



Department of Pesticide Regulation



Paul Helliker
Director

MEMORANDUM

Gray Davis
Governor
Winston H. Hickox
Secretary, California
Environmental
Protection Agency

TO: Joseph Frank, Senior Toxicologist
Worker Health and Safety Branch **HSM-03020**

FROM: Sheryl Beauvais, Staff Toxicologist, Specialist *[original signed by S. Beauvais]*
445-4268

DATE: July 25, 2003

SUBJECT: REVIEW OF DERMAL ABSORPTION DATA FOR INDOXACARB
(DATA PACKAGES 200486, 200487, 200488)

At my request for dermal absorption (DA) data for indoxacarb, DuPont submitted two studies, an *in vivo* study in which DA of the 30% WDG formulation was determined in rats (Fasano, 2002a), and an *in vitro* study using the same formulation and rat and human skin (Fasano, 2002c). In addition, DuPont sent two other reports that are based on these studies. Data from the *in vivo* study, which had an exposure duration of 6 hours (Fasano, 2002a), were extrapolated using the Exponential Saturation Model (Thongsinthusak *et al.*, 1999) to obtain an estimate of maximum DA (Fasano, 2002b). Results of the *in vivo* and *in vitro* studies were evaluated by Frame (2003) to determine an estimate of *in vivo* DA in humans. In this memo, all available data are reviewed and an estimate of dermal absorption for use the exposure assessment is provided.

***In Vivo* Study**

The *in vivo* study was performed according to European draft test guidelines, specifically the OECD 2002 Draft Guideline 427 (Fasano, 2002a). Male rats, aged 6 – 8 weeks and weighing 190 – 225 g, were used. Four animals were in each group, for a total of 16 in the study. Indoxacarb was administered in a 30% active ingredient (AI) water dispersible granular (WDG) formulation, radiochemical purity > 99%. This formulation was either applied directly to the skin at a rate of 5 mg/cm² (1.5 g AI/cm²) after application of physiologic saline (0.9%) at the rate of 10 µL/cm², or in an aqueous solution of 1.33 g AI/L at the rate of 10 µL/cm² (Fasano, 2002a).

Both applications were made to a shaved area of 10 cm², resulting in nominal doses of 2,000 µg AI/cm² (“high dose”) and 13.3 µg AI/cm² (“low dose”), respectively (actual doses were 1,850 and 11.0 µg AI/cm²). Doses were left in place for six hours; then skin was washed and half of the animals were euthanized (i.e., the exposure period was 6 hours for all animals). The other eight animals (two per group) had a new rigid mesh cover applied to the dose site, held in place with body gauze. These animals were maintained for an additional 162 hours post-dose, then they were euthanized.

After animals were euthanized, the dose site was excised and tape-stripped. Fasano (2002a) did not consider residues removed from skin with tape to be available. Absorbed dose was considered to include residues in the following: urinary and fecal excretion; whole blood



removed by cardiac puncture; red blood cells from sub-sampled blood; plasma from sub-sampled blood; carcass; cage wash; residual feed contaminated with urine and feces. Fasano (2002a) considered residue in stripped skin to be absorbable; residue stripped by the tape was considered to be non-absorbable. Other non-absorbable components include the gauze body wrap, mesh cover, O-ring, and skin wash.

Table 1 summarizes mean percent recovery for each component listed above, from Table 3 and Table 5 of Fasano (2002a). In Table 1, dose categories (absorbed, absorbable and unabsorbed) are reported as in Fasano (2002a). Total absorbable dermal values for rats euthanized at the end of the exposure period (0 hours post-dose) were 0.94% and 0.41% for the low and high doses. At 162 hours post-dose 4.91% of the low dose and 0.88% of the high dose were estimated to be absorbable (Table 1).

