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Review

Approaches to assess the oral bioaccessibility of persistent organic pollutants: A critical review

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Abstract

Oral bioaccessibility, also known as *in vitro* gastrointestinal extraction or the physiologically based extraction test (PBET), is an important tool when assessing the risk to humans from persistent organic pollutants (POPs) (and metals). The approach seeks to mimic the processes of human food digestion and thereby assess the bioavailability of POPs (and metals) from materials consumed either accidentally or intentionally in the diet. *In vitro* conditions are created to simulate various actions in the stomach and intestines (although some methods also include the mouth compartment). This paper reviews the current status of oral bioaccessibility with respect to the release of POPs from soil and related samples of environmental importance. Particular emphasis is placed on the parameters that influence gastrointestinal extraction including gastric and intestinal pH, enzymes, bile salts, food constituents and residence time. In addition, important developments in the use of *in vitro* gastrointestinal extraction are highlighted. These developments include the use of epithelial Caco-2 cells to mimic the intestinal cell lining, the potential for biotransformation of POPs into estrogenic metabolites as a result of colon microbiota, and the use of *in vivo* studies to validate existing approaches.

Keywords: Bioaccessibility; *In vitro* gastrointestinal extraction; Physiologically based extraction test; Persistent organic pollutants; Soils

Contents

1. Introduction

Persistent organic pollutants (POPs) are a class of organic compounds that have known toxic effects on humans (Jones and de Voogt, 1999; Orris et al., 2000). Historically, the use of dichlorodiphenyltrichloroethane (DDT) as a generic insecticide for the control of disease was an effective agent. However, it became apparent after approximately 20 years of use that it was persistent i.e. would not degrade significantly within a short time scale to become ineffective and harmless (Beard, 2006). It is

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now well known that DDT degrades into two metabolites (DDD and DDE), both of which are also persistent in the environment. This persistence has led to the banning of DDT in the developed world as a pesticide for control of insects. However, the legacy of DDT, and other such chemicals, has led scientists to explore the extent to which a chemical (e.g. POP) can be absorbed by a living organism i.e. its bioavailability. In this context a range of approaches have been considered to assess the bioavailability of POPs from the environment e.g. soil. Some of these approaches have evolved from the use of extraction methodology normally found in the analytical laboratory for the recovery of total POPs from soil (Dean, 2003). Examples include Soxhlet extraction, ultrasonic extraction, shake flask extraction as well as instrumental approaches including supercritical fluid extraction, microwave-assisted extraction and pressurized fluid extraction (Dean, 2003). In each of these approaches the common denominator is the choice of organic solvent to affect the recovery of POPs from soil (the exception is supercritical fluid extraction in which it is the combination of temperature and pressure alongside the use of carbon dioxide that affects recovery). Approaches to assess the bioavailability of POPs from soil have therefore relied on the choice of solvent (and extraction conditions) to partially recover the 'available' and 'unavailable' fractions. Examples include the use of mild organic solvents to affect the recovery of POPs from soil (Kelsey et al., 1997; Chung and Alexander, 1998; Liste and Alexander, 2002; Scott and Dean, 2003), the use of β-cyclodextrin to recover polycyclic aromatic hydrocarbons (PAHs) from soil (Reid et al., 2000b; Cuypers et al., 2002; Swindell and Reid, 2006), and the use of selective supercritical fluid extraction to assess polychlorinated biphenyl (PCB) and PAH bioavailability from soils and sediments (Bjorklund et al., 1999; Hawthorne and Grabanski, 2000; Nilsson et al., 2003). Alternative approaches have sought to mimic the 'bio' aspect more closely by using earthworms as indicators of POP bioavailability from soils (Morrison et al., 2000; Jager et al., 2005). Recent reviews have highlighted developments in this area of bioavailability (Reid et al., 2000a; Dean and Scott, 2004). This review however is concerned with developments in the oral bioaccessibility of POPs from soil (and related materials). Bioaccessibility can be defined as 'fraction of a chemical solubilized from a soil sample using *in vitro* test methods that simulate gastrointestinal conditions' (Kelley et al., 2002). This critical review assesses the different sample preparation approaches that have been developed to undertake oral bioaccessibility studies.

2. Physiology of the human digestive tract

In order to appreciate the stages that have to be considered in the development of an *in vitro* gastrointestinal extraction method requires an understanding of human physiology. A schematic diagram of the human digestive system (and associated accessory organs) is shown in Fig. 1. In the digestion process food enters via the mouth and exits via the anus. Therefore it is possible to identify seven specialist and different functions of the gastrointestinal tract (Dean, 2007; Wikipedia, 2007). They are as follows:

- **Ingestion.** The intake of material via the mouth.
- **Mastication.** The pulverization of material by chewing in the presence of saliva.
- **Deglutition**. The action of swallowing of the material which allows it to pass from the mouth to the stomach.
- **Digestion.** The mechanical and chemical breakdown of materials in the stomach.
- **Absorption**. The transfer of materials through the mucous membrane of the intestines into the blood stream.
- **Peristalsis.** The rhythmic, wavelike contractions that move material through the digestive tract.
- **Defecation.** The discharge of indigestible waste products from the body.

