Clearance and Tissue Distribution of Hexachlorobenzene in Rats

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Hexachlorobenzene (HCB) has been widely used as a fungicide on cereal grains and an additive for rubber products. HCB was associated with large scale of human poisoning in Turkey during the year 1955-1959(CAM and NIGOGOSYAN 1963). Recently MORLEY(1973) reported a case of porphyria in a worker occupationally exposed to HCB.

The presence of HCB was cofirmed in birds(VOS et. al. 1968),fish(HOLDEN 1970; ZITKO 1971; JOHNSON et.al. 1974) and human tissues(ACKER and SCHULTE 1972; BRADY and SIYALI 1972; SIYALI 1972; NEWTON and GREENE 1972; MORITA et.al.1975a) as well as foods such as cereals (STIJVE 1971), milk(GOURSAUD et.al. 1972),beef and chicken(MORITA et.al.1975b). It seemed to be necessary to study the metabolism, distribution, clearance, effect on reproduction, etc. of HCB in mammals if we consider the human poisoning and persistency of this material. This paper will report on the distribution and clearance rate of HCB in rats.

Materials and Methods

Wistar male rats, 7 weeks of age and weighing 150-160g were used. 30 rats were given single dose of 2.0mg of HCB per rat. HCB was dissolved in olive oil in the concentration of 1000 ppm and dosed intraperitoneally. The same volume of olive oil was given to the control rats. Water and food were given ad libitum. HCB level in food was 1.3 ng/g. Total intake of HCB during 56 days was estimated to be less than 2μg. Therefore HCB contamination of food was neglected.

The animals were killed by decapitation and blood, brain, heart, lungs, liver, kidneys, testes and adipose tissue were collcted. Each tissues were weighed and homogenized with 5g of anhydrous sodium sulfate in 25ml of n-hexane containing 5% of acetone. N-hexane layer (0.2ml) was diluted with 10ml of n-hexane and vigorously shaken with concentrated sulfuric acid. Supernatant hexane layer was injected to gas liquid chromatography (GLC) equipped with electron capture detector.

To determine the total amount in the whole body, carcass eliminated with organs was added to 500ml of

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warmed concentrated sulfuric acid(c.a. 60°C). The carcass was dissolved exothermally and yielded a black viscous liquid. To prevent the loss of HCB by evaporation, the solution was maintained below 90°C by cooling with cold water bath. 0.5mg of pentachlorobenzene was added to the mixture as an internal standard to calibrate the the recovery and the detector sensitivity. The mixture was extracted with 500 ml of n-hexane and shaken vigourously with concentrated sulfuric acid and then injected to the GLC.

GLC condition was as follows: Column Bentone 34 (5%)+DC 200(5%) on Gaschrom Q(100/120) Temp. 205°C , Carrier gas N_2 Flow rate 120ml/min., Detector ECD(Ni^{63}, 10mCi) Temp. 250°C, Injection Temp. 250°C.

Results and Discussion

Analyses of tissues indicated that HCB was located mainly in fat. Lower concentration were found in other organs: brain, heart, lungs, liver, kidneys and testes (TABLE 1).

TABLE 1
Concentration of HCB in various organs of rats 56 days after administration

Organs	HCB*	Organs	HCB*
Blood	0.30±0.00**	Liver	0.32±0.04
Brain	0.42±0.24	Kidneys	1.74±0.58
Heart	0.42±0.43	Testes	0.21±0.05
Lungs	2.09±0.61	Fat	18.76±1.54
Whole body	2.83±0.21		

* μg/g (on wet basis)
** Mean±SE

In most organs, HCB concentration reached to maximum within 6 or 12 hours and then decreased rapidly for 14 days (TABLE 2). From 14 days to 56 days, HCB content in each organs (HCB in organ per HCB total amount in the whole body) reached to nearly constant values. This value was 8, 5, 37, 40, and 41 ppm for brain, heart, liver, kidneys and testes, respectively. The pattern of distribution of HCB is different from either that of PCB(GRANT et.al.1971) or of PCT(SOSA-LUCERA et.al.1973).

Close correlation was demonstrable between HCB level in blood and total HCB amount found in the whole body($n=11$, $r=0.79$, $p<0.01$). HCB level in brain($n=12$, $r=0.78$, $p<0.01$), testes($n=12$, $r=0.88$, $p<0.01$) and fat ($n=9$, $r=0.71$, $p<0.05$) were also correlated to the total HCB amount in the period of 14-56 days. Furthermore the levels in brain($n=12$, $r=0.92$, $p<0.01$) and testes($n=12$, $r=0.94$, $p<0.01$) were remarkably well correlated to the

