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Effect of fatty acids and the aqueous diffusion barrier on the uptake and transport of polychlorinated biphenyls in Caco-2 cells

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Abstract Polychlorinated biphenyls (PCBs) dissolved in dietary fat are absorbed in the gastrointestinal tract by the enterocytes in combination with the fatty acids proceeding from the lipid hydrolysis in the gut lumen. The effect of fatty acid absorption on the uptake and transport of 14 PCBs in enterocytes was studied using monolayers of the human intestinal Caco-2 cell line as a model system. The diffusive resistance of the unstirred water layer and the facilitating role of mixed bile salt micelles on the PCB uptake were examined by varying the thickness of the unstirred water layer adjacent to the apical membrane. In additional experiments, the polarity of the PCB uptake and transport in Caco-2 cells was determined. The solubility of PCBs in the mixed bile salt–fatty acid micelles was 2.7- to 4.8-fold higher than the solubility in plain bile salt micelles. Both the uptake and transport of PCBs in Caco-2 cells were significantly higher (up to 10-fold) when the PCBs were presented mixed with fatty acids. Reducing the thickness of the unstirred water layer resulted in an increased uptake of PCBs. The PCB uptake in Caco-2 cells exceeded the uptake as expected from monomer diffusion only, indicating that bile salt micelles facilitate the PCB transport over the unstirred water layer. Concentrations of dichlorobiphenyls accumulating in the basolateral medium stayed unexpectedly low, suggesting that Caco-2 cells might possess the capability of metabolizing lower chlorinated biphenyls. Uptake of PCBs into the Caco-2 cells was not significantly different whether the PCBs were presented at the apical side or at the basolateral side. However, transport of PCBs over the cell monolayer was significantly higher when the PCBs were presented at the apical side as compared to the basolateral side, suggesting that the unidirectional transport of lipids and lipoproteins affected the PCB transport as well. Our studies indicate that monolayers of the Caco-2 cell line offer a useful model system for studying the intestinal uptake and transport processes of hydrophobic xenobiotics such as polychlorinated biphenyls.—**Dulfer, W. J., J. P. Groten, and H. A. J. Govers.** Effect of fatty acids and the aqueous diffusion barrier on the uptake and transport of polychlorinated biphenyls in Caco-2 cells. *J. Lipid Res.* 1996. **37:** 950–961.

Supplementary key words enterocytes • xenobiotics • triglyceride • lipoproteins • metabolism

Although the production of polychlorinated biphenyls (PCBs) has been banned for almost two decades now, PCBs are still widespread pollutants that have been found even in very remote areas (1). Due to their extremely hydrophobic character (n-octanol/water partition coefficients, log K_{ow}, larger than 5) and high resistance to biodegradation, many PCBs show bioaccumulation in food chains resulting in very high and toxic concentrations in animals of the higher trophic levels and in humans (2–4). The uptake of PCBs in terrestrial mammals and birds, but also in predatory fish, occurs predominantly by the food in which the PCBs are distributed, preferentially in the lipid fraction, and is considered to be a passive diffusion process (2, 5, 6).

As the major part of the hydrophobics that enter the gastrointestinal tract is dissolved in lipids, and because of the very low solubilities of these compounds in the aqueous environment of the gut lumen, it has been proposed that lipid assimilation might interfere with the uptake of extremely hydrophobic xenobiotics such as polyaromatic hydrocarbons (PAHs) and PCBs (7–9). This model suggests that after the dispersion and the hydrolysis of the dietary fats into fatty acids, the bile salt micelles in the lumen of the gut provide a more attractive hydrophobic phase to hydrophobic contaminants than the aqueous environment. Parallel with the fatty

Abbreviations: PCB, polychlorinated biphenyl; VLDL, very low density lipoprotein; PAH, polyaromatic hydrocarbon; Bap, benzo(a)pyrene; TC, sodium taurocholate; OA, oleic acid 18:1; FID, flame ionization detector; ECD, electron capture detector.

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acids, the hydrophobics partition into the micelles. In this way the uptake of hydrophobic xenobiotics in the absorptive intestinal cells is facilitated by the enhancement of the concentration gradient and by overcoming the resistance of the unstirred water layer adjacent to the brush border membrane of the enterocytes. Similar to the fatty acids, the xenobiotics are absorbed as monomers and must partition from the micelles via the water phase into the brush border membrane of the enterocytes. This process is believed to be mediated by the dissociation of the micelles in the low pH microclimate near the brush border membrane (10). Inside the enterocyte the fatty acids are resynthesized into triglycerides, which aggregate as fat vacuoles that are covered with phospholipids and apolipoproteins. These particles enter the lymph circulation in the form of chylomicrons or very low density lipoproteins (VLDL) by exocytosis (11–13).

In our previous work we showed high PCB affinity of bile salt micelles and membrane lipid vesicles (14, 15). High solubilization capacities of aqueous solutions of mixed bile salt micelles for PAHs and PCBs were also observed by Laher and Barrowman (16). Rahman and Barrowman (17) showed that for the uptake of PAHs with very low aqueous solubilities, the unstirred water layer forms the major resistance in the intestine of the rat, and incorporation in bile salt micelles enhanced the bioavailability of these chemicals significantly. High affinities of membrane vesicles for various chlorinated hydrocarbons and the hydrophobic pesticide DDT were observed by Omann and Lackowicz (18) and by Antunes-Madeira, Almeida, and Madeira (19), respectively. The high lipoprotein solubilization capacity for hydrophobic contaminants is supported by the observation that lipoproteins form the major binding side in hepatic cytosol and transport vehicle in the plasma for hydrophobic xenobiotics (20–23).

In recent studies, monolayers of Caco-2 cell cultures, isolated from a human colon carcinoma, have been used as a model system to examine the processes of lipid assimilation and lipoprotein production. Although lipid absorption in Caco-2 cells functions much less effectively, fatty acid uptake, triglyceride synthesis, and lipoprotein production show many similarities to the lipid transport properties typical of the mammalian small intestine enterocytes (24–29). Despite their colonic origin, Caco-2 cells spontaneously differentiate into polarized, columnar cells that show many morphological and physiological characteristics of mature enterocytes of the small intestine. Differentiated post-confluent Caco-2 cells exhibit well-developed microvilli and, grown on semi-permeable supports, they form tight monolayers with a polarized distribution of brush border enzymes (26). Similar to the enterocytes in the rat intestine, Caco-2 cells perform a fatty acid dose-dependent accumulation of triglyceride-rich lipoproteins at the basolateral membrane. Triglycerides are excreted by the Caco-2 cells mainly in the form of VLDL (24, 25, 27, 28). In Caco-2 cells, unsaturated fatty acids such as oleic acid 18:1 and linoleic acid 18:2 are shown to be the most potent stimulators of triglyceride secretion, whereas saturated fatty acids are much less potent (24, 28). The capability of Caco-2 cell monolayers of *trans*-cellular transport of hydrophobic drugs like felodipine and testosterone (log K_{ow} 3.48 and 3.31, respectively) was confirmed by the study of Artursson and Karlsson (30). The transport data they determined compared very well with data obtained in vivo.

