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Dermal Absorption and Distribution of Topically Dosed Jet Fuels Jet-A, JP-8, and JP-8(100)

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Dermal exposure to jet fuels has received increased attention with the recent release of newer fuels with novel performance additives. The purpose of these studies was to assess the percutaneous absorption and cutaneous disposition of topically applied (25 μl/5 cm²) neat Jet-A, JP-8, and JP-8(100) jet fuels by monitoring the absorptive flux of the marker Components ^{14}C naphthalene and ^{3}H dodecane simultaneously applied nonoccluded to isolated perfused porcine skin Raps (IPPSF) ($n = 4$). Absorption of ^{14}C hexadecane was estimated from JP-8 fuel. Absorption and disposition of naphthalene and dodecane were also monitored using a nonvolatile JP-8 fraction reflecting exposure to residual fuel that might occur 24 h after a jet fuel spill. In all studies, perfusate, stratum corneum, and skin concentrations were measured over 5 h. Naphthalene absorption had a clear peak absorptive flux at less than 1 h, while dodecane and hexadecane had prolonged, albeit significantly lower, absorption flux profiles. Within JP-8, the rank order of absorption for all marker components was (mean ± SEM % dose) naphthalene (1.17 ± 0.07) > dodecane (0.63 ± 0.04) > hexadecane (0.18 ± 0.08). In contrast, deposition within dosed skin showed the reverse pattern. Naphthalene absorption into perfusate was similar across all fuel types, however total penetration into and through skin was highest with JP-8(100). Dodecane absorption and total penetration was greatest from JP-8. Absorption of both markers from aged JP-8 was lower than other fuels, yet the ratio of skin deposition to absorption was greatest for this treatment group. In most exposure scenarios, absorption into perfusate did not directly correlate to residual skin concentrations. These studies demonstrated different absorption profiles for the three marker compounds, differential effects of jet fuel types on naphthalene and dodecane absorption, and uncoupling of perfusate absorption from skin disposition. © 1999 Academic Press

Key Words: jet fuel; naphthalene; dodecane; hexadecane; percutaneous/dermal absorption; skin.

Jet fuels used in commercial and military aircraft are a complex multicomponent mixture of aromatic and aliphatic hydrocarbons. Due to their natural petroleum base, their chemical composition is not defined and the fuels are classified according to broad performance criteria such as boiling and flash points. Military fuels are designated as members of the JP (jet propellant) series with JP-4 and JP-7 being the primary fuels used in earlier years. Recently, the military has fielded two new fuels [JP-8 and JP-8(100)] containing several performance additives, which result in fuels with higher flash points and improved heat sink characteristics.

The primary military jet fuel is JP-8 which is essentially a kerosene-cut commercial jet fuel base, Jet-A, to which additives are added to make up <2% of the formulation [a corrosion inhibitor DC1-4A, an antistatic compound Stats 450, and an icing inhibitor diethylene glycol monomethyl ether (DIEGME)]. In the base fuel, 13 components, present at greater than 1% v/v concentrations, comprise 29% of the formulation (see Table 1). JP-8(100) is JP-8 with an additional additive package consisting of antioxidant, chelator, detergent, and dispersant components. Because of the chemical complexity of these fuels, one cannot assess the absorption of the entire fuel, but must instead monitor absorption of individual so-called marker components.

Although there is a relatively large reviewed database on the toxicology and absorption after inhalational exposure to early jet fuels (Jet-A, JP-4, and JP-7) (ATSDR, 1995), and more recently to JP-8 (ATSDR, 1996; Pfaff et *al.*, 1995; Harris et al., 1997), there is no information on the percutaneous absorption after topical exposure to any jet fuels (ATSDR, 1995, 1996). Skin sensitization assays have demonstrated some level of imtation after exposure to the older fuels that may lead to chemical bums (Clark et *al.*, 1988; ATSDR, 1995, 1996), providing indirect evidence of dermal penetration. In reality, the potential for percutaneous absorption leading to dermal and systemic tissue exposure to components of jet fuels has not been systematically investigated.

To quantitate the dermal absorption and cutaneous distribution of these fuels, we utilized a radiolabeled aromatic (^{14}C-naphthalene) and an aliphatic (^{3}H-dodecane) marker from

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TABLE 1
Major Components of JetA

Class	Component	Percent of total	No. of carbons	No. of hydrogens	Mol. wt
Alkane	Dodecane*	4.7%	12	26	170
Alkane	Tridecane	4.4%	13	28	184
Alkane	Undecane	4.1%	11	24	156
Alkane	Tetradecane	3.0%	14	30	198
Alkane	2.6-Dimethylundecane	2.1%	13	28	184
Alkane	Pentadecane	1.6%	15	32	212
Alkane	2-Methylundecane	1.2%	12	26	170
Alkane	Heptylcyclohexane	1.0%	13	26	182
Aromatic	1methylnaphthalene	1.8%	11	10	142
Aromatic	2-methylnaphthalene	1.5%	11	10	142
Aromatic	2.6-Dimeth ylnapthalene	1.3%	12	12	156
Aromatic	Naphthalene;	1.1%	10	8	128
Aromatic	1,2,3,4-Tetramethylbenzene	1.1%	10	14	134
	Total	28.9%			

Note Jet fuel is composed of some 228 components and these 13 only represent 28.9% of the total fuel. The marker compounds studied represent only 5.8% of total.

* Chosen as a marker compound in this study.

among the most prevalent components of JP-8 listed in Table 1. To determine a constituent effect, we studied the absorption, penetration, and cutaneous distribution of these marker compounds in the *in* vitro isolated perfused porcine skin flap (IPPSF) when dosed from Jet-A, JP-8, and JP-8(100). This model system has been successfully utilized to model the mechanism of percutaneous chemical absorption (Riviere and Monteiro-Riviere, 1991; Riviere et al., 1995b; Qiao et al., 1996) and to extrapolate these results to humans (Riviere et al., 1992; Wester et *al.*, 1998; Williams et al., 1990). We also assessed the absorption of naphthalene and dodecane from a fraction of a so-called aged JP-8 "puddle" which was produced by allowing JP-8 to evaporate from an open glass petri dish left in a fume hood for 24 h. After the more volatile components evaporated, the remaining mixture should resemble residual jet fuel remaining 24 h after a jet fuel spill. Finally, to further probe the absorption characteristics of the primary fuel JP-8, we also studied the absorption of a longer chain aliphatic hydrocarbon, ^{14}C-hexadecane after exposure in JP-8.

MATERIALS AND METHODS

Chemicals. Radiolabeled "C-naphthalene (specific activity = 8.1 mCi/mmol) and ^{3}H-dodecane (specific activity = 10,000 mCi/mmol) in methylene chloride were custom labeled by the NCI Chemical Carcinogen Reference Standard Repository (Chemsyn Science Laboratories, Lenexa. KS). The ^{14}C-hexadecane (specific activity = 4.1 mCi/mmol) was obtained from Sigma Chemical (St. Louis, MO). Radiochemical purity of both compounds ranged from 98 to 100%.

All jet fuels. Jet-A. JP-8, and JP-8(100), were kindly supplied by Major T. Miller from Wright Patterson Air Force Base. To produce an aged JP-8 (puddle), JP-8 was allowed to evaporate in an open glass petri dish within a fume hood for 24 h (n = 2). The decline in mass was recorded using an analytical balance over the course of the 24-h period. The shape of the two evaporation curves was similar with no discernible "plateau" evident. As expected, there was a first-order decay to approximately 30% of the initial mass. This remnant solution was then spiked with the radiotracer markers and used for all exposures.

IPPSF *studies.* The IPPSF procedure has been previously documented (Riviere *et al.*, 1986; Riviere and Monteiro-Riviere, 1991; Riviere er *al.*, 1995b). In these studies. jet fuel mixtures were applied nonoccluded to mimic field exposure conditions, and experiments were conducted for a total of 5 h in IPPSFs with 4 replicates per treatment condition. A 1 x 5 cm dosing area was drawn on the surface of the skin flap with a surgery marker. A dose, containing 25 μl of the specified jet fuel containing approximately 2 μCi of ^{14}C-naphthalene plus 10 μCi of ^{3}H-dodecane, was applied directly to the surface of the skin flap. The specific activities of the marker compounds were sufficient that the added radiolabeled compounds had little effect on the final concentration of naphthalene (1.21% instead of 1.1%) and dodecane (4.701% instead of 4.7%). Single label studies were initially conducted and compared to the dual-label results to test whether using this dual-label experimental design had any effect on marker absorption. No effect was detected.

