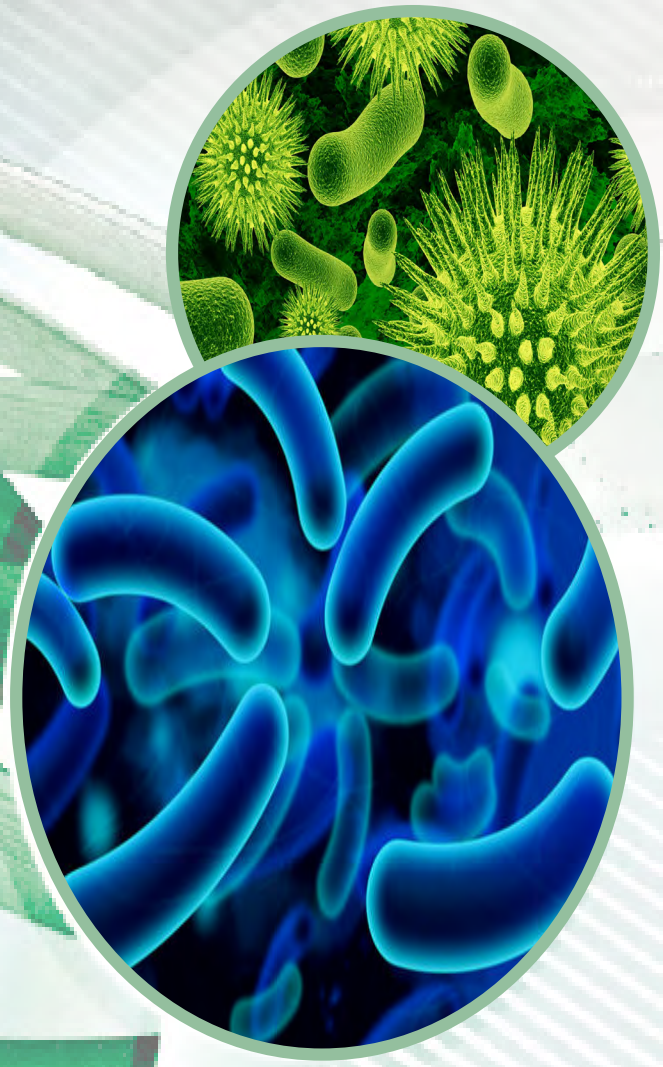
ACTIVE MICRO TECHNOLOGIES

Technical Dossier



ability natural rowan technology Activity
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Improving solar choice antimicrobial

AMTicide® VAF

Code Number: M14004

INCI Name: Bacillus Ferment & Saccharomyces Ferment Filtrate

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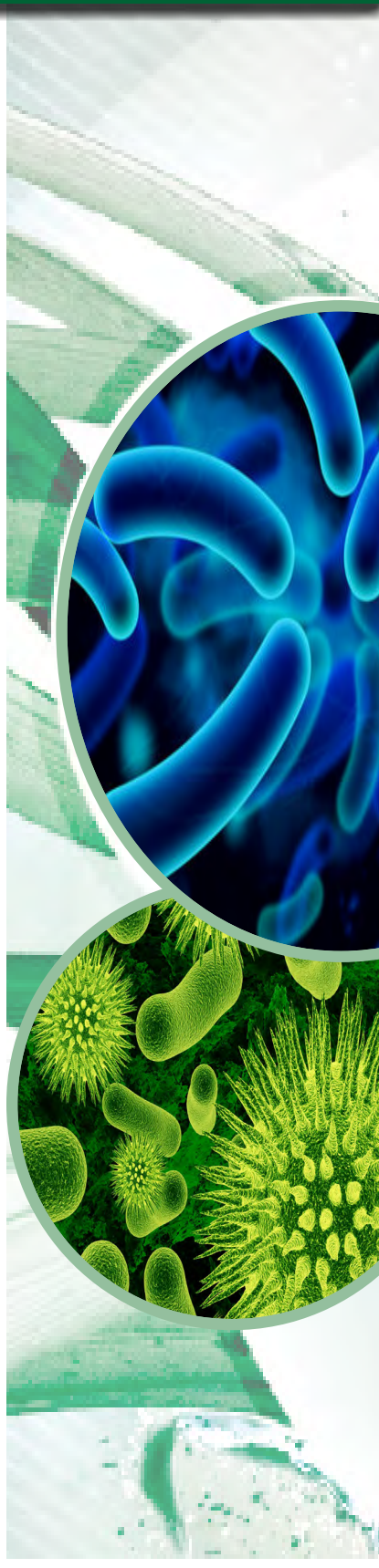