In the present study we used the Caco-2 cell line to study the uptake and transport of a selection of 14 representative PCBs with 2–10 chlorine atoms and log K_{ow} values in a range of 5.0–8.5. We examined the effect of the addition of oleic acid 18:1 on the uptake and transport rates of the PCBs. By means of varying the thickness of the unstirred water layer, we studied the facilitating role of bile salt micelles in overcoming the resistance of this diffusive barrier. In addition, we determined the polarity of the PCB transport directed from the apical side towards the basolateral side by comparison of the uptake and transport when the PCBs were presented to the apical side versus when the PCBs were presented to the basolateral side of the Caco-2 cell monolayers.

EXPERIMENTAL PROCEDURES

Cell culture

Dulbecco's modified Eagle's medium (DMEM), trypsin, glutamine, EDTA, phosphate-buffered saline (PBS), fetal calf serum (FCS), nonessential amino acids, and gentamicin were purchased from Flow Laboratories (Irvine, Scotland). Transwell permeable polycarbonate filter inserts (4.71 cm^2, 0.4 μm pore size) were purchased from Costar (Cambridge, MA). Caco-2 cells, originating from a human colorectal carcinoma, were obtained from ATCC (Rockville, MD). The cell line was maintained in DMEM containing 5% inactivated FCS, 1% nonessential amino acids, 50 μg/ml gentamicin, and 2 mM glutamine at 37°C in a humidified atmosphere containing 95% air and 5% CO_2. Trypsinization and freezing of the cells were performed according to the standard operating procedures of the ATCC company. Transfer medium contained 0.25% trypsin–0.02 M EDTA and cells were routinely frozen in culture medium containing 10% DMSO (Sigma Chemical Co., St. Louis, MO). Cells were seeded on the filter inserts at

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passages 37–38 and grown for 12 days after confluency was reached.

Before transport studies could be performed, the confluency of the cell monolayers was first established by measuring the transepithelial electric resistance with a Mitchell-ERS Epithelial Voltohmmeter (Millipore Co., Bedford, MA). Monolayers of cells showed a transepithelial resistance of ± 500 Ω · cm^2. At this resistance the cells are almost impermeable for hydrophilic macromolecules such as polyethylene glycol and arginine-vasopressin (30).

Preparation of PCB-containing media

Sodium taurocholate (TC) and oleic acid 18:1 (OA) were supplied by Sigma Chemical Co., and chromosorb G(AW) 60–80 mesh was from Chrompack (The Netherlands). The experiments were conducted with 14 PCBs of which 4,4′-, 2,4,6-, 2,2′,4,5′-, 2,2′,3,3′,4,4′-, 2,2′,4,4′,6,6′-, 2,2′,3,4,4′,5,5′-, 2,2′,3,3′,4,5,5′,6-, and decachlorobiphenyl were purchased from Promochem (Germany); 2,2′,3,3′-, 2,3,4,5-, 2,3,4,5,6-, and 2,2′,3,3′,6,6′-chlorobiphenyl were from Ultra Scientific (North Kingstown, RI), and 3,5,- and 2,4,5-chlorobiphenyl were from Dr. S. Ehrensdorfer (Germany). Literature data on the physical–chemical properties and IUPAC numbers of the selected PCBs are listed in Table 1 in the sequence of column elution (14, 31, 32). Recovery and internal analytical standards (1,2,3,5-tetrachlorobenzene and pentachlorobenzene, respectively) were from Ultra Scientific. The solvents acetone, pentane (Merck, Germany) and 2,2,4-trimethylpentane (Rathburn, Scotland) were pro analysis and HPLC grade, respectively, distilled and controlled for purity by means of FID and ECD chromatography, and proved to be more than 99.9% pure. Chlorobenzenes, TC, OA, and PCBs were used as received from their suppliers. The water used was distilled twice.

Experimental procedures to dissolve the PCBs in the media were similar to the procedures as described in extension previously (33), but in short the applied method was as follows. Appropriate amounts of PCBs were dissolved in pentane and, under continuous stirring and mild vacuum, the solvent was evaporated in a rotavapor to coat the chromosorb homogeneously with the PCBs. From the same batch of coated chromosorb, equal portions were added to serum-free DMEM containing 0.5% bovine serum albumin (BSA), 10 mM TC, and either with or without 0.5 mM OA. As model fatty acid OA was chosen because OA is a major product proceeding from dietary lipid hydrolysis and OA proved to be the strongest inducer of triglyceride synthesis and lipoprotein production in Caco-2 cells (28). The pH was adjusted to 7.0 and the media were stirred for 24 h at 25°C with the chromosorb to reach equilibrium.

Incubation of the cells with PCBs and analysis

All experiments were conducted under continuous shaking at 37°C in a humidified atmosphere containing 95% air and 5% CO_2. At 12 days post-confluence, Caco-2 cells were placed in Costar wells containing the prepared media. In the apical exposure experiments the apical side contained the PCBs dissolved in either TC micelles or mixed TC-OA micelles, and in the basolateral exposure experiments the basolateral compartment

TABLE 1. Applied IUPAC number, literature data on aqueous solubility in phosphate-buffered saline ($S_{(PBS)}$) and log K_{ow}, and experimental solubility data in media containing sodium taurocholate (TC) micelles and sodium taurocholate–oleic acid 18:1 (TC-OA) mixed micelles of the selected polychlorinated biphenyl congeners

Structure	IUPAC No.	$S_{(PBS)}$[a,b]	Log K_{ow}[a]	$S_{(TC)}$[c]	$S_{(TC\text{-}OA)}$[c]
		nmol/L		*nmol/ml*	*nmol/ml*
3,5-	14	285.2	5.37[d]	1.89 ± 0.12	5.19 ± 0.16
2,4,6-	30	1140	5.5[e]	11.26 ± 0.15	30.33 ± 0.93
4,4′-	15	320.9	5.3[e]	4.31 ± 0.13	12.16 ± 0.33
2,4,5-	29	642.4	5.6[e]	11.08 ± 0.24	32.12 ± 1.01
2,2′,4,5′-	49	28.03	6.1[e]	4.39 ± 0.10	14.70 ± 0.48
2,2′,3,3′-	40	85.24	5.6[e]	5.89 ± 0.10	18.11 ± 0.60
2,3,4,5-	61	48.00	5.9[e]	4.85 ± 0.09	14.21 ± 0.48
2,2′,4,4′,6,6′-	155	3.74	7[e]	1.18 ± 0.03	3.36 ± 0.09
2,3,4,5,6-	116	12.01	6.3[e]	4.02 ± 0.06	13.11 ± 0.43
2,2′,3,3′,6,6′-	136	4.85	6.7[e]	0.91 ± 0.02	3.29 ± 0.09
2,2′,3,3′,4,4′-	128	5.74	7[e]	1.01 ± 0.01	3.17 ± 0.09
2,2′,3,4,4′,5,5′-	180	2.34	7.21[d]	1.54 ± 0.01	5.79 ± 0.21
2,2′,3,3′,4,5,5′,6-	198	0.72	7.43[d]	0.13 ± 0.002	0.61 ± 0.02
2,2′,3,3′,4,4′,5,5′,6,6′-	209	0.02	8.26[e]	nd	0.05 ± 0.002

[a]Number of decimals as given in the references.
[b]From reference 14.
[c]Values ± standard deviations are for the means of triplicate samples; nd, not detected.
[d]From reference 32.
[e]From reference 31.

