Dermal absorption of tralomethrin, metam-sodium, tribufos, azinphos-methyl, and amitraz in rats

By

Thomas Thongsinthusak, Staff Toxicologist
John H. Ross, Senior Toxicologist

HS - 1770

June 19, 1998

California Environmental Protection Agency
Department of Pesticide Regulation
Worker Health and Safety Branch,
1020 N Street, Sacramento
California 95814-5624

This technical report contains five memoranda with the following titles:

1. Dermal Absorption of Tralomethrin in Male Rats (preliminary and definitive phases) (50665-068) (pages 2-7)
2. Metam Sodium: *In vivo* Dermal Absorption in the Rat and Stability Determination of Aqueous Solutions (pages 8-14)
3. DEF 6: Dermal Absorption in Rats (pages 15-20)
4. Guthion: Dermal Absorption Study (pages 21-26)
5. Amitraz: Dermal Absorption in the Rat (pages 27-34)

Fred Bundock, Registration Specialist
Pesticide Registration Branch

July 7, 1993

Sacramento

4-0455

-Tian Thongsinthusak, Staff Toxicologist
Worker Health and Safety Branch

PRODUCT NAME: Tralomethrin
ACTIVE INGREDIENT: Tralomethrin
COMPANY NAME: Roussel UCLAF Corporation
I.D. NUMBER: 140995-E
DOCUMENT NUMBER: 50665-068; 069
EPA REGISTRATION NUMBER: 432-0-
TITLE: Dermal absorption of tralomethrin in male rats (preliminary and definitive phases) (50665-068)

A dermal absorption study of tralomethrin in male rats was conducted by Hazleton Wisconsin, Inc. for Roussel UCLAF Corporation. This study was performed according to the study protocol which was reviewed by the Worker Health and Safety Branch, Department of Pesticide Regulation. The study was also conducted in compliance with Good Laboratory Practice standards. The Quality Assurance officer inspected various aspects of the study and signed the final report. A summary of this dermal absorption study and the evaluation of the results from the definitive phase of the study are presented below.

A. Preparation of Animals

Six week old male Sprague-Dawley rats (Charles River Crl:CD®BR) were used in the definitive phase of the study. Body weights of these rats ranged from 220 to 242 grams. These animals were inspected and acclimatized prior to the study. One day prior to the study, the backs and shoulders of each rat were shaved and the shaved areas were washed with acetone. A plastic enclosure was affixed to the shaved area to define the treated area which was approximately 12.5 cm^2. An Elizabethan collar was placed on the animal's neck to prevent it from licking the treated skin site.

B. Administration of the Doses

Three dose levels and one control were used in this study. The dose was prepared by adding the appropriate amounts of high purity ^{14}C-tralomethrin (>95% pure) and non-labeled tralomethrin to a blank formulation and suspended in Megapure water. The final doses were 0.037, 0.378, and 1.18 mg/animal equivalent to 3, 30, and 95 ug/cm^2. After administration of the test material, the treated skin site was covered with a nonocclusive cover made with filter paper.

C. Sample Collection and Analysis

The sacrifice times for the definitive phase of the study were: 0.5, 1, 2, 4, 10, 24, and 120 hours. For the 24- and 120-hour sacrifice times, treated skin sites were washed with 2% Ivory soap solution ten hours after exposure; daily urine and feces samples were collected and analyzed separately. Samples collected for analysis were: nonocclusive cover, dose enclosures, skin wash, treated skin site, cage wash, cage wipe, carcass, feces, and urine.

D. Determination of the Results

Percent of doses found in cage wash and cage wipe (CW/CW), carcass, feces, urine, and treated skin site are shown in Table 1. The results of the study indicated that dermal absorption of tralomethrin is dose dependent, i.e., percent dose absorbed for the low dose is higher than that for the medium and high doses. Furthermore, percent dose absorbed is greater for the longer exposure and sacrifice times for the same dose level. A low percent of dose (range 0.07-0.78) was observed in carcass for all dose groups and sacrifice times. The highest percent dose in the treated skin site was 24.3% at 10-hour sacrifice time for the low dose, whereas, for medium and high doses were 6.71 and 4.97% 10-hour after dosing, respectively.

Table 1. Percent dose in cage wash/cage wipe, carcass, feces, urine and treated skin site.

		Percent of radioactive dose					
	Sac. time (h)	CW/CW	Carcass	Feces	Urine	Treated skin	% Recovery
A. 0.037 mg/animal ($3\ ug/cm^2$)	0.5	ND	0.42	ND	ND	5.35	97.3
	1	ND	0.07	ND	0.01	3.68	96.8
	2	ND	0.46	ND	0.02	14.7	96.8
	4	ND	0.12	ND	0.05	12.1	96.9
	10	ND	0.65	ND	0.26	24.3	95.4
	24	0.08	0.56	0.1	0.51	22.2	94.9
	120	1.74	0.37	1.05	2.3	15.6	94.6
B. 0.378 mg/animal ($30\ ug/cm^2$)	0.5	ND	0.1	ND	0.01	3.42	103.6
	1	ND	0.27	ND	0.01	3.27	102.2
	2	ND	0.55	0.01	0.01	4.8	104
	4	ND	0.78	ND	0.04	6.03	103
	10	0.01	0.17	0.01	0.1	6.71	102
	24	0.07	0.23	0.05	0.27	3.14	98.2
	120	1.1	0.31	0.47	1.26	3.19	99.8

Table 1 (cont.). Percent dose in cage wash/cage wipe, carcass, feces, urine and treated skin site.

		Percent of radioactive dose					
	Sac. time (h)	CW/CW	Carcass	Feces	Urine	Treated skin	% Recovery
C. 1.18 mg/animal	0.5	ND	0.15	ND	0.01	2.38	101
(95 ug/cm^2)	1	ND	0.26	ND	0.01	2.77	103
	2	0.01	0.3	ND	0.01	2.65	100
	4	0.01	0.73	ND	0.08	4.86	101
	10	0.01	0.4	0.01	0.24	4.97	94.7
	24	0.16	0.28	0.08	0.38	3.49	97.2
	120	0.56	0.36	0.54	1.13	2.63	97.7

Notes: Percent Recovery includes % dose found in nonocclusive cover, dose enclosures, skin wash, treated skin site, cage wash, cage wipe, carcass, feces, and urine. Percent dose in blood was negligible.

Excretion kinetics of tralomethrin in urine and feces from treated rats were observed over 120 hours after dosing. Percent of dose excreted at different time intervals were tabulated (Table 2). In order to resolve the issue of bound skin residue, cumulative percent doses in urine and feces for different time intervals extrapolated beyond 120 hours were used for estimating the asymptote by employing an exponential saturation model with lag time. An equation representing this model is: Y = A*(1-EXP(-B*(X+C))) or Recov = Max*(1-EXP(-Rate*(Time+Lag))). An example of the plots for the lowest dose and the outputs are shown in Figure 1. The dermal absorption value is the sum of percent dose at asymptote (maximum or "A" term) and percent of dose recovered in carcass, blood, and cage wash/cage wipe. Percent of dose in blood in this case is negligible. Table 3 summarizes the dermal absorption values for low, medium and high doses obtained from this study. The corrected dermal absorption values for the lowest dose (3 ug/cm^2) with 10-hour exposure time is appropriate to be used in the exposure estimates because this dose should be representative of exposure experienced by the agricultural workers.

Table 2. Excretion of administered tralomethrin in urine and feces of male rats.

A. Dose: 0.037 mg/animal (3 ug/cm^2)

	Mean % dose excreted in urine and feces					
Time(h)	Feces	Urine		F + U	Cumulative	CW/CW
24	0.1	0.56		0.66	0.66	
48	0.36	0.61		0.97	1.63	
72	0.25	0.54		0.79	2.42	
96	0.2	0.33		0.53	2.95	
120	0.15	0.28		0.43	3.38	1.74
			sum			
Total	1.06	2.32	3.38	3.38		

B. Dose: 0.378 mg/animal (30 ug/cm^2)

Time(h)	Feces	Urine		F + U	Cumulative	CW/CW
24	0.07	0.41		0.48	0.48	
48	0.17	0.47		0.64	1.12	
72	0.11	0.26		0.37	1.49	
96	0.07	0.07		0.14	1.63	
120	0.05	0.05		0.1	1.73	1.09
			sum			
Total	0.47	1.26	1.73	1.73		

C. Dose: 1.18 mg/animal (95 ug/cm^2)

	Mean % dose excreted in urine and feces					
Time(h)	Feces	Urine		F + U	Cumulative	CW/CW
24	0.08	0.38		0.46	0.46	
48	0.18	0.39		0.57	1.03	
72	0.09	0.13		0.22	1.25	
96	0.08	0.13		0.21	1.46	
120	0.11	0.1		0.21	1.67	0.56
			sum			
Total	0.54	1.13	1.67	1.67		

CW/CW = cage wash/cage wipe; ND = Not Detected.

Table 3. Summary of tralomethrin dermal absorption values for low, mid, and high doses.

