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# Distribution of Some Organochlorine Compounds (PCB, CBz, and DDE) in Beeswax and Honey

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Organochlorines are ranked among the class of prevalent and environmentally persistent synthetic chemicals. Honey bees, beeswax, and honey could be indicators for monitoring environmental pollution by organochlorines such as polychlorobiphenyls (PCBs) and organochloro pesticides (Bromenshenk et al. 1991; Davis 1989; Gayger and Dustmann 1985; Morse et al. 1990). Scarcely any data were reported (Gayger and Dustmann 1985) on the distribution of organochloro compounds between beeswax and honey. Physicochemical factors such as adsorption, volatilization (Henry's law constant), lipophilicity (octanol-water partition coefficient) and metabolic stability (Isnard and Lambert 1989; Mackey 1982; Sedlak and Andren 1991) can influence the level of individual organochlorine compounds in beeswax and honey. During wax and honey formation metabolic attack by different enzymes can degrade pollutants (Davis 1987; Winston 1987). In the PCB and chlorobenzene (CBz) series, biodegradation decreases and bioconcentration increases with increasing degree of chlorine substitution (Hardman 1991; Kohli et al. 1976; Sedlak and Andren 1991). Regarding the composition of honey (sugars, water, and some organic material and particles such as pollen, organic acid and essential oils in traces), and of beeswax (esters, hydrocarbons, acids and some natural wax from plants as minor components), it is expected that beeswax is more lipophilic and organochlorines could be more enriched in beeswax. However, the presence of particulate matters (e.g.pollen) in honey can increase the level of nonpolar compounds in honey due to sorption processes. This effect has been demonstrated in a similar system (Schrap and Opperhuizen 1990) where suspended particles can influence the partition coefficient.

In this contribution (i) the partition between beeswax and honey of some organochlorine compounds (PCB and CBz isomers, DDE) and (ii) bioconcentration in beeswax and honey from a feeding experiment by administration to honey bees of feed fortified with these compounds is presented and discussed.

## MATERIALS AND METHODS

Partitioning of organochloro compounds between beeswax and

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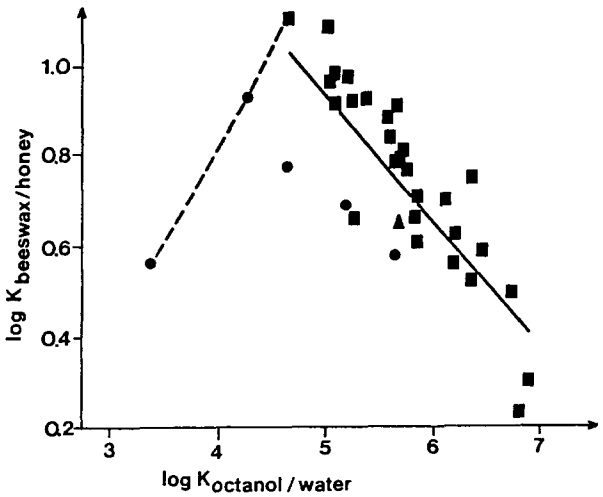


Figure 1. Relationship between beeswax/honey partition coefficients and 1-octanol/water partition coefficients of organochlorine compounds : CBz ( ), PCB ( ) and 4,4'-DDE ( ). $K_{ow}$ values from Hawker and Connell (1988); Isnard and Lambert (1989).

honey was performed at 70°C, above the mp of beeswax, in an ultrasonic bath for 15 min.

One honey bee colony (Apis Mellifera Carnica) received feed - 60 % sugar syrup with citrus essential oil as additive. One bee colony consumed on average 0.4 L sugar syrup daily for 5 weeks. The feed was spiked with the following level of organochlorines: PCB (as Aroclor 1242) 6,500; 4,4'-DDE 1,400; 1,4-diCBz 3,500; 1,2,3,-triCBz 350; 1,2,3,4-tetraCBz 220; 1,2,3,5-tetraCBz 220; pentaCBz 37; and hexaCBz 4 ppb on a fresh wt basis, respectively.