TABLE 2

Time course of HCB in verious organs following a single intraperitoneal dose*

Time	Brain	Heart	Lungs	Liver	Kidneys	Testes	Blood
2hr	1.78±0.17** (0.14)***	1.23±0.18 (0.04)	2.45±1.90 (0.18)	27.70±3.33 (2.46)	8.19±4.19 (0.37)	6.29±3.89 (0.37)	0.81±0.20****
6hr	2.53±0.32 (0.21)	2.33±0.30 (0.10)	5.63±1.18 (0.33)	10.72±1.44 (3.40)	12.53±2.46 (0.84)	5.35±0.54 (0.36)	1.66±0.06
12hr	2.04±0.43 (0.20)	2.95±0.49 (0.12)	3.39±1.00 (0.23)	4.92±2.33 (2.33)	7.33±1.28 (0.70)	5.16±2.20 (0.41)	1.07±0.31
24hr	2.43±0.63 (0.20)	3.37±0.83 (0.14)	3.98±0.89 (0.23)	5.05±2.52 (2.03)	9.72±1.03 (0.76)	5.35±0.88 (0.37)	2.02±0.40
48hr	3.17±0.93 (0.21)	1.93±0.18 (0.08)	3.60±0.47 (0.25)	2.93±0.45 (1.26)	7.52±0.23 (0.67)	2.86±0.67 (0.22)	1.82±0.14
7days	1.50±0.05 (0.15)	1.35±0.10 (0.06)	3.63±0.45 (0.29)	2.30±0.31 (1.36)	6.44±0.34 (0.75)	0.99±0.18 (0.12)	1.34±0.04
14days	0.80±0.19 (0.12)	1.06±0.30 (0.09)	3.26±1.39 (0.23)	0.84±0.29 (0.71)	2.43±0.13 (0.46)	0.52±0.16 (0.10)	0.79±0.22
28days	0.62±0.09 (0.09)	0.72±0.21 (0.10)	1.56±0.06 (0.16)	0.77±0.15 (0.75)	1.77±0.38 (0.32)	0.44±0.10 (0.11)	0.60±0.09
56days	0.43±0.02	0.42±0.04	2.09±0.61	0.32±0.04	1.74±0.58	0.07±0.01	0.30±0.00

* Dose---2mg per rat ; 3 rats/observation.
** Mean±standard error (µg/organ).
*** The parts per million of the total HCB found in the whole body.
****Mean concentration±standard error (µg/g).

level in blood. On the other hand, the level in other organs (liver, kidneys, lungs and herat) correlated well neither to the total amount nor the level in blood.

Clearance of HCB in brain is shown in Fig.1. following a single intraperitoneal injection in the period of 8 weeks.

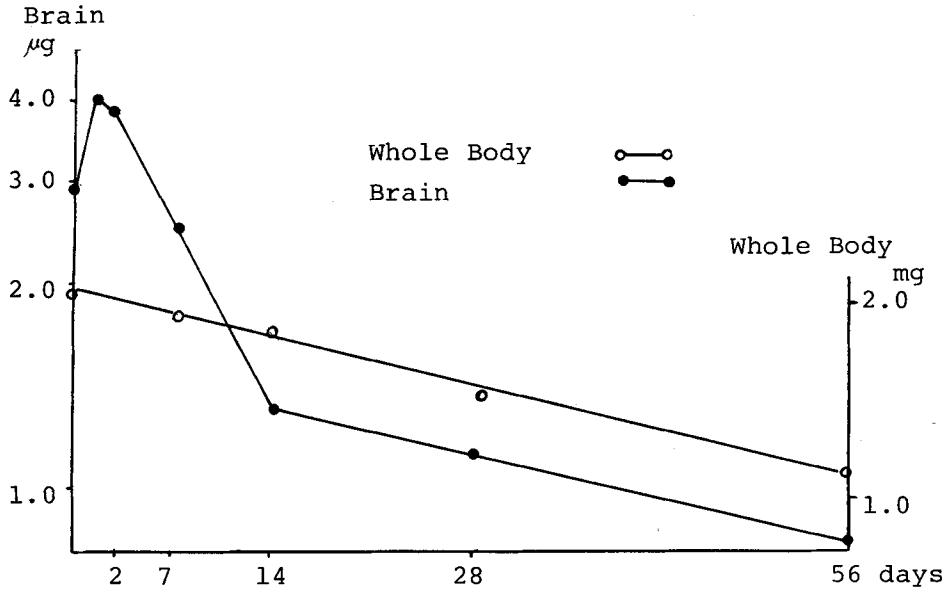

Fig. 1. Clearance of HCB in brain and whole body of rats.

HCB in brain reached to the maximum at 24 hours after administration and decreased slowly. Clearance seemed to occur in two steps. One is a less slow process found in the period from one day to 14 days and the other is a more slow process found thereafter. Half life corresponding to both processes were calculated for each organ and shown in TABLE 3. The presence of two components in the decline curve was noted by FRIES et. al.(1972) who examined PCB and DDE in cow.

The first process may be a redistribution of HCB among tissues of body. This clearance occured most rapidly in brain and proceed almost at the same speed in other organs. This fact coincide with the result of PCBs by GRANT et.al.(1971) that PCBs were most easily excreted from brain.

In contrast to the HCB level in organs, total HCB in the whole body diminished in a sigle step(Fig. 1). Half life for clearance was calculated to be 60±9 days

which coincide well with the second half life found in various organs except for lungs and kidneys. Because of large standard deviation, half life could not be determined in the above two organs.

TABLE 3
Estimated half life for clearance of HCB in various organs and whole body

Organs	First half life	Second half life
Brain	10 days	57days
Heart	15	c.a.50
Lungs	13	---*
Kidneys	16	---*
Testes	15	62
Whole body	---	60

*Because of large deviation in experimental values, half life was unable to determine.

STIJVE(1971) suggested that pentachlorobenzene might be a metabolic breakdown product of hexachlorobenzene from his observation that pentachlorobenzene was detected in chicken fat. However, pentachlorobenzene was not detected in all the organs and fat of rats examined in this experiment.

8 weeks after administration, rats were killed and compared with control group of rats. No significant difference was demonstrable between two groups in body weight,organ weights and pathological findings.

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