Department of Pesticide Regulation

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MEMORANDUM

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HSM-04027

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(original signed by S. Beauvais)

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SUBJECT: REVIEW OF DERMAL ABSORPTION DATA FOR CARBOFURAN

For carbofuran, no *in vivo* human dermal absorption studies are available to the Department of Pesticide Regulation (DPR), although reports of two *in vivo* and two *in vitro* dermal absorption studies have been published in the scientific literature. The first *in vivo* study examined dermal penetration rates of several pesticides in mice (Shah *et al*., 1981). The second *in vivo* study compared dermal penetration of carbofuran in young and adult female rats (Shah *et al.*, 1987a; 1987b). The first *in vitro* study compared dermal penetration of several pesticides, including carbofuran, through human foreskin pieces mounted in a static diffusion chamber (Shehata-Karam *et al*., 1988). The second *in vitro* study compared dermal penetration of three pesticides, including carbofuran, through rat abdominal skin mounted in a static diffusion chamber (Liu and Kim, 2003).

This memo summarizes evaluations of the available studies and justifies the value used for dermal absorption in the exposure assessment for carbofuran.

In Vivo Studies

In the first study (Shah *et al*., 1981), female mice aged seven to eight weeks were used. Radiolabeled pesticides dissolved in acetone were applied at a rate of 1 mg/kg to shaved skin areas of 1 cm^2. The carbofuran used in this study was ring-labeled (specific activity 2.85 mCi/mmol). Mice were kept in metabolism cages with CO_2-trapping devices after dosing. The dose site was unprotected, though mice were not observed to groom during the study. Groups of three mice were euthanized following intervals of 1, 5, 15, 60, 480 and 2880 min. Following euthanasia, 3- to 4-cm^2 patches of skin were excised (not washed first) to determine the 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 from the dose site. Shah *et al.* (1981) concluded their data showed that carbofuran penetrated mouse skin rapidly. At 5 min post-dose, 32.6% of recovered radioactivity had been absorbed, and at 15 min post-dose, 71.7% of recovered radioactivity had been absorbed. Shah *et al.* (1981) estimated the half-life for dermal penetration of carbofuran in mice to be 7.7 min; at 8 hours, 94.7% had penetrated (geometric mean of three animals).

The second study was described in detail in Shah *et al*. (1987a); selected data from the study were used in a comparison of dermal penetration of fourteen pesticides in Shah *et al*. (1987b). Briefly, young (33-day-old) and mature (82-day-old) female Fisher 344 rats were used, and dermal penetration was studied via both *in vivo* and *in vitro* methods. In the *in vivo* study, ring-labeled ^{14}C carbofuran (specific activity 39.4 mCi/mmol), diluted with 100 µl and 200 µl of acetone for the young and adults respectively, was assayed at doses of 28 (6.1 µg), 285 (63 µg), 535 (118 µg), and 2680 (593 µg) nmol/cm^2, following an exposure duration of 72 hours; also, the penetration of one dose (285 nmol/cm^2) was reported following multiple exposure durations (6, 24, 48, 72, and 120 hours). Each dose was applied to a 1-cm^2 area of shaved and cleaned skin. The dose site was protected by perforated plastic blister glued to the site. Following euthanasia, 3- to 4-cm^2 patches of skin were excised (not washed first) to determine the amount of unabsorbed radioactivity. Dermal penetration was calculated by subtracting the radioactivity recovered from the application site from total radioactivity recovered from all tissues; because of this, bound skin residues were considered unabsorbed. Two major results of this study were that dermal penetration in young animals exceeded that in adults (Shah *et al*., 1987a), and that dermal penetration was inversely proportional to the applied dose (Shah *et al*., 1987b). At 120 hours, the *in vivo* dermal penetration of a mid-level dose (285 nmol/cm^2) was 43% in young rats and 18% in adults (Shah *et al*., 1987a). At 72 hours, dermal penetration in mature rats ranged from 83% of the low dose to 6% of the high dose (Shah *et al*., 1987b).