Perfusate samples (3 ml) were collected every 5 min for the first 40 min, then every 10 min until 1.5 h, and then every 15 min until termination at 5 h. At termination, several samples were taken for mass balance of the marker compounds. The surface of the dose area was swabbed twice with a 1% soap solution and gauze, and then 12 stratum corneum tape strips were collected using cellophane tape (3M Corporation, Minneapolis, MN). The entire dose area was removed. A 1 × 1 cm core of the dose area was removed and frozen for subsequent depth of penetration studies. This consisted of laying the core sample epidermal side down in an aluminum foil boat and embedding in Tissue-Tek OCT compound (Miles, Inc., Elkhart, IN), snap freezing in liquid nitrogen. followed by sectioning (40 μm) on a Reichart-Jung Model 1800 Cryocut (Warner Lambert, Buffalo, NY). The remaining dosed area as well as the surrounding skin was separated from the fat and held for analysis. All samples (including swabs, tape strips, core sections. skin, fat, mass balance samples, etc.) were dissolved separately in Soluene. A representative volume of each sample was oxidized completely via a Packard Model 307 Tissue Oxidizer. The ^{3}H and ^{14}C samples were counted separately on a Packard Model 1900TR TriCarb Scintillation Counter.

Data analysis. Data was entered into a custom IPPSF database and the resulting analysis reported. Since all experiments were conducted using the

identical marker doses across all fuels. and the absolute concentrations of these marker compounds were similar. these results are expressed as percentage applied dose to give a representative assessment of the absorption and cutaneous penetration of a complex mixture such as jet fuel. This is appropriate since the absolute concentrations of jet fuel hydrocarbons is not fixed across all fuels due to differences that arise from the natural source of the petroleum and different refining processes. Area under the curve (AUC) in the perfusate was calculated using the trapezoidal method. Peak flux was the maximum flux (% dose/min) observed at any one time point.

Figure 1 depicts the experimental compartments which were analyzed in these studies where the following definitions apply. (1) Surface is the residue removed by washing the surface of the IPPSF at termination of the experiment plus the residues remaining in the dosing template. (2) Stratum corneum is the residue extracted from the outermost stratum corneum via 12 tape strips at the termination of the experiment. (3) Dosed skin is the residue that remained in the dosed skin plus the depth of penetration core taken at termination. (4) Absorption is the cumulative amount of the marker compound collected in the effluent over the course of the 5-h experiment. (5) Fat is the residue remaining in the fat when it was separated from the dermis at the end of the experiment. (6) Penetration is the summation of the label in the effluent plus skin plus fat, but not stratum corneum nor surface. (7) Evaporative loss is that label which was lost to evaporation. Our previous studies in the IPPSF indicated that the penetration estimate is the best empirical correlate to predict eventual *in vivo* absorption in humans (Wester *et al.*, 1998).

Statistical significance of absorption and penetration parameters were determined using ANOVA or by a priori-defined orthogonal contrasts where appropriate (SAS 6.12 for Windows; SAS Institute, Cary, NC) at the 0.05 level of significance. A least significance difference (LSD) procedure was used for multiple comparisons on overall tissue disposition.

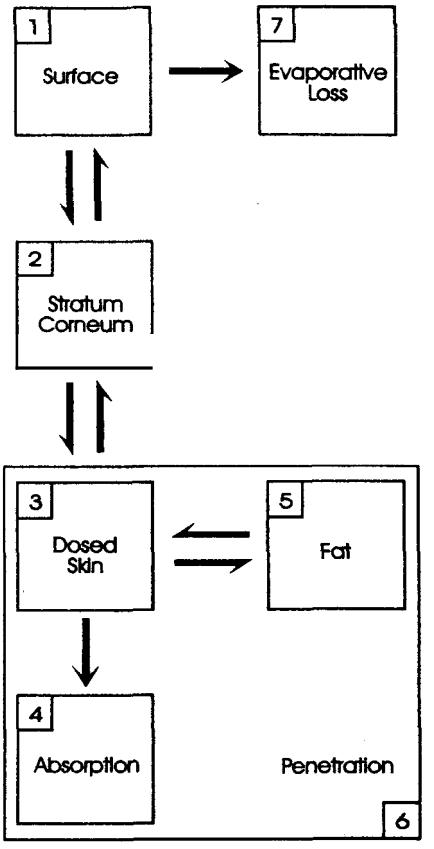

FIG. 1. Schematic depicting sampling sites for which absorption and disposition data are collected. (1) Surface is the residue removed by washing the surface of the IPPSF at termination of the experiment plus the residues remaining in the dosing template. (2) Stratum corneum is the residue extracted from the stratum corneum via 12 tape strips at the termination of the experiment. (3) Dosed skin is the residue that remained in the dosed skin plus the depth of penetration core taken at termination. (4) Absorption is the cumulative amount of the marker compound collected in the effluent over the course of the 5-h experiment. (5) Fat is the residue remaining in the fat when it was separated from the dermis at the end of the experiment. (6) Penetration is the summation of the label in the effluent plus skin plus fat, but not stratum corneum nor surface. (7) Evaporative loss is the unaccounted label.

RESULTS

There are two primary comparisons that are of interest in this study. The first is the relative absorption of the individual marker compounds from jet fuel, and the second is the effect of a specific jet fuel's composition on the absorption of a specific marker.

Absorption of Marker Compounds

Figure 2 compares the absorption flux profiles for naphthalene and dodecane across all fuels, while Fig. 3 depicts all three markers in JP-8. Naphthalene absorption peaks at less than 1 h while the aliphatic compounds are characterized by an absorption plateau at later time points. The primary effect seen in these data is this differential absorption of the individual chemical entities, an effect which overshadows any differences seen as a result of individual fuel composition effects. Table 2 summarizes the absorption parameters of naphthalene, dodecane, and hexadecane from *JP-8*. It is clear from these data that the rank order of marker absorption (% dose mean ± SEM) is naphthalene (1.17 ± 0.07) > dcdecane (0.63 ± 0.04) > hexadecane (0.18 ± 0.08) ($p < 0.05$). Similarly, AUC and *peak* flux are both significantly different among all three markers.

However, a different perspective is achieved when the cutaneous deposition of these markers after dosing in JP-8 is compared. There are differences in the depth of penetration profiles evident (Fig. 4), with the rank order of compounds now reversed at the outermost layers of skin: hexadecane > dodecane > naphthalene. This is especially evident with the hexadecane data. A similar pattern is seen when the disposition of the markers in all skin compartments are examined (Fig. 5). Naphthalene absorption is highest in the perfusate but paradoxically is lowest on the surface and in the stratum corneum and dosed skin. Naphthalene total penetration, which in the IPPSF should correlate to overall absorption *in vivo* in humans, is still marginally greater than the other markers, primarily due to a significantly greater ($p < 0.05$) partitioning into the fat compartment (0.22 vs 0.08 and 0.12 for naphthalene, dodecane, and hexadecane, respectively). These data highlight the importance of the experimental perspective. If an estimate of systemic exposure is desired, then naphthalene has the greatest potential of the three markers studied. If local cutaneous toxicity is the desired endpoint, then the reverse is true since higher amounts of the aliphatic markers remain deposited in the skin at the termination

