Intestinal lead uptake and transport in relation to speciation: an in vitro study

Agnes G. Oomen, Johannes Tolls, Adrienne J.A.M. Sips, and John P. Groten

submitted

Abstract

Children might be exposed substantially to contaminants such as lead via soil ingestion. In risk assessment of soil contaminants there is a need for information on oral bioavailability of soil-borne lead. Oral bioavailability can be seen as the result of four steps 1) soil ingestion, 2) mobilization from soil during digestion, i.e. bioaccessibility, 3) absorption from the intestinal lumen, and 4) first-pass effect. Lead bioaccessibility and speciation in artificial human small intestinal fluid, i.e. chyme, have been investigated in previous studies. In the present study, intestinal lead absorption was investigated using the Caco-2 cell line. Cell monolayers were exposed to (diluted) artificial chyme. In 24 h, approximately 27% of the lead accumulated into the cells and 3% were transported across the cell monolayer, without signs of approaching equilibrium. Lead accumulation into the cells showed a linear relationship with the total amount of lead in the system. Bile levels did not affect the fraction of lead that accumulated in the Caco-2 cells. Extrapolation of the lead flux across the cell monolayer to in vivo absorption indicates that absorption of bioaccessible lead is incomplete. Furthermore, the results indicate that, as the free Pb$^{2+}$ concentration in chyme was negligible, also lead species other than the free metal ion must have contributed to the lead flux towards the cells. On the basis of lead speciation in chyme, this can be attributed to dissociation of labile lead species such as lead phosphate and lead bile complexes, and subsequent transport of the released free metal ions across the intestinal membrane. The incomplete absorption of bioaccessible lead indicates that less than the bioaccessible fraction of soil-borne lead becomes bioavailable.
INTRODUCTION

Children ingest soil, either accidentally via hand-to-mouth behavior or deliberately. In this manner, a child ingests on average between 50 and 200 mg soil/day, although amounts of as much as 60 g/day have also been observed (66,67,69). Hence, soil ingestion can be a main route of exposure to soil-borne contaminants to children. An ubiquitous soil-borne pollutant that may cause health hazards is the heavy metal lead (1,56). In current Dutch risk assessment of contaminated soils, oral bioavailability of soil-borne lead is set equal to oral bioavailability of food-borne lead (1). However, several studies using test animals demonstrated that absorption and toxicity for lead ingested with soil is lower than for lead ingested with food or aqueous solution (11-14,17). In order to gain insight into the health risk associated to lead exposure via soil ingestion, the critical steps determining oral bioavailability of soil-borne contaminants should be investigated.

Oral bioavailability of a soil-borne contaminant is defined as the contaminant fraction that reaches the systemic circulation. Four steps can be distinguished before a contaminant becomes bioavailable: 1) soil ingestion, 2) mobilization of the contaminant from soil during digestion, i.e. bioaccessibility, 3) absorption of the mobilized contaminants, and 4) first-pass effect. Information on oral bioavailability of soil-borne lead is required to assess the health risk associated to soil ingestion. The first step can be considered as a given daily amount of soil that is ingested. The last step, first-pass effect consists of biotransformation and excretion of the contaminant in the intestine or liver. Heavy metals are not biotransformed, but may undergo some biliary excretion (18,96). Nevertheless, the first-pass effect is of minor importance for heavy metals such as lead. Hence, knowledge on the second and third steps, bioaccessibility and absorption, can provide insight into oral bioavailability of soil-borne lead.

