Determining the Dermal Absorption of [^{14}C]-Amitraz Using the Triple Pack Approach

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1 ABSTRACT

Human dermal absorption is often overestimated by *in vivo* experiments in rats. Rat *in vivo* dermal absorption (TG 427) data can be corrected to better estimate human absorption based on *in vitro* skin absorption assays using rat and human skin (TG 428) (the "triple pack"). Human dermal absorption of [^{14}C]-Amitraz was evaluated using the triple pack approach. Dermal doses equivalent to 20, 60, and 100 µg/cm^2 of [^{14}C]-Amitraz were applied to a 10-cm^2 area; after a 6.5-hour exposure period radioactivity slowly penetrated the skin of male rats. After washing, a mean of 37% of the applied dose was considered to be absorbed or absorbable among all dose groups. Absorption pathways did not appear to be saturated. During *in vitro* experiments, dermal doses equivalent to 20, 60, and 100 µg/cm^2 were applied to a 1-cm^2 area for 6 hours. The *in vitro* dermal absorption of Amitraz in corn oil was 1 ± 0.3%, 0.9 ± 0.4%, and 0.8 ± 0.2% through human skin and 4 ± 2%, 6 ± 3%, and 7 ± 3% through rat skin and results in a human/rat absorption ratio of approximately 1:4, 1:6.7 and 1:8.8 at concentrations of 2, 6 and 10 mg Amitraz/mL, respectively. The human skin absorption values are 9.25% (37%/4) at 2 mg/mL, 5.4% (36%/6.7) at 6 mg/mL and 4.3% (38%/8.8) at 10 mg/mL. The triple pack approach to estimating human dermal absorption has the advantage of addressing differences in absorption between rats and humans without requiring the use of human subjects.

2 *IN VIVO* METHODS - 1 SPECIES

- 4 rats per dose per time point
- Doses: 2 mg/mL (20 µg/cm^2); 6 mg/mL (60 µg/cm^2), or 10 mg/mL (100 µg/cm^2) and 8 to 9 µCi of [^{14}C]-Amitraz in corn oil
- Dose area: 10-cm^2, non-occluded
- Housed in individual metabolism cages for separate collection of urine and feces
- Exposure Period: 6.5 hours; at the end of exposure unabsorbed radioactivity removed with soap and water
- Termination Time Points: 6.5, 12, 24, and 72 hours post-dosing
- Sample Collection: skin from the dose site, skin from the surrounding area, tape strips, blood, urine from the bladder, and carcass
- Analysis: Liquid Scintillation Counting

3 *IN VITRO* METHODS – 2 SPECIES

At least 7 skin discs from at least 4 rats and 3 human donors.
Doses: 2 mg/mL (20 µg/cm^2); 6 mg/mL (60 µg/cm^2), or 10 mg/mL (100 µg/cm^2) and ~4 µCi of [^{14}C]-Amitraz in corn oil
Dose area: 1-cm^2, non-occluded
Exposure Period: 6 hours; at the end of exposure unabsorbed radioactivity removed with soap and water
Receptor fluid: Phosphate buffered saline 0.01M pH 7 containing 6% (w/v) polyoxyethylene 20 oleyl ether
Collection times: 0-1, 1-2, 2-4, 4-6, 6-8, 8-10, 10-12, 12-16, 16-20, 20-24 hours
Sample Collection: receptor fluid; skin and tape strips following the final collection of receptor fluid
Analysis: Liquid Scintillation Counting

4 *IN VIVO* CONCLUSIONS

Amitraz slowly penetrated the skin of male rats and was subsequently eliminated in the feces and urine.

- The mean percent of dose absorbed systemically after a 6.5-hour exposure was 2% for all dose levels (Table 1, Figure 1).
- For animals that were exposed for 6.5 hours, but not euthanized until 72 hours following administration, the mean percent of dose absorbed was approximately 12%, 17%, and 15% for the low, mid, and high dose levels (Table 1, Figure 1).
- The mean mass absorbed was approximately 23 µg, 98 µg, and 144 µg for the low, mid, and high dose levels by 72 hours post-dosing (Figure 2).
- At the time of washing, approximately 33% of the applied dose was absorbable (e.g. was not removed by washing).
- Approximately 53% to 68% of the radioactivity that was associated with the skin after 6.5 hours of exposure was absorbed by the end of the post-exposure phase (72 hours post dosing).
- The consistent percent of dose absorbed and similar absorption rate constants among dose groups (Table 2) indicate that the absorption processes were not saturated.