AMTicide® VAF Code Number: M14004
INCI Name: Bacillus Ferment & Saccharomyces Ferment Filtrate

AMTicide® VAF

Technical Data Sheet

BACKGROUND

Consumer demand and ever-changing regulations has driven the market from synthetic material to focus on natural solutions. Active Micro Technologies prides itself in developing and supplying effective, natural products that provide skin and hair conditioning benefits, along with providing natural antimicrobial activity. **AMTicide® VAF** is developed by co-fermenting *Bacillus subtilis* with *Saccharomyces boulardii* in a defined growth media to deliver a non-irritating, effective, multifunctional product. This highly marketable product can provide antioxidant benefits and is capable of preventing the growth of fungus in packaging headspace, making it the perfect addition to any formulation.

Code Number: M14004
INCI Nomenclature: Bacillus Ferment & Saccharomyces Ferment Filtrate
INCI Status: Approved
REACH Status: Fully Compliant
CAS Number: 92128-81-9 (or) 68582-99-0 & 8013-01-2
EINECS Number: 295-779-9 (or) N/A & 232-387-9
Origin: Biotechnology
Processing:
GMO Free
No Ethoxylation
No Irradiation
No Sulphonation
No Ethylene Oxide treatment
No Hydrogenation
Additives: None
Preservatives: None
Antioxidants: None
Other additives: None
Solvents used: Propanediol
Appearance: Hazy Liquid
Soluble/Miscible: Water
Suggested Use Levels: 1.0% maximum
Suggested Applications:
Skin Conditioning, Antifungal

SCIENCE

Active Micro Technologies has identified a gap in the market for a naturally derived volatile antifungal to prevent microbial growth associated with packaging. Packaging is a critical component for the successful preservation of cosmetic and personal care products. For a variety of packaging options, including jars, ensuring that the packaging headspace remains free of contamination is essential to prevent contamination of the entire formulation. Packaging processes are able to create a favorable growth environment for yeast and mold.

For example, leaving space between the contents of the packaging and the lid of the packaging allows sufficient room for microbial growth after a hot poured formulation has cooled. In addition, each time the packaging container is opened and the product is used, the headspace in the packaging increases, exposing more surface area of the formulation to the air. Phenoxyethanol, a synthetic volatile antimicrobial, has been commonly utilized to prevent headspace microbial growth. However, the potential for sensitization and sensory irritation of the skin associated with the use of phenoxyethanol[1], as well as stricter worldwide regulations on the material, pushes formulators to explore alternative options. Active Micro Technologies has successfully provided a natural volatile antifungal solution with the development of **AMTicide® VAF**.

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AMTicide® VAF

AMTicide® VAF is a product of the co-fermentation of *Bacillus subtilis* and *Saccharomyces boulardii* in a defined growth medium. *Bacillus* spp. are well known rhizosphere residents of many crops, including tomato, corn, and soybeans, and produce nonvolatile and volatile secondary metabolites that exhibit antifungal activity as a mechanism of biocontrol to promote plant growth.[2,3] The volatile organic metabolites produced by *Bacillus subtilis* have been known to naturally reduce and prevent plant diseases caused by fungi.

Saccharomyces boulardii is a probiotic strain of yeast, first isolated from lychee and mangosteen fruit in 1934 by a French scientist by the name of Henri Boulard. *Saccharomyces boulardii* has sparked interest around the world, specifically due to its wide variety of interactions with other microbes and ability to disable gastrointestinal disorder and symptoms of gastrointestinal distress. Current research in the food industry has examined the ability of *Saccharomyces* spp. and lactic acid bacteria, such as *Bacillus* spp., to enhance the production of volatile compounds when co-inoculated.[4,5]

Active Micro Technologies has included the inoculation of *Saccharomyces boulardii* in the fermentation process to enhance the bioactivity of the *Bacillus subtilis* volatile metabolites. Using bio-fermentation and various filtration techniques, the volatile organic metabolites are isolated and extracted from the bacteria cell to deliver high potency volatile antifungal activity. Active Micro Technologies has been able to successfully produce a naturally derived, high potency volatile material that delivers moisturizing and antifungal activity for effective headspace protection!