In a previous study, bioaccessibility of soil-borne lead has been investigated using a physiologically based in vitro digestion model (162). Digestions mimicking fasting conditions were performed with artificial standard soil, i.e. OECD-medium, spiked with 530 mg Pb/kg dry weight (162). It was shown that, for conditions representing the luminal content of the small intestine, about 23% of the lead were bioaccessible. Furthermore, lead speciation in artificial human intestinal fluid, i.e. chyme, was investigated. Speciation is the distribution of a compound among different physicochemical forms. Important lead forms in chyme were lead phosphate and lead bile complexes, while the free Pb$^{2+}$ fraction was negligible (162). These findings are in agreement with several other studies that discuss the (possible) formation of lead phosphate (154,155,160) and lead bile (96,161) complexes. It was also shown that the lead phosphate complexes are labile within a voltammetric time scale (162), indicating that the equilibrium with Pb$^{2+}$ is dynamic: dissociation of lead phosphate complexes occurs within
tenths of seconds. In addition, the results suggest that lead bile complexes are volt-
labile ( ), which is in agreement with studies of Feroci (161) that showed that lead bile
complexes are slightly soluble and voltammetrically labile.

In the present study, intestinal absorption of b
purpose, in vitro -2 cells were employed as a model to simulate human intestinal
(63,139 141)
more recently, to assess absorption of environmental contaminants 79,163,164) compounds that is transported across a Caco-
in vivo absorption 165) -2 cells originate from a c
confluency on a filter, they differentiate into polarized cells that show many morphological and
physiological characteristics of mature enterocytes of the small intestine. Experimentally, the
lation into and transport across Caco-
concentration dependency and chyme of different bile content were investigated. The data are
interpreted in terms of lead speciation in chyme as determined in previous studies 162)

The aim of the present study was to investigate 1) to what extent bioaccessible lead is
insight into the processes determining oral bioavailability of soil-
lead.

MATERIALS AND METHOD

-medium was used as standardized artificial
-medium contained 10% peat, 20% kaolin clay, and 70% sand and was
-guideline 207 98)
with a ground and solid lead nitrate. Subsequently, the peat and clay fractions were added. After further stirring, 50 mass% of water were added. OECD medium was spiked at 1, 3 or 5
times the current Dutch (37 , and stored at 5 °C.

Artificial Digestion

The physiologically based digestion model designed et al. 104 was
et al. ( )
process was based on physiological constituents and transit times for fasting conditions of . The digestion model is schematically presented in Figure 1. In short, synthetic saliva,
medium and rotated at 60 rpm for 5 min at 37 °C. Subsequently, the 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. the digested OECD-medium) and about 58.5 ml of supernatant (i.e. the artificial chyme). Important chyme components were freeze-dried chicken bile (0.9 g/l) and phosphate (2.6 mM). The ionic strength of chyme was 0.14 M. The pH of chyme increased from 5.7 (±0.2) directly after digestion, to approximately 6.5 upon storage for several days in the freezer.

![Figure 1. Schematic representation of the procedure of an artificial digestion, exposure of Caco-2 cells to a mixture of DMEM and chyme (1:1, v:v), i.e. DMEM/chyme, and analysis.](image)

**Cell Culture**

Monolayers of Caco-2 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 consists 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) and 2 mM glutamine, and 50 mg/l gentamicine.
Exposure

Caco 2 cells could not be exposed to pure chyme because it affects the cell viability. d with 1% NEAA, 2

The conditions used were similar to those employed during cell culture, except for stirring the m is schematically presented in Figure blood and lymph drain, respectively. Lead was always presented at the apical side in 2 ml compartment 2 ml uncontaminated DMEM/chyme was 7 and 7.5. Unless mentioned otherwise, chyme of standard bile concentration (0.9 g/l) was .45 g/l bile). Furthermore, the chyme was obtained from - times intervention value of 530 mg/kg, and had been stored in the freezer for several days.

Figure 2. -view of a well with Caco 2 cells.

