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# Department of Pesticide Regulation

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## MEMORANDUM

TO: Karen Fletcher, Registration Specialist  
Pesticide Registration Branch HSM-99010

FROM: Tom Thongsinthusak, Staff Toxicologist  
Worker Health and Safety Branch  
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DATE: April 12, 1999

SUBJECT:  
BRAND NAME: Amitraz  
ACTIVE INGREDIENT: Amitraz  
COMPANY NAME: AgrEvo USA Company  
I.D. NUMBER: SBRA-176250-E  
RECORD NUMBER (RN): 167403, 167404  
DATA PACKAGE NUMBER (DPN): 287-142, 287-143  
EPA REGISTRATION NUMBER: 45639-0-  
TITLE: AMITRAZ: RATES OF PENETRATION OF (<sup>14</sup>C)-AMITRAZ, AS  
MITAC W, THROUGH HUMAN AND RAT SKIN USING AN *IN VITRO* SYSTEM

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The above report (DPN 287-143, RN 167404) indicated that the penetration study of amitraz in rat and human skin was conducted in accordance with Covance Standard Operating Procedures, the UK Principles of Good Laboratory Practice (GLP), and the OECD Good Laboratory Practice in the Testing of Chemicals. The Quality Assurance Statement was also included in the submitted report. Details of test conditions for the dermal penetration study of amitraz using isolated rat and human skin are shown in Table 1.

The accompanying document, AgrEvo USA position statement on Cal-EPA occupational risk assessment for amitraz (DPN 287-142, RN 167403) has been previously reviewed and opinions were addressed in HSM-99007 (Thongsinthusak, 1999).

Table 1. Details of test conditions used in the dermal penetration study of amitraz in isolated rat and human skin.

Test conditions	Details	
	<i>In vitro</i> test using rat skin	<i>In vitro</i> test using human skin
Source of skin	Female Sprague Dawley rats (CrI:CD BR) from Charles Rivers.	2 male and 4 female donors (cadavers or patients undergoing surgery) from suppliers in the USA and the UK.
Age	3-5 weeks old on arrival.	28-82 (59 ± 20) years old.
Body weight	79-112 (97 ± 11) g.	NA
Necropsy method	Carbon dioxide asphyxiation followed by cervical dislocation.	NA. Conditions of donors were not known.
Skin area	Shaved dorso-lumbar skin. The skin was washed with acetone.	An area with intact epidermal layer at excision. The donors had not received medical treatment.
Storage temperature	<i>ca</i> -20 °C.	<i>ca</i> -20 °C.
Skin preparation	Soaked the skin in 2 M NaBr for <i>ca</i> 16 h and then rinsed in deionized water. The epidermis was isolated from the dermis. The epidermis (stratum corneum uppermost) was floated on deionized water and stored at <i>ca</i> 4 °C until use.	Placed the skin in hot water (60 ± 2 °C) for <i>ca</i> 1 min and then put the skin on tissue paper. The epidermis was isolated from the dermis. The epidermis (stratum corneum uppermost) was floated on deionized water and stored at <i>ca</i> 4 °C until use.
<i>In vitro</i> dermal penetration cell	Franz type static dermal penetration cell	Franz type static dermal penetration cell
Surface area	<i>ca</i> 2.545 cm <sup>2</sup> (1.8 cm diameter)	<i>ca</i> 2.545 cm <sup>2</sup> (1.8 cm diameter)
Receptor fluid	Ethanol:water (1:1, v/v)	Ethanol:water (1:1, v/v)
Temperature of the Franz cell	32 ± 2 °C.	32 ± 2 °C.
Rate of altered membrane integrity	>5 µL <sup>3</sup> H <sub>2</sub> O/cm <sup>2</sup> /2 h	>2 µL <sup>3</sup> H <sub>2</sub> O/cm <sup>2</sup> /2 h
Applied dose level (mg/cm <sup>2</sup> )	<sup>14</sup> C-amitraz + Mitac W (50% a.i.) (1:1 by a.i., w/w): 9.8 (Group A), 2.1 (Group B), 0.2 (Group C)	<sup>14</sup> C-amitraz + Mitac W (50% a.i.) (1:1 by a.i., w/w): 9.8 (Group D), 2.1 (Group E), 0.2 (Group F)
Replicate/dose	10 (Excluded altered membrane integrity & excessively high rate of penetration)	10 (Excluded altered membrane integrity & excessively high rate of penetration)
Receptor fluid sampling	Duplicate aliquots of 0.5 mL at 0, 1, 2, 4, 6, 10, and 24 h.	Duplicate aliquots of 0.5 mL at 0, 1, 2, 4, 6, 10, and 24 h.
Samples collected for analysis	Sampled receptor fluid, fluid in the cell, epidermal membranes, rinsate.	Sampled receptor fluid, fluid in the cell, epidermal membranes, rinsate.
Instrument for radioassay	LKB, Beckman or Packard Tri-Carb liquid scintillation Counter.	LKB, Beckman or Packard Tri-Carb liquid scintillation Counter.

Results:

*In vitro* penetration rates of amitraz through isolated human and rat skin are shown in Table 2. The penetration rate in rat skin applied with the high dose was about 3-fold higher than those from the low and medium doses. The penetration rates for the medium and low doses were very similar. A similar penetration pattern was also observed for isolated human skin. Penetration ratios between isolated rat and human skin for high, medium and low doses are also shown in Table 2. Generally, the isolated rat skin yielded about 5- to 6-fold higher penetration rate than the isolated human skin.

Table 2. Mean penetration rates of amitraz following a single application to preparations of isolated rat and human skin.