AMTicide® VAF was developed to be used in conjunction with one of our broad-spectrum antimicrobials, however it can be used alongside any preservative package for extra protection against yeast and mold associated with packaging.

BENEFITS

One of the first steps in the development of this product was to determine the products potential ability to inhibit the growth of yeast and mold. Using standard serial dilution protocols in growth media, the Minimum Inhibitory Concentrations (MICs) for **AMTicide® Coconut** were determined for both yeast and mold organisms. The results of these tests are shown in Figure 1.

Microorganism Tested	MIC (%)
E. coli	4.00
P. aeruginosa	2.00
S. aureus	2.00
A. brasiliensis	0.50
C. albicans	0.50

Figure 1. MIC Data for **AMTicide® VAF.**

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AMTicide® VAF

A skin moisturization study was performed using an untreated control, generic cream base, and an experimental with the same cream base containing 1.0% **AMTicide® VAF**. Comparative moisturization results from this study are shown in Figure 2. As demonstrated by the results of this study, the addition of 1.0% **AMTicide® VAF** improved moisture levels by 16.50% after 24 hours and by 34.0% after four weeks when compared to the untreated control. When compared to the base cream AMTicide® VAF improved moisturization by 3.96% and after 24 hours and by 9.88% after four weeks. Results indicate that **AMTicide® VAF** is capable of increasing moisturization when compared to both the untreated control as well as the base lotion.

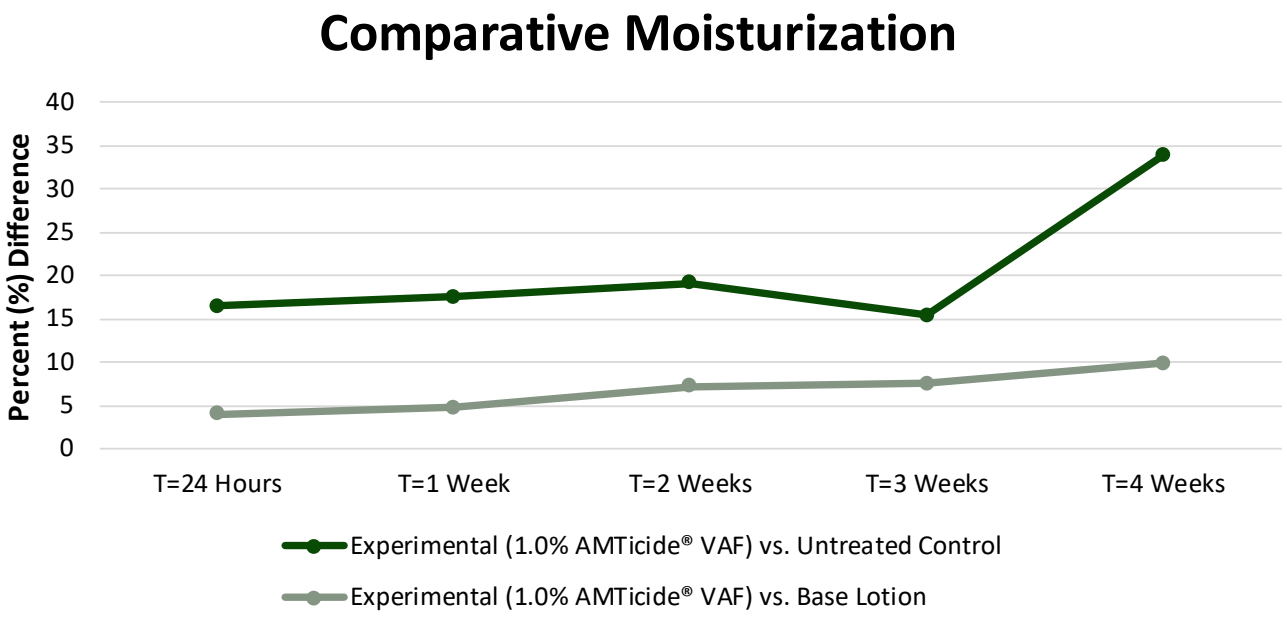

Figure 2. Comparative Moisturization.