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contained PCBs dissolved in mixed TC-OA micelles. The cells were incubated in triplicate for 15 min, 30 min, 1 h, 6 h, and 24 h. To investigate the effect of the aqueous diffusion layer on PCB uptake and transport at 1 h, shaking of the wells was increased from 35 rpm to 135 rpm in order to decrease the thickness of the unstirred water layer. The comparison of apical to basolateral PCB uptake and transport over the Caco-2 cell monolayer was conducted at 24 h.

In a separate experiment using TC and mixed TC-OA micelles, the distribution of PCBs between the VLDL fraction d < 1.006 g/ml and d > 1.006 g/ml in the basolateral compartment after 24 h of exposure was determined. For the separation of the VLDL fraction from the higher density lipoproteins, 2-ml aliquots of basolateral medium were overlayered with 1 ml of NaCl solution of d 1.006 g/ml. In a swing rotor ultracentrifuge type TFT 41.14 (Kontron Instruments, Milan, Italy) the samples were centrifuged for 42 min at 100,000 *g* (34). After centrifuging, the tubes were sliced at a fixed position and the upper 0.5 ml supernatant and the bottom fraction were collected separately. Tubes were rinsed twice with ethanol 96%.

The toxicity of PCBs and TC was examined at concentrations as indicated for the transport studies. After the 24-h incubation experiment, the medium was removed and kept on ice for measurement of lactate dehydrogenase (LDH). Cells were rinsed twice with PBS and finally exposed to neutral red in culture medium for 3 h. Measurement of LDH activity and neutral red uptake was carried out as described previously (35). At the experimental concentrations no cytotoxicity for PCBs was evident. TC slightly affected cytotoxicity, but the effect was not significant in comparison to controls.

After the incubation time the cell monolayers were rinsed 5 times with ice-cold phosphate-buffered saline containing 1% BSA and the filters were suspended in 2 ml of ethanol 96% in order to kill and disrupt the cells. Apical and basolateral media were collected. From the cell samples the PCBs were extracted with 5 ml of pentane, 3 ml of 2,2,4-trimethylpentane, 1 ml of KOH 10 M, and 300 ng recovery standard was added. PCBs were extracted from all basolateral and apical media with 2 ml of pentane again after addition of 300 ng recovery standard. Wells and filter supports were rinsed with 2,2,4-trimethylpentane that was combined with the respective samples afterwards. Control media were extracted with 3 ml of 2,2,4-trimethylpentane and 300 ng recovery standard. In order to clear the samples from organic lipids and other interfering compounds, the samples were shaken with 2 ml of H_2SO_4 18 M. Under a flow of nitrogen the pentane was evaporated and the analytical standard was added. Spike recoveries usually were between 80% and 120% for the recovery standard and always higher than 95% for the analytical standard.

Sample analysis was performed on a 30-m DB-5 column with a diameter of 0.32 mm in an HP-5890 GC equipped with a ^{63}Ni electron capture detector and on-column injector. The carrier gas was helium (30 cm/s flow and 84 kPa pressure), and the makeup gas was argon/methane. All 14 PCBs were separated by the following T-program: 80°C–2 min–10°C/min → 140°C– 5 min–3°C/min → 240°C–30°C/min → 325°C–5 min; the temperature of the detector was 350°C. Quantification of PCB peaks was done by integration of the peak areas and multilevel calibration consisting of six levels. Calibration levels ranged from 1 to 1000 pg/injection. Samples (0.5 μL) were injected in triplicate; standard deviations of the triplicate analysis of one sample were smaller than 3%. Standard deviations of the mean due to analytical error between triple samples were within 5%, except for 4,4′-dichlorobiphenyl which exhibited standard deviations up to 10% due to its irregular elution behavior.

Calculation of uptake and transport

Accumulation of PCBs in the cells and the media is measured as the concentration PCB in nmol per either filter or ml. Maximum net uptake fluxes, J_u, were calculated by extrapolation of the measured net uptake fluxes to zero time, and are expressed as the amount of pmoles of PCB that was absorbed per second per cm^2 cell monolayer. Net transport fluxes are expressed as the amount of pmoles of PCBs that appeared in the basolateral compartment per second per cm^2 cell monolayer. Steady-state transport fluxes were measured after 24 h of incubation. Data are expressed as mean values for triplicate experiments (± standard deviations). Statistical analysis of the data was conducted with Excel version 4.0 from Microsoft Corporation. The mean differences between groups were calculated by one-tailed, paired two-sample Student's *t*-tests to obtain *P* values.

RESULTS

PCB solubility and uptake

PCBs coated on the chromosorb were solubilized in the prepared media in the presence of TC micelles or mixed TC-OA micelles. No traceable amounts of PCBs were found in the control chromosorb, medium, or Costar wells. For all PCBs, significantly ($P < 0.0005$) higher amounts were dissolved in the medium containing 0.5 mM OA than in the OA-free medium (Table 1). The amount of PCB 209 that was dissolved in the OA-free medium was lower than its detection limit of 2.25 pmol/ml. As an example, in **Fig. 1** the upper panel

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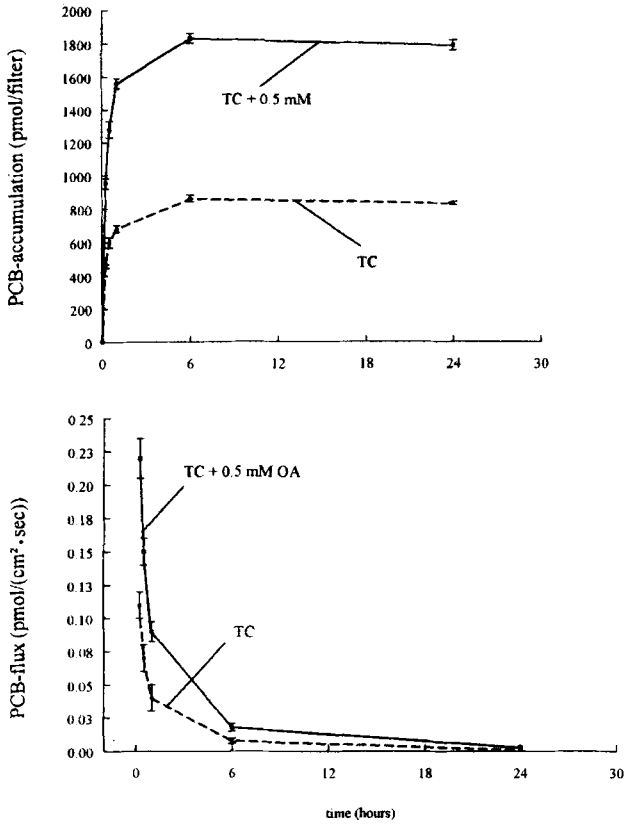

