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MEMORANDUM

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DATE: December 3, 2015

SUBJECT: STUDY REVIEWS: DICROTOPHOS TRIPLE-PACK DERMAL ABSORPTION, DATA PACKAGE ID# 260917

Summary
This memorandum is a review of two dermal absorption studies submitted by AMVAC Chemical Corporation in support of the 24© SLN registration of BIDRIN® 8 for use on cotton plants. Based on these studies, a recommendation regarding the human dermal absorption factor for estimating exposures to persons who come in contact with dicrotophos under label uses is provided.

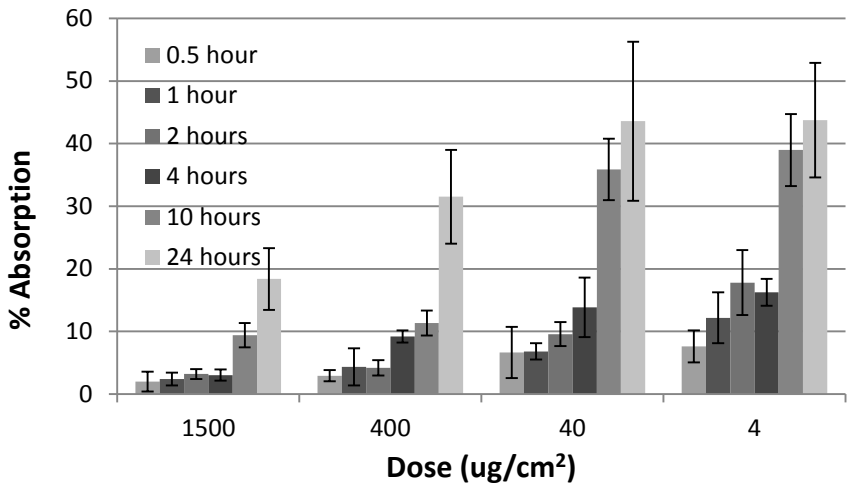
BIDRIN® 8 contains 82% dicrotophos, or 8 lbs per gallon, as the active ingredient in a water miscible concentrate formulation. Dicrotophos, an organophosphate pesticide with broad spectrum activity, is both a Federally- and California-restricted pesticide delivered by closed system aerial and ground equipment.

The two studies under review are "Dicrotophos: *In Vivo* Dermal Penetration Study in the Rat," hereafter referred to as "*In Vivo* Rat Study" (Gledhill, 1999), and "Dicrotophos: *In Vitro* Absorption Through Human and Rat Epidermis," hereafter referred to as "*In Vitro* Human and Rat Study" (Davies, 1999). These studies fulfilled the "triple-pack" requirements for determining human skin absorption of dicrotophos.

Overall, this assessment recommends an upper 95% confidence interval of the dermal absorption value of 26.3% to be used in human health exposure and risk estimates for BIDRIN® 8. This value is derived from the above-mentioned studies with consideration being given to both absorbed and skin-bound residues (i.e., those bound to the *Stratum Corneum* of the epidermis). Specifically, the "triple-pack" approach was employed for relating *in vitro* and *in vivo* animal data and applied the ratio to *in vitro* human data to derive a human *in vivo* dermal absorption value. The calculated 95% confidence interval approximates a human *in vivo* dermal absorption in the range of 11.8% to 26.3%.

Background
Percent absorption typically increases with decreasing dermal dose, as shown in **Figure 1,** which summarizes data from the *In Vivo* Rat Study (Gledhill, 1999). This decrease in dermal absorption at higher doses is thought to be the results of inundated absorption mechanisms. Although less clear, the *In Vitro* Human and Rat Study also showed a similar dose-response trend (Davies, 1999, data not shown).

Figure 1. *In Vivo* Rat Absorption of Dicrotophos



Based on an earlier evaluation of other studies finding absorption to be greater at lower doses, the lowest test dose recommended for use in experimental studies on dermal absorption is in the 1 to 6 µg/cm^2 range (Thongsinthusak, 1994). The lowest dose tested in the *In Vivo* Rat Study was 4 µg/cm^2. While the lowest nominal dose for the *In Vitro* Human and Rat Study was intended to be 4 µg/cm^2, the actual test dose was 9.66 µg/cm^2, making comparisons between the *in vivo* and *in vitro* studies less favorable. However, the percent absorption at 24 hours did not differ significantly between the two lowest doses *in vivo* (4 and 40 µg/cm^2), and between the two lowest doses *in vitro* for both rat and human tissues (9.66 and 43 µg/cm^2). For this reason, this assessment will consider the *in vitro* data for use in estimating the *in vivo* human dermal absorption value.

