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Enhanced Systemic Tissue Distribution after Dermal versus Intravenous 3,3′,4,4′-Tetrachlorobiphenyl Exposure: Limited Utility of Radiolabel Blood Area under the Curve and Excretion Data in Dermal Absorption Calculations and Tissue Exposure Assessment

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As a dioxin-like polychlorinated biphenyl (PCB), 3,3′,4,4′-tetrachlorobiphenyl (TCB) is receiving increasing research and regulatory interest due to its high toxicity and persistence in the environment. ^{14}C-TCB was administered at an identical dose of 300 μg via the intravenous (iv) or dermal route to swine to examine the exposure route dependency of the relationship between tissue exposure and blood area under the curve (AUC) and the relationship between dermal absorption and excretion of radiolabel. After iv and dermal exposure, blood, urine, and feces samples were collected during the 11-day *in vivo* studies. At the end of the experiments, full mass balance studies were conducted to characterize tissue distribution of label. On average, over 70% of the applied dermal and iv doses were recovered. As expected, more than a 10-fold increase in blood AUC (0.49 vs 0.031, h · % dose/ml), plasma AUC (0.40 vs 0.038, h · % dose/ml), urine excretion (29 vs 2.3% of the applied dose), and fecal (30 vs 3.0% of the applied dose) excretion was determined after iv exposure compared to dermal exposure. However, we unexpectedly found that the tissue residue following iv exposure (8.0% of the applied dose) was only half that following dermal exposure (16% of the applied dose). Significantly larger (20- to 30-fold) ratios of blood AUC: tissue residue and excretion:tissue residue were observed after iv exposure compared to dermal exposure. This may indicate a route-related concentration–dependent blood-to-tissue partition process of pooled label, unique skin metabolism, or saturable hepatic metabolism of TCB. Thus, a long-term, low-input exposure pattern similar to this dermal exposure could be more harmful to systemic tissues than a short-term, high-dose exposure similar to this iv exposure. One should be aware that greater absorption, higher blood concentrations, greater blood and plasma AUCs, and greater excretion of label do not necessarily result in a greater overall tissue exposure and that some conventional approaches using label determination in blood and excreta without full mass balance studies may underestimate dermal absorption of chemicals similar to TCB. © 2001 Academic Press

Skin exposure to occupational and environmental chemicals is recognized to be a significant route by which xenobiotics gain systemic access (absorption) and then cause systemic adverse health effects. Recently, it has been demonstrated that workers dermally exposed to some chemicals (e.g., isocyanates) may suffer systemic diseases (e.g., allergic asthma) without significant systemic absorption of the chemical (Kimber, 1996; Zhang, 1997) due to the fact that chemicals react with the antigen presenting cells in the skin. In addition, the skin often is the direct target organ of chemicals inducing acute irritation, dermatitis, and cancer. Chemical transcutaneous movement is perhaps the most difficult process to quantify and predict (Hinz *et al.*, 1991), since so many variables associated with the biological system, ambient environment, and exposure conditions can independently or interactively exert their influences on cutaneous disposition.

Polychlorinated biphenyls (PCBs), first synthesized over 100 years ago, were massively used until their production was banned in the late 1970s (WHO, 1993; Wolff, 1985). 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 are frequently found as complex mixtures of isomers in almost every component of the global ecosystem (Safe, 1993), including air, water, soil, dust, and surfaces in industrial and residential settings, and in animal and human tissues where they tend to

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have a long half-life and cause a high tissue burden (WHO, 1993).

The considerable health threat from PCB exposure via various routes has been widely recognized by regulators, researchers, and the general public. The internal dose from occupational exposure can be as high as 10 to 1000 times that of nonoccupational exposure (Wolff, 1985). Areas of study include pharmacokinetics, metabolism, health effects, and related cellular or molecular mechanisms for gene regulation, enzymatic induction/activation (e.g., P450 1A1/1A2), and endocrine effects of individual PCBs/metabolites (Brown *et al.*, 1994; DeVito *et al.*, 1993; Garner *et al.*, 1999; Jackson *et al.*, 1993; Safe, 1993).

More highly chlorinated PCBs have slower dermal absorption, less metabolic elimination, lower DNA binding, and less acute toxicity, but longer tissue residence times, greater body burdens, and higher cancer risk (Safe, 1989) than less-chlorinated PCBs. As one of the highly toxic dioxin-like PCBs, 3,3′,4,4′-tetrachlorobiphenyl (TCB) is suitable for dermal pharmacokinetic (PK) studies due to its acceptable half-life and residence time in the body. Dermal absorption of TCB has been studied in monkeys (Wester *et al.*, 1993) and pigs (Qiao and Riviere, 2000). The relationship between octanol-water partitioning coefficient and dermal absorption of PCBs (Garner and Matthews, 1998), including TCB (Jackson *et al.*, 1993; Safe, 1993), has been studied. It was found that highly chlorinated PCBs were retained in rat skin and slowly released into systemic circulation (Garner and Matthews, 1998).

The pig has been proven to be an excellent animal model for estimating human dermal absorption of a large variety of chemicals under various exposure conditions (Bartek *et al.*, 1972; Qiao *et al.*, 1993; Reifenrath and Hawkins, 1986). The histological (Monteiro-Riviere and Stromberg, 1985), physiological, biochemical, and pharmacological similarities between pig and human skin (Simon and Maibach, 2000), either for passive dermal absorption (Carver and Riviere, 1989; Qiao *et al.*, 1993, 1994, 1996; Reinfenrath *et al.*, 1984) or for iontophoretic transdermal drug delivery (Riviere *et al.*, 1992), are well documented.

When one considers potential concentration-dependent chemical transport from skin to blood and then from blood to systemic tissues and metabolism (enzyme spectrum, specific activity, and concentration dependence) variations in skin and in systemic organs following dermal vs iv exposure, it is reasonable to hypothesize that the tissue profiles and blood:tissue ratio may be exposure route dependent. Because of this, the area under the curve (AUC) of blood radiolabel concentration profiles or excretion may not serve as an absolutely useful measurement for systemic tissue risk analysis or for dermal absorption. Due to the inherent limitation of human study, systemic tissue concentrations are often unobtainable and thus human tissue risk assessment has to be largely or sometime solely based on indirectly "calculated" (blood AUC methods, excretion methods) rather than directly "determined" (full mass balance method) dermal absorption values of total label. Dermal absorption is commonly expressed as total label excretion or blood AUC being calibrated by iv data assuming the blood:tissue, tissue:excretion, and blood:excretion ratios are route independent. If this assumption cannot be satisfied, the risk assessment conclusions based on indirect dermal absorption data can be unreliable. Limited full mass balance studies in rats (Garner and Mathews, personal communication) and pigs (unpublished data from the authors of this work) suggest that reliable PCB dermal absorption data may not be obtained through traditional label excretion or blood AUC ratio methods, and thus PCB dermal risk assessment may not be achievable using traditional approaches. This is true especially when considering the systemic tissue risk from dermal exposure to PCBs. Therefore, a need may exist to reassess some approaches used in dermal absorption and risk analysis for toxicants similar to PCBs.