Fig. 1. Uptake of PCB 49 in Caco-2 cells; each point and error bar represents the value and standard deviation for the mean of a triplicate experiment. Upper panel: accumulation of PCB 49 in Caco-2 cells after 15 min, 30 min, 1 h, 6 h, and 24 h incubation with sodium taurocholate micelles (TC) or sodium taurocholate-oleic acid 18:1 mixed micelles (TC-OA). Lower panel: net uptake flux of PCB 49 over the unstirred water layer into Caco-2 cells. At the start of the experiments the fluxes were at the maximum and decreased to almost zero at 24 h, resulting in a constant PCB 49 concentration in the cells after 6 h.

shows that the uptake of PCB 49 is fast and at 1 h already 75% to 90% of the final amount was accumulated in the cells. At 6 h a maximum level was reached that stayed constant till the end of the experiment at 24 h. The final uptake in the presence of mixed TC-OA micelles was 2.25-fold greater than with pure TC micelles. For all PCBs the uptake shows a similar pattern and the uptake in the experiments with mixed TC-OA micelles was significantly ($P < 0.0005$ at 24 h) larger than in the experiments without OA (**Fig. 2**).

In the lower panel of Fig. 1 it can also be seen that the net uptake flux of PCB 49 from the apical medium into the Caco-2 cells was at its maximum at the beginning of the experiment, after which it decreased towards the end of the experiment when steady-state concentrations were reached. All PCBs showed their highest net uptake fluxes at 15 min, except for PCB 198 that exhibited the highest flux in the experiments without OA at 30 min, and for PCB 209 that reached its maximum uptake fluxes at 30 min and 6 h in the experiments with and without OA, respectively. Maximum net uptake fluxes are listed in **Table 2.** In the experiments where mixed TC-OA micelles were applied, the maximum net uptake fluxes were significantly ($P < 0.0005$) higher than in the experiments with pure TC micelles. Comparison of the Tables 1 and 2 shows that PCB uptake in the course of the experiments was relatively small (i.e. < 10%) compared to the total PCB amount in the apical compartment, which means that the experiments can be considered to be performed under sink conditions.

Effect of the unstirred water layer on the uptake of PCBs

To determine the effect of the diffusive resistance of the unstirred water layer on the uptake of the PCBs, the shaking of the Costar wells was increased almost 4-fold. The experiments were conducted in the presence of 0.5 mM OA. As is shown in **Fig. 3,** the net uptake flux at 1 h was enhanced significantly ($P < 0.001$) indeed in the experiments with increased shaking, up to 2.5-fold depending on the PCB congener.

Transport of PCBs from the apical side to the basolateral side of Caco-2 cells

Most PCBs presented in the apical medium of the Caco-2 cells did not appear in the basolateral compartment before 1 h. After that time period a time-linear increase of the PCB concentration in the basolateral medium was observed. As shown in **Fig. 4** for PCB 49, presentation in mixed TC-OA micelles enhanced the transport of PCB 49 to the basolateral side 2.7-fold at 24 h, as compared to the transport in the experiments with TC micelles only. Final PCB accumulations (in pmol/ml) in the basolateral compartment at 24 h are shown in the upper panel of **Fig. 5.** All PCB concentrations in the basolateral medium were above their solubilities as determined at 25°C in phosphate-buffered saline media (Table 1), except for PCB 14 and PCB 15, which exhibited remarkably lower concentrations.

Depending on the congener, after 24 h of incubation 10%–20% of the PCB present in the basolateral compartment was found in the lipoprotein fraction d < 1.006 g/ml, compared to 80%–90% in the d > 1.006 g/ml fraction. No significant difference in distribution could be found between the experiments using pure TC micelles and mixed TC-OA micelles. Also no relationship was observed between the PCB chlorination pattern and PCB distribution.

PCB net transport fluxes over the Caco-2 cells increased rapidly after the appearance in the basolateral compartment. After reaching a maximum they decreased again relatively rapidly to a steady-state level that

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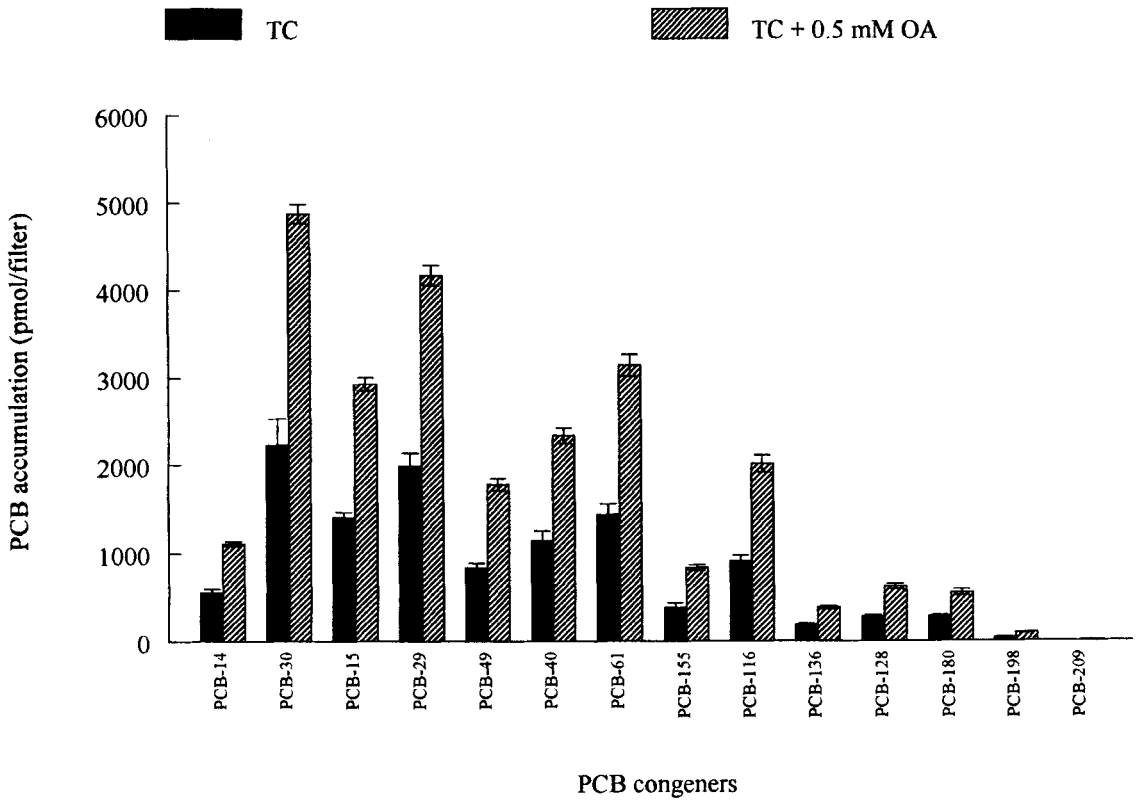

