

Fig. 1 Photography of a polar bear (*Ursus maritimus*) and three cubs on tundra. *a*, Normal black and white photograph in the visible portion of the electromagnetic spectrum (Kodak Double-X Aerographic 2405 film, Hasselblad 150 mm Sonnar lens, f/16, 1/500 s). *b*, Ultraviolet photograph (Kodak Aerographic Double-X 2405 film, Hasselblad 105 mm UV-Sonnar lens, Kodak 18A filter, f/5.6, 1/500 s).

This observation of 'black' polar bears seems to have further implications. At present, the biological significance of white coat coloration in animals is not clear. Whiteness in arctic climates obviously provides camouflage for many birds and mammals. Theoretical analysis and quantitative measurements suggest that some white pelts transfer solar energy more efficiently to the skin than dark pelts[3,4]. Thus white coloration may also play an important role in thermoregulation for a number of species including the white-coated pups of the harp seal (*Pagophilus groenlandicus*)[5]. The older view that dark pelts absorb solar radiation while white pelts reflect it and thus reduce radiation absorption[6] is probably incorrect. Regardless, the fact that some white pelts absorb ultraviolet radiation while others, such as the arctic hare reflect it[7], suggests that any discussion of "life's color code"[8] is incomplete without further consideration of the near ultraviolet component in solar radiation.

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Metabolic hydroxylation of a chlorobiphenyl containing only isolated unsubstituted positions—2,2′,4,4′,5,5′- hexachlorobiphenyl

RECENT investigations on the metabolism of chlorobiphenyls of known structure have shown that compounds containing four chlorine atoms or less are hydroxylated by rats, rabbits and pigeons[1–3]. All the compounds investigated have two unsubstituted carbon atoms in ortho positions to each other. It has been suggested that this substitution pattern is required for the metabolic breakdown of chlorobiphenyls[4]. A thorough investigation of the chlorobiphenyl residues found in man and in higher animals partly confirms this suggestion[5].

Hutzinger *et al.*[3] have investigated the fate of a chlorobiphenyl containing only isolated, unsubstituted carbon atoms—2,2′,4,4′,5,5′-hexachlorobiphenyl—in rats, pigeons and trout. No hydroxy-containing metabolites were detected in the excreta by the methods used. This chlorobiphenyl is one of the major components in technical PCB with chlorine contents above 50%. Furthermore, it is found in relatively high amounts in all species investigated including man[1].

It has been shown, however, that rabbits given 1,2,3-, 1,2,4- or 1,3,5-trichlorobenzene always excreted chlorinated monophenols. The yields over 5 d were 78, 42 and 5%, respectively[4].

The fact that even 1,3,5-trichlorobenzene (containing only isolated unsubstituted carbon atoms) is hydroxylated, led us to reinvestigate the behaviour of 2,2′,4,4′,5,5′-hexachlorobiphenyl in some animal species.

Rats were given the chlorobiphenyl[9] in peanut oil (20 mg ml^{-1}) and an amount corresponding to about 15 mg was consumed during the night by each animal. Faeces were collected daily, dried and extracted with ether (Soxhlet extraction). The extracts were divided into two parts, one of which was treated with diazomethane. After evaporation of solvent the residue was dissolved in hexane and shaken with sulphuric acid before analysis by gas chromatography using an electron capture detector[1]. The acid treatment removes most unwanted extractives but does not affect chlorobiphenyls and methoxy-chlorobiphenyls.

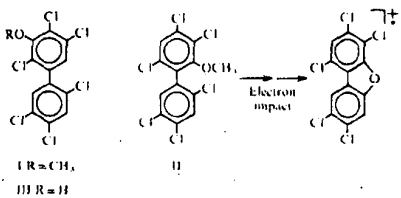


Fig. 1 Alternative structures I and II of the methylated metabolite of 2,2′,4,4′,5,5′-hexachlorobiphenyl in rats and mice. The suggested cyclisation reaction (see text) of II upon electron impact in the mass spectrometer is indicated.

In addition to unchanged hexachlorobiphenyl a new compound was found in the methylated extract after 2 d. When analysed by combined gas chromatography–mass spectrometry (quadropole instrument) this substance showed a molecular ion at 388 mass units (m.u.) (6 Cl) corresponding to a methoxyhexachlorobiphenyl. The structure of this compound (Fig. 1), obviously derived from a phenolic metabolite of 2,2′,4,4′,5,5′-hexachlorobiphenyl, must be I or II provided no migrations of chlorine atoms have taken place.