Both studies are considered unacceptable for several reasons. One of the most serious difficulties with these studies is that results were presented on a wet-weight basis, while organ weights were not given, preventing back calculations of dermal absorption values. Other serious issues involve deviations from procedures currently recommended by DPR (Thongsinthusak, 1994) and U.S. Environmental Protection Agency (U.S. EPA) (1998).

The reasons for rejecting both *in vivo* studies are listed below:

- Reporting of results on a wet-weight basis, with no conversion between wet and dry weights. Organs were dried and powdered before analysis, yet no data are given to show how measured radioactivity was related back to wet weights. Different organs would have differing water content, and it's unclear if this was considered in the calculations.
- Use of acetone as the vehicle. U.S. EPA (1998) recommends that the vehicle used should be the same as that "under which field exposure occurs." Organic solvents "must not be used." Studies should usually be conducted with formulated product or water as the vehicle.
- The treated skin was not washed off after the exposure period (e.g., 8-10 hours). Residues on and in the skin at the dose site were all considered to be unavailable. However, residues at the dose site are usually differentiated into those that are potentially available for absorption and those that are not. As explained by Thongsinthusak *et al*. (1999a), "the dermal absorption value is defined as the total quantity of the dose recovered from washed skin,

blood, urine, feces, cage wash/cage wipe, organs (if collected), expired air (if present), and the remaining carcass." In the absence of data on the availability of bound skin residues, DPR policy is to consider residues on washed skin at the treatment site as part of a dermal absorption value. Insufficient information was provided in either of these studies to determine residues on washed skin.

- The doses tested for durations approximating a workday (8 hours) were too high. Evaluation of studies done with other AI suggests that a higher dermal absorption may often occur with lower dose, as seen in this study (Thongsinthusak *et al*., 1999b).
- Treated areas measured 2.8 cm^2 for the juveniles and 5.6 cm^2 for the adults rather than the recommended 10 cm^2. U.S. EPA (1998) notes that smaller areas do not allow application of a consistent thin film, which may result in an underestimate of absorption.
- The treated skin was covered with a perforated plastic blister in the second study (no cover was used in the first study). This is possibly an occlusive cover, depending on the number and size of perforations. Nonocclusive covers described by U.S. EPA (1998) include a spacer (such as a plastic, glass, or rubber ring or other shape) glued to the skin, to which is attached a cover of filter paper or gauze.

In Vitro Studies

In the first study, *in vitro* dermal penetration was studied using foreskin segments from newborn humans (Shehata-Karam *et al*., 1988). Briefly, the tissue was obtained immediately after circumcision, kept moist on ice until used, and then mounted in a modified static diffusion chamber with nutrient media. Tests were run at 37°C. Pesticides were applied at a dose of 38 $\mu g/cm^2$, dissolved in 1 µl of acetone. Samples were collected from the media at intervals of 1, 6, 24, and 48 hours. The dermal penetration of carbofuran at 48 hours was 82%, a value which agrees with the 72-hr low-dose *in vivo* absorption result of Shah *et al*. (1987b).

In the second study, *in vitro* dermal penetration was studied using strips of abdominal skin obtained from male Sprague-Dawley rats that were 5-6 weeks old (Liu and Kim, 2003). Skin membranes (3.14 cm^2) were placed into diffusion chambers with physiological saline media immediately after they were obtained. Technical grade pesticides were applied in varying amounts ranging from 2 mg to 150 mg, dissolved in 100 µL of acetone. Tests were run at 32°C, with continuous shaking at 600 rpm for 48 hours. Samples were collected from the media at intervals of 6, 12, 24, 36, and 48 hours. The limit of detection was 0.1 ppm for all pesticides. The dermal penetration rate was estimated by plotting percent absorbed by time, and fitting a least-squares regression to the steady-state linear portion of the curve. For carbofuran, the steady-state linear equation was 1.05 $\mu g/cm^2$ per hour (Liu and Kim, 2003).

The use of *in vitro* studies to determine dermal absorption is problematic because the extent of compound solubility in receptor solutions may affect results and because 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 (Franklin *et al*., 1989; Wester and Maibach, 2000). Therefore, DPR does not, by standard practice, rely on *in vitro* studies to determine dermal absorption.

