

Evaluation of Gastrointestinal Solubilization of Petroleum Hydrocarbon Residues in Soil Using an In Vitro Physiologically Based Model

HOI-YING N. HOLMAN,*
REGINE GOTH-GOLDSTEIN,
DAVID ASTON, MAO YUN, AND
JENNY KENGSOONTRA

*E.O. Lawrence Berkeley National Laboratory,
1 Cyclotron Road, Berkeley, California 94720*

Petroleum hydrocarbon residues in weathered soils may pose risks to humans through the ingestion pathway. To understand the factors controlling their gastrointestinal (GI) absorption, a newly developed experimental extraction protocol was used to model the GI solubility of total petroleum hydrocarbon (TPH) residues in highly weathered soils from different sites. The GI solubility of TPH residues was significantly higher for soil contaminated with diesel than with crude oil. Compared to the solubility of TPH residues during fasted state, the solubility of TPH residues during fat digestion was much greater. Diesel solubility increased from an average of 8% during the "gallbladder empty" phase of fasting (and less than 0.2% during the other fasting phase) to an average of 16% during fat digestion. For crude oil, the solubility increased from an average of 1.2% during the gallbladder empty phase of fasting (and undetectable during the other fasting phase) to an average of 4.5% during fat digestion. Increasing the concentration of bile salts also increased GI solubility. GI solubility was reduced by soil organic carbon but enhanced by the TPH content.

Introduction

Soil ingestion is a potential exposure pathway that must be evaluated to establish risk-based cleanup levels for hydrocarbon residues at petroleum-contaminated sites. Petroleum hydrocarbon residues are hydrophobic molecules that remain tightly bound to soil particles after weathering. However, they can be solubilized in the aqueous gastrointestinal (GI) environments by mixed bile salts (MBS) and thus made available for uptake by epithelial absorptive cells (enterocytes) of the intestine (1). Food-induced mixed intestinal lipids (MIL), such as monolein and long-chain fatty acids, enhance GI solubilization of total petroleum hydrocarbon (TPH) residues. TPH uptake has been observed in the GI tract of small animals (2–7) and is likely to be comparable in humans.

Solubilization of TPH residues in the GI environment is prerequisite for their becoming available for absorption and entering blood circulation. In this study, a recently developed physiologically based small intestine (PSI) extraction model (8) was used to model human GI solubilization of TPH

residues in soils that were undergoing remediation. The formulation of this experimental model accords with known physiological data and existing in vitro methods of dissolution used for pharmacology/pharmacokinetics. We model only the upper small intestine, unlike other experimental measurements for bioavailability of soil-bound contaminants that mimic both the stomach and the small intestine (9–13). We do so because most solubilization of soil-bound TPH occurs in the upper small intestine because of the "detergency" characteristics of bile salts that enter this GI compartment from the gallbladder. The mechanical motions of the oral cavity, the contractile activity of the upper alimentary canal (esophagus and the stomach), and the gastric pH of the stomach merely alter the texture of the ingested soil by reducing the large soil aggregates to millimeter-sized particles (14, 15). However, Bost and Cuchens (16) did report that, for pure aromatic hydrocarbons, bypassing the stomach may alter their final absorption/distribution patterns within the lymphoid organs.

The extraction procedure of our present PSI model is built upon the premise that solubilization and absorption of TPH residues in GI environments are similar to the more thoroughly tested dissolution and uptake of hydrophobic drugs (17–21). Under this premise, the mixing of bile salts into the digestive fluid drastically enhances the solubility of hydrophobic petroleum hydrocarbons, both by lowering their surface tension and by combining with them to form "mixed micelles". These mixed micelles move in this form to and are absorbed by numerous epithelial cells (enterocytes) that line the surface of each of the fingerlike projections (villi) of the mucosa of the small intestine. A five-step solubilization mechanism, which is similar to the mechanism of detergency in an oily soil system (22), involves bile salt molecules (i) diffusing to the soil surface, (ii) absorbing onto the surface, and (iii) reacting with petroleum hydrocarbons to form mixed micelles and (iv) micelles containing petroleum hydrocarbons desorbing from soil surface and (v) diffusing into the lumen's bulk fluid (Figure 1A). Then a three-step absorption mechanism involves (i) micelles penetrating the unstirred layer, (ii) hydrocarbons adsorbing to the microvilli (small projections from each enterocytes), and (iii) hydrocarbons diffusing across enterocytes and entering portal blood and lymph circulations (Figure 1B).

The present PSI model also incorporates the effect of micelle size on the GI solubility of TPH. The size distribution of mixed micelles in the upper small intestine can vary from less than 0.040 to 0.7 μm , depending on the agitation intensity and the contents of the digestive fluid (15, 23, 24). Size is important because only micelles smaller than 0.5 μm can pass between the microvilli to reach the large surface area of the enterocytes (25, 26).

The synthetic upper small intestinal (SUSI) digestive fluid used in the model includes MBS and MIL but not the digestive enzymes. This strategy is based on results from our earlier batch experiments that detected no interactions between petroleum hydrocarbons and endogenous digestive enzymes. This interesting result implies that the structures of petroleum hydrocarbons may be xenobiologic, which precludes their interactions with endogenous digestive enzymes according to the structure–reactivity relationships proposed by Feaster et al. (27) and Carriere et al. (28). The purpose of this study was to utilize this in vitro model, based primarily on pharmaceutical and pharmacokinetic approaches and measurements, to elucidate factors controlling the GI solubility of total petroleum hydrocarbon (TPH) residues in highly weathered soils from different sites.

