Availability of Polychlorinated Biphenyls (PCBs) and Lindane for Uptake by Intestinal Caco-2 Cells

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Children may ingest contaminated soil from hand to mouth. To assess this exposure route, we need to know the oral bioavailability of the contaminants. Two determining steps in bioavailability of soil-borne contaminants are mobilization from soil during digestion, which is followed by 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), whereas only a small fraction is freely dissolved. In this study, we examine the second step 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. The uptake flux seemed to be 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 toward the cells. These results can be extrapolated qualitatively to *in vivo* conditions. Because the sorbed contaminants should be considered available for absorption, the first step of mobilization from soil is the most important step for oral bioavailability of the presently investigated soil-borne contaminants. ***Key words:*** **bile, bioavailability, fatty acid, freely dissolved concentration, intestinal absorption, lindane, PCB, protein, soil-borne contaminant.** ***Environ Health Perspect*** **109:731–737 (2001). [Online 11 July 2001]**
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For children, ingestion of contaminated soil from hand to mouth 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, we must assess their oral bioavailability. Several steps can be distinguished for bioavailability of soil-borne contaminants: *a*) soil ingestion, which averages 50–200 mg/day (*1–3*); *b*) mobilization from soil and distribution among different physicochemical contaminant forms in digestive fluid; *c*) intestinal absorption; and *d*) liver metabolism. In this study, we consider soil ingestion a given fact, while liver metabolism is not relevant for the presently used contaminants. In a previous study, we investigated the second step using a physiologically based *in vitro* digestion model (*4*). We also studied the distribution of several PCB congeners and lindane among constituents of artificial human intestinal fluid (i.e., chyme) and digested soil (*4*). 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 (*4,5*). More hydrophobic organic contaminants (HOCs) were mobilized from soil when more bile or protein was added during the artificial digestion (*4*). Other studies showed that HOC mobilization from soil depends on the soil type (*6,7*), and that addition of dry whole milk increases PCB mobilization from soil (*6*). Therefore, we can conclude that sorbing phases may increase the contaminant mobilization from soil during the digestion.

In studies using *in vitro* digestion models, the amount of contaminant that is mobilized from soil represents the maximum amount available for intestinal absorption. *In vivo* studies in rats indeed showed that intestinal absorption of PCBs administered via spiked soil is lower than that of PCBs ingested via corn oil (*8*). Yet it is unclear to what extent mobilized HOCs are absorbed, and what the effect is of constituents such as bile, proteins, and fatty acids in chyme 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, we investigated the third step of oral bioavailability—transport of the mobilized contaminants across the intestinal wall—using *in vitro* intestinal cells. To that end, we investigated 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. Intestinal absorption is assumed to be the predominant uptake pathway (*9,10*), because the surface area of the stomach is negligible compared to the intestinal surface area, and probably a small fraction of the HOCs is mobilized from the soil in the stomach. We used the Caco-2 cell line as a model system to simulate human intestinal absorption. Caco-2 cells originate from an epithelial colon cancer, and after growing to confluence on a filter they start to differentiate into polarized, columnar cells that show many morphologic and physiologic characteristics of mature enterocytes of the small intestine. These cells are used extensively in drug absorption research (*11–14*). Extrapolation to *in vivo* absorption only provides an indication: good, moderate, or poor absorption. However, the controllable exposure conditions allow for mechanistic research.

Experimentally, the composition of the exposure medium was varied at similar or increasing total HOC concentrations. The amount of HOC left in the exposure medium, the HOC uptake by the cells, and transport across the cell monolayer were measured in time. These time curves were described by rate constants, allowing quantitative discrimination.

Thus, the aim of the present study is to investigate *a*) to what extent PCBs and lindane are absorbed by *in vitro* intestinal Caco-2 cells; *b*) the effect of sorbing constituents on absorption of the HOCs, including whether HOCs mobilized from soil during digestion contribute to uptake

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into the intestinal cells; and *c*) which factors are likely to have the largest impact on oral bioavailability of the soil-borne HOCs.

Materials and Methods

Chemicals. We used 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)] 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 (*15,16*). 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 analytic grade.