Table 1. Mean Percent Indoxacarb Dose Applied to Rats ^a

Component	Low Dose (11.0 µg AI/cm ²)		High Dose (1,850 µg AI/cm ²)	
	0 hours post-dose	162 hours post-dose	0 hours post-dose	162 hours post-dose
Absorbed Dose ^b				
Urine + Feces	0.02	3.80	< LOD/LOQ	0.49
Cage Wash + Feed	< LOD/LOQ ^c	0.63	0.04	0.23
Non-dosed Skin	< LOD/LOQ	< LOD/LOQ	0.05	0.01
Carcass	0.26	0.35	0.21	0.18
Blood ^d	< LOD/LOQ	< LOD/LOQ	< LOD/LOQ	< LOD/LOQ
Total Absorbed	0.08	4.76	0.19	0.87
Absorbable Dose				
Tape-stripped skin	0.86	0.16	0.23	0.02
Total Absorbable	0.94	4.91	0.41	0.88
Unabsorbed Dose				
Body Wrap + Cover	0.31	0.78	5.23	3.28
Skin Wash	83.15	75.05	82.16	85.11
O-Ring	0.68	1.64	3.14	5.50
Tape strips	13.98	15.77	6.29	2.14
Total Unabsorbed	97.88	93.68	96.83	96.02
Total Recovered	98.82	98.59	97.24	96.90
^a Data from Fasano (2002a), Table 3 and Table 5. ^b Categories described in Fasano (2002a). ^c Below limit of detection (LOD) or limit of quantitation (LOQ) No values were given for LOD or LOQ. All data were reported as % administered dose, except those that were stated to be <LOD/LOQ. ^d Includes whole blood, as well as sub-samples of plasma and red blood cells				

This study was not done according to U.S. EPA (1998) DA test guidelines, although it met many of the requirements of those guidelines. The test material used, test animals, test substance administration, animal processing and sample analysis all met requirements of U.S. EPA (1998). Where the study deviated from U.S. EPA (1998) was in the use of fewer test animals (although numbers per exposure duration were acceptable) and fewer dose levels (two, including the concentrated WDG applied over saline and the aqueous dilution) and single exposure duration (6 hours). The mean total recoveries in each dose group were all high (96.9 – 98.8%).

The high dose, 1,850 $\mu\text{g AI/cm}^2$, is above the amount recommended by U.S. EPA (1998) for a maximum dose in any study (1,000 $\mu\text{g AI/cm}^2$), because of concern that such a high dose will “fall off the skin or exceed saturation of the absorption process.” Data were not provided to show that the high dose did not exceed saturation; this remains a concern about this study. The high dose is within the range predicted for handler exposure to indoxacarb based on assumptions used in the draft exposure assessment (10.9 – 2,190 $\mu\text{g AI/cm}^2$; Table 2), although it is well above the range predicted for reentry exposure (0.070 – 1.06 $\mu\text{g AI/cm}^2$; Table 3). Additionally, the fact that the dose in the study was applied at once rather than accumulated throughout a workday is problematic (Kissel and Fenske, 2000). However, in DA studies it is typical to apply each dose at the beginning of the study, and this is a concern for all such studies.

The low dose, 11.0 $\mu\text{g AI/cm}^2$, is equivalent to the lowest 8-hour exposure predicted for handlers, based on assumptions used in the draft exposure assessment (10.9 – 2,190 $\mu\text{g AI/cm}^2$; Table 2), although it is still above the range predicted for reentry exposure (0.070 – 1.06 $\mu\text{g AI/cm}^2$; Table 3). Evaluation of studies done with other AIs suggests that a higher DA may often occur with lower dose, as seen in this study (Thongsinthusak *et al.*, 1999b). Differences in DA between high and low doses seen in this study suggest that the lower doses anticipated during and following pesticide product use might be absorbed at a higher rate than is indicated by Fasano (2002a).

Tape stripping of the application site was intended to remove residues that are held in the upper layers of the skin. However, tape-stripping is not a standard technique in DA studies (although a different tape stripping method than was used here is described by Wester and Maibach (2000)). U.S. EPA (1998) recommends determining absorbable dose as “quantity in/on the washed skin.” Because tape-stripping followed skin washes, residues on tape were considered absorbable. The best estimate of DA relies on data from the low dose, 162 hours post-dose. Residues on tape strips collected at that time were 15.77% (Table 1). Adding 15.77% to the total absorbable dose reported in Table 1, 4.91%, gives an estimate of 20.68%. The best estimate of DA based on these data is 21%. This is the estimate used by the Department of Pesticide Regulation (DPR) in the indoxacarb exposure assessment.

Table 2. Estimated Potential Indoxacarb Exposure for Handlers^a

Work Task	Dermal Exposure ^b (µg/lb AI handled)	Acres per Day ^c	Rate ^c (lbs/acre)	Acute Exposure (µg/cm ²)
Aerial M/L, WDG ^e	578	350	0.11	1,270
Aerial M/L, liquid ^e	996	350	0.11	2,190
Aerial Applicator	14.6	350	0.11	32.1
Aerial Flagger	17.0	350	0.11	46.8
GB M/L, WDG ^e	578	80	0.11	363
GB M/L, liquid ^e	996	80	0.11	626
GB Applicator ^e	21.7	80	0.11	10.9
LPHW M/L/A ^e	9,970	5	0.11	313
Airblast M/L, WDG ^e	578	40	0.11	145
Airblast Applicator	1,270	40	0.11	319

^a Adapted from draft exposure assessment document (Beauvais and Goodbrod, 2003).

