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SUBJECT: DERMAL ABSORPTION OF CARBARYL

This memo summarizes dermal absorption data evaluated for carbaryl, and recommends a dermal absorption value to be used in the exposure assessment document (EAD).

In Vivo Data

The dermal absorption of carbaryl has been investigated in humans, rats, and mice. Details of study designs are summarized in Table 1. Results from a well-conducted human study would be anticipated to be predictive of dermal absorption in persons exposed to carbaryl. However, in the case of carbaryl the only human study available (Feldmann and Maibach, 1974) was conducted with an inappropriate solvent vehicle (acetone) and had low recovery of the radiolabel. This study is discussed first.

As part of a study involving several pesticides, Feldmann and Maibach (1974) measured dermal penetration of carbaryl in six human subjects. Applications were on the ventral side of the forearm at a dose of 4 $\mu g/cm^2$ over an area of 2.8 – 20 cm^2 with ^{14}C-carbaryl in 0.1 ml acetone (the position of the radiolabel on the molecule was unspecified). The dose site was not protected, and subjects were asked not to wash the area for 24 hours. Urine collections lasted 120 hours. To correct the dermal absorption for incomplete elimination, an intravenous (IV) dose of carbaryl was administered to the subjects at another time; an average of just 7.4% of the IV dose was excreted in urine. Mean urinary excretion of the dermally applied dose, corrected for urinary excretion of the IV-applied dose, was 73.9% (5.5% uncorrected), with a standard deviation of 21.0%.

The use of acetone as a vehicle by Feldmann and Maibach (1974) might be anticipated to result in an overestimate of dermal absorption. Acetone has been shown to increase dermal absorption of several compounds, including pesticides (Moody *et al*., 1992; Baynes *et al*., 1997; Baynes and Riviere, 1998; Tsai *et al*., 2001). Organic solvents can damage the skin barrier properties, artificially increasing dermal penetration (Scheuplein and Ross, 1970; Fartasch, 1997). For this reason, U.S. EPA (1998) recommends that the vehicle used in dermal penetration studies should be the same as that "under which field exposure occurs," and states that organic solvents "must not be used."

Table 1. Study Designs of *In Vivo* Dermal Absorption Studies for Carbaryl

Subjects	N [a]	Label Position [b]	Dose [c]	Vehicle	Exposure [d]	Total [d]
Human, Male [e]	6	Not specified	4	Acetone, 0.1 ml	24	120
Rat, Male [f]	3	Naphthyl-1	4	Acetone, 0.1 ml	4 – 120	120
Mouse, Female [g]	3	Methyl	1 mg/kg	Acetone, 0.1 ml	< 1 – 48	< 1 – 48
Rat, Male [h]	4	Naphthyl-1	43.5	Acetone, 0.2 ml	0.5 – 168	0.5 - 168
Rat, Female [i]	3	Naphthyl-1	31/37, 109, 539	Acetone, 0.1 ml	72	72
Rat, Female [j]	2 – 5	^{3}H, Ring	0.9	Benzene	< 1 – 24	< 1 – 24
Rat, Male [k]	3	Methyl	0.19	Ethyl alcohol	4	20
Rat, Male [l]	4	Naphthyl-1	35.6, 403, 3,450	CMC	0.5 – 24	0.5 – 24

[a] Number of replicates per dose and duration.
[b] Position of radiolabel on molecule. Radiolabel was ^{14}C and radiopurity of all labeled chemicals was > 99%, unless otherwise specified.
[c] Applied dose of carbaryl in µg/cm^2.
[d] Exposure duration and total test duration in hours. Total test duration includes exposure and subsequent observation, until test termination.
[e] Feldmann and Maibach (1974). Radiopurity not reported.
[f] Shah and Guthrie (1983). Doses not washed off until animals euthanized at 4, 8, 12, 24, 48, and 120 hours post-dose.
[g] Shah *et al.* (1981). Dose reported as mg/kg, applied to 1-cm^2 area. Doses not washed off until animals euthanized at 1, 5, 15, 60, 480, and 2880 minutes post-dose.
[h] Knaak *et al.* (1984). Radiopurity was > 98%. Doses not washed off until animals euthanized at 0.5, 1, 4, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hours post-dose.
[i] Shah *et al.* (1987a).
[j] O'Brien and Dannelley (1965). Radiolabel was ^{3}H; doses were applied to shaved belly of each animal. Doses not washed off until animals euthanized at 3 minutes and 1, 3, 4, 6, 18, and 24 hours post-dose. In another experiment, vehicle was 0.1 ml of either acetone, benzene, or corn oil; test duration 3 hours.
[k] Tos-Luty *et al.* (2001). Tail soaked in solution of carbaryl dissolved in ethyl alcohol.
[l] Cheng (1995). Vehicle was 1.0% carboxymethyl cellulose. Doses not washed off until animals euthanized at 0.5, 1, 2, 4, 10, and 24 hours post-dose.