	Percent of dose excretion at asymptote					
Dose (ug/cm^2)	CW/CW	Carcass		Total	% Recovery	Corrected* % dermal absorption
3	1.74	0.37	4.66	6.77	94.6	7.16
30	1.09	0.31	1.82	3.22	99.8	3.23
95	0.56	0.36	1.89	2.81	97.7	2.88

* Corrected for % recovery

Recommendation:

1. The dermal absorption study of tralomethrin in male rats as reported in document number 50665-068 is acceptable. The results are used to determine dermal absorption values for human exposure assessment.

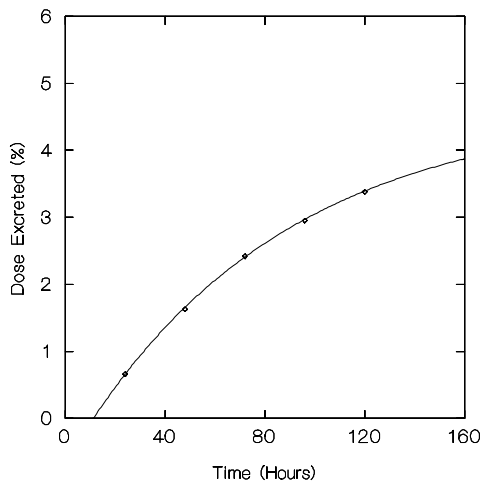
2. Dermal absorption of 7.2 percent will be used to estimate absorbed dosages of persons exposed to tralomethrin.

cc: Joshua Johnson (1 original, 5 copies)
James Herota

(TCW/dml-abs/tralome3)

Figure 1. Asymptotic plot of cumulative excretion of dose of tralomethrin in urine and feces at different time intervals for 3 ug/cm^2 dose.

$$Y = 4.6549*(1-EXP(-0.0119*(X-11.2803)))$$



Statistics:

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>MODEL RECOV=Max*(1-EXP(-Rate*(Time+Lag)))
>ESTIMATE/ ITER= 25 START= 4,.011,.1 SIMPLEX SCALE

FRI 6/04/93 1:37:18 PM   C:\TCSYS\TRALOM02.SYS

 ITERATION      LOSS       PARAMETER VALUES
    0      .4423074D+00  .4000D+01 .1100D-01 .1000D+00
    1      .2924814D+00  .4873D+01 .8706D-02 .1538D+00
    2      .1058626D+00  .6004D+01 .6795D-02 .1650D+00
    3      .7113534D-01  .6571D+01 .6012D-02 .1847D+00
    4      .6071989D-01  .7566D+01 .5059D-02-.4267D-02
    5      .5877466D-01  .8229D+01 .4550D-02-.1789D+00
    6      .5832284D-01  .8565D+01 .4349D-02-.4303D+00
    7      .5710997D-01  .8394D+01 .4531D-02-.1194D+01
    8      .4308329D-01  .7452D+01 .5442D-02-.4603D+01
    9      .3753377D-01  .6969D+01 .6101D-02-.6778D+01
   10      .3108027D-01  .6429D+01 .6682D-02-.5969D+01
   11      .2390916D-01  .6266D+01 .7173D-02-.7450D+01
   12      .1822321D-01  .5625D+01 .8358D-02-.8481D+01
   13      .1037538D-01  .5273D+01 .9430D-02-.8637D+01
   14      .3036110D-02  .4697D+01 .1154D-01-.1062D+02
   15      .1298963D-02  .4684D+01 .1174D-01-.1104D+02
   16      .1027378D-02  .4642D+01 .1199D-01-.1136D+02
   17      .1013248D-02  .4654D+01 .1192D-01-.1129D+02
   18      .1013029D-02  .4655D+01 .1191D-01-.1128D+02
   19      .1013022D-02  .4655D+01 .1191D-01-.1128D+02
   20      .1013022D-02  .4655D+01 .1191D-01-.1128D+02
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(TCW\dml-abs\Tralome4)

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DEPENDENT VARIABLE IS    RECOV
  SOURCE   SUM-OF-SQUARES    DF  MEAN-SQUARE

 REGRESSION       29.0748     3       9.6916
   RESIDUAL        0.0010     2       0.0005

     TOTAL       29.0758     5
 CORRECTED        4.6995     4

     RAW R-SQUARED (1-RESIDUAL/TOTAL)     =       1.0000
CORRECTED R-SQUARED (1-RESIDUAL/CORRECTED) =       0.9998
STANDARD ERRORS OF PARAMETERS ARE RESCALED


PARAMETER  ESTIMATE   A.S.E.    LOWER  <95%> UPPER
    MAX        4.6549      3.0174     -1.2591      10.5689
   RATE        0.0119      0.0147     -0.0169       0.0408
    LAG      -11.2803     18.2102    -46.9716      24.4111