Several series of exposure experiments were performed with the intestinal cell cultures (Table 1). First, the time course of lead uptake by and transport across Caco 2 monolayers was determined. Samples obtained, which allowed for comparison between two spiking methods and between fresh and stored chyme. To that end, two exposure media were prepared with freshly made chyme, either minated via digestion with spiked OECD-solution. Furthermore, another exposure medium was prepared with chyme that had been stored
in the freezer for several days and subsequently was spiked with a lead nitrate solution. In the second series of experiments, the concentration dependency of lead uptake by Caco-2 cells was investigated. To that end, DMEM/chyme was prepared with chyme from in vitro digestion with OECD-medium of different spiking levels. OECD-medium spiked with 1×, 3× and 5× the current Dutch intervention value of 530 mg Pb/kg dry was used. In the last series of experiments, the contribution of lead bile complexes to the lead flux towards the cells was examined. For that purpose, DMEM/chyme was prepared with chyme of different bile concentrations: 0×, 0.25×, 0.5×, 0.75×, 1× and 1.5× the default concentration of bile in chyme (0.9 g/l) was employed. For the latter two series of experiments only samples after 24 h of exposure were taken. Three wells were used for each combination of time and medium.

Table 1. Overview of the experiments with Caco-2 cells. The exposure media consist of one part of chyme diluted with one part of DMEM.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Chyme employed for exposure media</th>
<th>Exposure time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time curve</td>
<td>Freshly prepared chyme spiked with lead nitrate</td>
<td>1, 3, 5 and 24 h</td>
</tr>
<tr>
<td></td>
<td>Freshly prepared chyme contaminated via digestion with spiked OECD-medium</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stored chyme spiked with lead nitrate</td>
<td></td>
</tr>
<tr>
<td>Concentration dependency</td>
<td>Stored chyme contaminated via digestion with OECD medium spiked with 1×, 3×, 5× intervention value lead (530 mg/kg dry matter soil).</td>
<td>24 h</td>
</tr>
<tr>
<td>Bile</td>
<td>Stored chyme with 0×, 0.25×, 0.5×, 0.75×, 1× and 1.5× the default level of bile (0.9 g/l).</td>
<td>24 h</td>
</tr>
</tbody>
</table>

**Sampling procedure and treatment**

After exposure, 1.2 ml of the apical and basolateral compartment of each well were transferred into a tube that contained 0.8 ml 1 M HNO₃ and 7.0 ml water. A correction was made for the volume of DMEM/chyme in the remaining apical and basolateral compartment, since the apical volume can decrease and the basolateral volume can increase, due to active transport of water across the cells. These remaining volumes were transferred into a vial and determined by weighing. Subsequently, the cells were rinsed twice with 2 ml phosphate buffered saline (PBS), and the rinses were discarded carefully so that all non-cellular lead was removed. Then, 0.8 ml 1 M HNO₃ was added to disrupt the cells, the cells were scraped off and transferred into an empty tube. Two rinse steps with 2 ml PBS were performed and the washes were added to the corresponding sample. Finally, 4.2 ml water were added to the tubes.
containing cell samples. The samples were frozen at -20°C until analysis by means of an 6000).

**Cell viability and monolayer integrity**
- 2 monolayers was checked by determination of the transepithelial ERS Epithelial Voltohmmeter, MA). Cell viability was assessed by lactate dehydrogenase (LDH) leakage (BM/Hitachi 911, using pyruvate as substrate) and neutral red uptake. LDH samples -exposure h to DMEM/chyme with low (5 µµM) lead content and compared to -exposure to uncontaminated culture medium. Furthermore, the 3H mannitol was studied after 24

The permeability coefficient, \( P \) (cm/s), of lead and 3 -mannitol is calculated according:

\[
P_{\text{coeff}} = \frac{A \times C_0}{P}
\]  

(6.1)

\( P \), the permeability flux (mol/s), represents the rate at which a compound is transferred across the Caco-2 monolayer into the basolateral compartment. \( C_0 \) is the initial apical concentration of the test compound (mol/ml), and \( A \) the surface area of the filter where the Caco-2 cells grow on (cm²).

