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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

MEMORANDUM

Date: June 26, 2008

SUBJECT: Picaridin: In vitro dermal penetration study using human and rat skin

PC Code: 070705
Decision No.: 361412, 389997
Petition No.: NA
Risk Assessment Type: NA
TXR No.: 0054846
MRID No.: 47340901, 47342201

DP Barcode: D350980, D350983
Registration No.: NA
Regulatory Action: NA
Case No.: NA
CAS No.: 119515-38-7
40 CFR: NA

Ver.Apr.08

FROM: **Yung G. Yang, Ph.D.**
Toxicology and Epidemiology Branch
Health Effects Division (7509P)

THROUGH: **Mary Manibusan, Acting Chief**
Toxicology and Epidemiology Branch
Health Effects Division (7509P)

TO: **Christina Swartz, Chief**
Registration Action Branch 2
Health Effects Division (7509P)
And
Kevin Sweeney, Risk Manager Reviewer
Insecticide Branch
Registration Division (7505P)

I. CONCLUSIONS

Under conditions of these studies, the in vitro dermal penetration studies using human and rat skin demonstrated that (1) the level of direct absorption of KBR 3023 (Picaridin) technical and in 15% ethanol through human skin was lower than that for rat skin; (2) the addition of sunscreen to the Cutter Insect repellent Forumula A did not increase dermal penetration through human skin.

JUL 1 RECD, 2008

II. ACTION REQUESTED

This is a PRIA action. The Registration Division (RD) requested the Health Effects Division (HED) to review two in vitro dermal penetration studies and to compare (1) in vitro dermal absorption of KBR 3023 (Picaridin) technical and KBR 3023 in 15% ethanol using human and rat skin and (2) in vitro dermal absorption of the Cutter Insect Repellent Formula A vs. Cutter Insect Repellent SS (with sunscreen) using human skin.

III. BACKGROUND

Previously, the Registrant submitted study protocols for an in vitro study using human skin to compare the dermal absorption of two formulations (Cutter Insect Repellent SS and Cutter Insect Repellent Formula A). The Registrant also agreed to conduct an in vitro dermal absorption study with KBR 3023 (neat) and KBR 3023 15% in ethanol through human and rat skin. The proposed protocol was stated to follow OECD Guideline No. 428 and in compliance with several GLP regulations including U.S. EPA. These protocols were approved by the Agency with some additional recommendations (TXR 0054457).

IV. RESULTS/DISCUSSION

Two in vitro dermal penetration studies were submitted as entitled (1) Cutter insect repellent SS and Cutter insect repellent formula A formulations: Comparative *in vitro* dermal absorption study using human skin (MRID 47340901) and (2) KBR 3023 technical and KBR 3023 15% in ethanol: Comparative *in vitro* dermal absorption study using human and rat skin (MRID 47342201). The Toxicology and Epidemiology Branch (TEB) has reviewed these studies and classified these studies as acceptable/non-guideline. Executive summaries are as follows.

EXECUTIVE SUMMARY: In a dermal penetration study (MRID 47340901), KBR 3023 in Cutter Insect Repellent SS (with sunscreen) and Cutter Insect Repellent Formulation A (without sunscreen) were tested to quantify and compare the dermal absorption using an *in vitro* test with human skin. Radio-labeled [hydroxyethyl-1-^{14}C]-KBR 3023 and non-radiolabeled KBR 3023 were incorporated into each blank formulation to achieve a final concentration of 15%. Using a flow-through diffusion cell system, these test formulations were applied at a nominal concentration of 0.63 mg/cm^2 (achieved dose 0.44-0.46 mg/cm^2) to 1 cm^2 of excised human dermatomed skin (6 replicates per formulation) for an 8-hour exposure period. After 8 hours, the remaining test material was washed off the skin, and absorption into the receptor fluid was measured at hourly intervals throughout the 24-hour duration of the study. At the end of the study (24 hours after application), the skin samples were swabbed again and then were tape-stripped to remove residual surface dose and the *stratum corneum*. The non-absorbed dose was defined as the amount in the swabs, donor chamber, and first two tape strips; the directly absorbed dose was the amount in the receptor fluid and receptor chamber; and the dose present at the dosing site was considered available for potential absorption. The diffusion cell components were retained and washed, and the washings, along with all other samples, were analyzed to establish a mass balance.

Initial dose analyses showed that the mean achieved doses were low (69.52 and 72.49% nominal in the SS and Formula A formulations, respectively). The investigators demonstrated that this was due to the high viscosity and low volume of the test material dispensed. Because these values were consistently low among replicates and between the two formulations, the investigators decided that it was appropriate to normalize the data by considering the sum of radioactivity for each cell to equal 100% of the applied dose. The reviewers agree that this would not affect the integrity of the study, especially given that the purpose is to compare the two formulations.

Higher mean dermal penetration in all skin layers (surface dose, *stratum corneum*, remaining skin, and receptor compartment) was observed in Formula A compared to the SS formulation. The mean percentage of [^{14}C]-KBR 3023 that was directly absorbed (i.e., present in the receptor chamber and fluid) was 2.1 fold higher in Cutter Insect Repellent Formula A (12.44% dose) compared to the SS Formula (5.92% dose). Similarly, the mean percentage of [^{14}C]-KBR 3023 that was potentially absorbable (i.e., in the skin excluding top two tape strips of *stratum corneum*) was 1.6 fold higher in Formula A (6.47% dose) compared to the SS Formula (3.93% dose). Thus, the sum of the mean percentage of [^{14}C]-KBR 3023 that was directly absorbed and that which remained in the skin (including lower *stratum corneum*) for potential absorption was 1.9 fold higher in Formula A (18.91% dose) compared to the SS Formula (9.85% dose).

Examination of mean hourly receptor fluid samples showed that absorption occurred earlier and to a greater extent in Formula A (without sunscreen) compared to the SS Formula. In the SS formulation, the mean hourly absorption rate gradually increased and did not reach its peak until 15 hours post-dose (0.325% dose/hour). However, in Formula A, the mean hourly absorption rate peaked earlier (at 6 hours) and at a higher rate (0.702% dose/hour).

The addition of sunscreen to the Cutter Insect Repellent Formula A did not increase dermal penetration through human skin in this *in vitro* experiment. In fact, the addition of sunscreen to the formulation decreased the absorption by a factor of two.

This study is classified as **acceptable/non-guideline**. Note that although there is no OPPTS guideline, this study does satisfy the OECD guideline requirements (OECD 428) for an *in vitro* dermal penetration study using human skin.

EXECUTIVE SUMMARY: In a dermal penetration study (MRID 47342201), KBR 3023 technical and KBR 3023 15% (w/v) in ethanol were tested to compare *in vitro* dermal penetration following a single dermal application to excised human and rat skin. [Hydroxyethyl-1-^{14}C]-KBR 3023 and non-radiolabeled KBR 3023 technical were combined and applied as either the technical formulation or at 15% (w/v) in ethanol. These test formulations were applied at a nominal concentration of 0.63 mg/cm^2 (achieved dose 0.44-0.54 mg/cm^2) to 1 cm^2 of excised human or rat dermatomed skin (6 replicates per formulation) for an 8-hour exposure period. After 8 hours, the remaining test material was washed off, and penetration into the receptor fluid was measured at hourly intervals over a 24-hour period. At the end of the study (24 hours after application), the skin samples were swabbed again and then were tape-stripped to remove residual surface dose and the *stratum corneum*. The non-absorbed dose was defined as the amount in the swabs, donor chamber, and first two tape strips; the directly absorbed dose was the

amount in the receptor fluid and receptor chamber; and the dose present at the dosing site was considered available for potential absorption. The diffusion cell components were retained and washed, and the washings, along with all other samples, were analyzed to establish a mass balance.

Mean total recoveries ranged from 100.1-115.1% of the applied dose. For the technical formulation, 7.3% of the applied dose was directly absorbed through human skin, while 21.8% of the applied dose was directly absorbed through rat skin (2.97-fold difference). For the 15% (w/v) ethanol formulation, 5.6% of the applied dose was directly absorbed through human skin, while 16.8% of the applied dose was directly absorbed through rat skin (3.01-fold difference). For the technical formulation, 10.7% of the applied dose was potentially absorbable through human skin, while 25.2% of the applied dose was potentially absorbable through rat skin (2.4-fold difference). For the 15% (w/v) ethanol formulation, 9.8% of the applied dose was potentially absorbable through human skin, while 22.3% of the applied dose was potentially absorbable through rat skin (2.3-fold difference).

