungary/ gas-chromatograph provided with an electron-capture detector. The partition phase was 4 % SE-30 on 80/100 mesh Chromosorb W, in a 1/4 inch x 6 foot glass column. Temperatures: inj. block = 190 °C, column = 195 °C, detector = 195 °C. Nitrogen was used as carrier gas, at a flow rate of 35 ml/min.

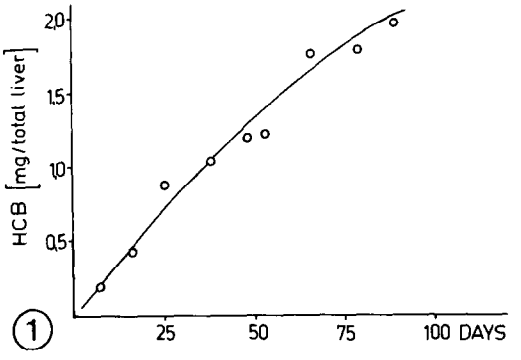
Determinations, including extraction, purification and chromatography, were carried out on parallel samples. As regards the entire procedure, recoveries of 80 ± 5 % were obtained for HCB in the faeces, 75 ± 7 % for the PCP in the droppings, 78 ± 6 % for the urinary PCP, 76 ± 8 % for the HCB in the liver tissue, and 77 ± 7 % for the HCB in the fatty tissue.

RESULTS

For measurement of the porphyrin, HCB and PCP contents of the excretions, examinations were initially made on 10 male and 10 female rats; from the 49th day on, this was reduced to 5 males and 5 females, because of the higher mortality rate among the females; in the 90-day measurements only 5 male and 2 female animals were examined; and after the 3rd month only male rats were available. Up to the 190th day 2 male rats remained alive. In all cases 3 parallel measurements were made on the excretions of the animal groups, and the averages of these are reported.

In the course of the poisoning, the development of the porphyric state of the animals was checked by analysis of the porphyrin excreted with the urine and the faeces. The results agreed well with the data of our earlier measurements, which indicated that with the lengthening of the poisoning period the initial coproporphyrinuria characteristic of the first weeks was gradually replaced by an excretion picture typical of HCB-porphyria: strongly elevated faecal proto- and coproporphyrin levels, and an elevated urinary porphyrin level, within this an increase in the proportion of uroporphyrin (10). From about the 2nd month on, this characteristic porphyrin excretion increased /to an ever smaller extent/ only as regards its overall quantity; the proportions of its constituents did not vary essentially. Thus, on the 150th day the faecal total porphyrin amounted to 510 μg/day/animal, distributed as follows: protoporphyrin: 296 μg, coproporphyrin: 120 μg, 5 COOH-: 5 μg, 6 COOH-: 30 μg, 7 COOH-: 49 μg, 8 COOH-porphyrin: 10 μg /part of the latter water-soluble fractions presumably passed with the urine into the collected faeces/; the urinary total porphyrin was 148 μg/day/animal, with the following distribution: coproporphyrin: 84 μg, 5 COOH-: 3 μg, 6 COOH-: 2 μg, 7 COOH-: 20 μg, 8 COOH-porphyrin: 39 μg.

The amounts of HCB accumulated in the liver and in the fatty tissue obtained from the abdominal cavity during the poisoning are illustrated in Figs. 1-2.



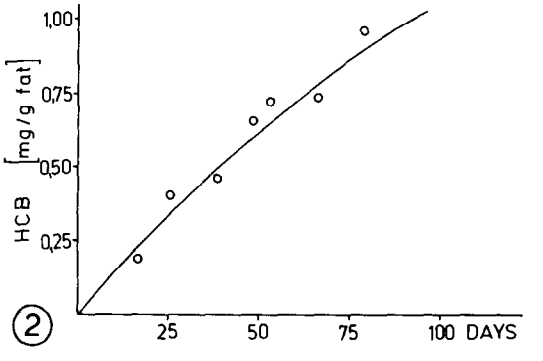


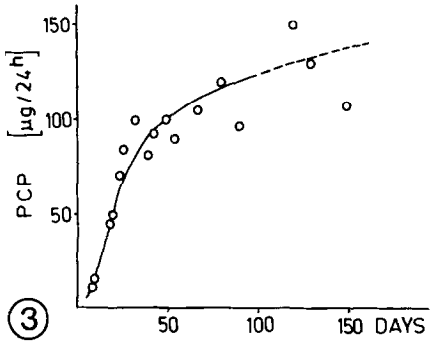
Fig.1. HCB accumulation in liver.
Fig.2. HCB accumulation in peritoneal fat.