The low recoveries of the IV and dermal doses decrease confidence in this estimate. The location of the radiolabel on the carbaryl molecule was unspecified, and the majority of its excretion was apparently not urinary. Use of a minor metabolite excreted in urine to estimate absorbed dose is undesirable because it results in exaggeration of measurement and extrapolation errors (Woollen, 1993).

Because of these difficulties, studies involving laboratory animals were also reviewed to determine whether they support results from Feldmann and Maibach (1974). These studies were evaluated based on guidelines in U.S. EPA (1998), which list criteria for the test substance, test animals, and procedure. Major deviations of studies from the guidance in U.S. EPA (1998) are discussed below.

Shah and Guthrie (1983) compared the indirect excretion method used by Feldmann and Maibach (1974) with the direct method used in most dermal absorption studies involving rats. In the direct method, animals are euthanized following multiple time intervals, and tissues and major excretory products are assayed. Shah and Guthrie (1983) used male rats weighing 175 – 200 g; carbaryl was one of three pesticides tested in this study. Six rats were given an intraperitoneal (IP) dose of 1 μCi (like an IV dose, an IP dose does not need to cross any external absorption barrier). For IP-dosed animals, urine and feces were collected at several time intervals up to 120 hours post-dose. For dermally dosed animals, ^{14}C-carbaryl was applied to the midback region (shaved 24 hours earlier), at a dose of 4 μg/cm^2 in 0.1 ml acetone. Groups of three rats were euthanized at 4, 8, 12, 24, 48, and 120 hours post-dose; urine and feces were collected separately. Blood, liver, application site (skin patch), and carcass were assayed for ^{14}C. Total radioactivity recovered in all treatment groups was > 90%; results were reported as % of recovered dose. This study deviated from guidance in U.S. EPA (1998) both in the number of animals per group (3 rather than the recommended 4) and in the use of acetone as a vehicle for dermal doses.

After 120 hours, mean excretion of ^{14}C from IP-dosed rats was 83.52%; time for 50% urinary clearance of the total dose was about 6.5 hours. The correction factor, based on the IP dose, was 1.2. Applying the correction factor to the indirect dermal dose, at 12, 24, and 120 hours the mean dermal absorption was 65.1%, 76.0%, and 91.3 %, respectively. In comparison, the direct dermal absorption at 12, 24, and 120 hours was 72.1%, 75.1%, and 95.7 %, respectively.

Three other studies are available in which dermal absorption of carbaryl dissolved in acetone was measured (Shah *et al*., 1981; Knaak *et al*., 1984; Shah *et al*., 1987a). All three featured higher doses than Shah and Guthrie (1983).

Shah *et al*. (1981) measured dermal absorption of several insecticides, including carbaryl, in female mice aged seven to eight weeks. Radiolabeled pesticides dissolved in acetone were applied at a rate of 1 mg/kg to shaved skin areas of 1 cm^2. Doses were not expressed as quantity per unit area of exposed skin as required by U.S. EPA (1998), and body weights of mice were not provided. If the body weights were in the range reported by Harkness and Wagner (1989) as typical for adult female mice (20 – 40 g), then the dose would be in the range of 20 – 40 μg/cm^2. The dose site was unprotected—also a deviation from U.S. EPA (1998)—though mice were not observed to groom during the study. The carbaryl used in this study was methyl-labeled (specific activity 57 mCi/mmol). Mice were kept in metabolism cages with CO_2-trapping devices after dosing. Groups of three mice were euthanized following intervals of 1, 5, 15, 60, 480 and 2880 min. Following euthanasia, patches of dosed skin were excised to determine amount of unabsorbed radioactivity. The percentage of radioactivity recovered (total >

90% for all compounds) from carcass, blood and urine was compared to that in skin patches. Based on these data, carbaryl penetrated mice skin rapidly. At 5 min post-dose, the geometric mean penetration was 31.5% of recovered radioactivity, and at 15 min post-dose, the geometric mean absorption was 56.0%. Shah *et al*. (1981) estimated the half-life for dermal penetration of carbaryl in mice to be 12.8 $\pm$ 4.1 min. At 480 min (8 hours), the geometric mean penetration was 88.5%; at 2880 min (48 hours), it was 98.9%.