Fig. 2. Effect of the presence of 0.5 mM oleic acid 18:1 (OA) in the apical medium on the final PCB concentrations in the Caco-2 cells after 24 h of incubation. Each bar and error bar represents the value and standard deviation for the mean of a triplicate experiment. In the experiments where the PCBs were solubilized in mixed taurocholate-oleic acid micelles, the accumulation of PCBs in the Caco-2 cells was significantly ($P < 0.0005$) higher than in the experiments with pure taurocholate micelles.

was maintained until the end of the experiments at 24 h as is shown in the lower panel of Fig. 4 for PCB 49. Comparison of the lower panels of Figs. 1 and 4 shows that in the beginning of the experiments net uptake fluxes of PCB 49 were about 50- to 100-fold higher than the net transport fluxes. At the end of the experiments they were of the same magnitude and most of the PCB that was taken up by the cell was transported immediately by the steady-state flux into the basolateral medium. In the lower panel of Fig. 5 the increase is shown in the steady-state net transport fluxes in the presence of OA from the apical compartment to the basolateral side at 24 h. Dependent on the PCB congener, the fluxes had increased by a factor of 2 to 2.5 ($P < 0.0002$), except for PCB 14 and PCB 15 that showed a 9- and 10-fold increase, respectively.

Polarity of PCB uptake and transport in Caco-2 cells

The effect of the side of presentation on the PCB uptake and transport rates at 24 h is shown in **Fig. 6.** The PCBs were dissolved in mixed TC-OA micelles and presented either to the basolateral side or to the apical side. The amount of PCBs that accumulated in the cells at 24 h was similar whether the PCBs were added to the basolateral or the apical media. However, the amount of PCBs that was transported over the Caco-2 cell monolayers was significantly ($P < 0.01$) lower when the PCBs were presented at the basolateral side as compared to the luminal or apical side.

DISCUSSION

In this study monolayers of Caco-2 cells were used in order to examine the uptake and transport of PCBs in the enterocytes of the gastrointestinal tract. In the small intestine, fatty acids proceeding from the hydrolysis of dietary fats are dissolved in bile salt micelles in order to overcome the resistance of the unstirred aqueous layer adjacent to the enterocyte brush border membrane. In accordance with the work of Laher and Barrowman (16) with PAHs and PCBs, our results show considerably higher PCB solubilities in the mixed micellar medium containing 0.5 mM OA as compared to the medium with pure TC micelles. Higher amounts of PCBs in the micelles result in a higher PCB monomer activity in the

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TABLE 2. Experimental maximum net uptake fluxes (J_u) of polychlorinated biphenyls into Caco-2 cells from media containing sodium taurocholate (TC) micelles and sodium taurocholate-oleic acid 18:1 (TC-OA) mixed micelles, and apparent thickness (d_{aq}) of the unstirred waterlayer adjacent to the Caco-2 cell brushborder membrane

IUPAC No.	J_u (TC)[a]	J_u (TC-OA)[a]	d_{aq}[b]
	pmol/(cm² · sec)		μm
14	0.059 ± 0.007	0.145 ± 0.012	16.17
30	0.333 ± 0.040	0.699 ± 0.056	12.46
15	0.130 ± 0.016	0.286 ± 0.023	9.39
29	0.257 ± 0.031	0.533 ± 0.043	9.18
49	0.109 ± 0.013	0.225 ± 0.018	0.97
40	0.127 ± 0.015	0.276 ± 0.022	2.35
61	0.128 ± 0.015	0.301 ± 0.024	1.12
155	0.029 ± 0.003	0.068 ± 0.005	0.38
116	0.093 ± 0.011	0.220 ± 0.018	0.36
136	0.024 ± 0.003	0.049 ± 0.004	0.69
128	0.024 ± 0.003	0.054 ± 0.004	0.76
180	0.022 ± 0.003	0.025 ± 0.002	0.65
198	0.003 ± 0.0003	0.006 ± 0.0004	0.83
209	$2.41 \cdot 10^{-5} \pm 2.9 \cdot 10^{-6}$	$4.17 \cdot 10^{-5} \pm 3.3 \cdot 10^{-6}$	2.77

[a]Maximum uptake fluxes were measured at 15 min, except for PCB 209 which showed the highest values at 30 min and 6 h in the experiments with and without OA, respectively. Values ± standard deviations are for the means of triplicate experiments.

[b]Data calculated for the experiments containing mixed TC-OA micelles.

vicinity of the brush border membrane, where a decrease in the pH causes the micelles to disintegrate (10). The diffusive uptake of extremely hydrophobic chemicals such as PCBs into the absorptive cell is considered to be totally dependent on the resistances of aqueous barriers. Rahman and Barrowman (17) demonstrated that the uptake of hydrophobic PAHs in the rat intestine was mediated by mixed bile salt micelles. In the present study the increased amount of PCB monomers available from the disintegrated mixed TC-OA micelles resulted in a significant higher monomer activity at the brush border membrane and increased PCB uptake in the Caco-2 cells as compared to the uptake from the plain TC micelles.