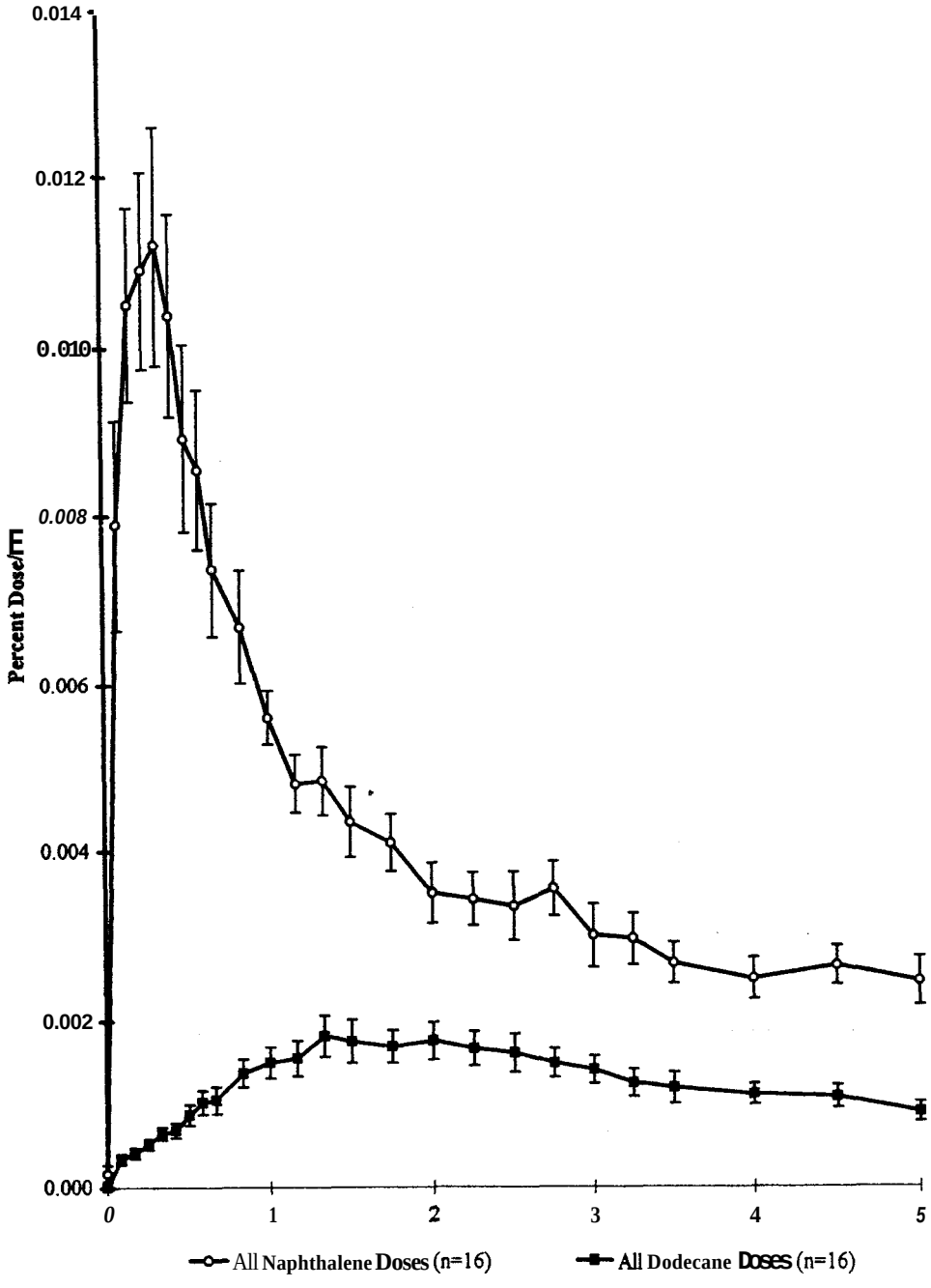

FIG. 2. Perfusate absorption profiles of naphthalene and dodecane (mean % dose/ml ± SEM) pooled from dosing in all jet fuel types.

of an experiment. However, it should be noted that if the compound present in the outermost stratum corneum, recovered in these studies by 12 tape strips, were totally available, then the rank order of penetration would be hexadecane (9.84% dose) > dodecane (6.24% dose) > naphthalene (2.12% dose).

Effect of Fuel Composition

The second phenomenon studied was the effect of the specific fuel formulation on absorption and deposition of the markers naphthalene and dodecane. The rank order of naphthalene perfusate absorption based on AUC, % dose absorbed, and peak flux was JP-8(100) > Jet-A > JP-8 > JP-8 (puddle) for naphthalene and JP-8 > JP-8(100) > Jet-A > JP-8 (puddle) for dodecane (Table 3). Figure 6 depicts the comparative perfusate absorption profiles, and Figure 7 illustrates overall tissue deposition. A dichotomy similar to that discussed above is seen when absorption parameters are compared to skin deposition. Naphthalene and dodecane disposition in skin com-

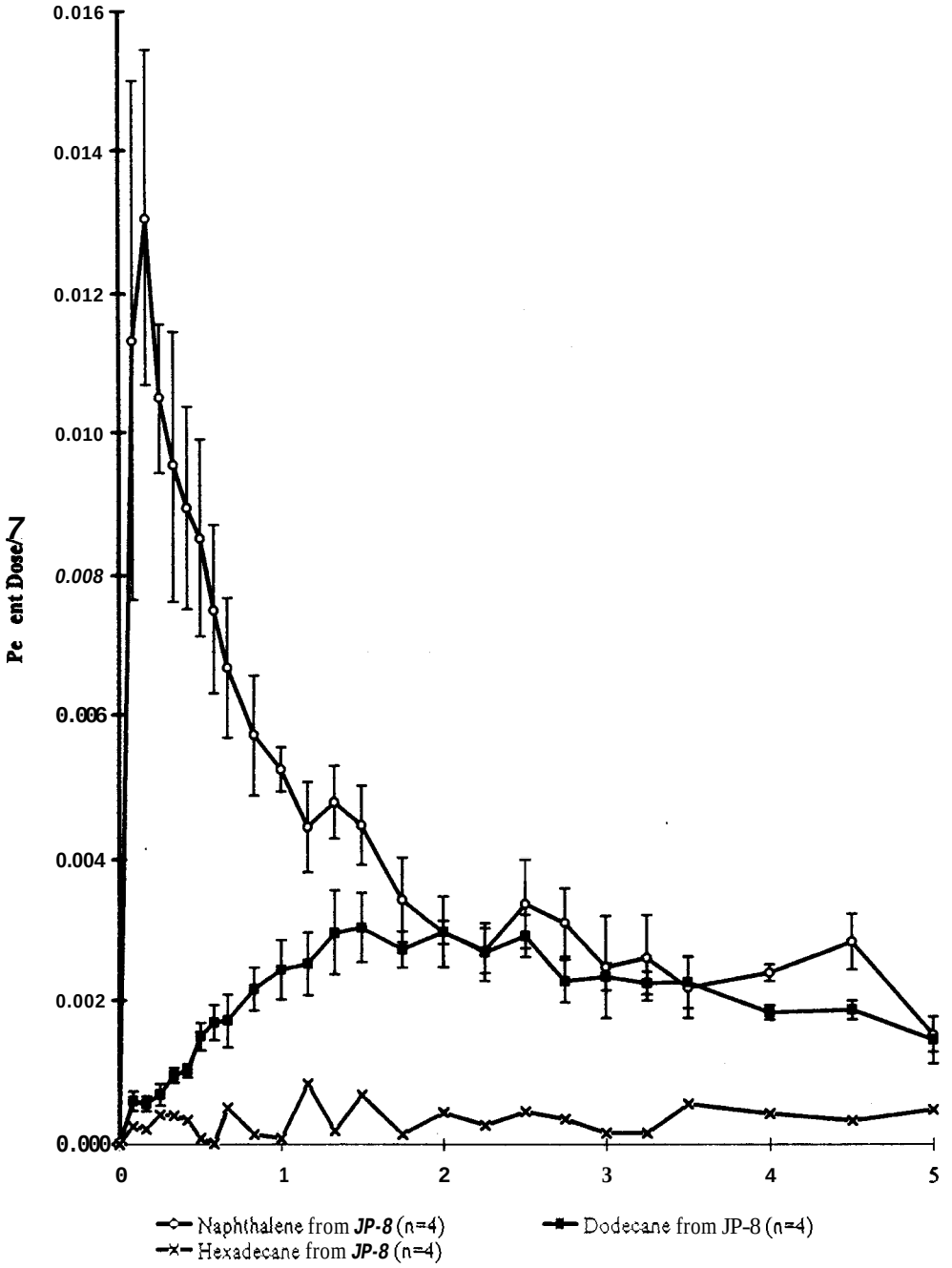

FIG. 3. Comparison of naphthalene, dodecane, and hexadecane absorption into perfusate (mean % dose/ml ± SEM) after dosing in JP-8 jet fuel.

partments did not exactly parallel that seen in perfusate. However, despite these differences in local skin disposition, overall penetration still tracks the pattern in the perfusate with naphthalene from JP-8(100) statistically greater ($p < 0.05$) than JP-8 or JP-8 (puddle). For dodecane, absorption from JP-8 is greater ($p < 0.05$) than the other fuels.

This apparent dichotomy between skin deposition and absorption is further explored in Fig. 8 which presents histograms of the dosed skin/absorption and absorption/dosed skin ratios for naphthalene and dodecane in Jet-A, JP-8, JP-8 (puddle), and JP-8 (100). The major effect seen with this analysis is the significantly different ratios for dodecane disposition from JP-8 (puddle), and again the differences between the naphthalene and dodecane markers.

