# Distribution of Polychlorinated Biphenyls: Structural Requirements for Accumulation in the Mouse Bronchial Mucosa[1]

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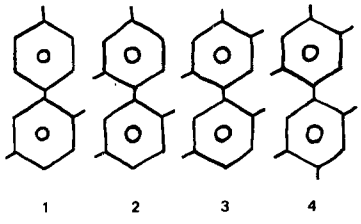
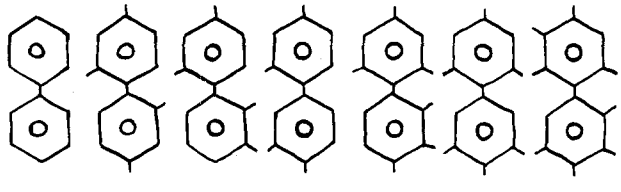
*Summary.* Autoradiography showed that labelled polychlorinated biphenyls with chlorine in positions 2, 4[1], 5 and hydrogen in positions 3, 3[1], 6, 6[1] in the molecule are accumulated in the mouse bronchial mucosa. Further testing of this observation showed that 2, 2[1], 4, 5[1]-tetrachlorbiphenyl-$^{14}$C, but not biphenyl-$^{14}$C, was taken up in the bronchi of mice.

Autoradiographic investigations have recently shown that 2, 2[1], 4, 4[1], 5, 5[1]-hexachlorobiphenyl-$^{14}$C and 2, 4[1], 5-trichlorobiphenyl-$^{14}$C are heavily accumulated and retained in the bronchial mucosa and in the mucosa of the trachea and larynx in mice[3,4]. Berlin et al.[5] reported a similar localization of 2, 2[1], 4[1], 5, 5[1]-pentachlorobiphenyl-$^{14}$C. This site of PCB-accumulation is so far unique. Previous studies found that a number of discrete PCBs were not taken up by the bronchi but were distributed very evenly throughout the lung parenchyma[4,6,7]. However, all PCBs investigated are more or less concentrated in the mucosa of the nasal cavities. When comparing the chlorine substitution patterns in the relatively few molecules so far studied, it appeared that the polychlorinated biphenyls with an affinity for the bronchi have the following structural characteristics in common: 1. they possess chlorine atoms in positions 2, 4[1], 5 in the biphenyl molecule (numbered from the biphenyl bond). 2. one *ortho-* and the diagonally situated *meta*-carbon atom in each ring (positions (3, 3[1], 6, 6[1]) are substituted with hydrogen).



1 2 3 4

**PCBs accumulating in bronchi**



5 6 7 8 9 10 11

**PCBs which do not accumulate in bronchi**

Eleven PCBs are classified according to their distribution patterns in the lung. Substances 1–4, which are accumulated and retained in the bronchial mucosa, are characterized by having chlorine in positions 2, 4[1], 5 in the molecule, whereas positions 3, 3[1], 6, 6[1] are substituted with hydrogen. Substances 5–11, which are not accumulated in the bronchi, do not fullfill the above structural criteria. Substances 1–2 and 4–11 were investigated in this laboratory (refs.[3,4] and[6,7]). Substance 3 was studied by Berlin et al.[5].

1 = 2, 4[1], 5-Trichlorobiphenyl; 2 = 2, 2[1], 4, 5[1]-Tetrachlorobiphenyl; 3 = 2, 2[1], 4[1], 5, 5[1]-Pentachlorobiphenyl; 4 = 2, 2[1], 4, 4[1], 5, 5[1]-Hexachlorobiphenyl. 5 = Biphenyl; 6 = 2, 2[1], 4, 4[1]-Tetrachlorobiphenyl; 7 = 2, 2[1], 3[1], 4-Tetrachlorobiphenyl; 8 = 2, 3[1], 4, 4[1]-Tetrachlorobiphenyl; 9 = 2, 2[1], 3, 4, 4[1], 6[1]-Hexachlorobiphenyl; 10 = 2, 3[1], 4, 4[1], 5[1], 6-Hexachlorobiphenyl; 11 = 2, 2[1], 3, 3[1], 4, 4[1], 5, 6[1]-Octachlorobiphenyl.