Dermal absorption of carbaryl reported by Shah *et al.* (1981) was higher than in any other study. However, the use of mice in this study is anticipated to result in an overestimate of dermal absorption, and is counter to the guidance of U.S. EPA (1998). Dermal absorption of all fourteen pesticides tested in mice by Shah *et al*. (1981) exceeded 65% at 8 hours, suggesting that these results were likely to overestimate dermal absorption through human skin. For four of the pesticides tested by Shah *et al*. (1981) in mice, Ross *et al.* (2001) reported human dermal absorption of 10% or less. In other studies involving pesticides, mice also showed higher dermal absorption than rats or humans (U.S. EPA, 1992; Baynes *et al.*, 1997). U.S. EPA (1998) states that the rat is the "required species" for dermal absorption studies. Data from this study were not used to estimate dermal absorption.

Knaak *et al*. (1984) tested absorption of carbaryl in 10-week old male Sprague-Dawley rats. Naphthyl-1-labeled ^{14}C carbaryl (specific activity 21.4 mCi/mmol) was diluted with acetone to give a solution concentration of 4.36 µCi/0.2 ml. Doses of 43.5 µg/cm^2 in 0.2 ml acetone were applied to 30.3-cm^2 shaved areas on the back of each rat. At the following intervals, three rats per dose were euthanized: 0.5, 1, 4, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hours. The radioactivity from treated skin, heart, liver, kidneys, carcass, acetone washing from treated skin, feces and urine was assayed. Just 65% of the applied dose was recovered at 165 hours. Of the recovered radioactivity, 7% was in bound skin residues and 57.7% in other tissues or excretions (39.3% in urine and 14.8% in feces). Results from intervals shorter than 168 hours were displayed in a figure in Knaak *et al*. (1984), but the values were not reported. The half-life of carbaryl dissipation from treated skin was 40.6 hours. Calculated skin absorption rate was 0.31 µg/hr/cm^2; however, the plot of carbaryl absorption over time did not appear to be linear. The poor recovery of radioactivity is puzzling, as the location of the radiolabel on the molecule is the same as in three other studies in which recovery exceeded 90% (Shah and Guthrie, 1983; Shah *et al*., 1987a; Cheng, 1995). However, as the material balance was so poor and results were not reported for intervals shorter than 165 hours, data from this study were not considered to reliably predict dermal absorption during a typical workday and were not used.

Shah *et al*. (1987a) compared dermal absorption of fourteen pesticides between young (33-day-old) and mature (82-day-old) female Fisher 344 rats. Treatment groups each had

three animals (Shah *et al*., 1987b), fewer than the four specified by U.S. EPA (1998). Naphthyl-1-labeled ^{14}C carbaryl (specific activity 5.8 mCi/mmol), diluted with 0.1 ml and 0.2 ml of acetone for the young and adults respectively, was assayed at doses of 31, 109, 539 μg/cm^2 (low dose for adults was 37 μmol/cm^2), following an exposure of 72 hours. Dermal penetration in young animals did not vary consistently from that in adults (i.e., no effect of age on penetration was apparent). In both young and adults, percent of dose absorbed was inversely proportional to the applied dose. Mean dermal absorption values in young rats for the low, middle, and high doses were 36.7, 12.2, and 4.8%, respectively; in adults, the values were 30.1, 19.8, and 4.0%.

O'Brien and Dannelly (1965) applied ring-labeled ^{3}H-carbaryl (specific activity 3.7 mCi/mol), diluted with 0.1 ml of benzene, to shaved areas on the bellies of female albino rats. Rats were anesthetized throughout the study, as multiple sections of skin were treated and then removed at specified intervals of 3 minutes and 1, 3, 4, 6, 18, and 24 hours. Half-life for dermal absorption was estimated at 14.5 hours. To determine the effect of solvent vehicle on dermal penetration, in a separate experiment carbaryl was applied to four or five rats per treatment, at a rate of 0.9 μg/cm^2 in 0.1 ml of either acetone, benzene, or corn oil. Animals were euthanized after 3 hours, and the percent of applied dose remaining in skin was determined. Acetone resulted in the greatest loss of carbaryl from skin, with a mean of 42.4% remaining on skin; a mean of 62.8% or 87.8% of applied carbaryl remained when benzene or corn oil, respectively, was the vehicle.

The solvent effect comparison done by O'Brien and Dannelly (1965) appears to support a conclusion that dermal penetration studies using acetone as the vehicle tend to overestimate dermal absorption. U.S. EPA (1998) states that in dermal absorption studies, organic solvents such as acetone or benzene "must not be used," to avoid affecting skin permeability. All the studies discussed to this point used acetone as a vehicle, and results of these studies are potentially confounded by the vehicle choice.

However, O'Brien and Dannelly (1965) can be considered as a pilot study at best, as the methods deviated profoundly in several important aspects from U.S. EPA (1998) guidance. Most importantly, O'Brien and Dannelly (1965) did not do a material balance. Rather than recovered radioactivity, results were reported as radioactivity remaining on skin. It is unclear whether doses were washed off before skin sections were removed, further confounding any relationship between radioactivity remaining on skin and dermal penetration. Additionally, doses were applied to 1.2-cm^2 areas on the skin, less than the 10-cm^2 area recommended by U.S. EPA (1998). Data from this study were not used to estimate dermal absorption.

