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Dermal Absorption and Tissue Disposition of 3,3′,4,4′-Tetrachlorobiphenyl (TCB) in an Ex-vivo Pig Model: Assessing the Impact of Dermal Exposure Variables

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TCB is one of the dioxin-like polychlorinated biphenyls (PCBs). This research was designed to help assess the risk of occupational and environmental TCB exposure. To evaluate exposure variables' effects on dermal absorption and cutaneous disposition, ^{14}C-TCB (40 μg/cm^2) in acetone, methylene chloride, a water–acetone mixture, and a soil-based mixture were applied in an ex-vivo pig-skin-flap model (n = 4–5/treatment). Dermal absorption (0.11–0.66%, 8 hr) and penetration (1.14–2.48%) varied according to exposure conditions. Acetone and methylene chloride vehicles differed in absorption profiles and skin penetration patterns but were similar in absorption amounts. Adding water to the acetone did not change absorption but did alter the penetration pattern. The non-occluded soil-based mixture showed more absorption than did the liquid vehicles ($p < 0.05$), but occlusion significantly ($p < 0.05$) decreased that absorption (0.66 →0.29%, 8 hr) and penetration (2.48 → 1.11%). In conclusion, dermal absorption data from liquid–organic or aqueous-organic mixtures may underestimate the risk of exposure to TCB-contaminated soil. *Key words:* 3,3′,4,4′-tetrachloro biphenyl (TCB); polychlorinated biphenyls (PCBs); skin; percutaneous absorption; exposure variables.

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Topical exposure to environmental and occupational chemicals is widely accepted as a primary route by which xenobiotics gain access to the systemic circulation. In addition, the skin is a direct target for chemicals that may produce toxic manifestations ranging from sensitization and acute irritation to proliferation, tumor promotion, and carcinogenesis.

The dermal transport process of chemicals is perhaps the most difficult to quantify and to predict,[1] since so many variables exert their influences on cutaneous disposition. In general, factors affecting cutaneous disposition involve the animal, the penetrant, the dosing vehicle, the exposure conditions (occlusion, dosage), and ambient environment. More importantly, interactions among these factors are critical to dermal absorption and risk assessment but have largely been ignored in the literature.

Polychlorinated biphenyls (PCBs) were first synthesized over 100 years ago and then massively produced and used until their production was banned in the late 1970s.[2,3] Their physicochemical stability, heat transfer, and electrical insulation properties led to their wide use, primarily in the electrical industry and secondarily in lubricants, papers, paints, inks, lacquers, varnishes, and pigments for plastics. Their widespread use and great resistance to environmental degradation resulted in extensive PCB contamination. PCBs were introduced into the environment in an unexpected way, mainly via industrial smoke and waste from the incineration of paper and plastic materials. PCBs have been detected in animal and human tissues in the absence of known industrial or occupational exposure, suggesting an obvious concern of environmental contamination. They are frequently found as complex mixtures of isomers in almost every component of the global ecosystem,[4] including air, water, soil, dust, and surface, in industrial and residential settings as well as in animal and human tissues, with a long half-life and a high tissue burden.[2]

The considerable health threat from PCB exposures via various routes has been widely recognized by regulators, researchers, and the general public. On average, occupational internal exposures doses can be as high as 10–1000 times that of non-occupational exposure.[3] Pharmacokinetics (PK), metabolism, health effects, and

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TABLE 1 Experimental Design

Vehicle Type	Application Vehicle Composition (65–70μL total)	Skin Occlusion	^{14}C-TCB Dose (μg/cm^2)	No.
Pure organic solvent	Acetone	—	40	4
	Methylene chloride	—	40	4
Organic + water mixture	Acetone + water (40/60%, v/v),	—	40	4
Soil-based mixture	Soil dust + acetone + water (54.7% + 30.6% + 14.7%, w/w/w	—	40	5
	Soil dust + acetone + water (54.7% + 30.6% + 14.7%, w/w)	+	40	4

related cellular or molecular mechanisms for gene regulation, enzymatic induction (e.g., P_{450} 1A1/1A2) and activation, and endocrine effects of individual PCB/metabolites have been studied.[4–8] PCBs can redistribute back into skin and adipose tissues after being absorbed into blood. Therefore, a high skin concentration and long residual time can be expected, with limited urinary and fecal excretion. It was found that highly chlorinated PBCs were retained in rat skin and slowly released into systemic circulation.[9]

As one of the dioxin-like PCBs, 3,3',4,4'-tetrachlorobiphenyl (TCB) is the most suitable for dermal PK studies while keeping its high toxicity in mind. Dermal absorption of TCB has been studied in various species, including pigs and monkeys.[10,11] Quite often, only one major metabolite can be derived from a PCB via oxidative dechlorination and conjugation (PCB → OH^- glucuronide conjugate). TCB may bind to the aryl hydrocarbon (Ah) receptor as an agonist.[4,12] The relationship between log Ko-w (octanol-water partitioning coefficient) and dermal absorption of TCB[4,7] and other PCBs[9] has been studied.

Generally, more highly chlorinated PCBs have slower dermal absorption, less metabolic elimination, lower DNA binding, and less acute toxicity, but longer tissue residue time, greater body burden, and higher cancer risk compared with PCBs with lower degrees of chlorination.[13] Dispositions of PCBs with various degrees of chlorination were kinetically compared in rats.[9] When selecting a PCB for dermal exposure assessment study, several factors have to be considered: 1) the potential for environmental and occupational dermal contact; 2) proper partition coefficient/PK properties; 3) metabolism rate; and 4) systemic and cutaneous toxicity. Obtaining data and techniques for extrapolating from hydrophilic and readily metabolized drugs to hydrophobic and slowly metabolized environmental toxicants such as PCBs has proven difficult.