The purposes of this study were to examine the exposure route dependency of the relationship between overall tissue exposure and blood AUC or total excretion of radiolabel following iv or topical TCB application, which reflects a short-term, large-dose or a long-term, low-dose systemic input, and to further explore the limitation of applying the blood AUC ratio or using excretion data of total label in dermal absorption calculation and tissue exposure assessment.

MATERIALS AND METHODS

Chemicals and reagents. [^{14}C-UL]TCB (12.7 mCi/mmol) and acetone (HPLC grade) were purchased from Sigma Chemical Co. (St. Louis, MO). HPLC grade ethyl acetate was ordered from Fisher Scientific (Fair Lawn, NJ). Ethanol (Absolute, 200 proof) was purchased from AAPER Alcohol and Chemical Co. (Shelbyville, KT).

Animals and TCB exposure. Eight- to 10-week-old female weanling Yorkshire–Landrace cross pigs (body wt range of 19.7–25.0 kg with a mean of 22.3 kg, $n = 6$, Neuhoff Farms, Inc., Greenville, NC) were used. Pigs were acclimated for 1 week and individually housed in metabolism cages at a standard temperature of 72°F and 12:12-h light:dark cycle during TCB exposure. Actual animal room temperature and relative humidity were monitored daily. Pigs were fed 15% protein Pig & Sow pellets (2–3 lbs/day, Wayne Feed Division, Continental Grain Co., Chicago, IL) and given free water access. Animals were handled according to the established North Carolina State University guidelines before, during, and after the proposed experiments. Six acclimated pigs were randomly assigned to either the iv or dermal group ($n = 3$/group). The jugular veins of anesthetized pigs were catheterized using our previously established procedure (Qiao *et al.*, 1993, 1994). In the iv study, a full dose of [^{14}C]TCB (10 μCi/300 μg/100 μl, in acetone) was injected into the blood circulation via the jugular cannula followed by 20-ml saline flush. In the dermal experiments, the selected lateral abdominal dosing site was first clipped 5–6 h prior to dosing. An iv-identical TCB dose of 10 μCi/300 μg/100 μl (in acetone) was evenly applied onto a 7.5-cm^2 circular dosing zone in the clipped area. The dosed skin was protected by a glass chamber with holes (50 mm diameter × 8 mm depth with 3 mm diameter holes), covered by nylon sieve screening (150-mesh, Cole-Parmer Instrument Co., Vernon Hills, IL), and secured by Elasticon tape wrapping. The 264-h experimental duration was selected for both iv and dermal exposures to ensure the data would be useful in PK data analysis.

Sampling. Blood samples (15–20 samples, 13 ml/sample) were withdrawn via the preinstalled jugular vein cannula. In order to compensate for the blood

TABLE 1
Key Disposition Parameters Following [^{14}C-UL]3,3′,4,4′-Tetrachlorobiphenyl iv and Dermal Exposures in Swine

Parameter	iv injection	Dermal exposure	Two-sided p value
Dermal absorption or iv dose found (% dose)	69 (36–99)	22 (15–29)	0.10*
Penetration (% dose)	N/A	23 (16–30)	N/A
AUC_p (% dose/ml · h)[a]	0.49 (0.26–0.71)	0.031 (0.028–0.036)	0.10*
AUC_b (% dose/ml · h)[b]	0.40 (0.25–0.56)	0.038 (0.021–0.059)	0.10*
Tissue residue (% dose)	8.0 (6.7–9.0)	16 (12–22)	0.10*
Excretion (urine + feces, % dose)	60 (26–89)	5.3 (3.4–6.9)	0.10*

Note. Values are means with ranges in parentheses. $n = 3$.
[a] AUC_p, area under the curve in plasma.
[b] AUC_b, AUC in blood.
* Significant differences were determined between iv and dermal exposure.

volume loss and to flush the cannula, 13 ml of heparinized saline was injected through the sampling port after each blood sample. An aliquot of 250 μl blood was collected, and then plasma was separated by centrifugation (1500*g*, 25 min). Urine was quantitatively collected by measuring the volume of each collection. "Pure feces" (on cage floor) and "urine-contaminated feces" (in urine tray of the metabolism cage) samples were collected daily, weighed, and homogenized with distilled water (~50:50, w/w) (Qiao *et al.*, 1993, 1994). The ^{14}C amount from the urine-contaminated fecal sample was theoretically divided into urinary and fecal contributions according to our published method (Qiao *et al.*, 1997). At the end of the observations, the pigs were euthanized and the following dosing materials were collected and extracted with ethyl acetate. Extraction volumes were as follows: Elasticon tape and screen (2 × 135 ml), dosing chamber (5 × 30 ml), and pooled cotton swabs (5 × 45 ml). The extraction intervals were 1 h for the chamber and swab and 2 h for the Elasticon tape and screen. The stratum corneum, isolated by tape stripping (10 times), was digested in ethyl acetate (15 ml/stripping). Skin, subcutaneous fat, and muscle samples at both the dosed site and the adjacent site (1-cm wide zone surrounding the dosed area) were separately excised, weighed, and assayed. Samples of most tissues/organs and excreta (nondosed skin, fat, and muscle at upper back site, diaphragm, liver, kidney, lung, heart, brain, spleen, ovary, uterus, gall bladder, urine bladder, stomach, stomach content, small intestine, small intestine content, large intestine, large intestine content, urine, and bile) were also collected and analyzed for ^{14}C concentrations in tissues and for mass balance calculations. Average organ weights expressed as percentage body weight were obtained from six previously euthanized pigs and were used to extrapolate the assayed ^{14}C tissue concentrations for mass balance calculations in this study.

^{14}C determination. Collected samples were stored at −20°C until analysis. An aliquot of 250–500 μl liquid or 100–700 mg solid sample was completely oxidized in a Packard tissue oxidizer and the absorbed gas was counted on a Packard 1900TR TriCarb Scintillation Counter.

Data analysis. Blood (or plasma) *AUC* was calculated as:

$$AUC = \sum_{i=1}^{m-1} \frac{(C_{i+1} + C_i)}{2}(t_{i+1} - t_i), \quad (1)$$

where $i = 1, 2, 3 \ldots m$ represents the blood sample number, C represents concentration, and t represents postexposure time. Total ^{14}C *in vivo* percutaneous absorption was defined as the ^{14}C absorbed into blood and redistributed from blood into other tissues. Thus, it was calculated as the total ^{14}C that appeared in the collected blood, urine, fecal samples, and the total tissue residues (except for the local dosed cutaneous tissues) during the 264-h *in vivo* studies. Total penetration was the sum of absorption and local dosed cutaneous tissue residues.