A Headspace Preservation Test was then conducted over a period of 28 days to evaluate the preservation adequacy of 1.0% **AMTicide® VAF** compared to phenoxyethanol (positive control) and an unpreserved generic cream base (negative control). *Penicillium brevicompactum* and *Fusarium* sp. were isolated from the environment via passive sedimentation, to observe a 'real-life' example of contamination from manufacturing areas, storage conditions, or consumer use.

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The base cream formula used to perform the test was poured into individual cosmetic containers at 45°C in order to create the best environment for mold to grow under the test conditions. Each plastic jar was filled to approximately 85% of its capacity (~25ml) leaving 25% capacity of each jar empty as headspace. 1.0% **AMTicide® VAF** and 1.0% phenoxyethanol were subsequently added separately. Each cap of the cosmetic containers was inoculated separately with *Penicillium brevicompactum* and *Fusarium* sp. Each cap was tested at 7, 14, 21 and 28 days after the initial inoculation. Figure 3 represents the percent reduction of viable organisms after being introduced into the test formulation.

Test Product	Testing Day	*Penicillium brevicompactum* 2.4 x 106 CFU/ml	*Fusarium* sp. 3.5 x 106 CFU/ml
1.0% AMTicide® VAF	Day 7	>99.999%	>99.999%
	Day 14	>99.999%	>99.999%
	Day 21	>99.999%	>99.999%
	Day 28	>99.999%	>99.999%
1.0% Phenoxyethanol	Day 7	68.785%	88.963%
	Day 14	75.263%	>99.999%
	Day 21	88.123%	>99.999%
	Day 28	>99.999%	>99.999%
Unpreserved Generic Cream	Day 7	2.631%	2.879%
	Day 14	3.820%	5.684%
	Day 21	4.631%	6.196%
	Day 28	5.287%	7.177%

Figure 3. Headspace preservation test results. Chart shows percent (%) reduction of viable organisms.

Under the conditions of this test, the **AMTicide® VAF** exhibited the best preservation efficacy of headspace in a cosmetic and/or personal care product container compared to phenoxyethanol. The results of this study indicate **AMTicide® VAF** is capable of effectively protecting products exposed to an open environment of mold contamination.

REFERENCES

1. Lee, E., An, S., Choi, D., Moon, S., and I. Chang. 2007. "Comparison of objective and sensory skin irritations of several cosmetic preservatives." Contact Dermatitis. 56: 131-136.
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Specification

Product Name: AMTicide® VAF
Code Number: M14004
CAS #'s: 92128-81-9 (or) 68582-99-0 & 8013-01-2
EINECS #'s: 295-779-9 (or) N/A & 232-387-9
INCI Name: Bacillus Ferment & Saccharomyces Ferment Filtrate

Specification	Parameter
Appearance	Hazy Liquid
Color (Gardner)	3 Maximum
Odor	Characteristic
pH (25°C)	4.0 – 6.0
Refractive Index (25°C)	1.400 – 1.500
Heavy Metals	< 20 ppm
Lead	< 10 ppm
Arsenic	< 2 ppm
Cadmium	< 1 ppm

DO NOT FREEZE; Store at or near room temperature;
Mix well prior to use; May Sediment upon Standing

Product may change appearance if exposed to cold temperatures during shipment or storage. If this happens, please gently warm to 45-50°C and mix until normal appearance is restored.

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Compositional Breakdown

AMTicide® VAF
Code: M14004

Compositional Breakdown:

Ingredient	%
Propanediol	80.00
Bacillus Ferment	10.00
Saccharomyces Ferment Filtrate	10.00

- **To our knowledge the above material is free of the following list of heavy metals:**
  - **Heavy Metals < 20 ppm (Max.)**
  - **Lead < 10 ppm (Max.)**
  - **Antimony < 5 ppm (Max.)**
  - **Arsenic < 2 ppm (Max.)**
  - **Mercury < 1 ppm (Max.)**
  - **Cadmium < 1 ppm (Max.)**

Compositional Breakdown

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This is to certify that AMTicide® VAF does not contain, neither directly nor through cross contamination, any of the 26 allergenic flavors or fragrances (Gas Chromatography-Mass Spectrometer Coupled):