This review finds the *In Vivo* Rat Study to be of high quality with acceptable and reliable data. Although the same conclusion cannot be expressed for the *In Vitro* Human and Rat Study, the data from this study may still be considered usable. It should be noted that, as opposed to the use of similar doses as employed in typical "triple pack" studies, these dermal absorption studies

were conducted with two rather different doses: 4 µg/cm^2 (*in vivo*) and of 10 µg/cm^2 (*in vitro*). The data are summarized in the following table, **Table 1**, with details provided in the rest of this memorandum.

U.S. EPA utilized route-specific toxicity studies as the basis for their steady-state points of departure (U.S. EPA, 2015) and therefore did not use absorption factors for exposure estimates. Nonetheless, U.S. EPA did adjust the dermal point of departure (2.1 mg/kg/day) using the ratio of the *in vitro* rat-to-*in vitro* human absorption (4.44) for an "adjusted dermal point of departure" of 9.33 mg/kg/day (2.1 mg/kg/day × 4.44).

As previously mentioned, HHA found the *In Vivo* Rat Study to be of high quality with reliable data. However, less confidence can be placed in the *In Vitro* Human and Rat Study due to absence of data points without providing explanation or justification and incomplete or inconsistent procedural and technical information. Since the *In Vitro* Human and Rat Study was completed in 1999, it also does not meet the requirement of demonstrating adequate solubility of the test compound in the receptor fluid (OECD, 2004). In addition, there appears to be a higher level of variability in the data values, with total recovery ranging from 79.9% to 132% throughout the study. For the lowest dose of 9.66 µg/cm^2, total recovery at 24 hours post-application ranged from 117% to 132% for the human tissue samples, with an average percent recovery of 124.7%. These high recovery values (i.e., > 110%) reduce the amount of confidence that can be placed on this study. One helpful aspect of the *In Vitro* Human and Rat Study is that the animal and human samples were conducted concurrently under the same experimental protocols. The use of the same test conditions is a fundamental principle in the "triple-pack" methodology, as *in vitro* test variables are recognized to greatly influence the test outcome.

Table 1. Dicrotophos Dermal Absorption Values Used by U.S. EPA and this Review.

	In Vitro Rat[a] (% Absorption)	*In Vitro* Human[a] (% Absorption)	*In Vivo* Rat[b] (% Absorption)	*In Vitro*[a] Rat to *In Vivo*[b] Rat	*In Vitro*[a] Rat to *In Vitro*[a] Human	"Equivalent" *In Vivo* Human (% Absorption)
U.S. EPA[c]	47.1	10.6	32.9	N/A	4.44	N/A
24 hours						
Reported "Absorbed" Dose[d]	37.2 ± 10.4	10.3 ± 3.0	32.9 ± 7.2	N/A	N/A	N/A
Residues in Epidermis or "skin"	16.7 ± 3.8	8.8 ± 0.6	2.9 ± 2.1	N/A	N/A	N/A
Residues in *Stratum Corneum*	N/A	N/A	7.9 ± 1.8	N/A	N/A	N/A
This Review[e]	53.9 ± 10.7	19.0 ± 2.8	43.7 ± 9.1	**0.88 – 1.6[f]**	N/A	**11.8 – 26.3[f]**
10 hours						
Reported "Absorbed" Dose[d]	53.1 ± 12.8	6.6 ± 7.5	28.0 ± 6.6	N/A	N/A	N/A
Residues in Epidermis or "skin"	17.6 ± 6.1	6.7 ± 1.9	3.3 ± 1.8	N/A	N/A	N/A
Residues in *Stratum Corneum*	N/A	N/A	7.8 ± 2.8	N/A	N/A	N/A
This Review[e]	70.6 ± 8.1	13.4 ± 9.1	39.0 ± 5.7	**1.5 – 2.1[g]**	N/A	**2.3 – 25.1[g]**

[a]Dermal dose was 9.66 µg/cm^2 (Davies, 1999); [b]Dermal dose was 4 µg/cm^2 (Gledhill, 1999); [c]Values used by U.S. EPA's risk assessment for dicrotophos (2014); *In vitro* values used by U.S. EPA appear to have been retrieved from a summary table of the study report, the values of which differed significantly from those derived using the raw data; [d]Absorbed values reported by the *In Vitro* Human and Rat Study (Davies, 1999) are concentrations detected in the receptor fluid, while values reported by the *In Vivo* Rat Study (Gledhill, 1999) are the sum of dicrotophos residues determined in the urine, feces, cage wash, carbon dioxide trap contents and charcoal trap extractions, GI tract contents, and carcass; [e]Values estimated from this review include residues detected in the *Stratum Corneum* and epidermis or application site skin, summed with the "absorbed" doses reported from respective study; [f]95% confidence interval calculated using N=6, t-value = 2.447 ; [g] same as "f", using N=5, t-value = 2.571; N/A = not applicable or not available.