These results indicate that Amitraz was slowly absorbed through the skin of male rats, and that absorption continued from the stratum corneum after the dose site was washed. There was a decrease in site skin concentration over time and an increase in cumulative absorption. Thus, the total rat dermal absorption value used in the calculation of human exposure should include the absorbed dose and the absorbable dose left in the skin and tape strips after washing.

Regardless of dose level or percent of dose absorbed, approximately 70% of the absorbed Amitraz was eliminated in the urine and up to 17% of the absorbed Amitraz was eliminated in the feces. The total amount of the absorbed dose that was eliminated was approximately 86% among all dose groups by 72 hours post-dosing (Table 3, Figure 3).

Table 1. Mean Percent Recovery of Amitraz Equivalents in Male Rats following Dermal Administration of [^{14}C]-Amitraz

Group	Euthanasia Time (h)	Not Absorbed	Absorbable	Absorbed	Total Recovery	Amount Absorbed	Amount Absorbed + Absorbable
100 µg/cm^2	6	55	35	2	91	2	37
	12	55	34	3	92	3	37
	24	56	32	6	94	6	38
	72	57	11	15	82	15	26

Table 2. Kinetics of the Dermal Absorption of Amitraz Equivalents in Male Rats following Dermal Administration of [^{14}C]-Amitraz

	Group 1 (20 µg/cm^2)	Group 2 (60 µg/cm^2)	Group 3 (100 µg/cm^2)
Mean Amount Absorbed (µg)*			
6h	3.95	10.67	14.98
12h	6.21	17.16	25.33
24h	12.52	24.45	54.05
72h	23.16	98.22	143.91
Absorption Kinetics (Linear Regression of log Amount vs. Time from 6 to 24 Hours Post-Dosing)			
Slope (b)	0.027	0.019	0.030
Y-Intercept (µg)	0.445	0.951	1.01
Coefficient of Determination (r^2)	0.996	0.927	0.993
Absorption Rate Constant (h^{-1})**	0.063	0.044	0.070

**Absorption Rate Constant = ln(10) × b

Table 3. Elimination of Amitraz Equivalents (µg) as a Percent of the Total Absorbed following Dermal Administration of [^{14}C]-Amitraz*

Group	Euthanasia Time (h)	Urine Mean %	Urine SD	Feces Mean %	Feces SD	Cumulative Percent of Absorbed Dose Mean %	Cumulative Percent of Absorbed Dose SD	Total Percent of Absorbed + Absorbable Dose Mean %	Total Percent of Absorbed + Absorbable Dose SD
100 ug/cm^2	6	20	5	2	3	22	6	1	0
	12	37	3	2	1	39	4	3	0
	24	58	7	6	2	64	7	9	2
	72	71	3	17	3	88	1	50	2

Figure 1. Mean Percent of Applied Dose Absorbed following Dermal Administration of [^{14}C]-Amitraz

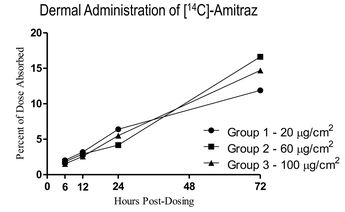


Figure 2. Mean Amitraz Equivalents Absorbed (µg) By Dose Group following Dermal Administration of [^{14}C]-Amitraz

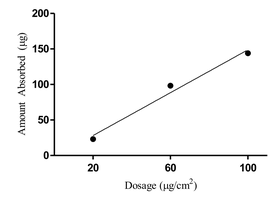
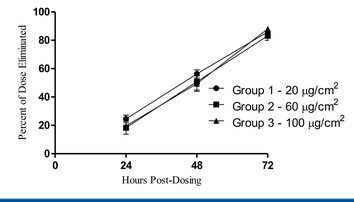