We used Organisation for Economic Co-operation and Development (OECD) medium as artificial standard soil. Dry OECD medium consisting of 10% peat, 20% kaolin clay, and 70% sand was prepared according to OECD guideline 207 (*17*). 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, we added lindane to the OECD medium as an aqueous solution that was prepared using the generator column technique (*18,19*). We spiked the OECD medium with a mixture of 7 mg PCB #52, 7 mg PCB #118, 14 mg PCB #153, 7 mg PCB #180, and 2 mg lindane/kg dry OECD medium, which is referred to as the reference contamination level, or with a 3- or 5-fold higher level. The concentration of lindane of 2 mg/kg represents the current Dutch ecotoxicologic intervention value (*20*). PCB #153 is environmentally abundant. Therefore, its level had to be higher than that of the other PCBs. The spiking levels of the PCBs are of environmental relevance (*21*), although relatively high for the PCBs relative to the current Dutch intervention value of 1 mg PCB/kg dry soil (*20*).

Artificial digestion. We used the physiologically based *in vitro* digestion model designed by Rotard et al. (*7*) in a modified version as described by Sips et al. (*22*). The digestion process was based on physiologic 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 hr. In the last digestion step duodenal juice and bile were added, and this mixture was rotated at 60 rpm for 2 hr. Finally, the mixture was centrifuged for 5 min at 3,000*g*, 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, pancreatine, 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 (Bedford, MA, USA) culture plate inserts of mixed cellulose esters (4.2 cm^2, 0.45 µm pore size) for 3–4 weeks. During this time the cells were maintained at 37°C in a humidified atmosphere containing 95% air and 5% CO_2, in culture medium. The culture medium consisted of Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Rockville, MD, USA), containing 25 mM HEPES and 4.5 g/L glucose, which was amended with 10% inactivated fetal calf serum (FCS), 1% nonessential amino acids (NEAA), 2 mM glutamine, and 50 mg/L gentamicin.

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. We employed similar conditions as during cell culture, 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 gentamicin was used for exposure experiments. For all experiments, we took care not to exceed the solubility of the HOCs, according to solubility data of Dulfer et al. (*23*) on DMEM with oleic acid and of Oomen et al. (*4*) on chyme. Losses of test compounds via the air and cross-contamination between and within the wells were prevented by closing the well and inserting a Teflon lid.

We performed several exposure experiments with Caco-2 cells (Table 1). First, we varied the composition of the apical

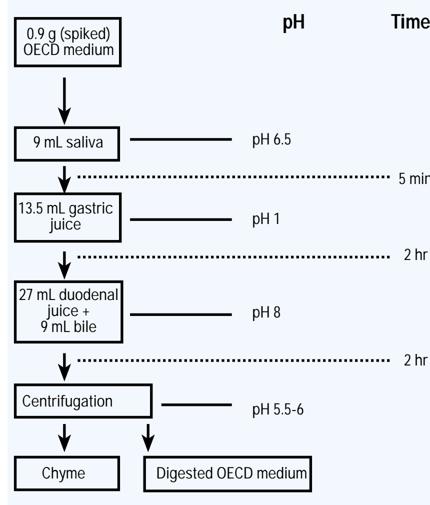

Figure 1. Schematic representation of the procedure of an artificial digestion.

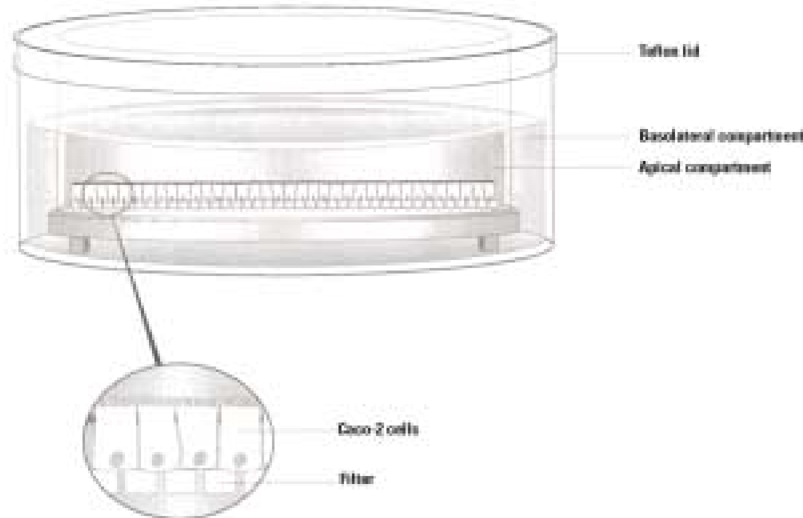

Figure 2. Schematic side view of a well with a monolayer of Caco-2 cells.

medium. To the apical compartment we added 2 mL of *a*) DMEM/chyme, *b*) DMEM that contained 0.5 mM oleic acid, or *c*) DMEM that contained 0.5 mM oleic acid and 0.9 g/L freeze-dried chicken bile. We chose oleic acid as model fatty acid because it is a major product from dietary lipid hydrolysis. We used an acetone solution with the test compounds to spike the different media.