ASYMPTOTIC CORRELATION MATRIX OF PARAMETERS

                MAX       RATE        LAG

    MAX          1.0000
   RATE         -0.9587     1.0000
    LAG          0.4454    -0.6272     1.0000
```

Kathy Wynn, Registration Specialist
Pesticide Registration Branch

July 26, 1993

Sacramento

4-0455

-Tian Thongsinthusak, Staff Toxicologist
Worker Health and Safety Branch

PRODUCT NAME: Metam-Sodium
ACTIVE INGREDIENT: Sodium N-methyldithiocarbamate
COMPANY NAME: Metam-Sodium Task Force c/o ICI Americas Inc.
I.D. NUMBER: SBC-139037-E
DOCUMENT NUMBER: 50150-095
EPA REGISTRATION NUMBER: 1051005-0-
TITLE: Metam Sodium: *In vivo* Dermal Absorption in the Rat and Stability Determination of Aqueous Solutions

There are two separate studies in this submitted document. The first study is on stability determination of aqueous solutions which was conducted by Zeneca Central Toxicology Laboratory and the second study is on i*n vivo* dermal absorption in the rat. The latter was performed by Hazleton UK. The first study was conducted to determine the appropriate dose levels prior to the dermal absorption study of metam-sodium in rats. Both studies were performed in compliance with Good Laboratory Practice standards. Summary of the two studies and the evaluation of dermal absorption values are presented below.

A. Metam-Sodium: Stability Determination of Aqueous Solutions

Stability of metam-sodium in water was conducted prior to the dermal absorption study in the rat. A mixture of high purity ^{14}C-metam sodium (between 94.4 and 97.4%) and nonlabeled metam-sodium (99.4%) were used in this and the dermal absorption study. The labeled position of [^{14}C] was the carbonyl carbon. ^{14}C-Metam sodium (7.66 mg) was mixed with nonlabeled metam-sodium (193.85 mg), evacuated, and dissolved in degassed HPLC grade water at about 200 mL to make a solution of 200 mg/mL. Dilutions were also done to prepare concentrations of 20, 2, and 0.2 mg/mL. All of the solutions were stored under nitrogen environment at room temperature in the dark. At different time intervals after the preparation of the solutions, samples were analyzed by HPLC using UV absorption at 241 nm with on-line radioactivity for [^{14}C] analysis.

The results showed that the 0.2 mg/mL solution is not stable because less than 63% of its initial analyzed concentration remained after three hours. For the 2 mg/mL solution, more than 93% of its initial concentration remained one hour after its preparation and 86% three hours after its preparation. Solutions of 200 and 20 mg/mL appeared to be stable for at least 20 hours. Due to

the instability of the lowest concentration solution of 0.2 mg/mL, this concentration was not used in the dermal absorption study in rats.

B. Metam-Sodium: *In vivo* Dermal Absorption in the Rat

B.1 Preparation of Animals

Six to ten-week old Charles River Crl:CD(SD)BR strain rats were used in the dermal absorption study. Body weights of these rats ranged from 178-274 grams. These animals were acclimatized 4-12 days prior to the study. An area of the dorso-lumbar skin was clipped free of hair using veterinary clippers, without abrading the skin. The shaved skin sites were washed with acetone. The treated skin site with an area of 11.6 cm^2 was enclosed with a glass saddle. The animals were placed in individual all glass metabolism cages that allowed the separate collection of urine, feces, and expired air.

B.2 Administration of the Doses

The test material was prepared as an aqueous solution in degassed HPLC grade water to give a nominal volume of 0.05 mL per animal. Three dose levels at nominal concentrations of 0.1, 1, and 10 mg/animal (equivalent to 8.6, 86.2, and 862 ug/cm^2) were applied dermally. After administration of the dosing solution, an activated charcoal filter, resting between two glass sinters, was placed in the saddle. The charcoal and sinters were secured by a foam disc, cotton gauze and an elastic band.

B.3 Sample Collection and Analysis

The sacrifice times were 1, 2, 10, 24, and 72 hours which corresponded to the exposure times 1, 2, 10, 24, and 10 hours, respectively. For the 72-hour sacrifice time, the treated skin sites and the inside of the glass saddles were swabbed thoroughly with liquid Ivory® soap solution and water and the animals were returned to the metabolism cages. Urine, feces and cage washings of animals for the 72-hour sacrifice time were collected at 10, 24, 48, and 72 hours. Samples collected for analysis were: nonocclusive cover (charcoal, charcoal washings and sinter washings), treated and untreated skin sites, cage washings, carcass, urine, feces, blood, air traps, and cage debris.

C. Determination of the Results

Results of the dermal absorption study are shown in Table 1. Radioactivity in the blood calculated as percent dose are shown in Table 2. The results indicated that metam-sodium and/or

its degradates are absorbed rapidly into the skin. Absorption in one hour after exposure is similar to that with longer exposure time. There is no obvious indication that absorption is dose or time dependent. This may be due to metam-sodium being rapidly degraded and the degradate(s) volatilized and are trapped in the charcoal or limited absorption of a radiolabeled impurity. Percent dose absorbed for 1, 2, 10, and 24-hour sacrifice times are presented in Table 1 as the sum of percent dose in treated skin, urine, feces, carcass, cage washings, air traps, and blood. These dermal absorption values were not used for worker exposure estimates because bioavailability of bound skin residues can be estimated from the 72-hour sacrifice time group.

In order to resolve the issue of bound skin residues, cumulative percent doses in urine, feces, and cage washings for different time intervals over 72 hours (Table 3) were used for estimating the asymptote by employing an exponential saturation model with lag time. An equation representing this model is: Y = A*(1-EXP(-B*(X+C))) or Recov = Max*(1-EXP(-Rate*(Time+Lag))). Example of the plots for the lowest dose and the outputs are shown in Figure 1. The dermal absorption value is the sum of percent dose at asymptote (maximum or "A" term) and percent of dose recovered in carcass, blood, air traps, and cage washings. Table 4 summarizes the dermal absorption values for low, medium and high doses used in this study. Percent doses excreted in three doses are similar, ranging from 0.89 to 1.18 percent. The obvious difference is percent dose in carcass for high and percent dose in air traps for medium dose. The corrected dermal absorption value of 2.5% for the lowest dose (8.6 ug/cm^2) is appropriate to be used in the worker exposure estimates because this dose should be representative of exposure experienced by the agricultural workers.

D. Recommendations

1. The dermal absorption study of metam-sodium in male rats as reported in this document is acceptable.

2. Dermal absorption of 2.5 percent will be used to estimate absorbed dosages of worker exposure to this pesticide. It is assumed that this percent dermal absorption is the combined absorption value of metam-sodium and its degradate(s), such as MITC.

3. It is assumed that the nature of exposure the workers will experience would resemble the manner in which this pesticide was administered to animals in the dermal absorption study. Nonocclusive patch used in the study would resemble the clothing the workers wear in their routine handling of this chemical.

cc: Joshua Johnson (1 original, 5 copies)
John Ross

(TCW/Dermal/Metam01)

Table 1. Percent recovery of administered dose of metam-sodium.

		Percent dose (mean)									
Dose	Sacrifice time (h)	Unab-sorbed*a	Cage debris	Treated skin*b	Urine	Feces	Carcass	CW	Air traps*c	Blood*d	Total recovery
0.1 mg/rat	1	92.8	0	2.738	0	0	0	0	1.791	0	97.329
(8.6 ug/cm2)				*(1-h) Absorbed dose (% dose)*e=*			*4.529*				
	2	87.61	0	3.575	0.018	0	0	0	2.161	0	93.364
				*(2-h) Absorbed dose (% dose)*e=*			*5.754*				
	10	91.73	0	7.741	0.451	0	0	0.081	0.824	0	100.827
				*(10-h) Absorbed dose (% dose)*e=*			*9.097*				
	24	71.29	0	8.423	1.002	0.035	0	0.015	1.93	0	82.695
				*(24-h) Absorbed dose (% dose)*e=*			*11.405*				
	72	92.95	0	2.794	1.049	0.006	0	0.1	1.276	0	98.175
1.0 mg/rat	1	82.71	0	3.623	0.011	0	0.144	0.022	1.707	0.06	88.277
(86.2 ug/cm2)				*(1-h) Absorbed dose (% dose)*e=*			*5.567*				
	2	78.22	0	6.777	0.044	0	0.266	0	2.777	0.07	88.154
				*(2-h) Absorbed dose (% dose)*e=*			*9.934*				
	10	90.16	0	4.31	0.749	0.026	0	0.087	2.823	0.02	98.175
				*(10-h) Absorbed dose (% dose)*e=*			*8.015*				
	24	76.37	0	4.107	1.321	0.701	0.815	0.161	1.725	0.02	85.22
				*(24-h) Absorbed dose (% dose)*e=*			*8.85*				
	72	87.65	0	1.633	0.697	0.016	0	0.129	2.325	0	92.45
10 mg/rat	1	64.11	0	14.88	0	0	0	0.064	0.324	0.05	79.428
(862 ug/cm2)				*(1-h) Absorbed dose (% dose)*e=*			*15.318*				
	2	79.34	0.03	1.754	0.079	0.044	0.432	0.062	0.682	0.04	82.463
				*(2-h) Absorbed dose (% dose)*e=*			*3.093*				
	10	80.82	0	1.31	0.489	0.021	0.342	0.106	0.556	0.03	83.674
				*(10-h) Absorbed dose (% dose)*e=*			*2.854*				
	24	80.1	0	2.472	0.753	0.459	0.541	0.157	0.325	0.02	84.827
				*(24-h) Absorbed dose (% dose)*e=*			*4.727*				
	72	79.07	0	0.7	0.942	0.109	1.217	0.145	1.101	0	83.284

Table 1. (cont.) Percent recovery of administered dose of metam-sodium.

*a Application site washings + charcoal + charcoal washings + sinter washings.
*b Includes untreated skin around treated skin site.
*c Trap (1) 2-ethoxyethanol to trap MITC + trap (2) ethanolamine:2-ethoxyethanol (91:3, v/v) to trap CO2 + trap (3) ethanol: diethylamine:triethanolamine (1000:1:20, v/v) to trap CS2. Due to unusually high percent dose recovered in trap (2) for 24-hour (0.1 mg/animal) and 2-hour (10 mg/animal) sacifice times, an average of % dose from trap (2) of other four sacrifice times of the same group was used.
*d Percent dose in blood was calculated based upon the assumption that one animal has 15 grams of whole blood.
*e When bioavailability of bound skin residues cannot be determined, dermal absorption value = % dose recovered from treated skin site + urine + feces + carcass + cage washings + air traps + blood.

(Note: "ND" shown in the submitted report is assumed = 0, CW = Cage washings)

Table 2. Percent dose of metam sodium in blood of experimental animals.

14C-metam sodium in blood

Sacrifice time	0.1 mg/animal			1 mg/animal			10 mg/animal		
(hour)	ug equiv./g	ug/animal	% dose	ug equiv./g	ug/animal	% dose	ug equiv./g	ug/animal	% dose
1	0	0	0	0.038	0.57	0.06	0.341	5.12	0.05
2	0	0	0	0.044	0.66	0.07	0.28	4.2	0.04
10	0	0	0	0.014	0.21	0.02	0.187	2.81	0.03
24	0	0	0	0.012	0.18	0.02	0.125	1.88	0.02
72	0	0	0	0	0	0	0	0	0

Notes:
1. Assume 15 grams of whole blood/animal.
2. Values were calculated from data in Tables 9.19-9.21 of Reg. Doc. No. 50150-095.

(TXL\Dermal\Metam05)

Table 3. Percent dose of metam-sodium excreted following 10-hour exposure.

A. 0.1 mg/animal or 8.6 ug/cm2 (Actual dose: 0.085 mg/animal or 7.3 ug/cm2)

	% Dose				
Time (h)	Urine	Feces	CW		Cumulative
10	0.351	0.006	0.094		0.451
24	0.591	0	0.007		1.049
48	0.082	0	0		1.131
72	0.025	0	0		1.156
				Total	
Subtotal	1.049	0.006	0.101	1.156	

B. 1 mg/animal or 86.2 ug/cm2 (Actual dose: 0.097 mg/animal or 83.8 ug/cm2)

	% Dose				
Time (h)	Urine	Feces	CW		Cumulative
10	0.356	0	0.112		0.468
24	0.154	0	0.011		0.633
48	0.145	0.016	0.006		0.8
72	0.043	0	0		0.843
				Total	
Subtotal	0.698	0.016	0.129	0.843	

C. 10 mg/animal or 862 ug/cm2 (Actual dose: 10.01 mg/animal or 863 ug/cm2)

	% Dose				
Time (h)	Urine	Feces	CW		Cumulative
10	0.342	0.011	0.094		0.447
24	0.379	0.05	0.042		0.918
48	0.13	0.024	0.009		1.081
72	0.092	0.024	0		1.197
				Total	
Subtotal	0.943	0.109	0.145	1.197	

Treated skin site = 11.6 cm2

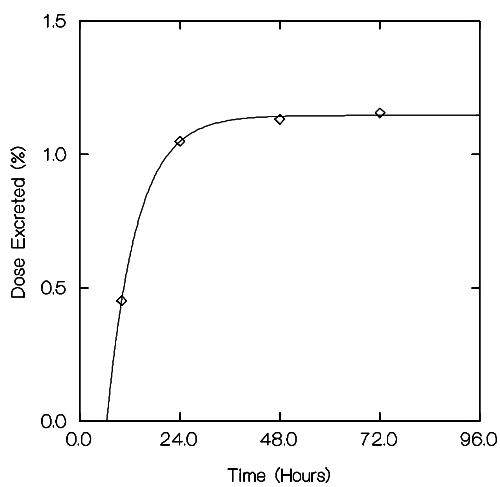
Table 4. Summary: Dermal absorption of metam-sodium in male rats.

	Percent of dose recovered						
Dose mg/animal (ug/cm2)	Excreted	Blood	Carcass	Expired air	Sub-total	Recovery	Total
0.1 (8.6)	1.15	0	0	1.28	2.43	98.18	2.48
1 (86.2)	0.89	0	0	2.33	3.22	92.45	3.48
10 (862)	1.18	0	1.22	1.1	3.5	83.28	4.20

(TXL/Dermal/Metam01)

Figure 1. Metam-sodium: Asymptotic plot of cumulative excretion of dose excreted in urine and feces at different time intervals for 0.1 mg/animal (8.6 ug/cm^2).

$$Y = 1.1457*(1-EXP(-0.1401*(X-6.4279)))$$



Statistics:

