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DATA EVALUATION RECORD

STUDY TYPE: Dermal Penetration - Rat
OPPTS Number: 870.7600 OPP Guideline Number: §85-3a
DP BARCODE: D279254 SUBMISSION CODE: S606344
P.C. CODE: 35001

TEST MATERIAL (RADIOCHEMICAL PURITY): Dimethoate (about 98% a.i.)

SYNONYMS: O,O-Dimethyl S-(N-methylcarbamoyl-methyl) phosphorodithioate-[l4C-methyl]

CITATION: Leibold, E., Study on the dermal penetration of ^{14}C-dimethoate in rats. Oct 9, 2001. Experimental toxicology and Ecology BASF. Project No. 01B0418/006016, MRID 45530501

SPONSOR: BASF Aktiengesellschaft, Germany

EXECUTIVE SUMMARY:

The study investigated dermal absorption in male rats following a single dermal administration of ^{14}C-Dimethoate (98% radiochemical purity) in formulation concentrate and in 10% and 0.5% aqueous dilutions thereof. The nominal dose preparations equated to doses of 0.02, 0.4 and 4.0 mg/cm^2. A total of 64 animals (16 per dose/4 per time group) were exposed for 1, 10, and 24 hour periods and sacrificed 1,10, 24 or 72 hours after exposure began. The 72 hour group underwent skin wash at 24 hours and were carried to 72 hours before termination.

Mean recoveries of applied radioactivity from all dose groups ranged from 94 to 107%. The following table summarizes mean percent absorption at each time period for the low and intermediate dose groups. Results from the high dose group are not presented because an excessive amount of the applied material was found on the application site cover and surrounding skin at all exposure periods for this dose group. Material on the cover and the skin around the application site is not available for absorption. Therefore data from the high dose group is unusable for determining the dermally absorbed portion of the applied dose. Material retention in the protective cover and surrounding skin was within expected ranges for all other doses/exposures.

Dose Level	Mean Percentage of Dose Absorbed			
	1 hour	10 hours	24 hours	72 hours*
0.02 mg/cm^2 (0.2 mg/animal)	5.68	28.15	38.06	41.81
0.4 mg/cm^2 (4.0 mg/animal)	5.68	24.98	25.33	31.69

* 72 hour value washed at 24 hours carried to 72 hours

Total radioactivity absorbed increased with increasing exposure time but decreased with increasing dose indicating saturation of penetration with increasing dose. The largest amount of radioactivity was found in the 10 and 24 skin washes from each dose (i.e., 50-60% recovered radioactivity). For both dose groups, the second skin wash (after the 72 hr exposure/observation period) contained significantly less applied radioactivity (1-4%) and proportionately more radioactivity was absorbed. The absorbed radioactivity was excreted rapidly and almost exclusively via the urine.

This dermal absorption study is classified acceptable/guideline and does satisfy the guideline requirement for a dermal absorption study (85-3) in rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

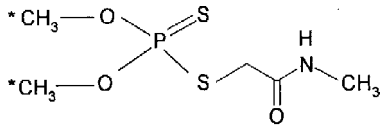
I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: The following is abstracted from the study report (MRID 45530501)

Radio labeled Test Substance
Test item: ^{14}C-DIMETHOATE
Chemical name: O,O-Dimethyl S-(N-methylcarbamoyi-methyl) phosphorodithioate-[l4C-methyl]
CAS-No.: 60-51-5 (unlabeled compound)

Chemical structure:



Molecular formula: $C_5H_{12}NO_3PS_2$
Origin: Wizard Labs. Inc. (USA) purified by Huntingdon Life Sciences (HLS), Huntingdon, Cambridgeshire, England
Batch/Lot No.: MEF24/8/TW
Specific activity: 42.56 mCi/mmol
Radiochemical purity: about 98 %; verified analytically prior to all experiments
Physical state: white crystalline solid
Storage: At -15ºC and in the dark
Stability: Verified analytically prior to all experiments (see raw data for details)

