DATA EVALUATION RECORD

CHLOROTHALONIL

Non-Guideline; *In Vitro* Dermal Penetration Study Using Human Skin

Work Assignment No. 3-01-91 A (MRID 46261901)

Prepared for
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DATA EVALUATION RECORD

STUDY TYPE: *In Vitro* Dermal Penetration Study - Human Skin; Non-guideline.

PC CODE: 081901 **DP BARCODE:** 301496

TEST MATERIAL (RADIOCHEMICAL PURITY): [^{14}C]Chlorothalonil (≥98%)

SYNONYMS: Tetrachloroisophthalonitrile; 2,4,5,6-tetrachloro-1,3-benzenedicarbonitrile

CITATION: Cage, S. (2004) [^{14}C]-Chlorothalonil: *in vitro* dermal penetration study using human skin. Huntingdon Life Sciences, Ltd., Alconbury, Huntington, Cambridgeshire, England. Laboratory Project ID: VCM/118, April 8, 2004. MRID 46261901. Unpublished

SPONSOR: Vischim Srl, Via Friuli, 55, 20031 Cesano Maderno, Milan, Italy

EXECUTIVE SUMMARY: In a non-guideline *in vitro* dermal penetration study (MRID 46261901), [^{14}C]-Chlorothalonil (>98% radiochemical purity, batch # 02BLY108) was suspended in either a suspension concentrate (SC) formulation or a water dispersable granule (WG) formulation and applied at one of two concentrations, to approximate exposure to the commercially supplied concentrate and to the minimum in-use spray dilution. The formulated test substance was applied to dermatomed human skin (0.64 cm^2) fixed in Scott-Dick flow-through diffusion cells for six h at dose levels of 62 or 5000 µg/cm^2. Receptor fluid (phosphate-buffered saline [pH 7.4] with 5% w/v bovine serum albumin) was passed through the receptor chamber at a flow rate of 1.5 mL/h, and fractions were collected for one h intervals up to 24 h. After six h, the skin was swabbed until radioactivity was no longer removed; after 24 h, the skin was tape-stripped for removal of residual surface radioactivity and the stratum corneum. All samples (receptor fluid samples, swabs, tape strips, and residual skin) were analyzed for the presence of radioactivity.

Recovery of the applied doses was similar for both dose levels and formulations (96.0-100.7% applied dose). The majority of the radioactivity was unabsorbed (89.1-97.5%). Skin swabs accounted for the largest portion of the applied dose, while surface tape strips removed a smaller fraction, and minor amounts of radioactivity were residual on the diffusion cells. Greater percentages of radioactivity were seen in fractions other than skin swabs in the low dose formulations than the high dose formulations.

Radioactivity was associated with the stratum corneum, but this material probably was not available for absorption. A greater proportion of the applied low dose formulations was associated with this compartment compared to the high dose formulations. Total absorbed radioactivity (stripped skin and receptor fluid) was similar in the low dose (0.7-0.8%) and high dose (0.13-0.14%) formulations, indicating that the different formulation types (SC and WG) had little effect on total absorption, or steady-state absorption rates (7.1-8.2 ng/cm^2/h for the low dose formulations; 151.1-231.2 ng/cm^{2}h for the high dose formulations).

This study is classified as **acceptable/non-guideline**.

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

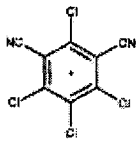
I. MATERIALS AND METHODS

A. MATERIALS

1. Test compound:

Radiolabeled test material 1: [^{14}C]-Chlorothalonil

- **Radiochemical Purity:** >98%
- **Specific Activity:** 66 mCi/mmol (9.1926MBq/mg)
- **Batch No.:** 02BLY108
- **Structure:**



*Uniformly labeled in the aromatic ring

Non-radiolabeled test material: Chlorothalonil

- **Description:** White powder
- **Batch No.:** C408
- **Purity:** 996 g/kg (99.6% w/w)
- **CAS # for TGAI:** 1897-45-6

Formulation 1: Chlorothalonil 500 SC (suspension concentrate)

- **Description:** White viscous liquid
- **Batch No.:** C411
- **Concentration of AI:** 505 g/L

Formulation 2: Chlorothalonil 500 SC Blank

- **Description:** Brown viscous liquid
- **Batch No.:** C412
- **Concentration of AI:** 0 g/L