The processes that occur within the gastrointestinal tract can therefore be described. Material is **ingested** into the **mouth** causing initial breakdown in the presence of saliva at a pH of 6.5. The material is **masticated** allowing larger components to be broken down into smaller fragments. This increases the surface area of the material for digestion in the stomach. This entire process is relatively short lasting from a few seconds to a few minutes. The material is then **swallowed** and transported, via the **oesophagus**, into the stomach in a few seconds. The oesophagus is approximately 25 cm long. By the action of wavelike muscular contractions (**peristalsis**) swallowed material is transferred

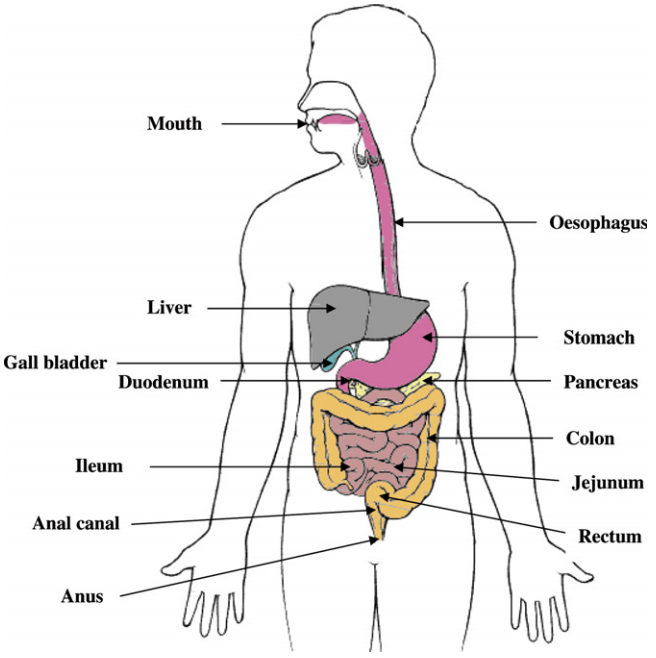

Fig. 1. The human digestive system including the gastrointestinal tract and associated organs.

from the mouth to the stomach. The **stomach** (a *J*-shaped pouch) stores material, initiates **digestion** of proteins, and transports it into the small intestine or duodenum. Cells within the stomach wall secrete the inactive enzyme pepsinogen and hydrochloric acid (pH < 2). The presence of hydrochloric acid has three functions: (1) proteins become denatured; (2) the enzyme pepsin is formed from pepsinogen; and (3) pepsin is more active at pH 2. The action of pepsin within the stomach can partially digest proteins (but not carbohydrates and fats). The **small intestine** is approximately 3 m long in the human body. It is composed of three parts the **duodenum** (20–30 cm long), the **jejunum** (approximately 110 cm long) and the **ileum** (approximately 165 cm long). The small intestines contain various enzymes including enterokinase which activates the protein-digesting enzyme trypsin which is secreted by the pancreas. The small intestines are the main site within the gastrointestinal tract where materials are **absorbed** including fats, carbohydrates, proteins, calcium, iron, vitamins, water and electrolytes. As well as having a regulatory effect on the chemical composition of blood, the liver produces and secretes bile which is initially stored in the gall bladder prior to its release into the duodenum. Bile is composed of bile salts (principally cholic acid and chenodeoxycholic acid), bile pigments (bilirubin), phospholipids (lecithin), cholesterol and inorganic ions (sodium, potassium, chloride and bicarbonate). The pancreas secretes pancreatic juice into the duodenum. Pancreatic juice contains water, bicarbonate as well as a range of digestive enzymes including amylase (digests starch), trypsin (digests protein) and lipase (digests triglyclerides). Water and electrolytes are absorbed from the food material in the large intestine or colon. After absorption of water and electrolytes the waste material that is left passes to the rectum. The build-up of rectal pressure, from the waste material, leads to the urge to **defecate**. At this point the waste products pass through the anal canal and exit the anus.

3. Considerations in the design and development of a simulated *in vitro* gastrointestinal extraction method

In vitro extraction methods are seeking to mimic the major processes that occur in the gastrointestinal tract in order to assess oral bioaccessibility. It is therefore possible to identify up to three distinct, but linked, areas of the human digestive system that are important when designing the extraction process (Dean, 2007; Wikipedia, 2007). These three areas are the mouth, stomach and (small) intestines (Fig. 2). While the mouth is an essential part of the entire human process of digestion in these *in vitro* approaches the fact that food remains in this compartment for a relatively short period of time means that it is often not included in simulated gastrointestinal extraction methods. In practice therefore *in vitro* gastrointestinal extraction approaches seek to mimic the human physiology of digestion by simulating the stomach where material is subject to pepsin at pH 2 for a few minutes (typically 8 min) to several hours (3 h). In addition, mimicking the small intestines (duodenum, jejunum and ileum) occurs by addition of intestinal juices composed of enzymes (trypsin, pancreatin and amylase), bile salts and bicarbonate.

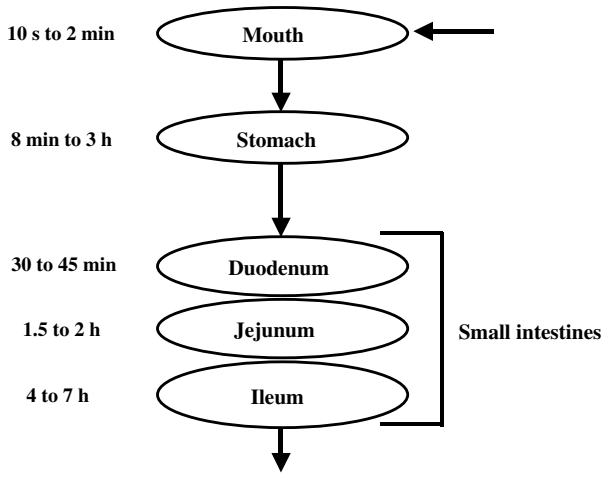

Fig. 2. Physiology of human contaminant uptake.