Statistical data analysis was done based on extensive consultations with experienced biostatisticians in the Biostatistics Branch of Health Effect Laboratory Division/National Institute for Occupational Safety and Health. Due to the small sample size ($n = 3$ in the *in vivo* pig study) and the unknown distribution of the data, we selected a nonparametric (distribution-independent) statistical approach (Wilcoxon–Mann–Whitney, NPAR1WAY, SAS, Cary, NC) to quantify the dosing route's effects on TCB disposition using Exact Two-Sided $p = 0.1$ as the significance level. The NPAR1WAY program can calculate the Wilcoxon–Mann–Whitney or Kruskal–Wallis statistic for studies with two treatments or with more than two treatments, respectively. These are the nonparametric analogs of the two-sample *t* test and ANOVA for parametric analyses. For similar reasons, the results are given as group means with data ranges, instead of means with standard errors/standard deviations.

RESULTS

TCB *in vivo* disposition parameters including total absorption, penetration, blood and plasma AUCs, overall excretion (urinary + fecal), and body burden (tissue residues) are summarized in Table 1. As expected, all parameters from iv-exposed animals are much higher than the corresponding ones from dermally exposed animals ($p < 0.1$), with the exception of the systemic tissue residue, for which the dermal exposure yielded a significantly higher value than the iv exposure ($p < 0.1$). This unexpected but very important finding may demonstrate the need to conduct *in vivo* full mass balance procedures in dermal absorption and tissue risk assessment studies.

After iv dosing, a total of 60% of the dose was excreted almost equally via urinary and fecal routes by 264 h, with 8% of the initial dose remaining in the body tissues (Tables 1 and 2). However, when TCB was given topically at an iv-identical dose, the total excretion (urinary + fecal) was only about 5% (vs 60%, iv) but with a much larger tissue residue of 16% (vs 8%, iv). Table 2 shows the full mass balance data at 264 h postexposure. Over 70% of the applied radioactive doses were similarly ($p > 0.1$) recovered from both the dermal and iv experiments; therefore the dermal and iv data can be directly compared without normalization by recovery. All the tissues that were sampled and analyzed in the mass balance study represented 90% of the body weight (bone is the major tissue

TABLE 2
Radiolabel Mass Balance Following [^{14}C-UL]3,3′,4,4′-Tetrachlorobiphenyl iv and Dermal Exposures in Swine

Parameter	iv injection (% dose)	Dermal exposure (% dose)	Two-sided *p* value
Tissue residue	8.0 (6.7–9.0)	16 (11–22)	0.10*
Urine collected	29 (9–58)	2.3 (2.0–2.9)	0.10*
Feces collected	30 (17–43)	3.0 (0.56–4.8)	0.10*
Plasma collected	0.38 (0.20–0.61)	0.010 (0.0096–0.011)	0.10*
Blood collected	0.64 (0.43–1.0)	0.026 (0.014–0.041)	0.10*
Dosing device[a]	1.1 (0.65–1.5)	17 (6.4–23)	N/A
Surface swabs	N/A	32 (24–39)	N/A
SC tape strips[b]	N/A	0.85 (0.60–1.2)	N/A
Recovery	70 (37–100)	71 (61–85)	1.0

Note. Values are means with ranges in parentheses. $n = 3$.
[a] iv, needle + catheter; topical, chamber + tape + screen.
[b] SC, stratum corneum.
* Significant differences were determined between iv and dermal exposure.

excluded due to technical difficulty in analysis), so it is unlikely that the nonrecovered doses from both routes were due to incomplete tissue collection. The AUC_{iv} in plasma (0.49 h · % dose/ml) and blood (0.40 h · % dose/ml) are much greater than the AUC_{dermal} (0.031 and 0.038 h · % dose/ml) as shown in Table 1.

Exposure route-dependent disposition parameter ratios are given and compared in Table 3. The excretion:tissue residue ratio for dermal exposure is 0.32, which is much smaller than the iv ratio of 7.7 (24-fold, $p < 0.1$, Table 3). Ratios of blood or plasma AUC:tissue residue were also significantly route-dependent ($p < 0.1$). The blood AUC:excretion ratio was route independent ($p > 0.1$).

Plasma and blood concentration profiles are illustrated in Figs. 1 and 2. As expected, label concentrations in both blood and plasma during the entire 264-h study were much higher after iv than after dermal exposure. To further explore the kinetic features of the plasma and blood profiles after dermal and iv exposure, semilog plots are presented (Figs. 1 and 2, insets). After iv application, exponential decline of the plasma and blood concentration was demonstrated, suggesting first-order elimination kinetics.

Cumulative urinary and fecal excretion curves are given in Figs. 3 and 4 to facilitate excretion data comparison with published TCB data at any given time point postapplication. As seen in Fig. 3, the urinary label excretion after iv administration was present throughout the entire 264-h experiment, with a higher excretion rate during the first 6 days than the rest of the experiment. After dermal exposure, cumulative urinary excretion was almost linear (Fig. 3), which was different from the iv curve due to different exposure/input pattern. As seen in Fig. 4, the cumulative fecal excretion after iv exposure was much faster during the first 3 days than the rest of the study (days 4–11). Fecal excretion after iv exposure was completed to an 80% degree by day 3 (fast phase) followed by a slow excretion phase. It is worth noting that the fecal excretion process was completed much faster (over 80% completion within 3 days, Fig. 4) than the urinary excretion (80% completion within 7 days, Fig. 3). Similar to the urine data, cumulative fecal excretion was almost linear after dermal exposure (Fig. 4).

Tissue radiolabel residues (% dose per organ/tissue, group means, and data ranges) after iv and dermal exposure are given in Table 4 for body burden estimation purpose. Tissue residues in 17 of 24 assayed organs/tissues were significantly higher ($p < 0.1$) after dermal than after iv application, and the remaining 7 organs/tissues showed a similar trend but did not reach statistical significance. The only exception was the gall bladder.

For tissue toxicity risk comparison purposes, radiolabel concentrations are given since toxicity may relate to concentration, rather than residue amount in an organ/tissue. Much higher tissue concentrations (% dose/gram of tissue, Fig. 5) were determined for nearly all the tissues assayed after dermal compared to iv exposure. The tissue:blood concentration ratios (Fig. 6) clearly showed that the labeled toxicants' blood–tissue partitioning was route dependent.

TABLE 3
Route-Dependent Disposition Ratios Following [^{14}C-UL]3,3′,4,4′-Tetrachlorobiphenyl iv and Dermal Exposures in Swine

Parameter	iv injection	Dermal exposure	Two-sided *p* value
Excretion:tissue residue	7.73 (2.9–11)	0.32 (0.30–0.36)	0.10*
AUC_b:tissue residue[a]	0.052 (0.028–0.067)	0.0022 (0.0018–0.0026)	0.10*
AUC_p:tissue residue[b]	0.063 (0.029–0.086)	0.0019 (0.0016–0.0024)	0.10*
AUC_b:excretion	0.0074 (0.0063–0.0095)	0.0069 (0.0061–0.0086)	0.40
AUC_p:excretion	0.0086 (0.0078–0.0099)	0.0062 (0.0051–0.0081)	0.40

Note. Values are means with ranges in parentheses. $n = 3$.
[a] AUC_b, area under the curve in blood.
[b] AUC_p, AUC in plasma.
* Significant differences were determined between iv and dermal exposure.