Tos-Luty *et al.* (2001) applied 1-naphthyl-N-methyl-^{14}C labeled carbaryl to tails of male Wistar rats. Another organic solvent, ethyl alcohol, was the vehicle in this study; the

vehicle choice, along with application to the tail rather than to shaved skin on the back, deviate from guidance in U.S. EPA (1998) and make data from this study difficult to interpret. Tails were allowed to soak in the solution for 4 hours. Following the 4-hour exposure, groups of three animals were euthanized immediately, 6 hours and 20 hours later. After 6 hours, 21.4% of the ^{14}C absorbed initially was recovered from the tail skin; after 20 hours, just 3.6% remained on the tail. ^{14}C was detected in liver, brain, lung, heart, kidney, and in the blood.

Just one study is available in which a formulated carbaryl product was used (Cheng, 1995). This study was performed in general accordance with U.S. EPA (1998). Doses were prepared by mixing naphthyl-1-labeled ^{14}C carbaryl (specific activity: 201 dpm/µg), formulated carbaryl product XLR Plus, and 1.0% carboxymethyl cellulose (CMC). Doses of 35.6, 403 and 3,450 µg/cm^2 in 0.1 ml of vehicle were applied to a shaved area of approximately 12.5 cm^2 on the back of each of four adult male Charles River Crl:CD®BR rats per dose-duration group. The dose site was defined with a plastic enclosure and covered with filter paper. Dosed skin was washed with 2% Ivory soap solution just before animals were euthanized at 0.5, 1, 2, 4, 10, and 24 hours later. Total radioactivity was assayed in urine, feces, the non-occlusive cover, enclosure, skin wash, blood, cage wash and wipe, and skin at application site.

Table 2 summarizes mean percent recovery for each component listed above following 10 and 24 hour exposures, from Tables 6 – 8 of Cheng (1995). In Table 2, dose categories (indirect absorbed and direct absorbed) are reported as in Cheng (1995). Overall mean recoveries for low, middle, and high doses, including all time points, were 95.8%, 94.0%, and 97.4%, respectively.

Total indirect absorbed radioactivity (which includes total direct absorbed) for rats exposed for 10 hours was 21.2%, 10.8%, and 2.50% for the low, middle, and high doses. Following 24 hours exposure, indirect absorbed radioactivity totaled 34.0% of the low dose, 24.9% of the middle dose, and 3.98% of the high dose (Table 2).

Table 2. Mean Percent Dermal Absorption of a Carbaryl Dose Applied to Rats [a]

	Low Dose (35.6 µg AI/cm^2)		Middle Dose (403 µg AI/cm^2)		High Dose (3,450 µg AI/cm^2)	
Component	10 hours	24 hours	10 hours	24 hours [c]	10 hours	24 hours
Direct Absorbed [b]						
Blood	0.08	0.03	0.10	0.06	0.01	0.01
Carcass	4.13	2.78	3.28	3.31	0.70	0.71
Cage Wash	0.78	2.79	0.56	1.66	0.14	0.22
Cage Wipe	0.37	1.26	0.46	1.63	0.03	0.24
Urine	7.28	16.4	3.02	13.3	1.04	1.72
Feces	0.08	1.60	0.03	2.10	0.01	0.28
Total Direct	**12.7**	**24.9**	**7.44**	**22.0**	**1.93**	**3.17**
Indirect Absorbed [b]						
Skin at application site	8.38	9.09	3.39	3.15	0.56	0.80
Total Indirect	**21.2**	**34.0**	**10.8**	**24.9**	**2.49**	**3.97**
Not Absorbed [b]						
Non-occlusive cover	0.26	1.00	0.20	0.49	0.03	0.09
Enclosure rinse	0.51	1.25	0.36	0.60	0.17	0.73
Skin wash	74.0	60.9	82.3	64.6	94.3	90.5
Total Radioactivity	96.0	97.1	93.7	93.5	97.0	95.3