The facilitating role of the micelles in carrying the PCBs over the unstirred water layer can also be demonstrated by estimation of the resistance of this aqueous diffusion barrier to the PCBs. This resistance is determined by the thickness and the surface area of the water layer overlying the brush border membrane of the Caco-2 cells. The apparent thickness of the unstirred water layer d_{aq} can be estimated from the product of the PCB aqueous diffusion coefficient D_{aq} and the aqueous concentration difference of the PCBs over the diffusive

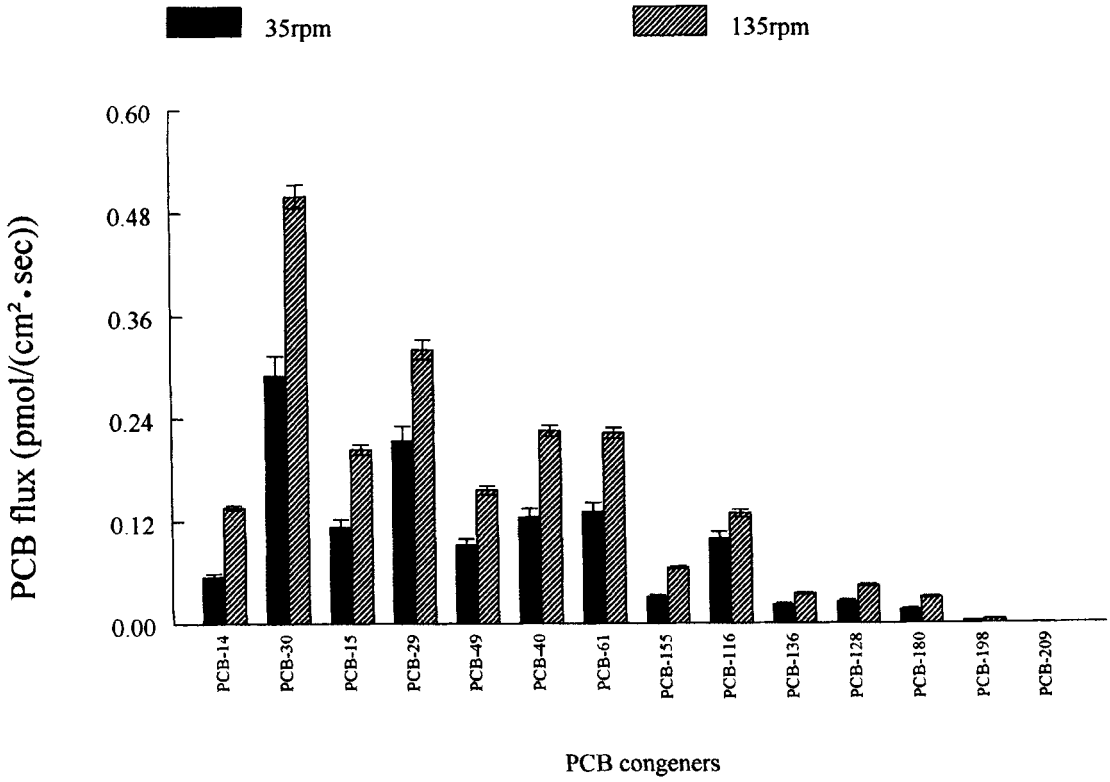

Fig. 3. Effect of shaking speed on the PCB net uptake flux over the unstirred water layer into the Caco-2 cell monolayer. Each bar and error bar represents the value and standard deviation for the mean of a triplicate experiment. In the experiments where the speed of shaking was increased from 35 rpm to 135 rpm, reducing the unstirred water layer adjacent to the Caco-2 cell brush border membrane by 30%–60%, net uptake fluxes of the PCBs increased significantly ($P < 0.001$).

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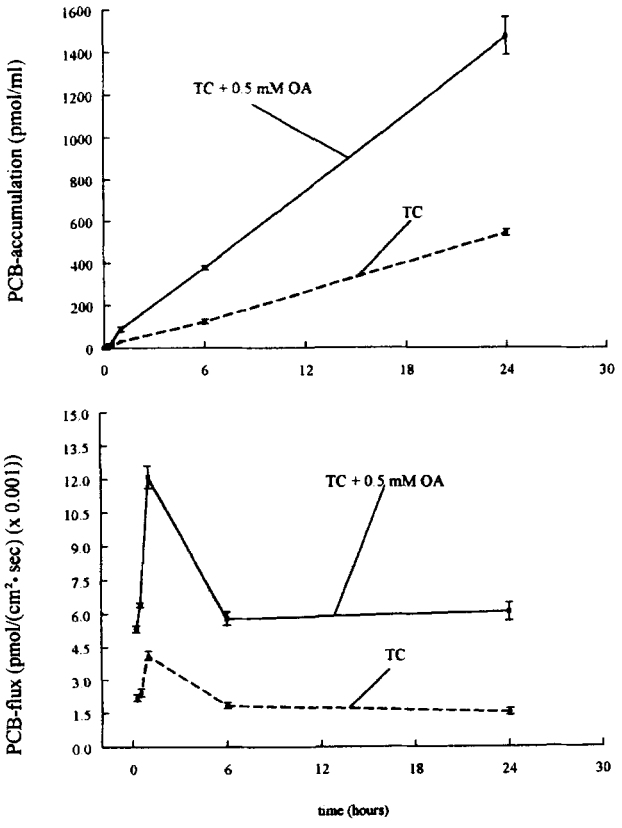

Fig. 4. Transport of PCB 49 over the Caco-2 cell monolayer into the basolateral compartment; each point and error bar represents the value and standard deviation for the mean of a triplicate experiment. Upper panel: accumulation of PCB 49 in the basolateral medium after 15 min, 30 min, 1 h, 6 h, and 24 h incubation with sodium taurocholate micelles (TC) or sodium taurocholate-oleic acid 18:1 mixed micelles (TC-OA). Lower panel: net transport flux of PCB 49 over the Caco-2 cell monolayer. After 6 h the net transport fluxes reached a steady-state level resulting in a linear increase of the PCB 49 concentrations in the basolateral compartment.

water layer divided by the PCB uptake flux J_u according to Stein and Lieb (36): $d_{aq} = D_{aq} (C_1 - C_2)/J_u$. The values of D_{aq} for the PCBs applied in this study are in the order of 7.25×10^{-6} cm^2/sec (37), and C_1 is the PCB's aqueous concentration in the bulk medium. As the media are saturated by the chromosorb, C_1 values were assumed to be equal to the aqueous solubilities as listed in Table 1, in spite of the fact that these solubilities were measured at 25°C, whereas the present experiments were conducted at 37°C. C_2 is the aqueous PCB concentration in the vicinity of the absorptive membrane that can be set at zero at the beginning of the experiments. Values for J_u at zero time are maximum values and can be derived from the extrapolation of the PCB uptake flux curves to zero time. For the experiments with mixed TC-OA micelles, the calculation of d_{aq} yields values for the apparent thickness of the unstirred water layer ranging from 0.35 μm for the larger more hydrophobic PCBs to 16 μm for the smaller PCBs with relative higher aqueous solubilities, and are listed in Table 2. These values are substantially less than the values reported for enterocytes in the rat intestine and the Caco-2 cell monolayer of 300 μm and 1000 μm, respectively, based on measurements with more hydrophilic probes (38, 39). Perhaps the order of magnitude as found in our study corresponds with the low pH micro climate area, where the bile salt micelles disintegrate and over which the PCBs diffuse as monomers. Our results suggest that hydrophobic PCBs in the gastrointestinal lumen are carried over the unstirred water layer predominantly in bile salt micelles, and that this effect is stronger for the more hydrophobic PCBs with lower aqueous solubilities, resulting in smaller values of the apparent aqueous resistance layer thickness. Though the micelles themselves exhibit much lower diffusion rates than the smaller monomers, these reduced diffusion rates are compensated for by the enhanced solubility capacity of the micelles. An inverse linear relationship exists between the apparent thickness of the diffusion barrier and the PCB uptake flux in the absorptive cells. The increase in the uptake flux by a factor of 1.5–2.5 in the experiments with a 4-fold increase in shaking the Caco-2 cell monolayers resulted in a reduction of the apparent diffusive barrier thickness of 30%–60%. This is in agreement with the results of Karlsson and Artursson (40) who estimated a reduction of the apparent barrier thickness from 500 μm to 300 μm with an increase in the shaking speed with a factor of 2.5.