It is therefore important in the development of an *in vitro* gastrointestinal method that due attention should be given to the following:

- **Temperature**. Extractions should take place at body temperature i.e. 37 °C.
- **Shaking or agitation**. In order to mimic the peristaltic actions of the oesophagus extractions should be performed by shaking or agitation.
- **Mouth compartment.** Due to the short time period that material may spend in the mouth this aspect may be optional. However, if included, the material should be subjected to saliva at pH 6.5 for approximately 2 min.
- **Stomach compartment.** Sample should be subjected to pepsin and hydrochloric acid at a pH of 1–4 for approximately 3 h.
- **Small intestines compartment.** Sample should be subjected to intestinal juices at a pH of 4–7.5 for approximately 7 h.

The typical chemical composition of saliva, gastric juice, duodenal juice and bile juice is shown in Table 1 (Wittsiepe et al., 2001). However, the approaches that have been developed tend to use a simpler combination of chemicals and procedures. These have recently been reviewed to highlight the major variables used in current *in vitro* gastrointestinal extraction methods for metals, but the data is analogous to that used for POPs (Intawongse and Dean, 2006). The main variables for *in vitro* gastrointestinal extraction are as follows:

- **Sample to solution ratio.** This varies between 1:2 and 1:150 (g ml^{-1}).
- **Mixing and incubation time.** In order to simulate gastrointestinal mixing, the procedures employ a variety of shaking techniques e.g. shaking water bath, mechanical

Table 1
Composition of saliva-gastro-duodenal-bile juices[a]

Juice	Organic constituents	Concentration (mg l^{-1})	Inorganic constituents	Concentration (mg l^{-1})
Saliva	α-amylase	145	KCl	895
	Mucin	5	KSCN	200
	Urea	200	Na_2SO_4	570
	Uric acid	15	NaCl	290
			NaH_2PO_4	885
Gastric	Glucosaminhydrochloride	330	$CaCl_2$	200
	Glucose	650	HCl	1380
	Glucuronic acid	20	KCl	820
	Mucin	1500	NaCl	2750
	N-acetylneuraminic acid	50	NaH_2PO_4	270
	Pepsin, porcine	1000	NH_4Cl	305
	Serum albumin, bovine	1000		
	Urea	85		
Duodenal	Lipase	500	$CaCl_2$	200
	Pancreatin	3000	KCl	560
	Serum albumin, bovine	1000	KH_2PO_4	80
	Stearic acid	5	$MgCl_2$	50
	Urea	100	NaCl	7010
			$NaHCO_3$	1800
Bile	Bile, chicken	3000	$CaCl_2$	220
	Serum albumin, bovine	1800	KCl	370
	Urea	250	NaCl	5250
			$NaHCO_3$	4200

[a] Adapted from Wittsiepe et al. (2001).

stirring, argon gas dispersion, end-over-end rotation or peristaltic movement. Samples are mixed for different periods of time. For gastric juice extraction between 1 and 4 h, whereas a 1–5 h incubation is used for intestinal juice extraction.

- **Addition of alimentary components.** Whole milk powder or cream may be added to the synthetic gastric juices to simulate the influence of food on the mobilization of the contaminants and be more representative of human digestion. However, the inclusion of these products has been shown to enhance the solubility of POPs (Hack and Selenka, 1996).
- **Mouth.** As the pH of saliva is close to neutral, it is not expected to facilitate significant dissolution from foodstuffs. Often this stage is not included.
- **Gastric juice.** The major enzyme involved in gastric juice extraction consists of a solution containing pepsin with concentrations in the range 1.25–10 mg ml^{-1}. The enzyme is prepared in a dilute hydrochloric acid solution with the pH of the solution being adjusted in the range 1.1–4.0.
- **Intestinal juice.** The main enzymes used in intestinal juice are pancreatin and bile salt. Pancreatin is a mixture of lipase (to dissolve fat), protease (to digest proteins) and amylase (to breakdown carbohydrates). The addition of bile salts acts to help dissolve fat and aid in absorption in the small intestine. The pH of intestinal juice is maintained in the range 5.0–8.0.
- **Analysis of *in vitro* extracts.** The choice of analytical technique, and any sample pre-treatment required, to determine the POP concentration for each simulated extraction step could influence the data reported e.g. the use of liquid–liquid extraction (or solid phase extraction) prior to gas/liquid chromatography for analysis.

A typical generalized form of the *in vitro* gastrointestinal extraction procedure is shown in Fig. 3.

4. Approaches to assess oral bioaccessibility

The application of *in vitro* gastrointestinal extraction prior to chromatographic analysis to assess the bioaccessibility of POPs is summarized in Table 2. Following *in vitro* digestion, typically, the filtered supernatants were subjected to liquid–liquid extraction using *n*-hexane or dichloromethane, often supplemented with acetone or (m)ethanol and solid NaCl. Additional cleanup steps were performed in some studies including column separation of impurities and esterification of lipids with methanolic KOH. Finally, the extracts were evaporated to a small volume or dryness (and then re-dissolved) for further enrichment prior to chromatographic analysis.