Formulation 3: Chlorothalonil 75% WG (water dispersable granules) Blank

- **Description:** Light brown powder
- **Batch Nos.:** C410
- **Concentration of AI:** 0 g/L

2. Relevance of test material to proposed formulation(s): The selected nominal concentrations for the SC formulation were equivalent to the commercially supplied concentrate (500 g/L) and the in-use application rate (6.2 g/L). The selected nominal concentrations for the WG formulation were the highest achievable dose concentration that could be accurately applied and had acceptable homogeneity (560 g/L) and the in-use application rate (6.2 g/L).

3. Skin source: Three full thickness human skin samples (International Institute for the Advancement of Medicine, Jessup, PA) taken from the backs of two men and one woman (ages

51-58 years old) were used for measurement of dermal penetration. The full thickness skin samples were stored at -20°C until use. No further information was provided.

B. STUDY DESIGN

1. Dose

Rationale: Dose levels were selected to represent those concentrations equivalent to the in-use application rate and commercially supplied concentrate in order to simulate exposures of loaders and applicators.

Nominal doses: The nominal doses were 62 and 5000 µg/cm^2 skin for the low and high doses, respectively (Table 1).

Actual doses: The mean achieved doses administered were 61.56 and 5324 µg/cm^2 for the low and high doses of the SC formulation, and 61.16 and 5623 µg/cm^2 for the WG formulation, respectively.

Dose volume: 6.4 µL of each dose level was applied to 0.64 cm^2 skin, resulting in a nominal dose volume of 10 µL/cm^2.

Duration of exposure (time from dose to skin wash): 6 h.

Sampling periods: receptor fluid fractions were collected for one h intervals for 24 h following dermal application of the test compound. At 24 h, the skin was tape-stripped to remove residual surface dose and the stratum corneum.

Number of skin samples/group prepared: seven skin samples/dose

2. Tissue preparation: Prior to use, the samples were thawed and any fat was removed. The skin was swabbed briefly with 70% ethanol to remove residual fat and blood, and then rehydrated with distilled water. The skin sample was pinned out on a dermatome board, and a mini-dermatome was used to cut slices of skin approximately 300 µm thick. These slices contained the epidermis and some dermis.

3. Dose preparation, administration, and quantification

Preparation: Aliquots of [^{14}C]-Chlorothalonil stock solution in acetonitrile were placed in glass vials, mixed with non-radioactive Chlorothalonil, and evaporated to dryness under a nitrogen stream. The appropriate formulation (Chlorothalonil 500 SC, Chlorothalonil 500 SC Blank, or Chlorothalonil 75% WG Blank) was added, stirred, vortexed, and/or sonicated. These dose preparations were then diluted with distilled water as needed. The achieved radiochemical and compound doses are given in Table 1.

Table 1. Dosing[a]

Dose groups	Nominal rate ($\mu g/cm^2$)	Actual dose ($\mu g/cm^2$)	Actual dose (μg)	Radiochemical dose (kBq)
High dose (500 g/L SC)	5000	5324	3408	26.00
Low dose (6.2 g/L SC)	62	61.56	39.40	24.42
High dose (75% WG)	5000	5623	3599	36.24
Low dose (6.2 g/L WG)	62	61.16	39.14	23.84

a Data were obtained from page 23 of the study report.

Homogeneity of the preparations was determined by sampling the top, middle, and bottom of the formulations and analyzing for radioactivity. All samples were found to be within 5% of the mean activity with no appreciable concentration gradient observed; therefore, the preparations were considered homogeneous.

Application: The skin samples were placed on the receptor chambers of stainless steel Scott-Dick flow-through diffusion cells (see Appendix). The upper donor chambers were then fixed in place, providing an exposure area of approximately 0.64 cm^2. The assembled apparatus was maintained at approximately 32°C. The receptor fluid was physiological phosphate-buffered saline, pH 7.4, with 5% (w/v) bovine serum albumin, and was pumped through the receptor chamber at a flow rate of 1.5 mL/h, allowing approximately six chamber content changes per h. Prior to application, it was determined that the solubility of the test material in the receptor fluid was 93.1 µg/mL. The dose preparations were applied to the skin membrane surface with a calibrated positive displacement pipette.