It was stated that in a previously performed *in vivo* dermal absorption study using human volunteers (MRID 44408738, not provided), the dermal absorption of [^{14}C]-KBR 3023 technical and a 15% ethanol formulation applied at dose levels similar to those used in the current study were 1.66% and 3.77% of the applied doses, respectively. Thus, the difference between *in vitro* and *in vivo* dermal absorption through human skin was 6.42-fold and 2.60-fold for the technical and 15% ethanol formulations, respectively. Similarly, in a previously performed *in vivo* dermal absorption study using male rats (MRID 44408737, not provided), the dermal absorption of [^{14}C]-KBR 3023 technical applied at a dose level similar to that used in the current study was 18.9% of the applied doses. Thus, the difference between *in vitro* and *in vivo* dermal absorption through rat skin was 1.33-fold for the technical formulations.

This study is classified as **acceptable/non-guideline;** however, it does satisfy the OECD guideline requirements (OECD 428) for an *in vitro* skin absorption study using excised skin.

DATA EVALUATION RECORD

KBR 3023 (PICARIDIN)

Study Type: Non-guideline; Comparative *In Vitro* Dermal Penetration Study Using Human Skin

Work Assignment No. 5-1-176 B (MRID 47340901)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2777 South Crystal Drive
Arlington, VA 22202

Prepared by

Pesticide Health Effects Group
Sciences Division
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1910 Sedwick Rd, Bldg. 100, Ste. B
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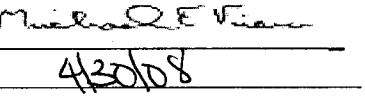
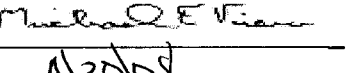
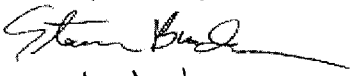
Secondary Reviewer: Michael E. Viana, Ph.D., D.A.B.T.	Signature:  Date: 4/30/08
Program Manager: Michael E. Viana, Ph.D., D.A.B.T.	Signature:  Date: 4/30/08
Quality Assurance: Steven Brecher, Ph.D., D.A.B.T.	Signature:  Date: 4/30/08

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

KBR 3023 (PICARIDIN)/PC Code 070705 OPPTS Non-guideline/ OECD 428

EPA Reviewer: Yung G. Yang, Ph.D. **Signature:** Yung G Yang

Toxicology Branch, Health Effects Division (7509P) **Date:** 6/26/2008

EPA Work Assignment Manager: Myron Ottley, Ph.D. **Signature:** MyOttley

Registration Action Branch 3, Health Effects Division (7509P) **Date:** 6/26/08

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: *In Vitro* Dermal Penetration Study Using Human Skin; Non-guideline; OECD 428.

PC CODE: 070705 **DP BARCODE:** D350983

TXR#: 0054846

TEST MATERIAL (RADIOCHEMICAL PURITY): KBR 3023 (>99%)

SYNONYMS: Icaridin; 1-methylpropyl 2-(2-hydroxyethyl)-1-piperidinecarboxylate

CITATION: Rascle, J.B. (2008) Cutter insect repellent SS and Cutter insect repellent formula A formulations: comparative *in vitro* dermal absorption study using human skin. Bayer CropScience, Sophia Antipolis Cedex, France. Laboratory Study No.: SA 07027, January 31, 2008. MRID 47340901. Unpublished.

SPONSOR: Regulatory & Government Affairs, Spectrum, Division of United Industries Corp. P.O. Box 142642, St. Louis, MO.

EXECUTIVE SUMMARY: In a dermal penetration study (MRID 47340901), KBR 3023 in Cutter Insect Repellent SS (with sunscreen) and Cutter Insect Repellent Formulation A (without sunscreen) were tested to quantify and compare the dermal absorption using an *in vitro* test with human skin. Radio-labeled [hydroxyethyl-1-^{14}C]-KBR 3023 and non-radiolabeled KBR 3023 were incorporated into each blank formulation to achieve a final concentration of 15%. Using a flow-through diffusion cell system, these test formulations were applied at a nominal concentration of 0.63 mg/cm^2 (achieved dose was 0.44-0.46 mg/cm^2) to 1 cm^2 of excised human dermatomed skin (6 replicates per formulation) for an 8-hour exposure period. After 8 hours, the remaining test material was washed off the skin, and absorption into the receptor fluid was measured at hourly intervals throughout the 24-hour duration of the study. At the end of the study (24 hours after application), the skin samples were swabbed again and then were tape-stripped to remove residual surface dose and the *stratum corneum*. The non-absorbed dose was defined as the amount in the swabs, donor chamber, and first two tape strips; the directly absorbed dose was the amount in the receptor fluid and receptor chamber; and the dose present at the dosing site was considered available for potential absorption. The diffusion cell components were retained and washed, and the washings, along with all other samples, were analyzed to establish a mass balance.

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Higher mean dermal penetration in all skin layers (surface dose, *stratum corneum*, remaining skin, and receptor compartment) was observed in Formula A compared to the SS formulation. The mean percentage of [^{14}C]-KBR 3023 that was directly absorbed (i.e., present in the receptor chamber and fluid) was 2.1 fold higher in Cutter Insect Repellent Formula A (12.44% dose) compared to the SS Formula (5.92% dose). Similarly, the mean percentage of [^{14}C]-KBR 3023 that was potentially absorbable (i.e., in the skin excluding top two tape strips of *stratum corneum*) was 1.6 fold higher in Formula A (6.47% dose) compared to the SS Formula (3.93% dose). Thus, the sum of the mean percentage of [^{14}C]-KBR 3023 that was directly absorbed and that which remained in the skin (including lower *stratum corneum*) for potential absorption was 1.9 fold higher in Formula A (18.91% dose) compared to the SS Formula (9.85% dose).

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The addition of sunscreen to the Cutter Insect Repellent Formula A did not increase dermal penetration through human skin in this *in vitro* experiment. In fact, the addition of sunscreen to the formulation decreased the absorption by a factor of two.

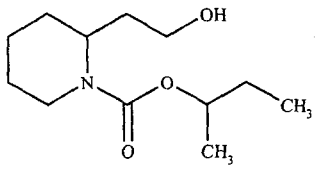
This study is classified as **acceptable/non-guideline**. Note that although there is no OPPTS guideline, this study does satisfy the OECD guideline requirements (OECD 428) for an *in vitro* dermal penetration study using human skin.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** KBR 3023 (Picaridin)

Description:	Technical; clear, colorless to brownish liquid
Batch #:	CHCAEC0069
Purity:	98.8% a.i.
Compound stability:	Not reported
CAS # for TGAI:	119515-38-7
Structure:	
Vehicle/Solvent used:	Blank commercial formulation for Cutter SS or Formula A (Spectrum, St. Louis, MO)
Radiolabelling:	The hydroxyethyl-1-C was uniformly radiolabeled.
Specific Activity:	3.55 MBq/mg (96 µCi/mg)
Radiochemical Purity:	>99%
Source:	Bayer CropScience AG, Product Technology, Isotope Chemistry (Wuppertal, Germany)

2. **Relevance of test material to proposed formulations:** The technical test material was incorporated into each of the blank commercial formulations at 15% a.i. Thus, the study was designed not only to mimic the actual commercially available formulations but also allowed comparison between the two formulations regarding dermal absorption through human skin.

B. STUDY DESIGN

1. **Objective:** The objective of this study was to quantify and compare the dermal absorption of KBR 3023 15% in Cutter Insect Repellent SS (with sunscreen) and Cutter Insect Repellent Formulation A (without sunscreen) using an *in vitro* test with human skin.

2. **Test system:** Dermatomed abdominal human skin (310-350 µM thick) from 5 different Caucasian female donors which was obtained from Biopredic Tissue Bank (Rennes, France) and stored at approximately -20°C until use. Human skin from male donors was not available at the time of the study.

3. **Diffusion cell design:** Absorption of the test formulations was measured using the Franz cell modified flow-through diffusion cell system (Gallas, France). Each diffusion cell chamber consisted of a donor chamber and a receptor chamber between which the skin was positioned, providing an exposure area of 1 cm^2. The fluid in the receptor chamber was maintained at 32 ± 2°C by a water bath in order to approximate normal skin temperature. The receptor fluid was pumped through the receptor chamber at a rate of 1.5 mL/h and stirred continuously by a magnetic stirring bar (400 rpm).