In order further to test the structural requirements for the uptake of PCBs in the bronchial mucosa, the distribution of 2, 2[1], 4, 5[1]-tetrachlorobiphenyl-$^{14}$C (which satisfies the above structural criteria) was studied using whole-body autoradiography. As it was considered to be of particular interest to provide some information also on the properties of the unsubstituted biphenyl nucleus, biphenyl-$^{14}$C was included in the investigation.

2, 2[1], 4, 5[1]-tetrachlorobiphenyl-$^{14}$C, uniformly labelled in the 2, 4-substituted ring, was synthetized as described by Sundström[8]. Biphenyl-$^{14}$C, previously prepared by several routes[9–13], was prepared from uniformly labelled anilin-$^{14}$C and benzene by a coupling reaction according to Cadogan[8,13]. Both compounds, of specific activity 25 mCi/mmol, were chromatographically pure (TLC-radiochromatogram scanning).

Two series of pregnant C57 Bl mice were i.v. injected with 0.2 µmol tetrachlorobiphenyl-$^{14}$C and biphenyl-$^{14}$C, respectively, in DMSO. Animals from each series were killed in carbon dioxide at intervals from 10 min up to 4 days after the injection. After being frozen to solid blocks in a gel of carboxymethyl cellulose and water, sagittal whole body sections were collected onto tape and autoradiograms prepared according to the Ullberg technique[14]. At the time of sacrifice, the mice were in the 17th day of gestation, the mating having been determined previously by means of vaginal plugs.

Four h after the injection of 2, 2[1], 4, 5[1]-tetrachlorobiphenyl-$^{14}$C, there was a strong uptake of radioactivity in the bronchial mucosa which roughly equalled the level in the body fat. After 4 days the bronchi showed by far the highest ocentration in the body, but the activity in the lung parenchyma was also high (Figure 1). At this

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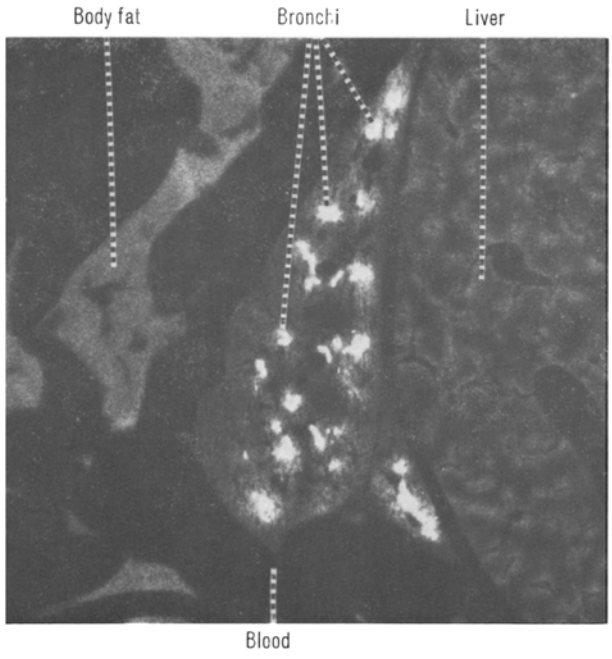


Fig. 1. Detail autoradiogram of a mouse 4 days after i.v. injection of 2,2¹,4,5¹-tetrachlorobiphenyl-¹⁴C. Note the strong uptake of radioactivity in the bronchi (white spots). The concentration in the body fat is considerably lower.

