In human risk assessment, ingestion of soil is considered a major route of exposure to many soil-borne contaminants (1-6). For that reason, absorption and toxicity of ingested contaminants have been studied extensively. It appears that less absorption and toxicity in test animals is observed when contaminants are ingested with soil compared to contaminants that are ingested with food or liquid (7-14). Furthermore, the chemical form of the ingested contaminant (13,15-18), the physicochemical conditions in the gastro-intestinal tract (18-23), and the nature of the matrix, i.e. type of soil, food, liquid etc. (18,24,25), may affect the amount absorbed and, consequently, toxicity. The conditions in the gastro-intestinal tract can be altered by factors such as the ingestion matrix, time after a meal, malnutrition and diseases.

The differences in absorption and toxicity between the presence and absence of soil during ingestion, and between different soils, show that soil and its characteristics should be taken into account in order to perform accurate risk assessment of soil-borne contaminants. However, it is not feasible to perform in vivo studies for each specific soil of a contaminated site. Furthermore, discrepancies between an in vivo test animal study and the actual situation in humans can occur since the biochemistry and physiology of the gastro-intestinal tract may be different. Therefore, insight into processes determining oral bioavailability of soil-borne contaminants is required as foundation for a proper approach to estimate the exposure via this route, and to establish critical factors in the exposure to soil-borne contaminants. The objective
of the present thesis is to gain insight into determinants of oral bioavailability of several soil-borne hydrophobic organic compounds (HOCs) and a heavy metal (lead). Oral bioavailability of soil-borne contaminants is defined as the contaminant fraction that reaches the systemic circulation.

This chapter will start with an introduction of the test compounds. Prerequisite for understanding of oral bioavailability of soil-borne contaminants is knowledge on the functioning of the gastro-intestinal tract. Hence, some background information on the physiology of the gastro-intestinal tract is given. Important differences between the fed and the fasted state are mentioned. Extra attention is paid to the anatomy of the small intestine and the possible routes of absorption. In the following section the reader is familiarized with the different processes that can be distinguished for oral bioavailability of soil-borne contaminants. Finally, the scope of this thesis is presented.

**Test compounds**

Lead (Pb), arsenic (As), polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and to a lesser extent lindane, are contaminants that are frequently present in soil at levels above the present intervention value. Of this series, lead, PCBs and lindane are chosen as test compounds. In addition, the PCBs and lindane are chosen as they cover a range of hydrophobicity, and more practical, these compounds can be studied simultaneously since the use of gas chromatography with electron capture detector (GC-ECD) allows for specific and sensitive analysis of these compounds. Hence, it is possible to generate data for a broad range of hydrophobicity with a limited number of experiments. Furthermore, an inorganic contaminant, lead, is examined. It is to be expected that organic and inorganic compounds are affected by different factors during digestion and absorption. The different physicochemical properties of the contaminants can be employed to extend our understanding of the processes that dominate oral bioavailability for different chemical classes.

**PCBs.** PCBs contain two phenyl rings substituted with different numbers of chlorine atoms at various positions, see Figure 1. Hence, 209 different PCB congeners exist that each have specific physicochemical properties, which are determined by their degree and position of chlorination. Their hydrophobicity is represented by the high octanol-water partition coefficient, $K_{ow}$, which, depending on the congener, ranges between $10^{3.9}$ and $10^{8.3}$ (26).

PCBs have been widely used as plasticizer, flame retardant, and as dielectric fluid in transformers and capacitors. PCB production in Europe increased from the latter half of the 1950s, peaked at the end of the 1960s, and ceased by the 1970s after the discovery of the presence of PCBs in all compartments of the environment (27-29). Due to their chemical stability, high levels of PCBs are still found all through the environment (30-34). Studies in
mammals have shown that these may compounds cause weight loss, reproductive impairment, thymus atrophy, immune disorders, teratogenesis and Ah receptor binding (35). Carcinogenic effects have also been shown (36). The current Dutch intervention value for the sum of PCBs is 1.0 mg/kg dry matter soil (37), which is based on potential risks to humans and ecosystems.