* Corresponding author phone: (510)486-5943; fax: (510)486-7152; e-mail: hyholman@lbl.gov.

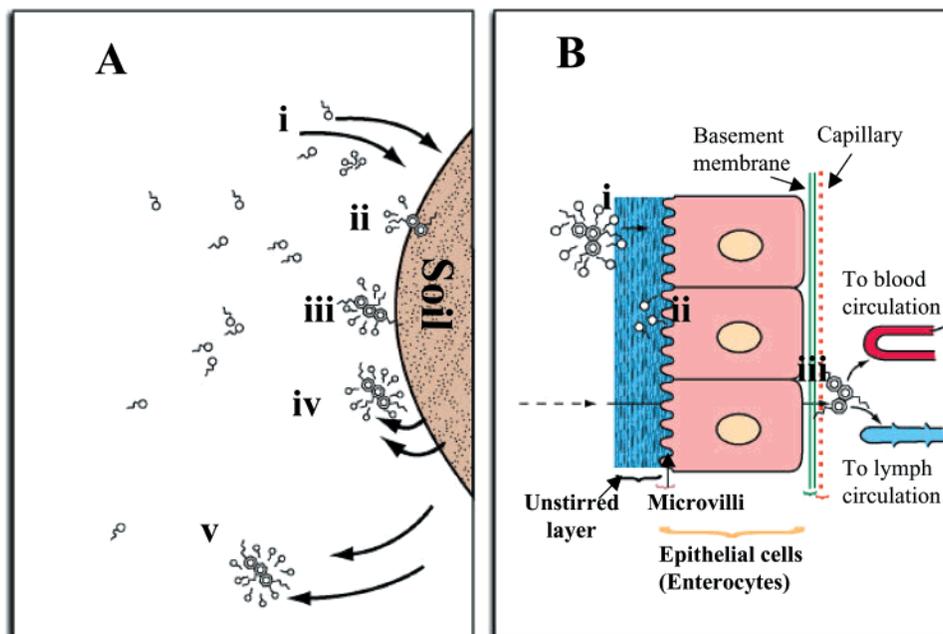


FIGURE 1. Schematic diagram illustrating mechanisms controlling (A) the solubilization and release of soil-bound petroleum hydrocarbons in the small intestinal lumen and (B) the transport of solubilized TPH from the lumen to the systemic circulation. The epithelial cells (enterocytes), which line the surface of each of the fingerlike projections (villi) of the mucosa of the small intestine, are replaced every 36 h as a result of the high mitotic activity of the cells at the base of the villi (67). See text for processes (i) through (v) in panel A and processes (i) through (iii) in panel B.

TABLE 1. Relevant Soil Properties

soil i.d.	clay/silt fraction (%) ^a	soil organic carbon (%) ^b	type of petroleum product	TPH (g/kg) ^c
A	95 ± 1.0	3.0 ± 0.1	diesel	11.7
B	65 ± 1.9	10.8 ± 0.4	crude oil	35.3
C	97 ± 0.5	5.5 ± 0.2	crude oil	10.2
D	48 ± 0.8	1.3 ± 0.1	diesel	12.8
E	40 ± 0.9	0.7 ± 0.1	diesel	5.0
F	37 ± 0.6	1.8 ± 0.2	diesel	5.2
I	33 ± 0.5	1.5 ± 0.1	diesel	5.2
J	54 ± 3.0	1.0 ± 0.1	crude oil	34.6
K	50 ± 1.0	6.8 ± 0.6	crude oil	9.8

^a Method of hydrometer (62); *n* = 2. ^b Modified Mebius Procedure (63); *n* = 2. ^c EPA SW-846 Method 8270 (47); *n* = 1.

Experimental Methods

All dissolution and extraction procedures were conducted at the human physiological temperature (37 °C). After incubating petroleum-contaminated soil in a continuous stirring reactor containing sterile SUSI digestive fluid for 4 h, the digestive fluid was filtered and analyzed for TPH. This TPH quantity was used to compute for the GI solubility of the soil's TPH residues. We varied the composition of the SUSI digestive fluid to mimic various digestion conditions. We measured relative GI solubility with respect to the digestion state, the petroleum products spilled, the soil organic carbon (SOC) concentration, and the silt/clay fraction in the soil.

Soil Samples. Highly weathered soils were collected from five diesel-contaminated and four crude oil-contaminated sites. Table 1 lists the soils chemical and physical properties. We utilized only soil particles that passed a 1-mm screen in order to mimic comminution in the mouth, esophagus, and stomach.

Chemicals. All chemicals were reagent grade and purchased from Sigma Chemical Co. (St. Louis, MO). Water was deionized, distilled, and then filtered to exclude dust particles larger than 0.1 μm. Anhydrous sodium phosphate (NaH₂-

PO₄) and sodium biphosphate (Na₂HPO₄) were used as received, while sodium chloride (NaCl) was used after being roasted at 600 °C in a muffle furnace for 4 h to oxidize and remove any organic impurity. The aqueous buffer was pH 6.5 (at 37 °C); it was prepared with 33 mM NaH₂PO₄ and Na₂HPO₄ in water.

We used 10 conjugated bile salts (namely, sodium glycocholate, sodium glycochenodeoxycholate, sodium glycodeoxycholate, sodium glycolithocholate, disodium glycolithocholate sulfate, sodium taurocholate, sodium taurochenodeoxycholate, sodium taurodeoxycholate, sodium tauroolithocholate, and disodium tauroolithocholate sulfate). They represent those found in human intestinal bile (24, 29). Although each bile salt has its own effect on the dissolution behavior of hydrophobic chemicals, their synergistic combination is extremely effective at solubilization (30–33). They were purified as described by Carey and Small (34) and then dried in vacuo for 36 h before use.