Figure 3. Mean Percent of Absorbed Dose Eliminated following Dermal Administration of [^{14}C]-Amitraz

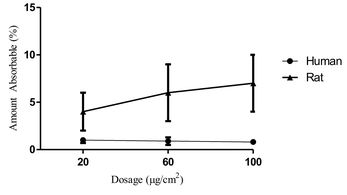


5 *IN VITRO* CONCLUSIONS

- The *in vitro* dermal absorption of Amitraz in corn oil in human skin was 1 ± 0.3% at 2 mg Amitraz/mL, 0.9 ± 0.4% at 6 mg Amitraz/mL, and 0.8 ± 0.2% at 10 mg Amitraz/mL (Table 4, Figure 4).
- The *in vitro* dermal absorption of Amitraz in rat skin was 4 ± 2% at 2 mg Amitraz/mL, 6 ± 3% at 6 mg Amitraz/mL, and 7 ± 3% at 10 mg Amitraz/mL (Table 5, Figure 4).

There appeared to be no dose-dependent differences in the fraction of test article absorbed for either species and absorption processes were not saturated. The rat skin discs showed higher permeability to Amitraz versus the human skin discs. A species difference in respect to absorption of Amitraz is also reflected by the flux.

Figure 4. Mean Amitraz Equivalents Absorbed (µg) By Dose Group following *in vitro* Dermal Administration of [^{14}C]-Amitraz



6 SCALED ABSORPTION

"Triple Pack" dermal absorption experiments are conducted in three parts – *in vivo* rat, *in vitro* human, and *in vitro* rat. In accord with regulatory recommendations, *in vivo* rat data was scaled to estimate human dermal exposure using *in vitro* ratios of human to rat dermal absorption. *In vitro* human/rat ratios of the absorption were about 1:4 at the low concentration of 2 mg Amitraz/mL, 1:6.7 at the middle concentration of 6 mg Amitraz/mL and 1:8.8 at the high concentration of 10 mg Amitraz/mL. Thus, the human skin absorption values are 9.25% (37%/4) at 2 mg/mL, 5.4% (36%/6.7) at 6 mg/mL and 4.3% (38%/8.8) at 10 mg/mL. These human skin absorption values provide a reasonable estimate of human exposure when performing risk assessments.

Table 4. Summary of the dermal absorption of Amitraz in corn oil at three dose levels using human skin *in vitro*

	2 mg Amitraz/mL		6 mg Amitraz/mL		10 mg Amitraz/mL	
Exposure	20.4 µg/cm^2		61.6 µg/cm^2		102 µg/cm^2	
Number of human skin discs	7		7		8	
Number of donors	3		4		6	
	Average % RAD	SD	Average % RAD	SD	Average[3)] % RAD	SD[3)]
Dermal absorption parameters						
Lag time (h)	1-2	NA	0	NA	0	NA
Maximum flux (µg/cm^2/h)	0.017	0.006	0.021	0.009	0.028	0.008
Surface compartment						
Total % non-absorbed[1)]	100	3	104	3	101	5
Overall absorption						
Total % potentially absorbable (excluding tape strips 1&2)[2)]	1	0.3	0.9	0.4	0.8	0.2
Recovery						
Total % recovery	102	3	105	2	102	5

Table 5. Summary of the dermal absorption of Amitraz in corn oil at three concentrations using rat skin *in vitro*

	2 mg Amitraz/mL		6 mg Amitraz/mL		10 mg Amitraz/mL	
Exposure	20.4 µg/cm^2		61.6 µg/cm^2		102 µg/cm^2	
Number of rat skin discs	7		8		8	
Number of different animals	5		4		4	
	Average[3)] % RAD	SD[3)]	Average % RAD	SD	Average % RAD	SD
Dermal absorption parameters						
Lag time (h)	1	NA	3	NA	3	NA
Maximum flux (µg/cm^2/h)	0.029	0.009	0.105	0.039	0.178	0.083
Surface compartment						
Total % non-absorbed[1)]	95	6	92	3	94	6
Overall absorption						
Total % potentially absorbable (excluding tape strips 1&2)[2)]	4	2	6	3	7	3
Total % recovery	99	5	101	3	102	4

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