Early work has sought to develop physiochemical approaches to assess POP bioaccessibility. The first exponents of the process were Rotard et al. (1992, 1995) in the early 1990s who studied the mobilization of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDDs/Fs) from an industriogenic soil i.e. red slag 'Kieselrot' used as a surface layer for playgrounds, sports field and pavements. A saliva-gastrointestinal (three compartment) model was formulated containing amylase, mucin, pepsin, pancreatin, bile and other permanent or potential compo-

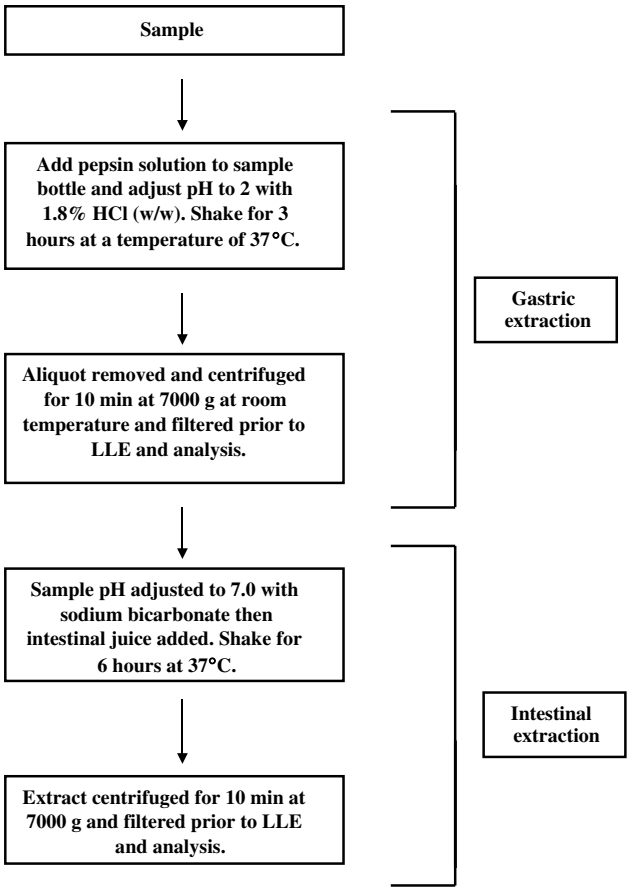

Fig. 3. Typical generalized form of the *in vitro* gastrointestinal extraction procedure (stomach and small intestines only).

nents of real digestive juices (detailed in Table 1); and less than 3% of individual congeners were solubilized. Subsequent modifications of the three compartment model by Oomen et al. (2000) allowed the availability of PCBs and lindane to be studied in a surrogate soil under fasting conditions. They also examined further uptake from the digests by intestinal epithelial Caco-2 cells (Oomen et al., 2001). Approximately 35% of the PCBs and 57% of lindane were bioaccessible after a default digestion; and based on a partitioning model, ~60% of the PCBs were sorbed to the soil, 25% to bile salt micelles and 15% to proteins (the respective values for lindane were 40%, 23% and 32%). The further uptake by intestinal epithelial cells was dose- and medium-dependent. Large fractions of PCBs and lindane accumulated in the cells indicating that pollutants sorbed to bile, oleic acid and digestive proteins contributed to the uptake flux toward the cells. The bioaccessibility increased 2–4 times when the bile content changed from none to 4-times the default level (Oomen et al., 2000). In a following study, however, bile of different animal origins showed little effect on the bioaccessibility of a PAH compound (Oomen et al., 2004).

Most approaches that have been investigated have ignored the first compartment i.e. the influence of saliva, due to the limited time that the sample is resident in the mouth compartment. A gastrointestinal model was developed to assess the influence of food materials on the mobilization of PAHs and PCBs by Hack and Selenka (1996). While the artificial gastric juice released between 3% and 22% of the pollutants from various samples (soil, sewage sludge, asphalt, metal scrap and blast sand), the gastricintestinal conditions with a bile concentration of $3\ g\ l^{-1}$ resulted in the mobilization of 5–40%, which further increased to 40–85% with the addition of lyophilized milk. A survey of 22 contaminated soils showed 7–95% of PAHs and 32–83% of PCBs were extracted. Wittsiepe et al. (2001) compared the two models for estimating PCDD/F bioaccessibility in the red slag 'Kieselrot', which ranged from less than 5% with a low bile content and in the absence of food material to more than 60% with a higher bile content and in the presence of whole milk powder. The presence of grape seed oil with a similar fat level to the milk powder increased the mobilization to a less extent. The degree of mobilization was dependent considerably on the bile concentration and supplementary lipophilic food material. It was also found to increase with the degree of chlorination (being generally higher for PCDFs than PCDDs), whereas it was noted the rate of absorption in animal digestive tract decreased in previous *in vivo* studies.

An upper small intestinal model was used by Holman et al. (2002) to evaluate the solubilization of total petroleum hydrocarbons (TPHs). The synthetic digestive fluid contained mixed bile salts and food-induced mixed intestinal lipids but not digestive enzymes because their previous work detected no interaction between TPHs and endogenous digestive enzymes. The TPH solubility was significantly higher for diesel than crude oil and much greater during fat digestion (bile salts, 20 mM) compared to during fasted states (bile salts, 0.1 or 5.0 mM), on average 16% versus <8% for diesel and 4.5% versus <1.2% for crude oil. The solubility increased with increasing concentration of bile salts and TPHs, but reduced with increasing soil organic carbon content. Nearly no effect of mixed intestinal lipids was found on pollutant solubilization particularly at higher bile concentration. Meanwhile Ruby et al. (2002) designed a physiologically based extraction test using artificial stomach and small intestinal fluids. The oral bioavailability of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and 16 other 2,3,7,8-substituted dioxin/furan congeners in soil ranged from 19 to 34% with an average of 25%, which was consistent with other animal studies (generally yielding 20–60% relative bioavailability for TCDD in soil). Bioaccessibility of individual congeners did not appear to be correlated with the degree of chlorination.

