Availability of polychlorinated biphenyls (PCBs) and lindane for uptake by intestinal Caco-2 cells

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Abstract

Children may ingest contaminated soil via hand-to-mouth behavior. To assess this exposure route, the oral bioavailability of the contaminants should be known. Two determining steps in bioavailability of soil-borne contaminants are: 1) mobilization from soil during digestion, which is followed by 2) intestinal absorption. The first step has been investigated in previous studies that showed that a substantial fraction of PCBs and lindane is mobilized from soil during artificial digestion. Furthermore, almost all contaminants are sorbed to constituents of artificial human small intestinal fluid (i.e. chyme), while only a small fraction is freely dissolved. In the present study, the second step is examined using intestinal epithelial Caco-2 cells. The composition of the apical exposure medium was varied by addition of artificial chyme, bile or oleic acid at similar or increasing total contaminant concentrations. The uptake curves were described by rate constants. It appeared that the uptake flux was dose-dependent. Furthermore, different exposure media with similar total contaminant concentrations resulted in various uptake rates. This can be attributed to different freely dissolved concentrations and carrier effects. In addition, the large fractions of contaminants in the cells indicate that PCBs and lindane sorbed to bile, oleic acid and digestive proteins contributed to the uptake flux towards the cells. These results can be qualitatively extrapolated to the in vivo situation. Since the sorbed contaminants should be considered available for absorption, the step of mobilization from soil, is the most important step for oral bioavailability of the presently investigated contaminants.
INTRODUCTION

For children, ingestion of contaminated soil via hand-to-mouth behavior can be a main route of exposure to contaminants such as polychlorinated biphenyls (PCBs) and lindane. To accurately assess reference values for soil-borne contaminants, the oral bioavailability has to be taken into account. Several steps can be distinguished for bioavailability of soil-borne contaminants: 1) soil ingestion, which is on average 50-200 mg/day (66,67,69), 2) mobilization from soil and distribution among different physicochemical contaminant forms in digestive fluid, 3) intestinal absorption, and 4) liver metabolism. In this study, soil ingestion is considered a given fact, while liver metabolism is not relevant or has been investigated extensively for the presently used contaminants. In a previous study, the second step has been investigated using a physiologically based in vitro digestion model (127). The distribution of several PCB congeners and lindane among constituents of artificial human intestinal fluid, i.e. chyme, and digested soil has been studied (127). It appeared that for fasting conditions, approximately 25% of the PCBs were sorbed to bile salt micelles, 15% to digestive proteins and 60% were still sorbed to the soil. The respective values for lindane were 23%, 32% and 40%. The percentage of contaminants that was freely dissolved was <1% for the PCBs and approximately 5% for lindane (111,127). More hydrophobic organic contaminants (HOCs) were mobilized from soil when more bile or protein was added during the artificial digestion (127). Other studies showed that HOC mobilization from soil is dependent on the soil type (103,104), and that addition of dry whole milk increases the PCB mobilization from soil (103). Therefore, it can be concluded that sorbing phases may increase the contaminant mobilization from soil during the digestion.

In studies utilizing in vitro digestion models, the amount of contaminant that is mobilized from soil is considered to represent the maximum amount that is available for intestinal absorption. In vivo studies in rat indeed showed that intestinal absorption of PCBs administered via spiked soil is lower than PCBs ingested via corn oil (9). Yet, it is unclear to what extent mobilized HOCs are absorbed, and what the effect of constituents such as bile, proteins and fatty acids in chyme is on intestinal absorption and bioavailability of HOCs. For example, the constituents may cause carrier effects, and may decrease the freely dissolved HOC concentration, which is the fraction that is at least available for absorption.

In the present study, the third step of oral bioavailability, i.e. transport of the mobilized contaminants across the intestinal wall, is investigated using in vitro differentiated intestinal cells. To that end, the effect of chyme (containing bile and digestive proteins), bile, and oleic acid as a fatty acid on the uptake of several PCB congeners and lindane into intestinal cells is investigated. Research is restricted to intestinal absorption since it is the predominant uptake...
pathway (23,58). The Caco-2 cell line was used as a model system to simulate human intestinal absorption. Caco-2 cells originate from an epithelial colon cancer and after growing to confluency on a filter, they start to differentiate into polarized, columnar cells that show many morphological and physiological characteristics of mature enterocytes of the small intestine. These cells are extensively used in drug absorption research (63,139-141). Experimentally, the composition of the exposure medium was varied at similar or increasing total HOC concentrations. The HOC uptake by the cells and transport over the cells was measured in time. These time curves were described by rate constants, allowing quantitative discrimination.