Statistical Comparisons

Table 4 provides an alternative perspective and statistical summary of the role of naphthalene and of dodecane seen in

TABLE 2
Comparison of Three Marker Compounds from JP-8
Mean (SEM) of Each Parameter

^{14}C-Naphthalene	(n = 4)
AUC (%D-h/ml)	0.0199(0.0020)^{A}
Absorbed (%D)	1.17 (0.07)^{A}
Peak flux (%D/min)	0.015 (0.003)^{A}
^{3}H-Dodecane	(n = 4)
AUC (%D-h/ml)	0.0107 (0.0009)^{B}
Absorbed (%D)	0.63 (0.04)'
Peak flux (%D/min)	0.0036(0.0004)^{B}
^{14}C-Hexadecane	(n = 4)
AUC (%D-h/ml)	0.0017 (0.0003)^{C}
Absorbed (%D)	0.18 (0.08)'
Peak flux (%D/min)	0.0011 (0.0002)^{B}

Note. Means with the same letter are not significantly different.

Fig. 7, A and B, respectively, and in Table 3. Two orthogonal contrasts on the four fuel treatments [Jet-A, JP-8, JP-8 (puddle), JP-8(100)] are used to examine two questions. (1) Is the mean response to Jet-A different from the average of the three JP-8 treatments, namely contrast 1 [3, −1, −1, −1]? (2) Is the mean response of JP-8 different from the mean of JP-8(100), namely contrast 2 [0, 1, 0, −1]? For answering these specific a priori questions based on the structure of the fuel treatments, orthogonal contrasts are statistically preferable to multiple comparison techniques such as LSD (Swallow, 1984; Hsu, 1996). While the LSD procedure allowed pairwise comparisons among the fuels, for question 1, Jet-A (a commercial fuel) is compared to the average of the three military fuels. For question 2, a specific pairwise comparison examines the role of additives in the JP-8 fuel series. The overall p value from the F statistic in ANOVA for the four jet fuels is also presented and denoted as ALL. Note that the overall F may fail to detect some differences that do exist, and that are indicated by the orthogonal contrasts.

In Table 4, the tissue disposition of naphthalene is analyzed using the two contrasts in columns 2 and 3. The only significant differences seen are for contrast 2 in fat and penetration. Surface indicates some difference ($p > 0.10$). For an overall effect (column 4), penetration and surface also have a $p < 0.10$. The use of log responses give a similar result. Note that the p value for surface and contrast 1 now becomes more significant at $p = 0.036$ as the variance is stabilized by the log transformation. In a similar manner, the last two rows (AUC and peak flux) are compared with Table 3. While the overall p value indicates a difference in AUC means, it is not primarily due to the two contrasts examined. For peak flux there is a bigger spread in the SEM and the overall p value becomes more significant after a log transformation, but again this is not due to the two contrasts of interest.

The last three columns in Table 4 examine the tissue disposition of dodecane in a similar manner. For contrast 1, all skin layers, AUC, and peak flux are significant with the exception of fat. For contrast 2, stratum corneum, absorption, penetration, AUC, and peak flux are significant. The overall p values are significant at all the places that contrast 2 is, but also indicate mean differences in dosed skin and evaporative loss. Similar results are found by the log responses. Note that the LSD approach in Fig. 7 and contrast 2 in Table 4 agree in indicating significant differences between JP-8 and JP8(100). However the LSD results from Fig. 7 are pairwise and cannot easily give the comparable results between Jet-A and the JP-8 fuels (contrast 1).

DISCUSSION

These studies highlight the complexities inherent in studying the percutaneous absorption of complex mixtures such as jet fuel. This complexity arises both from the heterogeneity of the chemicals comprising fuel as well as from the different interpretations obtained if systemic absorption vs local cutaneous disposition are the endpoints of concern.

Based on analyses of the fuel stock used in these studies, dodecane is a primary component present at 4.7% (v/v). Naphthalene is a primary aromatic constituent of jet fuel at 1.1%. In contrast, hexadecane is a minor component being present at less than 1%. There are a total of 228 characterized major nonadditive hydrocarbon constituents of these fuels. It is impossible to characterize the absorption of even a fraction of these constituents at the level of detail presented in this investigation. Being relatively nonvolatile, the long-chain hydrocarbons typified by dodecane and hexadecane make up a large percentage of this mixture. As seen in these studies, they have the greatest surface concentrations at the end of an experiment (Fig. 5) and tend to have a larger fraction of dose deposited in the skin. However, they have a decreased systemic absorption as characterized by perfusate fluxes and total penetration. Because the fractional absorption is almost an order of magnitude greater for naphthalene than for dodecane, absolute naphthalene mass absorbed (% dose × concentration in fuel) is still two-fold greater than dodecane even if the latter compound is present at close to 5 times the concentration. A similar ordering of naphthalene and dodecane was reported in preliminary *in vitro* rat absorption studies of JP-8 components (McDougal and Miller, 1998). The magnitude of the differences in total absorption between naphthalene and dodecane overshadow any effect that fuel composition has on marker absorption as can be appreciated by examining the composite absorption profiles in Fig. 2.

A limitation of any short duration study of this design is that steady-state conditions are impossible to achieve due to biological limitations of the model coupled with the volatility of

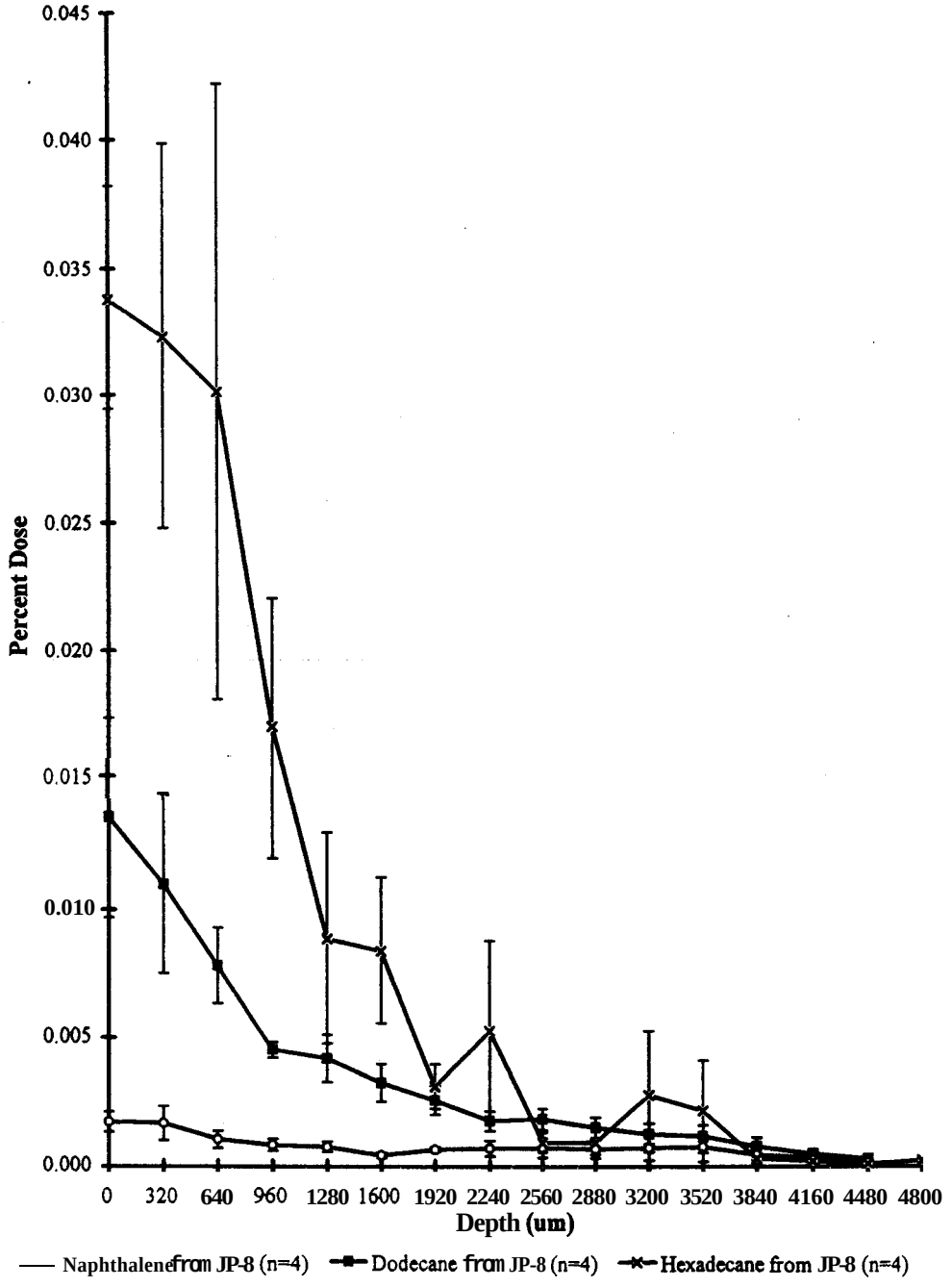

FIG. 4. Depth of penetration of naphthalene, dodecane, and hexadecane into skin (mean % dose ± SEM) after dosing in JP-8.