In the second series of experiments, we used different apical concentrations of PCBs and lindane in DMEM/chyme. Therefore, we prepared artificial chyme with OECD medium that was spiked with one, three, or five times the reference contamination level. Thus, we obtained three chyme solutions with increasing concentrations of mobilized HOCs. In addition, we added PCB congeners other than our test compounds 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 about 1.5 µM). In this manner, we obtained a DMEM/chyme solution with a total PCB concentration of 50 µM.

Sampling procedure. We incubated the cells with PCBs and lindane for various periods of time up to 24 hr. We took a 250 µL aliquot of the apical medium to determine lactate dehydrogenase (LDH) leakage of the cells. Subsequently, we took several samples of each well 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 internal standards were used to correct for volume differences between samples and losses of the PCBs and lindane during cleanup. The apical and basolateral compartments were rinsed twice with 2 mL phosphate-buffered saline (PBS); the basolateral compartment was also rinsed with 2 mL hexane. The washes were added to the corresponding samples. The apical volume can decrease and the basolateral volume can increase through active transport across the cells. Therefore, we corrected 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. We rinsed the remaining filter once with 2 mL PBS and once with 2 mL hexane and added the washes 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. We added 2 mL 18 M sulfuric acid to each sample to degrade organic interferences. After extensive stirring, we added about 10 mL water and restirred each sample. Subsequently, the water phase was frozen by storage at –25°C, after which the liquid hexane phase could easily be collected. Then we added about 2 mL new hexane to the aqueous sample. The sample was melted, stirred again, and stored at –25°C. We performed this procedure thrice. The combined hexane extracts were evaporated under a gentle nitrogen stream to approximately 100 to 500 µL, before analysis by gas chromatograph–electron capture detector.

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, USA). Hence, the curves could be compared quantitatively. We included an exponential loss term from the apical medium to account for losses of test compounds during the experiment.

$$\frac{dC_{\text{med}}}{dt} = k_{cm} \times C_{\text{cell}} - k_{mc} \times C_{\text{med}} - k_{ml} \times C_{\text{med}} \quad [1]$$

$$\frac{dC_{\text{cell}}}{dt} = k_{mc} \times C_{\text{med}} - k_{cm} \times C_{\text{cell}} \quad [2]$$

Equation 1 represents the change in concentration of an HOC in the exposure medium, C_{med}, in time, *t*. Equation 2 represents the change in concentration of an HOC in the cell compartment, C_{cell}, in time, *t*. Several rate constants are involved, representing transport from the apical medium to the cell compartment (k_{mc}), transport from the cell to the apical medium compartment (k_{cm}), and losses from the apical medium (k_{ml}). We estimated a cell volume of 10.6 µL/well, based on a cell height of 25 µm (*12*) and the filter surface. The standard deviations (SD) 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 SD of both constants do not overlap. The maximum uptake flux, $J_{\text{u,max}}$, can be calculated by extrapolation of the uptake flux to zero time, based on equation 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:

$$J_{\text{u,max}} = \frac{\left(k_{mc} \times C_{\text{med},t=0}\right)}{A_{\text{filter}}} \quad [3]$$

Quality control. We investigated the experimental validity in several ways. We calculated the mass balance of the test compounds by summing up the amounts of each compound measured in the apical, basolateral, and cell compartment. We compared these summed amounts 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. We took samples of uncontaminated media, chyme, PBS, and hexane, and of cells exposed to uncontaminated DMEM/chyme. Also, we determined the HOC content of some filters, which had been employed during exposure experiments and had been sampled.