```
>MODEL RECOV=Max*(1-EXP(-Rate*(Time+Lag)))
>ESTIMATE/ SIMPLEX

TUE 7/20/93 2:04:56 PM

 ITERATION      LOSS       PARAMETER VALUES
    0      .3247758D+01  .1000D+00 .1000D+00 .1000D+00
    1      .3886383D+00  .8715D+00 .2561D+00-.4200D+00
    2      .5451171D-01  .1167D+01 .6549D-01-.5273D+00
    3      .2375178D-01  .1200D+01 .6319D-01-.5593D+00
    4      .2329867D-01  .1200D+01 .6507D-01-.5711D+00
    5      .2322394D-01  .1200D+01 .6498D-01-.6019D+00
    6      .2199541D-01  .1187D+01 .7630D-01-.2021D+01
    7      .6108829D-02  .1178D+01 .9316D-01-.4637D+01
    8      .4324411D-02  .1158D+01 .1081D+00-.5498D+01
    9      .8641067D-03  .1141D+01 .1537D+00-.6789D+01
   10       .2488689D-03  .1145D+01 .1405D+00-.6439D+01
   11       .2377677D-03  .1146D+01 .1399D+00-.6422D+01
   12       .2375677D-03  .1146D+01 .1401D+00-.6428D+01
   13       .2375526D-03  .1146D+01 .1401D+00-.6428D+01
   14       .2375521D-03  .1146D+01 .1401D+00-.6428D+01
```

```
DEPENDENT VARIABLE IS    RECOV

  SOURCE   SUM-OF-SQUARES    DF  MEAN-SQUARE

 REGRESSION        3.9191     3      1.3064
  RESIDUAL         0.0002     1      0.0002

    TOTAL         3.9193     4
 CORRECTED        0.3340     3

    RAW R-SQUARED (1-RESIDUAL/TOTAL)     =       0.9999
CORRECTED R-SQUARED (1-RESIDUAL/CORRECTED) =       0.9993


PARAMETER  ESTIMATE  A.S.E.  LOWER  <95%> UPPER
   MAX        1.1457      0.0115      0.9999      1.2915
  RATE        0.1401      0.0140     -0.0378      0.3180
   LAG       -6.4279      0.3857    -11.3293     -1.5265


