# Interaction of Methylsulfonyl-Containing PCB with Mammalian Carrier Proteins

**G.L. Larsen and Å. Bergman***
U.S. Department of Agriculture, Agricultural Research Service, Biosciences Research Laboratory, Fargo, ND 58105-5674 USA; *University of Stockholm, Wallenberg Lab, Stockholm, SWEDEN

Because of their chemical stability, polychlorinated biphenyls (PCB) have been used extensively as industrial chemicals in electrical equipment worldwide. This stability translates into a persistence in the environment which causes PCB to be ubiquitous environmental pollutants.[1] PCB are metabolized in mammals to sulfur-containing metabolites such as methyl sulfides and methyl sulfones.[2] The latter type of PCB metabolites known to accumulate in mammals and some PCB-methyl sulfones are also selectively retained in certain tissues.[3][4][5] Introduction of methylthio- methylsulfinyl- and methylsulfonyl- groups into PCB is known to involve the mercapturic acid pathway.[6][7]

Previously, we have shown that 4,4'-bis(methylsulfonyl)-2,2',5,5'-tetrachlorobiphenyl formed a complex with the following carrier proteins: $\alpha_{2u}$-globulin ($\alpha_{2u}$) in kidney and urine in male rats, major urinary protein in mouse urine,[8] liver fatty acid binding protein (FABP) isolated from rat intestinal mucosa[9] and FABP from liver and intestinal mucosa of the chicken.[10]

The following chlorinated biphenyls (CBs) were studied to determine their association with $\alpha_{2u}$ and FABP in various tissues in the rat: 3,3',4,4'-tetrachloro-[$^{14}$C]biphenyl (CB-77[11]), 3,3',4,4',5-pentachloro-[$^{14}$C]biphenyl (CB-126) and 2,2',4,5,5'-pentachloro-[$^{14}$C]biphenyl (CB-101). The two 3-methylsulfonyl- and 4-methylsulfonyl-2,2',4',5,5'-pentachloro-[$^{14}$C]biphenyl (3-$CH_3SO_2$-CB-101 and 4-$CH_3SO_2$-CB-101, respectively, were studied in a similar way. The five compounds listed above were synthesized labelled with $^{14}$C [UL ring $^{14}$C]. In separate studies four male Sprague-Dawley rats (250-350 g) were each dosed orally with one of the five compounds (2.2-6.6 µCi, 52-133 µg depending on the compound). Feces and urine were collected for two days. For isolation of radioactive-protein complexes from kidney, rats were killed 48 h after dosing. Tissue samples were homogenized, centrifuged and chromatographed on Sephadex G-75 (G-75) and Sephacryl S-200 (S-200) as previously described.[12] Binding studies of the 14,000 Da protein isolated from various tissues were conducted using oleic acid as previously described to characterize the 14,000 Da protein as a FABP.[12] The method for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using 13% acrylamide is that of Mazel.[13]

The proteins of approximately 14,000 Da isolated from various tissues were characterized to be FABPS from their MW as determined by SDS-PAGE and capacity to bind oleic acid. Binding of the CB metabolites to $\alpha_{2u}$ or FABP from various tissues and urine was determined by isolation and co-chromatography on the G-75 and S-200

columns. Kidneys from male rats dosed with all compounds, except CB-126 (because of low levels of $^{14}C$), contained two metabolite/protein complexes which were isolated and characterized by SDS-PAGE to be $\alpha_{2u}$ and a FABP (MW ca 14,000 Da). Table I shows the distribution of $^{14}C$ in the rat from each of the PCBs listed for those tissues studied. *Table II shows an estimate of the percent of dose bound to carrier proteins in kidney,* urine, lung and liver, with the percent bound to the particular protein within the organ given in parenthesis.