Quantitation: To determine the quantity of radioactivity administered to each cell, quality control doses of 6.4 µL for each dose group were dispensed throughout the dose administration. These doses were diluted with acetone, soniciated, and the radioactivity in duplicate aliquots was determined by liquid scintillation counting (LSC). Additionally, the pipette tips for both actual and quality control doses were soaked in acetone, sonicated, and residual radioactivity was determined by LSC. The applied dose was determined with the following calculation:

Applied dose = total quality control activity for each dose group - dose pipette tip residue for each cell

4. **Skin membrane integrity and selection:** The integrity of each skin membrane was determined by measuring the dermal penetration of ^3H_2O. A 250 µL aliquot was applied to the skin surface, and the receptor chamber was perfused with distilled water at a flow rate of 1.5 mL/h. Fractions were collected for 30 minutes for a total of five h, at which time the residual ^3H_2O was removed from the skin surface. The skin surface was then washed with distilled water, followed by an overnight incubation of the skin surface with distilled water while perfusing the

receptor chamber with distilled water. Prior to dosing, samples of receptor fluid were taken and analyzed for residual ^3H_2O; it was stated that all cells used had acceptably low radioactivity levels. The permeability coefficient (Kp) was calculated for each skin membrane by dividing the absorption rate by the applied concentration of radioactivity (dpm/mL).

5. Sample collection: Receptor fluid fractions were collected for one h intervals up to 24 h following dermal application of the test compound. At six h after application, the skin surface was swabbed with aqueous 1% (v/v) Tween-80 on cotton wool buds until no radioactivity was removed (confirmed with a Geiger-Müller monitor). A dry cotton wool bud was used to remove any residual swabbing solution. At 24 h after application, the skin was tape-stripped to remove residual radioactivity and the stratum corneum. The initial tape strips (1-2) were collected separately and represented non-absorbed dose. Subsequent strips containing the stratum corneum were pooled and analyzed in groups of three. The remaining skin membrane was retained separately. After removal of the skin membrane, the receptor fluid remaining in the receptor chamber and outlet tubing was collected.

6. Sample preparation and analysis: All receptor fluid samples were mixed directly with scintillation fluid for LSC. The diffusion cell components were soaked in acetone and sonicated, and duplicate aliquots were analyzed for radioactivity. The cotton wool buds were extracted by sonicating them in acetone, and duplicate aliquots were taken for measurement of radioactivity. The extraction process was repeated once. The tape strips were solubilized by incubation at 60°C in a mixture of 6:3:1 (v/v/v) distilled water, methanol, and Triton X-405 containing 80 g/L sodium hydroxide, and analyzing duplicate aliquots for radioactivity. The residual skin was solubilized with Soluene-350 at 60°C and analyzed for radioactivity. It was stated that the limit of detection was derived statistically from the background counts so that there was a 99% certainty that samples with a mean value greater than the limit of detection contained radioactivity from the [^{14}C]-Chlorothalonil, and that the limit of detection throughout the study was approximately 6 dpm.

II. RESULTS

A. SKIN MEMBRANE INTEGRITY: It was stated that on examination of the test material absorption data, it was considered that if the total absorption and absorption profiles from skin membranes with Kp values >3.5 x 10^{-3} cm/h were similar to those from skin membranes with Kp values <3.5 x 10^{-3} cm/h, these data would be considered acceptable. With these criteria in mind, the data for Cell 4 (500 g/L SC) and Cell 14 (6.2 g/L SC) were excluded due to a high level of absorption of the test material relative to the other cells in the respective groups. These membranes were considered to have been damaged.

B. DERMAL ABSORPTION: Recovery of the applied doses was similar for both dose levels and formulations (96.04-100.7% applied dose; Table 2). Regardless of dose level or formulation, the majority of the applied dose was unabsorbed. Skin swabs accounted for 72.14-84.92% of the applied dose for the low dose formulations, and 92.67-96.69% for the high dose formulations. The surface tape strips removed a smaller portion of the applied dose, with a greater proportion of the low dose formulations (7.016-15.95%) than the high dose formulations (0.685-2.139%)

accounted for in this fraction. Minor amounts of radioactivity were residual on the diffusion cells (0.090-0.963%).