ASYMPTOTIC CORRELATION MATRIX OF PARAMETERS

                MAX       RATE        LAG

   MAX          1.0000
  RATE         -0.5788     1.0000
   LAG          0.4184    -0.9045     1.0000
```

(TCW\Dermal\Metam02)

John Inouye, Registration Specialist
Pesticide Registration Branch

August 10, 1993

Sacramento

4-0455

-Tian Thongsinthusak, Staff Toxicologist
Worker Health and Safety Branch

PRODUCT NAME: DEF 6
ACTIVE INGREDIENT: Tribufos
COMPANY NAME: Mobay Chemical Corporation
I.D. NUMBER: 135093-ER
DOCUMENT NUMBER: 272-074
EPA REGISTRATION NUMBER: 3125-0-
TITLE: DEF 6: Dermal Absorption in Rats

The submitted final report on a dermal absorption study of tribufos (DEF 6) in male rats has been reviewed and evaluated. This study was conducted in compliance with the Good Laboratory Practice standards by Miles Inc. The study protocol was previously reviewed by the Worker Health and Safety Branch. A report on quality assurance (QA) inspections was signed by the QA officer and was included in this submission. A summary of this study and the evaluation of dermal absorption values are presented below.

A. Preparation of Animals

Adult male Sprague-Dawley derived rats were used in this dermal absorption study. Body weights of these animals ranged from 200 to 238 grams. These animals were acclimatized at least 6 days prior to the study. During this holding period, rats were inspected daily for any changes in general appearance and for gross abnormalities. The back of each animal was clipped with electric clippers about 24 hours prior to dosing and the shaved skin site was washed with acetone. The treated skin site with an area of 15 cm^2 was enclosed with a rubber ring. The animals were placed in individual metabolism cages that allowed the separate collection of urine and feces during the study.

B. Administration of the Doses

The dosing solution was prepared in distilled water by mixing appropriate amounts of tribufos-1-^{14}C (radiochemical purity of 98.9%), DEF 6 blank formulation and nonlabeled tribufos technical (if needed) for each dose level. The volume used for each animal for the two high doses was 150 µl, whereas for the low dose was 75 µl. Three dose levels at nominal concentrations of 0.374, 0.903, and 9.89 mg a.i./mL were applied dermally using a micropipet. The treated skin sites were

protected with nonocclusive covers made of a Teflon-laminated filter and a carbon-impregnated material. Protective collars were also used to restrict movement of animals.

C. Sample Collection and Analysis

The sacrifice times were 1, 4, 10, and 168 hours which corresponded to the exposure times 1, 4, 10, and 10 hours, respectively. Four animals per dose were used for each sacrifice time. For the 168-hour sacrifice time, the treated skin site was lightly wiped twice with a gauze pad moistened with a 5% water solution of Ivory® dishwashing detergent, twice with a gauze pad moistened with distilled water, and once with a dry gauze pad. The animals were returned to the metabolism cages for additional observation. Urine and feces of animals for the 168-hour sacrifice time were collected at 24, 48, 72, 96, 120, 144, and 168 hours. Samples collected for analysis were: nonocclusive cover, ring wash, skin wash, treated skin sites, cage wash, carcass, urine, feces, and blood.

D. Determination of the Results

Results of the dermal absorption study are shown in Table 1. Recoveries of administered doses range from 92 to 106% for low dose, 89 to 105% for mid dose, and 101 to 107 % for high dose. Percent doses of tribufos excreted following a 10-hour exposure are shown in Table 2. Tribufos was apparently readily absorbed and saturated because skin residues for all doses and exposure times (1-10 hours) were similar. Dermal absorption values for 1-, 4-, and 10-hour sacrifice times are presented in Table 1 as the sum of percent dose in treated skin site, urine, feces, carcass, cage wash, and blood. These dermal absorption values range from 33.4 to 52.3% for low dose, 33.1 to 49.4% for mid dose, and 30.7 to 45.5% for high dose. These dermal absorption values are not used for worker exposure estimates because bioavailability of bound skin residues can be estimated from the 168-hour sacrifice time group.

Cumulative percent doses in urine and feces for different time intervals over 168 hours (Table 2) and from 10-hour sacrifice time were used for estimating the asymptote by employing an exponential saturation model with lag time. An equation representing this model is: Y = A*(1-EXP(-B*(X+C))) or Recov = Max*(1-EXP(-Rate*(Time+Lag))). Example of the plots for the low dose and the outputs are shown in Figure 1. The dermal absorption value is calculated as the sum of percent dose at asymptote (maximum or "A" term) and percent dose recovered in carcass, blood, and cage wash. Table 3 summarizes the dermal absorption values for low, mid and high doses used in this study. Dermal absorption values for three doses range from 34.4 to 43.6 percent. The corrected dermal absorption value of 47.5% for the low dose (1.93 µg a.i./cm^2) is appropriate to be used in the worker exposure estimates because this dose should be representative of exposure experienced by the agricultural workers.

E. Recommendations

1. The dermal absorption study of tribufos in male rats as reported in this document is acceptable.

2. Dermal absorption of 47.5 percent will be used to estimate absorbed dosages of worker exposure to tribufos. However, in case of a very high exposure, e.g. over 100 $\mu g/cm^2$, a dermal absorption value of 33.9 will be considered.

cc: Joshua Johnson (1 original, 5 copies)
John Ross
Tareq Formoli

(TCW/Dermal/DEF03)

Table 1. Percent recovery of administered dose of tribufos.

	Percent dose (mean)								
Dose (a.i.)	Sacrifice time (h)	Unab-sorbed*a	Treated skin site	Blood	*Urine*	Feces	Carcas	Cage wash	Total recovery
1.93 ug/cm2	1	72.66	32.52	0.01	0.03	0	0.83	0.03	106.08
(1.87 ug/cm2)*b			*(1-h) Absorbed dose (% dose)*c =*		*33.42*				
	4	57.43	38.05	0.1	1.07	0.1	7.58	0.13	104.46
			*(4-h) Absorbed dose (% dose)*c =*		*47.03*				
	10	51.66	33.64	0.14	5.89	0.44	11.41	0.8	103.98
			*(10-h) Absorbed dose (% dose)*c=*		*52.32*				
	168	44.5	2.82	0.02	36.03	3.05	2.49	2.89	91.8
12.4 ug/cm2	1	66.59	32.24	0.01	0.02	0	0.85	0	99.71
(9.03 ug/cm2)*b			*(1-h) Absorbed dose (% dose)*c =*		*33.12*				
	4	58.9	39.12	0.07	0.54	0.04	6.26	0.29	105.22
			*(4-h) Absorbed dose (% dose)*c =*		*46.32*				
	10	51.09	36.02	0.11	4.48	0.11	8.26	0.43	100.5
			*(10-h) Absorbed dose (% dose)*c=*		*49.41*				
	168	43.4	1.6	0.02	33.94	3.71	2.49	3.55	88.71
100 ug/cm2	1	75.85	29.75	0	0.01	0	0.91	0	106.52
(98.9 ug/cm2)*b			*(1-h) Absorbed dose (% dose)* =*		*30.67*				
	4	65.32	34.29	0.03	0.25	0.09	3.03	0.06	103.07
			*(4-h) Absorbed dose (% dose)*c =*		*37.75*				
	10	60.71	32.53	0.12	3.9	0.14	8.41	0.36	106.17
			*(10-h) Absorbed dose (% dose)*c=*		*45.46*				
	168	64	2.29	0.01	25.75	3.36	1.9	3.75	101.06

*a Protective cover + ring wash + skin wash

*b Nominal dose

*c When bioavailability of bound skin residues cannot be determined, demal absorption values = % dose recovered from treated skin site + blood + urine + carcass + cage wash.

(TXL\Dermal\DEF01)

Table 2. Percent dose of tribufos excreted following 10-hour exposure.

A. Actual dose: 1.93 ug/cm2

	Percent dose (mean)				
Time (h)	Urine (U)	Feces (F)	U + F		Cumulative
24	18.7	0.6	19.3		19.3
48	7.4	0.8	8.2		27.5
72	3.7	0.4	4.1		31.6
96	2.4	0.3	2.7		34.3
120	1.6	0.3	1.9		36.2
144	1.2	0.3	1.5		37.7
168	1	0.5	1.5		39.2
Total	36	3.2	39.2		

B. Actual dose: 12.4 ug/cm2

	Percent dose (mean)				
Time (h)	Urine (U)	Feces (F)	U + F		Cumulative
24	16.9	1.1	18		18
48	8.3	1.1	9.4		27.4
72	3.7	0.5	4.2		31.6
96	2	0.3	2.3		33.9
120	1.5	0.2	1.7		35.6
144	0.8	0.2	1		36.6
168	0.9	0.2	1.1		37.7
Total	34.1	3.6	37.7		

C. Actual dose: 100 ug/cm2

	Percent dose (mean)				
Time (h)	Urine (U)	Feces (F)	U + F		Cumulative
24	13.3	0.7	14		14
48	6.2	1	7.2		21.2
72	2.6	0.7	3.3		24.5
96	1.4	0.4	1.8		26.3
120	1.1	0.3	1.4		27.7
144	0.7	0.2	0.9		28.6
168	0.5	0.2	0.7		29.3
Total	25.8	3.5	29.3		

Table 3. Summary: Dermal absorption of tribufos in male rats*a.

Actual dose	Percent dose (mean)						
mg/animal (ug/cm2)	Excreted*b	Blood	Carcass	CW	Sub-total	Recovery	Adj. total
1.93	38.16	0.02	2.49	2.89	43.56	91.8	47.45
12.4	36.42	0.02	2.49	3.55	42.48	88.71	47.89
100	28.73	0.01	1.9	3.75	34.39	101.6	33.85

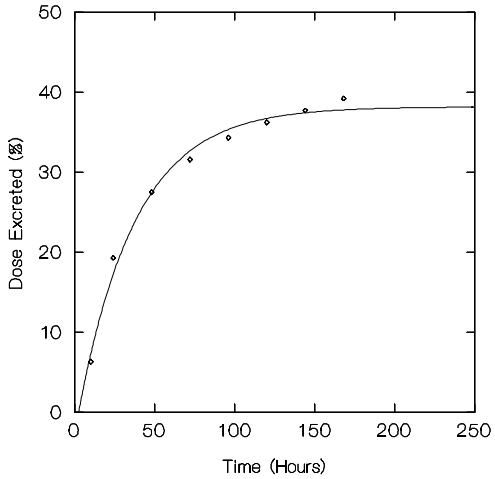
*a Percent doses: excreted + blood + carcass + cage wash.

*b At asymptote using an exponential saturation model.

(TXL\Dermal\DEF02)

Figure 1. Tribufos: Asymptotic plot of cumulative excretion of dose in urine and feces at different time intervals for 1.93 µg/cm^2.

$$Y = 38.1595*(1-EXP(-0.0278*(X-2.2599)))$$



Statistics:

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>NONLIN
>MODEL RECOV=Max*(1-EXP(-Rate*(Time+Lag)))
>ESTIMATE/ ITER= 25 START= 5,.02,1 SIMPLEX


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 ITERATION      LOSS      PARAMETER VALUES
    0      .5865028D+04  .5000D+01 .2000D-01 .1000D+01
    1      .1355948D+03  .3487D+02 .4471D-01-.2730D+01
    2      .1468310D+02  .3719D+02 .2993D-01-.1971D+01
    3      .9754106D+01  .3833D+02 .2719D-01-.2097D+01
    4      .9687465D+01  .3817D+02 .2775D-01-.2150D+01
    5      .9680877D+01  .3815D+02 .2787D-01-.2283D+01
    6      .9678270D+01  .3816D+02 .2782D-01-.2260D+01
    7      .9678233D+01  .3816D+02 .2783D-01-.2261D+01
    8      .9678232D+01  .3816D+02 .2782D-01-.2260D+01
    9      .9678232D+01  .3816D+02 .2782D-01-.2260D+01
   10      .9678232D+01  .3816D+02 .2782D-01-.2260D+01

 DEPENDENT VARIABLE IS    RECOV

   SOURCE   SUM-OF-SQUARES    DF  MEAN-SQUARE

 REGRESSION      7602.5514     3    2534.1838
  RESIDUAL        9.6782     5       1.9356

    TOTAL      7612.2289     8
 CORRECTED       876.6868     7

    RAW R-SQUARED (1-RESIDUAL/TOTAL)     =       0.9987
 CORRECTED R-SQUARED (1-RESIDUAL/CORRECTED) =
0.9890


PARAMETER      ESTIMATE      A.S.E.      LOWER  <95%> UPPER
    MAX         38.1595       1.0803      35.3826      40.9364
   RATE          0.0278       0.0040       0.0175       0.0382
    LAG         -2.2599       2.1909      -7.8919       3.3721


ASYMPTOTIC CORRELATION MATRIX OF PARAMETERS

                MAX        RATE        LAG

    MAX          1.0000
   RATE         -0.7954      1.0000
    LAG          0.4808     -0.7797      1.0000
```

(TCW\Dermal\DEF02)

John Inouye, Registration Specialist
Pesticide Registration Branch

February 25, 1994

Sacramento

4-0455

-Tom Thongsinthusak, Staff Toxicologist
Worker Health and Safety Branch

PRODUCT NAME: Guthion
ACTIVE INGREDIENT: Azinphos-methyl
COMPANY NAME: Mobay Chemical Corp.
I.D. NUMBER: SBRA-133980-E
DOCUMENT NUMBER: 154-261
EPA REGISTRATION NUMBER: 3125-0-
TITLE: Guthion: Dermal Absorption Study

A dermal absorption study of azinphos-methyl (Guthion) in male rats was conducted by Miles, Inc. This study was performed according to the study protocol which was reviewed and commented on by the Worker Health and Safety Branch, Department of Pesticide Regulation. The study was also conducted in compliance with Good Laboratory Practice standards. The Quality Assurance officer inspected various phases of the study and signed the final report. A summary of this dermal absorption study and the evaluation of the results are presented below.

A. Preparation of Animals

Adult male Sprague-Dawley derived rats (*Rattus norvegicus*) were used in this dermal absorption study. Body weights of these rats ranged from 202 to 275 grams. These animals were inspected and acclimatized prior to the study. The room temperature was set at 65 to 78 ^{o}F with 40 to 70% relative humidity. The photo period was 12 hours of light/12 hours of dark cycle. Approximately one day prior to the study, the backs of animals were clipped with electric clippers and the clipped areas were washed with acetone.

B. Administration of the Doses

Three dose levels and untreated and sham-treated controls were used in this study. The dose was prepared by adding the appropriate amounts of high purity [phenyl-UL-^{14}C] azinphos-methyl (99.3% pure) and non-labeled azinphos-methyl to a blank Guthion formulation (35% WP) and suspended in deionized water. The dose solution was applied at a volume of 150 uL per 15 cm^2 of treated skin site. The nominal doses were 56, 560, and 5,600 ug of azinphos-methyl/kg body weight equivalent to 0.93, 9.3, and 93 ug azinphos-methyl/cm^2. Four animals were used per

sacrifice time of each dose. After administration of the test material, the treated skin sites were protected with a nonocclusive cover made with a rubber ring (5.0 cm ID) and filter paper. Protective collars were used to restrict movement of animals from accessing the treated skin sites. The animals were housed individually during the study in stainless steel cages that allowed collection of urine and feces samples.