Table I. Distribution of $^{14}C$ residues in rats dosed with the following CBs and $CH_3SO_2$-CBs in urine and tissues studied.

| | CB-77 (%) | CB-126 (%) | CB-101 (%) | 3-$CH_3SO_2$-CB-101 (%) | 4-$CH_3SO_2$-CB-101 (%) |
|---|---|---|---|---|---|
| Urine | | | | | |
| 0-24 h | 0.88 | 0.04 | 0,42 | 0.06 | 0.06 |
| 24-48 h | 0.47 | 0.05 | 0.18 | 0.02 | 0.06 |
| Tissues | | | | | |
| liver | 2.47 | 81.2 | 2.14 | 7.10 | 7.05 |
| kidney | 0.24 | 0.23 | 0.46 | 0.39 | 0.90 |
| lung | 0.24 | 0.09 | 0.57 | 0.23 | 2.06 |

Table II. Binding of CBs and $CH_3SO_2$-CBs to carrier proteins as a percent of dose.

| | CB-77 (%) | CB-126 (%) | CB-101 (%) | 3-$CH_3SO_2$-CB-101 (%) | 4-$CH_3SO_2$-CB-101 (%) |
|---|---|---|---|---|---|
| $\alpha_{2u}$ | | | | | |
| 0-24 h urine | 0.36(41) | 0.02(50) | 0.39(93) | 0 | 0 |
| 24-48 h urine | 0.27(58) | 0.01(20) | 0.16(89) | 0 | 0.01(14) |
| kidney | 0.11(46) | N.D. | 0.22(48) | 0.39(100) | 0 |
| 14,000 Da FABP | | | | | |
| liver | 0.08(3.4) | 5.7(7) | 0.40(19) | 4.8(68) | 4.3(61) |
| kidney | 0.14(46) | N.D. | 0.12(27) | 0.39(100) | 0.03(3.7) |
| 14,000 Da carrier protein | | | | | |
| lung | 0.20(84) | N.D. | 0.54(95.1) | N.D. | 2.1(100) |

(% $^{14}C$ bound by $\alpha_{2u}$ or fatty acid binding protein in urine or tissue.)
N.D. - not determined

Low levels of $^{14}C$ were excreted in urine (0.06-0.9% of these two, Table I) with the two methylsulfonyl-CBs being the lowest. Likewise, very little binding of these two CB-metabolites to $\alpha_{2u}$ was observed in urine from the rats (Table II). With the rats dosed with other CBs most of the $^{14}C$ excreted in the urine was bound to $\alpha_{2u}$, especially in the case of CB-101 (93% bound for 0-24 h and 89% for 24-48 h, Table II).

Of tissues analyzed the liver contained the highest levels of $^{14}C$-residues (2.14-81.2%, Table I). Very high level of $^{14}C$-residues were found in the livers of CB-126 dosed rats (81.2%, Table I) with 7% binding to FABP, Table II. High levels were also found with the 3- and 4-$CH_3SO_2$-CB-101 dosed rats (7.0-7.1%, Table I). These were also found to have high levels of binding to FABP (4.3% and 4.8% for 4-$CH_3SO_2$-CB-101 and 3-$CH_3SO_2$-CB-101, respectively, Table II). Generally, after 48 h low levels of $^{14}C$ (0.23-0.90% of the dose, Table I) were found in the kidney with CB-101 (0.42%) and 4-$CH_3SO_2$-CB-101 (0.9%) being the highest. High levels of CB metabolite binding to $\alpha_{2u}$ were observed with CB-77, 3-$CH_3SO_2$-CB-101 and CB-101 (27-100%, Table II). Binding to FABP in the kidney was observed with all the compounds except CB-126 where levels of $^{14}C$-residue were too low to be detected. In the lung most of the $^{14}C$-residues from CB-77, CB-101, and 4-$CH_3SO_2$-CB-101 were bound to a 14,000 Da protein which is believed to be the uteroglobulin-like protein reported by Lund, et al. (1988).[14] Notably, higher levels of $^{14}C$-residues/14,000 Da protein complex were found in 4-$CH_3SO_2$-CB-101 dosed rat lung.

In the rat, FABP is thought to be involved in absorption, intracellular transport, compartmentalization, and metabolism of free fatty acids and their acyl-CoA esters.[15)16)] Thus, FABP in the liver and kidney may be involved in the uptake, intracellular transport, storage, and metabolism of PCB. The 14,000 Da protein in lung may also belong to the family of FABP carrier proteins and also function similarly in the lung. Ultimately, these processes may be responsible for the accumulation of a particular PCB metabolite (notably, methylsulfonyl-containing PCB) in a specific tissue. In addition, these PCB metabolites which bind to FABPs may interfere with the metabolism, storage, and transport of fatty acids in the cell by associating with FABP. This association could adversely affect reproductive efficiency because studies have shown that PCB can induce reproductive insufficiencies in mink[17)] and is probably the cause of poor reproductive success in seals and otters[18)19)].

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