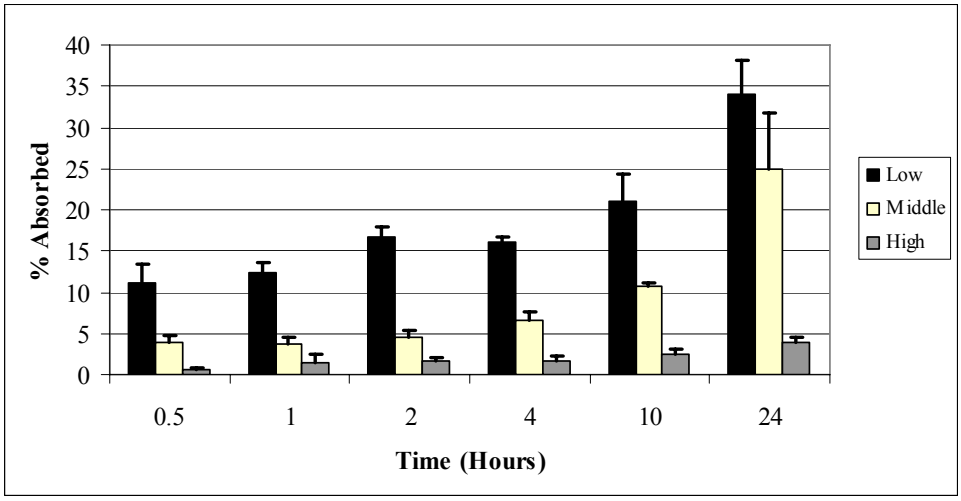
[a] Reported as percent applied dose. Data from Tables 6 – 8 in Cheng (1995).
[b] Categories described in Cheng (1995). Indirect absorbed includes total direct absorbed residues plus amount in skin at application site following skin wash.
[c] Means reported in Cheng (1995) for 24-hour samples of the middle dose were higher than reported here due to exclusion of one replicate because of a low result in urine (4.82%). Cheng (1995) suspected the low result meant that the urine sample was incomplete, but no evidence was provided to support that belief and the weight of the urine sample was in the range of other samples. Mean percent urine, total direct, and total indirect results reported by Cheng (1995) were 16.1%, 24.7%, and 27.8%, respectively.

The high dose, 3,450 µg AI/cm^2, is above the amount recommended by U.S. EPA (1998) for a maximum dose in any study (1,000 µg AI/cm^2), because of concern that such a high dose will "fall off the skin or exceed saturation of the absorption process." The high dose is apparently above the level of saturation (Cheng, 1995). Evaluation of studies done with other AIs suggests that a higher penetration may often occur with lower dose (Thongsinthusak *et al*., 1999), consistent with results of this study. Furthermore, comparison of doses used by Cheng (1995) with other *in vivo* studies reported in Table 1 shows that even the lowest dose (35.6 µg/cm^2) was greater than doses used in many of the other studies. With the exception of Shah *et al.* (1981), Shah and Guthrie (1983), and Shah *et al.* (1987a), the low-level doses were 4 µg/cm^2 or less.

U.S. EPA (1998) guidance on dose selection states that doses "should be at log intervals (i.e. 1.0, 0.1, 0.01, and 0.001 mg/cm^2) and span the range of doses expected in field exposure." Application of higher doses than those anticipated in the field can result in underestimating dermal absorption (Thongsinthusak *et al*., 1999). Preliminary estimates of dermal exposure to handlers, calculated according to DPR policy and assuming a body surface area of 18,150 cm^2, suggests that anticipated dermal exposures range from approximately 0.8 µg/cm^2 to about 800 µg/cm^2. Reentry exposures would be anticipated to be in the range of 0.09 µg/cm^2 to 20 µg/cm^2 (estimates not shown). These expected field exposures suggest that doses in the range of 0.1 µg/cm^2 and 1.0 µg/cm^2 are appropriate.

Figure 1 is a plot of percent total indirect absorption for each of the times sampled, including intervals omitted from Table 2. At each time interval, the percent of applied dose absorbed is greater for the low dose than for the middle dose, with even lower absorption of the high dose. This suggests that there is a dose effect for carbaryl, in which the dermal absorption is greater at lower doses than at higher ones.

Figure 1. Dermal Absorption of Carbaryl Doses Applied to Rats [a]