Triple-Pack Dermal Absorption Method

The "triple-pack" approach correlates *in vitro* and *in vivo* animal, as well as *in vitro* human data to make inferences for an appropriate human dermal absorption factor value to be used in human health risk assessment. Various regulatory bodies take slightly different approaches to how to ratio or relate these *in vivo* and *in vitro* data. In this assessment, the ratio of *in vitro* animal data to *in vivo* animal data presents a means of determining the reliability of the *in vitro* test conditions to predict *in vivo* absorption.

$$\text{IF} \quad \frac{\text{Animal } \textit{in vitro}}{\text{Animal } \textit{in vivo}} \approx 1 \qquad \text{THEN} \quad \text{Human } \textit{in vitro} \approx \text{Human } \textit{in vivo}$$

This "triple-pack" approach suggests that the ratio of animal *in vitro* to *in vivo* dermal absorption is essentially one for the human *in vitro* data to be considered equivalent to *in vivo* human dermal absorption. The question then arises, as to how close to the value of one must this ratio be for the human *in vitro* data to be acceptable and representative of human *in vivo* absorption. This would require a limit or range to be defined for acceptable ratio values. At this time, we are proposing to use a 95% confidence interval (CI) to describe the uncertainty associated with relating *in vitro* to in *vivo* data (computational details are provided in the appendix).

Calculation of a 95% CI will be performed twice in the "triple-pack" approach. First, in defining the CI for the ratio of the mean values of *in vitro* to *in vivo* animal absorption, it can be determined, with 95% confidence, whether the ratio value statistically overlaps with the value of one. If this CI for the animal ratio satisfies the criterion of approximating the value of one, then the assumption is that the conditions of the *in vitro* assay are appropriate for estimating observed *in vivo* dermal penetration. Subsequent to satisfying this criterion, a second 95% CI is calculated for the mean *in vitro* human absorption. This calculation incorporates the relative errors from the animal data with the relative error from the *in vitro* human data to derive a range in which *in vivo* human dermal penetration is expected for dicrotophos. Data quality is accounted for to some degree, in that the sample size and data variance can affect the confidence interval size. For example, although the 95% CI of the *in vitro* to *in vivo* animal absorption ratio may overlap with the value of one, if the standard error of any of the datasets is large, the resultant upper-bound estimate for *in vivo* human absorption is increased.

Additionally, in this assessment, any residues determined in the *Stratum Corneum*, epidermis, and dermis are considered to be absorbed. By contrast, neither the study authors nor U.S. EPA used the skin-bound residues to calculate the dermal equivalent dose. By including the skin-

bound residues, the estimated dermal absorption is significantly increased from "absorbed" values reported in the study (**Table 1)**.

An exposure period of 24 hours was implemented in the *In Vitro* Human and Rat Study, with samples collected at 0.5, 1, 2, 4, 10, and 24 hours (these were exposure and sampling times for the *In Vivo* Rat Study). A sampling period of 24 hours is typically required to suitably characterize the absorption profile (OECD, 2004). For the 1:1000 dilution (4 or 9.66 $\mu g/cm^2$) test dose, the test dose with the most relevance to dermal exposure scenarios under the proposed uses, the absorption at 24 hours post-exposure was utilized for this assessment. Although it may be argued that 10 hours is a more relevant time-point for consideration of some scenarios, such as an 8 hour work day, it should be noted that the absorption values at 10 hours did not differ significantly from those at 24 hours at the two lowest doses (4 and 40 $\mu g/cm^2$ for the *In Vivo* Rat Study, and 9.66 and 43 $\mu g/cm^2$ the *In Vitro* Human and Rat Study). Furthermore, calculations for the 95% CI for the study data did not meet the "triple-pack" criteria of overlapping the value of one.

References

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Appendix

I. Application of the 95% Confidence Interval for Deriving Human Dermal Absorption

The 95% confidence interval (CI) calculations were developed by Kwok (2015) based on the principal of error propagation (Bevington and Robinson, 2003). The 95% CI was calculated for the ratio of the animal data and for the mean *in vitro* human dermal absorption. These mean values were determined from the data of submitted dermal absorption studies. The relative errors from the animal studies are incorporated with the relative error of the human *in vitro* data to calculate the total error and interval of values for the human *in vivo* dermal absorption estimate. An example of how this 95% CI is applied to *in vitro* human absorption values is provided at the end of this appendix.