MIL consisted of cholesterol monohydrate, lecithin, oleic acid (as fatty acid), monoolein (as racemic monoacylglycerol), and diolein (as diacylglycerol). Cholesterol monohydrate and lecithin are normally found in human bile, while oleic acid, monoolein, and diolein normally exist as the end products from enzymatic action of pancreatic lipase during the digestion of dietary fat and triglycerides (24, 29, 35). They were stored according to manufacturers' instructions until use.

Preparation of MBS Stock Solution. The MBS stock solution was prepared by dissolving in the aqueous buffer 23.5 mM sodium glycocholate, 23.5 mM sodium glycochenodeoxycholate, 16 mM sodium glycodeoxycholate, 0.7 mM sodium glycolithocholate, 3 mM disodium glycolithocholate sulfate, 12 mM sodium taurocholate, 12 mM sodium taurochenodeoxycholate, 8 mM sodium taurodeoxycholate, 0.3 mM sodium tauroolithocholate, and 1 mM disodium tauroolithocholate sulfate. The final stock solution was 100 mM with respect to the total bile salt concentration.

Preparation of SUSI Digestive Fluid. Table 2 summarizes the composition of fasted and fat digestion state digestive fluids, which are in accord with published data (20, 24, 36–

TABLE 2. Composition of Fasted and Fat Digestion State Digestive Fluid Systems

	component	concn (mM)	
fasting	MBS	0.1	
	Na ⁺ (from NaCl)	150.0	
fasting (gallbladder empty phase)	MBS	5.0	
	Na ⁺	150.0	
fat digestion state	MBS	20.0 (5.0–25.0)	
	MIL	30.0	
	Na ⁺	150.0	
mixed bile salts (MBS)		100.0	
	sodium glycocholate	23.5	
	sodium glycochenodeoxycholate	23.5	
	sodium glycodeoxycholate	16.0	
	sodium glycolithocholate	0.7	
	disodium glycolithocholate sulfate	3.0	
	sodium taurocholate	12.0	
	taurochenodeoxycholate	12.0	
	sodium taurodeoxycholate	8.0	
	sodium tauroolithocholate	0.3	
	disodium tauroolithocholate sulfate	1.0	
	mixed intestinal lipids (MIL)	cholesterol monohydrate	1.9
		oleic acid	19.5
		monoolein	3.9
diolein		0.8	
lecithin		3.9	

39). The fasted state refers to the state similar to between meals and overnight fasting. The SUSI digestive fluid for fasting was the aqueous buffer medium with 0.1 mM MBS (40, 41) and a final Na⁺ concentration of 150 mM (24). Since the gallbladder also empties bile salts into the small intestine during the “intestinal housekeeping” phase of fasting, we also prepared the digestive fluid for this extraordinary event with the aqueous buffer medium with 5 mM MBS and again a final Na⁺ concentration of 150 mM. A 5 mM MBS is consistent with known bile salt mass–time profiles (39).

For the fat digestion state, the digestive fluid approximated the aqueous lipid compositions of the upper small intestine content after a meal of two fried eggs, bacon, toast with butter, and milk (24, 34). It was prepared first by dissolving into 200 mL of MBS stock solution 19.5 mmol of oleic acid, 3.9 mmol of monoolein, 0.8 mmol of diolein, 3.9 mmol of lecithin, and 1.9 mmol of cholesterol monohydrate. Aqueous buffer medium was then added to the mixture in sufficient quantity to produce a 1000-mL SUSI digestive fluid containing 20 mM MBS and 30 mM total intestinal lipids with a physiological fatty acid:monoacylglycerol molar ratio of 5:1. Sufficient NaCl was also added to the fluid to give a final Na⁺ concentration of 150 mM. Batch preparation of mixtures of MBS and MIL was used because the output of bile salts from the gallbladder in a human is stimulated by the ingestion of food and occurs by emptying from the gallbladder (42). This is equivalent to MBS output occurring as a (food) bolus that entered the upper small intestine concurrently with mixed intestinal lipids.

The mixture was mechanically stirred about a vertical axis at 20 rotations per minute for 2 days under sterile conditions using a flotation stirrer and a precision stirring apparatus (Thermolyne Type 45600 Cellgro stirrer). This stirring speed approximates the agitation intensity in the human GI tract; it is based on a comparison of results from our earlier in vitro solubility study of acetaminophen and the in vivo measurements presented by Katori et al. (43). A 2-day mixing was needed to attain an equilibrium size distribution of mixed micellar particles (24), which is prerequisite to reproducible experimental conditions.

In Vitro Gastrointestinal Solubilization Experiments.

Each experiment was duplicated. Each began by adding about 0.4 g (by dry weight) of soil into a continuous stirring reactor containing 250 mL of sterile SUSI digestive fluid. The daily dose estimated by EPA (44) was 0.4 g. Although the average volume of a meal is in the range of 300–500 mL (15), we used only 250 mL of digestive fluid. This is partly because 250 mL is about the aqueous volume used for experimentally measuring stomach and small intestinal uptake of environmental contaminants in ingested soil (9). More importantly, this is a sufficient fluid volume to preclude capacity limitations on the solubilization of TPH residues in our GI–soil system.