the hydrocarbons being investigated. This is especially evident when interpreting tissue concentrations at a single 5 h time point at the termination of a study. Previous IPPSF studies have taught that an accurate estimate of potential in vivo absorption is obtained by combining skin and fat residues with absorbed perfusate flux (Wester et ai., 1998; Williams et ai., 1990). This is the penetration parameter reported in this work. Furthermore, skin concentrations at any single time point must be interpreted in the context of a continuum (Williams and Riviere, 1995; Manitz et ai., 1998). Finally, it is expected that some fraction of dose in skin will remain nonabsorbed since a concentration gradient may no longer exist to deliver the compound if the compound preferentially partitions in the skin and forms a depot. These residual skin concentrations could have toxicological significance for a bioactive molecule. The full prediction and description of skin concentrations is obviously more complex. Various pharmacokinetic models are being developed to assess such phenomenon (Manitz *et* al., 1998;

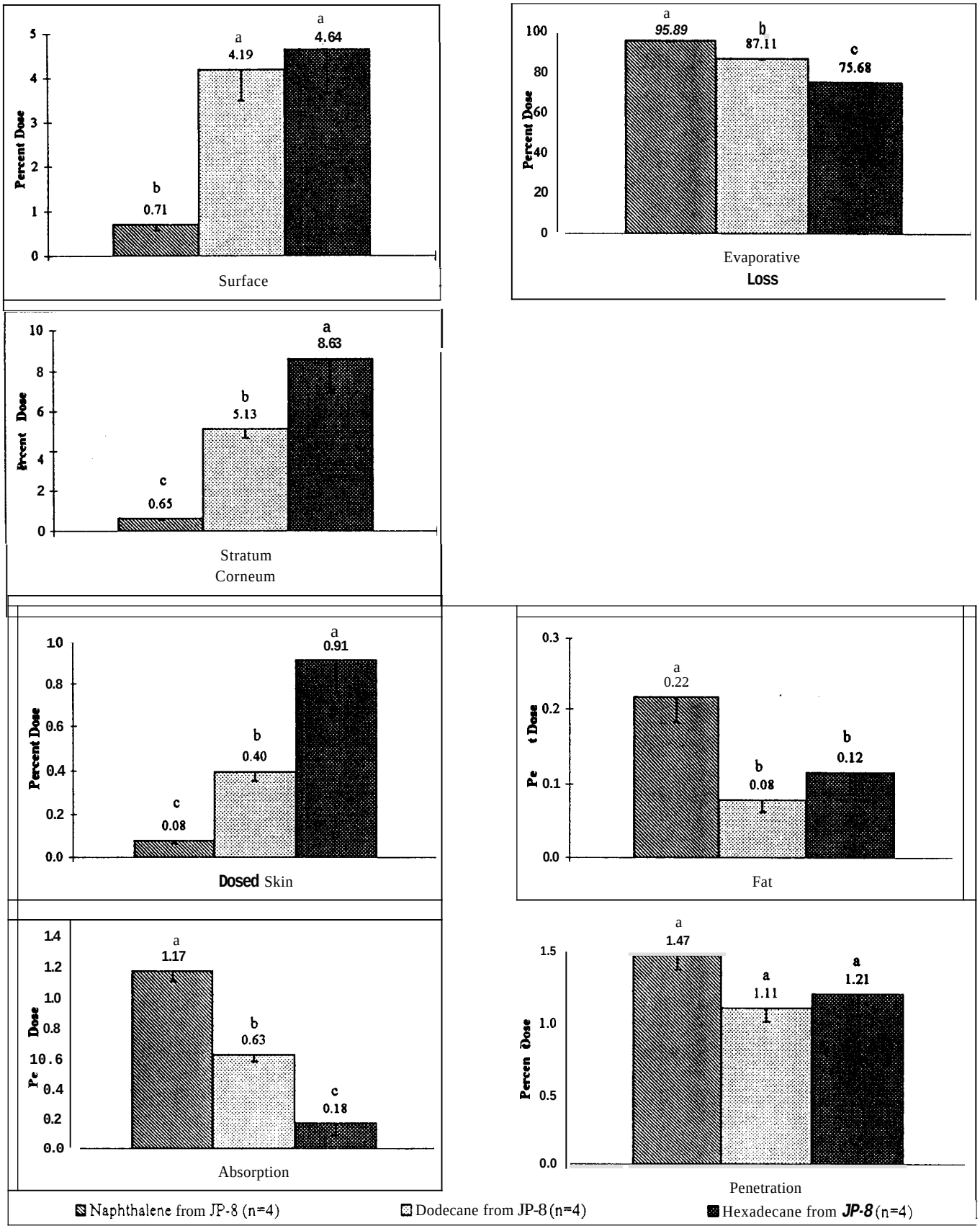

FIG. 5. Pattern of tissue deposition and absorption of naphthalene. dodecane. and hexadecane dosed in JP-8 fuel. All values are expressed as mean % dose ± SEM. Values with different letters are significantly different ($p < 0.05$).

TABLE 3
Jet Fuel Comparison Mean (SEM) of Each Parameter

	Jet-A (n = 4)	JP-8 (n = 4)	JP-8(Puddle) (n = 4)	JP-8(100) (n = 4)	All 16 Doses (n = 16)
^{14}C-Naphthalene					
AUC (%D-h/ml)	0.025 (0.002)^{c}	0.020 (0.002)^{c}	0.015 (0.003)^{b}	0.026 (0.003)^{a}	0.021 (0.002)^{A}
Absorbed (%D)	**1.49 (0.18)**^{a}	1.17 (0.07)^{a}	1.11 (0.16)^{a}	1.63 (0.29)^{a}	1.35 (0.10)^{A}
Peak flux (%D/min)	**0.015** (0.001)^{ab}	**0.015** (0.003)^{ab}	0.008 (0.001)^{b}	0.016 (0.003)^{a}	0.013 (0.001)^{A}
^{3}H-Dodecane					
AUC (%D-h/ml)	0.0048 (0.0005)^{bc}	**0.0107** (0.0009)^{a}	0.0039 (0.0004)^{c}	0.0061 (0.0007)^{b}	0.0064 (0.0008)^{B}
Absorbed (%D)	**0.29** (0.04)^{b}	0.63 (0.04)^{a}	**0.27** (0.07)^{b}	0.35 (0.04)^{b}	0.38 (0.04)^{a}
Peak flux (%D/min)	0.0017 (0.0002)^{bc}	0.0036 (0.0004)^{a}	0.0014 (0.0002)^{c}	0.0024 (0.0001)^{b}	0.0023 (0.0002)^{B}

Note. **Means with the same letter are not significantly different. Lowercase letters represent comparisons within parameters across fuel types. Uppercase letters represent comparisons only within the last column across markers.**

McCarley and Bunge, 1998; Riviere *et al.,* 1995a; Williams and Riviere, 1995), however a discussion of such kinetic interactions is beyond the scope of the present work. The important point to be taken from these studies is that compound absorption and penetration must be assessed in all skin compartments since only looking at the absorbed fraction (e.g., perfusate) may produce very misleading conclusions.

One can appreciate the difference seen for a rapidly penetrating compound like naphthalene vs slower penetrating substances such as dodecane and hexadecane. It is not surprising that a low molecular weight aromatic such as naphthalene would have an enhanced absorption compared to less volatile long-chain aliphatic hydrocarbons such as dodecane and hexadecane. Based on data for these three compounds, the percutaneous absorption is relatively low with the greatest estimated penetration being only 1.5% of the applied dose. However, estimating total *in* vivo absorption is not only a function of the applied dose, but also of the surface area of skin to which the jet fuel is exposed. Thus exposure to large surface areas for prolonged contact times could potentially result in significant systemic absorption.