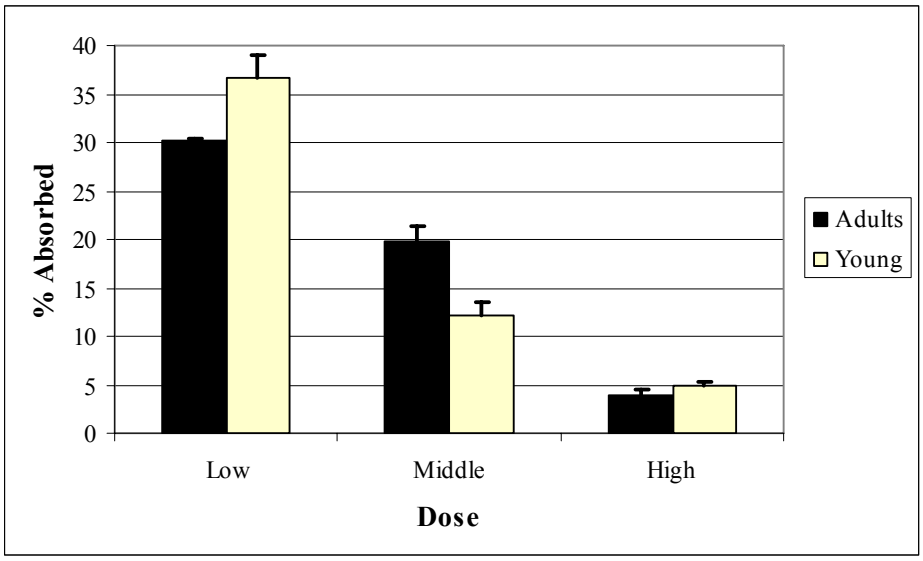


[a] Data from Cheng (1995). Each bar is the group mean ± standard deviation of 4 replicates following dermally applied doses of 34.5 µg/cm^2 (dark bars), 403 µg/cm^2 (light bars), or 3,450 µg/cm^2 (gray bars).

Thus, in the *in vivo* studies determining dermal absorption of carbaryl, potential confounding occurs due to both vehicle (i.e., organic solvent or formulation) and dose levels. Comparison of data from Cheng (1995) with dermal absorption from other studies in which acetone was the vehicle can help clarify the extent of vehicle and dose level effects. Figure 2 summarizes percent dermal absorption following 72 hours exposure to one of three different dose levels. The low dose shown in Figure 2 (31 µg/cm^2 for adults)

is about the same as the low dose shown in Figure 1 (34.5 μg/cm^2), and about eight-fold higher than the 4 μg/cm^2 dose applied to human subjects by Feldmann and Maibach (1974).

Figure 2. Percent Absorbed at 72 Hours of Carbaryl Dose Applied to Female Rats [a]

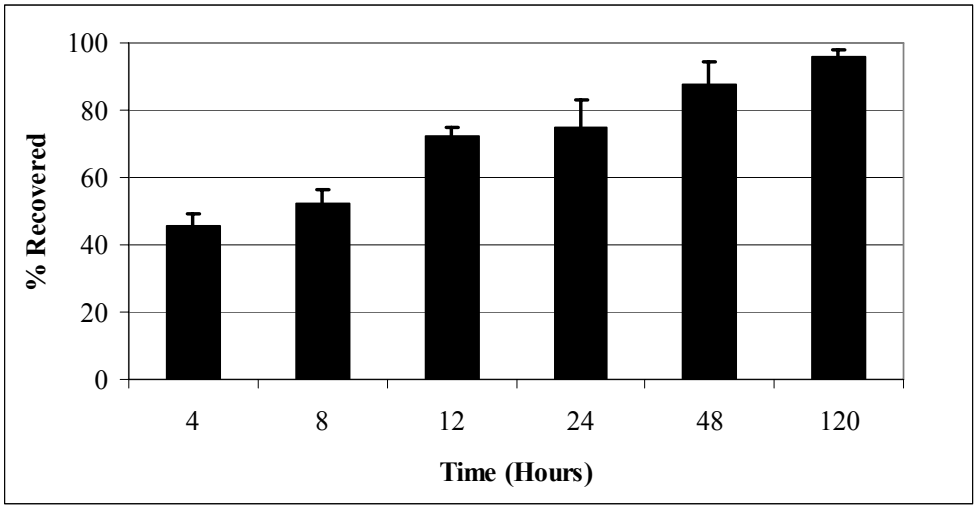


[a] Data from Shah *et al*. (1987a). Each bar is the group mean ± standard deviation of 3 adult (dark bars) or young (light bars). Dermally applied doses were 37 μg/cm^2 for young rats and 31 μg/cm2 for adults (low dose), 108 μg/cm^2 (middle dose), or 538 μg/cm^2 (high dose). Doses were applied in an acetone vehicle.

Data from Shah and Guthrie (1983) can be used to assess whether applying a lower dermal dose correlates with a higher percentage of dose absorbed. Figure 3 summarizes the percent of carbaryl absorbed at several time intervals following an applied dose of 4 μg/cm^2 in acetone to adult male rats (Shah and Guthrie, 1983). Following 8 hours of exposure, the mean absorbed carbaryl dose was 52.2%, and after 12 hours of exposure, the mean absorbed carbaryl dose was 72.1%.

Comparison of Figure 3 with Figures 1 and 2 suggests that the applied dose greatly affected dermal absorption in the range of doses tested in all three studies. Greater absorption of the 4 μg/cm^2 dose applied by Shah and Guthrie (1983) was reported after 4 hours (45.7%) than was reported for absorption of a higher dose (31 – 34.5 μg/cm^2) over longer exposure durations of up to 24 or 72 hours by either Cheng (1995) or Shah *et al.* (1987a).