4. **Receptor fluid:** The receptor fluid was comprised of Eagle's medium (pH of 7.3 to 7.4) supplemented with 5% bovine serum albumin and gentamycin. The solubility of [^{14}C]-KBR 3023 in the receptor fluid was verified prior to the study by dissolving the volume of [^{14}C]-KBR 3023 corresponding to the maximum quantity of the test material applied to the cell (0.63 mg) in approximately 3 mL of receptor fluid, corresponding to the total volume of the cell. This procedure simulated the conditions of maximum absorption through the skin. The amount of radioactivity found: immediately after dilution; 72 hours after dilution; and after centrifugation were similar and ranged from 111.0-116.1% nominal, indicating that [^{14}C]-KBR 3023 was fully soluble in the receptor fluid.

5. **Skin membrane selection and integrity:** Prior to dose application, the integrity of the skin samples was assessed by measuring the trans-epidermal water loss (TEWL) from the *stratum corneum*. An evaporator probe (Cortex Technology, Hadsund, Denmark) was placed on top of the donor chamber and the rate of water diffusing through the skin was measured in g/h m^2. Skin samples with a TEWL of >40 g/h m^2 were considered potentially damaged and replaced. The TEWL for skin samples used in this study ranged from 1.9-23.6 g/h m^2. Thus, no samples used in this study needed to be replaced.

6. **Dose preparation, application, and quantification**

Dose preparation: The two formulations of the test substance were prepared at the performing laboratory by incorporation into the blank formulations the appropriate amounts of [^{14}C]-KBR 3023 and non-radiolabeled technical test material to achieve the final concentration of 15% active ingredient in the two formulations (Table 1). Test formulations were stored at -20°C in the dark until application.

TABLE 1. Dosing [a]

Dose level (µg/cm^2)	Amount compound in dosing solution (mg)		Final Specific activity (MBq/mg) [c]	Nominal dose (mg/cm^2)	Achieved dose (mg/cm^2) [d]
	Radio-labeled [b]	Non-labeled			
KBR 15% in Cutter SS	0.28	29.7	0.033	0.63	0.44 (70) [e]
KBR 15% in Formula A	0.28	29.8	0.033	0.63	0.46 (73)

a Data were obtained from page 17 and from Table 1e on page 47 of the study report. Non-labeled weights (in mg) and radio-labeled volumes (in µL) of the test material were provided on page 47. The nominal dose was provided on page 17.

b The radio-labeled weight (in mg) was verified by the reviewers as follows: 189.5 µL x 0.00528 MBq per µL/ x 1 mg per 3.55 MBq.

c The actual specific activity of the isotopically diluted test solutions was calculated by the reviewers by dividing the weight of the radio-labeled test material by the sum of the radio-labeled and non-labeled.

d The achieved dose was only approximately 70-73% of the nominal dose. This finding was demonstrated to be due to the high viscosity and small volume dispensed instead of low recovery. The low achieved doses were not considered to affect this study because they were consistent among replicates and between the two formulations and because the objective was to compare the two formulations.

e The achieved dose, expressed as the % nominal dose is included in parentheses.

Dose application: The dose preparation was applied to the skin sample with a micro-pipette at a nominal concentration of 0.63 mg/cm^2 and left on the skin for 8 hours.

Dose quantification: The actual doses applied were determined by taking three surrogate dose samples: one prior to the first application; another during dosing (after the third application); and a final sample after the last application. Each of these 4.2 µL samples was diluted with 1 mL of acetonitrile, and each pipette tip was thoroughly rinsed into the solvent. Triplicate aliquots (100 µL) were taken for measurement of radioactivity using liquid scintillation counting (LSC).

The **achieved doses** were uniformly low, ranging from 50.2-80.8% nominal. In an attempt to interpret these data, the investigators noted that the test solutions were highly viscous and that a small dose volume was applied. They hypothesized that the low achieved doses were attributed to the fact that the pipette was not rinsed onto the skin application site during dosing (a single gentle deposit with the pipette on the skin), but was rinsed into the vial during surrogate sample checks. In order to quantify this pipetting bias, 4.2 µL of each [^{14}C]-KBR 3023 formulation was pipetted either into vials (with thorough rinsing of the pipette tip) or onto human skin samples (a single deposit). These results (n = 5) indicated that the achieved dose via the application on the skin without rinsing the pipette was 18-23% lower than the dose dispensed and rinsed into the vials. These results demonstrate that the low achieved dose was not due to low recovery, but most likely attributed to the fact that the actual amount deposited on the skin samples maintained in the flow through cells was lower than the amount deposited into the vials when performing the dose checks. On that basis, the investigators considered it appropriate to normalize the data by considering the sum of radioactivity for each cell to equal 100% of the applied dose. The normalization factor was based on the mean total % nominal values of 69.52 and 72.49% for the Cutter SS and Formula A formulations, respectively.

Homogeneity of the radiochemical dose was determined in five samples of the dose formulations taken before application. Homogeneity results were 89.6-100.2% nominal; 2.18-2.25% coefficient of variation.

Thus, the analytical data indicated that the mixing procedure was adequate, and the achieved doses (after normalization) were considered acceptable to achieve the objectives of the study.

7. **Experiment procedure and sample collection:** The formulations were applied onto the dermatomed skin samples for 8 hours, after which time the remaining dose was washed off the skin with 1% v/v Tween 80 in phosphate buffered saline (PBS) using natural sponge swabs until no radioactivity was detected by a Geiger-Müller monitor. Receptor fluid samples were collected at hourly intervals for the duration of the study (24 hours). Samples were collected into glass vials held in a fraction collector, which was started after the dose application for each group was complete. At the end of the study (24 hours after application), the skin samples were swabbed again and then were tape-stripped to remove residual surface dose and the *stratum corneum*. Skin was tape-stripped by applying Monaderm adhesive tape (Monaderm, Monaco) for 5 seconds and carefully removing it against the direction of hair growth. This procedure was repeated until a shiny appearance of the epidermis was evident, which indicated that the *stratum corneum* had been removed. The area of skin surrounding the application site was also swabbed (surrounding swabs). After tape-stripping, the remaining skin was removed, and the skin surrounding the application site was separated

from the treated skin. Both compartments (treated skin and surrounding skin) were saved for analysis. The receptor fluid remaining in the cell and outlet tubing at the end of the experiment was collected and analyzed. The diffusion cell components were retained and washed, and the washings were analyzed to establish a mass balance.

8. **Sample preparation and analysis:** The weights of each of the collected samples were obtained either directly (tared measurement) or indirectly (by difference between the full and empty container). Whenever possible, the samples were processed as they were collected. The remaining samples were stored at approximately -20°C in the dark until processing and analysis. The amounts of radioactivity in the various samples were determined using LSC. The limit of detection was taken to be twice the background values for blank samples. Total amounts of radioactivity in samples were reported as a percentage of the total dose (normalized to 100%).

   Receptor fluid samples for each interval were weighed. Scintillation fluid was added directly to each receptor fluid sample and to the receptor fluid/out tubing contents and analyzed. Receptor fluid at study termination (terminal receptor fluid) was weighed, and duplicate weighed aliquots were taken for analyses.

   With the exception of the final three swabs used at 8 hours to wipe the application site, swabs taken at 8 hours were weighed and solubilized using an appropriate volume of Soluene. Triplicate aliquots were taken for analysis. Each of the following swabs was solubilized using 2 mL of Soluene, and scintillation fluid was added directly to each vial for analysis: the last three swabs at the end of the 8-hour exposure period; the terminal swabs (at 24 hours) from the application site; and surrounding swabs at 24 hours.

   Each tape strip was individually solubilized using tetrahydrofuran, and scintillation fluid was added directly to each sample for analysis.

   Each treated skin or surrounding skin sample was solubilized separately using Soluene, and scintillation fluid was added directly to each vial for analysis.

   The diffusion cell components were sampled in the following manner: the receptor chamber (including the stirrer bar and outlet tubing), the donor chamber (including securing screws), the cell and outlet tubing were soaked separately in a mixture of acetonitrile/distilled water (50:50 v/v) for 12 hours. The diffusion cell components were then removed and monitored for radioactivity using a Geiger-Müller monitor. The washings were weighed, and duplicate weighed aliquots were taken for analysis.