C. Sample Collection and Analysis

The sacrifice times for various groups of animals were: 1, 4, 10, 24, 72 and 168 hours. For the 24, 72 and 168-hour sacrifice times, treated skin sites were lightly wiped twice with gauze pads moistened with a 5% aqueous detergent (Contrad 70) solution and once with a dry gauze pad ten hours after exposure. These animals were returned to their cages for further monitoring. Daily urine and feces samples were collected and analyzed separately. Samples collected for analysis were: application device, nonocclusive cover, skin wash, treated skin site, cage wash, blood, carcasses, feces, and urine.

D. Determination of the Results

Recovery of administered doses of azinphos-methyl for the 1, 4, 10, and 168-hour sacrifice times are shown in Table 1. The results of the study indicated that dermal absorption of azinphos-methyl is dose dependent, i.e. percent dose absorbed for the low dose is higher than that for the medium and high doses. Furthermore, percent dose absorbed is greater for the longer exposure and sacrifice times for the same dose level. The highest percent dose in the treated skin site was 36.44% at 1-hour sacrifice time for the low dose, whereas, for medium and high doses were 22.08 and 23.71% 10 hours after dosing, respectively.

Excretion kinetics of azinphos-methyl in urine and feces from treated animals were observed over 168 hours after dosing. Percent of dose excreted at different time intervals were tabulated (Table 2). Excretion of the dose for the 24 and 72-hour sacrifice times for all dose groups were similar to those with the same sample collection times for the 168-hour sacrifice time. This indicated a consistency in study procedures and the analysis of samples.

In order to resolve the issue of bound skin residue, cumulative percent doses in urine and feces for different time intervals extrapolated beyond 168 hours were used for estimating the asymptote by employing an exponential saturation model with lag time. An equation representing this model is: Y = A*(1-EXP(-B*(X+C))) or Recov = Max*(1-EXP(-Rate*(Time+Lag))). An example of the plots for the lowest dose and the outputs are shown in Figure 1. The dermal absorption value is the sum of percent dose at asymptote (maximum or "A" term) and percent of dose recovered in carcasses, blood, and cage washes. Table 3 summarizes the dermal absorption values for low,

medium and high doses obtained from this study. The corrected dermal absorption values for the lowest dose (0.93 ug/cm^2) with 10-hour exposure time is appropriate to be used in the exposure estimates because this dose should be representative of exposure experienced by agricultural workers.

Plasma and erythrocyte cholinesterase levels in test animals were also determined in this study. For all dose groups, azinphos-methyl did not result in a plasma or erythrocyte cholinesterase depression that exceeded 20% of the control level. However, a dose of 93 ug/cm^2 caused erythrocyte cholinesterase depression ranging from 9 to 17% compared to a control group.

Recommendations:

1. The dermal absorption study of azinphos-methyl in male rats as reported in document number 154-261 is acceptable.

2. Dermal absorption of 44.2 percent may be used to estimate absorbed dosages of persons exposed to azinphos-methyl unless human dermal absorption data is available.

cc: Joshua Johnson (1 original, 5 copies)
John Ross
Tareq Formoli

(TCW/dml-abs/Guthion1)

Table 1. Recovery of administered dose of azinphos-methyl.

	Percent dose (mean)								
Dose (a.i.)	Sacrifice time (h)	Unab-sorbed*a	Treated skin site	Blood	Urine	Feces	Carcass	Cage wash	Total recovery
0.84 ug/cm2	1	55.53	36.44	0.17	0.34	0.04	8.78	0.08	101.38
(0.93 ug/cm2)*b			(1-h) Absorbed dose (%dose)*c		=	45.85			
	4	52.72	25.78	0.43	2.37	0.31	19.55	0.37	101.53
			(4-h) Absorbed dose (%dose)*c		=	48.81			
	10	48.26	32.24	0.36	4.86	0.38	16.44	0.66	103.2
			(10-h) Absorbed dose (%dose)*c		=	54.94			
	168	39.98	15.82	0.08	26.24	9.8	1.87	3.65	97.44
8.78 ug/cm2	1	58.41	19.16	0.05	0.14	0.01	3.43	0.04	81.24
(9.3 ug/cm2)*b			(1-h) Absorbed dose (%dose)*c		=	22.83			
	4	60.13	21.57	0.06	0.34	0.04	4.32	0.14	86.6
			(4-h) Absorbed dose (%dose)*c		=	26.47			
	10	46.31	22.08	0.22	2.82	0.36	11.19	0.57	83.55
			(10-h) Absorbed dose (%dose)*c		=	37.24			
	168	53.77	5.43	0.04	11.77	5.83	1.64	2.57	81.05
98.7 ug/cm2	1	76.06	19.74	0.01	0.02	0	0.45	0	96.28
(93.0 ug/cm2)*b			(1-h) Absorbed dose (%dose)*c		=	20.22			
	4	78.72	14.61	0.02	0.14	0.01	1.08	0.02	94.6
			(4-h) Absorbed dose (%dose)*c		=	15.88			
	10	68.91	23.71	0.04	0.63	0.02	2.12	0.05	95.48
			(10-h) Absorbed dose (%dose)*c		=	26.57			
	168	67.41	7.06	0.03	10.14	5.07	1.33	1.76	92.8

*a Protective cover + ring wash + skin wash
*b Nominal dose
*c When bioavailability of bound skin residues cannot be determined, dermal absorption values = % dose recovered from treated skin site + blood + urine + feces + carcass + cage wash.

(TXL/Dermal/Guthion1)

Table 2. Percent dose of azinphos-methyl excreted following 10-hour exposure.

A. Actual dose: 0.84 ug/cm2

	Percent dose (mean)				
Time (h)	Urine (U)	Feces (F)	U + F		Cumulative
24	15	2.7	17.7		17.7
48	4.9	3.6	8.5		26.2
72	1.4	1	2.4		28.6
96	1.9	1.2	3.1		31.7
120	2	1.2	3.2		34.9
144	1.2	0.6	1.8		36.7
168	1	0.6	1.6		38.3
Total	27.4	10.9	38.3		

B. Actual dose: 8.78 ug/cm2

	Percent dose (mean)				
Time (h)	Urine (U)	Feces (F)	U + F		Cumulative
24	6.7	1.5	8.2		8.2
48	3.1	1.6	4.7		12.9
72	1.1	0.6	1.7		14.6
96	0.3	0.2	0.5		15.1
120	0.6	0.4	1		16.1
144	0.8	0.4	1.2		17.3
168	0.9	0.7	1.6		18.9
Total	13.5	5.4	18.9		

C. Actual dose: 98.7 ug/cm2

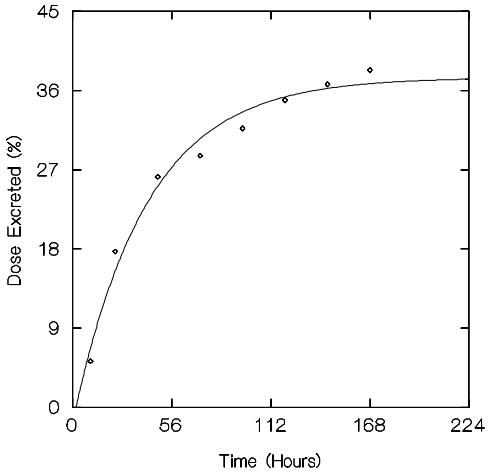
	Percent dose (mean)				
Time (h)	Urine (U)	Feces (F)	U + F		Cumulative
24	2.3	0.5	2.8		2.8
48	2	1.1	3.1		5.9
72	1.2	0.8	2		7.9
96	1.1	0.6	1.7		9.6
120	1	0.5	1.5		11.1
144	0.8	0.5	1.3		12.4
168	0.7	0.5	1.2		13.6
Total	9.1	4.5	13.6		

Table 3. Summary: Dermal absorption of azinphos-methyl in male rats*a.

Actual dose	Nominal dose	Percent dose (mean)						
(ug/cm2)	(ug/cm2)	Excreted*b	Blood	Carcass	Cage wash	Sub-total	Recovery	Adj. total
0.84	0.93	37.5	0.08	1.87	3.65	43.1	97.44	44.23
8.78	9.3	14.7	0.04	1.64	2.57	18.95	81.05	23.38
98.7	93	17.4	0.03	1.33	1.76	20.52	92.8	22.11

*a Percent doses: excreted + blood + carcass + cage wash.
*b At asymptote using an exponential saturation model. (TXL\Dermal\Guthion2)

Figure 1. Azinphos-methyl: Asymptotic plot of cumulative excretion of dose in urine and feces at different time intervals for 0.84 ug/cm^2

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>NONLIN
>MODEL RECOV=Max*(1-Exp(-Rate*(Time+Lag)))
>ESTIMATE/ QUASI

WED 2/23/94 9:30:35 AM   C:\TCSYS\GUTHION1.SYS

 ITERATION      LOSS       PARAMETER VALUES
    0      .6838779D+04  .1000D+00 .1000D+00 .1000D+00
    1      .8680116D+03  .2742D+02 .9789D+00 .1047D+00
    2      .8350973D+03  .2744D+02 .3579D+00 .4448D-01
    3      .6359906D+03  .2744D+02 .1569D+00 .3734D-01
    4      .3578665D+03  .2745D+02 .6488D-01 .3601D-01
    5      .3144805D+03  .2745D+02 .4349D-01 .3589D-01
    6      .8168787D+02  .3184D+02 .4752D-01-.6914D+01
    7      .5060506D+02  .3335D+02 .3415D-01-.5450D+01
    8      .2681820D+02  .3552D+02 .3242D-01-.5824D+01
    9      .2350264D+02  .3673D+02 .2440D-01-.3539D+01
   10      .1970548D+02  .3660D+02 .2634D-01-.3563D+01
   11      .1846508D+02  .3720D+02 .2522D-01-.2210D+01
   12      .1813839D+02  .3728D+02 .2456D-01-.2086D+01
   13      .1807941D+02  .3742D+02 .2392D-01-.1819D+01
   14      .1807333D+02  .3746D+02 .2388D-01-.1736D+01
   15      .1807267D+02  .3747D+02 .2385D-01-.1730D+01
   16      .1807267D+02  .3747D+02 .2385D-01-.1730D+01