There is limited data in the literature on the percutaneous absorption of these three hydrocarbons in other experimental models or vehicles. Percutaneous absorption studies of neat naphthalene in humans or laboratory animals had not been reported in a recent toxicological profile (ATSDR, 1993). Absorption was inferred from the occurrence of toxicity after dermal application. A study of neat naphthalene absorption in rats suggested that 50% of the applied dose was excreted in urine by 12 h, with the predominant urinary metabolites being 2,7- and 1,2-dihydroxynaphthalene (Turkall *et al.*. 1994). Naphthalene was also included in an *in virro* monkey skin study comparing the percutaneous absorption in acetone using a series of aliphatic compounds (Sartorelli *et al.,* 1998). Log octanol/water partition coefficients were correlated to permeability constant, with vapor pressure and molecular weight not adding any significant increase in correlation. In the context of the present studies, these correlations would be to the observed perfusate absorption. Evaluation of perfusate fluxes alone, a procedure often done with *in vitro* diffusion cell studies, would mask the significant absorption which ultimately could occur with dodecane and especially hexadecane when skin depots are factored in.

These experimental data suggest that both aliphatic compounds have a propensity for partitioning into skin and forming relatively slowly equilibrating pools that ultimately could be absorbed. Previously, hexadecane has been reported to bind to the stratum corneum (Singh, 1998), a phenomenon which would explain the elevated hexadecane levels seen in stratum corneum (Fig. *5).* In contrast, naphthalene is rapidly absorbed and has minimal propensity to stay in skin, although it tends to partition into subcutaneous fat after absorption. The volatility of these compounds could also be an important factor in determining skin concentrations. Both dodecane and hexadecane have significantly higher residual surface concentrations which result in longer surface residence times on skin, and thus in effect longer dosing times. These different mechanisms of absorption and deposition impact on interpreting what absorption and penetration mean. Compounds with enhanced skin residence, if inherently bioactive, might be expected to have a greater propensity for inducing skin irritation than those compounds which do not form skin depots. All of these hydrocarbons, and especially the composite fuels, have the potential to extract epidermal lipids thereby altering the barrier properties of skin. Such an effect was reported in a preliminary study of JP-8 toxicity (Singh, 1998) and is consistent with toxicological findings in our laboratory that transepidermal water loss (TEWL) increased with chronic jet fuel exposure (unpublished data).

These findings suggest that if toxicology studies of individual components are conducted, the disposition of an aromatic such as naphthalene cannot be used to extrapolate absorption estimates to a long-chain aliphatic such as dodecane. The optimal approach would be to select marker compounds that are characteristic of groups of fuel components based on structure–activity relationships, and then assess absorption, cutane-

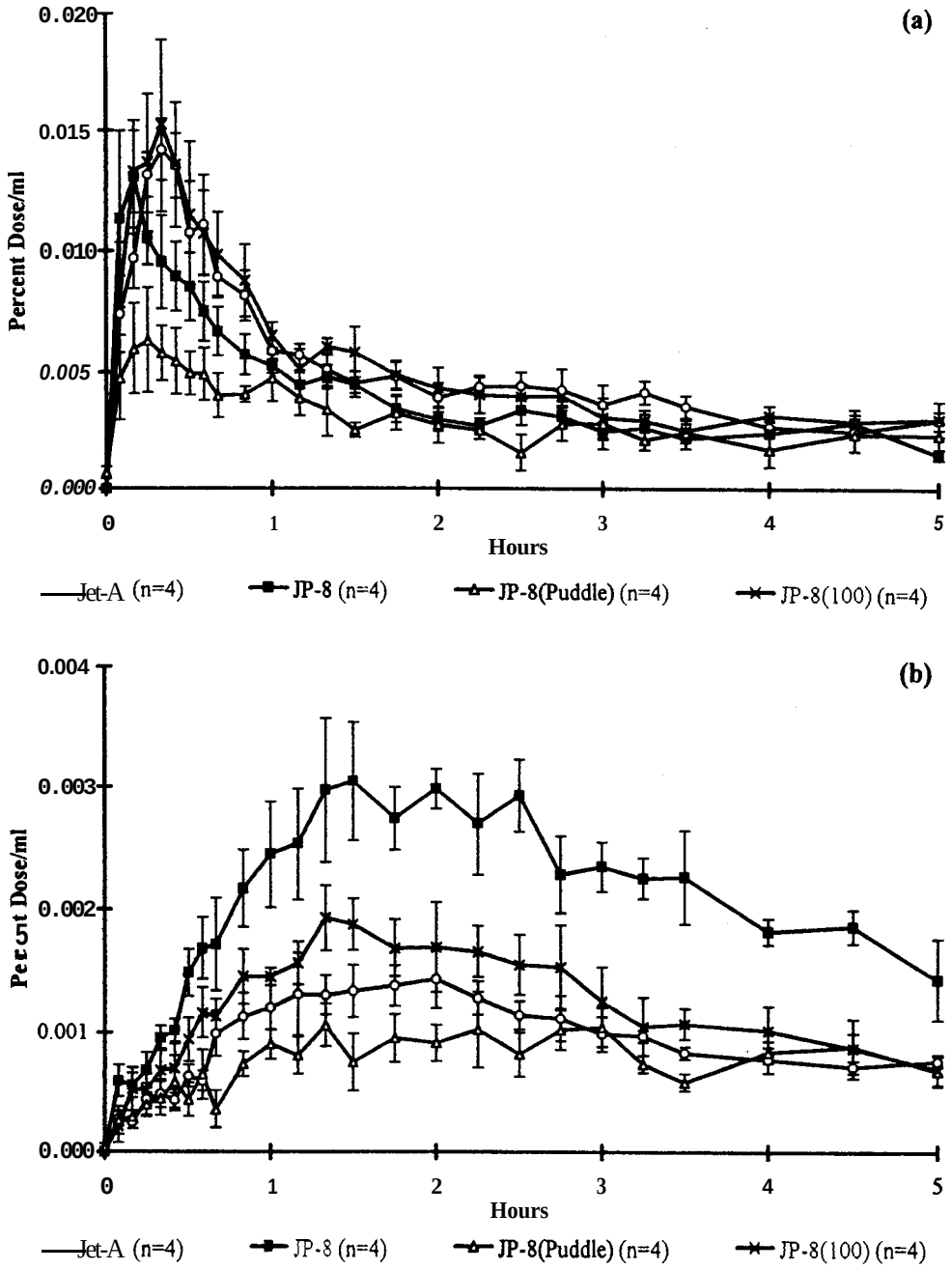

FIG. 6. Naphthalene (a) and dodecane (b) perfusate absorption (mean % dose/ml ± SEM) after dosing in Jet-A, JP-8, JP-8(puddle), and JP-8(100) jet fuels.

ous disposition, as well as toxicological parameters (Basak and Grunwald, 1998).

A surprising finding was the difference in naphthalene and dodecane absorption and skin deposition between the three fuels studied, since these fuels are predominantly composed of similar hydrocarbon components with different additive combinations. Absorption of dodecane was greater from JP-8 than from Jet-A or JP-8(100), while overall penetration of naphthalene was greatest from JP-8(100). Aging of the JP-8 by 24 h evaporation decreased the absorption for both of these markers compared to the other fuel types.