9. **Calculations:** Based upon the quantitative analyses of radioactivity from the different media, the following parameters were calculated:

   Non-absorbed = amount in the swabs, donor chamber, and first two tape strips

   Directly absorbed = amount in the receptor fluid and receptor chamber

Potentially absorbed (present at the dosing site) = tape strips (excluding the first two) and in the skin (treated skin + untreated skin) at the application site

II. RESULTS

A. SUMMARY TABLE: Higher mean dermal penetration in all skin layers (surface dose, *stratum corneum*, remaining skin, and receptor compartment) was observed in Formula A compared to the SS formulation (Table 2). The mean percentage of [^{14}C]-KBR 3023 that was directly absorbed (i.e., present in the receptor chamber and fluid) was 2.1 fold higher in Cutter Insect Repellent Formula A (12.44% dose) compared to the SS Formula (5.92% dose). Similarly, the mean percentage of [^{14}C]-KBR 3023 that was potentially absorbable (i.e., in the skin excluding top two tape strips of *stratum corneum*) was 1.6 fold higher in Formula A (6.47% dose) compared to the SS Formula (3.93% dose). Thus, the sum of the mean percentage of [^{14}C]-KBR 3023 that was directly absorbed and that which remained in the skin (including lower *stratum corneum*) for potential absorption was 1.9 fold higher in Formula A (18.91% dose) compared to the SS Formula (9.85% dose).

TABLE 2. Mean (± SD) *in vitro* dermal absorption of [^{14}C]-KBR 3023 (% dose) in human skin after 24 hr [a]

Sample	Cutter Insect Repellent SS Formulation	Cutter Insect Repellent Formulation A
	n = 6	n = 5 [b]
Surface Compartment		
Swabs (8 hr + 24 hr + surrounding swabs)	81.903 ± 12.161	68.305 ± 16.665
Surface dose (Tape strips 1 and 2) [c]	2.765 ± 1.855	8.374 ± 6.078
Dose remaining in donor chamber	5.350 ± 3.374	3.873 ± 3.110
Total % non-absorbed	**90.017 ± 7.032**	**80.552 ± 8.219**
Skin Compartment		
Skin after tape-stripping + surrounding skin	2.114 ± 1.673	3.909 ± 5.416
Stratum corneum (exclude tape strips 1 and 2) [c]	1.817 ± 1.335	2.560 ± 2.205
Total % at dose site (potentially absorbable)	**3.931 ± 2.916**	**6.470 ± 7.438**
Receptor Compartment		
Receptor fluid (0-24 hr)	5.023 ± 3.589	10.543 ± 3.605
Receptor fluid terminal	0.491 ± 0.365	0.924 ± 0.375
Receptor chamber wash	0.410 ± 0.493	0.971 ± 0.638
Total % directly absorbed	**5.924 ± 4.299**	**12.439 ± 3.989**
Skin + Receptor Compartments		
Total % directly absorbed + potentially absorbable	**9.854 ± 5.689**	**18.908 ± 8.269**
Total recovery (normalized)	**100**	**100**

a. Data were obtained from page 12 of the study report. Percent dose values were normalized so that the total recovered dose = 100%.

b Cell H11 was excluded due to a technical problem during receptor fluid collection.

c Tape strips 1 and 2 were considered to be non-absorbed because test substance desquamated in upper part of stratum corneum.

B. INDIVIDUAL DATA TABLES: Data for each sample, including the hourly receptor fluid samples, are presented in Table 4a (Cutter SS) and Table 4b (Formula A) of the study report, included as an attachment to this DER. In the SS formulation, the mean hourly absorption rate gradually increased and did not reach its peak until 15 hours post-dose (0.325% dose/hour). However, in Formula A, the mean hourly absorption rate peaked earlier (at 6 hours) and at a higher rate (0.702% dose/hour).

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS'S CONCLUSIONS: Taking into account inter-individual variability, a tendency towards higher dermal penetration in all skin layers (surface dose, *stratum corneum*, skin, receptor compartment) was observed in Formula A compared to the SS formulation (between 1.4 and 3.0 fold difference). After 24 hours, the mean percentage of [^{14}C]-KBR 3023 considered to be directly absorbed was 5.92% and 12.44% of the applied dose (normalized to 100% recovery) for the SS and Formula A. formulations, respectively, yielding a factor difference of 2.1 between the two formulations. The mean percentage of [^{14}C]-KBR 3023 considered to be potentially absorbable (i.e., total mean % directly absorbed + total mean % at dose site) from the SS formulation was 9.85% and 18.91% of the applied dose for the SS and Formula A. formulations, respectively, yielding a factor difference of 1.9 between the two formulations.

B. REVIEWER COMMENTS: Higher mean dermal penetration in all skin layers (surface dose, *stratum corneum*, remaining skin, and receptor compartment) was observed in Formula A compared to the SS formulation. The mean percentage of [^{14}C]-KBR 3023 that was directly absorbed (i.e., present in the receptor chamber and fluid) was 2.1 fold higher in Cutter Insect Repellent Formula A (12.44% dose) compared to the SS Formula (5.92% dose). Similarly, the mean percentage of [^{14}C]-KBR 3023 that was potentially absorbable (i.e., in the skin excluding top two tape strips of *stratum corneum*) was 1.6 fold higher in Formula A (6.47% dose) compared to the SS Formula (3.93% dose). Thus, the sum of the mean percentage of [^{14}C]-KBR 3023 that was directly absorbed and that which remained in the skin (including lower *stratum corneum*) for potential absorption was 1.9 fold higher in Formula A (18.91% dose) compared to the SS Formula (9.85% dose).

Examination of mean hourly receptor fluid samples showed that absorption occurred earlier and to a greater extent in Formula A (without sunscreen) compared to the SS Formula. In the SS formulation, the mean hourly absorption rate gradually increased and did not reach its peak until 15 hours post-dose (0.325% dose/hour). However, in Formula A, the mean hourly absorption rate peaked earlier (at 6 hours) and at a higher rate (0.702% dose/hour).

It appears that the addition of sunscreen to the Cutter Insect Repellent Formula A did not increase dermal penetration through human skin in this *in vitro* experiment. In fact, the addition of sunscreen to the formulation decreased the absorption by a factor of two. It is possible that the sunscreen additive(s) reduced absorption by providing a barrier or by binding to the KBR 3023. Additionally, the reviewers noted that the Formulation A is comprised of 60.6% ethanol, whereas the SS formulation is 91.4% ethanol, which may affect

absorption.

C. **STUDY DEFICIENCIES:** The following minor deficiencies were noted under the OECD Guideline 428, but were not considered to affect the interpretation or acceptability of this study:

- Achieved doses were low (69.52 and 72.49% nominal in the SS and Formula A formulations, respectively). The study author stated that this was due to the high viscosity and low volume used for dosing. Because this finding was similar among replicates and between formulations, it did not impact the study's purpose of comparing absorption between the two formulations.
- The skin samples used in this study came from only 5 donors. However, it was ensured that the skin from each donor was used for each formulation, thus equalizing the variability between the two formulation treatment groups.
- The application volume of 4.2 µl appeared to be small compared with 10 µl which is routinely applied. The small volume may have underestimated the dermal penetration results; however, since the identical volume was used for both formulations, the differences would not have significant effect on the comparison of two formulations.

ATTACHMENT

The following data are Tables 4a and 4b on pages 57 & 59 of the study report.

