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# Metabolism and Pharmacokinetics of Pentachlorobenzene in the Rhesus Monkey

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Pentachlorobenzene (PCBz) is a major contaminant of commercial hexachlorobenzene (HCBz) which was widely used as a fungicide, plasticizer, flame retardant and additive to lubricants. PCBz is also one of the metabolites of HCBz in mammals (ROZMAN et al. 1975, 1977). This experiment was designed to establish the pharmacokinetics and metabolic break down of PCBz in the rhesus monkey. LEBER et al. (1977) published some results on this subject. However, their data are not consistent with the results obtained in our study.

## MATERIALS AND METHODS

Two male (5.2 and 3.6 kg) and two female (5.5 and 3.9 kg) rhesus monkeys received a single oral dose of 0.5 mg/kg bodyweight $^{14}C$-labeled PCBz by stomach tube (spec. act.: 3.6 mCi/mmol). Animals were housed in metabolism cages with access to 1 L water daily. Daily food intake consisted of 150 - 200 g Purina monkey chow supplemented with fruit. Urine and feces were collected daily. Blood was taken from the animals on several occasions. After 40 days one male and one female monkey were sacrificed and the body distribution of PCBz and/or metabolites determined.

Urinary and fecal excretion of radioactivity was measured separately for each animal. Urine was extracted with ether in a liquid-liquid extractor for 24 h, acidified to pH 1 and extracted for an additional 24 h. Feces was blended with $MgSO_4$ and extracted for 48 h in a Soxhlet extractor with methanol. Blood was hemolized and extracted with benzene in a separatory funnel. Liver and kidney were each homogenized separately, blended with $MgSO_4$ and extracted in a Soxhlet extractor with benzene for 48 h. Clean-up procedures of the various extracts consisted of repeated column chromatography on 60 - 200 mesh silical gel and deactivated (5 % $H_2O$) neutral aluminum oxide. Solvents used in column chromatography were: hexane, benzene, benzene : ethyl acetate = 9 : 1, benzene : ethyl acetate = 7 : 3, chloroform : methanol = 4 : 1, chloroform : methanol = 1 : 1, acetonitrile, and

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methanol. Final purification was achieved by TLC on silica gel G type 60 (Merck) and aluminum oxide PF-254 (Merck). Tissue samples were combusted in a Packard Tri-Carb sample oxidizer. Samples containing radioactive material were counted in a Packard Tri-Carb scintillation spectrometer. The radioactive zones on the TLC-plates were located with a Berthold DC-scanner. Structural elucidation was carried out with a Finnigan 3000 D gas chromatograph/mass spectrometer (column: 3 % OV-1 on Chromosorb W-HP 80/100; carrier gas: helium ca. 40 mL/min.; temperature: 160°C). Quantitative determination of PCBz and metabolites was performed with a gas chromatograph ($^{63}$Ni-EC-detector; column: 3 % OV-1 on Chromosorb W-HP 80/100; carrier gas: nitrogen ca. 40 mL/min.; temperature: 160°C). For gas chromatography the phenols were methylated with diazomethane.

## RESULTS

Absorption: at least 95 %, indicated by the fecal excretion of the first four days (Table 1).

Excretion: fecal excretion is about twice the amount of the urinary excretion (Table 1, Figure 1). The estimated half-life of PCBz in rhesus monkey is 2 - 3 mo.

Blood Levels: rising to peak levels between 2 and 4 ½ h, subsequently declining slowly (Table 2).

Body Distribution: fat and bone marrow contain the highest concentrations, followed by the lymph nodes, thymus, adrenal cortex and the large intestine (Table 3).

Metabolisation: Pentachlorophenol (PCPh)
2,3,4,5-Tetrachlorophenol (2,3,4,5-TCPh)
2,3,5,6-Tetrachlorophenol (2,3,5,6-TCPh)
1,2,3,4-Tetrachlorobenzene (1,2,3,4-TCBz)
were identified as metabolites of PCBz (Table 4). There was no significant sex related difference in the metabolisation pattern of rhesus monkeys.