^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/cm²) = [(dermal exposure) x (multiplier) x (rate) x (acres/day)]/(18,150 µg/cm²).

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 cm². 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. WDG = water dispersible granular. M/L/A = mixer/loader/applicator.

Exponential Saturation Model Output

To address the concern that 162 hours post-exposure (168 hours post-dose) provides an insufficient time for absorption of bound residues, Fasano (2002b) estimated exposure using the Exponential Saturation Model described in Thongsinthusak *et al.* (1999a). Excreta (urine and feces) collected from rats at intervals through 162 hours post-exposure were used to estimate maximum elimination of the applied dose. The model is of the form $RECOV = MAX \times [1 - EXP(-RATE \times (TIME - LAG))]$, in which RECOV (Y) is the cumulative percentage of dose recovered in excreta (urine and/or feces); TIME (X) is the time postadministration of the dose, MAX (A) is the maximum excretion of administered dose at asymptote as determined from the model, RATE (B) is the first-order rate constant for excretion as determined from the model, and LAG (C) is the estimated time from the administration to the initial excretion as determined from the model. The model predicted that at 14 days post-dose the absorption of the low dose (11.0

$\mu\text{g AI/cm}^2$) would be 6.67%. The high dose was predicted to have 1.06% absorption (Fasano, 2002b).

Table 3. Estimated Potential Indoxacarb Reentry Exposures^a

Work Task	DFR ^b ($\mu\text{g/cm}^2$)	TC ^c (cm^2/hr)	Acute Exposure ^d ($\mu\text{g/cm}^2$)
Thinning Apple/Pear	0.68	3,000	0.898
Hand Harvesting Apple/Pear	0.32	3,000	0.422
Hand Harvesting Brassica/Lettuce	0.48	5,000	1.06
Scouting Brassica/Lettuce	0.62	2,000	0.546
Hand Harvesting Sweet Corn	0.12	17,000	0.898
Scouting Sweet Corn	0.32	1,000	0.141
Scouting Cotton	0.32	2,000	0.282
Staking/Tying Tomato	0.32	1,000	0.141
Weed Potato	0.32	500	0.070

^a Adapted from draft exposure assessment document (EAD; Beauvais and Goodbrod, 2003).
^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 ($\mu\text{g/cm}^2$) = [(DFR) x (TC) x (8 hours/day)]/(18,150 $\mu\text{g/cm}^2$).
 • Body surface area = 18,150 cm^2 (U.S. EPA, 1997)

T. Thongsinthusak used the same data in the Exponential Saturation Model and arrived at maximum absorption values of 8.27% and 1.29%, respectively (T. Thongsinthusak, DPR, personal communication, May 27, 2003). These values do not include residues on tape strips. It is not appropriate to include these residues, as the purpose of the Exponential Saturation Model is to estimate percent absorption of bound skin residues (Thongsinthusak *et al.*, 1999a). The bound skin residues in this study are considered to include residues in tape strips and in stripped skin; it is appropriate to either assume all residues are available (as in the estimate from the previous section, 21%), or to estimate the percent available.

Thongsinthusak *et al.* (1999a) recommend using the Exponential Saturation Model only with studies where the exposure duration is at least 10 hours, and excreta are collected at least 7 days or 10 urinary excretion half-lives. Although the 7-day excretion collection in the study by Fasano (2002a) met the second criterion, the study used a shorter exposure duration (6 hours). This, along with the fact that only two doses were used, both of which were on the high end of the range of anticipated exposures, favor estimates that include all bound skin residues rather

than a portion of them. Therefore, the Exponential Saturation Model was not used by DPR in estimating DA of indoxacarb.

In Vitro Study

The *in vitro* study was performed according to European draft test guidelines, specifically the OECD 2002 Draft Guideline 428 (Fasano, 2002c). The same formulations and rates as used in the *in vivo* study (Fasano, 2002a) were applied to samples of shaved rat dorsal skin and samples of human skin (specific information about human skin was not reported) mounted in static *in vitro* diffusion cells. The exposure area in the cells was 0.64 cm². The receptor solution was 50% (v/v) ethanol in deionized water. The exposure duration was 6 hours. In each dose group, four skin preparations were terminated at the end of the exposure period (0 hours post-exposure) and four skins were terminated 18 hours later. The mean total recovery rates in dose groups were all high, ranging 90.3 – 105.5%. The mean total DA (receptor fluid plus skin sample) for rat skin to which the low dose was applied was 15.8% at the end of the 6-hour exposure period; mean total DA for human skin was 0.36%. The values following the 18-hour post-exposure period were 15.2% and 0.87%, respectively. Consistent with the *in vivo* study, absorption values for the high dose were lower: following the 18-hour post-exposure period, total absorption was 0.38% for rat skin and 0.08% for human skin. Fasano (2002c) concluded that DA was greater in rat skin than in human skin, and that penetration of indoxacarb through human skin would be negligible.