Exposure conditions such as the vehicle (organic vs aqueous, liquid vs solid or semi-solid; single chemical vs mixture, etc.), occlusion, dosage, and even skin anatomic site may be critical to the cutaneous disposition, dermal absorption, and overall risk of a toxicant. In addition, dermal absorption model complexity in terms of histologic structure and physiologic function and ambient factor changes are also important issues in dermal data extrapolation.

The pig has been widely accepted as an animal model for studying human percutaneous absorption of a large variety of chemicals under various experimental conditions[14–16] because of the well-documented histologic,[17] physiologic, biochemical, and pharmacologic similarities between pig skin and human skin. Additionally, in terms of passive percutaneous absorption[15,18–21] as well as of iontophoretic transdermal drug delivery,[22] the pig appears to be an excellent animal model. An anatomically intact, viable, isolated, perfused, tubed ex-vivo skin preparation, known as isolated perfused porcine skin flap[23] (IPPSF), can overcome many of the limitations associated with traditional in-vivo animal and in-vitro diffusion-cell models.

The aim of this IPPSF study was to evaluate the effects of dermal exposure variables (application vehicle and skin occlusion) on TCB dermal absorption and cutaneous tissue disposition in order to facilitate dermal data extrapolation in environmental and occupational risk assessment of TCB and other PCBs.

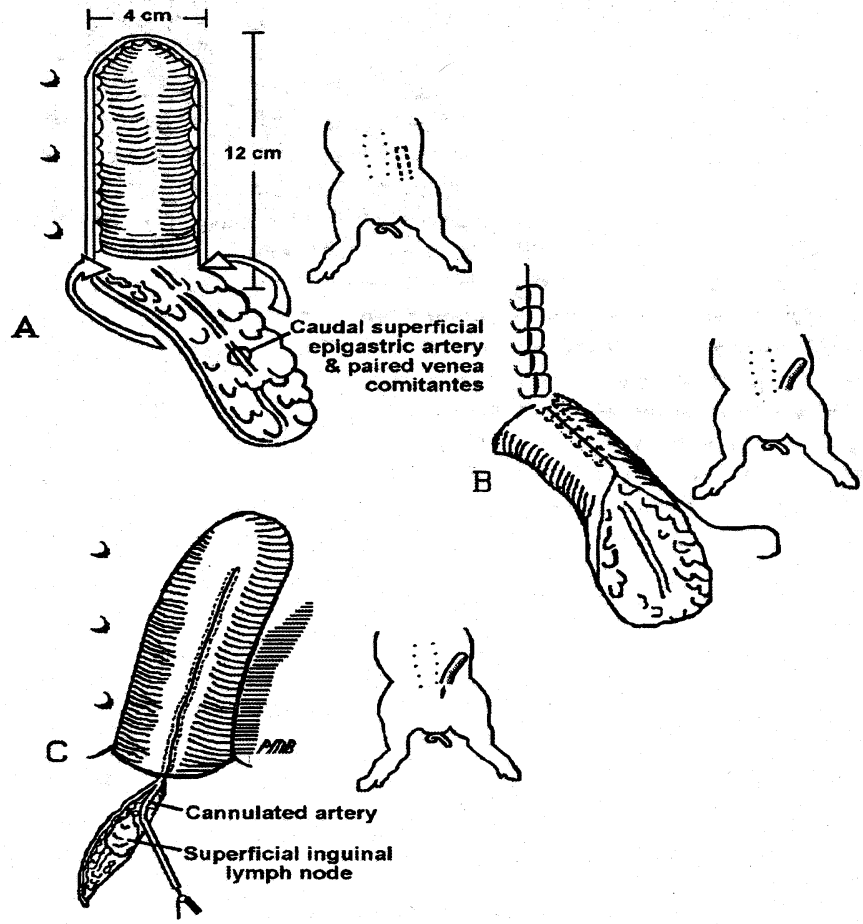
MATERIALS AND METHODS

Chemicals. [^{14}C-UL]TCB (12.7 mCi/mmol) and application vehicles including acetone and methylene chloride were purchased from Sigma Chemical Co. (LC grade, St. Louis, MO). Ethyl acetate (LC grade, Fisher Scientific, Fair Lawn, NJ) was used as a sample extraction solvent.

Dose formulations and dosages. ^{14}C-TCB was formulated into either a single acetone, a methylene chloride, an acetone–water (40/60%,v/v), or a soil-based mixture (acetone–water–soil dust) vehicle for topical application (Table 1). A representative Cecil soil sample, common in the Piedmont region of North Carolina and other southeastern states of the United States, was obtained from West Raleigh, North Carolina. Soil analysis showed that the acidic (pH 5.3) soil sample contained 31.2% sand, 16.8% silt, 53.0% clay (90% kaolinite), 0.3% organic matter, and 1.0% water

(air-dried). The soil dose using non-sterilized dust was prepared several hours before topical application and contained air-dried soil + water + acetone (54.7% + 30.6% + 14.7%, w/w/w) and TCB at 0.1 μCi/mg. TCB dosage in all formulations was uniformly selected as 40 μg/cm^2 skin.

Animals. Eight- to ten-week-old female weanling Yorkshire–Landrace cross pigs (~20 kg, Neuhoff Farms, Inc., Greenville, NC) were acclimated a week before harvest of the ex-vivo skin flap from an abdominal site. The animals were given 15% (protein) Pig-and-Sow pellets (~2 lb/pig/day, Wayne Feed Division, Continental