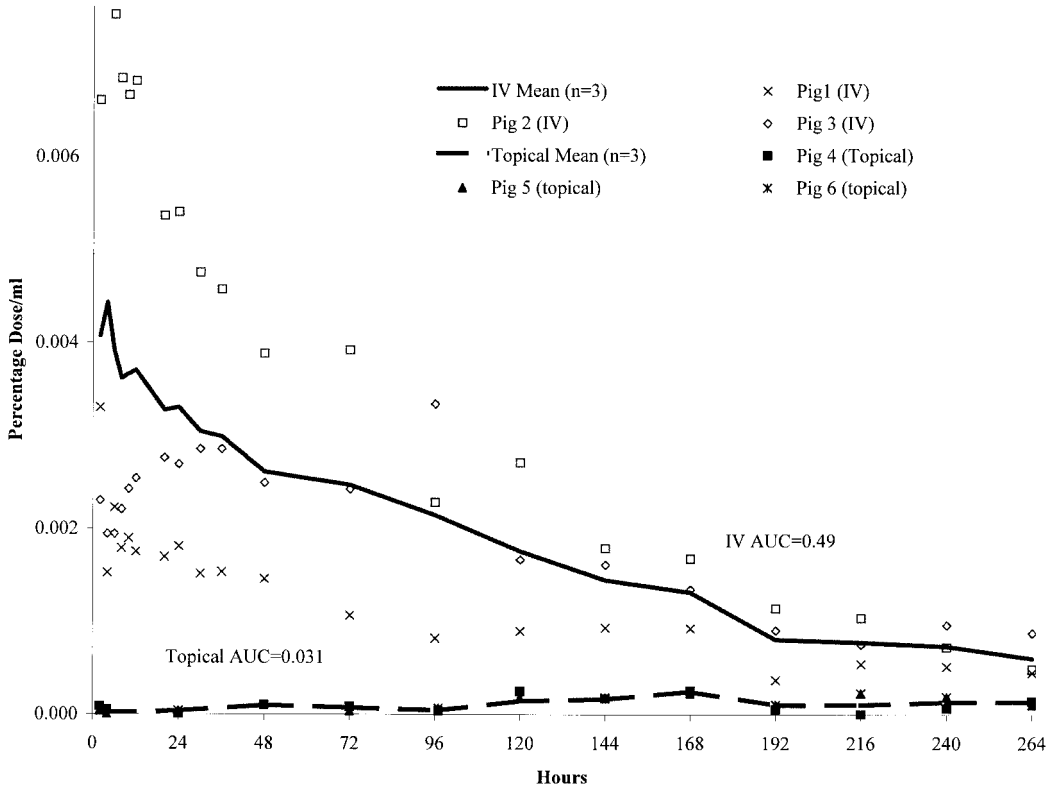

FIG. 1. ^{14}C plasma concentration profiles following [^{14}C-UL]3,3′,4,4′-tetrachlorobiphenyl iv vs dermal application in the *in vivo* swine model.

DISCUSSION

Limited Utilization of Radiolabel Blood AUC and Excretion Data in Dermal Absorption Calculations and Tissue Exposure Assessment

By definition, "systemic absorption or absolute bioavailability" of a chemical is the fraction of the applied dose reaching the systemic blood circulation intact after extravascular administration (Roberts and Walters, 1998). However, in reality, dermal absorption studies are often done with a labeled compound, for which a certain radioisotope is incorporated into a stable structural moiety of a molecule to ensure the total label count will reflect the moles of the chemical or its metabolites absorbed (total dermal absorption). This is because the low dermal dose, low absorption rate, and large *in vivo* dilution factor often yield a chemical concentration too low to measure directly for either parent chemical or metabolites. Thus, dermal absorption of a chemical is often experimentally measured by pooled (total) radiolabel appearing in different biosamples such as blood, excreta, and/or tissues. This indicates the distinction between the theoretical definition and the experimental approach of dermal absorption.

Most of the methods in determining the *in vivo* and *in vitro* dermal absorption of a topically exposed chemical were reviewed (EPA, 1992) and some new methods based on comprehensive PK modeling with metabolic analysis have been developed since that time (Qiao and Riviere, 1995).

Direct Methods for Dermal Absorption Calculation

An *in vivo* full mass balance study is considered the direct dermal absorption method (Shah and Guthrie, 1983; Yang *et al.*, 1986), which should be the most reliable determination of dermal absorption. Although good agreement between direct and indirect methods (see below) can be found in a specific study, we cannot extend the conclusion to other cases without considering the metabolism and tissue residue characteristics.

Indirect Methods for Dermal Absorption Calculation

Indirect methods include the blood (or plasma) AUC method, the total radiolabel excretion method, and other methods based on limited indirect measurements of tissue residue or biological activities related to dermal absorption. These methods are briefly discussed below.

Blood (or plasma) AUC method. Blood AUC of a single chemical (or more often the pooled radiolabel as a trace of the chemical and its metabolites) is often referred to as its dermal absorption after a simple calibration by the corresponding iv value from the same dosage (Feldmann and Maibach, 1969, 1970, 1974; Qiao *et al.*, 1993; Qiao and Riviere, 1995; Wester and Maibach, 1983). Blood AUC is often used alone in calcu-