Mass spectral studies performed in this laboratory using about 35 synthetic methoxychlorobiphenyls (S. J. and G. S., unpublished) have shown that compounds containing a methoxy group in the two position, II, for example, readily lose CH_3Cl (50 m.u.) upon electron impact. This results in very abundant peaks in the range 83–234 m.u. of the molecular ion at M^+-50 m.u. which can be explained by the formation of cyclic structures (Fig. 1).

Similar fragmentation patterns have previously been

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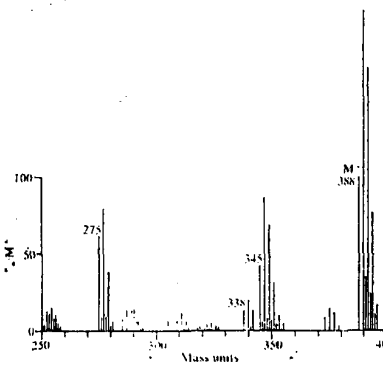


Fig. 2 Part of the mass spectrum of the methylated metabolite.

observed in the mass spectra of 2-methoxychlorodiphenyl ethers[9,11].

The loss of CH_3Cl in the mass spectra of all 3- or 4-methoxychlorobiphenyls investigated so far have on the other hand, never exceeded 30% of the molecular ion. The mass spectrum of the methyl ether of the present metabolite (Fig. 2) shows a peak at M⁺-50 m.u. which is only 13% of the molecular ion. This fragmentation strongly favours structure III of the original phenol. Final proof for the structure, however, must await comparison with synthetic material of known structure.

The elimination rate of unchanged hexachlorobiphenyl and the metabolite (assuming equal detector response on GLC) is shown in Fig. 3. The total amount of metabolite excreted during 7 d was calculated to be about 1.3% of the dose. The corresponding figure for unchanged biphenyl was about 7%. No trace of III or conjugates thereof were found in the urine.

Our experiments do not discriminate between microsomal or microbial hydroxylation of the chlorobiphenyl as no bile cannulation experiments have been performed. But the presence of the metabolite in the faeces—and not in the urine—is in accordance with the results of other investigations concerning the metabolism of PCB in rats[3,4].

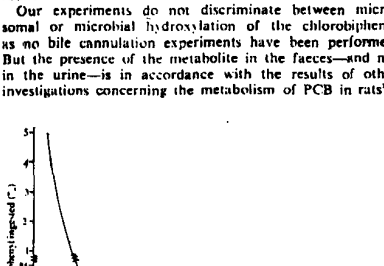


Fig. 3 Excretion rate (faeces) of 2,2',4,4',5,5' hexachlorobiphenyl (——) and its metabolite (– – –) from a rat as % hexachlorobiphenyl ingested.

Control experiments showed that bacterial action during the drying of the faeces was not responsible for the hydroxylation.

Mice given the hexachlorobiphenyl also excreted III (GLC) in the faeces. When the substance was given to quails (about 11 mg per animal) no metabolite could be detected in the excreta. It was, however, found that large amounts of unchanged chlorobiphenyl were excreted in both faeces and eggs—about 9 and 33%, respectively, during 10 d.

The results presented here show that a chlorobiphenyl containing only isolated unsubstituted positions can be metabolised by mammals albeit at a lower rate than chlorobiphenyls containing vicinal hydrogen atoms. It seems, therefore, that most of the high chlorinated compounds in PCB can be excreted not only unchanged but also as metabolites. The latter route implies that the chlorobiphenyls are not further circulated in the environment but that new compounds are released the persistence and toxicity of which are not sufficiently known.

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Sexual maturation of Japanese eel and production of eel larvae in the aquarium

THE life history of the eel is complex and despite many investigations little is known about its embryonic development. Some investigators such as Grassi and Calandriccio (1896 cited in ref. 1) and Fish[1], described the supposed early development of eels, using developing eggs collected by a trawl for young fish in suspected spawning grounds of eels. The time of fertilisation of these eggs, however, was unknown, and the species involved was uncertain. Artificial maturation of eels by hormonal treatment is another approach which has been attempted by many workers[2–6]. They all, however, failed to obtain mature eggs and sperm and thus to clarify the embryonic development of eels. Here we describe the first success with this approach.