The mixture was stirred continuously at 37 °C for 4 h. Four hours is the average intestinal transit time through the small intestine (depending on the food contents and individuals), although clinical studies have shown that food-induced transit time is about half of the fasting transit time (45, 46). The mixture then was centrifuged at 1100g for 45 min at 37 °C to separate large soil particles, and the supernatant permeated gently through a 0.45-μm filter (of cellulose acetate polymeric membranes). This filtration was to simulate the diffusion of only those TPH-bearing micelles that were sufficiently small to pass between the brush-like microvilli and thereby reach beyond their tips to the much larger absorptive areas (enterocytes) along their sides (see Figure 1).

TPH Analysis. TPH in the filtrate was solvent extracted according to U.S. EPA Method 3550b (47). TPH remains in the soil was extracted using the accelerated solvent extraction technique (EPA Method 3545). TPH concentrations in the extract were measured by gas chromatography/mass spectrometry (GC/MS) using the procedure of EPA Method 8270 modified for TPH analysis. All analyses were performed by Sequoia Analytical Laboratory (Petaluma, CA). The detection limit was 1.5 μg/mL.

Calculation of In Vitro Gastrointestinal Solubility. The gastrointestinal solubility of TPH was expressed as a percentage of that present in the soil sample:

$$\text{solubility (\%)} = \frac{M_{\text{filtrate}}}{C_o W_{\text{dry}}} \times 100\%$$

where M_{filtrate} is the total mass of solubilized TPH (μg) in the filtrate, C_o is the concentration of TPH in the soil sample (μg/g), and W_{dry} is the dry weight (g) of the soil sample.

Mass Balance Analysis. The mass balance of the procedure ranged from 94% to 102% for the fat digestion state and from 96% to 100% for the fasted state.

Results and Discussion

Comparisons of GI Solubility of Different TPH during the Fat Digestion and Fasted States. Values of GI solubility of residual TPH in test soils are shown for fat digestion and gallbladder empty phase of fasting in Figure 2. An average of 12% of TPH residues in all soil samples were GI soluble in the fat digestion state, but less than 5% were GI soluble during the gallbladder empty phase of fasting. GI solubility was undetectable during the other phase of fasting. The composition of the three different types of synthetic digestive fluids used in this study were formulated on the basis of available physiological data to represent the fed and two different fasted states. Systems for the fed and gallbladder empty phase of fasting were comprised of the dominant surface-active species found in the upper small intestine at physiologically relevant concentrations. The system for the other phase of fasting was free of surface-active species. Our results indicate that the significant difference in the wetting properties of the synthetic digestive fluid for the fed state

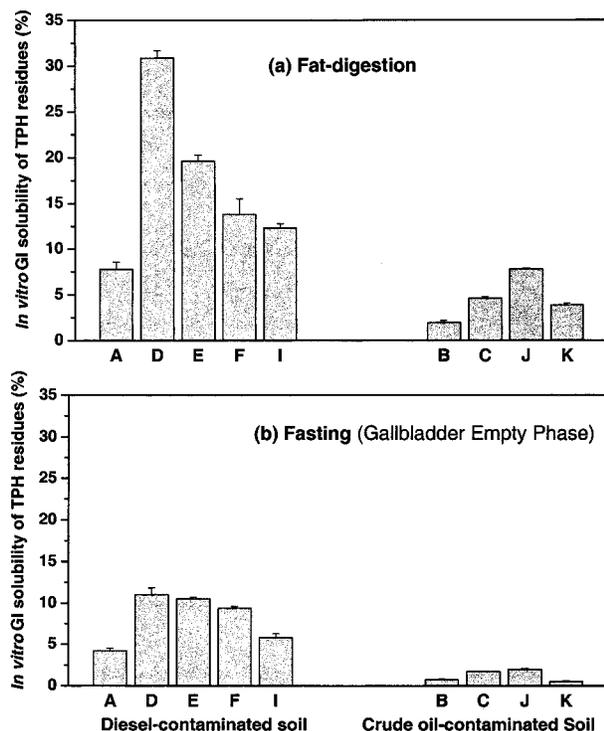


FIGURE 2. Comparisons of the averaged in vitro solubility of TPH residues ($n = 2$) in soils contaminated with diesel and crude oil during simulated (a) fat digestion state and (b) gallbladder emptying phase of fasting. No solubility was detected for the other phase of fasting. The error bar represents the range of the measured solubility.

relative to the different phases of fasting had substantially increased the solubilization of TPH relative to the fasted states. This enhancement of the solubility of hydrophobic petroleum hydrocarbons was accomplished by lowering their surface tension and by their combining with surface-active species to form "mixed micelles" that move away from the soil particles. A surfactant-like mechanism was also reported by Hack and Selenka (12), who observed a 2–4-fold increase in in vitro releases of PAH and PCB when their extraction fluid was supplemented with bile salts and lyophilized milk. Similar differences between fasting and fat digestion have been reported for the solubility and uptake of poorly water-soluble drugs by humans (18–20, 26, 40, 41, 48).

TPH residues from diesel-contaminated soils are more GI soluble than those from soils contaminated by crude oil. During fat digestion (Figure 2a), the average in vitro GI solubility was 4.5% (ranging from 2 to 7.8%) for crude oil-contaminated soils as compared to 16% (ranging from 7.7 to 31%) for all diesel-contaminated soils. Similarly, during the gallbladder empty phase of fasting (Figure 2b), the average in vitro GI solubility was 1.2% (ranging from 0.5 to 2%) for crude oil-contaminated soils as compared to 8% (ranging from 4 to 11%) for diesel-contaminated soils. More specifically, although the general properties of the diesel-contaminated soil D and crude oil-contaminated soil J are similar (Table 1), the solubility of TPH residues in soil D was 4–5-fold higher. In part, we attribute this contrast to the presence of high molecular weight hydrocarbons of extremely low solubility in crude oil (49). Comparisons of drug absorption profiles with drug hydrophobicity and drug molecular weights have shown that absorption of hydrophobic drugs declines at larger molecular weights (50, 51).