A sample of honey (15 g) was dissolved in water (50 mL) and organochlorine compounds were extracted with hexane. A sample of beeswax (5 g) was melted and ultrasonically extracted twice with acetonitrile (30 mL) at 70°C for 15 min. To the combined acetonitrile extracts water was added and organochlorines extracted into hexane. The hexane extract was cleaned-up over silica, with sulphuric acid, alkaline hydrolysis, and chomatography over Florisil before GC analysis.

The hexane eluate was analysed by high resolution gas chromatography (HRGC) with $^{63}Ni$ electron capture detection (ECD) and with split-splitless injection. HRGC-ECD was performed on a Hewlett-Packard 5890 instrument, using a 60 m x 0.25 mm (id) fused silica capillary column, coated (film thickness 0.25 µm) with SPB-5 (Supelco) and hydrogen carrier gas at a flow rate of 38 cm/sec (at 200°C). The make up gas was nitrogen. The chromatographic conditions were as follows: injector 240°C, detector 280°C, column 55°C for 1.1 min then to 200°C at 15 °C/min and to 235°C at 1.5°C/min. Purge activation time was 1.5 min. Quantification was

Table 1. Physicochemical properties of some organochlorine compounds and their bioconcentration in beeswax and honey from organochlorine compounds administered to bees.

| compound | chlorine substitution | log $K_{ow}$* | HLC**[a] | relative metabol.*** | bioconcentration ($x10^3$) beeswax | honey |
|---|---|---|---|---|---|---|
| PCB-6 | 2,3 | 5.06 | 3.486 | 1.00 | 36 | 1 |
| PCB-18 | 2,2',5 | 5.24 | 3.495 | 0.86 | 100 | 3 |
| PCB-24 | 2,3,6 | 5.35 | 3.507 | 0.94 | 89 | 9 |
| PCB-28 | 2,4,4' | 5.67 | 3.544 | 0.90 | 230 | 11 |
| PCB-40 | 2,2',3,3' | 5.66 | 3.738 | 0.76 | 294 | 7 |
| PCB-74 | 2,4,4',5 | 6.20 | 3.668 | 0.67 | 372 | 15 |
| PCB-101 | 2,2',4,5,5' | 6.38 | 3.610 | 0.57 | 482 | 16 |
| diCBz | 1,4 | 3.38 | 2.59 | | 9 | 2 |
| triCBz | 1,2,3 | 4.27 | 2.70 | | 94 | 20 |
| tetraCBz | 1,2,3,4 | 4.65 | 3.16 | | 14 | 8 |
| pentaCBz | 1,2,3,4,5 | 5.19 | 3.15 | | 22 | 12 |
| hexaCBz | 1,2,3,4,5,6 | 5.66 | 3.24 | | 130 | 4 |

a -log HLC in atm x $m^3$/mol
* data from Hawker and Connell (1988); Isnard and Lambert (1989)
** data from Dannenfelser et al. (1991); Dunnivant et al. (1992)
*** data from Sedlak and Andren (1991)

based on external standards. Quantification of PCBs was done as the sum of 31 PCB isomers (presented in Fig. 2). Recoveries were in the range of 78 to 103 %, depending on the compound. CBz isomers and 4,4'-DDE were supplied by Fluka (Bucks, Switzerland). PCB isomers were identified by comparison with a reference solution of PCB standards (CIL, Promochem) and/or from data on Aroclor 1242 (Mullin et al. 1984; Capell et al. 1985) under similar conditions. IUPAC numbers were used for the assignation of PCB isomers (Ballschmiter and Zell 1980).

## RESULTS AND DISCUSSION

In Fig. 1 partitioning of organochloro compounds (PCB, CBz isomers, DDE) between beeswax and honey was correlated with the 1-octanol-water partition coefficient ($K_{ow}$). From similar relationships (Hawker and Connell 1988; Isnard and Lambert 1989; Mackay 1982)