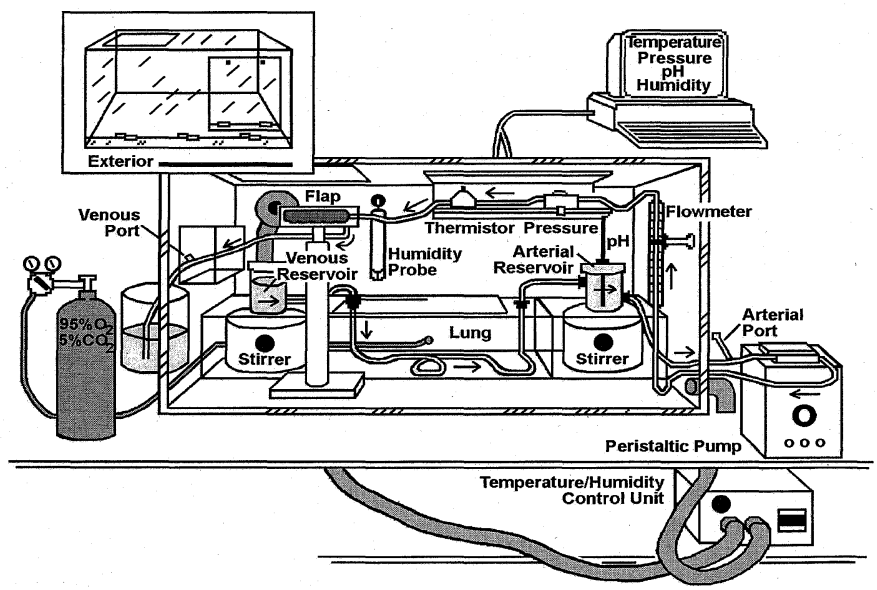


Figure 1—Isolated perfused porcine skin flap (IPPSF) model and perfusion chamber.

Grain Co., Chicago, IL) and free access to drinking water. All animals were humanely handled according to preapproved animal use and care protocols during the entire process.

Skin flap. IPPSF creation procedures and perfusion experimental protocols are described elsewhere.[23] In brief, an acclimated female pig was aseptically prepared for surgery in the caudal abdominal and inguinal areas. As showed in Figure 1, a 4 × 12-cm^2 skin area was marked on each side of the ventral abdomen. Following incision and scalpel dissection of the subcutaneous tissue, the caudal incision was apposed and sutured, and the tubed skin flap edges trimmed of fat and closed. The flap areas were protected by a bandage. Two days later, a second surgical procedure was performed to cannulate the caudal superficial epigastric artery perfusing the flap area and to harvest/isolate the flap. The flap was transferred to the temperature- and humidity-regulated perfusion chamber, where it was placed on a cradle for perfusion (Figure 1). A one-hour pre-dosing perfusion for system stabilization and an eight-hour post-dosing perfusion for data collection were allowed. Perfusate flow rate, pH, temperature, and relative humidity (RH%) were monitored and constantly maintained at 1 mL/min/flap (3–7 mL/min/100 g), 7.4, 37° C, and 60–80%, respectively, using a non-recirculating perfusion system configuration. Flow rate and perfusion pressure (mean arterial pressure 30–70 mm Hg) were recorded every 30 minutes during the experiments. The perfusion medium was a Krebs–Ringer bicarbonate buffer (pH 7.4, 350 mOsm/kg), containing albumin (45 g/L) and glucose (80–120 mg/dL) to facilitate chemical partitioning from tissue into perfusate and to provide an energy source for the living skin.

Pre-dosing stabilization. During the one-hour pre-dosing perfusion, 1.0-mL arterial and 6.0-mL venous perfusate samples were collected at 30 and 60 minutes to confirm adequate glucose utilization (arterial and venous) and acceptable background TCB (venous) levels. After confirming flap viability (glucose utilization > 0.2 mg/g tissue/hr or arterial–venous glucose concentration difference of > 10 mg/dL), perfusion was interrupted and the flap was temporarily removed from the chamber for dosing preparation.

Dosing. A Stomahesive® template was glued onto the flap using Skin Bond, leaving a 1 cm × 5 cm skin area uncovered for TCB application. The flap was then returned to the chamber and perfusion was resumed. The PCP dose of 65–70 μL liquid or 65–70 mg soil containing 200 μg of ^{14}C-TCB (40 μg/cm^2) was evenly applied onto the 5-cm^2 skin surface within 2 minutes using a Hamilton syringe or a dosing rod. The dosed skin area was either left open (non-occlusive) or closed with Parafilm (occlusive). TCB dosing solutions and soil-based paste were analyzed before and after dosing procedures to confirm the dosage applied.

Post-dosing perfusion. After dosing, 1.0-mL arterial samples (perfusate before entering the IPPSF) were taken hourly. Venous samples (6.0 mL each) were collected every 30 minutes for eight hours. Each sample was then aliquotted into four tubes containing 1, 1, 1, and 3 mL, respectively. Hourly samples were analyzed for glucose utilization immediately after sampling (1-mL arterial and 3-mL venous) and all other samples were analyzed for TCB only.

Perfusion termination and full mass balance procedures are described elsewhere.[20] The flap and cradle were removed from the chamber and the dosing template (patch) was removed from the flap. The dosed skin surface was twice swabbed with 2 × 2 inch gauze wetted with 5% aqueous soap. Each swab was placed in a vial containing 15 mL ethyl acetate. The stratum corneum within the dosed skin area was isolated by 12 Scotch-tape strippings. Each two successive tape strips were digested in 15 mL ethyl acetate. The underside of the flap and the cradle were rinsed with 10 mL water into a vial. The cradle was rerinsed with an additional 10 mL water as described above. The dosed skin was excised and then a small center portion of this sample (core) was collected for tissue sectioning in the penetration pattern study (see below). The remaining dosed skin was weighed and digested in 15 mL Soluene at 50° C (water bath). The skin samples under the patch and the skin under the patch opposite the dose site were cut out, weighed, and digested as above. The remainder of the flap was frozen in liquid N_2 and stored at −20° C. The pooled waste venous effluent was assayed for ^{14}C. All vials/tubes were capped, sealed with Parafilm, and stored at −20° C until further analysis.

Cutaneous penetration determination. The core skin samples were prepared according to the procedures for routine frozen tissue sectioning. In brief, a skin sample was first reshaped (trimmed) to ensure the same size/volume of each tissue section. All the tissue trimmed off was collected for ^{14}C assay. The reshaped tissue sample was continuously sectioned from the epidermis surface all the way down to the dermis and subcutaneous tissues (0 to 3,300 μm) at a thickness of 80 μm for each section. The tissue sections were separately oxidized for total ^{14}C analysis as described below.