Nonradiolabeled Test Substance
Test item: DIMETHOATE 400 g/l EC
Origin: Cheminova A/S, Lemvig, DenM2rk
CAS-No: 60-51-5 (refers to active ingredient)
Batch/Lot No.: 70917-00
Test substance No.: 00/0417-1
Chemical purity stability: 38 % active ingredient (stability guaranteed until Sept.2001)
Appearance: Colorless liquid
Storage: In refrigerator (ca. 4ºC)

2. Vehicle:
high-dose: formulation concentrate
intermediate-dose: 1/10 aqueous dilution
low-dose: 1/200 dilution formulation concentrate.

3. Test Animals:

Animals: rats
Strains: Crl:Wl (GLX/BRL/HAN)IGS BR
Origin: Charles River Laboratories, Sulzfeld, Germany
Sex: Male
Age: About 14 weeks at application
Weight: Ca. 266 - 328 g (weight was measured prior to dosing; see also tables 4-15)
Rationale: Recognized by international guidelines as the recommended test system (EPA 870.7600 and OECD guidelines).

B. STUDY DESIGN AND METHODS

1. Dose Selection: ^{14}C-Dimethoate was applied at 3 concentrations. The nominal doses of 4.0 mg/cm^2 (formulation concentrate), 0.4 mg/cm^2 (1/10 aqueous dilution of formulation concentrate) and 0.02 mg/cm^2 (1/200 aqueous dilution of formulation concentrate) were selected to simulate user specific exposure scenarios and to cover a broad range of potential exposures. Calculation of dermal dose was based on a 100 µl dose of the test item preparation applied to a total area of about 10 cm^2. The following table summarizes dose selection and grouping.

Dose Group	Dose (Nominal) (mg/cm$^{2)}$	Number of Animals	Exposure Duration (hrs)	Termination Time (hrs)*
I	0.02	16	1, 10 or 24	1, 10, 24, 72
II	0.4	16	1, 10 or 24	1, 10, 24, 72

* 72 hour value washed at 24 hours carried to 72 hours

2. Dose Preparation: A stock solution of the radio labeled test item was prepared in acetone. For the formulation concentrate dose (target radioactivity per animal of about 1-2 Mbq/animal), a respective aliquots of the of the stock solution were taken and the acetone evaporated to dryness. The residue was dissolved and filled up to the final volume with the formulation concentrate. Dilutions of formulation concentrate (target radioactivity of about 0. 1 -2 Mbq/animal) were prepared in the same manner except that after the residue was dissolved in the respective volume of the formulation concentrate, this mixture was filled up to the final volume with the respective volume of doubly distilled water. The preparations were stirred to ensure a homogeneous preparation. Samples were taken before and after dosing to determine the amount of radioactivity in the preparation and demonstrate the homogeneity and correctness of the concentration.

3. Animal Preparation and Dosing: An approximate area of about10 cm^2 of the back skin of the rats was clipped free of hair and washed with acetone 24 h prior to dosing. A silicone rubber ring was glued onto the skin with tissue glue. The respective test material (about10 µl/cm^2) was administered with a syringe which was weighed before and after application. A nylon mesh was then glued to the surface of the silicone ring and a porous (semi-occlusive) bandage was wrapped around the trunk of the animal. After dosing, animals are placed in metabolism cages for up to 72 hours.

4. Animal Observations and Sample Collection: After the respective exposure period the protective cover was removed and the exposed skin was washed with a mild soap solution. For animals with a post-observation period, a new gauze and a new bandage was applied and a second skin wash performed before sacrifice. Animals were sacrificed at the end of the various collection periods and the following specimens/tissues were checked for remaining radioactivity: excreta (urine, feces), blood, carcass, skin (treated = application site and non-treated = surrounding skin), and skin wash(es). For mass balance estimates the cage wash as well as the protective cover (including the gauze with bandage and the silicone rubber ring) were also checked for radioactivity.
5. Data Analysis: Means and standard deviation were calculated using computer spreadsheets. Group mean and individual data are rounded to two significant digits in the results tables. The total amount of test compound absorbed by each animal is the sum of the quantity found in the excreta (urine, feces), organs/tissues, carcass and cage wash.