**Lindane.** Lindane is the γ-isomer of hexachlorocyclohexane, and its structure is presented in Figure 1. Lindane has a $K_{ow}$ of $10^{3.8}$ (38), indicating the hydrophobic nature of the compound. Eight isomers of hexachlorocyclohexane exist, but the γ-isomer is the active compound due to its insecticidal activity. It has widely been used in veterinary medicine and as a household, agricultural and gardening pesticide. In the Netherlands lindane is presently almost abolished, with a few exceptions for crop protection. Lindane is degraded rapidly relative to PCBs, both in soil by action of microorganisms (39,40), and by the liver of higher organisms (41-43). Interconversion to other isomers can take place (39). Toxicity exerted by the hexachlorocyclohexane isomers has been related to interference with GABA receptors in the central nervous system (44). Lindane may act as a convulsant agent, and may cause both acute and chronic neurotoxic effects (45-48). In addition, hepatotoxic (47,49) and uterotoxic (47,50) effects have been observed. The β-isomer of hexachlorocyclohexane is known for its endocrine activity (51), and lindane might also act as an endocrine disrupter (52). The current Dutch ecotoxicological and human toxicological intervention value for lindane are 2.0 and 21.1 mg/kg dry matter soil, respectively (37).

![Figure 1. The general structure of PCBs and the structure of lindane.](image)

**Lead.** Many locations in the Netherlands contain relatively high levels of the heavy metal lead, mostly due to historic input (53). Several major sources of lead contamination can be identified, including leaded gasoline, leaded paint, shooting and stationary sources such as mining. Leaded gasoline represents the dominant diffuse source (54,55). The alkyl lead addition to gasoline was reduced from the 1970s throughout the 1980s and 1990s, and presently is ongoing in many countries all over the world (55). Although the anthropogenic lead emission
is declining, lead is still a high priority hazardous substance. Lead may elicit symptoms of the peripheral and central nervous system (2,56), while also the kidneys and gastro-intestinal tract may be affected (56). Lead is an animal carcinogen, but conclusive evidence for carcinogenesis in humans is lacking (56). In human subjects without obvious clinical signs, nonspecific symptoms such as fatigue, impaired concentration, loss of memory etc. may occur (2,56). Of main concern is mental retardation of children caused by chronic lead exposure (1,2). The current Dutch intervention value based on potential risks to humans and ecosystems is 530 mg/kg dry matter soil (37).

Physiology of gastro-intestinal tract

The gastro-intestinal tract is evolved in order to digest and absorb food, and can be divided into compartments with different functions. In the mouth the food is chewed to smaller pieces and homogenized with saliva, while also the enzyme α-amylase is excreted. This already starts the degradation process of starch. Subsequently, the homogenate is transported through the esophagus to the stomach. Here, it is acidified and enzymatic protein and fat digestion begins due to the action of pepsine and gastric lipase. The food homogenate is stored in the stomach until, depending on the size of the food particles, it is delivered to the small intestine. Bicarbonate secretions, both from the pancreas and from the gall bladder, neutralize the contents. These secretions are addressed in the present thesis as duodenal juice and bile, respectively. The resulting fluid in the small intestine is referred to as chyme. The enzymatic degradation process is continued, while the small intestine is also the site where absorption mainly takes place (23,57,58). Fat absorption is facilitated by accumulation of its degradation products, i.e. fatty acids, glycerol etc, in the interior of mixed micelles. The interface of mixed micelles is formed by bile salts, as these are amphipathic molecules. In this manner, fat is solubilized and its flux towards the intestinal cells is increased (23,58). The small intestine merges into the large intestine. The role of the large intestine is primarily to reabsorb water and to store unabsorbed material, while further degradation of remaining food components can take place due to microbial activity.