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

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

Finally, we studied the effect of DMEM/chyme on transport of the reference

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/kg dry OECD medium.

compounds ^{3}H-mannitol, fluorescein isothiocyanate dextran FD4 (Mw 4000), and fluorescein across the Caco-2 cell monolayers.

Results and Discussion

Artificial digestion. During 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 (*4*).

Quality control. **Recovery.** We recovered 95% (± 18%) by summation of the amounts of contaminants in the apical, basolateral, and cell compartments after 0.5 hr of exposure, compared to the amounts of contaminants measured in the exposure media. In some cases we observed a gradual decrease in the recovered HOC amount in the system during the 24 hr 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 hr of exposure, compared to 0.5 hr 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. None of the blank samples, the filters, and the uncontaminated solutions and compartments of unspiked wells showed 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 hr 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: We measured < 2% of PCB #118, PCB #153, and PCB #180, and < 10% of lindane and PCB #52 in the basolateral compartment after 24 hr of exposure. This is in line with a similarly low basolateral HOC concentration after 24 hr of exposure with a Caco-2 cell layer (Figures 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 (*23,24*), and subsequently enter the lymph flow (*9,24,25*). Although the cell line presently used expresses some lipid transport, the extent is probably not fully comparable to *in vivo* conditions (*26*). Therefore, the present experimental set-up is limited 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 Equation 1.

Cell viability and integrity. The TEER was approximately 500 Ω/cm^2. This shows that the enterocyte cells were mature and that no holes were present in the cell monolayers (*12*).

Neutral red uptake by cells after preexposure for 24 hr to DMEM/chyme was the same as the uptake by cells after preexposure for 24 hr 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 cell viability was not compromised by the different media or HOCs. In general, < 5% of the total amount of cells were disrupted after 24 hr of exposure, which we consider 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 for mannitol and FD4 approximated a factor of 5 to 7 after 2 hr of exposure to both media. The apparent permeability for mannitol was increased by a factor of 15 after 24 hr of preexposure to DMEM/chyme and subsequent exposure to mannitol for 1 hr, compared to exposure to culture medium only. In a similar experiment the apparent permeability for fluorescein was increased by a factor of 40. The reference compounds are known to be transported paracellularly, i.e., through 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 (*9*). Therefore, we regard this state of the monolayer as realistic and workable.

P-glycoprotein pump. P-glycoprotein may pump xenobiotics such as the presently used compounds from inside the cells back into the apical medium. According to results of Dulfer et al. (*23*), who studied PCB uptake by and transport across the Caco-2 cell monolayers, P-glycoprotein is not likely to largely influence PCB uptake by the Caco-2 cells.

In the two series of experiments, we varied *a*) the composition and *b*) the contaminant concentration of the apical medium. 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 article, PCB #153 is 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.

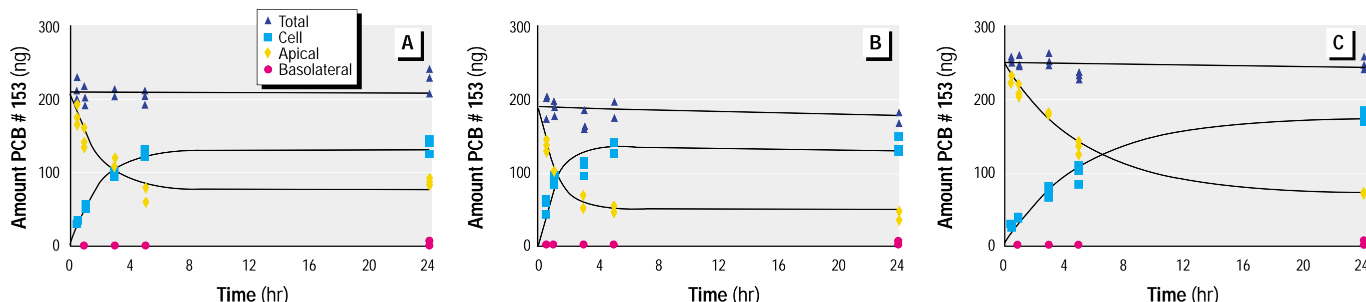

Figure 3. Time curves for PCB #153 for different compositions of the apical medium. (*A*) A mixture of DMEM and chyme (1:1, v:v), i.e., DMEM/chyme. (*B*) DMEM with 0.5 mM oleic acid. (*C*) DMEM with 0.5 mM oleic acid + 0.9 g/L bile.