Aim
The aim of the present study is to investigate 1) to what extent PCBs and lindane are absorbed by in vitro intestinal Caco-2 cells, 2) the effect of sorbing constituents on absorption of the HOCs, which includes the issue whether HOCs that are mobilized from soil during digestion contribute to the uptake into the intestinal cells, and 3) which factors have the largest impact on oral bioavailability of the soil-borne HOCs.

MATERIALS AND METHODS

Chemicals
PCB congeners 2,2',5,5'-tetrachlorobiphenyl (IUPAC PCB #52), 2,3',4,4',5-pentachlorobiphenyl (IUPAC PCB #118), 2,2',4,4',5,5'-hexachlorobiphenyl (IUPAC PCB #153), 2,2',3,4,4',5,5'-heptachlorobiphenyl (IUPAC PCB #180) and lindane (γ-hexachlorocyclohexane, γ-HCH) were used as test compounds. The logarithms of their octanol-water partition coefficients, log $K_{ow}$, are 6.1, 6.2-6.5, 6.9, 7.2 and 3.8, respectively (26,38). The internal standards for the PCBs were 2,3,3',5,6-pentachlorobiphenyl (IUPAC PCB #112) and 2,2',4,4',6,6'-hexachlorobiphenyl (IUPAC PCB #155), and for lindane α-hexachlorocyclohexane (α-HCH). All chemicals were of analytical grade.

OECD-medium was employed as artificial standard soil. Dry OECD-medium consisting of 10% peat, 20% kaolin clay, and 70% sand was prepared according to OECD-guideline 207 (98). The appropriate amounts of PCBs dissolved in hexane were added to dry, uncontaminated OECD-medium. The hexane was evaporated under continuous shaking. To prevent losses of lindane during spiking, lindane was added to the OECD-medium as an aqueous solution that was prepared using the generator column technique (106,130). The OECD-medium was spiked with a mixture of 7 mg PCB #52, 7 mg PCB #118, 14 mg PCB #153, 7 mg PCB #180 and 2 mg lindane per kg dry OECD-medium, which is referred to as the reference contamination level, or with a three or five fold higher level. The concentration of lindane of 2 mg/kg
represents the current Dutch ecotoxicological intervention value (37). PCB #153 is environmentally abundant. Therefore, its level was chosen higher than that of the other PCBs. The spiking levels of the PCBs are of environmental relevance (107), although relatively high for the PCBs in the perspective of the current Dutch intervention value of 1 mg PCB/kg dry soil (37).

![Chemical digestion process](chart)

**Figure 1.** Schematic representation of the procedure of an artificial digestion.

**Artificial digestion**

The physiologically based *in vitro* digestion model designed by Rotard *et al.* (104) was employed in this study in a modified version as described by Sips *et al.* (110). The digestion process was based on physiological constituents and transit times for fasting conditions of children. The digestion model is schematically presented in Figure 1. In short, synthetic saliva, gastric juice, duodenal juice and bile were prepared. The saliva was added to 0.9 g OECD-medium and rotated at 60 rpm for 5 min at 37 °C. Subsequently, gastric juice was added, and the mixture was rotated at 60 rpm for 2 h. In the last digestion step duodenal juice and bile were added, and this mixture was rotated at 60 rpm for 2 more hours. Finally, the mixture was centrifuged for 5 min at 3000g, yielding a pellet (i.e. digested OECD-medium) and about 58.5 ml of supernatant (i.e. artificial chyme). Important constituents of the digestion were freeze-dried chicken bile, bovine serum albumin (BSA), mucine, pancreatin, pepsin and urea. After the artificial digestion, 0.9 g/l bile, 15.4 g/l OECD-medium and 3.7 g/l protein were present in the system. The ionic strength of the chyme was 0.14 M and the pH was 5.5 (±0.2). Freshly prepared chyme was used in all exposure experiments.
Cell Culture

Cells from passage 30-45 were grown on Millipore culture plate inserts of mixed cellulose esters (4.2 cm², 0.45 µm pore size) for 3 to 4 weeks. During this time the cells were maintained at 37 °C in a humidified atmosphere containing 95% air and 5% CO₂, in culture medium. Culture medium consist of Dulbecco’s Modified Eagle’s Medium (DMEM), containing 25 mM Hepes and 4.5 g/l glucose, which was amended with 10% inactivated fetal calf serum (FCS), 1% non essential amino acids (NEAA), 2 mM glutamine, and 50 mg/l gentamicine.