Field, Albright, and Mathur (28) showed that secretion of triglyceride-rich lipoproteins in Caco-2 cells exhibited a maximum in response to an excess of fatty acid uptake as result of the saturable metabolic pathway in the enterocyte's cytosol. After the uptake of fatty acids, a time interval occurred before newly synthesized triglycerides were secreted over the basolateral membrane, caused by the time required to esterify the triglycerides, to assemble the lipoprotein particles, and to transfer the particles to the basolateral membrane for secretion (28). In the present study, a similar time interval can be seen in the PCB production in the basolateral compartment. It took most PCBs between 30 min and 1 h to appear in the basolateral medium. This time-delay effect was more pronounced in the experiments without OA, probably as a result of a lower lipoprotein production rate (29). From the moment of appearance the basolateral PCB concentration increased in a time-linear manner, corresponding with a steady-state net flux over the Caco-2 cell monolayer, keeping the PCB concentration in the cells constant as is shown for PCB 49 in the upper panel of Fig. 1.

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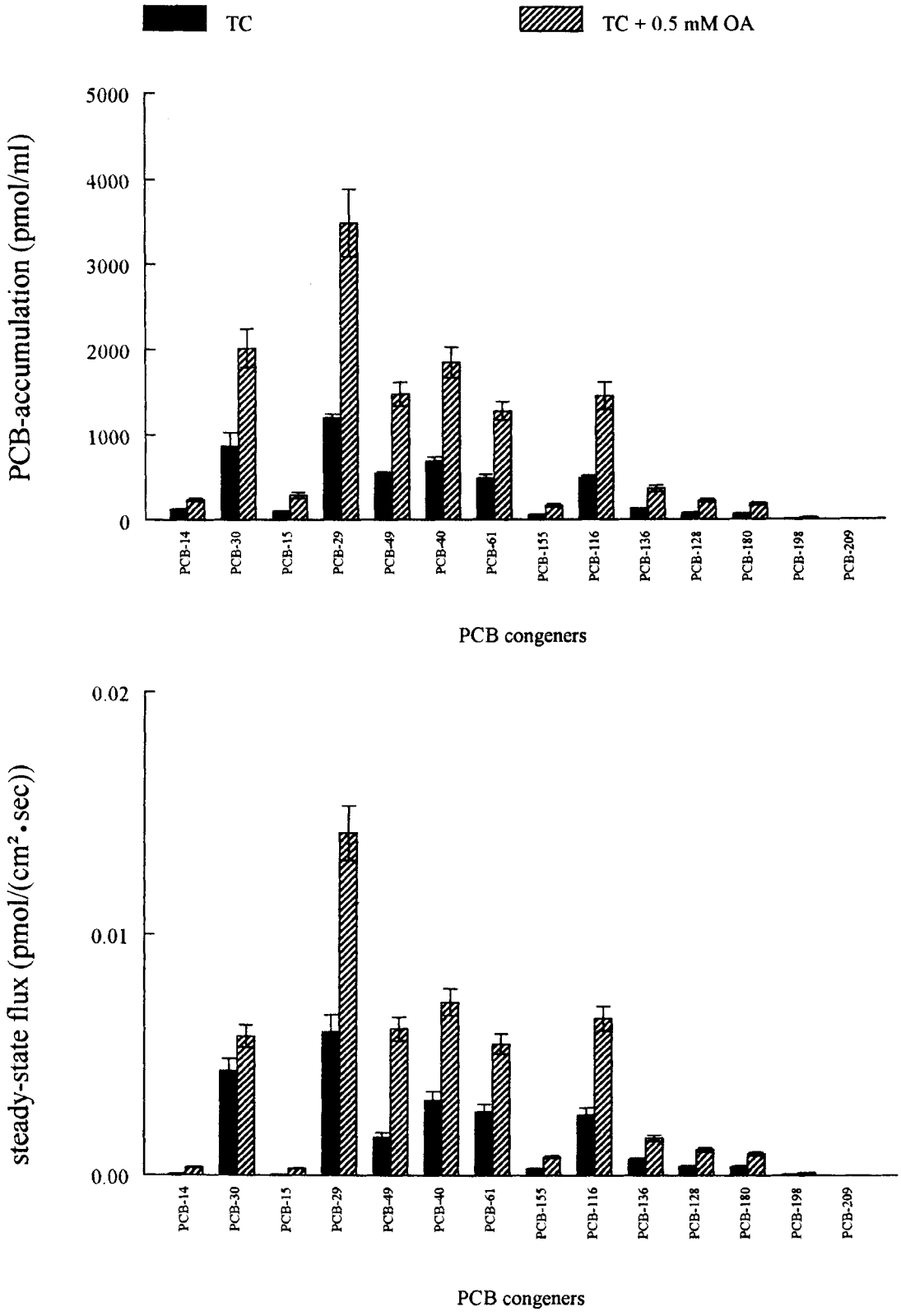

Fig. 5. Effect of the presence of 0.5 mM oleic acid 18:1 (OA) in the apical medium on the accumulation (upper panel) and the PCB steady-state fluxes over the Caco-2 cell monolayer (lower panel) into the basolateral compartment after 24 h of incubation. Each bar and error bar represents the value and standard deviation for the mean of a triplicate experiment. In the experiments where the PCBs were solubilized in mixed taurocholate-oleic acid micelles, the accumulation and steady-state fluxes of PCBs in the Caco-2 cells were significantly ($P < 0.0002$) higher than in the experiments with pure taurocholate micelles.