TABLE 1

Cumulative Urinary and Fecal Excretion on PCBz and Metabolites during 40 Days Following a Single Oral Dose of 0,5 mg/kg Bodyweight PCBz in the Rhesus Monkey

| | Day | Males[a] | Females[a] |
|---|---|---|---|
| Urine | 4 | 1.9 | 2.4 |
| | 10 | 4.8 | 4.3 |
| | 20 | 8.6 | 7.8 |
| | 30 | 11.3 | 10.0 |
| | 40 | 13.2 | 11.4 |
| Feces | 4 | 6.3 | 4.4 |
| | 10 | 11.5 | 8.3 |
| | 20 | 19.3 | 16.4 |
| | 30 | 23.6 | 19.8 |
| | 40 | 27.0 | 21.8 |
| Total | | 40.2 | 33.2 |

[a]Expressed in % of the total administered dose

TABLE 2

Blood Levels of PCBz and/or Metabolites during 40 Days Following a Single Oral Dose of 0,5 mg/kg Bodyweight in the Rhesus Monkey

| Time | Males ppm | Females ppm |
|---|---|---|
| 5 Min | 0.01 | 0.01 |
| 15 Min | 0.01 | 0.01 |
| 1 Hour | 0.01 | 0.02 |
| 2 Hours | 0.05 | 0.07 |
| 4 ½ Hours | 0.07 | 0.06 |
| 24 Hours | 0.05 | 0.05 |
| 72 Hours | 0.05 | 0.06 |
| 3 Days | 0.05 | 0.06 |
| 9 Days | 0.04 | 0.07 |
| 20 Days | 0.05 | 0.05 |
| 30 Days | 0.03 | 0.04 |
| 40 Days | 0.04 | 0.04 |

TABLE 3

Body Distribution of PCBz and/or Metabolites on the 40th Day Following a Single Oral Dose of 0.5 mg/kg Bodyweight PCBz in the Rhesus Monkey

| Organ | Male ppm | Female ppm |
|---|---|---|
| Fat[a] | 1.86 | 2.68 |
| Bone Marrow[a] | 1.10 | 2.35 |
| Lymph Nodes[a] | 0.35 | 0.79 |
| Thymus | 0.50 | 0.61 |
| Adrenal Cortex | 0.31 | 0.56 |
| Adrenal Medulla | 0.18 | 0.07 |
| Skin | 0.26 | 0.26 |
| Liver | 0.19 | 0.17 |
| Kidney | 0.09 | 0.10 |
| Lungs | 0.06 | 0.06 |
| Spleen | 0.04 | 0.04 |
| Heart | 0.07 | 0.12 |
| Bile | 0.09 | 0.09 |
| Stomach | 0.06 | 0.06 |
| Duodenum | 0.11 | 0.06 |
| Cecum | 0.24 | 0.18 |
| Large Intestine | 0.31 | 0.33 |
| Small Intestine | 0.17 | 0.07 |
| Brain | 0.05 | 0.06 |
| Cerebellum | 0.05 | 0.06 |

[a]Average value from 5 different parts of the body

TABLE 4

PCBz and its Metabolites Identified in Urine, Feces, and Various Organs of Rhesus Monkeys Dosed 0,5 mg/kg Bodyweight PCBz

| | PCBz | 1.2.3.4-TCBz | PCPh | 2.3.4.5-TCPh | 2.3.5.6-TCPh |
|---|---|---|---|---|---|
| Liver | 99.0% | 1.0% | -- | -- | -- |
| Bile | nonpolar | compound(s) | -- | -- | -- |
| Feces | 99.0% | 1.0% | -- | -- | -- |
| Blood | 45.8% | -- | 54.2% | -- | -- |
| Kidney | 51.3% | -- | 48.7% polar compound(s) | | |
| Urine | -- | -- | 58.1% | 32.2% | 9.7% |