Study SA 07027
Normalized recovery of radioactivity (% dose applied)
Cutter Insect Repellent SS formulation

Sample	Timepoint	H01	H02	H03	H04	H05	H06	mean	SD
Receptor Fluid	1 m	0.006	0.010	0.005	0.003	0.003	0.012	0.006	0.004
Receptor Fluid	1 h	0.018	0.032	0.019	0.014	0.018	0.045	0.024	0.012
Receptor Fluid	2 h	0.024	0.049	0.018	0.020	0.040	0.041	0.032	0.013
Receptor Fluid	3 h	0.036	0.062	0.018	0.033	0.075	0.035	0.047	0.026
Receptor Fluid	4 h	0.052	0.126	0.022	0.049	0.120	0.032	0.067	0.045
Receptor Fluid	5 h	0.080	0.176	0.027	0.066	0.170	0.035	0.092	0.065
Receptor Fluid	6 h	0.106	0.225	0.034	0.090	0.208	0.035	0.115	0.084
Receptor Fluid	7 h	0.142	0.274	0.043	0.090	0.241	0.037	0.138	0.101
Receptor Fluid	8 h	0.211	0.446	0.089	0.112	0.324	0.046	0.205	0.155
Receptor Fluid	9 h	0.267	0.489	0.114	0.130	0.343	0.057	0.234	0.164
Receptor Fluid	10 h	0.281	0.514	0.118	0.136	0.331	0.052	0.239	0.171
Receptor Fluid	11 h	0.313	0.547	0.136	0.143	0.367	0.048	0.259	0.185
Receptor Fluid	12 h	0.333	0.598	0.162	0.146	0.390	0.044	0.279	0.202
Receptor Fluid	13 h	0.351	0.629	0.170	0.143	0.406	0.041	0.290	0.215
Receptor Fluid	14 h	0.383	0.685	0.294	0.152	0.432	0.040	0.331	0.226
Receptor Fluid	15 h	0.381	0.699	0.261	0.145	0.432	0.033	0.325	0.235
Receptor Fluid	16 h	0.365	0.699	0.229	0.148	0.447	0.032	0.320	0.238
Receptor Fluid	17 h	0.383	0.677	0.203	0.142	0.431	0.028	0.311	0.234
Receptor Fluid	18 h	0.381	0.651	0.188	0.142	0.435	0.027	0.304	0.228
Receptor Fluid	19 h	0.369	0.635	0.183	0.140	0.439	0.025	0.298	0.224
Receptor Fluid	20 h	0.349	0.590	0.172	0.138	0.431	0.021	0.283	0.211
Receptor Fluid	21 h	0.329	0.577	0.170	0.138	0.425	0.020	0.276	0.208
Receptor Fluid	22 h	0.283	0.535	0.160	0.128	0.419	0.017	0.257	0.194
Receptor Fluid	23 h	0.132	0.253	0.074	0.062	0.198	0.008	0.121	0.092
Receptor Fluid	24 h	0.169	0.369	0.110	0.089	0.253	0.012	0.170	0.128
	Subtotal	*5.762*	*10.567*	*3.018*	*2.588*	*7.377*	*0.822*	*5.023*	*3.589*
Rec. Fluid Term	24 h	0.536	0.935	0.293	0.259	0.892	0.028	0.491	0.365
Receptor chamber	24 h	0.000	1.160	0.773	0.000	0.529	0.000	0.410	0.493
Total % directly absorbed		**6.299**	**12.662**	**4.084**	**2.848**	**8.798**	**0.850**	**5.924**	**4.299**
Swabs	8 h	79.481	59.825	84.688	87.208	74.072	97.173	80.408	12.730
Swabs X	8 h	0.209	0.832	0.443	0.143	0.518	0.161	0.384	0.268
Swabs Y	8 h	0.257	0.264	0.352	0.317	0.577	0.165	0.325	0.139
Swabs Z	8 h	0.171	0.500	0.219	0.615	0.609	0.173	0.381	0.216
Swabs term	24 h	0.473	0.587	0.274	0.370	0.505	0.045	0.376	0.195
Surrounding swabs	24 h	0.088	0.035	0.009	0.037	0.004	0.000	0.029	0.033
	Subtotal	*80.679*	*62.063*	*85.985*	*88.690*	*76.285*	*97.716*	*81.903*	*12.161*
Donor chamber	24 h	6.181	10.956	3.605	4.014	6.346	0.995	5.350	3.374
SC1	24 h	1.300	2.285	1.822	1.390	2.584	0.052	1.572	0.896
SC2	24 h	1.121	3.356	0.529	0.879	1.254	0.024	1.192	1.149
	Subtotal	*8.602*	*16.597*	*5.948*	*6.283*	*10.184*	*1.071*	*8.114*	*5.180*
Total % non-absorbed		**89.281**	**78.660**	**91.932**	**94.973**	**86.469**	**98.787**	**90.017**	**7.032**
Skin	24 h	0.576	4.095	2.565	0.674	1.468	0.279	1.626	1.458
Surrounding skin	24 h	1.366	0.924	0.022	0.033	0.575	0.008	0.488	0.570
	Subtotal	*2.042*	*5.019*	*2.587*	*0.707*	*2.043*	*0.287*	*2.114*	*1.673*
SC3	24 h	0.602	0.667	0.498	0.234	0.581	0.013	0.433	0.255
SC4	24 h	0.435	1.117	0.500	0.301	1.354	0.009	0.624	0.520
SC5	24 h	0.279	0.235	0.399	0.168	0.724	0.008	0.302	0.243
SC6	24 h	0.211	0.172	N.S.	N.S.	N.S.	0.011	0.131	0.106
SC7	24 h	0.229	1.190	N.S.	N.S.	N.S.	0.005	0.475	0.629
SC8	24 h	0.144	0.108	N.S.	N.S.	N.S.	0.004	0.085	0.072
SC9	24 h	0.124	0.171	N.S.	N.S.	N.S.	0.005	0.100	0.085
SC 10	24 h	0.080	N.S.	N.S.	N.S.	N.S.	0.019	0.049	0.043
SC 11	24 h	0.275	N.S.	N.S.	N.S.	N.S.	N.S.		
SC 12	24 h	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.		
SC 13	24 h	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.		
SC 14	24 h	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.		
SC 15	24 h	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.		
SC 16	24 h	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.		
SC 17	24 h	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.		
SC 18	24 h	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.		
SC 19	24 h	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.		
SC 20	24 h	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.		
	Subtotal	*2.379*	*3.658*	*1.397*	*0.702*	*2.689*	*0.076*	*1.817*	*1.335*
Total % at dose site (skin + stratum corneum)		**4.420**	**8.677**	**3.984**	**1.409**	**4.732**	**0.363**	**3.931**	**2.916**
Total Potentially absorbable		**10.719**	**21.340**	**8.068**	**4.256**	**13.531**	**1.213**	**9.854**	**5.889**
Total recovery		100.000	100.000	100.000	100.000	100.000	100.000		

Study SA 07027
Normalized recovery of radioactivity (% dose applied).
Cutter Insect Repellent Formula A formulation