**RESULTS**

**Cell integrity and viability**
- The TEER of the cultured cell monolayers was approximately 500 Ω/cm², indicating that the enterocyte cells were mature and that no holes were present in the cell layers after 3 to 4 weeks of culture (140).
- The active uptake of neutral red by Caco-2 cells after pre-exposure for 24 h to DMEM/chyme for both Pb levels was the same as uptake by the cells after pre-exposure for 24 h to culture medium. In addition, the LDH values increased with exposure time to about 5% or less of the maximum LDH level. The LDH values did not differ for wells exposed to DMEM/chyme or culture medium. Hence, both neutral red and LDH results indicate that
neither DMEM/chyme nor lead compromised the cell viability under the present test conditions.

The $P_{\text{coeff}}$ for $^3$H-mannitol was $0.9\times10^{-7}$ cm/s after exposure to culture medium only, and was increased by a factor 16 after 24 h of pre-exposure to DMEM/chyme. The increase in mannitol transport across the cell layer, without concomitant increase in LDH release, indicates that chyme most likely affected the cell-cell junctions, without clear signs of cellular toxicity. Since alterations of the permeability of the intestinal wall are also triggered by bile salts in vivo ((166,167) and references in (23)), we regard the observed increase in transport rate of the reference compounds as a normal physiological response. Therefore, the cell integrity and viability data indicate that the present experimental set-up functions in analogy to the physiological reality.

Time curve

As can be seen in Figure 3, the amount of lead in the apical compartment decreased by approximately one-fourth in 24 h. Within the same time interval, a corresponding increase was observed in the amount of lead in the cells. An increase of the cellular lead accumulation was observed after each time point. This indicates that the cells were not saturated with lead within 24 h of exposure. Although not always significant (considered significant if $\alpha\leq0.05$), the cellular accumulation tended to go faster between 5 and 24 hours than between 0 and 5 hours. This slightly increasing rate might have been caused by increased membrane permeability due to the chyme in the exposure medium. However, the effect of chyme on the cellular lead accumulation probably is small, since the cellular lead accumulation in time does not largely deviate from linearity.

A small fraction of lead was transported into the basolateral compartment. No large differences in the lead distribution among the compartments were observed between spiking of DMEM/chyme with a lead nitrate solution or employing contaminated chyme that was obtained from an artificial digestion with spiked OECD-medium (Figure 3a versus 3b). Furthermore, no differences were observed between the time curves of DMEM/chyme with fresh or stored chyme (Figure 3b versus 3c). This indicates that stored chyme could be employed for further experiments.
Figure 3. Time curves of lead uptake by and transport through Caco-2 monolayers after exposure to DMEM/chyme. To that end, chyne was employed that was, a) freshly prepared and contaminated with lead via an artificial digestion with spiked OECD-medium at 5× intervention value, b) freshly prepared and spiked with a lead nitrate solution, and c) stored (few days in freezer) and spiked as in b).
Concentration dependency

Figure 4 presents the lead distribution among the different compartments after 24 h of exposure to DMEM/chyme with increasing lead concentration. The amount of lead in the apical and cell compartments after 24 h showed a linear relationship with the total amount of lead in the well. The $r^2$ values were 0.999 and 0.997, respectively. Relationships with the amount of lead in the basolateral compartment could not be determined, since the amount of lead in the basolateral compartment was hardly measurable.

Figure 4. Distribution of lead among the apical, basolateral and cell compartment after 24 h of exposure of Caco-2 cells to DMEM/chyme with different lead concentrations. To that end, chyme was employed that was prepared by artificial digestion with OECD-medium spiked with 1×, 3× or 5× the current Dutch intervention value of 530 mg Pb/kg dry.

Figure 5. Relative distribution of lead among the apical, basolateral and cell compartment after 24 h of exposure to DMEM/chyme with increasing bile content. The x-axis expresses the bile concentration relative to the bile concentration in DMEM/chyme after a default situation (0.45 g/l).
Bile

Figure 5 shows the relative distribution of lead among the different compartments after exposure for 24 h to DMEM/chyme with varying bile concentrations. No increasing or decreasing trend with increasing bile concentration could be observed for all compartments within the experimental variation.