Total ^{14}C determination. After complete digestion, 100 μL of each Soluene tissue digestion sample or ethyl acetate tape-strip sample and 250 μL of each venous effluent or ethyl acetate extract were pipetted into combusto-cones with pads to be oxidized (Packard Tissue Oxidizer, Model 307) and counted on a Packard TR1900 Scintillation Counter.

Data processing and statistical analysis. Al real-time data were handled by our copyrighted computer database to facilitate data analysis (Roundup®, North Carolina State University, Raleigh, NC). Total ^{14}C percutaneous absorption was determined as the percentage of the topically applied radioactive dose appearing in the venous

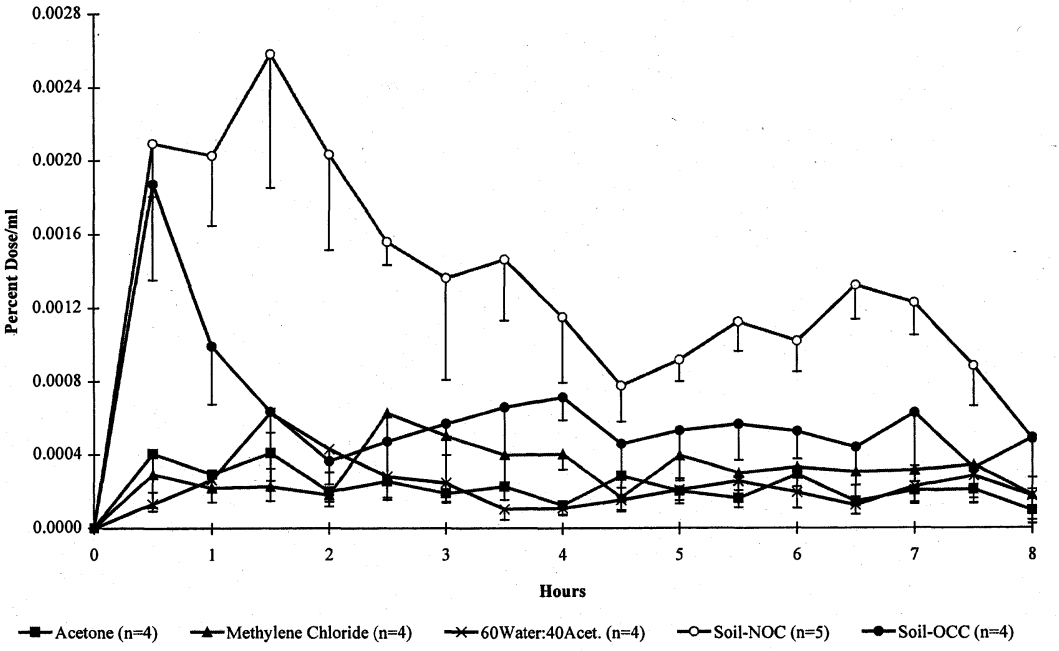


Figure 2—^{14}C concentration profiles in perfusate (mean ± SEM) following (^{14}C-UL)TCB topical application in the ex-vivo IPPSF model.

effluent (perfusate) over the entire eight-hour experimental period. Penetration is defined as the sum of absorption and local tissue residues excluding stratum corneum residue. All parameters were determined for each individual flap and the group means were calculated and statistically compared across treatments through General Linear Model Procedure with LSD multi-comparison test at $\alpha = 0.05$ (SAS Institute, Cary, NC).

RESULTS

TCB dermal absorption time courses, as illustrated by the perfusate ^{14}C concentration profiles, are shown in Figure 2. Vehicle-dependent *dermal absorption profiles* were observed. Comparing the two pure organic vehicles, it was found that TCB in acetone showed a higher dermal absorption during the first two hours, but methylene chloride gave a larger absorption rate from the second hour through the eighth hour after TCB application. Adding water to the acetone solvent slowed the dermal absorption during the first hour but accelerated it from hour 1 to hour 3. After the third hour, dermal absorption profiles were similar for the acetone and acetone–water mixture vehicles. Although addition of water delayed the dermal absorption peak, the maximal absorption peak was higher with the acetone–water mixture than with acetone only. In the case of TCB exposure in a soil-based matrix (dust, moisture, and organic solvent), the dermal uptake was much higher than that in any of the liquid formulations tested. The perfusate TCB concentration was enhanced as much as 4–13-fold

TABLE 2 Cutaneous Disposition Parameters of [^{14}C]-TCB Following Topical Application in the Ex-vivo IPPSF Model

	Acetone ($n = 4$)	Methylene Chloride ($n = 4$)	Water ($n = 4$)	Soil—Non-occlusive ($n = 5$)	Soil—Occlusive ($n = 4$)
Dosing device (%D)	13.14 (3.13)^{a*}	10.73 (1.23)^{a}	12.74 (1.23)^{a}	3.33 (0.79)^{b}	1.33 (0.31)^{b}
Surface swabs (%D)	31.90 (1.57)^{c}	64.01 (7.81)^{a}	55.74 (4.57)^{ab}	56.67 (4.68)^{ab}	35.24 (6.59)^{bc}
Stratum corneum (%D)	5.72 (1.84)^{a}	3.93 (0.98)^{ab}	1.74 (0.46)^{bc}	0.90 (0.09)^{bc}	0.50 0.05)^{c}
Dosed skin (%D)	0.22 (0.09)^{ab}	0.41 (0.12)^{a}	0.21 (0.04)^{ab}	0.18 (0.01)^{b}	0.14 0.01)^{b}
Tissue total (%D)	1.02 (0.39)^{a}	1.13 (0.16)^{a}	1.42 (0.34)^{a}	1.67 (0.42)^{a}	0.82 (0.18)^{a}
Absorption (%D)	0.11 (0.01)^{b}	0.14 (0.03)^{b}	0.14 (0.02)^{b}	0.66 (0.06)^{a}	0.29 (0.07)^{b}
Penetration (%D)	1.14 (0.39)^{b}	1.28 (0.15)^{ab}	1.57 (0.33)^{ab}	2.48 (0.44)^{a}	1.11 (0.21)^{b}
Absorption/ penetration ratio	0.12 (0.03)^{b}	0.12 (0.02)^{b}	0.10 (0.02)^{b}	0.28 (0.03)^{a}	0.27 (0.05)^{a}
Recovery (%D)	52.20 (2.00)^{b}	80.49 (8.77)^{a}	72.28 (4.09)^{a}	64.67 (4.98)^{ab}	39.48 (6.13)^{b}
Mean absorption time (hour)	3.76 (0.42)^{a}	4.10 (0.14)^{a}	4.05 (0.17)^{a}	3.80 (0.32)^{a}	3.52 (0.36)^{a}
Peak flux (%D/min/cm^2)	0.00014 (0.00004)^{a}	0.00012 (0.00002)^{a}	0.00013 (0.00002)^{a}	0.00133 (0.00064)^{a}	0.00037 (0.00007)^{a}