ALLERGENS listed in Annex III of EU Cosmetic Regulation(EC) No. 1223/2009 amending EU Directive 2003/15/EC		
INCI NAME	CAS NUMBER	Limit (ppm)
Alpha-IsoMethyl Ionone	127-51-5	< 0.02
Amyl Cinnamal	122-40-7	< 0.10
Anise Alcohol	105-13-5	< 0.00
Benzyl Alcohol	100-51-6	< 0.01
Benzyl Benzoate	120-51-4	< 0.09
Benzyl Cinnamate	103-41-3	< 0.30
Benzyl Salicylate	118-58-1	< 0.06
Butylphenyl Methylpropional	80-54-6	< 0.50
Cinnamal	104-55-2	< 0.01
Cinnamyl Alcohol	104-54-1	< 0.30
Citral	5392-40-5	< 1.00
Citronellol	106-22-9	< 1.00
Coumarin	91-64-5	< 0.00
Eugenol	97-53-0	< 0.70
Farnesol	4602-84-0	< 0.04
Geraniol	106-24-1	< 0.08
Hexyl Cinnamal	101-86-0	< 0.40
Hydroxycitronellal	107-75-5	< 1.00
Hydroxymethylpentyl 3-Cyclohexene carboxaldehyde	31906-04-4	< 0.00
Isoeugenol	97-54-1	< 0.06
Limonene	5989-27-5	< 0.05
Linalool	78-70-6	< 0.00
Methyl 2-Octynoate	111-12-6	< 0.20
Evernia prunastri extract (Oak moss extract)	90028-68-5	< 0.00
Evernia furfuracea extract (Treemoss extract)	90028-67-4	< 0.00
Amylcinnamyl Alcohol	101-85-9	< 1.00

Compositional Breakdown

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This is to certify that AMTicide® VAF does not contain pesticide levels exceeding the following (Reverse Phase High Performance Liquid Chromatography-Mass Spectrometer Coupled):

EPA Pesticide Levels	
INCI NAME	LIMIT (mg/kg)
Alachlor	< 0.02
Aldrin and Dieldrin	< 0 .05
Azinphos-methyl	< 1. 00
Bromopropylate	< 3.0 0
Chlordane(cis and trans)	< 0.05
Chlorfenvinphos	< 0.50
Chlorpyrifos	< 0.20
Chlorpyrifos-methyl	< 0.10
Cypermethrin	< 1.00
DDT	< 1.00
Deltamethrin	< 0.50
Diazinon	< 0.50
Dichlorvos	< 1.00
Dithiocarbamates	< 2.00
Endosulfan	< 3.00
Endrin	< 0.05
Ethion	< 2.00
Fenitrothion	< 0.50
Fenvalerate	< 1.50
Fonofos	< 0.05
Heptachlor	< 0.05
Hexachlorobenzene	< 0.10
Hexachlorocyclohexane	< 0.30
Lindane	< 0.60
Malathion	< 1.00
Methidathion	< 0.20
Parathion	< 0.50

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Compositional Breakdown

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Parathion-methyl	< 0.20
Permethrin	< 1.00
Phosalone	< 0.10
Piperonyl butoxide	< 3.00
Pirimiphos-methyl	< 4.00
Pyrethrins	< 3.00
Quintozene(sum of 3 items)	< 1.00

Moisturization/Hydration Assay

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Tradename: AMTicide® VAF

Code: M14004

CAS #: 92128-81-9 (or) 68582-99-0 & 8013-01-2

Test Request Form #: 4057

Lot #: NC171207-C

Sponsor: *Active Micro Technologies, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Danaher*
Principle Investigator: *Jennifer Goodman*

Test Performed:
Moisturization/Hydration Assay

Introduction

An *in-vivo* study was conducted over a period of four weeks to evaluate the moisturization benefits **AMTicide® VAF**. 10 M/F subjects between the ages of 23-45 participated in the study. Results indicate that this material is capable of significantly increasing moisturization compared to the control.

The moisturization assay was conducted to assess the moisturizing ability of **AMTicide® VAF.**

Materials

A. Equipment: DermaLab Skin Combo (Hydration/ Moisture Pin Probe)

The moisture module provides information about the skin's hydration by measuring the conducting properties of the upper skin layers when subjected to an alternating voltage. The method is referred to as a conductance measurement and the output is presented in the unit of uSiemens (uS). A moisture pin probe is the tool used to gather hydration values.