Figure 3. Percent Absorbed of 4 µg/cm^2 Carbaryl Dose Applied to Male Rats [a]



[a] Data from Shah and Guthrie (1983). Each bar is the group mean ± standard deviation of 3 replicates. Dermally applied doses were 4 µg/cm^2, applied in an acetone vehicle.

Furthermore, the low dose of 34.5 µg/cm^2 applied by Cheng (1995) was about the same as the low dose of 31 µg/cm^2 applied to adult rats by Shah *et al.* (1987a). The 34.0% absorption of the low dose in rats studied by Cheng (1995) following a 24-hour exposure was also similar to the 30.1% absorption of the low dose reported by Shah *et al.* (1987a) after 72 hours. These data suggest that use of acetone as a vehicle by Shah *et al.* (1987a) did not substantially increase dermal absorption of carbaryl.

The greater absorption reported by Shah and Guthrie (1983) than by Cheng (1995) appears to be related more to differences in dose (4 µg/cm^2 vs. 35.6 µg/cm^2) than to the use of acetone as a vehicle. This in turn suggests that dermal absorption reported by Cheng (1995) might underestimate that occurring in the field.

In Vitro Data

A total of four studies are available, three using porcine skin sections and one using rodent skin.

Chang *et al*. (1994) assessed the dermal penetration of four pesticides (carbaryl, lindane, malathion, parathion) in perfused-porcine skin flap. Administration of the compounds occurred in ethanol solution at a dose rate of 40 ug/cm^2. Monitoring of the absorption from the afferent venous system took place for 8 hours, after which 4.22% of the applied carbaryl dose had crossed the membrane. These results were incorporated into a

pharmacokinetic model to extrapolate absorption data to 6 days, giving a predicted 6-day absorption estimate for carbaryl of 33.2% (Chang *et al*., 1994).

Two studies (Baynes *et al*., 1997; Baynes and Riviere, 1998) examined mixtures of carbaryl and *N,N*-diethyl-*m*-toluamide (DEET), along with other chemicals and solvents, in flow-through diffusion cells. In these experiments, porcine skin disks with an exposed area of 3.2 cm^2 were perfused at a flow rate of 4.0 mL/hour with Krebs-Ringer bicarbonate buffer. Perfusate samples were collected hourly for 8 hours; at the end of the 8-hour interval skin sections was swabbed twice with a soapy solution and tape-stripped six times. Each mixture was replicated at least four to seven times.

Baynes *et al*. (1997) applied 40 $\mu g/cm^2$ of ^{14}C-carbaryl in acetone or dimethylsulfoxide (DMSO), with or without DEET (15% concentration). Vehicle (acetone or DMSO) concentrations were 40% or 80%. Mean dermal penetration of carbaryl in acetone ranged 8.51% to 20.4%. DMSO enhanced penetration; mean dermal penetration of carbaryl in DMSO ranged 21.3% to 36.9%. With both vehicles, penetration was decreased at the high vehicle concentration (80%) than the lower (40%); DEET decreased penetration further.

Baynes and Riviere (1998) used the same 8-hour flow-through study design, and added sodium lauryl sulfate (0%, 1% or 5%), piperonyl butoxide (0% or 2%), and 1-naphthol (0 or 40 $\mu g/cm^2$) to the mixtures. Data from Baynes *et al*. (1997) are also reported in Baynes and Riviere (1998). Sodium lauryl sulfate, 1-naphthol, and piperonyl butoxide all tended to increase carbaryl penetration in the mixtures tested.

MacPherson *et al.* (1991) investigated dermal penetration and metabolism of carbaryl in the dorsal skin of rats, using an open-top static *in vitr*o system. Square sections of dorsal skin about 2 cm^2 were mounted in a diffusion cell, with about 1.18 cm^2 exposed to the air in the donor cell. The exposed portion received 40 $\mu g/cm^2$ of ^{14}C-carbaryl in 0.01 ml of acetone. In various trials, receptor solutions were either 50% ethyl alcohol in water, 0.9% sterile saline, or tissue culture medium prepared from Earle's salt base. Receptor fluid was sampled (0.05 ml) and replaced with fresh solution at intervals ranging between 0 and 7 hours after carbaryl application. At 7.5 hours post-application, the test was terminated and skin and receptor solution analyzed. Of the three receptor solutions, ethyl alcohol acquired the most ^{14}C, with a mean of 2.76% of the applied dose. Saline and Earle's medium acquired averages ^{14}C of 0.77% and 0.95% of the applied dose, respectively. Conversely, bound-skin residues of carbaryl were lower when the receiving solution was ethyl alcohol, 31% of the applied dose compared to 41% and 35.5% for saline and Earle's medium. In this experiment, there was no evidence of skin metabolism of carbaryl. However, in other experiments, when homogenates of skin were incubated

with carbaryl, both with and without cofactors, metabolic products indicative of hydrolysis and ring hydroxylation followed by conjugation were detected.