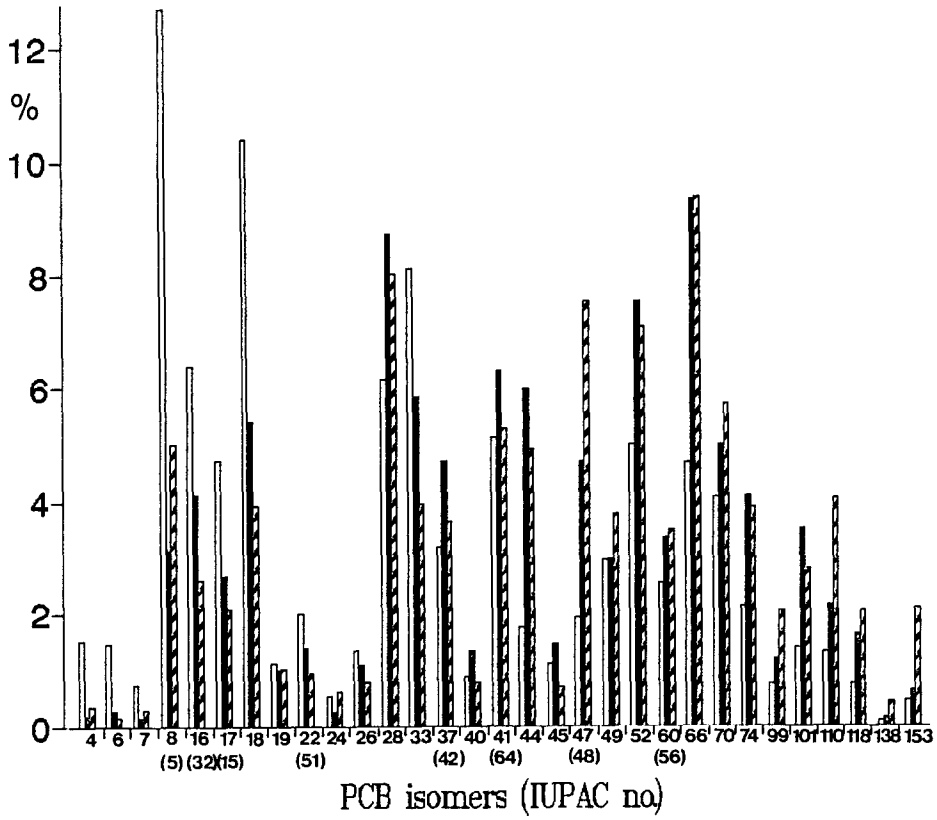


Figure 2. Relative composition (wt %) of PCB isomers in feed (□), beeswax (■) and honey (▨) administered to bees. In brackets the isomer coeluted in GC peak is given.

a slope with a positive bioconcentration of organochlorine compounds is expected. The beeswax-honey partitioning followed a linear relationship with $K_{ow}$ up to 4.5. More lipophilic compounds (higher $K_{ow}$) follow a negative slope in this relationship. Reduction of the beeswax-honey partition coefficient could be the result of sorption of the compounds on suspended particles (e.g. pollen). This reduction is significant for components with a high sorption coefficient or $K_{ow}$.

The proportion of different PCB isomers in beeswax and honey produced by honeybees fed contaminated food are presented in Fig. 2. The following fresh weight levels were present in feed, beeswax and honey, respectively: total PCBs (sum of 31 isomers) 6,500; 1,300; 50 ppb, 4,4'-DDE 1,400; 740; 40 ppb, 1,4-diCBz 3,500; 31; 7 ppb, 1,2,3-triCBz 350; 33; 7 ppb, 1,2,3,4-tetraCBz 220; 3.2; 1.7 ppb, 1,2,3,5-tetraCBz 220; 5.9; 1.8 ppb, pentaCBz 37; 0.82; 0.46 ppb, hexaCBz 4; 0.54; 0.015 ppb. In Fig. 3 the HRGC patterns of organochloro compounds in beeswax and honey are shown. The depletion (Fig.3) of lightly chlorinated compounds in beeswax and honey