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 contaminant 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 (Tables 2 and 3). Therefore, at steady state, the concentration of an 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 about 5% (*4,5*). 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.

Steady state distributions for different apical media were comparable. Apparently, the capacity of the cells for HOCs mainly determines the steady state situation. Uptake into Caco-2 cells occurred faster 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} hr^{-1}, and k_{cm} between 6×10^{-7} and 28×10^{-7} hr^{-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.

Table 2. Rate constants ± SD of HOC transport into Caco-2 cells for different apical exposure media.

Compound	Apical medium	(k_{mc} ± SD) $\times 10^{-4}$ [hr^{-1}][a]	(k_{cm} ± SD) $\times 10^{-7}$ [hr^{-1}][b]	(k_{ml} ± SD) $\times 10^{-5}$ [hr^{-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 are considered significantly different if the average value (± 2 × SD) of both constants do not overlap, which represents $\alpha \leq 0.05$.
[a]Rate constants represent HOC transport from the apical medium to the Caco-2 cells. [b]Rate constants represent HOC transport from the cells to the apical medium. [c]Rate constants represent losses from the apical medium.

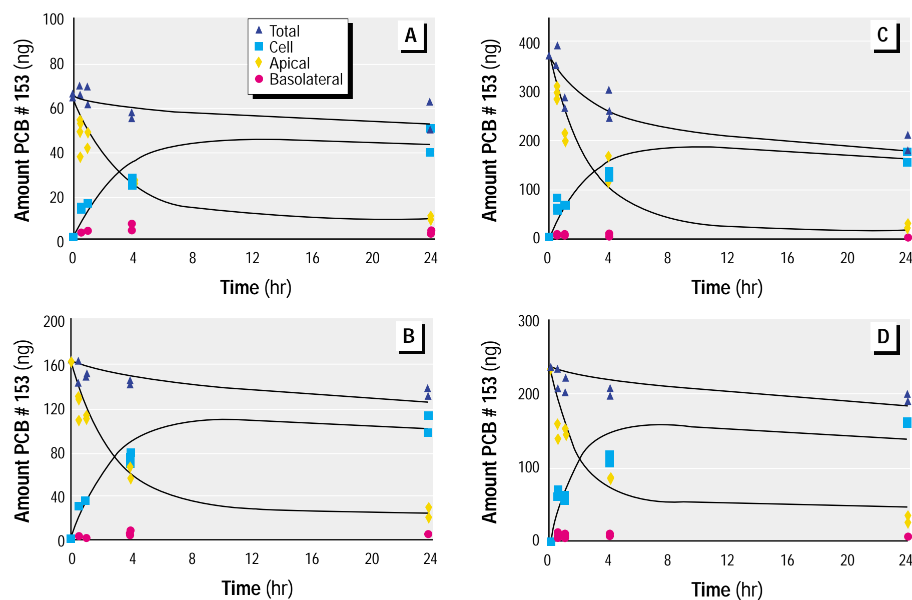

Figure 4. Time curves for PCB #153 for increasing apical exposure concentrations in DMEM/chyme. To that end, the chyme was contaminated via an artificial digestion with spiked OECD medium. The OECD medium was spiked with (*A*) 1×, (*B*) 3×, (*C*) 5×, and (*D*) 5× the reference mixture: 7 mg PCB #52, 7 mg PCB #118, 14 mg PCB #153, 7 mg PCB #180, and 2 mg lindane/kg dry OECD medium. For (*D*) 50 µM of other PCB congeners were added via acetone spike. For t = 1 hr and t = 4 hr, two replicate wells were used.