![Figure 2. Schematic side-view of a well with a monolayer of Caco-2 cells.](image)

Experiments

The exposure system is schematically presented in Figure 2. The system has an apical and a basolateral side, which represent the intestinal lumen and the blood and lymph drain, respectively. The test compounds were always presented at the apical side, and in all experiments an uncontaminated mixture of DMEM and chyme (1:1, v:v) was added to the basolateral compartment. This mixture of DMEM and chyme will be referred to as DMEM/chyme. Similar conditions as during cell culture were employed, except that the well plates were stirred at approximately 60 rpm. Furthermore, unless mentioned otherwise, DMEM amended with 1% NEAA, 2 mM glutamine and 150 mg/l gentamicine was used for exposure experiments. For all experiments, care was taken not to exceed the solubility of the HOCs based on solubility data of a study of Dulfer et al. on DMEM with oleic acid (79) and of Oomen et al. on chyme (127). Losses of test compounds via the air and cross-contamination between and within the wells were prevented by closing the well and insert with a Teflon lid.
Table 1. Overview of the experimental variations.

| Apical exposure medium | Spike apical medium (µM) |
|------------------------|-------------------------|
| DMEM/chyme             | 0.2-0.3 µM by acetone spike |
| DMEM + 0.5 mM oleic acid | 0.2-0.4 µM by acetone spike |
| DMEM + 0.5 mM oleic acid + 0.9 g/l bile | 0.2-0.4 µM by acetone spike |
| DMEM/chyme             | 1×, 3× and 5× reference level \(a\), by artificial digestion |
| DMEM/chyme             | 5× reference level \(a\) by artificial digestion + 50 µM total PCB by acetone spike |

\(a\) DMEM/chyme was prepared with chyme that was obtained from an artificial digestion with spiked OECD-medium. The reference HOC level in OECD-medium was: 7 mg PCB #52, 7 mg PCB #118, 14 mg PCB #153, 7 mg PCB #180 and 2 mg lindane per kg dry OECD-medium.

Several exposure experiments were performed with Caco-2 cells, see Table 1. First, the composition of the apical medium was varied. To the apical compartment 2 ml of 1) DMEM/chyme, 2) DMEM that contained 0.5 mM oleic acid, or 3) DMEM that contained 0.5 mM oleic acid and 0.9 g/l freeze dried chicken bile was added. Oleic acid was chosen as model fatty acid since it is a major product from dietary lipid hydrolysis. An acetone solution with the test compounds was used to spike the different media. In the second series of experiments, different apical concentrations of PCBs and lindane in DMEM/chyme were utilized. Therefore, chyme was artificially prepared with OECD-medium that was spiked with one, three or five times the reference contamination level. In that manner, three chyme solutions with increasing concentrations of mobilized HOCs were obtained. In addition, PCB congeners other than our test compounds were added to the exposure medium with chyme from an artificial digestion with OECD-medium that was spiked with five times the mentioned HOC mixture (total PCB concentration ±1.5 µM). In this manner, a DMEM/chyme solution with a total PCB concentration of 50 µM was obtained.

**Sampling procedure**

The cells were incubated with PCBs and lindane for various periods of time up to 24 h. A 250 µl aliquot of the apical medium was taken to determine lactate dehydrogenase (LDH) leakage of the cells. Subsequently, several samples of each well were taken for HOC determination. The complete basolateral medium and the rest of the apical medium were sampled and transferred to separate glass tubes that contained 2 ml hexane and internal standards. The apical and basolateral compartments were rinsed twice with 2 ml phosphate buffered saline (PBS) and the basolateral compartment also with 2 ml hexane. The washes were added to the corresponding samples. The apical volume can decrease and the basolateral volume can increase, due to active transport across the cells. Therefore, a correction was performed for the amount of test compound in the LDH sample based on volume determination.
via weighing of the samples for HOC determination. The cells were disrupted by 2 ml ethanol and resuspended. This solution was subsequently transferred into a glass sample tube. The remaining filter was rinsed once with 2 ml PBS and once with 2 ml hexane. The washes were added to the cell sample. Thus, all compartments (apical, basolateral and cell) were sampled from each well. In addition, three wells were used for each combination of exposure time and medium.

**Sample treatment**

2 ml 18 M H$_2$SO$_4$ were added to each sample in order to degrade organic interferences. After extensive stirring, about 10 ml water were added, and each sample was restirred. Subsequently, the water phase was frozen by storage at –25 °C, and the liquid hexane phase was collected. Then, about 2 ml new hexane was added to the aqueous sample. The sample was melted, stirred again and stored at –25 °C. This procedure was performed thrice. The combined hexane extracts were evaporated under a gentle nitrogen stream to approximately 100 to 500 µl, prior to analysis by GC-ECD.