Dermal Absorption Estimate Used in Exposure Assessment

As no acceptable data are available, the DPR default value of 50% will be used in the exposure assessment for calculations of absorbed dermal doses. This default value is based on a review of data from several chemicals, as documented in Donahue (1996).

Recommendations for Additional Studies

Additional study data would be needed to support lower dermal absorption estimates. Additional studies should be done according to the "Significant quantity of residue remaining on the washed skin" recommendations in U.S. EPA (1998). That is, exposure durations should be 10 hours, and daily excretion collections should continue for a minimum of 14 days and a maximum of 21 days.

Dose levels should be lower than those used in Shah *et al*. (1987a), and should be logarithmically spaced. Exposure estimates shown below in Tables 1 and 2 suggest that dose levels should include 1 and 0.1 $\mu g/cm^2$.

Alternately, if analytical methods do not support use of dose levels as low as 0.1 $\mu g/cm^2$, then at least three doses, logarithmically spaced (e.g., 1, 10, 100 $\mu g/cm^2$), can be used in a study conducted according to recommendations in U.S. EPA (1998). In addition to providing a better supported estimate of dermal absorption, such a study would explore the relationship, if any, between dose and dermal absorption, and could be used to show that at doses anticipated to occur during and following use, dermal absorption is not greater at lower doses.

If additional studies are contemplated, DPR welcomes the opportunity to review and discuss study protocols before such studies are initiated (Thongsinthusak, 1994).

Table 1. Estimated Potential Carbofuran Exposure for Handlers [a]

Work Task	Dermal Exposure [b] (µg/lb AI handled)	Acres per Day [c]	Rate [c] (lbs/acre)	Acute Exposure [d] (µg/cm^2)
Aerial M/L, Liquid [e]	77.3	1,000	1.0	4.26
Aerial Applicator	14.6	1,000	1.0	49.1
Aerial Flagger	17.0	1,000	1.0	8.37
M/L, Chemigation	77.3	350	6.0	8.94
GB M/L, liquid [e]	77.3	540	10.0	23.0
GB Applicator [e]	102	540	10.0	30.3
LPHW M/L/A [e]	9,480	1	0.025	0.0131

[a] Adapted from draft exposure assessment document (Beauvais and Johnson, 2004).

[b] Calculated from surrogate data using PHED database and software (PHED, 1995). Values from PHED were rounded to three significant figures.

[c] Maximum acres/day based on default (Haskell, 1998). Application rate (lbs/acre) is maximum label rate. NA = Not applicable.

[d] Acute Exposure (µg/cm^2) = [(dermal exposure) x (multiplier) x (rate) x (acres/day)]/(18,150 µg/cm^2).

Values in calculation derived from include those described in previous footnote and the following:

- Multipliers are explained in Powell (2002). Briefly, they are intended to address uncertainty in how well PHED subsets correspond to exposure scenarios they are intended to represent.
- Body surface area = 18,150 cm^2. Mean of 50th percentile male and female total body surface area from Table 6-2 and Table 6-3 (U.S. EPA, 1997)

[e] GB = groundboom. LPHW = low pressure handwand. M/L = mixer/loader. M/L/A = mixer/loader/applicator.

Table 2. Estimated Potential Carbofuran Reentry Exposures [a]

Work Task	DFR [b] (µg/cm^2)	TC [c] (cm^2/hr)	Acute Exposure [d] (µg/cm^2)
Scouting Cotton	0.057	2,000	0.050
Pruning/Thinning Artichokes	3.27	1,000	1.44

[a] Adapted from draft exposure assessment document (EAD; Beauvais and Johnson, 2004).

[b] Dislodgeable foliar residue (DFR) estimated for appropriate restricted entry interval. Sources listed in EAD.

[c] Transfer coefficient (TC) is rate of skin contact with treated surfaces.

[d] Acute Exposure (µg/cm^2) = [(DFR) x (TC) x (8 hours/day)]/(18,150 µg/cm^2).

- Body surface area = 18,150 cm^2 (U.S. EPA, 1997)

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