*Means with the same superscript letter are not significantly different across the five treatment groups.

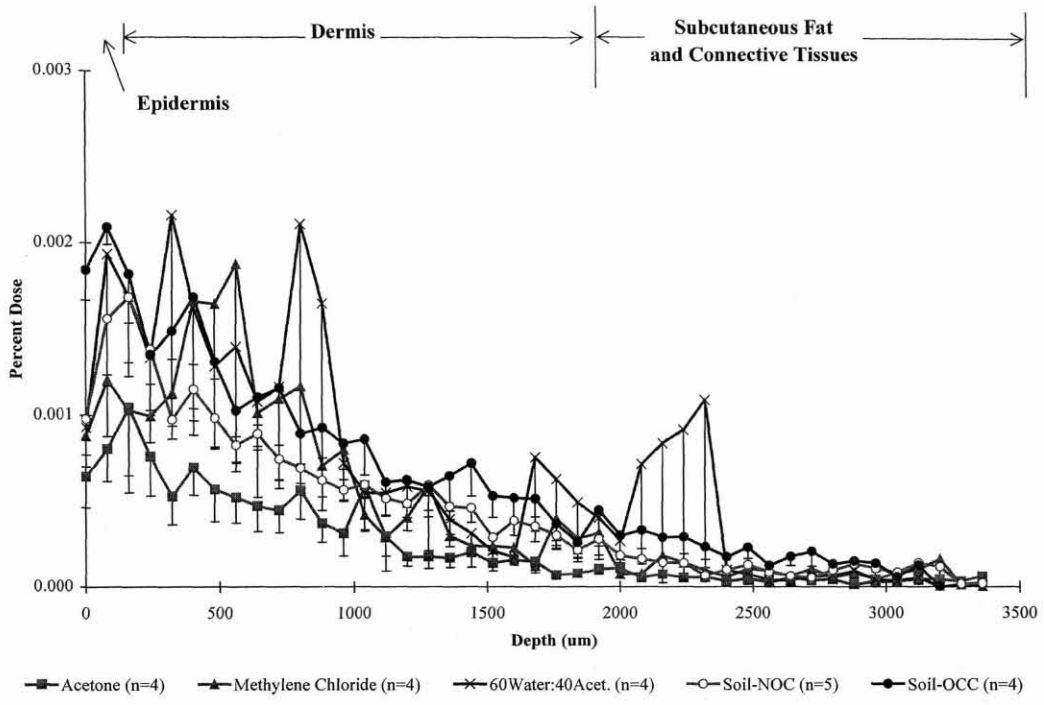


Figure 3—Depth of ^{14}C penetration (mean ± SEM) following (^{14}C-UL)TCB topical application in the ex-vivo IPPSF model.

(t = 1.5 hour, Figure 2). TCB dermal absorption data from liquid formulations may considerably underestimate the risk of exposure to TCB in a soil matrix. This was also observed in other absorption model systems we used under identical exposure conditions for TCB (to be published).

Occlusion of the applied soil-based TCB dose did not show any dermal absorption effect during the first 30 minutes. However, occlusion decreased the dermal uptake rate of the total radioactivity over eight hours. This was unlike our dermal absorption data for PCP in soil[19] or parathion in ethanol.[15] A low recovery may contribute to a smaller absorption amount in general, but even if the TCB dermal absorption data were normalized by total recovery, such an effect was still obvious (0.66% in non-occluded soil dose vs 0.47% in occluded