**Data handling**

The time course of the HOC amounts in the different compartments was fitted to a first-order two compartment model (the apical and cell compartment) with the Scientist program of ChemSW™ (Fairfield, Ca). Hence, the curves could be compared quantitatively. An exponential loss term from the apical medium was included in order to account for losses of test compounds during the experiment.

\[
\frac{dC_{med}}{dt} = k_{cm} \times C_{cell} - k_{mc} \times C_{med} - k_{ml} \times C_{med}
\]  
(4.1)

\[
\frac{dC_{cell}}{dt} = k_{mc} \times C_{med} - k_{cm} \times C_{cell}
\]  
(4.2)

Eq 4.1 represents the change in concentration of a HOC in the exposure medium, C$_{med}$, in time, t. Eq 4.2 represents the change in concentration of a HOC in the cell compartment, C$_{cell}$, in time, t. Several rate constants are involved: a constant that represents transport from the apical medium to the cell compartment ($k_{mc}$), from the cell to the apical medium compartment ($k_{cm}$), and losses from the apical medium ($k_{ml}$). A cell volume of 10.6 µl per well was estimated, based on a cell height of 25 µm (140) and the filter surface. The standard deviations of the rate constants were estimated by the fit program. Two rate constants are considered to be significantly different ($\alpha \leq 0.05$) if the average values plus or minus two times the standard deviation of both constants do not overlap. The maximum uptake flux, J$_{u,max}$, can be calculated by extrapolation of the uptake flux to zero time, based on eq 4.2. A$_{filter}$ represents the surface
area of the filter, and $C_{\text{med},t=0}$ the contaminant concentration in the apical compartment at zero time:

$$J_{u, \text{max}} = \frac{(k_{mc} \times C_{\text{med},t=0})}{A_{\text{filter}}}$$  \hspace{1cm} (4.3)

**Quality control**

The experimental validity was investigated in several manners. The mass balance of the test compounds was calculated by summing up the amounts of each compound measured in the apical, basolateral and cell compartment. These summed amounts were compared to amounts of the corresponding test compounds in the exposure media that were added to cells at the beginning of the experiment.

Furthermore, blanks were always included. Samples of uncontaminated media, chyme, PBS and hexane, and of cells exposed to uncontaminated DMEM/chyme were taken. Also the HOC content of some filters was determined, which had been employed during exposure experiments and had been sampled.

In addition, several system control experiments were performed. First, transfer of the test compounds in time over a filter without Caco-2 cells was determined. Second, sorption of the HOCs to the insert wall was investigated.

The integrity of the cell monolayer of each well was checked by determination of the transepithelial electric resistance (TEER) at room temperature with a Mitchell-ERS Epithelial Voltohmmeter (Millipore Co., Bedford, MA). Cell viability and toxicity after exposure to DMEM/chyme was assessed by means of neutral red uptake by the cells, and by means of determination of the LDH leakage (BM/Hitachi 911, using pyruvate as substrate) from the cells into the apical medium, which is a measure of cell disruption. The LDH leakage was sampled in all wells after exposure and therefore also assessed toxicity due to the other exposure media and contaminants.

Finally, the effect of DMEM/chyme on transport of the reference compounds $^3$H-mannitol, fluorescein isothiocyanate dextran FD4 (Mw 4000), and fluorescein across Caco-2 monolayers was studied.
RESULTS AND DISCUSSION

Artificial Digestion

During the artificial digestion 54% of lindane and between 30 and 47% of the PCBs were mobilized from spiked OECD-medium. This percentage is similar to results of previous experiments (127).

Quality Control

Recovery. 95% (± 18%) were recovered by summation of the amounts of contaminants in the apical, basolateral and cell compartments after 0.5 h of exposure, compared to the amounts of contaminants that were measured in the exposure media. In some cases a gradual decrease in the recovered HOC amount in the system was observed during the 24 h of exposure. However, this decrease was in general only a minor fraction: in most cases more than 70% of each HOC was present after 24 h of exposure compared to 0.5 h of exposure. This indicates that no major losses of test compounds occurred during exposure. We consider the mass balance to be satisfying, especially in light of the extreme hydrophobicity of the contaminants, which results in a high tendency of the HOCs to evaporate and to sorb to surfaces.

Blanks. All blank samples, the filters, and the uncontaminated solutions and compartments of unspiked wells, did not show traces of test compounds. Therefore, the measured test compounds in the different compartments originated from exposure media only, and the sampling procedure was appropriate to remove all HOCs from the filter. In addition, the absence of test compounds in the blank well compartments after 24 h of exposure indicates that no cross-contamination between wells took place.

Filter. Transport of the HOCs over the insert filter without a cell layer appeared to be low: <2% of PCB #118, PCB #153 and PCB #180, and <10% of lindane and PCB #52 were measured in the basolateral compartment after 24 h of exposure. This is in line with a similarly low basolateral HOC concentration after 24 h of exposure with a Caco-2 cell layer (see Figure 3 and 4). Therefore, the filter itself is a major barrier for the HOCs.