The presence of food can markedly alter the physicochemical conditions in the gastro-intestinal tract (22,23,57,59-62). An important difference is that the gastric pH is low for the fasted state and much higher for the fed state, the pH can be as low as 1 and as high as 6 for the respective conditions. Furthermore, the secretions of the gastric juice, duodenal juice and bile increase for the fed state, while the presence of food also delays the gastric emptying. To the contrary, both the human small intestinal pH of 5.5 to 7.5 and the intestinal transit time of about 2 to 5 h are hardly affected by the presence of food (57,59-62).
Small intestinal anatomy. The small intestine requires some further attention, since absorption occurs mainly here. The small intestinal epithelium is composed of an enormous number of small, fingerlike projections, which are directed into the lumen, see Figure 2. These projections, which are about one millimeter long, are called villi. Inside each villus are blood and lymphatic capillaries. The capillaries provide the routes by which compounds are transported to the rest of the body. The surface of each villus consists of a heterogeneous population of cells, which include enterocytes or absorptive cells, goblet cells, which secrete mucin, endocrine cells and several more cell types.

The enterocyte is the most common cell and is predominantly responsible for intestinal absorption. The luminal membrane of each enterocyte contains microvilli, each about 1 µm long and 0.1 µm in diameter. The microvilli, villi and large ridges in the intestinal tissue called plicae circularis attribute to an enormous magnification of the total surface area of the small intestine, of up to 200 m² for adults. This large surface area facilitates mass transfer from the intestinal lumen to enterocytes and thereby to the blood and lymph flow.

Intestinal absorption routes. Compounds can be absorbed across the intestinal epithelium either along the cells, i.e. the paracellular route, or through the cells, the transcellular route (63), see Figure 3. Transport via the paracellular route is reserved for small hydrophilic molecules only, due to the presence of tight junctions between the cells. Transcellular transport of a molecule can take place by passive diffusion, or by a specific carrier, either active or facilitated. Another possibility is that compounds are absorbed via transcytosis, which means
that a small volume of the intestinal fluid is invaginated by the cell membrane to form an endocytotic vesicle. This route is also referred to as pinocytosis.

\[\text{Figure 3. Schematic representation of the transepithelial permeation routes in the intestine.}\]

**ORAL BIOAVAILABILITY OF SOIL-BORNE CONTAMINANTS**

In the present thesis oral bioavailability is defined as the fraction of an orally administered dose that reaches the systemic circulation. The flow chart describes the different steps of oral bioavailability of soil-borne contaminants. After soil ingestion, contaminants can be partially or totally released from soil during digestion in the gastro-intestinal tract. The fraction of contaminant that is mobilized from soil into chyme is defined as the bioaccessible fraction. This fraction is considered to represent the maximum amount of contaminant available for intestinal absorption. Bioaccessible contaminants can subsequently be absorbed, i.e. transported across the intestinal wall, and transferred into the blood or lymph stream. The compounds may be biotransformed and excreted in the intestinal epithelium or liver. This is referred to as first-pass effect. After these steps, the contaminants reach the systemic circulation and thereby the rest of the body, and may exert system toxicity. Consequently, oral bioavailability of soil-borne contaminants is the resultant of the four steps of the flow chart: soil ingestion, bioaccessibility, absorption, and first-pass effect.
The different steps of exposure to soil-borne contaminants are discussed in more detail below.

**Soil ingestion**

Obviously, the amount of soil that is ingested largely determines the exposure to soil-borne contaminants. Both children and adults ingest soil via food. Sheppard and Evenden estimated the soil loads for leafy tissues after normal washing at 20 mg soil/kg dry weight, and for fruits at 2 mg soil/kg dry weight (64). Furthermore, soil is also ingested via hand-to-mouth behavior. Thereby, soil and dust particles that stick to an object or fingers are put into the mouth and are ingested. This is of special importance for children as they typically play outside and display hand-to-mouth behavior (65). Several studies have been performed to estimate the amount of soil that is ingested by children. These studies indicate that, in general, between 50 and 200 mg soil per day is ingested (66-70). Besides this normal hand-to-mouth behavior, some children deliberately ingest soil. Via this so-called pica behavior ingestion of several grams of soil on a single day, up to 60 g, has been observed (67,70,71).

For risk assessment purposes, the Dutch “National Institute of Public Health and the Environment” assumes that the daily intake of soil is 150 mg for children and 50 mg for adults (1). Other countries use similar values (1). For example, the U.S. Environmental Protection Agency (U.S. EPA) has assumed that 95 percent of children ingest 200 mg of soil per day or less, references in (1,67,71).