II. RESULTS

A comparison of skin penetration at the two usable dose applications showed that the relative amount of radioactivity absorbed increased with increased exposure. With increasing dose, the percentage of radioactivity absorbed decreased indicating saturation of skin penetration after a 24 h exposure period. Only a minor part the radioactivity that remained in the skin at the application site at the end of 24 h exposure was absorbed during the 48 h post-exposure observation period. A tabular summary of results is provided in attached tables 1, 2, and 3.

Low Dose

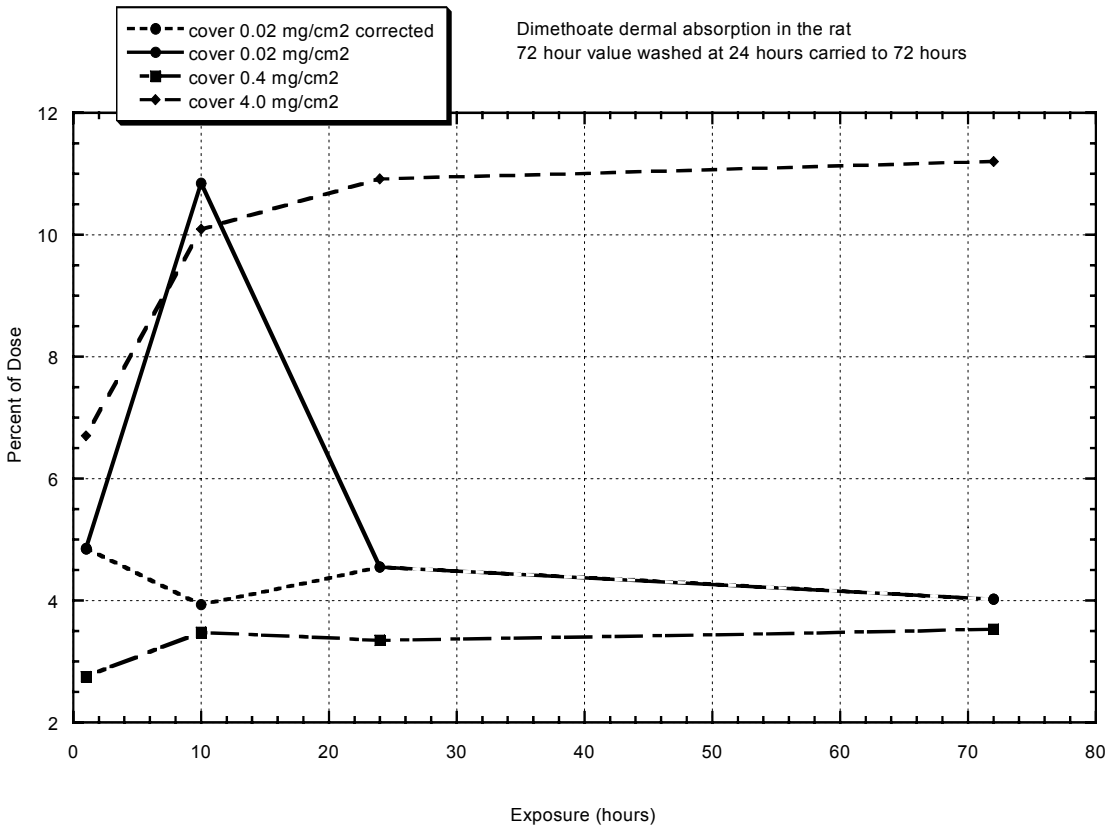
Results provided in the submitted study for the 10 hour exposure group have been revised to remove anomalous data. A review of the results for individual animals revealed that data on percent of material retained on the protective cover from one animal in this dose group (#38) was clearly outside the norm (32% vs. 2-6%). Data from that animal was therefore removed and results for the low dose 10 hour exposure group was recalculated without it, resulting in a significant reduction in the mean amount of material on the protective cover (from 11% to 4%). A graph of the results with and without the outlying data is provided below. The 10 hour results were also revised to correct an error in the reported value for percent material found in/on the surrounding skin. The reported value was 4.56%; the correct amount (from the raw data) is 0.50%. The following summarizes the revised results.