U.S. EPA Estimate of Dermal Absorption of Carbaryl

U.S. EPA's Hazard Identification Assessment Review Committee (HIARC) established a dermal absorption value to be used in the carbaryl risk assessment of 12.7% (Dobozy, 2002). HIARC determined that value based on the absorption of the low dose from Cheng (1995), following 10 hours exposure (see Table 2). U.S. EPA excluded bound skin residues in estimating dermal penetration.

Dermal Absorption Estimate Recommended for Use in the Exposure Assessment

Data from *in vitro* studies were not used to determine dermal absorption for the purpose of exposure assessment. Use of *in vitro* studies to estimate dermal absorption is problematic because: 1) the extent of compound solubility in receptor solutions may affect results; 2) relationships between *in vivo* and *in vitro* test results have not been reliably established for many classes of compounds, and have been shown to vary for compounds that have been tested; and 3) the viability of membranes used with *in vitro* systems may be affected by preparation and storage (Franklin *et al*., 1989; Wester and Maibach, 2000; Zendzian and Dellarco, 2003).

Table 3 compares dermal absorption values among selected *in vivo* studies. Comparisons were done of dermal absorption values pertinent to a typical occupational exposure period, about 10 hours (this assumes that workers do not shower until reaching home at the end of a workday). Studies that did not report data for exposure intervals of 24 hours or less were omitted from this comparison (Knaak *et al*., 1984; Shah *et al*., 1987a). One other study was omitted from this comparison (Tos-Luty *et al.*, 2001) because the study design did not match the other studies.

The study with the highest dermal absorption (88.5%; Shah *et al.*, 1981) was the only study done using mice. In other studies, mice exhibited a higher dermal absorption than rats or humans (U.S. EPA, 1992; Baynes *et al.*, 1997), suggesting that including a mouse study would result in overestimating dermal absorption. Furthermore, this was also the only study in which the application site was unprotected, possibly resulting in overestimated absorption due to ingestion during grooming. Although grooming was not observed to occur, the possibility could not be excluded over an 8-hour period. For these reasons, data from Shah *et al.* (1981) were not used to estimate dermal absorption.

Table 3. Comparison of Selected Results of Carbaryl Dermal Absorption Studies [a]

Subjects	Dose [b]	Vehicle	Exposure Duration [c]	% [d]
Human, Male [e]	4	Acetone, 0.1 ml	24	73.9
Rat, Male [f]	4	Acetone, 0.1 ml	12	72.1
Mouse, Female [g]	1 mg/kg	Acetone, 0.1 ml	8	88.5
Rat, Female [h]	0.9	Acetone/Benzene/Corn oil, 0.1 ml	3	***57.6/37.2/12.2***
Rat, Male [i]	35.6	1.0% Carboxymethyl cellulose	10	21.2

[a] Comparison of *in vivo* studies only. Study details are given in Table 1.
[b] Applied dose of carbaryl in μg/cm^2, unless stated otherwise.
[c] Exposure duration in hours.
[d] Dermal absorption, reported as percent of applied dose. For studies with more than one dose or exposure duration, the lowest dose and duration closest to 10 hours were selected for this comparison. Italicized values were calculated indirectly as described in text, and are probably not directly comparable to other studies.
[e] Feldmann and Maibach (1974).
[f] Shah and Guthrie (1983).
[g] Shah *et al.* (1981). Dose reported as mg/kg, applied to 1-cm^2 area. Body weights of mice were not provided, but if they were in the range of 20 – 40 g, then the dose would be in the range of 20 – 40 μg/cm^2. Percent penetration reported as geometric mean. The application site was unprotected, and although grooming was not observed to occur the possibility could not be excluded.
[h] O'Brien and Dannelley (1965). Dermal absorption calculated by subtracting amount recovered from skin from 100%.
[i] Cheng (1995).

The dermal absorption estimate recommended for use in the exposure assessment is 70%, based on the dermal absorption in humans following 24 hours exposure to carbaryl (Feldmann and Maibach, 1974), and in rats following 12 hours exposure to carbaryl (Shah and Guthrie, 1983). These data might overestimate dermal absorption in humans following a full workday, but are considered to be the best available for occupational exposure.

Additional study data would be needed to support lower dermal absorption estimates. Ideally, data from the well-conducted study of Cheng (1995) should be supplemented by additional dose levels of 4 μg/cm^2 or below to counter the low-dose dermal absorption reported by Shah and Guthrie (1983). The expected field exposures for handlers and reentry workers suggest that doses in the range of 0.1 μg/cm^2 and 1.0 μg/cm^2 are needed.

If additional studies are contemplated, DPR welcomes the opportunity to review and discuss study protocols before such studies are initiated.

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