Effect of Mixed Bile Salts (MBS). During fat digestion state the in vivo concentration of MBS in the digestive fluid of the human small intestine depends on a meal's content and the individual. Even within normal physiological condi-

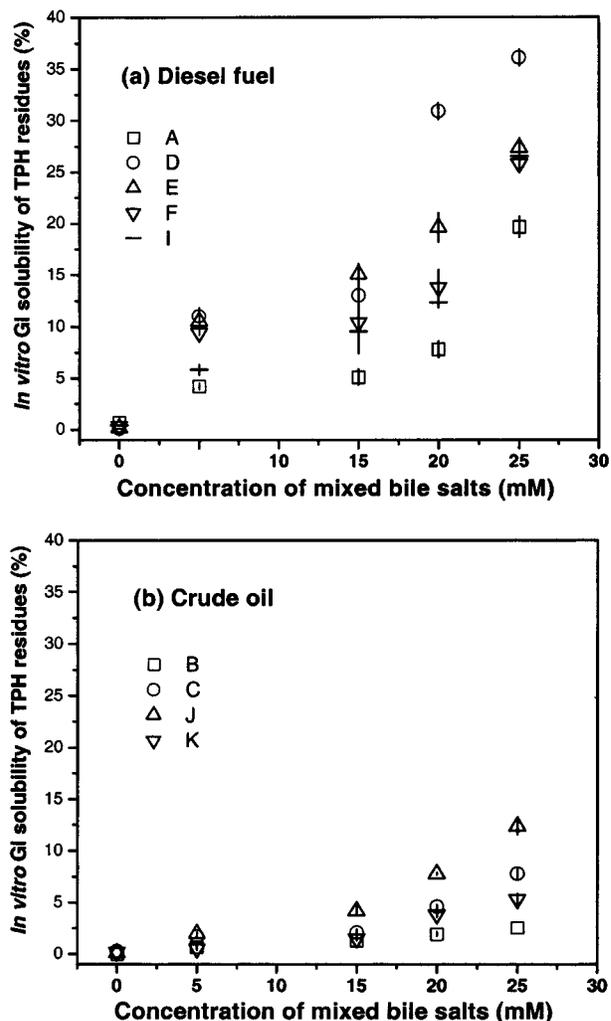


FIGURE 3. Comparisons of the averaged in vitro solubility of TPH residues ($n = 2$) at MBS concentrations of 0, 5, 15, 20, and 25 mM during simulated fat digestion state for soils contaminated with (a) diesel and (b) crude oil. The error bar represents the range of the measured solubility.

tions, values can range from less than 5 to about 25 mM (29, 42, 52, 53). In extreme cases, the MBS concentration can be up to 65 mM (29). To gain insight into their impact on TPH residue solubility, in vitro solubility measurements were made at MBS concentrations of 0, 5, 15, 20, and 25 mM. Figure 3 summarizes the results for soils from both diesel- and crude oil-contaminated sites. All (except soil B) show a slowly increasing TPH solubility between 5 and 15 mM and a rapid increase in TPH solubility through MBS concentrations from 15 to 25 mM. The abrupt change in slope near 15 mM reflects the critical micelle concentration (cmc) for these GI–diesel– or –crude oil–soil systems; 15 mM is within the range of cmc values that have been reported for bile salts in biorelevant media by Luner et al. (20) and Bates et al. (54). Soil B, the exception, was unusually rich in soil organic carbon (10.8%), to which TPH is bound more tightly. The initial increase in the TPH solubility below 5 mM MBS may indicate the enhancement from mixing intestinal lipids. We shall return to this in the next section.

Effect of Mixed Intestinal Lipids (MIL). The role of MIL on the GI solubility of TPH has been uncertain. Monoolein and lecithin, the key components of MIL, are insoluble swelling amphiphiles (20, 55, 56) that may enhance the solubilization of TPH in bile salt solutions. Oleic acid, on the contrary, may compete for micellar solubilization and hinder

