Bull. Environm. Contam. Toxicol. 21, 243-248 (1979)

Hexachlorobenzene Ingestion by Female Rhesus Monkeys: Tissue Distribution and Clinical Symptomatology

V. Knauf and W. Hobson

International Center of Environmental Safety, Albany Medical College, P.O. Box 1027, Holloman Air Force Base, New Mexico 88330

Hexachlorobenzene (HCB) is a chemically stable benzene, substituted at every carbon atom with chlorine; the molecule's considerable biopersistence is directly related to its aromaticity and high degree of chlorination. HCB is commonly used as a fungicidal dressing on cereal grains. Accidental ingestion (SCHMID 1960, CAM and NIGOGOSYAN 1963) as well as rising levels in the general environment (KOEMAN et al. 1969, BURNS and MILLER 1975) point to its potential as a pollutant and health hazard. This study was undertaken to investigate the susceptibility of rhesus monkeys (Macaca Mulatta) to HCB poisoning. Findings of clinical chemistry are presented herein; results of histopathological examination were presented separately (IATROPOULOS et al. 1976).

METHODS

Adult, female rhesus monkeys were individually housed, fed a controlled daily diet including monkey biscuits (250 g) and fresh fruit, and given water ad libitum. After a 30-day quarantine and an additional 30-day period in which to obtain baseline hematology and clinical chemistry values, eight animals were assigned to the study: Two animals received 128 mg HCB per kg body weight per day, one monkey 64 mg/kg/day, another 32 mg/kg/day and two monkeys received 8 mg/kg/day. HCB (Fisher) was recrystalized twice in benzene. Doses were suspended in one-percent methyl cellulose in distilled water and administered daily by gastric intubation. Two animals received methyl cellulose solution only and served as vehicle controls. All monkeys were dosed daily for 60 days.

Animals were examined twice daily for behavior and apparent health. Serum samples were obtained from physically restrained monkyes by venipuncture at two-week intervals beginning four weeks prior to the first dose (Week 0). Serum samples were analyzed

0007-4861/79/0021-0243 $01.20

for HCB content, sodium, potassium, urea nitrogen, total protein, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), lactic dehydrogenase (LDH) and total cholesterol. Whole blood tests included red blood cell counts, total white blood cell counts, hemoglobin and hematocrit.

All animals except one control and one receiving 8 mg/kg/day HCB were sacrificed at Day 60 of dosing. Representative tissue samples were taken at autopsy to determine HCB content. Serum samples for HCB determination were extracted in hexane (10:1 hexane:serum) and analyzed directly by gas chromatography. Fat and bone marrow were homogenized in five baths of hexane. Each extract was taken to dryness and redissolved in a known amount of hexane. Sample cleanup, if necessary, was accomplished by addition of 1-2 ml concentrated sulfuric acid before gas chromatography. Extraction recoveries ranged from 90 to 100%.

RESULTS

Five of the six monkeys receiving HCB began to lose weight by the fourth week of dosing. No significant changes were seen in hematologic parameters. Cholesterol was significantly ($p<0.01$) depressed at Weeks 3, 5 and 8 in 5 animals receiving HCB (Table 1). Samples taken from #618 (128 mg/kg) at Day 60 revealed depressed serum potassium (2.7 mEq/l), elevated GOT (65 units/ml) and elevated blood urea nitrogen (140 mg/100 ml serum) compared to this animal's pretreatment control mean values of 4.1 mEq/l, 26 units/ml and 14 mg/100 ml serum respectively. Monkey #627 (64 mg/kg/day) suffered severe tremors and muscular weakness Day 58-60, whereas #618 showed marked lethargy and weakness by Day 52.

Serum HCB levels (Table 2) did not appear directly related to the amount of HCB ingested, although levels generally increased during the duration of dosing. Tissue levels of HCB showed higher levels in fat and bone marrow than other tissues and seletively higher levels in the adrenal cortex than in the medulla.

TABLE 1

Cholesterol (mg/100 ml)

Monkey	Dose	Mean of Values Weeks 3,5,& 8 ± SE	
613	128	100[a]	9.5
618	128	80[ab]	19.3
627	64	70[a]	3.5
817	32	59[a]	2.7
1163	8	86[a]	21.2
1184	8	140	7.3
1826	Control	121	10.5
1315	Control	159	6.1

[a] Significantly different ($p<0.01$) than a mean of all monkeys pretreatment cholesterol determinations (153 mg/100 ml)

[b] No value for Week 8

DISCUSSION

As might be expected, the greatest amounts of HCB were generally found in tissues containing higher lipid concentrations. Adrenal cortex concentrations of HCB were selectively higher than adrenal medulla suggesting the possibility of glucocorticoid response to stress caused by HCB ingestion (WASSERMAN et al. 1973). Tissue distribution patterns found in this study were in excellent agreement with previous patterns (ROZMAN et al. 1975). Although extensive ovarian changes were found in the monkeys in the present study (IATROPOULOS 1976), HCB was not preferentially concentrated in the ovaries.