A. Confidence Interval of *In Vitro*-to-*In Vivo* Animal Dermal Absorption Ratio (R)

$$\textit{In Vitro}\text{: }\textit{In Vivo}\text{ Ratio (R)} = \frac{\textit{In Vitro}\text{ Dermal Absorption (x)}}{\textit{In Vivo}\text{ Dermal Absorption (y)}}$$

The error of R can be expressed approximately as

$$\Delta R = \frac{\partial R}{\partial x}\Delta x + \frac{\partial R}{\partial y}\Delta y$$

Partial derivatives of each variable are the function of the other variable

$$\frac{\partial R}{\partial x} = \frac{1}{y} \text{ and } \frac{\partial R}{\partial y} = \frac{-x}{y^2}$$

Therefore,

$$\Delta R = \frac{\partial x}{y} - \frac{x\partial y}{y^2}$$

$$\Delta R = \frac{\partial x}{x}\frac{x}{y} - \frac{\partial y}{y}\frac{x}{y}$$

$$\Delta R = \frac{\partial x}{x}R - \frac{\partial y}{y}R$$

$$\Delta R = R\left(\frac{\partial x}{x} - \frac{\partial y}{y}\right)$$

$$\left(\frac{\Delta R}{R}\right)^2 = \left(\frac{\partial x}{x} - \frac{\partial y}{y}\right)^2$$

$$\left(\frac{\Delta R}{R}\right)^2 = \left(\frac{\partial x}{x}\right)^2 + \left(\frac{\partial y}{y}\right)^2 - 2\left(\frac{\partial x}{x}\right)\left(\frac{\partial y}{y}\right)$$

can be expressed as

$$\frac{\sigma_R^2}{R^2} = \frac{\sigma_x^2}{x^2} + \frac{\sigma_y^2}{y^2} - 2\frac{\sigma_{xy}^2}{xy}$$

If the covariance is equal to zero, then

$$\left(\frac{\Delta R}{R}\right)^2 = \left(\frac{\partial x}{x}\right)^2 + \left(\frac{\partial y}{y}\right)^2$$

$$\sigma_R^2 = R^2\left(\frac{\sigma_x^2}{x^2} + \frac{\sigma_y^2}{y^2}\right)$$

$$\sigma_R = R\sqrt{\left(\frac{\sigma_x}{x}\right)^2 + \left(\frac{\sigma_y}{y}\right)^2}$$

Since $\frac{\sigma_x}{x}$ and $\frac{\sigma_y}{y}$ can be considered as relative uncertainties or errors (Err) of x and y, respectively, therefore the total error of R is given by:

$$\text{Err (R)} = \left(\frac{x}{y}\right)\sqrt{(\text{Err}[x])^2 + (\text{Err}[y])^2}$$

For *in vitro*-to-*in vivo* dermal absorption in rats, the 95% CI of R_{rat} is given by

$$R_{rat} \pm t^* \left(\frac{\sigma_R}{\sqrt{N}}\right)$$

where:
t* = the critical value of t at 95th confidence level
N = sample size

B. Confidence Interval of *In Vivo* Dermal Absorption in Humans

Assuming that *in vitro*-to-*in vivo* dermal absorption ratios in humans and animals are the same, i.e., $R_{rat} \approx 1 \approx R_{human}$, using the estimated Err (R_{rat}) and experimentally determined Err (x) (*in vitro* dermal absorption in humans), the estimated Err (y) (*in vivo* dermal absorption in humans) can be expressed as:

$$\text{Err (y)}=(y)\sqrt{(\text{Err[x]})^2+(\text{Err[R]})^2}$$

For *in vivo* dermal absorption in humans, the 95% CI of y is given by

$$y \pm t^* \left(\frac{\sigma_y}{\sqrt{N}}\right)$$

where:
t* = the critical value of t at 95% confidence level
N = sample size

I. Example for Application of 95% CI in Deriving Human Dermal Absorption

A. Confidence Interval of *In Vitro*-to-*In Vivo* Dermal Absorption Ratio (R)

Given: ***In vitro* rat absorption (%): 53.9 ± 10.7 (**data from triple pack studies)
***In vivo* rat absorption (%): 43.7 ± 9.1 (**data from triple pack studies)

Err[x] = 10.7/53.9 = 0.198
Err[y] = 9.1/43.7 = 0.208
R = x/y = 53.9/43.7 = 1.23

$$\text{Err (R)} = \left(\frac{x}{y}\right)\sqrt{(\text{Err}[x])^2+(\text{Err}[y])^2}$$

Therefore, Err[R] = 0.35

The 95% CI of R: 0.88 – 1.59
(for N = 6 and t* = 2.447)

B. Confidence Interval of *In Vivo* Dermal Absorption in Humans

Given: ***In vitro* human absorption (%): 19.0 ± 2.8 (**data from triple pack studies)

Err [x] = 2.8/19.0 = 0.147
Err[R] = 0.35 (from above calculations)

Assuming that R ≈ 1, the *in vivo* human absorption would be 19%

$$\text{Err (y)} = (y)\sqrt{(\text{Err}[x])^2+(\text{Err}[R])^2}$$

Therefore, Err [y] = 7.2
the 95% CI of y: 11.8 – 26.3%
(for N = 6 and t* = 2.447)