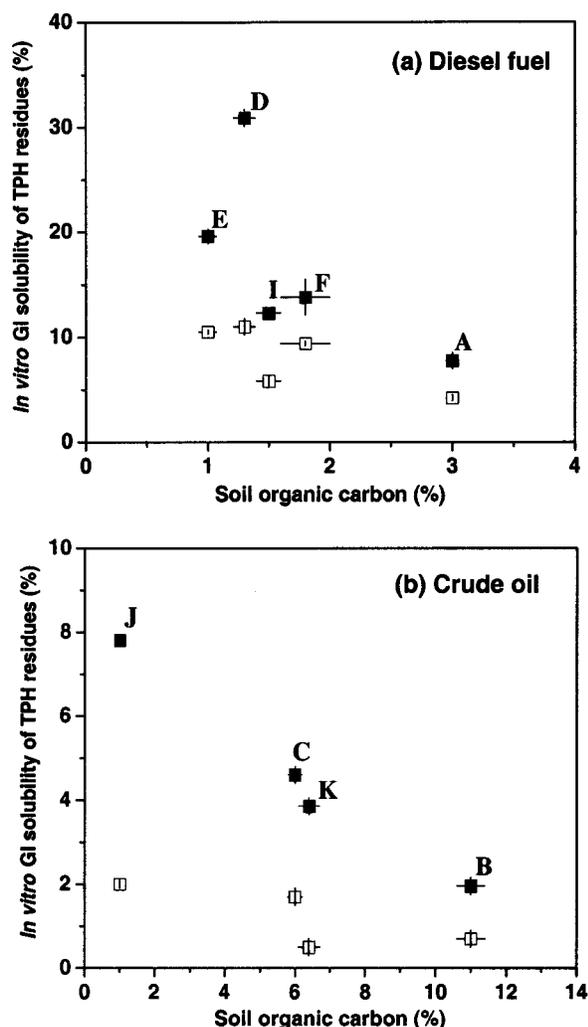


FIGURE 4. Averaged in vitro solubility of TPH residues ($n = 2$) as a function of SOC during simulated fat digestion state (■) and gallbladder emptying phase of fasting (□) for soils contaminated with (a) diesel and (b) crude oil. The error bar represents the range of the measured solubility.

the solubilization of TPH (53). To gain insights into the combined effects of food-induced MIL and MBS on the GI solubility of TPH residues, we repeated the in vitro experiments for the diesel-contaminated soil A and crude oil-contaminated soil J, using instead a MIL-free digestive fluid that contained 5 and 20 mM of MBS, respectively. When compared to our earlier results, MIL appears to enhance TPH solubility in diesel-contaminated soils but only at low concentrations of MBS. In the crude oil-contaminated soils, to the contrary, we found no effect. The average in vitro GI solubility of TPH residues at a concentration of 5 mM MBS was 2.3% ($\pm 0.2\%$) for soil A without the presence of MIL and 1.8% ($\pm 0.1\%$) for soil J. Our earlier measurements using a SUSI digestive fluid with both MBS and MIL (Figure 2) were nearly twice as great for soil A ($4.2 \pm 0.3\%$) and similar for soil J ($2 \pm 0.2\%$). But at the higher 20 mM MBS concentration, values of GI solubility were quite similar with or without the presence of MIL.

Effects of Soil Organic Carbons and Degrees of Contamination. The wide range of concentrations of soil organic carbon (SOC) and silt/clay fraction in our samples (see Table 1) allowed us to evaluate their influence on the GI solubility of TPH residues. For diesel-contaminated soils (Figure 4a) (which had SOC concentrations of 1–3%), GI solubility decreased with increasing SOC for both fat digestion and

fasted states. The correlation coefficients $r^2 = 0.46$ and 0.62 , respectively. Similar behavior was observed for crude oil-contaminated soils with a correlation coefficient $r^2 = 0.82$ for the fat digestion and 0.46 for fasted states (Figure 4b).

However, these soil samples were not equally contaminated. Our data, especially those for diesel-contaminated soils, suggest that there could also be a relationship between the concentration of TPH and their GI solubility. For example, the most heavily diesel-contaminated soil (D) also had the highest TPH solubility. To accurately assess the significance of SOC, one must remove the influence of different degrees of contamination among the soil samples. This was done by plotting the GI solubility of TPH of each soil sample against the SOC content normalized by the petroleum concentrations. This yielded better correlation coefficients, but only for diesel ($r^2 = 0.85$) in the fat digestion state and not for crude oil or fasting.

The unambiguous evidence of inverse relation between the in vitro GI solubility for diesel and SOC concentration is consistent with the known strong role of SOC in controlling the solid-liquid partitioning of hydrophobic organic chemicals (57–59). This is also consistent with the reported influence of SOC on the bioavailability behavior of hydrophobic organic compounds in animals (5, 60).

Significance in Risk-Based Assessment of Petroleum Contaminated Soils. Petroleum hydrocarbon residues pose risks through ingestion to humans only if they are solubilized in the GI lumen and became available for GI absorption. Our physiologically based extraction model of upper small intestine has shown that even though TPH residues are often tightly bound to soil particles, a portion can be solubilized in human GI environments and become available for absorption. The in vitro measurements indicate that an average of 12% (2–31%) of the TPH residues in these nine soils can be solubilized in human GI environments. Our measurements also indicate that the GI solubility of TPH is profoundly influenced by the digestion state, the type, and the concentration of petroleum hydrocarbons present and modified by other site-specific conditions such as the relative abundance of SOC.

GI solubility is not merely a prerequisite to uptake by blood or lymph but the dominant factor affecting the bioavailability of TPH (1). Our in vitro approach of upper small intestine facilitates its estimation for use in calculating site-specific risk-based cleanup levels. For example, assuming that all solubilized TPH residues in soil samples D, J, and B became bioavailable, the site-specific risk-based cleanup levels can be calculated using an ingestion absorption factor of 31% for site D, 8% for site J, and 2% for site B. These are upperbounds as it is unlikely that all the solubilized TPH inside enterocytes will be completely partitioned into the portal blood and lymph circulations within 36 h. Enterocytes are continually replaced as a result of the high mitotic activity of the cells at the base of the villi. The new cells migrate from the base to the top replacing older enterocytes, which disintegrate and are discharged into the lumen of the intestine. The entire epithelium of the intestine is replaced every 36 h (61). It is likely that part of the solubilized TPH will be discharged together with the epithelium lining as biological wastes.

Although the model was calibrated, in part, against human data for drug uptake, it is important that the in vitro measurements of solubilization of TPH can be related to their in vivo bioavailability. Because of the concerns of using human subjects in this type of research, we are currently conducting in vivo mouse experiments to evaluate the overall integrity of the in vitro model. Preliminary results indicate that the solubility values from in vitro model relate well with in vivo bioavailability data. As more in vitro/in vivo com-

parisons are completed, the underlying usefulness of our in vitro method in the site-specific risk assessment will be elucidated.