10 volunteers M/F between the ages of 23 and 45 and who were known to be free of any skin pathologies participated in this study. A Dermalab Corneometer was used to measure the moisture levels on the subject's volar forearms. The Corneometer is an instrument that measures the amount of water within the skin. The presence of moisture in the skin improves conductance therefore results in higher readings than dry skin. Therefore the higher the levels of moisture, the higher the readings from the Corneometer will be. Baseline moisturization readings were taken on day one of the study.

Following initial measurements, all subjects were asked to apply 2 mg of each test material on their volar forearms. Measurements were taken immediately after application of test materials and then weekly for 4 weeks. The test material consisted of 1.0% **AMTicide® VAF** in a base lotion.

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For added perspective, measurements of an untreated test site and a site treated with a base lotion (Cetaphil Moisturizing for All Skin Types) were recorded.

Results

AMTicide® VAF showed high moisturizing capabilities at a 1.0% concentration. Please note that each value is an average of three consecutive readings per test site.

Moisturization		T = 0	T= 24 Hours	= 1 Week	T = 2 Weeks	T= 3 Weeks	T= 4 Weeks
Panelist 1	Experimental	61	63	70	84	71	82
	Base Lotion	74	65	69	84	70	86
	Untreated	55	57	89	71	81	80
Panelist 2	Experimental	152	155	156	152	153	159
	Base Lotion	110	112	117	119	126	132
	Untreated	89	99	97	85	99	93
Panelist 3	Experimental	48	68	64	37	79	43
	Base Lotion	51	63	79	53	83	45
	Untreated	91	74	69	82	81	65
Panelist 4	Experimental	82	106	114	90	94	80
	Base Lotion	89	103	101	92	105	88
	Untreated	82	79	80	85	83	84
Panelist 5	Experimental	52	81	79	74	101	65
	Base Lotion	65	75	80	81	91	60
	Untreated	47	55	57	66	70	85
Panelist 6	Experimental	66	88	112	90	78	72
	Base Lotion	80	115	110	108	101	85
	Untreated	62	90	91	93	122	91
Panelist 7	Experimental	74	98	98	101	104	113
	Base Lotion	78	80	87	68	72	56
	Untreated	53	96	96	99	80	50
Panelist 8	Experimental	83	107	114	123	132	133
	Base Lotion	55	110	112	105	113	119
	Untreated	50	96	98	94	141	50
Panelist 9	Experimental	83	107	114	123	128	132
	Base Lotion	55	110	121	105	104	119
	Untreated	52	96	98	52	50	50
Panelist 10	Experimental	67	66	51	48	48	55
	Base Lotion	65	70	52	45	54	60
	Untreated	55	64	52	47	49	49
Number of Panelists		10	9	10	10	10	10

Table 1. Participant impedance values.

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Averages	T = 0	T = 24 Hours	T = 1 Week	T = 2 Weeks	T = 3 Weeks	T = 4 Weeks
Experimental (1.0% AMTicide® VAF) in Base Lotion	76.8	93.9	97.2	92.2	98.8	93.4
Base Lotion Control	72.2	90.3	92.8	86	91.9	85
Untreated Control	63.6	80.6	82.7	77.4	85.6	69.7

Table 2. Average moisturization values.

Percent (%) Change	T = 0	T = 24 Hours	T = 1 Week	T = 2 Weeks	T = 3 Weeks	T = 4 Weeks
Base Lotion vs. Untreated Control	13.52	12.03	12.21	11.11	7.36	21.95
Experimental (1.0% AMTicide® VAF) vs. Untreated Control	20.75	16.50	17.53	19.12	15.42	34.00
Experimental (1.0% AMTicide® VAF) vs. Base Lotion	6.37	3.98	4.74	7.21	7.51	9.88

Table 3. Percent chance in moisturization.