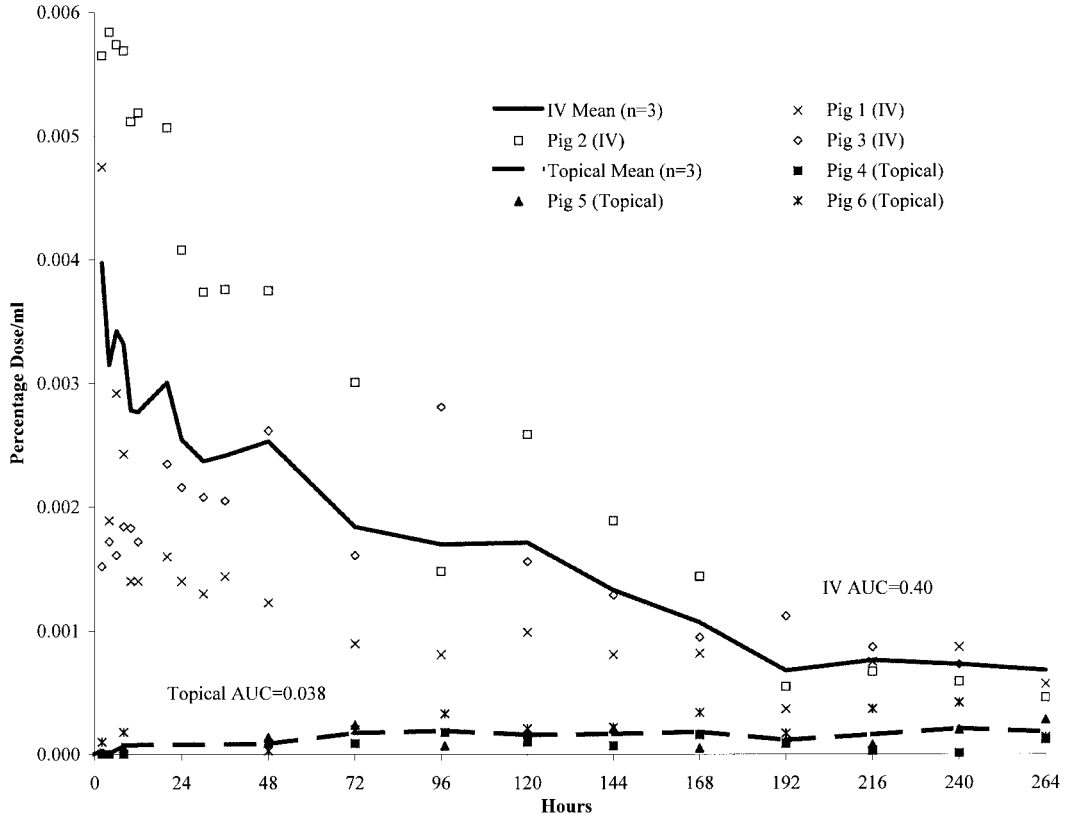

FIG. 2. ^{14}C blood concentration profiles following [^{14}C-UL]3,3′,4,4′-tetrachlorobiphenyl iv vs dermal application in the *in vivo* swine model.

lating the bioavailability of a drug in pharmaceutical fields as well. However, the dermal absorption result could be inaccurate if a pooled label AUC is used, as demonstrated by this report. When dealing with pooled label, this method can be utilized only if all the labeled chemicals (parent chemical and its metabolites) show similar blood–tissue partitioning coefficients and blood:excretion ratios.

In this method, dermal absorption can often be determined (Gibaldi, 1991; Gibaldi and Perrier, 1982; Qiao and Riviere, 1995; Wester and Maibach, 1987) as

$$\text{Dermal absorption} = \left(\frac{AUC_{\text{dermal}}}{AUC_{\text{iv}}}\right)\left(\frac{\text{Dose}_{\text{iv}}}{\text{Dose}_{\text{dermal}}}\right) \times 100\%. \quad (2)$$

The dermal absorption data given in Table 1 were directly determined through the full mass balance analysis and will be compared to the values derived from Equation (2).

Total radiolabel excretion method. *In vivo* dermal absorption is also often calculated as the total label excretion in both urine and feces after calibration by iv excretion data for any incomplete excretion (dose–recovery difference or direct mass balance analysis) or by employing the established "bioavailability–excretion" or "bioavailability–bioavailability" approach (Qiao and Riviere, 1995) depending on the skin metabolism and systemic tissue residue characterization. Any incomplete excretion of an iv dose should be due to significant systemic tissue residues because the full iv dose is instantly introduced into the systemic circulation via iv bolus injection, bypassing any absorption barriers and thus yielding 100% "absorption". By assuming the excretion:tissue residue ratio to be application route independent and an identical dose to be applied intravenously or topically, dermal absorption is calculated as:

$$\text{Dermal absorption} = \left[\frac{\text{Excretion (urine + feces)}_{\text{dermal}}}{\text{Excretion (urine + feces)}_{\text{iv}}}\right] \times 100\%. \quad (3)$$

This might be the easiest measurement and interpretation with many test chemicals and should be used in cases where there is no way to check the excretion:systemic tissue residue ratio (e.g., *in vivo* human study).

Other methods for dermal absorption calculation. Other methods have been used to calculate dermal absorption including (1) monitoring specific tissue concentrations either in the liver (Shu *et al.*, 1988) or in the stratum corneum (by tape

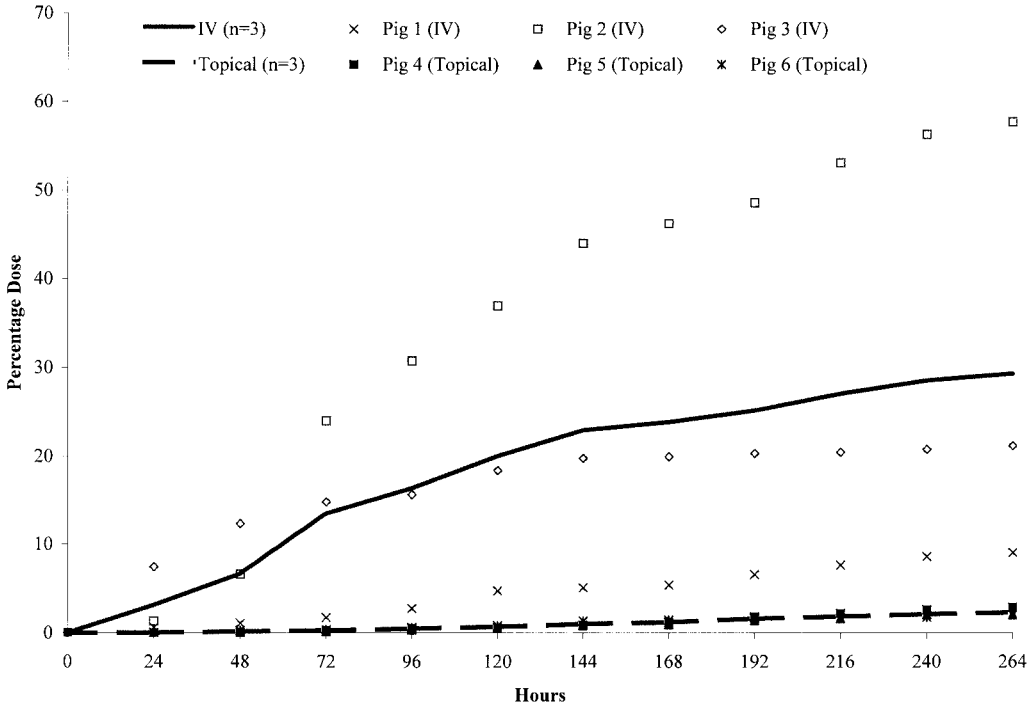

FIG. 3. ^{14}C cumulative urinary excretion profiles following [^{14}C-UL]3,3′,4,4′-tetrachlorobiphenyl iv vs dermal application in the *in vivo* swine model.

stripping, Rougier *et al.*, 1987); (2) quantifying chemical disappearance from the dosed skin surface (Fredericksson, 1962) or from donor solution (Dutkiewizc and Tyras, 1967); and (3) quantifying bioresponse after nicotinate dermal application using Laser Doppler Flowmetry measurement as relative dermal absorption (Guy *et al.*, 1985; Kohli *et al.*, 1987). However,