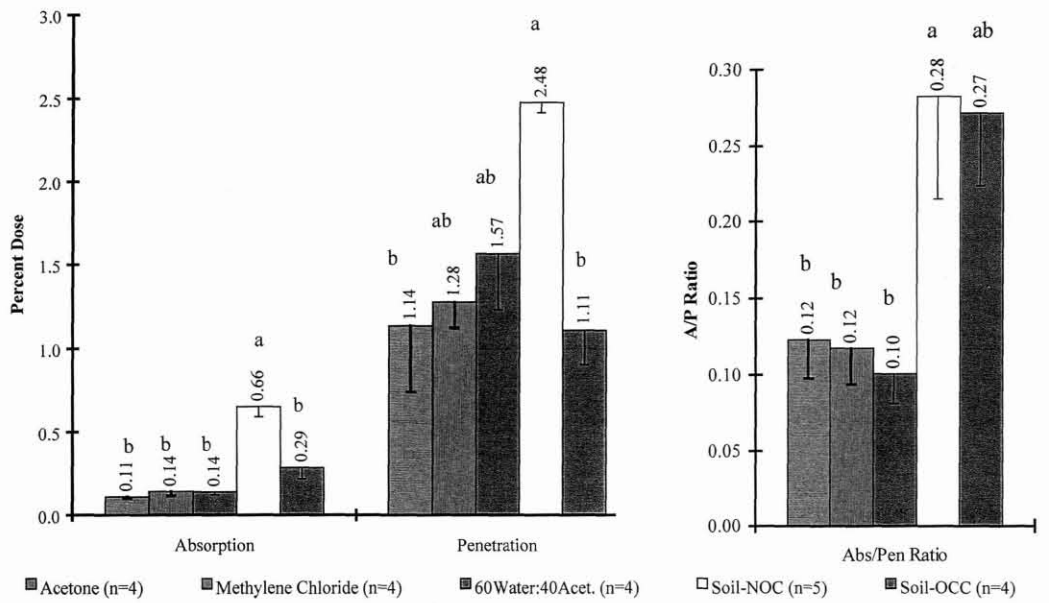


Figure 4—^{14}C absorption, penetration, and A/P ratio following (^{14}C-UL)TCB topical application in the ex-vivo IPPSF model. Means with the same superscript letter are not significantly different across the five treatment groups.

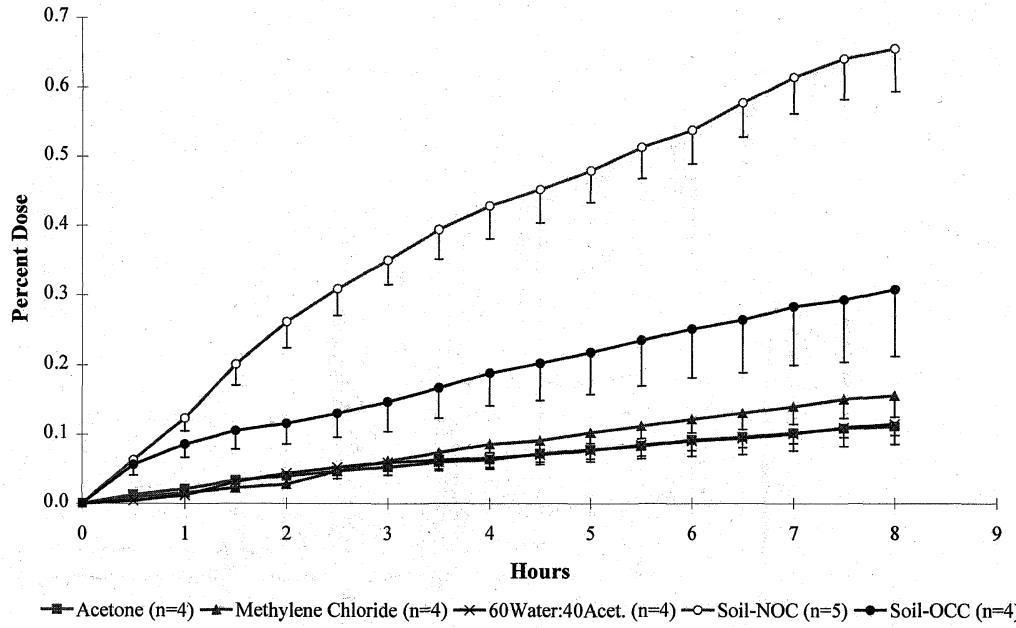


Figure 5—^{14}C cumulative absorption profiles in perfusate following (^{14}C-UL)TCB topical application in the ex-vivo IPPSF model.

soil dose, Table 2). The mechanisms for this and other interesting disposition characteristics of TCB need to be further elucidated.

In Figure 3, the cutaneous *penetration depth/tissue distribution* pattern is illustrated. TCB in acetone vehicle showed the lowest skin tissue concentrations at different depths at the end of the eight-hour IPPSF perfusion studies. The highest concentrations were found in the junction area of the viable epidermis and the dermis (~160 μm). However, methylene chloride showed much higher tissue concentrations in the upper dermis than in the epidermis. The highest cutaneous tissue residue due to methylene chloride was found in the upper dermis, where the microvasculature network for dermal absorption of label into the systemic circulation is located. The concentrations of radiolabel at 400–500 μm depth from the skin surface were two to three times higher with methylene chloride than with acetone. Adding water to the acetone solvent enhanced dermal penetration (two- to threefold over acetone alone), although the total eight-hour dermal absorption increase was insignificant (0.11 to 0.14%, $p > 0.05$, Table 2). The soil-based mixture vehicle generated a skin-penetration pattern between those of the methylene chloride and acetone–water vehicles. Occlusion of the soil TCB dose gave a 1.5- to twofold skin tissue concentration increase with a tissue distribution pattern similar to the non-occlusion pattern (Figure 3).

Dermal *absorption* into blood vessels, *penetration* into viable cutaneous tissues (dermal absorption + local tissue residues), and absorption/penetration ratios under various exposure conditions are compared in Figure 4. The total eight-hour short-term dermal absorption levels of TCB ranged from 0.11% to 0.66% of the initial radioactive topical dose. Absorption from the soil-based matrix was demonstrated to be much higher than in liquid vehicles (five- to six-fold, $p < 0.05$). Occlusion significantly ($p < 0.05$) decreased dermal absorption of TCB exposed in the soil mixture. The occlusive soil TCB showed an insignificantly higher dermal absorption when compared with other liquid doses (0.29 vs. 0.11–0.14%, $p > 0.05$). TCB penetration into and through viable tissues by a non-occlusive soil dose was much higher than with acetone and occlusive soil doses ($p < 0.05$, Figure 4). The absorption/penetration ratio reflects dermal absorption efficiency after TCB and its dermally-derived labeled metabolites have passed through the primary skin-penetration barrier of the stratum corneum. The results suggest that TCB could be more efficiently absorbed into blood circulation from a soil matrix than from liquid solutions ($p < 0.05$). Occlusion of the soil TCB did not change the ratio ($p > 0.05$, Figure 4).