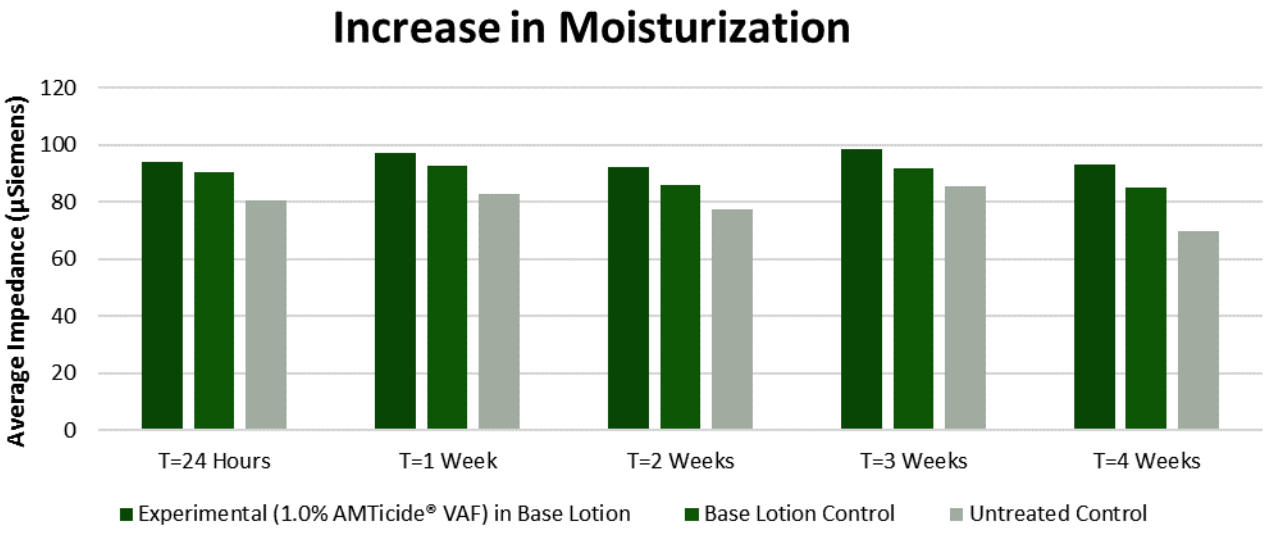

Figure 1. Average increase in moisturization.

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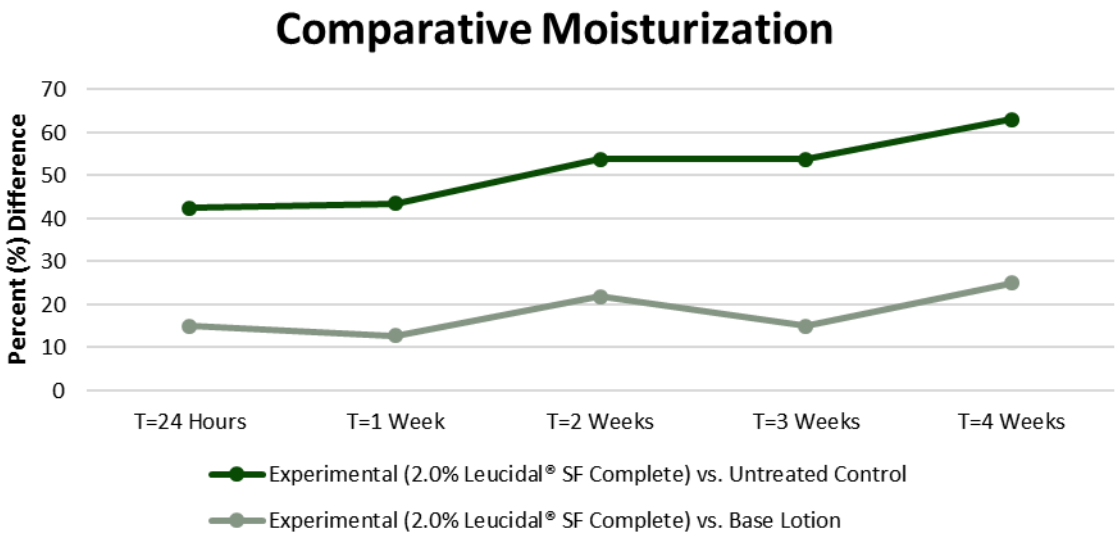

Figure 2. Percent difference in moisturization.