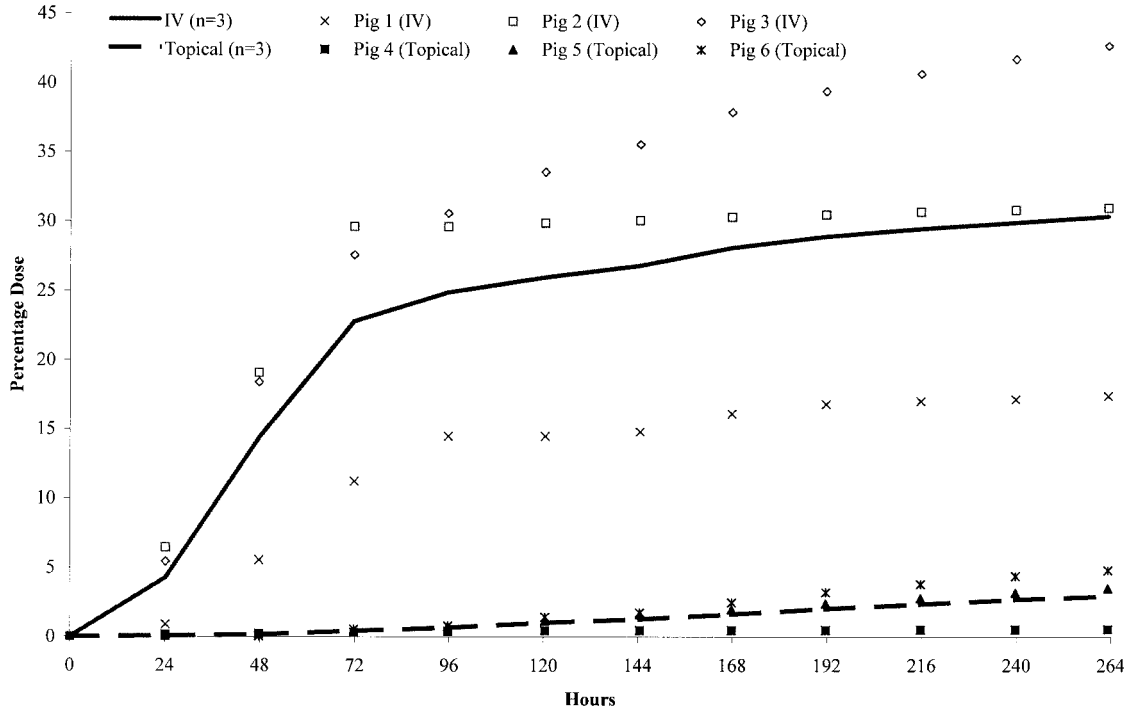

FIG. 4. ^{14}C cumulative fecal excretion profiles following [^{14}C-UL]3,3′,4,4′-tetrachlorobiphenyl iv vs dermal application in the *in vivo* swine model.

TABLE 4
^{14}C Tissue Residues Following [^{14}C-UL]3,3′4,4′-Tetrachlorobiphenyl iv and Dermal Exposures in Swine

Organ/tissue	%Dose		Two-sided *p* value
	iv	Topical	
Liver	0.38 (0.33–0.42)	0.77 (0.45–1.1)	0.10*
Kidney	0.031 (0.026–0.041)	0.21 (0.17–0.23)	0.10*
Lung	0.32 (0.29–034)	0.35 (0.082–0.61)	0.70
Heart	0.020 (0.016–0.024)	0.058 (0.051–0.064)	0.10*
Diaphragm	0.020 (0.0083–0.034)	0.064 (0.038–0.099)	0.10*
Brain	0.0007 (0–0.0022)	0.029 (0.023–0.033)	0.10*
Spleen	0.0096 (0.0007–0.022)	0.048 (0.036–0.054)	0.10*
Small intestine	0.11 (0.086–0.13)	0.19 (0.14–0.26)	0.10*
Small intestine contents	0.11 (0–0.22)	0.30 (0.28–0.35)	0.10*
Large intestine	0.17 (0.13–0.22)	0.33 (0.15–0.45)	0.40
Large intestine contents	0.29 (0.21–0.41)	0.32 (0.11–0.44)	1.0
Stomach	0.036 (0–0.077)	0.091 (0.042–0.15)	0.40
Stomach contents	0.071 (0.040–0.12)	0.39 (0.22–0.66)	0.10*
Bile	0.049 (0.026–0.063)	0.060 (0.034–0.086)	0.70
Gall bladder	0.0051 (0.0039–0.0062)	0.0021 (0.0015–0.0035)	0.10*
Urinary bladder	0.0034 (0–0.0087)	0.019 (0.015–0.023)	0.10*
Ovary	0.0001 (0.00002–0.00011)	0.0022 (0.0015–0.0032)	0.10*
Uterus	0.0036 (0.0010–0.0078)	0.067 (0.012–0.14)	0.10*
Control skin	1.56 (0.97–2.2)	2.8 (2.6–2.9)	0.10*
Control fat	3.0 (3.0–3.1)	4.4 (2.6–5.7)	0.70
Control muscle	1.78 (0.35–3.7)	4.6 (2.5–6.9)	0.20
Dosed skin	N/A	0.46 (0.27–0.76)	N/A
Dosed fat	N/A	0.13 (0.024–0.30)	N/A
Dosed muscle	N/A	0.0081 (0.0032–0.012)	N/A
Adjacent skin	0.0008 (0.00016–0.0012)	0.70 (0.23–1.2)	0.10*
Adjacent fat	0.0018 (0.0015–0.0021)	0.015 (0.0086–0.024)	0.10*
Adjacent muscle	0.0005 (0.00035–0.00066)	0.0029 (0.0014–0.0057)	0.10*
Tissue total	8.0 (6.7–9.0)	16 (12–22)	0.10*

Note. Values are means with ranges in parentheses. $n = 3$. *Indicates a significant level at $p = 0.1$.

those methods should only be used when a dose–response relationship has been well characterized under the defined exposures.

Since the absorbed dose can be present in blood, tissues, excreta, and exhaled air, one must carefully consider the chemical amounts/profiles in those physiological spaces and properly include them when calculating dermal absorption. If the excretion:tissue residue ratio is route dependent (Table 3) due to any mechanisms, the traditional dermal absorption calculation method using iv-calibrated excretion data may be invalid. This is especially important when systemic tissue risk or overall body burden is the center of the experimental test.

As seen in Table 1, the absolute or direct TCB dermal absorption (by actual mass balance measurement, not by simply comparing the dermal excretion data with the iv data) was determined to be 22%. If tissues had not been assayed in this study, a calculated (indirect) dermal absorption using the traditional excretion approach would have been 2.3/29 = 7.9% (urine ratio method), 3.0/30 = 10% (fecal ratio method), or 5.3/60 = 8.8% (total excretion method) on average (Tables 1 and 2), which would obviously underestimate the actual TCB dermal absorption of 22%. Similarly, the results obtained using the blood AUC (6.3%) or plasma AUC (9.5%) method (Table 1) are not better than those obtained using the excretion approaches. Therefore, an erroneous calculated dermal absorption of 6.3–10% would have been obtained for TCB in this pig model if no mass balance study had been conducted. Thus, the calculation method for dermal absorption simply using iv-calibrated total label excretion or blood AUC may not always be appropriate, since no application route dependency for biotransformation, tissue:excretion ratio, or blood-to-tissue partitioning has been taken into consideration. This needs to be brought to the attention of researchers and risk assessors, especially when dealing with toxicants similar to TCB. Similarly, this would significantly impact the calculation of slaughter withdrawal times in food-producing animals exposed to such compounds.