Sample	Timepoint	H07	H08	H09	H10	H12	mean	SD
Receptor Fluid	1 m	0.014	0.000	0.005	0.000	0.003	0.004	0.006
Receptor Fluid	1 h	0.085	0.000	0.021	0.009	0.021	0.027	0.034
Receptor Fluid	2 h	0.182	0.000	0.224	0.035	0.065	0.101	0.087
Receptor Fluid	3 h	0.664	0.000	0.634	0.078	0.196	0.315	0.314
Receptor Fluid	4 h	0.713	0.000	0.561	0.128	0.434	0.367	0.297
Receptor Fluid	5 h	1.008	0.004	0.518	0.179	0.764	0.495	0.412
Receptor Fluid	6 h	1.302	0.154	0.563	0.260	1.238	0.702	0.538
Receptor Fluid	7 h	0.939	0.629	0.477	0.257	1.030	0.666	0.321
Receptor Fluid	8 h	0.831	0.520	0.604	0.378	0.958	0.658	0.235
Receptor Fluid	9 h	0.659	0.413	0.420	0.381	0.780	0.531	0.178
Receptor Fluid	10 h	0.572	0.363	0.555	0.408	1.004	0.580	0.254
Receptor Fluid	11 h	0.528	0.322	0.566	0.430	1.186	0.607	0.337
Receptor Fluid	12 h	0.492	0.317	0.572	0.451	1.196	0.606	0.343
Receptor Fluid	13 h	0.463	0.298	0.551	0.473	1.169	0.591	0.336
Receptor Fluid	14 h	0.427	0.000	0.531	0.488	1.190	0.527	0.427
Receptor Fluid	15 h	0.433	0.285	0.523	0.513	1.153	0.581	0.333
Receptor Fluid	16 h	0.427	0.281	0.496	0.528	1.135	0.573	0.328
Receptor Fluid	17 h	0.402	0.277	0.469	0.535	0.541	0.445	0.109
Receptor Fluid	18 h	0.376	0.278	0.439	0.552	0.223	0.374	0.130
Receptor Fluid	19 h	0.348	0.268	0.423	0.557	0.294	0.378	0.116
Receptor Fluid	20 h	0.345	0.260	0.397	0.565	0.178	0.349	0.147
Receptor Fluid	21 h	0.333	0.253	0.380	0.663	0.204	0.347	0.139
Receptor Fluid	22 h	0.324	0.249	0.353	0.589	0.094	0.317	0.173
Receptor Fluid	23 h	0.145	0.106	0.146	0.249	0.049	0.139	0.073
Receptor Fluid	24 h	0.236	0.187	0.277	0.557	0.056	0.263	0.184
	Subtotal	*12.251*	*5.463*	*10.702*	*9.143*	*15.157*	*10.543*	*3.606*
Rec. Fluid Term	24 h	0.708	0.557	0.715	1.221	1.422	0.924	0.375
Receptor chamber	24 h	0.419	0.682	0.520	1.936	1.296	0.971	0.638
Total % directly absorbed		**13.378**	**6.702**	**11.937**	**12.300**	**17.877**	**12.439**	**3.989**
Swabs	8 h	67.065	80.262	80.970	39.990	55.142	64.686	17.424
Swabs X	8 h	1.987	0.326	0.342	0.210	0.278	0.625	0.752
Swabs Y	8 h	1.175	0.189	0.076	0.044	0.117	0.316	0.482
Swabs Z	8 h	0.689	0.207	0.690	0.277	0.263	0.425	0.243
Swabs term	24 h	1.344	0.553	0.315	0.184	0.212	0.521	0.482
Surrounding swabs	24 h	0.361	0.718	0.493	2.605	4.480	1.731	1.786
	Subtotal	*72.602*	*82.235*	*82.985*	*43.310*	*60.493*	*68.305*	*16.665*
Donor chamber	24 h	1.589	1.984	1.332	6.562	7.900	3.873	3.110
SC1	24 h	5.017	2.748	1.604	15.358	8.358	6.217	5.439
SC2	24 h	1.834	1.806	1.144	2.919	3.082	2.157	0.820
	Subtotal	*8.440*	*6.538*	*4.080*	*24.839*	*17.335*	*4.013*	*8.635*
Total % non-absorbed		**81.042**	**88.774**	**86.965**	**68.149**	**77.830**	**80.552**	**8.219**
Skin	24 h	0.900	1.456	0.869	13.493	2.570	3.858	5.430
Surrounding skin	24 h	0.004	0.005	0.011	0.004	0.235	0.051	0.103
	Subtotal	*0.905*	*1.461*	*0.880*	*13.497*	*2.805*	*3.909*	*5.416*
SC3	24 h	2.379	2.273	0.060	2.718	0.838	1.654	1.145
SC4	24 h	0.798	0.455	0.070	0.853	0.472	0.530	0.315
SC5	24 h	0.332	0.235	0.038	0.836	0.177	0.324	0.306
SC6	24 h	0.677	0.100	0.049	0.558	N.S	0.346	0.318
SC7	24 h	0.491	N.S	N.S	0.259	N.S	0.371	
SC8	24 h	N.S.	N.S.	N.S.	0.327	N.S.		
SC9	24 h	N.S.	N.S	N.S.	0.347	N.S.		
SC 10	24 h	N.S.	N.S	N.S	0.099	N.S.		
SC 11	24 h	N.S.	N.S	N.S	0.065	N.S.		
SC 12	24 h	N.S.	N.S	N.S	N.S.	N.S.		
SC 13	24 h	N.S	N.S	N.S	N.S.	N.S.		
SC 14	24 h	N.S	N.S.	N.S.	N.S.	N.S.		
SC 15	24 h	N.S.	N.S.	N.S	N.S.	N.S		
SC 16	24 h	N.S.	N.S	N.S.	N.S	N.S		
SC 17	24 h	N.S	N.S	N.S	N.S	N.S		
SC 18	24 h	N.S.	N.S.	N.S	N.S.	N.S.		
SC 19	24 h	N.S.	N.S.	N.S	N.S.	N.S		
SC 20	24 h	N.S.	N.S.	N.S.	N.S.	N.S.		
	Subtotal	*0.218*	*3.064*	*1.979*	*6.055*	*1.487*	*2.560*	*2.205*
Total % at dose site (skin + stratum corneum)		**1.121**	**4.525**	**2.859**	**19.552**	**4.292**	**6.470**	**7.438**
Total % potentially absorbable		**14.499**	**11.226**	**14.796**	**31.851**	**22.170**	**18.908**	**8.269**
Total % recovery		100.00	100.00	100.00	100.00	100.00		

DATA EVALUATION RECORD

KBR 3023 (PICARIDIN)

Study Type: Non-guideline; Comparative *In Vitro* Dermal Penetration Study Using Human and Rat Skin

Work Assignment No. 5-1-176 A (MRID 47342201)

Prepared for

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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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EPA Work Assignment Manager: Myron Ottley, Ph.D. **Signature:** Myron Ottley
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 6/26/08
Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Comparative *In Vitro* Dermal Absorption Study Using Human and Rat Skin; Non-guideline, OPPTS None; OECD 428

PC CODE: 070705 **DP BARCODE:** D350980
TXR#: 0054846

TEST MATERIAL (RADIOCHEMICAL PURITY): [^{14}C]-KBR 3023 (>99%)

SYNONYMS: Picaridin; Icaridin; 1-methylpropyl 2-(2-hydroxyethyl)-1-piperidinecarboxylate

CITATION: Rascle, J. B. (2008) KBR 3023 technical and KBR 3023 15% in ethanol: Comparative *in vitro* dermal absorption study using human and rat skin. Bayer CropScience, Sophia, Antipolis, Cedex, France. Laboratory Study No.: SA 07026, January 11, 2008. MRID 47342201. Unpublished

SPONSOR: Saltigo GmbH (Lanxess Group), Q 18-2, Raum 758, Leverkusen, Germany

EXECUTIVE SUMMARY: In a dermal penetration study (MRID 47342201), KBR 3023 technical and KBR 3023 15% (w/v) in ethanol were tested to compare *in vitro* dermal penetration following a single dermal application to excised human and rat skin. [Hydroxyethyl-1-^{14}C]-KBR 3023 and non-radiolabeled KBR 3023 technical were combined and applied as either the technical formulation or at 15% (w/v) in ethanol. These test formulations were applied at a nominal concentration of 0.63 mg/cm^2 (achieved dose was 0.44 – 0.54 mg/cm$^{2)}$ to 1 cm^2 of excised human or rat dermatomed skin (6 replicates per formulation) for an 8-hour exposure period. After 8 hours, the remaining test material was washed off, and penetration into the receptor fluid was measured at hourly intervals over a 24-hour period. At the end of the study (24 hours after application), the skin samples were swabbed again and then were tape-stripped to remove residual surface dose and the *stratum corneum*. The non-absorbed dose was defined as the amount in the swabs, donor chamber, and first two tape strips; the directly absorbed dose was the amount in the receptor fluid and receptor chamber; and the dose present at the dosing site was considered available for potential absorption. The diffusion cell components were retained and washed, and the washings, along with all other samples, were analyzed to establish a mass balance.

Mean total recoveries ranged from 100.1-115.1% of the applied dose. For the technical formulation, 7.3% of the applied dose was directly absorbed through human skin, while 21.8% of the applied dose was directly absorbed through rat skin (2.97-fold difference). For the 15% (w/v) ethanol formulation, 5.6% of the applied dose was directly absorbed through human skin, while

16.8% of the applied dose was directly absorbed through rat skin (3.01-fold difference). For the technical formulation, 10.7% of the applied dose was potentially absorbable through human skin, while 25.2% of the applied dose was potentially absorbable through rat skin (2.4-fold difference). For the 15% (w/v) ethanol formulation, 9.8% of the applied dose was potentially absorbable through human skin, while 22.3% of the applied dose was potentially absorbable through rat skin (2.3-fold difference).

It was stated that in a previously performed *in vivo* dermal absorption study using human volunteers (MRID 44408738, not provided), the dermal absorption of [^{14}C]-KBR 3023 technical and a 15% ethanol formulation applied at dose levels similar to those used in the current study were 1.66% and 3.77% of the applied doses, respectively. Thus, the difference between *in vitro* and *in vivo* dermal absorption through human skin was 6.42-fold and 2.60-fold for the technical and 15% ethanol formulations, respectively. Similarly, in a previously performed *in vivo* dermal absorption study using male rats (MRID 44408737, not provided), the dermal absorption of [^{14}C]-KBR 3023 technical applied at a dose level similar to that used in the current study was 18.9% of the applied doses. Thus, the difference between *in vitro* and *in vivo* dermal absorption through rat skin was 1.33-fold for the technical formulations.