Following a single dermal administration of ^{14}C-Dimethoate in a 0.5% dilution of formulation concentrate at a nominal dose of 0.02 mg/cm^2, mean recovery of radioactivity was 93 to 101%. In all groups, the largest proportion of radioactivity was recovered from the skin wash. In the 1 h exposure, 77 % of the applied radioactivity was recovered from the skin wash. Skin wash from the 10 and 24 h exposure groups contained 49 and 38% of the applied radioactivity, respectively. In the 24 h exposure/48 h post-exposure observation group, 37% of the applied dose was found in the 24 h skin wash and about 2% in the 72 h wash. Decrease in radioactivity in skin wash with increased exposure time was reflected in increased absorbed radioactivity. The

protective cover contained 4 to 5% of applied radioactivity. Radioactivity extracted from the application site ranged from 9 to 14%. Radioactivity on the skin adjacent to the application site was between 0.2 and 3.4%.

Total radioactivity absorbed (including excreta, cage wash, blood and carcass) was 6 and 28, and 38% of the applied dose at 1, 10, and 24 h exposures, respectively. Total absorption after the 24 h exposure/48 h post-exposure observation was 42%. These data show that only a minor part of the radioactivity remaining in the skin after the 24 h skin wash was absorbed during post-observation. Absorbed radioactivity was excreted rapidly and almost exclusively via the urine.

Low Dose Dermal Absorption with and without Outlying Data at the 10 hr Exposure Duration



Intermediate Dose

Following a single dermal administration of ^{14}C-Dimethoate in a 10% aqueous dilution of the formulation concentrate at a nominal dose of 0.4 mg/cm^2, the mean recovery of applied radioactivity in the exposure groups was between 98 and 106%. In all groups, the largest proportion of radioactivity was recovered from the skin wash. In the 1 h exposure group, 89% of the applied radioactivity was found in the skin wash. After 1, 10 and 24 h exposures, skin wash contained 89, 64, and 65% of the applied radioactivity respectively. In the group with 24 h exposure/ 48 h post-exposure observation, 52% was found in the 24 h skin wash and 2% in the 72 h skin wash. The decrease in radioactivity in skin wash with increased exposure time was reflected in increased radioactivity absorbed. The protective cover contained 3 - 3.5 % of the applied radioactivity. Radioactivity at the application site ranged from 3 to 6 %. Radioactivity in the skin adjacent to the application site was between 0.2 and 5% of the applied dose.

Total radioactivity absorbed was about 6% after 1 h, and 25 % after both the 10 and 24 h. exposure periods. Total absorption after the 24 h exposure/48 h post-observation was about 32%. The 72 h absorption value was higher than expected based on 72 h results at other dose levels, i.e., the amount absorbed during the 48 h post-exposure observation was 4% for the low dose and about 1% for the high dose; this compares with 7% for the intermiediate dose. This is probably because all animals of this group removed part of the protective cover and so were therefore able to lick off and ingest some of the radioactivity remaining in and on the skin. The absorbed radioactivity was excreted rapidly and almost exclusively via the urine.

<u>High Dose</u>

Results from the high dose group are discussed here but should not be included in the analysis because an excessive amount of the applied material was found on the application site cover and surrounding skin at all exposure periods for this dose group. Data from this group is unusable for determining the dermally absorbed portion of the applied dose because material on the cover and the skin around the application site is not available for absorption.