These findings suggest that one or more of the additives combined with Jet-A to make JP-8 modulated dodecane absorption but had minimal effect on naphthalene absorption. The total additive package [corrosion inhibitor DCI-4A, antistatic compound Stats **450.** the deicing additive diethylene glycol monomethyl ether (DIEGME)] composes only a small percentage of the final fuel. A common attribute of these three

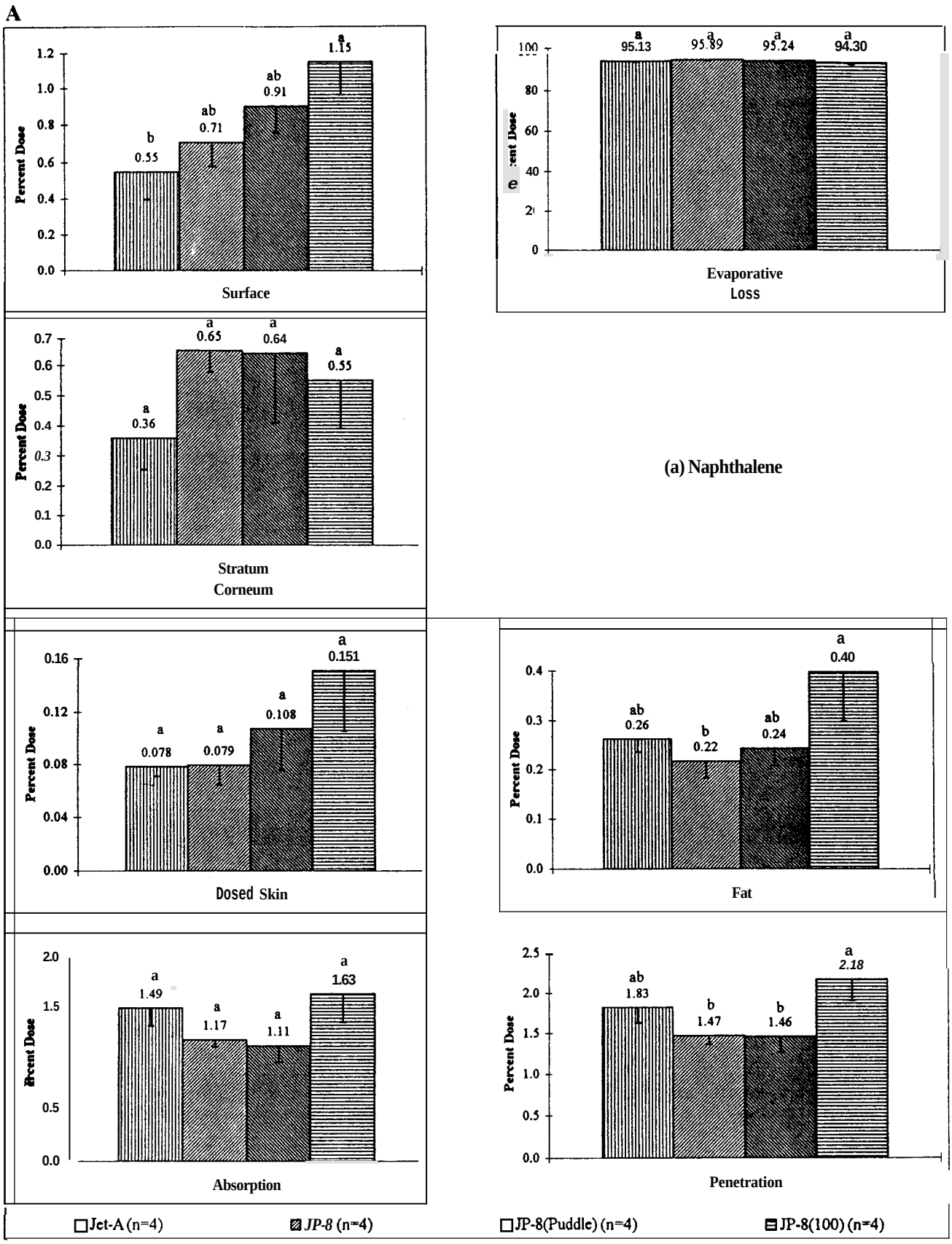

FIG. 7. Pattern of tissue deposition and absorption of naphthalene (a) and dodecane (b) dosed in Jet-A. JP-8, JP-8(puddle), and JP-8(100) jet fuels. All values are expressed as mean % dose ± SEM. Values with different letters are significantly different ($p < 0.05$).