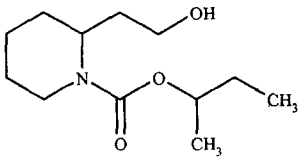
This study is classified as **acceptable/non-guideline;** however, it does satisfy the OECD guideline requirements (OECD 428) for an *in vitro* skin absorption study using excised skin.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: KBR 3023 (Picaridin)

Description:	Clear colorless to brownish liquid
Batch #:	CHCAEC0069
Purity:	98.8% a.i.
Compound stability:	Not reported
CAS # for TGAI:	119515-38-7
Structure:	
Receptor fluid:	Eagle's medium (pH 7.3-7.4) supplemented with 5% bovine serum albumin and gentamycin
Radiolabelled material:	[Hydroxyethyl-1-^{14}C]-KBR 3023
Specific Activity:	3.55 MBq/mg (96 µCi/mg)
Radiochemical Purity:	>99% (HPLC and TLC)
Source:	Bayer CropScience AG, Product Technology, Isotope Chemistry (Wuppertal, Germany)

2. Dose level rationale: The concentration selected (0.63 mg/cm^2 KBR 3023) was equivalent to the dose used in a previously conducted human volunteer study, and was considered to reflect the anticipated human exposure level to the product in commercial formulations.

B. STUDY DESIGN

1. Objectives: The objectives of this study were:

- To determine and compare the dermal absorption of KBR 3023 technical and the dermal absorption of KBR 3023 15% (w/v) in ethanol by monitoring the radioactivity in an *in vitro* system using flow-through diffusion cells over a period of 24 hours.
- To quantify the amount of radioactivity remaining on the skin surface (unabsorbed) at 8 hours post-application.
- To quantify the amount of radioactivity remaining on and in the skin at 24 hours post-application.
- To compare the permeability of human and rat skin to KBR 3023.

2. Test system

Human: Dermatomed abdominal human skin (310-350 µM thick) from three different Caucasian female donors was obtained from Biopredic Tissue Bank (Rennes, France) or from a local hospital (CHU, Nice, France) and stored at approximately -20°C until use. Subject ages were 33, 64, and 66 years old. It was stated that human skin from male donors was not available at the time of study performance.

Rat

Species:	Rat (male only)
Strain:	Wistar (Rj:WI (IOPS HAN)
Age at study initiation:	Young adult (protocol specified 6-8 weeks)
Body weight at study initiation:	237-483 g
Source:	R. Janvier (Le Genest St Isle, France)
Housing:	Group housed in a wire-mesh bottomed stainless steel cage during acclimation
Acclimation period:	At least 5 days

Following acclimation, each rat was killed by cervical dislocation, and an area of dorsal skin was clipped and excised. The skin was dermatomed to a thickness of 320-350 µM, similar to the human skin samples used in the study.

3. **Diffusion cell design:** Absorption of the test compound formulations was measured using a flow-through diffusion cell system (modified Franz cell; Gallas, France). Each diffusion cell chamber consisted of a donor chamber and a receptor chamber between which the skin was positioned, providing an exposure area of 1 cm^2. The receptor chamber and fluid were maintained at 32 ± 2°C by a water bath in order to approximate normal skin temperature. The receptor fluid was pumped through the receptor chamber at a rate of 1.5 mL/h and stirred continuously in the chamber by a magnetic stirring bar (400 rpm).

4. **Receptor fluid:** The receptor fluid was Eagle's medium (pH 7.3-7.4) supplemented with 5% bovine serum albumin and gentamycin. The solubility of [^{14}C]-KBR 3023 in the receptor fluid was verified prior to the study by dissolving the volume of [^{14}C]-KBR 3023 corresponding to the maximum quantity of the test material applied to the cell (0.63 mg) in approximately 3 mL of receptor fluid, corresponding to the total volume of the cell. This procedure simulated the conditions of maximum absorption through the skin. The amount of radioactivity found: immediately after dilution; 72 hours after dilution; and after centrifugation were similar and ranged from 105.7-109.6% of the initial amount, indicating that [^{14}C]-KBR 3023 was fully soluble in the receptor fluid.

5. **Skin membrane selection and integrity:** Prior to dose application, the integrity of the skin samples was assessed by measuring the trans-epidermal water loss (TEWL) from the *stratum corneum*. An evaporator probe (Cortex Technology, Hadsund, Denmark) was placed on top of the donor chamber and the rate of water diffusing through the skin was measured in g/m^2h. It was stated that skin samples with a TEWL of >40 g/m^2h were considered potentially damaged and were not used. The TEWL for skin samples used in this study ranged from 1.0-27.7 g/m^2h for human skin and 7.4-39.2 g/m^2h for rat skin. Therefore, all samples were in the acceptable range for use in the study.

6. Dose preparation, application, and quantification

Dose preparation: Two formulations of the test substance were prepared at the performing laboratory by combining appropriate amounts of [^{14}C]-KBR 3023 and non-radiolabeled technical test material. One formulation was prepared undiluted and applied at a dose volume of 0.63 µL/cm^2; the other was prepared as a 15% (w/v) dilution in ethanol and applied at a dose volume of 4.2 µL/cm^2 (Table 1). Test formulations were stored at -20°C in the dark until application.

TABLE 1. Dosing [a]

Dose level (µg/cm^2)	Amount compound in dosing solution (mg)		Final Specific activity (MBq/mg) [b]	Nominal dose (mg/cm^2)	Achieved dose (KBq)	Achieved dose (mg/cm^2) [c]
	Radio-labeled	Non-labeled				
KBR 3023 technical	2.8	197	0.050	0.63	21.9	0.44
KBR 3023 15% (w/v) in ethanol	0.6	59	0.067	0.63	35.9	0.54

a Data were obtained from pages 19 and 24, and Tables 1d and 1e on pages 60 and 62 of the study report.
b The actual specific activity of the isotopically diluted test solutions was calculated by the reviewers by dividing the weight of the radio-labeled test material by the sum of the radio-labeled and non-labeled.
c Calculated by the reviewers by dividing the achieved doses of radioactivity by the final specific activity.

Dose application: The dose preparations were applied to the skin samples using a micro-pipette to achieve a nominal concentration of 0.63 mg/cm^2 and left on the skin for eight hours.

Dose quantification: The actual doses applied were determined by taking three surrogate dose samples: one prior to the first application; another during dosing (after the third application); and a final sample after the last application. Each of these samples (0.60 µL for the technical formulation; 4.2 µL for the 15% ethanol formulation) were diluted with acetonitrile, and triplicate aliquots were taken for measurement of radioactivity using liquid scintillation counting (LSC). It was stated that the achieved doses were considered acceptable to achieve the objectives of this study.

Homogeneity of the radiochemical doses were determined in five samples of each dose formulation taken before application. Homogeneity results were 89.8-99.0% nominal; 5.87-8.27% coefficient of variation. Thus, the analytical data indicated that the mixing procedure was adequate.

7. **Experimental procedure and sample collection:** The study design used six human and six rat skin samples for each formulation. The formulations were applied to the *stratum corneum* of the skin for eight hours, after which time the remaining dose was washed off the skin with 1% v/v Tween 80 in phosphate buffered saline (PBS) using natural sponge swabs until no radioactivity was detected by a Geiger-Müller monitor. Receptor fluid samples were collected at hourly intervals for the duration of the study (24 hours after application), starting after the dose application for each group was complete. At the end of the study, the skin samples were swabbed again and then were tape-stripped to remove the residual surface

dose and the *stratum corneum*. Skin was tape-stripped by applying Monaderm adhesive tape (Monaderm, Monaco) for 5 seconds and carefully removing it against the direction of hair growth. This procedure was repeated until a shiny appearance of the epidermis was evident, indicating the *stratum corneum* had been removed. The area of skin surrounding the application site was also swabbed (surrounding swabs). After tape-stripping, the remaining skin was removed, and the skin surrounding the application site was separated from the treated skin. Both compartments (treated skin and surrounding skin) were saved for analysis. The receptor fluid remaining in the cell and outlet tubing at the end of the experiment was collected and analyzed. The diffusion cell components were retained and washed, and the washings were analyzed to establish a mass balance.

8. **Sample preparation and analysis:** The weights of each of the collected samples were obtained either directly (tared measurement) or indirectly (by difference between the full and empty container). Whenever possible, the samples were processed as they were collected. The remaining samples were stored at approximately -20°C in the dark. The amounts of radioactivity in the various samples were determined using LSC. Quenching effects were determined using an external standard and spectral quench parameter (tSIE) method. The limit of detection was taken to be twice the background values for blank samples. Total amounts of radioactivity in samples were reported as a percentage of the total.