Following a single dermal administration of ^{14}C-Dimethoate in the formulation concentrate at a nominal dose of 4.0 mg/cm^2, the mean recovery of radioactivity in the exposure groups was between 94 and 97%. In all groups, the largest proportion of radioactivity was recovered from the skin wash. In the 1 h exposure group 70% of the applied radioactivity was recovered from the skin wash. After 10 and 24 h exposures, skin wash contained 62% and 59% of the applied radioactivity, respectively. In the 24 hour exposure/48 hour post-observation group, 57% of applied dose was found in the 24 hour skin wash and 4% of the 72 hour skin wash. The decrease in radioactivity in skin wash with increasing exposure time was reflected in an increase in radioactivity absorbed. The protective cover contained 7 - 11% of the radioactivity applied. Amount of radioactivity extracted from the application site ranged from 3 to 8%. The penetration of radioactivity into the skin adjacent to the application site was between 5 and 12%.

Total radioactivity absorbed was 1, 6, and 12% after 1, 10, and 24 h exposures

respectively. After the 72 h exposure/observation period, 13% of the applied radioactivity was absorbed. These data show that only a minor part of the radioactivity remaining in the skin after the 24 h skin wash was absorbed during post-observation and most of the remaining radioactivity could be washed off at 72 h. The absorbed radioactivity was excreted rapidly and almost exclusively via the urine.

III. DISCUSSION

A. Investigator's Conclusion: Following single dermal administration of radiolabeled Dimethoate in the formulation concentrate and at 1/10 and 1/200 aqueous dilutions thereof, systemic absorption accounted up to 42% of the applied radioactivity after 24 h exposure and 72 h post-exposure observation. After 10 hours of exposure, up to 25% of the radioactivity was absorbed. With increasing dose, the percentage of radioactivity absorbed decreased indicating saturation of skin penetration with increasing dose

B. Reviewer's Discussion: Results from the high dose group of animals cannot be used to estimate dermal absorption at that dose level. An excessive amount (10-20%) of the applied high dose was found on the application site cover and surrounding skin after all exposure periods. The protective cover consisted of a silicon rubber ring which was glued onto the skin with tissue glue, a nylon mesh which was glued to the surface of the silicone ring and a porous (semiocclusive) bandage which encircled the trunk of the animal. Material that is wicked up by the protective cover and/or spread outside the application site is not available for absorption. Therefore data from the high dose group is unusable for determining the dermally absorbed portion of the applied dose. Material retention in the protective cover (3-4%) and on the surrounding skin (0.2-4%) was within expected ranges for all other doses/exposures.

Results provided in the submitted study for the low dose 10 hour exposure group have been revised to remove anomalous data. A review of the results for individual animals from this group showed that data on the protective cover from one animal (#38) was clearly outside the norm. Removal of the outlying data results in a significant reduction in the mean amount of material retained on the protective cover for the 10 hour exposure period (from 11% to 4%).

The study appears to overestimate absorption at the intermediate dose for the 72 h exposure/observation period. Absorbed radioactivity at 72 h was higher than expected for this group. For the intermediate dose group, an additional 7% radioactivity was absorbed during the 48 h post-exposure observation period (25% absorbed at 24 h, 32% at 72 h). This compares to 4% radioactivity absorbed during the 48 h follow-up for the low dose group and 1% absorbed for the high dose group. Absorption for the intermediate dose should reasonably be expected to be between 4% and 1%. The absorption spike found for the middle dose group after 72 h exposure and follow-up probably occurred because all animals in this group removed the protective cover so that the animals could lick and ingest material which remained on the skin.

Attachment

Table 1: Mean excretion and retention of radioactivity after a single dermal administrati of ^{14}C-DIMETHOATE to rats at a nominal dose level of 4.0 mg/cm^2 (40.0 mg/animal).

Results expressed as % of the radioactivity administered.