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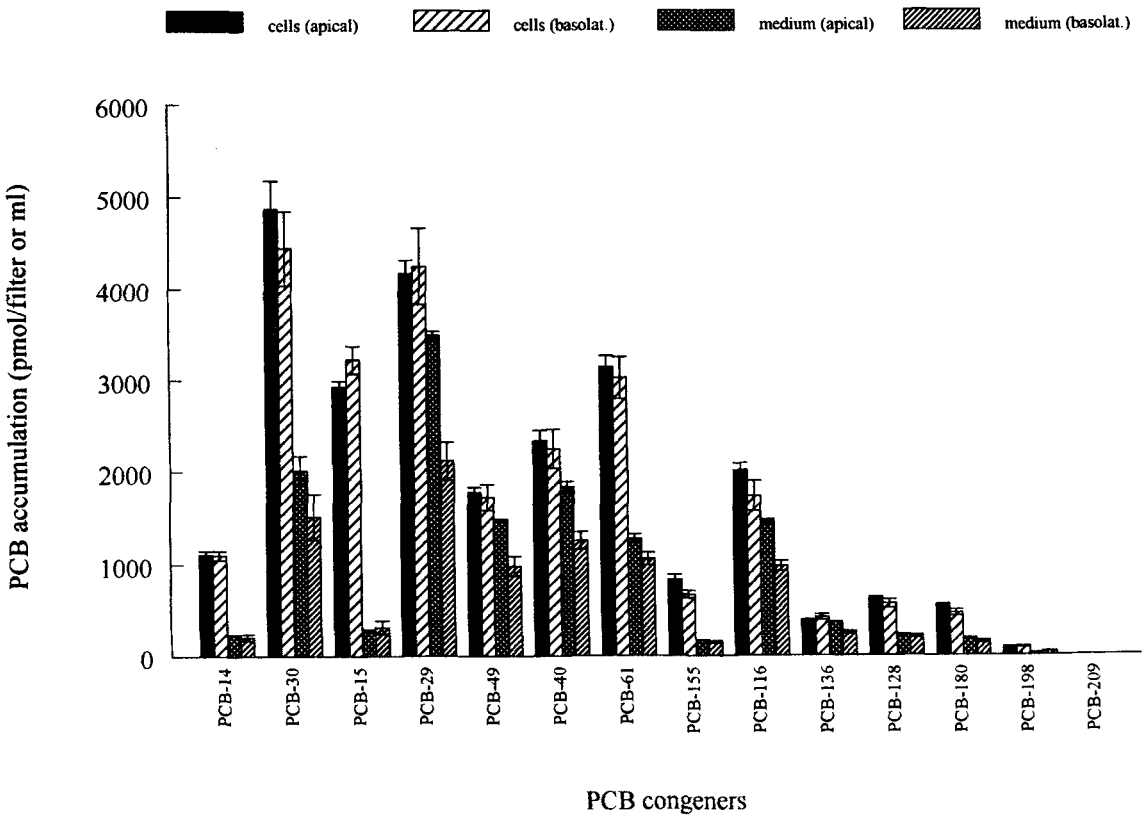

Fig. 6. Polarity of uptake and transport of PCBs in Caco-2 cell monolayers. The PCBs were presented in mixed taurocholate-oleic acid micelles. Each bar and error bar represents the value and standard deviation for the mean of a triplicate experiment. Concentrations of the PCBs in the cells at 24 h were similar whether the PCBs were presented apically or basolaterally. Concentrations of the PCBs in the opposite compartments were significantly ($P < 0.01$) larger when the PCBs were presented at the apical membrane as compared to when they were presented at the basolateral membrane.

Also, the observation that PCB concentrations in the basolateral medium exceeded their aqueous solubilities in phosphate-buffered saline media supports the model that hydrophobics are released in the lymph in combination with the triglyceride core of lipoproteins (8, 21). The PCB concentration enhancement in the presence of lipoproteins was more pronounced for the more highly chlorinated PCBs with lower aqueous solubilities than for the smaller, less chlorinated, PCBs. The two dichlorobiphenyls applied in this study showed basolateral concentrations that are even lower than their aqueous solubilities. Compared to the other PCBs their accumulation in the basolateral compartment was remarkably low, whereas their uptake in the cells was in agreement with the rest of the PCBs. An explanation for this phenomenon might be that similar to the enterocytes in the intestine (7, 41, 42), Caco-2 cells seem to possess the capability to metabolize the lower chlorinated biphenyl isomers into more polar metabolites that can be separated from the triglyceride vacuoles in the cytosol. In future experiments this impression should be studied further.

In the present study the results show that the amount of PCBs that is accumulated in the Caco-2 cells is similar whether they are presented at the apical side or at the basolateral side. However, the transport of PCBs over the cells was significantly polar, and directed from the luminal side towards the basolateral side. These results suggest that in addition to the facilitating role of the bile salt micelles in overcoming the resistance of the unstirred water layer, the polarity of the physiological lipid absorption processes affects the transport of PCBs as well.

The distribution of PCBs in the VLDL fraction d < 1.006 g/ml in the basolateral compartment after 24 h was much lower than expected. In the course of the experiments it did not become clear whether the lack of PCBs in the VLDLs compared to the higher density fraction was the result of reduced production of VLDLs in our Caco-2 cells or was caused by the low association of PCBs with VLDL particles compared to the HDL fraction as was observed in the plasma of pigeons (43). Apart from the problem of PCB redistribution in the basolateral compartment, the small amounts of PCBs secreted and the accompanying limits of detection make it technically difficult to determine in what lipoprotein

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fraction the PCBs were excreted exactly. However, to understand the details of xenobiotic transport in enterocytes, we will try to elucidate this problem further in future experiments with use of ^{14}C-labeled PCB.

In Caco-2 cell monolayers the unidirectional transport of fat is less efficient than in rat enterocytes and considerable amounts of synthesized triglycerides proceeding from fatty acids presented at the apical side are found apically instead of basolaterally (25, 29). Hence, in vivo the polarity of PCB transport is expected to be even more significant. The unidirectional transport of lipids over the gastrointestinal wall will result in a reduction of the resistance of the PCB transport from the gut lumen to the portal system and the lymph system, whereas the resistance of the transport vice versa remains high. Bioaccumulation of hydrophobic xenobiotics is considered to be a partitioning process, eventually resulting in an internal body concentration that is in equilibrium with the environment. Hence, reduction of the uptake resistance will not alter the final equilibrium, but the time required to reach this equilibrium will be reduced. However, for chemicals that undergo elimination by the organism, the final body burden might increase due to the increase of the uptake rate.

Still, little is known about the role of lipid assimilation on the uptake and transport of hydrophobic contaminants in the intestinal enterocytes. The results in the present study on the effect of bile salts, fatty acids, and the aqueous diffusion barrier on the uptake and transport of PCBs in Caco-2 cells and on the polarity of this transport are in agreement with the results from studies in fish and mammals (7–9, 17). Moreover, recent studies showed many similarities between lipid uptake and transport processes in Caco-2 cells and lipid assimilation in vivo (24–29). Hence, we believe that monolayers of Caco-2 cells might offer an appropriate model system for further studies on the uptake and transport processes of hydrophobic xenobiotics in the intestinal enterocytes. Though their lipid assimilation is less efficient than that of enterocytes in vivo, Caco-2 cells are easy to handle and provide reliable results, which is important especially in studies with extremely hydrophobic xenobiotics at very low concentrations.

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