   Receptor fluid samples for each interval were weighed. Scintillation fluid was added directly to each receptor fluid sample and to the receptor fluid/outlet tubing contents and analyzed. Receptor fluid at study termination (terminal receptor fluid) was weighed, and duplicate weighed aliquots were taken for analyses.

   With the exception of the final three swabs used at eight hours to wipe the application site, swabs taken at eight hours were weighed and solubilized using an appropriate volume of Soluene. Triplicate aliquots were taken for analysis. Each of the following swabs was solubilized using 2 mL of Soluene, and scintillation fluid was added directly to each vial for analysis: the last three swabs at the end of the 8-hour exposure period; the terminal swabs (at 24 hours) from the application site; and surrounding swabs at 24 hours.

   Each tape strip was individually solubilized using tetrahydrofuran, and scintillation fluid was added directly to each sample for analysis.

   Each treated skin or surrounding skin sample was solubilized separately using Soluene, and scintillation fluid was added directly to each vial for analysis.

   The diffusion cell components were sampled in the following manner: the receptor chamber (including the stirrer bar and outlet tubing), the donor chamber (including securing screws), the cell and outlet tubing were soaked separately in a mixture of acetonitrile/distilled water (50:50 v/v) for 12 hours. The diffusion cell components were then removed and monitored for radioactivity using a Geiger-Müller monitor. The washings were weighed, and duplicate weighed aliquots were taken for analysis.

9. **Calculations:** Based upon the quantitative analyses of radioactivity from the different media, the following parameters were calculated:

   Non-absorbed = amount in the swabs, donor chamber, and first two tape strips

   Directly absorbed = amount in the receptor fluid and receptor chamber
   Potentially absorbed (present at the dosing site) = tape strips (excluding the first two) and in the skin (treated skin + untreated skin) at the application site

II. RESULTS

A. **SUMMARY TABLE:** Mean total recoveries ranged from 100.1-115.1% of the applied dose (Table 2). For the technical formulation, 7.3% of the applied dose was directly absorbed through human skin, while 21.8% of the applied dose was directly absorbed through rat skin (2.97-fold difference). For the 15% (w/v) ethanol formulation, 5.6% of the applied dose was directly absorbed through human skin, while 16.8% of the applied dose was directly absorbed through rat skin (3.01-fold difference). For the technical formulation, 10.7% of the applied dose was potentially absorbable through human skin, while 25.2% of the applied dose was potentially absorbable through rat skin (2.4-fold difference). For the 15% (w/v) ethanol formulation, 9.8% of the applied dose was potentially absorbable through human skin, while 22.3% of the applied dose was potentially absorbable through rat skin (2.3-fold difference).

TABLE 2. Mean (± SD) *in vitro* dermal absorption of [^{14}C]-KBR 3023 (% dose) in human and rat skin after 24 hr [a]				
Sample	KBR 3023 Technical		KBR 3023 15% (w/v) in ethanol	
	Human (n=5) [b]	Rat (n=4) [c]	Human (n=5) [d]	Rat (n=4) [e]
Surface Compartment				
Surface dose (tape strips 1 and 2)	1.399±1.195	0.438±0.571	1.123±0.711	0.289±0.286
Skin swabs	84.646±6.742	89.100±19.116	87.004±11.971	78.997±12.543
Donor chamber	3.382±3.845	0.383±0.424	2.362±1.689	5.795±10.455
Total % non-absorbed	**89.427±8.879**	**89.921±18.838**	**90.460±11.030**	**85.081±6.588**
Skin Compartment				
Skin [f]	2.112±1.351	1.571±2.461	**2.084±0.857**	4.796±5.511
Stratum corneum [g]	1.211±0.965	1.829±2.867	2.157±2.042	0.783±0.654
Total % at dose site	**3.322±2.283**	**3.401±5.321**	**4.242±2.407**	**5.580±6.041**
Receptor Compartment				
Total % directly absorbed [h]	**7.336±3.831**	**21.760±12.364**	**5.571±1.291**	**16.763±6.158**
Total Absorbable				
Total % directly absorbed	**7.336±3.831**	**21.760±12.364**	**5.571±1.291**	**16.763±6.158**
Total % at dose site	**3.322±2.283**	**3.401±5.321**	**4.242±2.407**	**5.580±6.041**
Total % potentially absorbable	**10.658±4.847**	**25.161±11.627**	**9.812±3.527**	**22.342±10.069**
Total % recovery	**100.085±11.437**	**115.082±9.564**	**100.302±8.872**	**107.423±7.534**

a Data were obtained from pages 12, 24, and 27 of the study report.

b Human skin data were obtained from five cells; sample H05 was excluded because the percentages of radioactivity found in the skin swabs at 8 h and the total % recovery were unusually high and much higher than the other cells of this group. Therefore, it is assumed that the application rate was too high.

c Rat skin data were obtained from four cells. Sample R01 was excluded because the % of radioactivity recovered in the receptor fluid was unusually high and much higher than the other cells of this group at the early time points; therefore, this membrane was probably damaged. Sample R05 was excluded because the total % recovery was unusually high and much higher than the other cells of this group.

d Human skin data were obtained from five cells; sample H08 was excluded because the total % recovery was unusually low compared to the other cells of this group.

e Rat skin data were obtained from four cells. Sample R07 was excluded because of a technical problem encountered during the collection of receptor fluid from this cell. Sample R12 was excluded because the percentages of radioactivity found in the swabs at 8 h and the total % recovery value were very low compared to the other cells of this group.

f After tape-stripping procedure

g Tape-strips excluding #s1 and 2, which are considered to be part of the non-absorbed dose

h Includes receptor fluid (0-24 h), receptor fluid at termination, and receptor chamber

B. COMPARISON OF *IN VIVO* AND *IN VITRO* DERMAL ABSORPTION OF KBR 3023: It was stated that in a previously performed *in vivo* dermal absorption study using human volunteers (MRID 44408738, not provided), the dermal absorption of [^{14}C]-KBR 3023 technical and a 15% ethanol formulation applied at dose levels similar to those used in the current study were 1.66% and 3.77% of the applied doses, respectively. Thus, the difference between *in vitro* and *in vivo* dermal absorption through human skin was 6.42-fold and 2.60-fold for the technical and 15% ethanol formulations, respectively. Similarly, in a previously performed *in vivo* dermal absorption study using male rats (MRID 44408737, not provided), the dermal absorption of [^{14}C]-KBR 3023 technical applied at a dose level similar to that used in the current study was 18.9% of the applied doses. Thus, the difference between *in vitro* and *in vivo* dermal absorption through rat skin was 1.33-fold for the technical formulations.

III. DISCUSSION AND CONCLUSIONS

A. **INVESTIGATOR'S CONCLUSIONS:** Following dermal application of [^{14}C]-KBR 3023 technical and 15% in ethanol, the results obtained were found to be relatively similar for the two formulations. The level of direct absorption of KBR 3023 technical and in 15% ethanol through human skin was approximately three times lower than for rat skin. When taking the conservative approach that radioactivity in the *stratum corneum* and in the skin could potentially be absorbed later, the level of potential absorption through human skin was 2.3-2.4 times lower than that of the rat skin.

B. **REVIEWER COMMENTS:** Mean total recoveries ranged from 100.1-115.1% of the applied dose. For the technical formulation, 7.3% of the applied dose was directly absorbed through human skin, while 21.8% of the applied dose was directly absorbed through rat skin (2.97-fold difference). For the 15% (w/v) ethanol formulation, 5.6% of the applied dose was directly absorbed through human skin, while 16.8% of the applied dose was directly absorbed through rat skin (3.01-fold difference). For the technical formulation, 10.7% of the applied dose was potentially absorbable through human skin, while 25.2% of the applied dose was potentially absorbable through rat skin (2.4-fold difference). For the 15% (w/v) ethanol formulation, 9.8% of the applied dose was potentially absorbable through human skin, while 22.3% of the applied dose was potentially absorbable through rat skin (2.3-fold difference).

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C. **STUDY DEFICIENCIES:**

- The application volume (0.63 or 4.2 µl) appeared to be small compared with 10 µl which is routinely applied. The small volume may have underestimated the dermal penetration results; however, since the identical volume was used, the differences would not have significant impact on the comparison of human and rat skin.

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Chemical Name: Picaridin

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