The bioavailability of PAHs in surface soils from various public areas was lately assessed by Tang et al. (2006) adopting the gastrointestinal extraction test. The bioaccessibility in small intestinal condition ranged from 9.2% to 60.5% of total PAHs in soil, constantly higher than that in gastric conditions which ranged from 3.9% to 54.9%; while the ratio of bioaccessibility between intestinal to gastric conditions varied from 1.1 to 9.7. The addition of bile

Table 2
Applications of *in vitro* gastrointestinal extraction to oral bioaccessibility assessment of POPs

Compounds	Simulated digestive extraction conditions	Analysis	Ref.
PCDDs/Fs	2 g sample added into 20 ml saliva (see Table 1) with or without 7 g milk powder and shaken at 200 rpm for 0.5 h at 37 °C 40 ml gastric juice (see Table 1) added and pH set at 2.0 with 1.8% HCl then shaken for 3 h 40 ml duodenal juice (see Table 1) added and pH set at 7.5 with $NaHCO_3$ then shaken for 1.5 h; 20 ml bile (see Table 1) added and shaken for 1.5 h Centrifuged at 7000*g* for 2 h at room temperature; supernatant filtrated through 5 μm sieve	GC-MS (high resolution)	Rotard et al. (1995), Wittsiepe et al. (2001)
PAHs and PCBs; PCDDs/Fs	1 g sample suspended in 105 ml distilled water and pH set at 2.0 with 1.8% HCl; 10 mg pepsin and 350 mg mucin added with no foodstuff or with 7 g whole milk powder or 1.82 g grape seed oil; suspension shaken at 200 rpm for 2 h at 37 °C Gastric digest neutralized (pH 7) with solid $NaHCO_3$; 10 mg trypsin, 350 mg pancreatin and 350 mg bile added; suspension incubated for 6 h Suspension centrifuged for 10 min at 7000*g* and room temperature; supernatant (~150 ml) filtered through 5 μm sieve	HPLC with fluorescence detector for PAHs and GC-ECD for PCBs; GC-MS for PCDDs/Fs.	Hack and Selenka (1996), Wittsiepe et al. (2001)
PCBs and lindane; Benzo[*a*]-pyrene (PAH)	0.6–0.9 g sample added into 9 ml saliva (290 mg l^{-1} α-amylase, 100 mg l^{-1} mucin and 30 mg l^{-1} uric acid plus inorganic and organic supplement) at pH 6.5 and rotated at 60 rpm for 5 min at 37 °C 13.5 ml gastric juice (2 g l^{-1} pepsin, 6 g l^{-1} mucin and 2 g l^{-1} BSA plus inorganic and organic supplement) at pH 1 added and rotated for 2 h 27 ml duodenal juice (6 g l^{-1} pancreatin, 2 g l^{-1} BSA and 1 g l^{-1} lipase plus inorganic and organic supplement) and 9 ml bile (12 g l^{-1} chicken bile and 3.6 g l^{-1} BSA plus inorganic and organic supplement) both at pH 8 added and rotated for 2 h Centrifuged at 3000*g* for 5 min	GC-ECD for PCBs and lindane; HPLC with fluorescence detector for PAH	Oomen et al. (2000, 2001, 2004)
TPHs	0.4 g sample added into 250 ml synthetic upper small intestinal digestive fluid at pH 6.5 containing 150 mM NaCl and 0.1/0.5/20 mM mixed bile salts (sodium salts of glycocholate, glycochenodeoxycholate, glycodeoxycholate, glycolithocholate, glycolithocholate sulfate, taurocholate, taurochenodeoxycholate, taurodeoxycholate, taurolithocholate and taurolithocholate sulfate) with or without 30 mM mixed intestinal lipids (cholesterol monohydrate, oleic acid, monoolein, diolein and lecithin); stirred at 37 °C for 4 h; centrifuged at 1100*g* for 45 min at 37 °C and supernatant permeated through a 0.45 μm filter	GC-MS	Holman et al. (2002)
PCDDs/Fs	10 g sample and 6 ml oleic acid added into 950 ml stomach fluid (2.5 g l^{-1} mucin, 1 g l^{-1} pepsin, 0.2 M glycine, 5 g l^{-1} BSA and 150 mM NaCl) at pH 1.5 and stirred for 1 h at 30 rpm and 37 °C pH adjusted to 7.2 with 10 ml of 50% NaOH; 600 mg porcine pancreatin and 4 g bovine bile added; stirred for 4 h Centrifuged at 3000*g* for 10 min	Not available	Ruby et al. (2002)
PAHs	20 g sample applied to 200 ml solution of 0.1 M $KHCO_3$ and 0.1 M NaCl; acidified to pH 1.5 with 1.3 ml of 5 M HCl; porcine pepsin added to a concentration of 10 mg l^{-1}; stirred at 150 rpm for 2 h at 37 °C Supplemented with 100 ml pancreatic juice (0.9 g l^{-1} porcine pancreatin powder, 12.5 g l^{-1} $NaHCO_3$ and 6 g l^{-1} Oxgall) at pH 6.3; stirred at 150 rpm for 5 h at 37 °C Supplemented with 100 ml microbiota suspension; stirred at 150 rpm for 18 h at 37 °C Centrifuged at 1500*g* (stomach and duodenum digests) or 3000*g* (colon digest) for 5 min	GC-MS	Van de Wiele et al. (2004, 2005)

Table 2 (*continued*)