Dose (mg/cm <sup>2</sup> )	Mean penetration rate, µg/cm <sup>2</sup> /h (Group)		Penetration ratio (Rat/human skin)
	Rat skin	Human skin	
High (9.8)	2.368 (A)	0.468 (D)	5.1:1
Medium (2.1)	0.912 (B)	0.146 (E)	6.2:1
Low (0.2)	0.915 (C)	0.168 (F)	5.4:1

Factors that could affect dermal penetration rates:

Ages of rats on arrival were about 3 to 5 weeks old. The body weight of these animals ranged from 79 to 112 (97 ± 11) g. These animals were acclimatized for about three days. Hence, the isolated rat skin used in the study was obtained from very young rats whereas the isolated human skin was obtained from older donors with 28 to 82 (59 ± 20) years of age.

Zendzian (1994) recommended young adult male rats with body weight in the range of 200 to 250 g. An *in vivo* dermal absorption study of amitraz used rats with ages on arrival ranging from 6 to 10 weeks old. Body weights of these animals ranged from 225 to 321 g (AgrEvo USA Company, 1994). This study was used to establish *in vivo* dermal absorption values for amitraz (Thongsinthusak, 1994). It is possible that the isolated skin from young rats could yield higher penetration rates than that from older rats. This would give a higher penetration ratio between isolated rat and human skin.

Brief literature review on the potential effect of age on dermal absorption in rats:

Bank *et al.* (1990) showed that the dermal absorption of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 2,3,4,7,8-pentachlorodibenzofuran (4PeCDF) was reduced in older age groups of rats. Old rats (36 to 120 weeks old) absorbed TCDD or 4PeCDF about 1- to 3-fold lower than young rats (10 weeks old). Shah *et al.* (1987a) revealed that chorpypirifos, carbaryl, chlordecone, atrazine, and PCB had a significantly higher fractional penetration in young compared to adult rats at some doses. However, old rats absorbed dinoseb, DSMA, folpet, MSMA, and parathion more than young rats. There was no conclusive effect of age to dermal absorption for carbofuran, captan, nicotine, PCB, and permethrin. Shah *et al.* (1987b) showed that an *in vitro* penetration rate of carbofuran in young rat skin was about 4.3-fold (flow system) and 1.4-fold (static system) higher than that in adult rats skin. The *in vivo* dermal absorption rate in young rats was about 3.4-fold higher than that in adult rats (Shah *et al.*, 1987b).

Because there was some uncertainty on the influence of age on dermal penetration rates of chemicals, we could not rule out the possibility that isolated skin from young rats could absorb amitraz at a higher rate than the skin from adult rats. In order to use the penetration ratios as shown in Table 2 to derive *in vivo* human dermal absorption, those ratios were corrected. A correction factor of 2.7-fold (an average of 1.5, 4.3, 1.4, and 3.4) was conservatively used to correct dermal penetration rates of the rat skin.

Estimation of *in vivo* human dermal absorption:

An equation used to derive an estimate of *in vivo* human dermal absorption was adopted from a guidance document (Thongsinthusak *et al.*, 1993) and is shown below:

$$\frac{\text{Rats}}{\text{Humans}} = \frac{\text{In vitro Absorption rate}^a}{\text{Absorption rate}^b} = \frac{\text{In vivo Dermal absorption}^c}{\text{Demal absorption}^d}$$

<sup>a/b</sup> Corrected penetration ratio of the isolated rat and human skin.

<sup>c</sup> *In vivo* dermal absorption of amitraz in rats was 13.8% (Thongsinthusak, 1994).

<sup>d</sup> *In vivo* human dermal absorption. The value was derived from the equation as shown below. Results are shown in Table 3.

$$\text{In vivo human dermal absorption} = \frac{13.8\%}{\text{Corrected in vitro penetration ratio (rat/human)}}$$

Table 3. Estimated *in vivo* human dermal absorption values.

Dose (mg/cm <sup>2</sup> )	Penetration ratio (rat/human skin)		<i>In vivo</i> human dermal absorption
	Uncorrected ratio	Corrected ratio <sup>a</sup>	
High (9.8)	5.1:1	1.9:1	7.3
Medium (2.1)	6.2:1	2.3:1	6.0
Low (0.2)	5.4:1	2.0:1	6.9

<sup>a</sup> Correction factor = 2.7

Some confounding factors related to the *in vitro* study:

The report mentioned that any samples which showed an excessively high rate of <sup>14</sup>C-amitraz penetration were not reported. However, the report did not define “an excessively high rate.” The report indicated that the generally poor recovery (about 77% for high dose, 83% for medium dose, and 89% for low dose) of radioactivity was probably due to the viscous nature of the dose solutions and their tendency to solidify on the surface of the skin and the edges of the Franz cell. Some attempt to estimate dose in the epidermis should be made either by using whole skin and skin stripping or quantitate epidermal concentration after washing.

Recommendations:

1. The estimated *in vivo* human dermal absorption of 6.9% for amitraz should be used in the calculation of absorbed dose instead of 13.8%.
2. The registrant may elect to do a new *in vitro* dermal absorption of amitraz. This will help to resolve the issue on the effect of age on dermal penetration rates. For this purpose, the isolated rat skin should be obtained from rats that have a body weight range of 200 to 250 g (6-10 weeks old). A concurrent study using the human skin should also be included.
3. Applied dose levels should be the same as those used in a previous *in vivo* dermal absorption study in rats, i.e., 10, 100, and 1,000 µg/cm<sup>2</sup>.
4. A study protocol should be submitted to DPR for review before conducting the study.
5. To answer the question about the utility of the human dermal NOEL study, we suggest including 2 low dose groups of technical amitraz only replicates to compare to the Mitac results.
6. Attempt to estimate residues in epidermis following skin stripping with tape.

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