The quantity of PCP excreted with the daily urine is shown in Fig. 3. In the majority of the urine samples traces of HCB were also detected, but even the highest values barely exceed 1 μg/day, and accordingly we do not attribute importance to this. The HCB found in the urine is presumably /at least in part/ only contamination arising during collection. The HCB and PCP contents of the faeces as functions of the poisoning time are demonstrated in Figs.4-5. Since the experimental results became more uncertain with the decrease in the number of animals, the second halves of the curves in the figures have been drawn with dashed lines.

In 4 untreated rats /2 males, 2 females/ a loading test was carried out with ca. half of the LD_{50} value of HCB: 0.2 g HCB, suspended in sunflower oil, was administered via a stomach tube to each animal, and the urine and faeces were then collected for 2 days. Meanwhile the animals received water ad libitum. The HCB and PCP excretions on the first and second days, referring to one animal, are listed in Table I.

DISCUSSION

Our data indicate that in the course of chronic poisoning the HCB level constantly rises /though to a decreasing extent/ in both the liver and the fatty tissue in rats with the passage of time. Even in the case of chronic



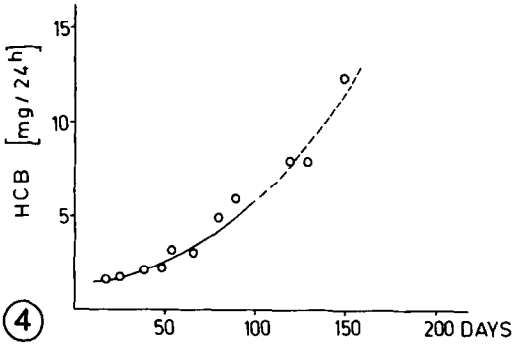


Fig.3. Daily urine PCP excretion µg/animal.
Fig.4. Daily fecal HCB excretion mg/animal.

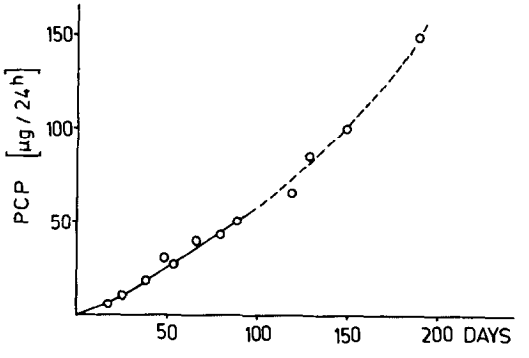


Fig.5. Daily fecal PCP excretion µg/animal.

Note: In all cases the rats /male and female/ were chronically fed a standard fodder containing 0.2 % HCB.

poisoning, the proportions of the HCB accumulation in these two types of tissue are by and large similar to those found by Mehendale et al. following a single poisoning (4). The curve depicting the HCB content of the liver /Fig. 1/, however, does not display such a pronounced flattening from the 30th-40th day of poisoning as in the data of Stonard (11); we observed only a later and somewhat less extensive decrease in the monotonous rise of the function. As regards the shapes of the curves relating to the accumulation, we did not detect any break at all around the 5th-7th week of poisoning, when

TABLE I. HCB and PCP excretion in rats following a single loading with 0.2 g HCB

	HCB		PCP	
	Urine	Faeces	Urine	Faeces
Day 1	32 μg	43260 μg	30 μg	< 1 μg
Day 2	27 μg	11912 μg	< 1 μg	< 1 μg

the porphyrin excretion picture characteristic of this experimentally-induced porphyria develops permanently in the animals.

Although HCB is a compound of fairly low reactivity, we too support the possibility of its catabolism in the organism. The catabolite PCP is to be found in the urine, and in lower quantities in the faeces /Figs. 3 and 5/. After the administration of a single large dose of HCB, besides HCB only PCP appeared in high concentration in the excretions, which is in contrast with the view of Mehendale et al. (4), and suggests the primary nature of this catabolite. After the 4th-5th week of the chronic poisoning, however, in both the urine and the faeces there were progressive accumulations of other apolar products and products more polar than PCP /tetrachlorobenzene, tetrachlorohydroquinone, pentachlorothiophenol/; the presence of these in the urine after chronic poisoning has also been referred to by Lui et al. and by Koss (6, 7). It is understandable, therefore, that Mehendale et al. did not find any catabolite at all in the faeces after administering a single oral dose of HCB (4).