Exposure Period Sacrifice	1 h 1 h	10 h 10 h	24 h 24 h	24 h 72 h
Urine	0.16	3.88	10.30	10.75
Feces	0.01	0.09	0.12	0.34
Cage wash	0.05	0.38	0.60	1.16
Blood	0.03	0.02	0.03	0.03
Carcass	0.79	1.40	1.01	0.90
% absorbed	1.03	5.78	12.06	13.17
Surrounding skin	11.13	9.14	11.56	5.28
Protective cover	6.70	10.09	10.92	11.20
Application site	7.84	8.28	3.63	2.99
Skin wash	70.37	62.24	59.03	57.12
2nd skin wash	---	---	---	4.13
Total	97.06	95.52	97.20	93.88

Table 2: Mean excretion and retention of radioactivity after a single dermal administration of ^{14}C-DIMETHOATE to rats at a nominal dose level of 0.4 mg/cm^2 (4.0 mg/animal).

Results expressed as % of the radioactivity administered.

Exposure Period Sacrifice	1 h 1 h	10 h 10 h	24 h 24 h	24 h 72 h
Urine	2.76	21.07	22.71	29.93
Feces	0.07	0.46	0.56	0.58
Cage wash	0.66	1.54	0.83	0.61
Blood	0.10	0.07	0.04	0.02
Carcass	2.78	1.85	1.20	0.55
% absorbed	5.68	24.98	25.33	31.69
Surrounding skin	3.65	5.05	1.26	0.19
Protective cover	2.75	3.48	3.35	3.53
Application site	5.58	3.24	5.32	4.21
Skin wash	88.89	63.91	65.31	57.27
2nd skin wash	---	---	---	1.57
Total	106.55	100.66	100.57	98.45

Original Table 3 from the submitted study:

Table 3: Mean excretion and retention of radioactivity after a single dermal administration of ^{14}C-DIMETHOATE to rats at a nominal dose level of 0.02 mg/cm^2 (0.2 mg/animal).

Results expressed as % of the radioactivity administered.

Exposure Period Sacrifice	1 h 1 h	10 h 10 h	24 h 24 h	24 h 72 h
Urine	1.89	20.36	35.77	40.04
Feces	0.01	0.04	0.73	0.58
Cage wash	0.43	2.43	0.94	0.75
Blood	0.33	0.06	0.03	0.03
Carcass	3.49	1.51	0.59	0.41
% absorbed	5.68	24.40	38.06	41.81
Surrounding skin	3.44	4.56	0.31	0.22
Protective cover	4.86	10.85	4.55	4.02
Application site	10.25	12.64	12.69	8.84
Skin wash	76.89	45.64	38.48	37.41
2nd skin wash	---	---	---	1.64
Total	101.12	98.08	94.09	93.94

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evised Table 3 - Mean % radioactivity retained/excreted at nominal dose level of 0.02 mg/cm^2 -- Results with outlying data from a single animal in the 10 hour dose group removed[1]

Exposure Period Sacrifice	1h 1h	10 h 10 h	24 h 24 h	24 h 72 h
Urine	1.89	23.43	35.77	40.04
Feces	0.01	0.05	0.73	0.58
Cage wash	0.43	3.00	0.94	0.75
Blood	0.33	0.07	0.03	0.03
Carcas	3.49	1.60	0.59	0.41
% absorbed	5.68	28.15	38.06	41.81
Surrounding skin	3.44	0.28	0.28	0.22
Protective cover	4.86	3.93	4.55	4.02
Application site	10.25	13.91	12.69	8.84
Second skin wash	76.89	48.56	38.48	37.41
	---	---	---	1.64
Total	101.12	94.84	94.09	93.94

[1] Results for the 10 hour exposure group have been revised to remove outlying data on percent of material retained on the protective cover from animal # 38. This revised results show a significant reduction in the mean amount on the protective cover (from 11% to 4%). The 10 hour results were also revised to correct an error in the reported value for % found in/on the surrounding skin . The reported value was 4.56%; the correct amount (from the raw data) is 0.50%. This correction, combined with removal of the outlying data, results in a revised value of 0.28% on surrounding skin for the 10 hour period.