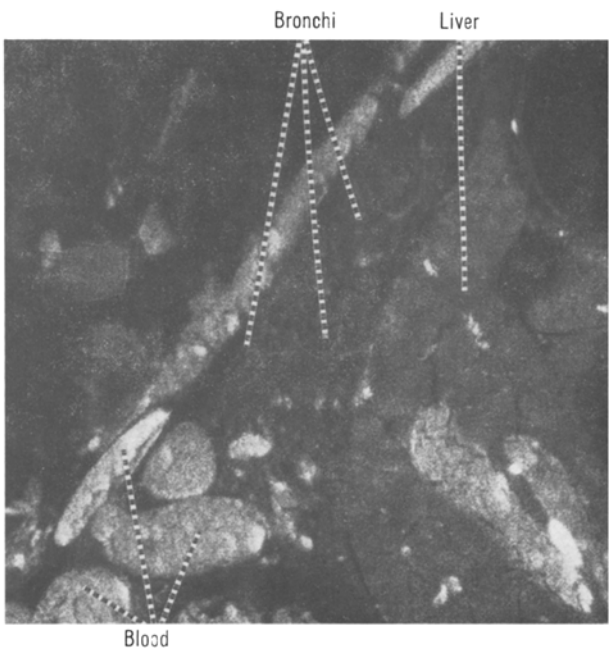


Fig. 2. Detail autoradiogram of a mouse 4 h after i.v. injection of biphenyl-¹⁴C. There is no site of accumulation in the lung. The activity in the blood is high.

time, a definite localization of label was observed also in the fetal bronchi. In contrast to the 2, 2¹, 4, 5¹-tetrachlorobiphenyl-¹⁴C, the unsubstituted biphenyl-¹⁴C was distributed very evenly throughout the lung (Figure 2), with no tendency towards concentration in specific structures.

The present results show that the ability to accumulate in the bronchi is not primarily bound to the biphenyl nucleus itself, but appears at chlorination of the molecule. They also further support the opinion that specific structural requirements exist – the fullfillment of which indicates substances with a high affinity for the bronchial tissue. Thus it was possible to predict the pulmonary behavior of 2, 2¹, 4, 5¹-tetrachlorobiphenyl in mice. The Table classifies some PCBs according to their pulmonary distribution pictures, and illustrates the differences in the chlorine positions. It is, however, possible that investigations into the mechanism of accumulation will give further information about the critical features in the substitution pattern of the biphenyls.

An important question in this respect is whether the radioactivity in the bronchial mucosa represents metabolized or unchanged PCB, or both. It has been demonstrated that intermediates to halogenated benzenes (e.g. chlorobenzene) covalently bind to the bronchial epithelium, where they cause tissue necrosis[15]. A similar mechanism of binding may be involved for the PCBs. Another possibility is that the bronchial concentration of radioactivity reflects a specific excretion pathway for certain PCB-structures or their metabolites in the lung.

The biological significance of these observations is at present unknown. Few investigations have been reported concerning chronic effects of structurally defined PCBs in laboratory animals, and pulmonary lesions have so far not been observed. It was recently shown that the uptake of 2, 2¹, 4, 4¹, 5, 5¹-hexachlorobiphenyl-¹⁴C in mice bronchi was almost completely blocked when the mice were pretreated with a large dose of the unlabelled isomer[3]. This may indicate that a possible adverse effect will not follow a simple dose-response relationship, and therefore not easily be provoked in short term high dose experiments. Obviously more attention should be paid to the late effects of defined PCBs in the different species. It is notable that 2, 2¹, 4, 4¹, 5, 5¹-hexachlorobiphenyl is a major PCB-component in human fat as well as in the fat of fisheating birds and seals[16].

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## Thermoregulatory Effects of Histamine

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In recent years there has been considerable neurochemical evidence, including regional distribution, cellular localization and the presence of synthetic enzymes, to implicate histamine as a putative central neurotransmitter. Central thermoregulatory mechanisms constitute one neuronal system on which a neurophysiological response to histamine can be demonstrated[2]. Thus, in the rat injection of histamine directly into the rostral hypothalamus causes a fall in body temperature which can be prevented by systemic administration of histamine $H_1$-