**Bioaccessibility**

After soil ingestion, contaminants can be mobilized from soil during digestion. This bioaccessible fraction of soil-borne contaminants is considered the fraction that is at maximum available for intestinal absorption. Different factors can affect bioaccessibility. For example, the bioaccessibility of metals and ionizable contaminants (acids and bases) from soil is expected to be highly dependent on the pH values in the different compartments of the gastro-intestinal tract (22,72,73).
Another factor that may affect bioaccessibility is the presence of food, which increases the transit time of the stomach. Therefore, the period in which mobilization can take place is increased, which may be important for compounds for which dissolution is rate limiting (23). Also an increased solubilizing capacity of the digestive mixture, due to an increased flow of the digestive juices or to the presence of food particles, may cause an increase in the mobilization of contaminants from soil (22,23). Especially bile is known to increase the solubilizing capacity for poorly water-soluble compounds, as bile salts form micelles that have an apolar interior (22,23). Furthermore, since bile salts have surfactant properties, they may increase the wetting and thereby the rate of mobilization from soil (22,23).

Absorption

It is generally accepted that at least the freely dissolved form is available for transport across a biological membrane (74-77), which is the case for the transcellular intestinal permeation route. Therefore, the freely dissolved contaminant concentration seems to be an important determinant for absorption. Besides this freely dissolved form, contaminants are present in the small intestine in other physicochemical forms. The distribution of compounds among different physicochemical species is referred to as speciation. The physicochemical properties of each form determine, together with properties of the intestinal cells, whether and to what extent a form can be absorbed. As precipitation and complexation reactions in the small intestine determine the contaminant speciation, they may affect absorption and oral bioavailability. Also, the flows of digestive juices determine the concentration of sorbing constituents and thereby influence the speciation, which in turn may change the absorption.

Extremely hydrophobic compounds such as PCBs are assumed to join the pathway for lipid transport. In line with fatty acid absorption, HOCs may accumulate in bile salt micelles, which can act as a transport vehicle towards the intestinal membrane. After dissociation from or degradation of the micelles, the PCBs probably traverse the luminal membrane by passive diffusion. The mechanism of HOC transport across the cells is not fully known, but this process is probably also comparable to the lipid pathway. Lipids are transported through the cells via very low density lipoproteins (78,79), and subsequently enter the lymph flow. Also highly hydrophobic compounds such as PCBs are almost exclusively transported to the lymph (23,78-80). As lipid assimilation is enhanced in the presence of food in the gastro-intestinal tract, transport of compounds following the lipid pathway may also be enhanced. Lindane can either follow the same route as the PCBs or cross the intestinal cells by passive diffusion only.
The exact mechanisms of absorption for lead are unknown and may follow the calcium pathway (18,81-84). Lead absorption is assumed to involve active and passive transport, both via the transcellular and paracellular permeation route (18,82). Transcellular metal uptake may first involve a binding step to the luminal membrane followed by internalization into the cell (82,85-88). Lead is mainly transported to the blood flow (80,82). Children are known to absorb more lead than adults do (2,82,89). Children have higher calcium absorption efficiencies than adults, which is induced by their elevated calcium demands for bone formation (90). Probably, for this reason is the efficiency of lead absorption higher for children than for adults.

It should be noted that the properties of the gastro-intestinal tract and the constituents that are present during digestion can affect both bioaccessibility and absorption.

**First-pass effect**

![First-pass effect diagram]

After absorption, the contaminants may be biotransformed and excreted by enzymes such as the cytochrome P-450s and P-glycoprotein pump in the intestinal cells (91-94). Contaminants that are transported from the intestine via the portal vein to the liver may be extracted and excreted into bile, or biotransformed by the liver, before the systemic circulation is reached. Consequently, less than the absorbed fraction of contaminants may pass the liver unchanged. This phenomenon of removing chemicals after oral absorption and before entering the systemic circulation is referred to as first-pass effect (95).