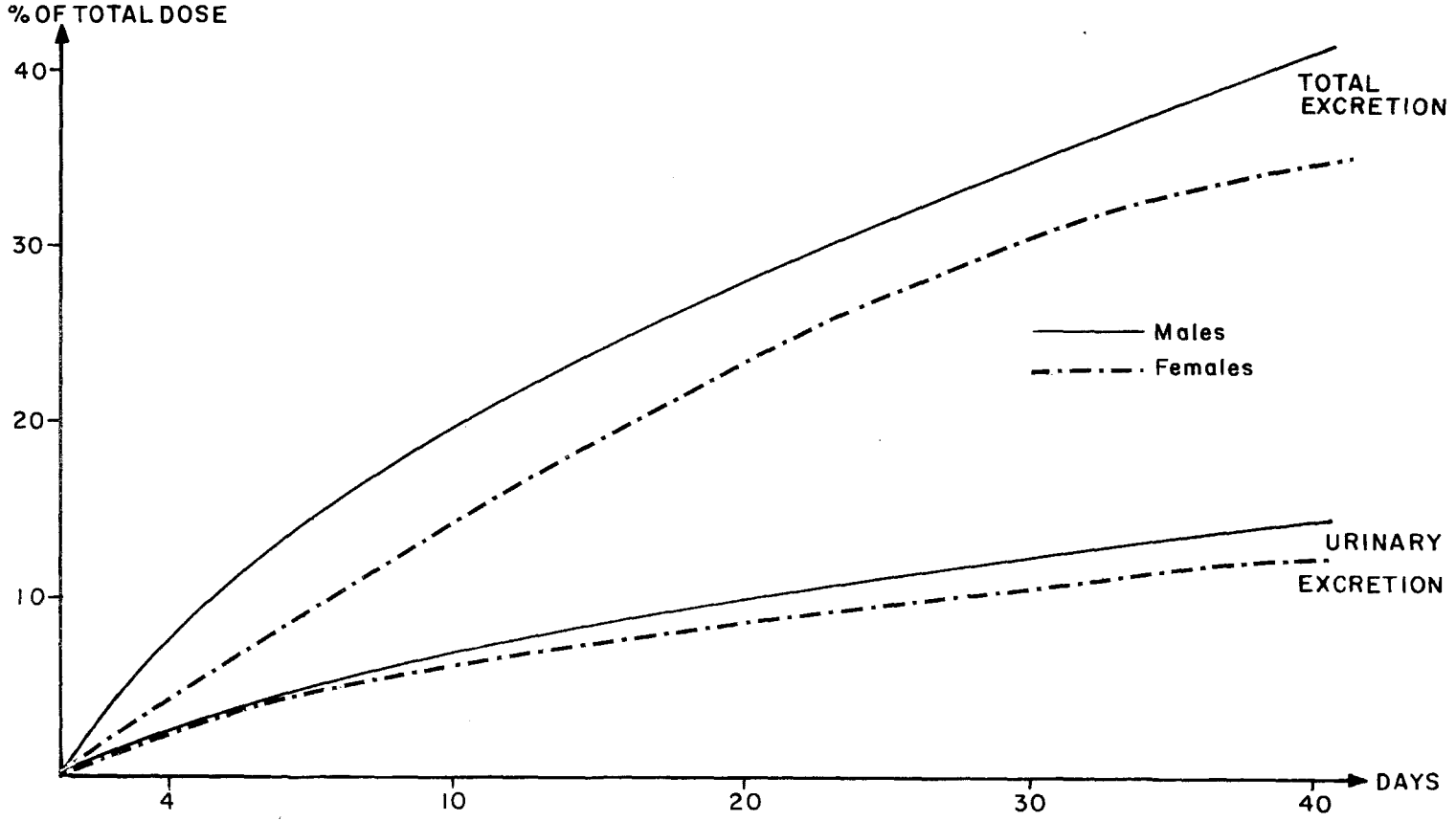


FIGURE 1: CUMULATIVE URINARY AND TOTAL EXCRETION OF PCBz AND METABOLITES DURING 40 DAYS FOLLOWING A SINGLE ORAL DOSE OF 0.5 mg/kg BODYWEIGHT PCBz IN THE RHESUS MONKEY

## CONCLUSIONS

Both PCBz and HCBz in the 0.5 mg/kg bodyweight range will be readily absorbed by the rhesus monkey. Blood and tissue levels of PCBz and/or metabolites bear strong resemblance to those of HCBz, indicating the involvement of the lymphatic system into the absorption process (M.IATROPOULOS et al. 1975). Urinary excretion is substantially higher than in the HCBz-study. However, the major excretory pathway appears to be the fecal. The biological half-life of PCBz is reduced by a factor of ca. 12 compared to HCBz. The metabolisation pattern shows some peculiarities. In neither study there could be found any evidence for the presence of "polar" (phenolic) metabolites in liver, bile, and feces. Phenolic metabolites seem to be restricted to blood, kidney, and urine. This raises some questions. If metabolisation takes place in the liver, why the lack of phenolic metabolites in liver, bile and feces? Why the strong representation of phenolic metabolites in blood, kidney, and urine only?

The evidence suggests that a metabolizing system other than hepatic cytochrome $P_{450}$ is involved in the hydroxylation of higher chlorinated benzenes.

Furthermore the presence of PCPh and the lack of tetrachlorobenzenes in the blood allows the possibility of two different hydroxylation pathways:

1) oxidation of PCBz to PCPh
2) nucleophilic displacement reaction of PCBz to TCPh-s

in metabolically active sites other than the liver.

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