B

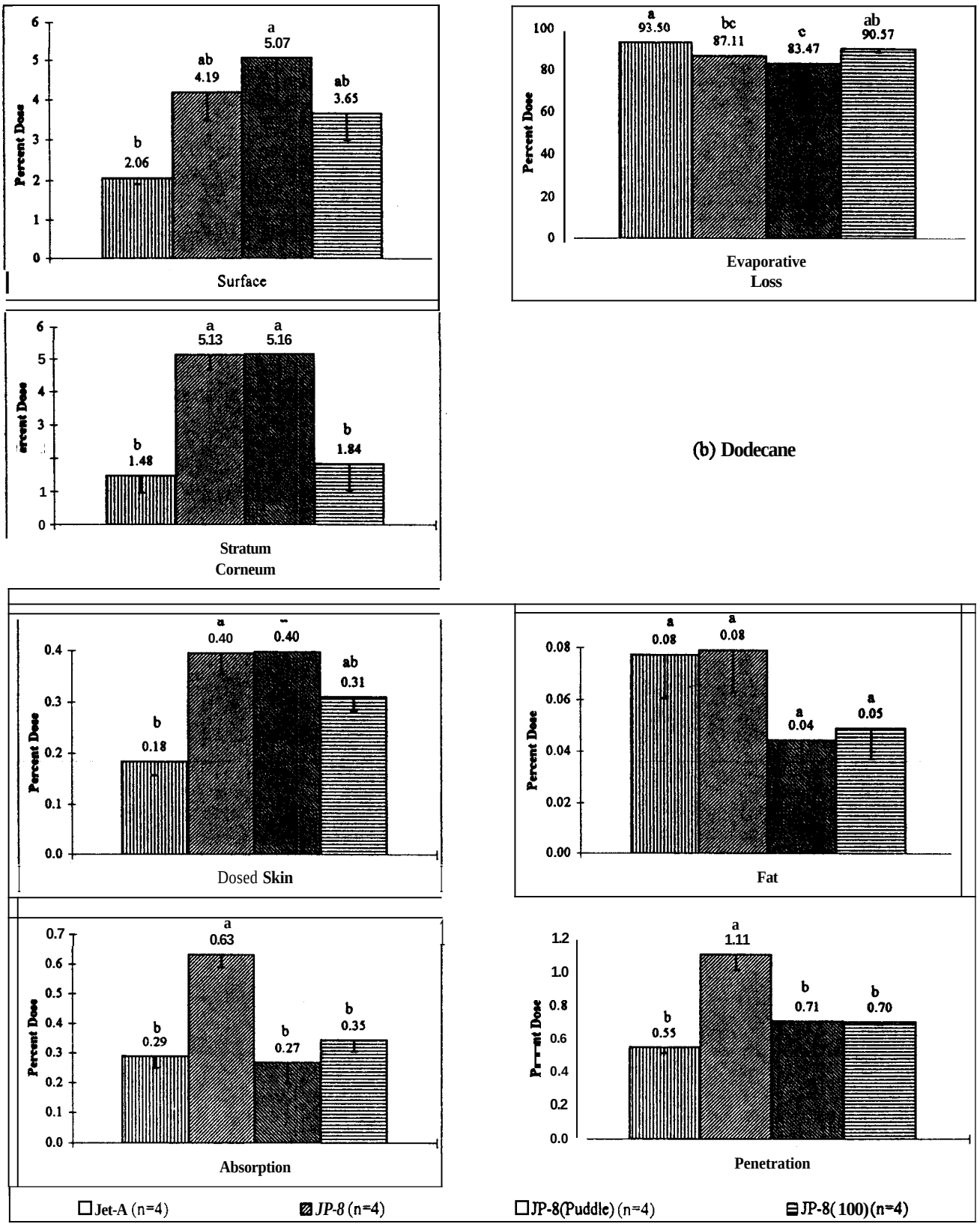

FIG. 7—*Continued*

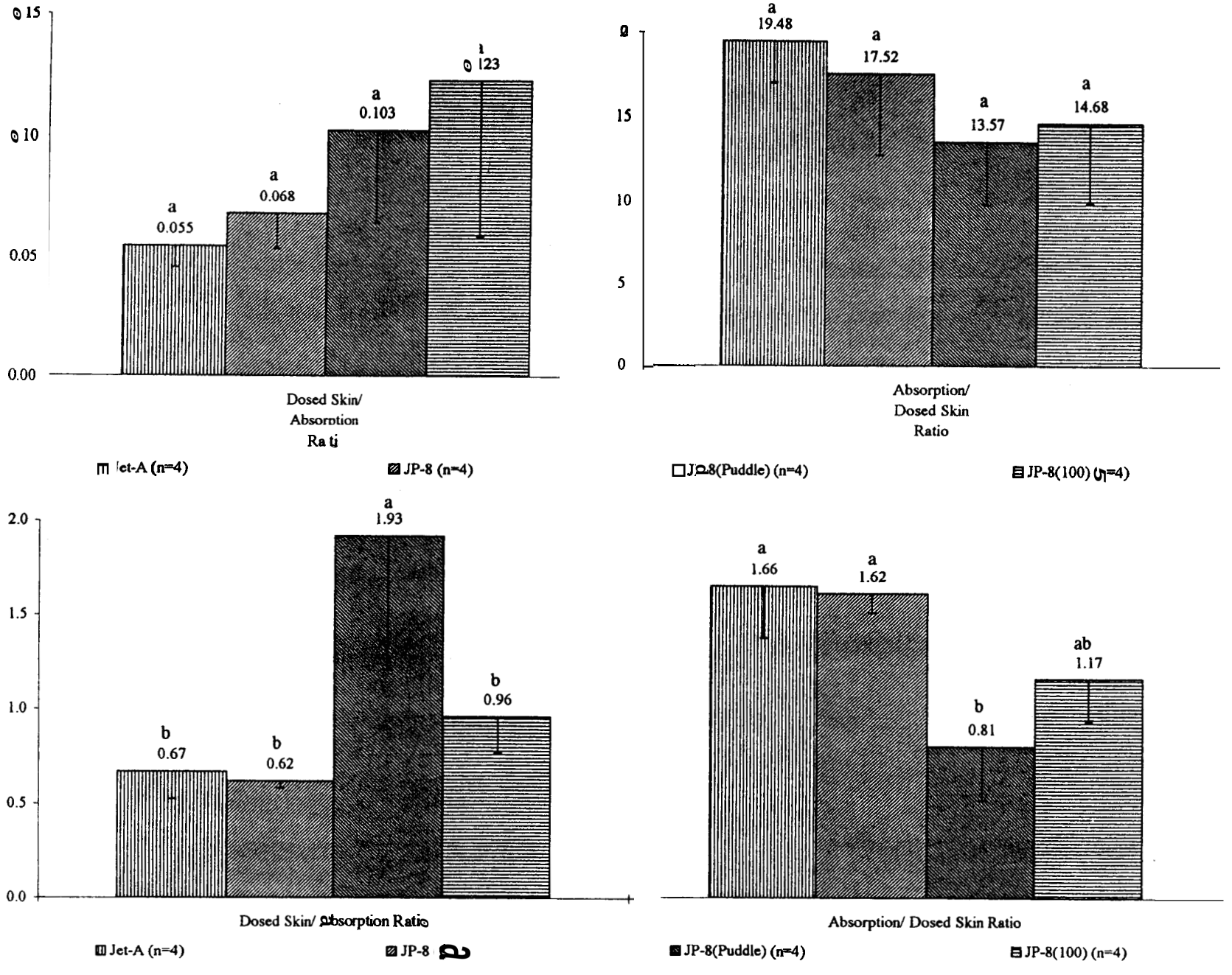

FIG. 8. Absorption to skin deposition ratios (mean ± SEM) of naphthalene (top) and dodecane (bottom) after dosing in Jet-A, JP-8, JP-8(Puddle), and JP-8(100) jet fuels. Values with different letters are significantly different ($p < 0.05$).

TABLE 4
Statistical Significance (p Values) of Napthalene and of Dodecane Absorption and Skin Deposition from Four Jet Fuels

	Napthalene		Contrasts	Dodecane		
	1	2	All	1	2	All
			Original Scale			
Surface	.06	.06	.08	**.021**	.61	.07
Stratum corneum	.18	**.66**	.53	**.030**	**.024**	**.017**
Dosed skin	.32	.11	.30	**.005**	.22	**.022**
Fat	.72	**.045**	.17	.24	.15	.21
Absorption	.43	.12	.22	**.048**	**.002**	**.0008**
Penetration	.61	**.026**	.07	**.001**	**.0005**	**.0002**
Evaporative loss	.98	.10	.38	**.003**	.13	**.003**
AUC	.11	.12	**.027**	**.013**	**.0002**	**.0001**
Peak flux	.49	.64	.10	**.017**	**.008**	**.0003**
			Ln scale			
Surface	**.036**	.13	.09	**.006**	.59	**.033**
Stratum corneum	.24	.38	.52	**.026**	**.012**	**.012**
Dosed skin	.44	.11	.35	**.001**	.25	**.006**
Fat	.96	.07	.28	.24	.16	.21
Absorption	.34	.17	.24	814	**.015**	**.005**
Penetration	.48	**.025**	.06	**.0007**	**.002**	**.0003**
Evaporative loss	.99	.09	.38	**.004**	.15	**.004**
AUC	.11	.20	**.027**	**.026**	**.001**	**.0001**
Peak flux	.27	.71	**.043**	**.015**	**.029**	**.0002**

Note. Contrasts: (Jet-A, JP-8, JP-8(Puddle), JP-8(100)); 1: Jet-A vs Others (3, −1, −1, −1); 2: JP-8 vs JP-8(100) (0, 1, 0, −1); All: Overall p value from ANOVA for four jet fuels. Bold p values are 50.05.

compounds is their relative hydrophilicity compared to the hydrophobic environment of the hydrocarbon jet fuel. DC1-4A is a linoleic acid derivative. Linoleic acid has previously been identified as a skin penetration enhancer for topically applied drugs (Aungst et al., 1986; Mahjour *et* al., 1989; Schneider et al., 1996). The potential for DIEGME to function as a pharmaceutic enhancer has not been investigated, although in the preliminary study quoted above (McDougal and Miller, 1998), DIEGME had an order of magnitude greater transdermal flux of any hydrocarbon component of JP-8. Enhancers generally have their greatest effect on more nonpolar compounds for which the stratum corneum is a true barrier to absorption. These studies would seem to suggest that dodecane would be more susceptible to enhancement than naphthalene. Individual component absorption studies would have to be assessed to further probe this effect.

Of equal significance is the finding that some of these effects (e.g., dodecane) were apparently reversed in JP-8(100), suggesting further interactions with this more complex additive package. For naphthalene, the additive package which transforms JP-8 into JP-8(100) seemed to have increased its penetration. These findings are not totally unexpected, since we have reported on similar complex interactions when absorption of various organics (parathion, carbaryl. benzidine) was studied from defined chemical mixtures (Baynes et al., 1996. 1997; Qiao *et al.,* 1996; Williams *et al.,* 1996). Possible interactions include chemical binding, chemical effects from surfactants, stratum corneum lipid interactions from enhancers, solvent-mediated lipid extraction, low level irritation, and vasomodulation. Dankovic (1989) had reported on prolonged dermal residence times for benzo[a]pyrene when applied in a mixture of 11 polycyclic aromatic hydrocarbons compared to application in a volatile solvent. All of this previous work suggests that such subtle changes in absorption from mixtures is not unexpected. Finally, the absorption of these additives was not investigated in this study, and their potential biological effects on skin could not be assessed. Since these additives are more polar than the hydrocarbon fuel constituents, they would be expected to be more sensitive to modulation by fuel composition. Further speculation on the mechanism of these jet fuel interactions, and on identifying responsible components, is not warranted at this time but requires further probing.

It must be stressed that these studies were designed only to probe whether absorption of two marker hydrocarbon components of jet fuel were affected by addition of performance additives. It is intriguing that there were some significant differences in absorption of the two markers studied. The absorption of both markers was reduced from the aged JP-8(puddle). This is not totally unexpected since the composition of this fuel would be expected to be the most different from the

other fuels since a large fraction of its most volatile components are lost. This could alter partitioning of the markers from the fuel into the stratum corneum lipids. Second, one must also stress that selection of naphthalene and dodecane was not based on any toxicological property, but only by their virtue of representing a fraction of fuel. It is possible that if any cutaneous toxicity were produced from jet fuel exposure that the additive components themselves could be responsible. If the markers studied, naphthalene and dodecane, were affected by fuel composition, it is entirely feasible that the disposition of the more polar additives could be modulated. This modulation could alter the propensity to induce cutaneous toxicity. This hypothesis has not been evaluated in this work.

In conclusion, assessment of dermal absorption of jet fuels is extremely complex. We have characterized the absorption of three different hydrocarbon marker compounds and demonstrated significant differences in individual component absorption and skin deposition. It must be stressed that we are only looking at 3 of the 228 hydrocarbon components that comprise these fuels and there is no reason to believe that these specific components are toxicologically significant. However, before a complete risk assessment can be made, some estimate of dermal absorption and deposition is required. Second, it is critical that when making these assessments, both absorption into perfusate as well as penetration into skin be assessed to obtain a complete picture of disposition. Finally, the effects of fuel additives on the disposition of naphthalene and dodecane suggests that all fuels cannot be treated as one and furthermore, the mechanisms of these specific interactions deserve clarification.

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