Acknowledgments

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Literature Cited

- (1) Hrudey, S. E.; Chen, W.; Rousseaux, C. G. *Bioavailability in Environmental Risk Assessment*; CRC Lewis Publishers: Boca Raton, FL, 1996.
- (2) van Schooten, F. J.; Moonen, E. J. E.; van der Wal, L.; Levels, P.; Kleinjans, J. C. S. *Arch. Environ. Contam. Toxicol.* **1997**, *31*, 317–322.
- (3) Koganti, A.; Spina, D. A.; Rozett, K.; Ma, B.-L.; Weyand, E. H.; Taylor, B. B.; Mauro, D. M. *Environ. Sci. Technol.* **1998**, *32*, 3104–3112.
- (4) Penry, D. L.; Weston, D. P. *Environ. Toxicol. Chem.* **1998**, *17*, 2254–2265.
- (5) Weston, D. P.; Mayer, L. M. *Environ. Toxicol. Chem.* **1998**, *17*, 830–840.
- (6) Fouchecourt, M. O.; Arnold, M.; Berny, P.; Videmann, B.; Rether, B.; Riviere, J. L. *Environ. Res.* **1999**, *80*, 330–339.
- (7) Roos, P. H.; Weissenfels, W. D.; van Afferden, M.; Pfeifer, F.; Hanstein, W. G. *Umweltwiss. Schadst.-Forsch.* **2000**, *12*, 13–19.
- (8) Holman, H.-Y. N. U.S. Patent 6040188, University of California, 2000.
- (9) Ruby, M. V.; Davis, A.; Kempton, J. H.; Drexler, J. W.; Bergstrom, P. D. *Environ. Sci. Technol.* **1992**, *26*, 1242–1248.
- (10) Ruby, M. V.; Davis, A.; Link, T. E.; Schoof, R.; Chaney, R. L.; Freeman, G. B.; Bergstrom, P. *Environ. Sci. Technol.* **1993**, *27*, 2870–2877.
- (11) Ruby, M. V.; Davis, A.; Schoof, R.; Eberle, S.; Sellstone, C. M. *Environ. Sci. Technol.* **1996**, *30*, 422–430.
- (12) Hack, A.; Selenka, F. *Toxicol. Lett. (Shannon)* **1996**, *88*, 199–210.
- (13) Williams, T. M.; Rawlins, B. G.; Smith, B.; Breward, N. *Environ. Geochem. Health* **1998**, *20*, 169–177.
- (14) Dressman, J. B. *Pharm. Res.* **1986**, *3*, 123–131.
- (15) Armand, M.; Borel, P.; Dubois, C.; Senft, M.; Peyrot, J.; Salducci, J.; Lafont, H.; Lairon, D. *Am. J. Physiol. (Gastrointest. Liver Physiol.)* **1994**, *266*, G372–G381.
- (16) Bost, K. L.; Cuchens, M. A. *Carcinogenesis* **1986**, *7*, 1251–1256.
- (17) Borgström, B.; Lundh, G.; Hofmann, A. *Gastroenterology* **1968**, *54* (Suppl.), 781–783.
- (18) Grisafe, J. A.; Hayton, W. L. *J. Pharm. Sci.* **1978**, *67*, 895–899.
- (19) Ritschel, W. A. *Methods Find. Exp. Clin. Pharmacol.* **1991**, *13*, 313–336.
- (20) Luner, P. E.; Babu, S. R.; Radebaugh, G. W. *Pharm. Res.* **1994**, *11*, 1755–1760.
- (21) Luner, P. E.; Babu, S. R.; Mehta, S. C. *Int. J. Pharm. (Amsterdam)* **1996**, *128*, 29–44.
- (22) Shaeiwitz, J. A.; Cussler, E. L.; Evans, D. F. *J. Colloid Interface Sci.* **1981**, *84*, 47–56.
- (23) Mazer, N. A.; Carey, M. C.; Kwasnick, R. F.; Benedek, G. B. *Biochemistry* **1979**, *18*, 3064–3075.
- (24) Staggers, J. E.; Hernell, O.; Stafford, R. J.; Carey, M. C. *Biochemistry* **1990**, *29*, 2028–2040.
- (25) Jennings, J. B. *Feeding, Digestion and Assimilation in Animals*; MacMillan St Martin's Press: London, 1972.
- (26) Muranishi, S. *Pharm. Res.* **1985**, *2*, 108–118.
- (27) Feaster, S. R.; Lee, K.; Baker, N.; Hui, D. Y.; Quinn, D. M. *Biochemistry* **1996**, *35*, 16723–16734.
- (28) Carriere, F.; Withers-Martinez, C.; Van Tilbeurgh, H.; Roussel, A.; Cambillau, C.; Verger, R. *Fett/Lipid* **1998**, *100*, 96–102.
- (29) Hernell, O.; Staggers, J. E.; Carey, M. C. *Biochemistry* **1990**, *29*, 2041–2056.
- (30) Stolk, M. F. J.; Van De Heijning, B. J. M.; Van Erpecum, K. J.; Van Den Broek, A. M. W. C.; Renooij, W.; Van Berge-Henegouwen, G. P. *J. Hepatol.* **1994**, *20*, 802–810.
- (31) Naylor, L. J.; Bakatselou, V.; Dressman, J. B. *Pharm. Res.* **1993**, *10*, 865–870.
- (32) Dangi, J. S.; Vyas, S. P.; Dixit, V. K. *Drug Dev. Ind. Pharm.* **1998**, *24*, 631–635.