Lead transport and cellular accumulation

Considering all experiments, after 24 h of exposure on average 70% (SD ±11%) of the lead was still present in the apical compartment, 27% (SD ±11%) in the cells and 3% (SD ±3%) in the basolateral compartment. From these values, the $P_{\text{coeff}}$ for lead can be calculated according to eq 6.1, and is $1.7 \times 10^{-7} \text{ cm/s}$.

DISCUSSION

Experimental conditions

In the present study, an effort is made to expose Caco-2 cells to lead of a speciation similar to the in vivo situation. To that end, chyme obtained from an in vitro digestion model was employed. In order to prevent toxicity of the Caco-2 cells, the chyme was diluted with DMEM (v:v, 1:1). DMEM contains high salt concentrations that may form complexes with lead and thereby affect the lead speciation, for example phosphate, chloride and carbonate can be present in the mM range. Nevertheless, we expect that the main features of lead speciation in DMEM/chyme and in chyme are similar, since these salt concentrations are similar or even higher in chyme ($110$). Hence, it can be assumed that in both solutions the free Pb$^{2+}$ fraction is negligible, and that important lead species are lead phosphate and lead bile complexes.

Chyme is a complex solution that may bring about some experimental difficulties. It contains many lead complexing agents such as phosphate, bile, carbonate and chloride. Especially lead phosphate complexes are extremely insoluble, and its formation is thermodynamically favored ($153, 154, 168-170$). Since the phosphate concentration in chyme is 2.6 mM, the solubility product of lead phosphate is most probably exceeded, as it also would be in most cell culturing media. This means that speciation studies with sparingly soluble metals are difficult to perform under conditions in which cells have to be kept viable. The presence of complexing agents in DMEM/chyme indicates that a suspension may be formed. Lead may precipitate if large particles are formed, possibly affecting the experimental results. For example, precipitation of macroscopic lead particles in DMEM/chyme or in chyme may explain the difference in the total amount of lead per well for 5× the intervention value for the first and second series of experiments (±12 µg in Figure 3 versus ±7 µg in Figure 4). Nevertheless, the presence of insoluble complexes is not an artifact, as lead phosphate
 complexes are expected to exist in vivo too. Hence, to our opinion, the presently used experimental set-up is a valid and physiological simulation of intestinal cells exposed to chyme containing lead.

**Lead speciation and cellular uptake**

The mechanism of lead absorption is supposed to involve both the transcellular and paracellular pathway (18,82,83). In the present section we consider which lead species have contributed to the transcellular route. Therefore, we focus on the results of lead determined in the cell compartment, as this lead must have traversed or has been complexed to the luminal membrane. Previous studies showed that the free Pb$^{2+}$ fraction in artificial chyme is negligibly small (162), and it can be assumed that free Pb$^{2+}$ is negligible in DMEM/chyme as well. At least free metal ions are able to bind to the intestinal membrane and/or traverse the membranes (74,76,77). Yet, the in vitro experiments with intestinal cells show that a considerable fraction, about 27% after 24 h, of the lead presented at the apical site was determined in the cell compartment, see Figures 3, 4 and 5. Thus, a larger lead fraction than the free Pb$^{2+}$ fraction had accumulated into the intestinal cells. This implies that lead species other than Pb$^{2+}$ must have contributed to the flux towards the Caco-2 cells.

Furthermore, the fraction of lead that accumulated into the Caco-2 cells appeared to be independent of the bile level in DMEM/chyme (see Figure 5). Nevertheless, the experimental variation of bile was expected to have a profound effect on the lead speciation in DMEM/chyme. Previous studies showed a clear effect of the absence of bile on the complexing capacity and stability constant of lead for chyme (162). The range of bile concentrations for the Caco-2 experiments (0 to 0.68 g/l) was similar to the range that was employed for the lead speciation experiments (0 to 0.9 g/l). Therefore, the variation of bile in DMEM/chyme can be assumed to affect the speciation of lead. Yet, the mechanism of lead uptake should be able to explain bile-level independent lead accumulation in the cells.