Potential Mechanisms for Enhanced Tissue Distribution after Dermal vs iv Exposure

The higher tissue burden resulting from dermal vs iv exposure suggests some new needs in risk assessment strategy

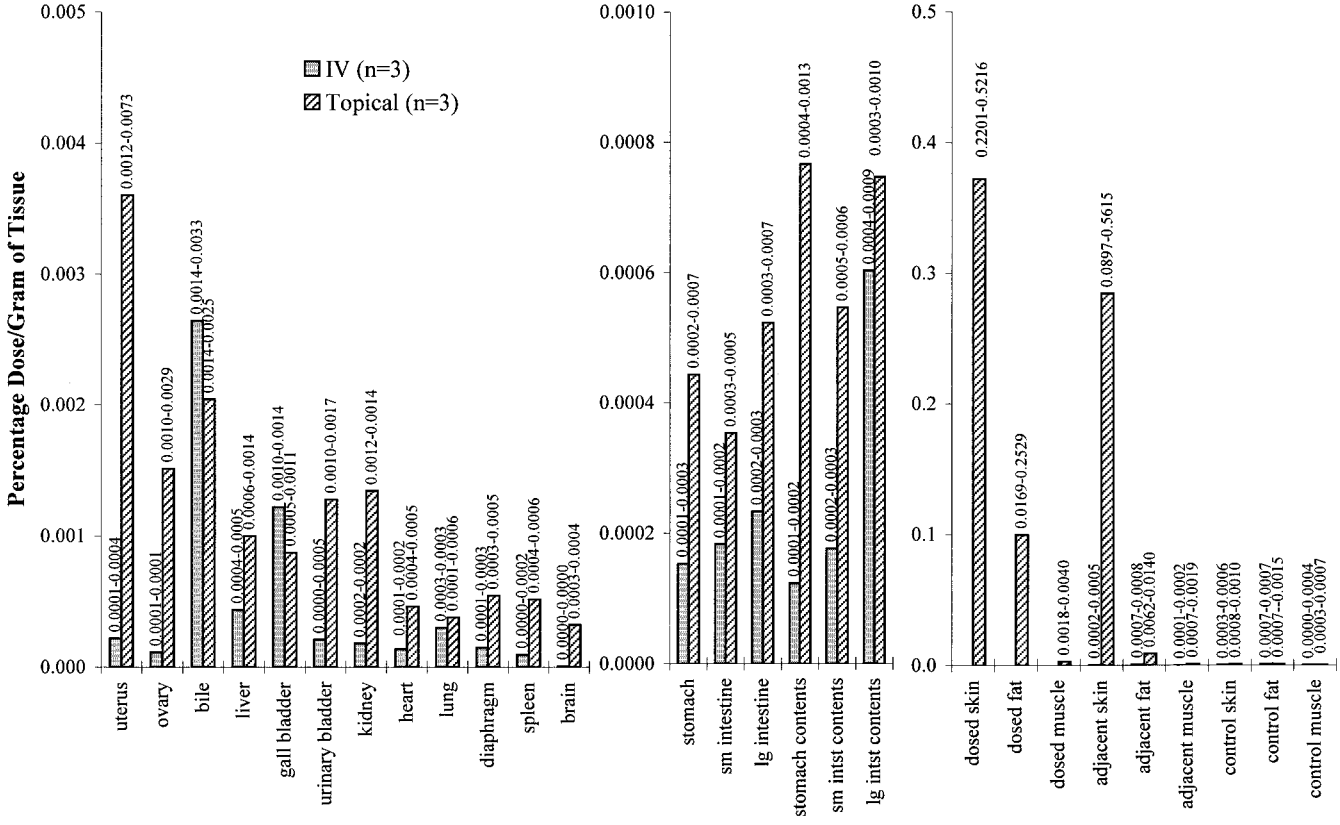

FIG. 5. ^{14}C tissue concentrations following [^{14}C-UL]3,3′,4,4′-tetrachlorobiphenyl iv vs dermal application in the *in vivo* swine model (mean, $n = 3$ with individual data range).

development for chemicals similar to TCB, especially concerning the systemic tissue risk from long-term, low-dose exposure (input rate similar to the dermal experiment in this study) vs short-term, high-dose exposure (input rate similar to the iv experiment in this study) with different pharmacokinetic characteristics. Similar to this *in vivo* study in the pig, Garner *et al.* (personal communication) at NIEHS carried out a totally independent *in vivo* study in the rat and obtained almost identical results of higher tissue residues following dermal rather than iv exposure. Since unique route-dependent blood:tissue and excretion:tissue disposition characteristics of TCB (and likely other PCBs) were identified by independent research groups in different animal species (pigs and rats), the results reported in this work should be very convincing, although the mechanisms may need to be elucidated so as to theoretically advance the science in this field.

Saturable blood-to-tissue transport may play a role in the enhanced tissue distribution after dermal vs iv exposure. The AUC ratio (non-iv over iv) is often used as a standard method for bioavailability (systemic absorption) calculations by the pharmaceutical industry. This method has to assume the blood:tissue and the blood:excretion (by all excretion mechanisms) ratios to be application route independent. However, those two ratios can be route dependent if a blood concentration-dependent partitioning (blood-to-tissue, blood-to-excreta) mechanism (e.g., saturation of a chemical transport carrier) exists, because different routes (iv and non-iv) yield very different blood concentration profiles as "mixtures" of different pharmacokinetic processes. If an identical dose is used, iv dosing often gives much higher blood concentrations at all time points postapplication than the non-iv route. In this case, a high concentration from an iv dose may saturate (overload) the blood-to-tissue transport system; this may not exist in a non-iv situation, and thus the blood:tissue ratio would be application route dependent. According to Borlakoglu *et al.* (1990), lipid-rich lipoproteins provide an excellent system to transport lipophilic PCBs to tissues. They have reported that the distribution of PCBs is more complex than can be explained solely by their solubility in the lipid components of plasma fractions in the pigeon and may suggest a complex association with apolipoproteins and plasma proteins that are important in transporting PCB to tissues. The identification of individual PCBs in a lipoprotein fraction provides evidence for their role in the transport of lipophilic xenobiotics in blood and it is suggested that PCBs associated with lipoproteins are taken up by cells as lipoprotein–PCB complexes (Borlakoglu *et al.*, 1990). The higher blood concentrations after iv than after dermal exposure may exceed the lipoprotein transport capacity and thus yield a