- (33) Dangi, J. S.; Vyas, S. P.; Dixit, V. K. *Drug Dev. Ind. Pharm.* **1998**, *24*, 681–684.
- (34) Carey, M. C.; Small, D. M. *J. Clin. Invest.* **1978**, *61*, 998–1026.
- (35) Andersson, L.; Bratt, C.; Arnoldsson, K. C.; Herslöf, B.; Olsson, N. U.; Sternby, B.; Nilsson, A. *J. Lipid Res.* **1995**, *36*, 1392–400.
- (36) Mansbach, C. M. D.; Cohen, R. S.; Leff, P. B. *J. Clin. Invest.* **1975**, *56*, 781–791.
- (37) Dressman, J. B.; Berardi, R. R.; Dermentzoglou, L. C.; Russell, T. L.; Schmaltz, S. P.; Barnett, J. L.; Jarvenpaa, K. M. *Pharm. Res.* **1990**, *7*, 756–761.
- (38) Kararli, T. T. *Biopharm. Drug Dispos.* **1994**, *16*, 351–389.
- (39) Luner, P. E.; Amidon, G. L. *J. Pharm. Sci.* **1993**, *82*, 311–318.
- (40) Luner, P. E.; Vander Kamp, D. *Int. J. Pharm.* **2001**, *212*, 81–91.
- (41) Luner, P. E.; Vander Kamp, D. *J. Pharm. Sci.* **2001**, *90*, 348–359.
- (42) van Berge-Henegouwen, G. P.; Hofmann, A. F. *Gastroenterology* **1978**, *75*, 879–85.
- (43) Katori, N.; Aoyagi, N.; Terao, T. *Pharm. Res.* **1995**, *12*, 237–243.
- (44) U.S. EPA. *Exposure Factors Handbook—Update to Exposure Factors Handbook*; EPA/600/8-89/043; Office of Research and Development, U.S. EPA: Washington, DC, 1996.
- (45) Malagelada, J. R.; Robertson, J. S.; Brown, M. L.; Reminton, M.; Duenes, J. A.; Thomforde, G. M.; Carryer, P. W. *Gastroenterology* **1984**, *87*, 1255–1263.
- (46) Molino, G.; Hofmann, A. F.; Cravetto, C.; Belforte, G.; Bona, B. *Eur. J. Clin. Invest.* **1986**, *16*, 397–414.
- (47) U.S. EPA. *Testing Methods for Evaluating Solid Waste: Laboratory Manual; Physical/Chemical Methods*; Office of Solid Waste and Emergency Response, U.S. EPA: Washington, DC, 1986.
- (48) Stella, V. J.; Martodihardjo, S.; Rao, V. M. *J. Pharm. Sci.* **1999**, *88*, 775–779.
- (49) Flaherty, J. M.; Jones, B. H.; Nakles, D. V.; Andes, R. P.; Barkan, C. P. L. *Comparison of analytical methods for use in evaluating the risk from petroleum hydrocarbons in soil*; Remediation Technologies, Inc.: Pittsburgh, PA, 1997; p 98.
- (50) Borgstrom, B. *J. Lipid Res.* **1967**, *8*, 598–608.
- (51) Kimura, T.; Sudo, K.; Kanzaki, Y.; Miki, K.; Takeichi, Y.; Kurosaki, Y.; Nakayama, T. *Biol. Pharm. Bull.* **1994**, *17*, 327–333.
- (52) Brunner, H.; Northfield, T. C.; Hofmann, A. F.; Go, V. L.; Summerskill, W. H. *Mayo Clinic Proc.* **1974**, *49*, 851–860.
- (53) Hofmann, A. F.; Molino, G.; Milanese, M.; Belforte, G. *J. Clin. Invest.* **1983**, *71*, 1003–1022.
- (54) Bates, T. R.; Gibaldi, M.; Kanig, J. L. *J. Pharm. Sci.* **1966**, *55*, 901–906.
- (55) Hofmann, A. F.; Borgstrom, B. *Fed. Proc.* **1962**, *21*, 43–50.
- (56) El-Arini, S. K.; Shiu, G. K.; Skelly, J. P. *Pharm. Res.* **1990**, *7*, 1134–1140.
- (57) Ressler, B. P.; Kneifel, H.; Winter, J. *Appl. Microbiol. Biotechnol.* **1999**, *53*, 85–91.
- (58) Ghosh, U.; Gillette, J. S.; Luthy, R. G.; Zare, R. N. *Environ. Sci. Technol.* **2000**, *34*, 1729–1736.
- (59) Mulder, H.; Breure, A. M.; Rulkens, W. H. *Chemosphere* **2001**, *42*, 285–299.
- (60) Weston, D. P.; Lawrence, M. M. *Environ. Toxicol. Chem.* **1998**, *17*, 820–829.
- (61) Vander, A. J.; Sherman, J. H.; Luciano, D. S. *Human Physiology*; McGraw-Hill: New York, 1994.
- (62) Gee, G. W.; Bauder, J. W. in *Methods of Soil Analysis: Part 1 Physical and Mineralogical Methods*; Klute, A., Ed.; American Society of Agronomy/Soil Science Society of America: Madison, WI, 1986; pp 383–412.
- (63) Nelson, D. W.; Sommers, L. E. in *Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties*; Page, A. L., Ed.; American Society of Agronomy/Soil Science Society of America: Madison, WI, 1986; pp 539–580.

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