(TCW\Dermal\Guthion2)
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DEPENDENT VARIABLE IS    RECOV

 SOURCE   SUM-OF-SQUARES    DF  MEAN-SQUARE

REGRESSION      6863.759     3     2287.920
  RESIDUAL        18.073     5        3.615

   TOTAL       6881.828     8
 CORRECTED       868.073     7

   RAW R-SQUARED (1-RESIDUAL/TOTAL)     =       0.997
CORRECTED R-SQUARED (1-RESIDUAL/CORRECTED) =       0.979

PARAMETER      ESTIMATE      A.S.E.      LOWER  <95%> UPPER
   MAX          37.466       1.771      32.913      42.019
  RATE           0.024       0.005       0.011       0.037
   LAG          -1.730       3.430     -10.546       7.087
```

Gary Varnado, Registration Specialist
Pesticide Registration Branch

October 28, 1994

Sacramento

445-4267

-Tom Thongsinthusak, Staff Toxicologist
Worker Health and Safety Branch

PRODUCT NAME: Mitac 50WP
ACTIVE INGREDIENT: Amitraz
COMPANY NAME: AgrEvo USA company
I.D. NUMBER: SBRA-149299-E
DOCUMENT NUMBER: 287-104
EPA REGISTRATION NUMBER: 45639-51
TITLE: Amitraz: Dermal Absorption in the Rat

A dermal absorption study of (^{14}C)-amitraz in male rats was conducted by Hazelton Europe. This study was conducted in compliance with US Good Laboratory Practice standards and UK Principles of Good Laboratory Practice. The Quality Assurance officer inspected various phases of the study and signed the final report. A summary of this dermal absorption study and the evaluation of the results are presented below.

A. Preparation of Animals

Adult male Crl:CD(SD)BR strain rats obtained from Charles River (UK) Ltd. were used in this dermal absorption study. Body weights of these rats ranged from 225 to 321 grams. These animals were inspected and acclimatized for about one week prior to the study. Experimental rooms were air conditioned to provide a minimum of 10 air changes per hour. The temperature was maintained at 19 to 23 ^{o}C and at a relative humidity of 40 to 70%. The photo period was 12 hours of light/12 hours of dark cycle. One to two days prior to the study, an area of the dorso-lumbar skin was clipped free of hair using veterinary clippers, without abrading the skin, and the shaved area was washed with acetone. A pair of contoured silicone rings ca 3.6 cm in diameter was glued to the middle of the back of each animal using Zap-A-Gap™. A porous bandage (Vetrap supplied by Animal Care Products/3M) was used to encircle the animal's trunk. The treated skin area per animal was approximately 10 cm^2.

B. Administration of the Doses

Three dose levels of amitraz were used in this study. The nominal doses were 0.1, 1.0 and 10 mg of amitraz per animal equivalent approximately to 10, 100, and 1,000 ug/cm^2, respectively. The dose was prepared by adding the appropriate amounts of high purity [phenyl-UL-^{14}C]-amitraz (>99% pure) and non-labeled amitraz (99.6% pure) to a blank amitraz formulation (Mitac 50WP) and suspended in deionized water. The dose solution was applied at a nominal dose volume of 100 uL per animal (about 10 cm^2). Four animals were used per sacrifice time of each dose. After administration of the test material, the treated skin sites were protected with nonocclusive covers made with nylon mesh. The animals were thereafter placed in individual all-glass metabolism cages suitable for the separate collection of urine and feces.

C. Sample Collection and Analysis

The exposure times for all dose groups of animals were: 0.5, 1, 2, 4, and 10 hours. The sacrifice times were: 0.5, 1, 2, 4, 10, 24, and 120 hour post-dose. For the 24- and 120-hour sacrifice times, the treated skin sites and silicone rings were swabbed thoroughly with detergent (Liquid Ivory soap) and warm water before being rinsed and dried with cotton wool swabs at ten hours after dosing. Fresh gauze was applied to the backs of the animals and they were returned to their cages for further monitoring. Daily urine and feces samples were collected and analyzed separately. Samples collected for analysis were: nonocclusive covers, back washings, treated skin sites, cage washings/debris, blood, carcasses, feces, and urine.

D. Determination of the Results

Recovery of administered doses of amitraz for the 0.5, 1, 2, 4, 10, 24, and 120-hour sacrifice times are shown in Table 1. The results of the study indicated that dermal absorption of amitraz, if percent dose in treated skin site is included, is dose dependent. For example, percent dose absorbed for the low dose (0.1 mg/animal) is higher than that for the medium and high doses. However, percent dose absorbed for the medium and high doses are similar. Percent dose absorbed is generally greater for the longer exposure and sacrifice times for the same dose level. The highest percent dose in the treated skin site was 12.1% at 4-hour sacrifice time for the low dose. Percent of dose in treated skin sites for the medium and high doses also peaked about 4 hour post-dose. Percent of dose in blood was estimated and shown in Table 2. It can be seen that percent of dose in blood is very low ranging from 0.015 to 0.03% for the low dose group.

Excretion kinetics of amitraz in urine and feces from treated animals were observed over 120 hours after dosing. Percent of dose excreted at different time intervals were tabulated (Table 3). In order to resolve the issue of bound skin residue, cumulative percent doses in urine and feces for different time intervals extrapolated beyond 120 hours were used for estimating the asymptote by employing an exponential saturation model with lag time. An equation representing this model is: Y = A*(1-EXP(-B*(X+C))) or Recov = Max*(1-EXP(-Rate*(Time+Lag))). An example of the plots for the low dose and the outputs are shown in Figure 1. The dermal absorption value is the sum of percent dose at asymptote (maximum or "A" term) and percent of dose recovered in carcasses, blood, and cage washings/debris.

Table 4 summarizes the dermal absorption values for low, medium and high doses obtained from this study. The adjusted dermal absorption values for the low dose with 10-hour exposure time is 13.8%. This dermal absorption value is appropriate to be used in the exposure estimates because this dose should be representative of exposure experienced by agricultural workers. A lower dermal absorption value of 6.6% may be used if a dermal dose for humans is comparable to the medium dose (about 100 ug/cm^2) used in this dermal absorption study.

Recommendations:

1. The dermal absorption study of amitraz in male rats as reported in pesticide registration document number 287-104 is acceptable.

2. Dermal absorption of 13.8 percent may be used to estimate absorbed dosages of persons exposed to amitraz unless human dermal absorption data is available.

cc: David Haskell
Joshua Johnson (1 original, 5 copies)

(TCW/dermalAmitraz1)

Table 1. Recovery of administered dose of amitraz.