The mechanism of HOC transport across the cells is not fully known. Extremely hydrophobic compounds such as PCBs are assumed to go along with the cellular pathway for lipid assimilation. Lipids are transported through the cells via very low density lipoproteins (VLDL) (78,79), and subsequently enter the lymph flow (23,78,80). Although the presently used cell line expresses some lipid transport, the extent is probably not fully comparable to the in vivo situation (142). These considerations suggest that the present experimental set-up allows us to study the first step for intestinal absorption, transport of HOCs from the apical medium into the cells.
Sorption to insert walls. Approximately 10-15% and 3% of the total amount in the apical compartment of respectively the PCBs and lindane were sorbed to insert walls. These are minor fractions that will not largely influence the time curves. Therefore, sorption to the insert wall is not considered further, although it might be partly represented by the undefined loss term in eq 4.1.

Cell viability and integrity. The TEER was approximately 500 Ω/cm². This shows that the enterocyte cells were mature and that no holes were present in the cell monolayers (140).

Neutral red uptake by cells after pre-exposure for 24 h to DMEM/chyme was the same as the uptake by cells after pre-exposure for 24 h to culture medium. This indicates that chyme did not decrease the active uptake of neutral red, which is a measure for cell viability. The LDH values increased with exposure time. However, this was also the case for wells that were exposed to culture medium only, indicating that the cell viability was not compromised by the different media or HOCs. In general, less than 5% of the total amount of cells were disrupted after 24 h of exposure, which we consider to be acceptable.

Reference compounds. The reference compounds showed a clear increase in the amount that was transported across the Caco-2 cells to the basolateral compartment when the cells were exposed to DMEM/chyme compared to culture medium only. The increase in the apparent permeability of mannitol and FD4 approximated a factor 5 to 7 after 2 h of exposure to both media. The apparent permeability of mannitol was increased by a factor 15 after 24 h of pre-exposure to DMEM/chyme and subsequent exposure to mannitol for 1 h, compared to exposure to culture medium only. In a similar experiment the apparent permeability for fluoresceïne was increased by a factor 40. The reference compounds are known to be transported paracellularly, i.e. through the tight junctions and intracellular spaces. The increase in the transport of reference compounds indicates that chyme most probably affected the cell-cell junctions, without clear signs of cellular toxicity. Also in vivo it is known that bile salts alter the intrinsic permeability of the intestinal membrane, leading to increased permeation via paracellular or transcellular routes (23). Therefore, we regard this state of the monolayer as realistic and workable.

In the two series of experiments 1) the composition and 2) the contaminant concentration of the apical medium were varied. Consequently, both the freely dissolved and the total HOC concentration in the apical compartment were manipulated.
Composition exposure medium

For the first series of experiments, the distribution of PCB #153 among the apical, basolateral and cell compartments as a function of exposure time is presented in Figure 3. Other test compounds showed similar patterns. In the remainder of this paper PCB #153 will be presented graphically as representative of all test compounds. As can be seen in Figure 3, at all time points hardly any contaminants were present in the basolateral compartment. Hence, the first-order two compartment model can be applied to fit the uptake of HOCs from the apical medium into the Caco-2 cells. Furthermore, the amount of contaminant in the cells increased rapidly to reach a steady state within a few hours. Steady state can be assumed when the lines for the apical, cell and total amounts run parallel. Meanwhile, the amount of contaminant in the apical compartment decreased within the same time frame. The amount of contaminants in the cell compartment at steady state is the largest contaminant fraction present in the system. This is illustrated by the ratio $k_{mc}/k_{cm}$, which represents the HOC concentration ratio over the cell and apical compartment at steady state. This ratio is approximately $10^3$, see Table 2 and 3. Therefore, at steady state, the concentration of a HOC is about a factor $10^3$ higher in the Caco-2 cells than in the apical medium. Previous studies showed that the freely dissolved concentration of the PCBs and lindane in chyme is small, respectively <1% and ± 5% (111,127). Thus, the freely dissolved HOC fraction is much smaller than the total fraction that accumulated into the cells, which was always more than 50%. This indicates that more than the freely dissolved compounds contributed to the uptake into the cells.
Figure 3. Time curves for PCB #153 for different composition of the apical medium. a) A mixture of DMEM and chyme (1:1, v:v), i.e. DMEM/chyme, b) DMEM with 0.5 mM oleic acid, and c) DMEM with 0.5 mM oleic acid + 0.9 g/l bile. The symbols represent the measured amounts of PCB #153 in the apical (open diamonds), cell (open squares), and basolateral (open circles) compartments, and the sum of these amounts (solid triangles). The lines represent the fitted curves.
Table 2. Rate constants ± standard deviation (±SD) of HOC transport into Caco-2 cells for different apical exposure media. Rate constants are considered to be significantly different if the average value (± 2×SD) of both constants do not overlap, which represents α≤0.05.