Compounds	Simulated digestive extraction conditions	Analysis	Ref.
Phenanthrene (PAH) and PCBs	1 g sample added into 9 ml saliva (4 g l^{-1} mucin, 1 g l^{-1} urea, 0.6 g l^{-1} Na_2HPO_4, 0.99 g l^{-1} $CaCl_2 \cdot 4H_2O$, 0.4 g l^{-1} KCl and 0.4 g l^{-1} NaCl) at pH 6.5 and incubated for 5 min at 37 °C 14 ml gastric juice (3.2 g l^{-1} pepsin, 2 g l^{-1} NaCl and 0.7% HCl) at pH 3.0 added and incubated for 2 h 27 ml duodenal juice (0.2 M $NaHCO_3$) plus 7 ml bile both at pH 7.0 added and incubated for 2 h	HPLC-UV for PAH; GC-ECD for PCBs	Pu et al. (2004, 2006)
PAHs	6 g sample added into 600 ml gastric solution (1.25 g l^{-1} pepsin, 8.8 g l^{-1} NaCl, 0.5 g l^{-1} citrate, 0.5 g l^{-1} malate, 0.04% lactic acid and 0.05% acetic acid) at pH 1.5 adjusted with 12 M HCl and stirred at 100 rpm and 37 °C for 1 h Gastric digest adjusted to pH 7.0 with saturated $NaHCO_3$; 1.2 g porcine bile extract and 0.36 g porcine pancreatin added and stirred for 4 h Suspensions centrifuged at 7000*g* for 10 min and supernatant filtered through 0.45 μm glass fiber filter	GC-MS	Tang et al. (2006)
Pesticides, phenols and base-neutral compounds	5 g sample added into 15 ml of 0.1–1% pepsin in saline (154 mM) at pH 1.8 adjusted with HCl and shaken at 100 rpm and 37 °C for 3 h; centrifuged at 3000 rpm for 10 min at room temperature and supernatant filtrated Gastric digest residue added into 5 ml of 3% pancreatin, 5 ml of 1% amylase and 5 ml of 0.15% bile salts all in saline; pH adjusted to 7.0 with saturated $NaHCO_3$ or diluted NaOH and shaken for 3 h; centrifuged at 3000 rpm for 10 min and supernatant filtrated	GC-MS	Scott and Dean (2005), Esteve-Turrillas et al. (2005)

extract was found to enhance the bioaccessibility (possibly due to the formation of micelles improving the solubility) of individual PAHs to different extents. The soil organic carbon normalized bioaccessibility of individual PAHs in general decreased with the increasing ring number in both gastric and small intestinal conditions, however, the bioaccessibility ratio of individual PAHs between small intestinal to gastric conditions increased with increasing ring number, indicating the pronounced effect of bile salt on improving bioaccessibility of PAHs with relatively high ring numbers.

Dean and Scott (2004) discussed various *in vitro* gastrointestinal extraction procedures for assessing oral bioaccessibility of POPs. In their study on pesticides (lindane, endosulfan I, endosulfan II, endrin, DDE and DDD), phenols (cresol, 2,4,6-trichlorophenol and pentachlorophenol) and base-neutral compounds (hexachloroethane, acenaphthene, dibenzofuran, fluorene and hexachlorobenzene) in soils, the bioavailability following extraction with gastric fluid ranged from 0.8% to 8.3% while following intestinal extraction ranged from 5.5% to 13.5% (Scott and Dean, 2005). The majority of POPs present (i.e. >75%) would not be absorbed in the gastrointestinal tract and be excreted if consumed by humans. As the uptake of POPs to humans is more likely via the consumption of food crops grown on contaminated soil, the study was extended to test the bioavailability of organochlorine pesticides, endosulfan compounds, in lettuce (Esteve-Turrillas et al., 2005). Despite its known low bioaccumulation, endosulfan compounds were found in the roots of all lettuce plants irrespective of the pollutant level in soil or age of plant, and present in the leaves only when plants were grown on soil with high pollutant level and for long periods of time. When lettuce samples were subjected to *in vitro* digestion, no endosulfan compounds were detected in the gastric extracts while small quantities were found by the intestinal extraction, indicating <3.5% bioavailability.

An important aspect in the development of *in vitro* gastrointestinal models for the assessment of the environmental risk of POPs to humans is the potential for microbial degradation in the intestine compartment. This particular aspect identifies the major difference in the development of *in vitro* gastrointestinal models for POPs compared to metals. The potential microbial degradation of POPs in the intestine compartment could lead to the production of organic contaminant metabolites that are more toxic, and hence a greater risk to humans, than the parent POPs. Initial work by Van de Wiele et al. (2004) simulated the human intestinal microbial ecosystem comprising the stomach, duodenal and colon compartments to assess PAH release from soil. The freely dissolved PAH fraction in the stomach digest supernatant after centrifugation was only 0.44% of the total PAHs in soil when tested with a low liquid-to-soil ratio (volume/mass) of 10 (comparable with the fasting conditions of pica children). PAH release was enhanced with an increasing liquid-to-soil ratio, 0.83% in the stomach digest with a ratio of 40 which was then increased to 1.4% by the addition of food compounds. The lower PAH concentrations in the duodenum (0.13%) and colon (0.30%) digests were attributed to combined complexation and precipitation with bile salts, dissolved organic matter or colon microbiota after desorption. Mathematical equations were

developed and validated using model compounds to predict PAH complexation processes in the gastrointestinal tract. Pollutant release and subsequent complexation in the gut was thought to be an important prerequisite to intestinal absorption and thus bioavailability. Subsequent work by the group (Van de Wiele et al., 2005) has shown that human intestinal microbiota (extracted from human faeces) can lead to the formation of estrogenic metabolites. In particular, it was reported that microbial PAH transformation of pyrene and benzo(a)pyrene resulted in the formation of 1-hydroxypyrene and 7-hydroxybenzo(a)pyrene in colon digests. These findings are particularly important in the on going development of *in vitro* models to assess the environmental risk to humans as most other current approaches are excluding the potential for microbial transformation in the intestines.