	Percent dose (mean)								
Dose (a.i.)	Sacrifice time (h)	Unab-sorbed*a	Treated skin site	Blood	Urine	Feces	Carcass	Cage wash	Total recovery
0.1 mg/animal	0.5	93.44	4.19	0.015	0	0	0	0.002	97.65
(10 ug/cm2)*b			*(.5-h) Absorbed dose (% dose)*c =*			*4.207*			
	1	105.7	7.89	0.015	0	0	0.143	0	113.75
			*(1-h) Absorbed dose (% dose)*c =*			*8.048*			
	2	103.49	8.47	0.015	0.025	0.001	0	0.012	112.01
			*(2-h) Absorbed dose (% dose)*c =*			*8.523*			
	4	87.59	12.09	0.03	0.075	0.001	0.629	0.032	100.45
			*(4-h) Absorbed dose (% dose)*c =*			*12.857*			
	10	95.45	7.57	0.03	1.01	0.04	1.118	0.202	105.42
			*(10-h) Absorbed dose (% dose)*c=*			*9.97*			
	24	91.43	2.83	0.03	4.226	0.896	1.289	0.771	101.47
			*(24-h) Absorbed dose (% dose)*c=*			*10.042*			
	120	81.03	0.48	0.015	8.689	2.303	0.242	1.412	94.17
			*(120-h) Absorbed dose (%dose)*c=*			*13.141*			
1 mg/animal	0.5	87.85	3.62	0.008	0	0	0	0.001	91.48
(100 ug/cm2)*b			*(.5-h) Absorbed dose (% dose)*c =*			*3.629*			
	1	105.22	3.23	0.006	0	0	0.234	0	108.69
			*(1-h) Absorbed dose (% dose)*c =*			*3.47*			
	2	87.84	5	0.009	0.007	0	0	0.003	92.86
			*(2-h) Absorbed dose (% dose)*c =*			*5.019*			
	4	116.98	6.64	0.018	0.053	0.015	0.186	0.006	123.90
			*(4-h) Absorbed dose (% dose)*c =*			*6.918*			
	10	86.83	7.13	0.017	1.618	0.007	0.162	0.174	95.94
			*(10-h) Absorbed dose (% dose)*c=*			*9.108*			
	24	80.69	2.05	0.023	1.166	0.232	0.759	0.179	85.10
			*(24-h) Absorbed dose (% dose)*c=*			*4.409*			
	120	97.41	0.39	0.023	4.712	1.092	0	0.735	104.36
			*(120-h) Absorbed dose (%dose)*c=*			*6.952*			

Table 1. (cont.) Percent recovery of administered dose of amitraz.

Dose (a.i.)	Sacrifice time (h)	Unab-sorbed*a	Treated skin site	Blood	Urine	Feces	Carcass	Cage wash	Total recovery
	Percent dose (mean)								
10 mg/animal	0.5	98.14	2.41	0.003	0	0	0	0.002	100.56
(1000 ug/cm2)*b			*(.5-h) Absorbed dose (% dose)*c=*			*2.415*			
	1	113.04	3.28	0.008	0	0.001	0.117	0.001	116.45
			*(1-h) Absorbed dose (% dose)*c =*			*3.407*			
	2	109.96	4.62	0.007	0.011	0	0	0.004	114.60
			*(2-h) Absorbed dose (% dose)*c =*			*4.642*			
	4	87.11	5.66	0.003	0.017	0.001	0	0.005	92.80
			*(4-h) Absorbed dose (% dose)*c =*			*5.686*			
	10	116.75	1.87	0.013	0.394	0.012	0	0.042	119.08
			*(10-h) Absorbed dose (% dose)*c=*			*2.331*			
	24	105.06	1.22	0.011	0.868	0.112	2.459	0.45	110.18
			*(24-h) Absorbed dose (% dose)*c=*			*5.12*			
	120	89.84	0.32	0.006	2.494	1.129	0.796	0.519	95.10
			*(120-h) Absorbed dose (%dose)*c=*			*5.264*			

*a % Washed off + % in dressings
*b Treated skin site was approximately 10 cm2
*c When bioavailability of bound skin residues cannot be determined, demal absorption values = % dose recovered from the treated skin site + blood + urine + feces + carcass + cage washings/debris.

Table 2. Percent dose of amitraz in blood.

14C-Amitraz in blood

Sacrifice time	0.1 mg/animal			1 mg/animal			10 mg/animal		
(hour)	ug equiv./g	ug/animal	% dose	ug equiv./g	ug/animal	% dose	ug equiv./g	ug/animal	% dose
0.5	0.001	0.015	0.015	0.005	0.075	0.0075	0.021	0.315	0.00315
1	0.001	0.015	0.015	0.004	0.06	0.006	0.05	0.75	0.0075
2	0.001	0.015	0.015	0.006	0.09	0.009	0.045	0.675	0.00675
4	0.002	0.03	0.03	0.012	0.18	0.018	0.023	0.345	0.00345
10	0.002	0.03	0.03	0.011	0.165	0.0165	0.087	1.305	0.01305
24	0.002	0.03	0.03	0.015	0.225	0.0225	0.076	1.14	0.0114
120	0.001	0.015	0.015	0.015	0.225	0.0225	0.038	0.57	0.0057

Notes:
1. Assumed 15 grams of whole blood/animal.
2. Values were calculated from data reported in Pesticide Registration Document Number 287-104.

(TXL\Dermal\Amitrz1)

Table 3. Percent dose of amitraz excreted following 10-hour exposure.

A. 0.1 mg/animal (10 ug/cm2)

	Percent dose (mean)				
Time (h)	Urine (U)	Feces (F)	U + F		Cumulative
10	1.347	0.066	1.413		1.41
24	3.157	0.541	3.698		5.11
48	2.006	0.586	2.592		7.70
72	1.331	0.602	1.933		9.64
96	0.551	0.294	0.845		10.48
120	0.297	0.214	0.511		10.99
Total	8.689	2.303	10.992		

B. 1 mg/animal (100 ug/cm2)

	Percent dose (mean)				
Time (h)	Urine (U)	Feces (F)	U + F		Cumulative
10	0.572	0.061	0.633		0.63
24	1.487	0.097	1.584		2.22
48	1.564	0.307	1.871		4.09
72	0.623	0.357	0.98		5.07
96	0.313	0.188	0.501		5.57
120	0.154	0.081	0.235		5.80
Total	4.713	1.091	5.804		

C. 10 mg/animal (1000 ug/cm2)

	Percent dose (mean)				
Time (h)	Urine (U)	Feces (F)	U + F		Cumulative
10	0.115	0.021	0.136		0.14
24	0.681	0.267	0.948		1.08
48	0.771	0.341	1.112		2.20
72	0.479	0.21	0.689		2.89
96	0.257	0.137	0.394		3.28
120	0.191	0.153	0.344		3.62
Total	2.494	1.129	3.623		

Table 4. Summary: Dermal absorption of amitraz in male rats*a.

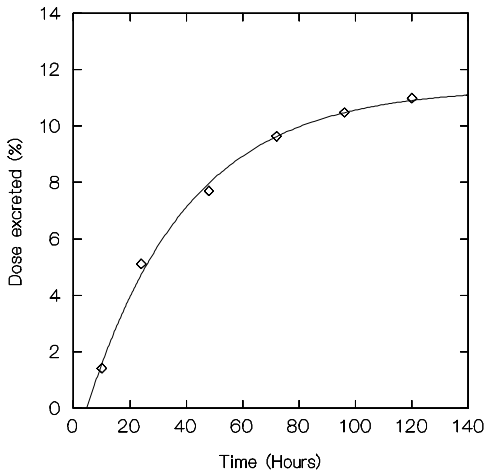
Dose	Percent dose (mean)*b						
(ug/cm2)	Excreted*c	Blood	Carcass	Cage wash	Sub-total	Recovery(%)	Total abs.*d
10	11.36	0.02	0.24	1.41	13.03	94.2	13.83
100	6.17	0.02	0	0.74	6.93	104.4	6.64
1000	4.06	0.01	0.8	0.52	5.39	95.1	5.67

*a Based on 10-hour exposure time and 120-hour sacrifice time.
*b Percent doses: excreted + blood + carcass + cage washings/debris.
*c At asymptote using an exponential saturation model.
*d Adjusted to reflect 100% recovery.

(TCXL/Dermal/Amitrz1a)

Figure 1. Asymptotic plot of percent dose excreted in urine and feces after topical administration of amitraz at 0.1 mg/animal (ca 10 µg/cm^2)

$$Y = 11.356*(1-EXP(-0.028*(X-4.738)))$$



Statistics:

WED 10/26/94 10:09:05 AM C:\TCSYS\AMITRZ1D.SYS

ITERATION	LOSS	PARAMETER VALUES			
0	.4021768D+03	.1000D+00	.1000D+00	.1000D+00	
1	.3014362D+03	.6667D+01	.1067D+01	-.4833D+01	
2	.6787968D+02	.7453D+01	.8223D+00	-.4994D+01	
3	.4288598D+02	.8078D+01	.1604D+00	-.4593D+01	
4	.1483351D+02	.8776D+01	.5133D-01	-.4969D+01	
5	.6958837D+01	.1015D+02	.5252D-01	-.5953D+01	
6	.5753622D+00	.1118D+02	.3192D-01	-.6662D+01	
7	.4512091D+00	.1104D+02	.3220D-01	-.6561D+01	
8	.4500136D+00	.1104D+02	.3225D-01	-.6565D+01	
9	.4496657D+00	.1104D+02	.3224D-01	-.6562D+01	
10	.4466517D+00	.1100D+02	.3214D-01	-.6468D+01	
11	.4342199D+00	.1092D+02	.3254D-01	-.6168D+01	
12	.3769090D+00	.1121D+02	.3089D-01	-.5815D+01	
13	.2552073D+00	.1135D+02	.2848D-01	-.4920D+01	
14	.2446734D+00	.1138D+02	.2785D-01	-.4646D+01	
15	.2429564D+00	.1135D+02	.2816D-01	-.4748D+01	
16	.2429082D+00	.1136D+02	.2811D-01	-.4737D+01	
17	.2429066D+00	.1136D+02	.2811D-01	-.4737D+01	
18	.2429065D+00	.1136D+02	.2811D-01	-.4738D+01	
19	.2429065D+00	.1136D+02	.2811D-01	-.4738D+01	

DEPENDENT VARIABLE IS RECOV

(TCW/Dermal/Amitrz1W)

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE
REGRESSION	410.741	3	136.914
RESIDUAL	0.243	3	0.081
TOTAL	410.983	6	
CORRECTED	68.424	5	

RAW R-SQUARED (1-RESIDUAL/TOTAL) = 0.999
CORRECTED R-SQUARED (1-RESIDUAL/CORRECTED) = 0.996

PARAMETER	ESTIMATE	A.S.E.	LOWER <95%>	UPPER
MAX	11.356	0.357	10.218	12.493
RATE	0.028	0.003	0.018	0.038
LAG	-4.738	1.272	-8.785	-0.691

ASYMPTOTIC CORRELATION MATRIX OF PARAMETERS

	MAX	RATE	LAG
MAX	1.000		
RATE	-0.872	1.000	
LAG	0.479	-0.712	1.000