Tissue and serum levels of HCB varied widely between monkeys that received similar amounts of HCB. Monkey #627 (64 mg/kg/day) had serum and selected tissue levels much higher than the two animals that received twice as much HCB. This variation seems in large part due to the amount of body fat individual animals possessed. Body fat appeared to serve as a "protective" reservoir. Monkey #613 (128 mg/kg/day) was a small, slight monkey whose fat stored more HCB on a "per mg" basis than #618 (64 mg/kg/day), although 618, an obese monkey, probably had a larger total amount of HCB. The thinnest animal #627 (64 mg/kg/day), had almost no adipose tissue and had the highest non-fat tissue and serum values. These data

TABLE 2

Tissue Levels of HCB (ppm)

Monkey No.	613	618	627	817	1163	1826
Dose (mg/kg/day)	128	128	64	32	8	0
Body Fat	930	215	540	250	580	1.1
Bone Marrow	460	175	1700	255	350	1.6
Adrenal Cortex	150	30	325	90	50	0.1
Adrenal Medulla	12	9	285	35	4	<0.1
Liver	20	50	365	40	30	<0.1
Kidney	18	19	258	11	3	<0.1
Brain	25	19	108	12	8	<0.1
Ovaries	6	23	133	3	1	<0.1
Muscle	4	21	24	7	2	<0.1
Serum	2.5	1.5	11.0	0.5	3.3	<0.1

and the correlation between high brain levels and the severe symptoms of neurological damage in 627 suggest that physically thin individuals may be more susceptible to HCB poisoning; and furthermore suggests that rapid weight loss of a chronically dosed animal could release amounts of HCB much larger than daily intake.

Findings of elevated blood urea nitrogen, elevated GOT, and depressed blood potassium levels in high dose animal #618 were associated with histopathological findings of liver and kidney damage (IATROPOULOS et al. 1976). It is not clear why HCB ingestion altered serum cholesterol levels (Table 1) although one might speculate that it was due to liver damage or unusual steroidogenic activity associated with changes in ovarian morphology (IATROPOULOS et al. 1976). It is possible that cholesterol levels may have changed in response to changes in food consumption. However, in a similar study of polychlorinated biphenyl ingestion undertaken at the same time, animals with appetites similarly depressed showed no such changes in cholesterol levels (KNAUF et al. 1978).

In general these data suggest: 1) primates can tolerate short term exposure to HCB at fairly high levels; 2) the first observable symptoms of HCB poisoning are lethargy and loss of appetite; 3) clinical symptomatology and testing cannot detect or predict toxicity at the lower dose levels which Iatropoulos et al. (1976) found to cause significant changes in the ovaries.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the expert assistance of L. Blevins, K. Diaz, and G. Salazar and the suggestions of Dr. Wolfgang Mueller of the Gesellschaft Fur Strahlen und Umweltforschung mbH Munich, Germany. This research was part of a joint program between the Gesellschaft Fur Strahlen und Umweltforschung mbH Munich, Germany and the Institute of Comparative and Human Toxicology, Albany Medical College, Albany, New York.

REFERENCES

BURNS, J.E. and F.M. MILLER: Arch. Env. Health 30, 44 (1975).

CAM, C. and G. NIGOGOSYAN: J. Amer. Med. Assoc. 183, 88 (1963).

IATROPOULOS, M., W. HOBSON, V. KNAUF, and H. ADAMS: Tox. Appl. Pharm. 37, 433 (1976).

KNAUF, V., W. MUELLER, G.B. FULLER, M. IOTROPOULOS, L. BALLHORN, F. COULSTON, and W. HOBSON: (In Preparation).

KOEMAN, J.J., M.C. ten NOEVER de BRAUN, and R.H. de VOS: Nature 221, 1126 (1969).

ROZMAN, K., W. MUELLER, F. COULSTON, and F. KORTE: Chemosphere, 5, 289 (1975).

SCHMID, R.: New Eng. J. Med. 263, 397 (1960).

STEELE, R.G.D. and J.H. TORRIE: Principles and Procedures of Statistics, New York; McGraw-Hill (1960).

WASSERMAN, D., M. WASSERMAN, SIMI CUCOS and M. DJAVAHERIAN: Environ. Res. 6, 338 (1973).