The acceptance of *in vitro* gastrointestinal extraction as an approach to assess environmental risk to humans of exposure to POPs requires justification. This can only be done via the use of *in vivo* methods. This has been recently reported by Pu et al. (2004, 2006). This group evaluated the *in vitro* saliva-gastrointestinal extraction test for phenanthrene and PCBs in soils, in comparison with an *in vivo* rat model (soil slurry administered by gavage). Soils with higher organic carbon and clay contents resulted in lower bioavailability of phenanthrene in both tests, while no considerable differences were detected for PCBs in the *in vivo* assay. A significant correlation was observed between the *in vitro* mobilized fraction of phenanthrene from soil and its *in vivo* bioavailability ($r = 0.73$), but not for the PCBs. The *in vitro* test underestimated PCBs released from a soil in the *in vivo* assay. These findings therefore indicate that more work is required in the development of *in vitro* approaches, in order that better correlation exists with respect to *in vivo* approaches.

5. Conclusions and future development

Simulated gastrointestinal extraction provides important information for risk assessment of contaminated soil, waste and other related materials. It is clear that a range of different models have been adopted to mimic gastrointestinal digestion of POPs in environmental matrices and results obtained largely depend on the conditions (e.g. digestive juice constituents and their concentrations, food substance, liquid-sample ratio, incubations times, pH, centrifugation and filtration regime). In terms of the development of this approach as the method to assess bioaccessibility this is not beneficial. Future development requires the establishment of a standard protocol for the assessment of bioaccessibility of POPs from environmental matrices. The method validation has so far been limited while method development has been solely based on spiked and aged soil samples. Large scale inter-laboratory comparative studies could identify a common procedure and lead to the production of a number of reference materials for various POPs at different concentrations in major sample types, which would then facilitate the method validation and quality control schemes in testing laboratories. Such developments would also warrant an increasing application of the approach. Furthermore, due to obvious ethical issues restraining human *in vivo* studies, human cell lines and mammalian animal models are valuable to check the validity of the approaches as illustrated by recent reports (Oomen et al., 2001; Pu et al., 2004, 2006).

References

Beard, J., 2006. DDT and human health. Sci. Total Environ. 355, 78–89.

Bjorklund, E., Bowadt, S., Mathiasson, L., Hawthorne, S.B., 1999. Determining PCB sorption/desorption behaviour on sediments using selective supercritical fluid extraction. 1. Desorption from historically contaminated samples. Environ. Sci. Technol. 33, 2193–2203.

Chung, N., Alexander, M., 1998. Differences in sequestration and bioavailability of organic compounds aged in dissimilar soils. Environ. Sci. Technol. 32, 855–860.

Cuypers, C., Pancras, T., Grotenhuis, T., Rulkens, W., 2002. The estimation of PAH bioavailability in contaminated sediments using hydroxypropyl-β-cyclodextrin and Triton X-100 extraction techniques. Chemosphere 46, 1235–1245.

Dean, J.R., 2003. Methods for Environmental Trace Analysis. Wiley, Chichester.

Dean, J.R., 2007. Bioavailability, Bioaccessibility and Mobility of Environmental Contaminants. Wiley, Chichester.

Dean, J.R., Scott, W.C., 2004. Recent developments in assessing the bioavailability of persistent organic pollutants in the environment. Trends Anal. Chem. 23, 609–618.

Esteve-Turrillas, F.A., Scott, W.C., Pastor, A., Dean, J.R., 2005. Uptake and bioavailability of endosulfan by lettuce grown in contaminated soil. J. Environ. Monit. 7, 1093–1098.

Hack, A., Selenka, F., 1996. Mobilization of PAH and PCB from contaminated soil using a digestive tract model. Toxicol. Lett. 88, 199–210.

Hawthorne, S.B., Grabanski, C.B., 2000. Correlating selective supercritical fluid extraction with bioremediation behaviour of PAHs in a field treatment plot. Environ. Sci. Technol. 34, 4103–4110.

Holman, H.N., Goth-Goldstein, R., Aston, D., Yun, M., Kengsoontra, J., 2002. Evaluation of gastrointestinal solubilization of petroleum hydrocarbon residues in soil using an *in vitro* physiologically based model. Environ. Sci. Technol. 36, 1281–1286.

Intawongse, M., Dean, J.R., 2006. *In vitro* testing for assessing oral bioaccessibility of trace metals from soil and food samples. Trends Anal. Chem. 25, 876–886.

Jager, T., Van Der Wal, L., Fleuren, R.H.L.J., Barendregt, A., Hermens, J.L.M., 2005. Bioaccumulation of organic chemicals in contaminated soils: evaluation of bioassays with earthworms. Environ. Sci. Technol. 39, 293–298.

Jones, K.C., de Voogt, P., 1999. Persistent organic pollutants (POPs): state of the science. Environ. Pollut. 100, 209–221.

Kelley, M.E., Brauning, S.E., Schoof, R.A., Ruby, M.V., 2002. Assessing oral bioavailability of metals in soil. Battelle Press, Columbus, OH.