For the purpose of data comparison, the *cumulative ^{14}C absorption profiles* are given in Figure 5. Amounts of dermal absorption of TCB from the three liquid formulations were constant, as indicated by the straight cumulative lines. However, in the case of TCB exposure in the soil matrix, the absorption rate was not constant and was much greater than that in liquid solutions (Figure 5).

The full set of *cutaneous disposition parameters* is summarized in Table 2. The largest amount was detected on the dosed skin surface, followed by the dosing device (the template, see Materials and Methods section), stratum corneum, and cutaneous tissue residues. The high stratum corneum residue indicated good penetration into the stratum corneum but low dermal absorption, probably due to TCB's extremely high lipophilicity. Total recoveries from the full mass balance procedures are

also included in Table 2. During the soil TCB exposure study, we always tried to recover all the radioactive samples for a higher recovery. Obviously, the occlusive soil dose recovery could be higher if the experimental procedures could be further refined.

DISCUSSION

TCB Dermal Absorption

From this study, using the eight-hour ex-vivo dermal absorption data (0.11–0.14% from liquid formulations), we would predict a 1.7–2.1% 120-hour absorption by linear extrapolation. In a 24-hour exposure–120-hour-absorption in-vivo rat study, 6–8% of the occlusive TCB topical dose in any of the four physical forms, including solid, aqueous paste, suspension, and ethanol solution was absorbed.[24] Those results seem consistent with the fact that rat skin is more permeable to most chemicals than human or pig skin.[25] It has been documented that the skins of humans, pigs, guinea pigs, rats, and rabbits are increasingly more permeable in vitro not only to ionic and covalent chemicals in aqueous solutions but also to organic penetrants.[25] In general, the permeability of pig skin is 1.5–2× higher than that of human skin under most common exposure conditions for many compounds. The high lipophilicity of TCB may result in low dermal absorption into blood vessels, with a larger percentage of the label trapped in the stratum corneum, other lipid components of the skin, and subcutaneous fat (4–6% in stratum corneum and 1–2% in local tissues from TCB doses in acetone and methylene chloride, Table 2). A similar phenomenon was observed with DDT (highly lipophilic) in pig, monkey, and human studies.[25] Estimates of the log Ko-w of TCB ranged from 5.45 to 6.63 as determined by various experimental or computer algorithms.[7,26] A strong inverse correlation was observed between Ko-w and single-dose dermal absorption with most chemicals tested.[7] This was also verified by in-vivo PCB dermal absorption in rats.[9]

Vehicle Effect

Many organic solvents such as acetone/ethanol are principal components at hazardous waste sites and are used as vehicles in dermal absorption and toxicity tests of a variety of industrial, agricultural, and environmental chemicals. Such solvents are also often used in transdermal drug delivery and iontophoresis studies of drugs and peptides. However, vehicle effects on dermal absorption and on local disposition have not been adequately studied. The vehicle issue may remain one of the major difficulties in physiologically-based PK modeling of dermal penetration, data extrapolation, and risk assessment.

Solvent may alter not only pre-penetration fates (evaporation and binding) but also dermal penetration, cutaneous tissue distribution, and percutaneous absorption. In this study, we used various vehicles as either neat solvents (acetone or methylene chloride) or mixtures (acetone + water, or soil-based), and their effects on evaporation and partitioning can be critical to the interpretation of penetration and absorption data. The vehicle can alter chemical partitioning between the dosed solution and the stratum corneum, the primary passive barrier to percutaneous absorption[27,28] and a reservoir for lipophilic agents.[29–31] Based on the biochemistry and biophysics of the stratum corneum lipids, most research has been focused on the mechanism by which a compound crosses the intercellular lipids of the stratum corneum. The key determinants of transport through the "brick (dead skin cells)-and-mortar (intercellular lipids)" structure of the stratum corneum are the apparent diffusivity of the penetrant and its solubility in the stratum corneum relative to the vehicle.[32,33] Solvent can delipidize the stratum corneum or alter stratum corneum lipid composition and/or conformation. It was demonstrated that pesticide partitioning from vehicle into stratum corneum is occlusion- and anatomic site-dependent.[34] Increased research attention has been directed to the correlation between partition coefficient/lipoactivity and percutaneous absorption.[35] Obviously, the nature (especially the lipoactivity) of a neat solvent or a multi-phase absorption vehicle such as the organic–aqueous mixture or soil mixture used in this study can be a key factor in determining the cutaneous disposition of a topically exposed toxicant. Recent work has also addressed the biologic modulation of epidermal barrier function by vehicles.[36–39] Interactions between topically applied chemicals and their vehicle during the percutaneous absorption process have not been widely studied but are pertinent to risk assessment.

In this report, a higher TCB dermal absorption was seen with acetone solvent during the first two hours, but methylene chloride gave a larger absorption rate from the second hour through the eighth hour (Figure 2). In a parathion-mixture absorption study in this skin-flap model, similar absorption profiles were observed in which acetone showed a larger initial absorption rate than dimethyl sulfoxide during the first two to three hours after dermal exposure.[20] In-vitro TCB absorption data in flow-through diffusion cells (to be published) with pig skin showed trends similar to those reported here. The high solubility of acetone in both lipid and aqueous phases of the skin and its high volatility may play a role in producing the larger initial but shorter-duration absorption profile when compared with other solvents. It was found that the enhancing effect of lipophilic vehicles on in-vivo percutaneous absorption of methyl nicotinate was due to an alteration of the lamellar structure of the stratum corneum lipid either by a fluidizing action or by dissolution/extraction of the lipids. The oil–water partition coefficient of a penetrant