The observed lead accumulation in the intestinal cells can be explained in several ways. Presently, the different possibilities are considered.

**Pinocytosis.** A contribution via pinocytosis of small volumes DMEM/chyme containing lead may have occurred. Although this cannot be fully excluded, pinocytosis is not a probable route for drug absorption in the intestine (63). Therefore, it is unlikely that about 27% of the lead was transferred from the apical medium into the Caco-2 cells in 24 h via this mechanism.

**Hydrophobic lead complexes.** In parallel to the free Pb$^{2+}$ ions, hydrophobic organometal complexes may diffuse across the intestinal membrane and contribute to the lead flux towards the intestinal cells. Lead species such as lead phosphate complexes are large (compared to Pb$^{2+}$) and hydrophilic and are therefore considered not able to traverse the membrane by passive diffusion. However, lead bile complexes may be able to diffuse across the luminal membrane.
In that case, it can be expected that the contribution of lead bile complexes to the lead flux into the intestinal cells is much larger than the contribution of Pb\(^{2+}\), as the free Pb\(^{2+}\) fraction is negligible. Therefore, based on this mechanism, an increasing lead accumulation into the cells is expected for increasing bile levels. However, although Figure 5 shows a lot of scatter, an increasing trend with increasing bile levels is not observed. This implies that a contribution to the lead accumulation into the intestinal cells caused by diffusion of lead bile complexes across the membrane does not play an important role.

**Labile lead complexes.** If transfer of Pb\(^{2+}\) across the membrane is fast compared to transport through the diffusion layer along the intestinal wall, the free metal ion concentration next to the membrane decreases due to lead uptake. Then, some complexes can dissociate within the experimental time scale and the free lead ions thus produced can be absorbed as well (147). Under these conditions, not only the free metal ions contribute to the metal flux across the biological membrane, but also these rapidly dissociating, i.e. labile, metal species (147), although only lead in the form of Pb\(^{2+}\) is transported across the luminal membrane.

The experimental results indicate that uptake took (mainly) place via dissociation of labile lead complexes that then can contribute to the lead flux towards Caco-2 cells. Lead species that may contribute are lead phosphate and lead bile complexes, as they are important lead forms in chyme and voltammetrically labile. Voltammetric lability indicates that these species can dissociate within the time scale of the voltammetric experiment, i.e. within tenths of a sec, and contribute to the lead flux towards a mercury surface used by this technique (162).

The experimental conditions for the transport studies with Caco-2 cells were different than for the voltammetric studies. The equilibria between Pb\(^{2+}\) and complexed lead species were disturbed in the entire exposure medium, as the Caco-2 cells were able to accumulate about 27% of the lead in 24 hours. Consequently, dissociation of lead complexes was not related to the time for lead to diffuse across the diffusion layer along the intestinal cells. This indicates that, compared to the voltammetric situation, even more slowly dissociating lead complexes may have been able to dissociate and contribute to the lead accumulation into the cells.

An explanation based on transport of Pb\(^{2+}\) across the intestinal membrane, which is buffered by labile complexes, can account for the bile-level independent lead accumulation in the cells, although lead bile complexes constitute an important lead fraction in chyme (162). The fraction of lead bile complexes can be assumed to increase for increasing bile concentrations in DMEM/chyme. Consequently, the free Pb\(^{2+}\) and lead phosphate fractions decrease. If lead bile complexes do not contribute to the uptake flux, a decrease in the lead fraction that accumulates into the Caco-2 cells is expected with increasing bile concentration. In contrast, if the lead bile complexes do contribute to the uptake flux, a less steep decrease, no decrease or an increase might be observed. Although Figure 5 displays a lot of scatter, the fraction of lead that
accumulated into the cells does not show a clear decreasing trend with the bile concentration. Hence, the experimental results suggest that lead bile complexes indeed contribute to the flux towards the intestinal cells.