| Compound | Apical medium                  | \((k_{mc} ± SD) \times 10^4\) (h\(^{-1}\)) \(^a\) | \((k_{cm} ± SD) \times 10^7\) (h\(^{-1}\)) \(^b\) | \((k_{ml} ± SD) \times 10^5\) (h\(^{-1}\)) \(^c\) |
|----------|--------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| Lindane  | DMEM/chyme                     | 15 ± 1                                        | 10 ± 3                                        |                                                |
|          | DMEM + oleic acid              | 12 ± 1                                        | 7 ± 3                                         |                                                |
|          | DMEM + oleic acid + bile       | 12 ± 1                                        | 9 ± 3                                         |                                                |
| PCB #52  | DMEM/chyme                     | 9 ± 1                                         | 10 ± 4                                        |                                                |
|          | DMEM + oleic acid              | 38 ± 4                                        | 56 ± 10                                       |                                                |
|          | DMEM + oleic acid + bile       | 5 ± 1                                         | 15 ± 5                                        |                                                |
| PCB #118 | DMEM/chyme                     | 8 ± 1                                         | 21 ± 3                                        | 6 ± 1                                          |
|          | DMEM + oleic acid              | 19 ± 1                                        | 32 ± 4                                        | 18 ± 2                                         |
|          | DMEM + oleic acid + bile       | 3 ± 0.2                                       | 8 ± 2                                         | 6 ± 1                                          |
| PCB #153 | DMEM/chyme                     | 7 ± 1                                         | 22 ± 3                                        |                                                |
|          | DMEM + oleic acid              | 14 ± 1                                        | 28 ± 4                                        | 2 ± 1                                          |
|          | DMEM + oleic acid + bile       | 3 ± 0.1                                       | 6 ± 1                                         | 0.5 ± 0.3                                      |
| PCB #180 | DMEM/chyme                     | 6 ± 1                                         | 19 ± 7                                        | 7 ± 2                                          |
|          | DMEM + oleic acid              | 7 ± 1                                         | 9 ± 4                                         | 15 ± 3                                         |
|          | DMEM + oleic acid + bile       | 2 ± 0.2                                       | 6 ± 1                                         | 2 ± 0.4                                        |

Rate constants representing \(^a\) HOC transport from the apical medium to the Caco-2 cells, \(^b\) from the cells to the apical medium, and \(^c\) losses from the apical medium.

Steady state distributions for different apical media were rather comparable. Apparently, the capacity of the cells for HOCs mainly determines the steady state situation. Uptake into Caco-2 cells occurred fastest for DMEM with oleic acid, than for DMEM/chyme, and slowest for DMEM with fatty acids and bile. This is illustrated by statistically different \(k_{mc}\) and \(k_{cm}\) values, which are presented in Table 2. The \(k_{mc}\) for PCB #153 varied between 3×10\(^4\) and 14×10\(^4\) h\(^{-1}\), and \(k_{cm}\) between 6×10\(^{-7}\) and 28×10\(^{-7}\) h\(^{-1}\). The difference in the values of the rate constants can be attributed to several counteracting processes that the sorbing constituents exert on the intestinal absorption of HOCs. These processes are presently addressed, assuming that only the freely dissolved HOCs can traverse the membrane.

Possible effects exerted by sorbing constituents. First, constituents such as micelles and proteins can sorb HOCs, and in this manner, decrease the freely dissolved concentration. If the sorbed contaminates do not dissociate, the contaminant fraction that is available for intestinal absorption is reduced. This can result in a lower absorption.