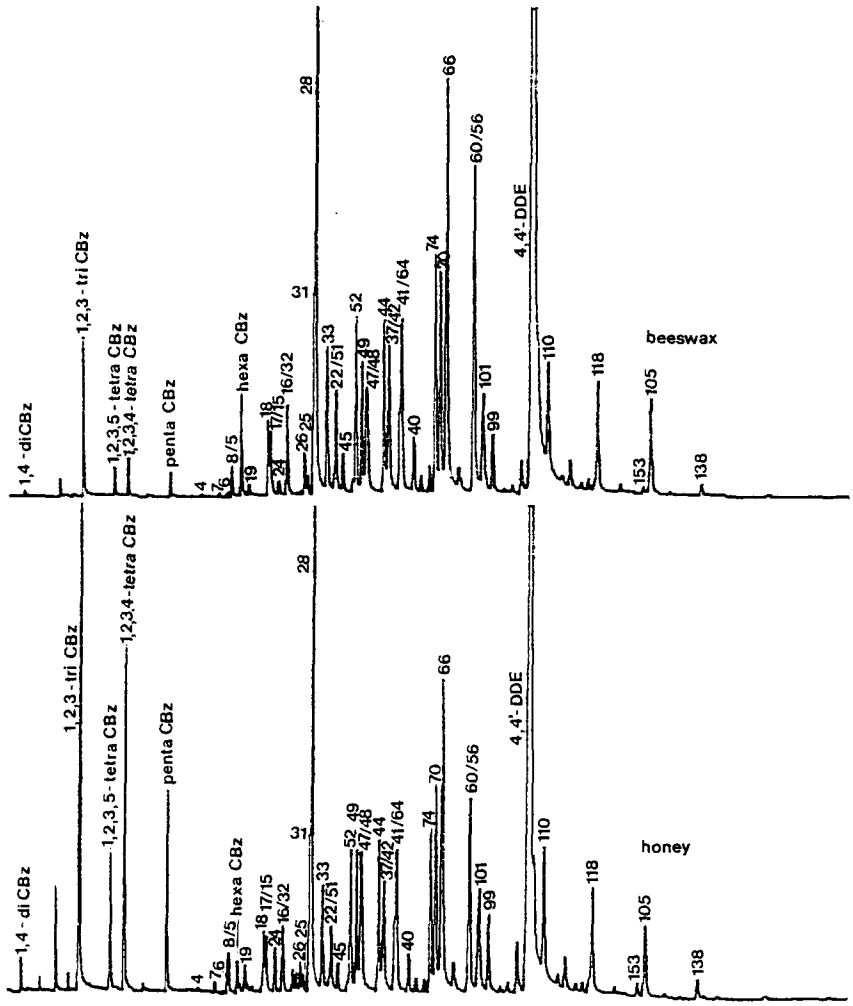


Figure 3. Gas chromatograms (EC detector), in the PCB, CBz and DDE region, of beeswax and honey made by bees from contaminated feed.

could be influenced by two factors: (I) Some enzymes during beeswax formation degrade lower chlorinated compounds (e.g.di-to tetraCBz, dichlorobiphenyls: PCB-4, PCB-6, PCB-7, PCB-8, and trichlorobiphenyls non para-chloro substituted: PCB-16, PCB-18, PCB-19, PCB-24); and (II), it is speculated that during honey formation depletion by volatilization of lower chlorinated compounds occurs. Lower chlorinated PCB and CBz have high vapour pressures and can evaporate from honey in view of their Henry's law constant (HLC). Loss by volatilization prevails over possible metabolic degradation in honey. The bioconcentration (Fig.2) of some individual compounds was calculated by comparing their abundance in beeswax and honey extracts with their abundance in the standard solution added to feed. In Table 1 some physicochemical parameters of individual organochloro compounds and their

bioconcentration into beeswax and honey (C wax or honey/C feed) through contaminated bees feed are presented. In beeswax hexa CBz, higher chlorinated PCB isomers from the technical Aroclor 1242 mixture and di-para chloro substituted biphenyls (e.g. PCB no. 28,74) which are stable to metabolic attack were enriched.

However, lower chlorinated -benzenes and -biphenyls were depleted. It was also evident by comparison of pairs of PCB isomers with the same chlorine content (PCB no. 33/28, 44/74, 52/66, 70/74), that isomers with higher para chlorine substitution (PCB no. 28, 66, 74) which are more resistent to metabolic degradation predominated in beeswax compared to the composition added to feed. Metabolic degradation is more pronounced in beeswax than honey.

The present data elucidate the influence of partitioning, volatilization, and metabolism on the levels of some organochlorines in beeswax and honey.

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