Furthermore, a pool of labile lead complexes that dissociate in order to restore the equilibrium with Pb\(^{2+}\) can explain the concentration independent accumulation into the cells. The free Pb\(^{2+}\) concentration can be considered as the driving force for lead transfer towards the intestinal cells. For higher total lead concentrations in DMEM/chyme, also higher Pb\(^{2+}\) concentrations are expected. The free Pb\(^{2+}\) concentration is not expected to change largely in time, since the labile lead complexes buffer the Pb\(^{2+}\) concentration. Hence, if the free Pb\(^{2+}\) concentration represents a constant fraction of the total bioaccessible lead concentration, the lead accumulation in the cells would display a linear relationship, as is measured.

As lead phosphate complexes are voltammetrically labile, they should be considered available for intestinal absorption. However, if macroscopic lead phosphate particles are formed, dissociation is expected to be slower than for microscopic lead phosphate complexes, making them less available for absorption. Therefore, if redissolution becomes slower, formation of macroscopic particles may decrease the lead flux towards intestinal cells.

These experimental results are in agreement with in situ studies by Cikrt and Tichý (96), and everted gut sac studies by Hilburn et al. (171), that showed that no decrease was observed for lead transport across the intestinal epithelium comparing the presence and the absence of bile. Toxicity caused by bile salts did probably not occur. The in situ study was performed at or below physiological bile levels as the experiments were performed with bile duct cannulated and bile duct non-cannulated rats (96). For the everted gut sac experiments low bile concentrations and short exposure times (compared to in vivo) were employed (171).

**In vitro versus in vivo**

For extrapolation from the present in vitro observations to in vivo absorption, the lead flux across the Caco-2 monolayer is employed. This gives a better indication of in vivo absorption than the cellular lead accumulation as it includes both the para- and transcellular permeation route. Artursson and Karlsson demonstrated that a good correlation was obtained between oral absorption in humans and P\(_{\text{coeff}}\) values determined in the Caco-2 model for a broad chemical range of compounds (165). The correlation shows that compounds with a P\(_{\text{coeff}}\) <1×10\(^{-7}\) are poorly absorbed, >10×10\(^{-7}\) cm/s are well absorbed, and in between are absorbed incompletely.

In the present study, the permeability of lead (1.7×10\(^{-7}\) cm/s) was 2-fold higher than the permeability of mannitol after exposure to culture medium (0.9×10\(^{-7}\) cm/s), and 8-fold lower than the permeability of mannitol after exposure to DMEM/chyme for 24 h (14×10\(^{-7}\) cm/s). These permeabilities are comparable to the P\(_{\text{coeff}}\) for ^3^H-mannitol (1.8×10\(^{-7}\) cm/s) found by
Artursson and Karlsson ( ). Hence, extrapolation to the situation suggests that lead is incompletely absorbed. This is in agreement in vivo studies, which typically show that salts, while children absorb more lead than adults do ( ).

**Oral bioavailability**

The incomplete bioaccessibility of lead determined by several physiologically based in vitro digestion models (for example ( ) ), demonstrates that mobilization from soil decreases the bioavailability of soil-borne lead. Oral bioavailability can cause a difference in the oral bioavailability of soil-borne lead is absorbed. Lead species that are likely to contribute to the lead flux towards intestinal cells larger experimental time scale also more slowly dissociating lead complexes may contribute to the intestinal absorption indicate that not all bioaccessible lead is absorbed. Therefore, also the mechanism of lead absorption, and the relation between lead absorption and lead speciation in bioaccessible lead from both matrices is incompletely absorbed. However, further research into

**Acknowledgement**

the Caco-Health and the Environment) for the assistance during digestions, and the Laboratory of Inorganic analytical Chemistry (National Institute of Public Health and the Environment) for analyzing the samples. Carolien Versantvoort is acknowledged for h manuscript. This work was supported by UTOX.