Further data are necessary to clarify the mechanism of HCB catabolism. Attention should be paid to the conception of Lui et al. that the catabolism proceeds via an epoxide compound intermediate (6). Nevertheless, we do not discount the possibility that there may be several routes in the catabolism.

The decomposition of the PCP formed is much more rapid than that of HCB /see the data of Ahlborg (12) relating to PCP decomposition/, and further it can be accepted in general that the more polar a product formed, the more

easily it may be expected to be excreted from the organism. If employed alone, neither PCP nor any of several compounds of chlorphenol type caused porphyria of the same type as did HCB. Again, if animals were fed chronically with one or other of these compounds simultaneously with HCB, the picture of porphyria produced by HCB alone was not altered by any of them. Accordingly, we do not ascribe a determining role to the polar decomposition products of HCB in the development of the known porphyric picture. /A detailed account of these combined poisoning will be presented in another publication./ The ability of HCB to accumulate is fairly high (13-17), as supported by our own experimental data /Figs. 1-2/. It is all the more interesting, therefore, to consider the "passive" role of the HCB itself, in that it is incorporated into the mitochondrium lipoid membrane structures, thereby causing some distortion in the orientation of the molecules constituting the membrane, and consequently influences the transport phenomena across the membrane (18, 19). In addition, it must not be left out of consideration that an increase in the activity of the aspecific enzymes /primarily haeme enzymes bound to the membranes of the organelles/ tending to eliminate the incorporated HCB may result in a locally chronic redox potential change, which is capable of influencing the energy-dependent transport. The other aetiopathogenetic conceptions assumed to date with regard to chronic hepatic porphyrias can be fitted into a comparatively uniform picture via precisely the energetic system, and hence the actual conditions of the existence of these can still not be excluded.

REFERENCES

1. Cam, C. (1958) Saglik Derg. 32, 215-216.
2. Ockner, R.K. and Schmid, R. (1961) Nature 189, 499.
3. Parke, D.V. and Williams, R.T. (1960) Biochem.J. 74, 5-9.
4. Mehendale, H.M., Fields, M. and Matthews, H.B. (1975) J.Agr.Food Chem. 23, 261-265.
5. Lui, H. and Sweeney, G.D. (1975) FEBS Lett. 51, 225-226.
6. Lui, H., Sampson, R. and Sweeney, G.D. (1976) in Porphyrins in Human Diseases /1st Int.Porphyrin Meet. Freiburg/Br. 1975/ /Doss, M., ed./, pp. 405-413, Karger, Basel.
7. Koss, G. (1976) in Porphyrins in Human Diseases /1st Int.Porphyrin Meet. Freiburg/Br. 1975/ /Doss, M., ed./, pp.414-417, Karger, Basel.

8. Doss, M. (1972)in Progress in Thin-Layer Chromatography and Related Methods /Niederwieser, A. and Pataki, G., eds./, Vol.3, pp. 147-176, Ann Arbor, Michigan.
9. Holden, A.V. and Marsden, K. (1969) J.Chromatog. 44, 481-492.
10. Simon, N. und Siklósi, Cs. (1974) Berufsdermatosen 22, 237-259.
11. Stonard, M.D. (1974) Brit.J.Haemat. 27, 617-625.
12. Ahlborg, U.G., Lindgren, J.E. and Mercier, M. (1974) Arch.Tox. 32, 271-276.
13. Koeman, J.H., Vink, J.A.J. and de Goeij, J.J.M. (1969) Ardea 57, 67-76.
14. Vos, J.G. and Koeman, J.H. (1970) Toxicol.Appl.Pharmacol. 17, 656-668.
15. Vos, J.G., Maas, H.L. van der, Müsch, A. and Ram, E. (1971) Toxicol. Appl. Pharmacol. 18, 944-957.
16. Vos, J.G., Botterweg, P.F., Strik, J.J.T.W.A. and Koeman, J.H. (1972) TNO Nieuws 27, 599-603.
17. Villeneuve, D.C. (1975) Toxicol.Appl.Pharmacol. 31, 313-319.
18. Kószó, F., Siklósi, Cs. and Simon, N. (1974) Biochim.Biophys.Acta 363, 182-189.
19. Simon, N., Siklósi, Cs. and Kószó, F. (1976) in Porphyrins in Human Diseases /1st Int.Porphyrin Meet. Freiburg/Br. 1975/ /Doss, M. ed./, pp.432-437, Karger, Basel.