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

Second, bile salt micelles can act as carriers for fatty acids and HOCs, which are able to traverse the unstirred water layer (UWL) along the intestinal wall (*9,23*). Thereby, the apparent thickness of the UWL is reduced, which may result in an uptake flux that is higher than that based on the concentration of freely dissolved contaminants. PCBs sorbed to chyme constituents can participate in the uptake flux toward a passive sorbing phase, a solid phase microextraction (SPME) fiber (*5*). 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 sorbing phase. HOCs may dissociate from the micelles and/or proteins in the UWL and subsequently be absorbed. The release can occur through restoration of the decrease in freely dissolved HOC concentration next to the sorbing 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 to restore this equilibrium. Release of HOCs in the UWL can also occur through a physiologically based degradation of micelles/proteins. The low pH microclimate near the intestinal membrane might induce micelles to disintegrate (*23*) and release sorbed HOCs (*27*). The digestion of proteins to di- and tripeptides may induce a release of HOCs. The magnitude of the contribution of sorbed HOCs to the uptake flux compared to the situation where 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 toward 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 (*4*), 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 4×10^{-4} and 7×10^{-4} hr^{-1}, and the k_{cm} values between 2×10^{-7} and 13×10^{-7} hr^{-1}. In addition, the corresponding maximum uptake fluxes were calculated according to Equation 3, and are presented in Table 4. The maximum uptake fluxes increased with increasing exposure concentration and varied for PCB #153 between 2.4×10^{-3} and 13.2×10^{-3} pmol/cm^2 × sec. This indicates that at higher HOC concentrations in the apical medium more HOCs accumulate into the cells, whereas steady state is reached within the same time interval, i.e., dose-dependent behavior.

Dulfer et al. (*23*) measured a similar pattern for PCB uptake by Caco-2 cells at higher PCB concentrations. They obtained a higher PCB concentration through 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. To investigate whether higher PCB concentrations caused an increased flux to the basolateral compartment, possibly caused by an increased permeability of the cells, we exposed the cells to 50 mM 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* because 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 (*8,28*). However, with the present knowledge, a quantitative extrapolation to *in vivo* conditions cannot be performed.

Furthermore, these high absorption efficiencies indicate that besides the freely dissolved HOCs, HOCs also sorbed to bile, proteins, and oleic acid contributed to the uptake flux toward the Caco-2 cells. It appeared that these constituents affected the intestinal uptake rate. Such processes will probably also affect *in vivo* intestinal absorption because environmentally relevant contaminant concentrations and physiologically constituent concentrations are employed. Therefore, HOCs that are mobilized from soil should be regarded as 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.

Table 3. Rate constants (± SD) for increasing apical HOC concentrations in DMEM/chyme.

Compound	Apical exposure concentration	(k_{mc} ± SD) × 10^{-4} [hr^{-1}]	(k_{cm} ± SD) × 10^{-7} [hr^{-1}]	(k_{ml} ± SD) × 10^{-5} [hr^{-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	

For explanation of reference levels, see Table 1. For explanation of statistics and rate constants, see Table 2.

Table 4. Maximum uptake fluxes $J_{u,max}$ (± SD) for increasing apical HOC concentrations.

	$J_{u,max}$ ± SD for 1× reference level [pmol/cm^2 × sec)]	$J_{u,max}$ ± SD for 3× reference level [pmol/(cm^2 × sec)]	$J_{u,max}$ ± SD for 5× reference level [pmol/(cm^2 × sec)]	$J_{u,max}$ ± SD for 5× reference level + 50 µM other PCBs [pmol/(cm^2 × sec)]
Lindane	$(1.3 \pm 0.2) \times 10^{-3}$	$(4.7 \pm 0.5) \times 10^{-3}$	$(5.8 \pm 0.7) \times 10^{-3}$	$(7.8 \pm 0.8) \times 10^{-3}$
PCB #52	$(2.5 \pm 0.5) \times 10^{-3}$	$(9.1 \pm 1.5) \times 10^{-3}$	$(12.0 \pm 2.1) \times 10^{-3}$	$(11.0 \pm 1.3) \times 10^{-3}$
PCB #118	$(3.0 \pm 0.6) \times 10^{-3}$	$(7.7 \pm 1.4) \times 10^{-3}$	$(8.7 \pm 0.9) \times 10^{-3}$	$(13.5 \pm 2.6) \times 10^{-3}$
PCB #153	$(2.4 \pm 0.4) \times 10^{-3}$	$(6.5 \pm 0.7) \times 10^{-3}$	$(11.3 \pm 1.6) \times 10^{-3}$	$(13.2 \pm 1.8) \times 10^{-3}$
PCB #180	$(2.3 \pm 0.5) \times 10^{-3}$	$(4.0 \pm 0.5) \times 10^{-3}$	$(6.8 \pm 0.9) \times 10^{-3}$	$(8.2 \pm 1.1) \times 10^{-3}$

For explanation of reference level, see Table 1. For explanation of statistics, see Table 2.

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