The use of *in vitro* studies to determine DA 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). Therefore, DPR does not, by standard practice, rely on *in vitro* studies to determine DA. Additionally, with just two doses it is difficult to say that the relationship between penetration of rat skin and human skin is linear, or what the best factor might be, which complicates use of these data. The intent of this study was apparently to provide a correction factor for the DA estimate generated by the *in vivo* study using rats, but this study only provides qualitative support for the assertion that DA data from rats results in an overestimate of DA in humans. Data in this study were not considered by DPR in estimating DA of indoxacarb.

Dermal Absorption Estimates Recommended by Registrants

Frame (2003) evaluated the studies of Fasano (2002a, 2002b, 2002c), and concluded that the DA of indoxacarb through human skin should be estimated at 0.19% for the undiluted concentrate (high dose) and 0.28% for the aqueous dilution (low dose). These values were obtained by using the following equation:

$$\text{Human DA (in vivo)} = \text{Rat DA (in vivo)} \times [\text{Human DA (in vitro)}/\text{Rat DA (in vitro)}]$$

$$\text{High dose: } 0.88\% \times 0.08/0.38 = 0.19\%$$

$$\text{Low dose: } 4.91\% \times 0.87/15.2 = 0.28\%$$

Interestingly, Frame (2003) used values corresponding to 6 days post-exposure for rat *in vivo* DA, rather than the estimates adjusted for 14 days from Fasano (2002b). However, as the calculations performed by Frame (2003) relied on *in vitro* data, DPR did not use this estimate for DA. DPR's estimate relied solely on *in vivo* data from Fasano (2002a).

U.S. EPA Estimate of Dermal Absorption of Indoxacarb

Prior to receiving these studies, U.S. EPA estimated DA using toxicity data (Copley, 1999). Dermal absorption was estimated by U.S. EPA to be 1%, based on a ratio of oral and dermal toxicity lowest observable adverse effect levels (LOAELs). Approximation of the DA by the ratio of oral LOAEL to dermal LOAEL is problematic because: 1) it depends on the assumption that all of the difference between oral and dermal lethal toxicity is due to DA, which may not be valid for most pesticides; 2) it depends on the assumption that 100% of an oral dose is absorbed; 3) LOAEL is a much higher dose than is typically of interest for DA and the ratio may not generalize to lower doses; and 4) dose determination in the studies on which LOAELs are based may not be sufficiently exact for determining DA.

Conclusions

1. The *in vivo* study suggests a maximum DA in rats of 4.91%, according to Fasano (2002a). Because the non-standard technique of tape stripping the skin was used, this value is not considered to be the best estimate for DA.
2. The estimate of DA extrapolated to 14 days post-dose (Fasano, 2002b) suggests a maximum DA in rats of 6.67%. Using the same data, T. Thongsinthusak (personal

communication) estimated a maximum DA of 8.27%. This value excludes residues tape-stripped from the skin and may underestimate DA of indoxacarb in rats.

3. Comparison in a static *in vitro* system of penetration of indoxacarb through rat and human skin samples (Fasano, 2002c) suggests that rats might overestimate human exposure to indoxacarb, at least at high doses. DPR did not consider these *in vitro* data in estimating DA.
4. The estimate to be used in exposure assessment is 21%, based on the inclusion of residues on tape strips to residues considered by Fasano (2002a) to be absorbable. This is considered to be the best estimate of DA for indoxacarb based on available data.

Recommendations for Additional Studies

Additional study data would be needed to support lower DA estimates as recommended by Fasano (2002a, 2002c) and Frame (2003). 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 (longer durations are suggested by metabolism studies of indoxacarb, which show long excretion half-lives). Dose levels should be lower than those used in Fasano (2002a), and should be logarithmically spaced. Exposure estimates shown in Tables 2 and 3 above suggest that dose levels should include 1 and 0.1 $\mu\text{g}/\text{cm}^2$.