Lindane can be biotransformed in the liver (41-43). Lead is not biotransformed, but may undergo some biliary secretion (18,96). PCBs can be excreted into the bile and are biotransformed to a small extent (95). However, most absorbed PCBs circumvent first-pass liver metabolism and excretion, since they almost completely enter the lymph flow (78,97), which joins the systemic circulation without first being transported towards the liver.

**SCOPE OF THIS THESIS**

The objective of the present thesis is to gain insight into determinants of oral bioavailability of soil-borne PCBs, lindane and lead for children. Children are the group at risk as they are exposed to higher contaminant doses than adults, especially in relation to body weight. Furthermore, children may display increased oral bioavailability due to their high demand of
specific compounds, and they may also be more vulnerable to contaminants as vital organs are growing.

We consider soil ingestion as a given fact, and first-pass effect is not relevant or has been studied extensively for the presently used contaminants. Therefore, we will focus on several aspects of the second and the third step of the flow chart, bioaccessibility and absorption.

In the present thesis, we aimed at employing reproducible methods and conditions in order to investigate mechanistic aspects of oral bioavailability of soil-borne contaminants. To that end, artificial soil, i.e. OECD-medium, was used to obtain comparable ingestion conditions. Furthermore, in vitro models were employed to obtain reproducible digestion and absorption conditions that also allow for specific variations. An in vitro digestion model based on the physiology of children simulated gastro-intestinal digestion. This model was applied to investigate the bioaccessibility of the soil-borne contaminants. Subsequently, in vitro differentiated intestinal cells were employed to mimic intestinal absorption. This model was employed to investigate the absorption of bioaccessible contaminants, and to explore the effects of the different physicochemical forms on the intestinal absorption.

The research approach to investigate several aspects of oral bioavailability of the soil-borne HOCs and lead was similar. Therefore, the present thesis consists of two parallel research lines. Chapter 2, 3 and 4 apply for the HOCs, Chapter 5 and 6 apply for lead.

**PCBs and lindane**

In Chapter 2, mobilization of PCBs and lindane from an artificial standard soil, i.e. OECD-medium, during in vitro digestion is investigated. Furthermore, the effect of bile, digestive proteins and OECD-medium on the bioaccessibility is examined. A partitioning based model was developed and employed in order to estimate the distribution of the PCBs and lindane among bile salt micelles, proteins and OECD-medium. The impact of these constituents on the bioaccessibility of PCBs and lindane is discussed.

Non-equilibrium solid-phase microextraction (SPME) is applied in Chapter 3 to artificial chyme in order to estimate the freely dissolved concentration of PCBs and lindane. The contribution of PCBs sorbed to digestive proteins to the accumulation into this passive
chemical sampling phase is discussed. The SPME fiber is a passive sampling phase for HOCs in chyme. The HOC accumulation into the SPME fiber can be compared to the active and biological HOC uptake by small intestinal cells from a chyme solution.

Chapter 4 describes the uptake of the PCBs and lindane into in vitro intestinal Caco-2 cells. The Caco-2 cells are exposed to HOCs in different apical exposure media to different concentrations of HOCs. In this way, the absorption by intestinal cells of the HOCs that are mobilized from the OECD-medium during artificial digestion is investigated.

Lead

In Chapter 5, the mobilization of lead from OECD-medium during artificial digestion is investigated. Furthermore, the fraction of the free metal ion, Pb\(^{2+}\), is estimated. Main physicochemical forms of lead in chyme are determined and their dissociation and association kinetics are investigated and commented.

Chapter 6 describes the lead accumulation into and transport across a monolayer of in vitro intestinal Caco-2 cells. These results are related to lead speciation in the chyme solution and to human in vivo lead absorption. The contribution of different lead forms to lead absorption is considered.

General discussion

In Chapter 7, contaminant bioaccessibility, accumulation into a passive sampler and the intestinal cells, and transport across the cell monolayer, are interpreted in the perspective of mass transfer processes. With use of this interpretation the main factors that determine oral bioavailability of soil-borne PCBs, lindane and lead are discussed. Also, the effect of the physiological status on the oral bioavailability is considered. The differences and similarities between the different contaminants are commented and implications for exposure assessment for soil ingestion with these compounds are given.