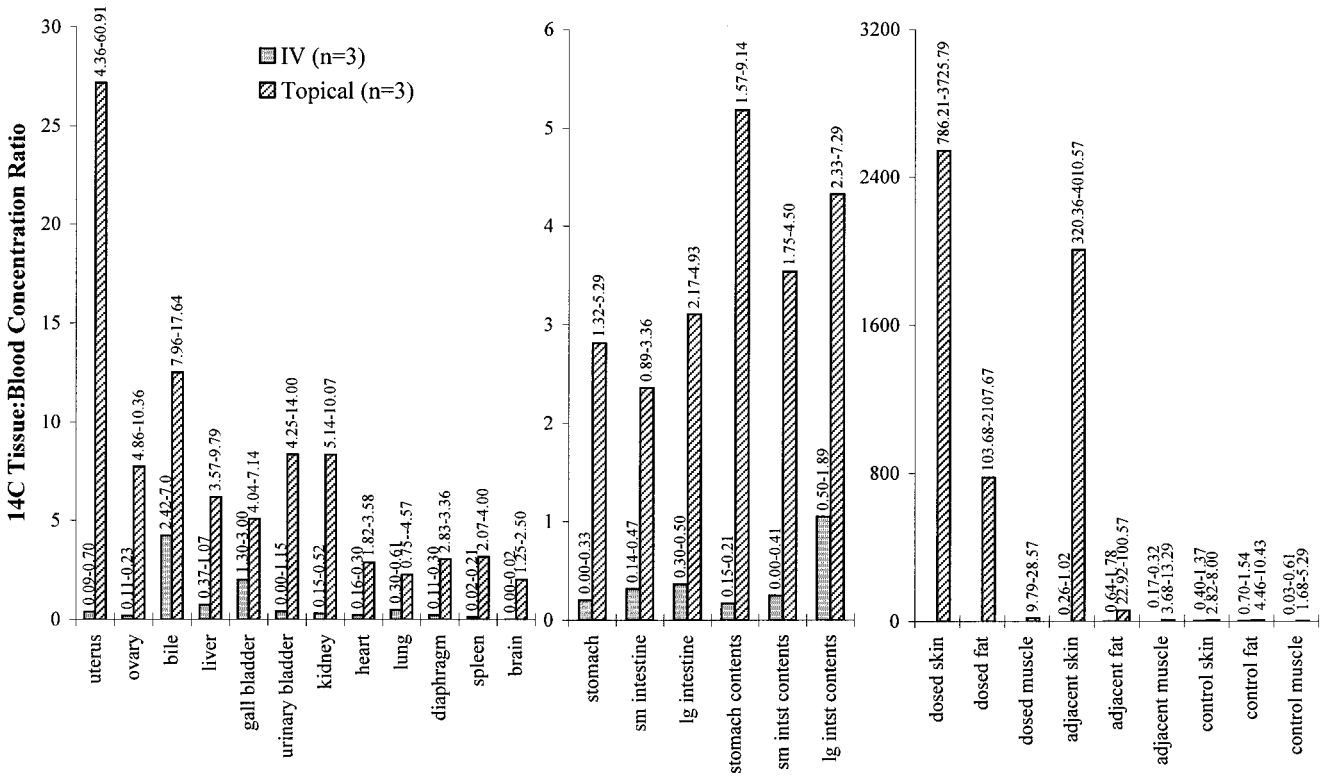

FIG. 6. ^{14}C tissue:blood concentration ratios following [^{14}C-UL]3,3′,4,4′-tetrachlorobiphenyl iv vs dermal application in the *in vivo* swine model (mean, $n = 3$ with individual data range).

lower tissue accumulation of TCB in pig tissues. To elucidate this mechanism, a low-rate iv infusion study (using the same dermal exposure duration) can be proposed to mimic the slow systemic input from a dermal application.

Another mechanism could be exposure route-dependent metabolism. This can be related to enzyme spectrum/relative activity differences between local skin tissue and liver, hepatic enzyme capacity saturation by high concentration of substrate due to quick and large systemic dose load, and/or enzyme induction/inhibition by exposed toxicant at the dermal application site. First, if the label in blood resides in a different profile of metabolites and the parent compound for the two routes, the blood–tissue partitioning and blood:excretion ratios from those application routes could be different. In this *in vivo* study, there could be a unique skin metabolism process, which might be either absent or exist at a lower metabolic rate in systemic metabolizing organs. In general, the likelihood for this seems extremely low, although it is possible. Second, a saturable hepatic TCB metabolism may be responsible, through which chemical species being more readily distributed into tissues, could be derived. An excessive hepatic input rate from iv exposure can hypothetically saturate the liver enzyme system and result in a lower tissue accumulation. However, considering the large capacity of liver metabolism and the low iv dose, the chance for this mechanism is also low.

Excretion Kinetics

In principle, iv bolus application often provides confirmative information about a chemical's distribution and elimination kinetics by bypassing the less-controlled absorption processes associated with non-iv routes. From the nonlinear cumulative excretion curves after iv exposure (Figs. 3 and 4), it was suggested that neither urine nor fecal excretion were zero-order rate processes. However, after dermal exposure, constant urinary and fecal excretion rates were observed, as indicated by the linear cumulative excretion curves. This suggests a "false" zero-order urinary or fecal excretion feature. Since the much higher systemic input and blood concentrations from the iv dose were unable to saturate the urinary and fecal excretion mechanisms (Figs. 3 and 4), the observed linear cumulative dermal urinary and fecal excretion curves cannot serve as evidence of zero-order excretion. Instead, it must be related to the rate-limiting dermal uptake, a phenomenon similar to rate-limited absorption utilized in sustained release pharmaceutical formulations where so-called "flip–flop" kinetics is observed.

Cross-route comparison of AUC:excretion ratios indicated insignificant route dependency ($p > 0.1$). Therefore, one can use blood or plasma AUC to estimate excretion or vice versa (Table 3). This also suggests the renal and fecal clearance was the same for the two exposure routes. However, strong route

dependency in the excretion:tissue residue ratio (a 20-fold difference, $p < 0.1$) and the AUC:tissue residue ratio (a 20- to 30-fold difference, $p < 0.1$) were demonstrated, suggesting neither the label excretion nor the blood AUC data can predict systemic tissue exposure.

In conclusion, TCB dermal absorption is considerable (22%) with a high body burden since 3/4 of the absorbed TCB still remains in the tissues 11 days after dermal exposure. Fecal and urinary mechanisms are equally important for TCB-derived label excretion. An exposure route-dependent relationship between the radiolabel found in the tissues and total excretion, blood AUC, or plasma AUC was determined. Much lower blood concentrations and urinary and fecal excretion during the 11-day study period, but much higher tissue concentrations on the 11th day postdosing were found after dermal compared to iv exposure, suggesting a precaution when using radiolabel blood AUC and excretion data in indirect dermal absorption calculations. Neither blood AUC nor total excretion of ^{14}C can accurately predict overall TCB dermal absorption due to the unique tissue disposition kinetics following dermal exposure to [^{14}C]TCB. This also indicates the necessity to conduct *in vivo* full mass balance studies in order to obtain reliable dermal absorption data, as well as to properly address the overall tissue risk. Indirect dermal absorption methods using label excretion ratios or blood AUC ratio (topical over iv) might be inappropriate if route-dependent tissue distribution kinetics and/or route-dependent metabolism exist.

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