is a useful predictor of dermal absorption, which also depends on stratum corneum delipidization and the penetrant concentration in the vehicle.[40] Therefore, vehicle effects on stratum corneum lipid can be a very important mechanism for altering chemical dermal penetration and absorption. In this study, acetone (miscible with water) and methylene chloride (not miscible with water) were selected as dosing vehicles for TCB, and we saw different absorption profiles and local skin-tissue penetration patterns (Figures 2 and 3). In general, acetone gave a quicker absorption peak than methylene chloride. Acetone is a known skin irritant, possibly inducing TNFα and inflammatory cytokine release and increasing skin permeation. Incubation of epidermal slices with acetone also increased epidermal cAMP content through activation of adenylate cyclase.[41] It may take more than 24 hours to fully restore acetone-perturbed stratum corneum barrier functions.[42] Dermal absorption of TCB dosed in solid, aqueous paste, suspension, and ethanol solution was examined in rats and obvious formulation form-dependence in dermal absorption was observed.[24] PCB in-vitro absorption with human skin did not correlate well with in-vivo monkey findings.[43] Vehicle effects on solute concentration change on the dosed skin surface and on stratum corneum lipid fluidity have been incorporated into a comprehensive PK model.[44] Significant effects of solvent (acetone and ethanol) on surface evaporation rates, tissue penetration patterns, and percutaneous absorption profiles of phenol, *p*-nitrophenol, PCP, and TCB have been demonstrated.[11,45,46]

Organic–aqueous mixture vehicle. A neat acetone vehicle showed a larger dermal absorption of 2,4-D-amine than did a water vehicle.[47] Addition of water to ethanol significantly enhanced PCP dermal uptake in various absorption models.[46] It has been demonstrated that 40% ethanol (in water) as a vehicle for orally dosed PCP significantly enhanced absorption when compared with water alone.[48] The effects of both organic and aqueous components in mechanistically-defined chemical mixtures were demonstrated in parathion and benzidine percutaneous-absorption studies in this flap model.[20,49] As is often seen, acetone can give a quick-and-large onset of dermal absorption but such a relatively large absorption rate lasts for a very short time when compared with rates seen with other vehicles. Mixing water into the acetone vehicle can potentially "dilute" the initial absorption-enhancing effect of acetone, and thus a delayed but larger absorption peak was observed (Figure 2). The addition of water to an organic solvent (e.g., acetone) may increase the systemic risk associated with topical exposure to TCB during the initial phase of exposure, as indicated by this model.

Soil-based mixture vehicle. Interestingly, this study showed that TCB absorption from a soil-mixture vehicle was much greater than from the other three (liquid) formulations. This was somewhat unexpected but is supported by data from other in-vivo and in-vitro pig and human studies to be published by the authors. Using an indirect calculation approach (excretion ratio of topical over IV doses) in an in-vivo monkey model, Wester et al. found that about 14% of two PCBs (Aroclors 1245 and 1254) dosed in soil non-occlusively (spiked with PCB in hexane:methylene chloride mixture solvent and then dried) was quickly and large absorbed using a 24-hour exposure time but a five-week sample-collection schedule.[10] This amount of absorption from soil is very similar to those seen with other dosing vehicles (acetone, trichlorobenzene, and mineral oil[41]). The measured partition coefficient result suggested a greater affinity of PCBs for powdered human stratum corneum than for soil. Thus, the transfer of PCBs from soil into skin occurred readily. PCBs residing in moist soil would be in a ripe environment for PCB delivery into skin and the systemic circulation.[10] This is very supportive to the finding of the large and rapid dermal absorption of TCB from a soil-based dose in this study. The data indicate that PCB dermal risk can be much higher with exposure in soil than with exposures in liquid solutions.

TCB molecules on the soil particles may be diffused better into the intercellular lipid region in the stratum corneum due to the soil organic matter and the particle's bridging effect. The liquid dose did not have such a mechanism once the liquid solvent evaporated from the dosed skin surface shortly after the exposure. This may partially explain the enhanced dermal absorption of lipophilic PCBs from soil compared with liquid formulations. Potential soil microbial degradation of TCB during the absorption study may provide another possibility of changing dermal absorption. Discussions of chemical transfer between soil matrix and skin can be found in the literature.[50] Mathematical modeling showed that the kinetics of soil desorption relative to dermal absorption is an important process, but more experiments are needed.[50] Soil organic carbon content inversely correlates with dermal absorption of TCB and other lipophilic compounds.[26,50] However, the mechanisms behind this phenomenon remain largely unknown and deserve further investigation.

Occlusion

In this study, we found that occlusion of the soil-dosed skin noticeably decreased percutaneous absorption and reshaped the absorption time course curve of TCB-derived radiolabel (Table 2 and Figure 2). Recovery-normalized absorption (non-occluded 0.66% vs occluded 0.47%) still indicated the absorption-inhibitory effect of skin occlusion. Again, this is another unexpected dermal disposition characteristic of TCB and potentially of other PCBs. However, occlusion greatly increased the local skin-tissue concentration of TCB-derived ^{14}C (1.5- to two-fold increases in different depths of the skin tissues, Figure 3). Different parti-

tioning and metabolism mechanisms may exist in TCB soil absorption when compared with other chemicals we have studied.[15,19,34,51] Occlusion of the dosed skin site is critical for data extrapolation and risk assessment, because occlusion may influence the penetrant's: 1) partition/penetration/distribution processes in the structures above the cutaneous vasculature,[15,34,51] 2) first-pass cutaneus metabolism,[34] 3) microorganism degradation on the skin surface,[19] and 4) other behavior on the dosed skin surface, such as evaporation and dosing-device binding rates.[15,34,51]

CONCLUSION

TCB dermal penetration is significant but has limited systemic uptake, suggesting considerable potential for local toxicity in the skin. Some unique disposition characteristics of TCB were observed in terms of organic–water mixing, liquid-vs-soil formulation, and skin-occlusion effects. Dermal absorption data from liquid TCB doses in organic or aqueous–organic mixture vehicles may underestimate the risk of exposures to TCB in contaminated soil. Skin occlusion and water mixing with organic solvents showed little risk of enhancing TCB dermal uptake. Such observed exposure-dependent dermal absorption and tissue-disposition profiles need to be taken into account in dermal-exposure and risk-assessment studies of TCB and other PCBs.

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