Radioactivity also was associated with the stratum corneum. For the low dose formulations, 7.474-10.57% of the applied dose was associated with this compartment; for the high dose formulations, 0.384-0.659% of the applied dose was recovered here.

Relatively low amounts of radioactivity were found in tape-stripped (residual) skin; 0.482-0.486% of the applied dose for the low dose formulations, and 0.067-0.075% of the dose for the high dose formulations. Additionally, for the low dose formulations, 0.240-0.271% of the applied dose was found in the receptor fluid, while 0.054-0.069% of the applied high dose formulations was accounted for in this compartment. The total absorbed dose was <0.8% of the applied dose.

From these data, the steady-state absorption rates were calculated. For the low dose formulations, the steady-state absorption rate was 7.142-8.232 ng/cm^2/h, while for the high dose formulations, rates of 151.1-231.2 ng/cm^2/h were determined.

Table 2. Mean recovery of radioactivity (% applied dose) following a 6 h exposure to a single dermal dose of [^{14}C]-Chlorothalonil at nominal dose levels of 62 and 5000 µg/cm^2.[a]

Sample	Formulation			
	6.2 g/L SC	500 g/L SC	6.2 g/L WG	75% WG
Receptor fluid[b]	0.240	0.069	0.271	0.054
Stripped skin	0.482	0.067	0.486	0.075
Total absorbed	**0.722**	**0.135**	**0.757**	**0.130**
Total stratum corneum	**7.474**	**0.384**	**10.57**	**0.659**
Skin swab	84.92	96.69	72.14	92.67
Surface tape strip	7.016	0.685	15.95	2.139
Residual on cell	0.600	0.090	0.963	0.448
Total unabsorbed	**92.54**	**97.47**	**89.05**	**95.25**
Total recovery	**100.7**	**97.99**	**100.4**	**96.04**
Absorption rate (ng/cm^2/h)	**7.142**	**231.2**	**8.232**	**151.1**

a Data were obtained from page 25 of the study report.
b Includes residual fluid in receptor chamber and outlet tubing
ND Not detected
NS No sample

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The absorption of Chlorothalonil through human skin was similar whether applied as a SC or WG formulation. By 24 h after application, the total amount of radioactive material absorbed from the SC formulation was 0.135% and 0.722% for the high and low dose levels, respectively. For the WG formulations, the total amount of radioactive material absorbed by 24 h was 0.130% and 0.757% at the high and low dose levels, respectively. The level of absorption for the SC and WG formulations were very similar at both

doses showing that the different formulation types do not significantly effect the absorption of Chlorothalonil.

B. REVIEWER COMMENTS: Recovery of the applied doses was similar for both dose levels and formulations (96.04-100.7% applied dose). The majority of the radioactivity was unabsorbed (89.05-97.47%). Skin swabs accounted for the largest portion of the applied dose, while surface tape strips removed a smaller fraction, and minor amounts of radioactivity were residual on the diffusion cells. Other than for skin swabs, the low dose formulations showed higher percentages of fractions than the high dose formulations.

Radioactivity also was associated with the stratum corneum. A greater proportion of the applied low dose formulations (7.474-10.57%) was associated with this compartment compared to the high dose formulations (0.384-0.659%). Similar findings were noted for tape-stripped (residual) skin, with 0.482-0.486% of the applied dose retained for the low dose formulations, and 0.067-0.075% of the dose retained for the high dose formulation. This indicated that radioactivity associated with the stratum corneum probably was not available for absorption. Additionally, losses due to desquammation did not occur in this *in vitro* system. Finally, 0.240-0.271% of the applied dose was found in the receptor fluid for the low dose formulations, while 0.054-0.069% of the applied high dose formulations was accounted for in this compartment. The total absorbed dose was <0.8% of the applied dose.

From these data, the steady-state absorption rates were calculated. For the low dose formulations, the steady-state absorption rate was 7.142-8.232 ng/cm^2/h, while for the high dose formulations, rates of 151.1-231.2 ng/cm^2/h were determined.

This study is classified as **acceptable/non-guideline**.

C. STUDY DEFICIENCIES: No deficiencies were noted.

APPENDIX

The following diagram is from page 68 of the study report, MRID 46261901

Diagrammatic representation of the flow through diffusion cell