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

References

Beauvais, S. and Goodbrod, J. 2003. Estimation of Exposure of Persons in California to Pesticide Products that Contain Indoxacarb. Draft, dated March 20. Sacramento, CA: Worker

Health and Safety Branch, California Department of Pesticide Regulation, California Environmental Protection Agency.

Copley, M. 1999. DPX-MP062 – Report of the Hazard Identification Assessment Review Committee. HED Doc. No. 013528. Memorandum dated June 24, 1999. Washington, DC: Health Effects Division, Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency.

Fasano, W.J., Sr. 2002a. DPX-MP062 30WG (300 g indoxacarb/kg): In Vivo Dermal Absorption in the Rat. Unpublished study submitted by E.I. du Pont de Nemours and Co., Wilmington, DE, Laboratory Project ID DuPont-11303. Report dated December 4. DPR Volume Number 52425-137.

Fasano, W.J., Sr. 2002b. DPX-MP062 30WG (300 g indoxacarb/kg): In Vivo Dermal Absorption in the Rat, Supplement 1. Unpublished study submitted by E.I. du Pont de Nemours and Co., Wilmington, DE, Laboratory Project ID DuPont-11303, Supplement 1. Report dated December 18. DPR Volume Number 52425-138.

Fasano, W.J., Sr. 2002c. DPX-MP062 30WG (300 g indoxacarb/kg): In Vitro Dermal Kinetics in Rat and Human Skin. Unpublished study submitted by E.I. du Pont de Nemours and Co., Wilmington, DE, Laboratory Project ID DuPont-11302. Report dated December 4. DPR Volume Number 52425-136.

Frame, S.R. 2003. Estimation of the Dermal Absorption of DPX-MP062 30WG in Humans. Unpublished study submitted by E.I. du Pont de Nemours and Co., Wilmington, DE, Laboratory Project ID DuPont-12896. Report dated April 10. DPR Volume Number 52425-139.

Franklin, C.A., Somers, D.A. and Chu, I. 1989. Use of percutaneous absorption data in risk assessment. *Journal of the American College of Toxicology* 8:815-827.

Haskell, D.E. 1998. Canada-United States Trade Agreement (CUSTA) Working Group, Final Draft of Position Paper for Issue Eight: Typical Workdays for Various Crops. Memo No. HSM-98001, dated June 19. Sacramento, CA: California Department of Pesticide Regulation, Worker Health and Safety Branch.

Kissel, J. and Fenske, R. 2000. Improved estimation of dermal pesticide dose to agricultural workers upon reentry. *Applied Occupational and Environmental Hygiene* 15:284-290.

PHED. 1995. The Pesticide Handlers Exposure Database, Version 1.1. Prepared for the PHED Task Force representing Health and Welfare Canada, U.S. Environmental Protection Agency,

Joseph Frank
July 25, 2003
Page 10

and the National Agricultural Chemicals Association; prepared by Versar, Inc., 6850 Versar Center, Springfield, VA 22151.

Powell, S. 2002. Approximating Confidence Limits for Upper Bound and Mean Exposure Estimates from the Pesticide Handlers Exposure Database (PHED V1.1). Memo No. HSM-02037, dated September 27. Sacramento, CA: California Department of Pesticide Regulation, Worker Health and Safety Branch.

Thongsinthusak, T. 1994. Determination of Dermal Absorption of Pesticides in Animals. Memo No. HSM-94001, dated April 7. Sacramento, CA: California Department of Pesticide Regulation, Worker Health and Safety Branch.

Thongsinthusak, T., Ross, J.H., Saiz, S.G. and Krieger, R.I. 1999a. Estimation of Dermal Absorption Using the Exponential Saturation Model. Regulatory Toxicology and Pharmacology 29:37-43.

Thongsinthusak, T., Ross, J.H. and Dong, M.H. 1999b. Significance of dermal dose levels in dermal absorption studies of pesticides. Report No. HS-1801. Sacramento, CA: California Department of Pesticide Regulation, Worker Health and Safety Branch.

U.S. EPA. 1997b. Exposure factors handbook. EPA/600/P-95/002Fa. Washington, D.C.: Office of Research and Development, U.S. Environmental Protection Agency.

U.S. EPA. 1998. Health effects test guidelines: Dermal penetration (OPPTS 870.7600). Washington, DC: Office of Prevention, Pesticides and Toxic Substances, U.S. Environmental Protection Agency.

Wester, R.C. and Maibach, H.I. 2000. Understanding percutaneous absorption for occupational health and safety. International Journal of Occupational and Environmental Health 6:86-92.

cc: Jim Goodbrod