Kelsey, J.W., Kottler, B.D., Alexander, M., 1997. Selective chemical extractants to predict bioavailability of soil-aged organic chemicals. Environ. Sci. Technol. 31, 214–217.

Liste, H.-H., Alexander, M., 2002. Butanol extraction to predict bioavailability of PAHs in soil. Chemosphere 46, 1011–1017.

Morrison, D.E., Robertson, B.K., Alexander, M., 2000. Bioavailability of earthworms of aged DDT, DDE and DDD, and dieldrin in soil. Environ. Sci. Technol. 34, 709–713.

Nilsson, T., Sporring, S., Bjorklund, E., 2003. Selective supercritical fluid extraction to estimate the fraction of PCB that is bioavailable to a benthic organism in a naturally contaminated sediment. Chemosphere 53, 1049–1052.

Oomen, A.G., Sips, A.J.A.M., Groten, J.P., Sijm, D.T.H.M., Tolls, J., 2000. Mobilization of PCBs and lindane from soil during *in vitro* digestion and their distribution among bile salt micelles and proteins of human digestive fluid and the soil. Environ. Sci. Technol. 34, 297–303.

Oomen, A.G., Tolls, J., Kruidenier, M., Bosgra, S.S.D., Sips, A.J.A.M., Groten, J.P., 2001. Availability of polychlorinated biphenyls (PCBs) and lindane for uptake by intestinal Caco-2 cells. Environ. Health Perspect. 109, 731–737.

Oomen, A.G., Rompelberg, C.J.M., Van de Kamp, E., Pereboom, D.P.K.H., De Zwart, L.L., Sips, A.J.A.M., 2004. Effect of bile type on the bioaccessibility of soil contaminants in an *in vitro* digestion model. Arch. Environ. Contam. Toxicol. 46, 183–188.

Orris, P., Chary, L.K., Perry, K., Asbury, J., 2000. Persistent organic pollutants (POPs) and human health. World Federation of Public Health Associations' POPs Project, Washington DC.

Pu, X., Lee, L.S., Galinsky, R.E., Carlson, G.P., 2004. Evaluation of a rat model versus a physiologically based extraction test for assessing phenanthrene bioavailability from soils. Toxicol. Sci. 79, 10–17.

Pu, X., Lee, L.S., Galinsky, R.E., Carlson, G.P., 2006. Bioavailability of 2,3′,4,4′,5-pentachlorobiphenyl (PCB118) and 2,2′,5,5′-tetrachlorobiphenyl (PCB52) from soils using a rat model and a physiologically based extraction test. Toxicol. 217, 14–21.

Reid, B.J., Jones, K.C., Semple, K.T., 2000a. Bioavailability of persistent organic pollutants in soils and sediments – a perspective on mechanisms, consequences and assessment. Environ. Pollut. 108, 103–112.

Reid, B.J., Stokes, J.D., Jones, K.C., Semple, K.T., 2000b. Non-exhaustive cyclodextrin-based extraction technique for the evaluation of PAH bioavailability. Environ. Sci. Technol. 34, 3174–3179.

Rotard, W., Christmann, W., Knoth, W., Mailahn, M., 1992. Investigation on the absorption availability of PCDD/PCDF from industriogenic soil. Model experiments on absorption assessment after oral ingestion. Organohalogen Compounds 10, 199–200.

Rotard, W., Christmann, W., Knoth, W., Mailahn, M., 1995. Determination of absorption availability of PCDD/PCDF from Kieselrot – simulation of the digestion of technogenic soil (in German). UWSF-Z. Umweltchem. Ökotox. 7, 3–9.

Ruby, M.V., Fehling, K.A., Paustenbach, D.J., Landenberger, B.D., Holsapple, M.P., 2002. Oral bioaccessibility of dioxins/furans at low concentrations (50–350 ppt toxicity equivalent) in soil. Environ. Sci. Technol. 36, 4905–4911.

Scott, W.C., Dean, J.R., 2003. Selective extraction of persistent organic pollutants from environmental matrices. J. Environ. Monit. 5, 724–731.

Scott, W.C., Dean, J.R., 2005. An assessment of the bioavailability of persistent organic pollutants from contaminated soil. J. Environ. Monit. 7, 710–715.

Swindell, A.L., Reid, B.J., 2006. Comparison of selected non-exhaustive extraction techniques to assess PAH availability in dissimilar soils. Chemosphere 62, 1126–1134.

Tang, X., Tang, L., Zhu, Y., Xing, B., Duan, J., Zheng, M., 2006. Assessment of the bioaccessibility of polycyclic aromatic hydrocarbons in soils from Beijing using an *in vitro* test. Environ. Pollut. 140, 279–285.

Van de Wiele, T.R., Verstraete, W., Siciliano, S.D., 2004. Polycyclic aromatic hydrocarbon release from a soil matrix in the *in vitro* gastrointestinal tract. J. Environ. Qual. 33, 1343–1353.

Van de Wiele, T.R., Vanhaecke, L., Boeckaert, C., Peru, K., Headley, J., Verstraete, W., Siciliano, S., 2005. Human colon microbiota transform polycyclic aromatic hydrocarbons to estrogenic metabolites. Environ. Health Perspect. 113, 6–10.

Wikipedia, 2007. <http://en.wikipedia.org/wiki/Digestion> (last accessed on 09.03.2007).

Wittsiepe, J., Schrey, P., Hack, A., Selenka, F., Wilhelm, M., 2001. Comparison of different digestive tract models for estimating bioaccessibility of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/F) from red slag 'Kieselrot'. Int. J. Hyg. Environ. Health 203, 263–273.