Second, bile salt micelles have been mentioned to act as carriers for fatty acids and HOCs, which are able to traverse the unstirred water layer (UWL) along the intestinal wall (23,79). Thereby, the apparent thickness of the UWL is reduced, which may result in an uptake flux that
is higher than based on the concentration of freely dissolved contaminants. It has been shown that PCBs sorbed to chyme constituents can participate in the uptake flux towards a passive sampling phase, a Solid Phase MicroExtraction (SPME) fiber (111). Probably, the rate-limiting step of HOC uptake for both the intestinal membrane and the SPME fiber is diffusion of the HOC through the UWL along the sampling phase. HOCs may dissociate from the micelles and/or proteins in the UWL and subsequently be absorbed. The release can occur due to 1) restoration of the decrease in freely dissolved HOC concentration next to the sampling phase. If the freely dissolved HOCs at the membrane surface are rapidly absorbed, the freely dissolved concentration decreases locally. Subsequently, if association and dissociation kinetics between sorbed HOCs and freely dissolved HOCs are dynamic, sorbed HOCs can dissociate in order to restore this equilibrium. 2) Release of HOCs in the UWL can also occur due to a physiologically based degradation of micelles/proteins. The low pH microclimate near the intestinal wall might induce micelles to disintegrate (79) and release sorbed HOCs (143). The digestion of proteins to di- and tri-peptides may induce a release of HOCs. The magnitude of the contribution of sorbed HOCs to the uptake flux compared to the situation that all HOCs were freely dissolved depends on two opposing processes. Carriers with sorbed HOCs have a lower diffusivity than the freely dissolved HOCs. This can reduce the transport of HOCs sorbed to bile salts, fatty acids and proteins towards the intestinal wall. On the other hand, these constituents may contain a relatively high load of HOCs. Hence, enough sorbed contaminants may be present in the UWL to maintain a high concentration gradient of the freely dissolved contaminant between the UWL and intestinal cells.

**Increasing exposure concentration**

Time curves for the second series of experiments with increasing apical exposure concentrations are presented in Figure 4. Since the distribution of the test compounds in chyme is based on partitioning (127), the concentration of freely dissolved contaminants and sorbed contaminants can be assumed to increase proportionally with increasing concentration of HOC. The total amount of PCB #153 in the different compartments increases, but the distribution among the compartments in time remains similar. This is also apparent from the rate constants \(k_{mc}\) and \(k_{cm}\) in Table 3, which are not significantly different. The \(k_{mc}\) for PCB #153 varied between \(4 \times 10^{-4}\) and \(7 \times 10^{-4}\) h\(^{-1}\), and the \(k_{cm}\) values between \(2 \times 10^{-7}\) and \(13 \times 10^{-7}\) h\(^{-1}\). In addition, the corresponding maximum uptake fluxes were calculated according to eq 4.3, and are presented in Table 4. The maximum uptake fluxes increased with increasing exposure concentration and varied for PCB #153 between \(2.4 \times 10^{-3}\) and \(13.2 \times 10^{-3}\) pmol/cm\(^2\)×s. This indicates that at higher concentrations in the apical medium more HOCs accumulate into the cells, whereas steady state is reached within the same time period: dose-dependent behavior.
Figure 4. Time curves for PCB #153 for increasing apical exposure concentrations in DMEM/chyme. To that end, chyme was employed that was contaminated via an artificial digestion with spiked OECD-medium. The OECD-medium was spiked with a) 1×, b) 3×, c) and d) 5× the reference mixture: 7 mg PCB #52, 7 mg PCB #118, 14 mg PCB #153, 7 mg PCB #180 and 2 mg lindane per kg dry OECD-medium. For situation d) 50 µM of other PCB congeners were added via an acetone spike. See Figure 3 for explanation of the symbols. For t=1 and t=4 h two replicate wells were used.
Table 3. Rate constants (±SD) for increasing apical HOC concentrations in DMEM/chyme. For explanation reference levels see Table 1. For explanation statistics and rate constants see Table 2.

| Compound     | Apical exposure concentration | \(k_{mc} \pm SD\) \(\times 10^4 \text{ h}^{-1}\) | \(k_{cm} \pm SD\) \(\times 10^7 \text{ h}^{-1}\) | \(k_{ml} \pm SD\) \(\times 10^5 \text{ h}^{-1}\) |
|--------------|-----------------------------|---------------------------------|---------------------------------|---------------------------------|
| Lindane      | 1× reference level          | 11 ± 2                          | 31 ± 8                          | 10 ± 2                          |
|              | 3× reference level          | 14 ± 1                          | 18 ± 4                          | 14 ± 3                          |
|              | 5× reference level          | 13 ± 1                          | 11 ± 4                          | 35 ± 7                          |
|              | 5× reference level + 50 µM | 19 ± 2                          | 25 ± 6                          | 12 ± 3                          |
| PCB #52      | 1× reference level          | 7 ± 1                           | 3 ± 4                           |                                 |
|              | 3× reference level          | 10 ± 2                          | 13 ± 5                          |                                 |
|              | 5× reference level          | 7 ± 1                           | 5 ± 3                           |                                 |
|              | 5× reference level + 50 µM | 9 ± 1                           | 8 ± 2                           | 7 ± 3                           |
| PCB #118     | 1× reference level          | 15 ± 3                          | 29 ± 11                         | 4 ± 3                           |
|              | 3× reference level          | 8 ± 1                           | 11 ± 5                          |                                 |
|              | 5× reference level          | 6 ± 2                           | 5 ± 2                           | 6 ± 2                           |
|              | 5× reference level + 50 µM | 10 ± 2                          | 17 ± 7                          | 5 ± 3                           |
| PCB #153     | 1× reference level          | 4 ± 1                           | 2 ± 2                           | 14 ± 4                          |
|              | 3× reference level          | 5 ± 1                           | 7 ± 2                           | 7 ± 1                           |
|              | 5× reference level          | 4 ± 1                           | 4 ± 2                           | 21 ± 3                          |
|              | 5× reference level + 50 µM | 7 ± 1                           | 13 ± 4                          | 4 ± 2                           |
| PCB #180     | 1× reference level          | 8 ± 2                           | 15 ± 7                          |                                 |
|              | 3× reference level          | 6 ± 1                           | 8 ± 3                           |                                 |
|              | 5× reference level          | 5 ± 1                           | 9 ± 3                           | 10 ± 2                          |
|              | 5× reference level + 50 µM | 9 ± 1                           | 14 ± 4                          |                                 |

Table 4. Maximum uptake fluxes \(J_{u,max}\) (±SD) for increasing apical HOC concentrations. For explanation reference level see Table 1, for explanation statistics see Table 2.

|          | \((J_{u,max} \pm SD) \times 10^3\), (pmol/(cm²×s)) | \((J_{u,max} \pm SD) \times 10^3\), (pmol/(cm²×s)) | \((J_{u,max} \pm SD) \times 10^3\), (pmol/(cm²×s)) | \((J_{u,max} \pm SD) \times 10^3\), (pmol/(cm²×s)) |
|----------|-----------------------------------------------------|-----------------------------------------------------|-----------------------------------------------------|-----------------------------------------------------|
|          | for 1× reference level                               | for 3× reference level                               | for 5× reference level                                | for 5× reference level + 50 µM other PCBs             |
| Lindane  | 1.3 ± 0.2                                           | 4.7 ± 0.5                                           | 5.8 ± 0.7                                           | 7.8 ± 0.8                                           |
| PCB #52  | 2.5 ± 0.5                                           | 9.1 ± 1.5                                           | 12.0 ± 2.1                                          | 11.0 ± 1.3                                          |
| PCB #118 | 3.0 ± 0.6                                           | 7.7 ± 1.4                                           | 8.7 ± 0.9                                           | 13.5 ± 2.6                                          |
| PCB #153 | 2.4 ± 0.4                                           | 6.5 ± 0.7                                           | 11.3 ± 1.6                                          | 13.2 ± 1.8                                          |
| PCB #180 | 2.3 ± 0.5                                           | 4.0 ± 0.5                                           | 6.8 ± 0.9                                           | 8.2 ± 1.1                                           |
Dulfer et al. (79) measured a similar pattern for PCB uptake by Caco-2 cells at higher PCB concentrations. They loaded their medium with PCBs to their solubility, and obtained a higher PCB concentration due to a higher solubility in the exposure medium caused by addition of oleic acid to a DMEM solution with sodium taurocholate, a bile salt. However, Dulfer et al. measured a PCB flux into the basolateral compartment, which is not found in the present study. The same test system was employed in both studies, except that Dulfer et al. used polycarbonate insert filters, higher PCB concentrations (35-100 times higher total PCB concentrations), and did not use Teflon lids. In order to investigate whether higher PCB concentrations caused an increased flux to the basolateral compartment, possibly due to an increased permeability of the cells, we exposed the cells to 50 µM extra PCBs. However, no significant differences were observed, see Table 3 and compare Figure 4c and 4d.

**Implications**

Most HOCs accumulated into the Caco-2 cells. Therefore, high absorption efficiencies can also be expected in vivo, probably even higher than in vitro since the contaminant concentration in intestinal cells will be lower due to HOC transport from the cells into the body. This is in accordance with rat in vivo studies that showed almost complete absorption of PCBs after ingestion in a corn oil matrix (9,100). However, with the present knowledge, a quantitative extrapolation to the in vivo situation cannot be performed.

Furthermore, these high absorption efficiencies indicate that besides the freely dissolved HOCs, also HOCs sorbed to bile, proteins and oleic acid contributed to the uptake flux towards the Caco-2 cells. It appeared that these constituents affected the intestinal uptake rate. Such processes will most probably also affect the in vivo intestinal absorption since environmentally relevant contaminant concentrations and physiologically constituent concentrations are employed. Nevertheless, HOCs that are mobilized from soil should be regarded available for intestinal absorption. Hence, mobilization of HOCs from soil during digestion is considered the most important step that determines the oral bioavailability of the presently used contaminants.

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