Discussion

As evidenced in a four-week efficacy study of **AMTicide® VAF**, moisture levels were improved by 16.50% after 24 hours and by 34.0% after four weeks when compared to the untreated control. When compared to the base cream **AMTicide® VAF** improved moisturization by 3.96% and after 24 hours and by 9.88% after four weeks. Results indicate that **AMTicide® VAF** is capable of increasing moisturization when compared to both the untreated control as well as the base lotion.

The present study confirms that **AMTicide® VAF** is capable of providing moisturizing and skin hydrating benefits when added to cosmetic applications.

Oxygen Radical Absorbance Capacity (ORAC) Assay

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Tradename: AMTicide® VAF

Code: M14004

CAS #: 92128-81-9 (or) 68582-99-0 & 8013-01-2

Test Request Form #: 4057

Lot #: NC171207-C

Sponsor: *Active Micro Technologies, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Danaher*
Principle Investigator: *Jennifer Goodman*

Test Performed:
Oxygen Radical Absorbance Capacity (ORAC)

Introduction

Reactive oxygen species (ROS) are generated by normal cellular processes, environmental stresses, and UV irradiation. ROS are dangerous to cellular structures and functional molecules (i.e., DNA, proteins, lipids) as they act as strong oxidizing agents or free radicals. The oxygen radical absorbance capacity (ORAC) assay is a standard method used to assess antioxidant capacity of physiological fluids, foods, beverages, and natural products. The assay quantitatively measures a sample's ability to quench free radicals that have the potential to react with and damage cellular components.

Oxygen Radical Absorbance Capacity (ORAC) assay was conducted to assess the antioxidant capacity of **AMTicide® VAF**.

Assay Principle

This assay is based upon the effect of peroxyl radicals generated from the thermal decomposition of 2, 2'-azobis-2-methyl-propanimidamide dihydrochloride (AAPH) on the signal intensity from the fluorescent probe, fluorescein, in the presence of an oxygen radical absorbing substance. The degree of change is indicative of the amount of radical damage and the presence of antioxidants results in an inhibition in the free radical damage to the fluorescein. The antioxidant protection of the sample can be calculated by comparing it to a set of known standards. Trolox®, a water soluble vitamin E analog, with known antioxidant capabilities is used in this ORAC assay as the standard for measuring the antioxidant capacity of unknown substances. ORAC values, expressed in µM of Trolox® equivalents (TE), are calculated using the area under the curves (AUC) of the test product, Trolox®, and the control materials. Trolox equivalency is used as the benchmark for antioxidant capacity of mixtures since it is difficult to measure individual components.

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Oxygen Radical Absorbance Capacity (ORAC) Assay

Materials

A. Equipment:	Synergy H1 Microplate reader (BioTek Instuments, Winooski, VT); Gen5 software (BioTek Instuments, Winooski, VT); Pipettes
B. Buffers:	75mM Potassium Phosphate (pH 7.4); Deionized H_2O
C. Reagents:	2,2′-Azobis(2-methylpropionamidine) dihydrochloride (AAPH) (153mM); 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox®); Fluorescein Sodium Salt (4nM)
D. Preparation:	Pre-heat (37˚C) Synergy H1 Microplate reader; Prepare Trolox® standards, sample dilutions, fluorescein solution, and AAPH.
E. Microtitre Plates:	Corning 96 Well Black Side/Clear Bottom Microplates

Methods

Solutions of **AMTicide® VAF** and Trolox® (positive control) were prepared in 75mM potassium phosphate buffer. Materials were prepared at three different concentrations/dilutions. Trolox® was used as a reference for antioxidant capacity and prepared a concentrations ranging from 12.5µM to 200µM in 75mM potassium phosphate buffer.

For the ORAC assay, 25µL of test material and Trolox® were combined with 150µL of fluorescein in 75mM potassium phosphate buffer and incubated in the Synergy HT Microplate reader at 37˚C for 30 minutes. At the end of the incubation period, 25µL of AAPH were pipetted into each well. Fluorescent measurements were then taken every 2 minutes for approximately 2 hours at an excitation wavelength of 485nm and an emissions wavelength of 520nm.

The AUC and Net AUC values of the standards and samples were determined using Gen5 2.0 Data Reduction Software (BioTek Instruments) using the below equations:

$$AUC = 0.5 + \frac{R2}{R1} + \frac{R3}{R1} + \frac{R4}{R1} + \cdots + \frac{Rn}{R1} \rightarrow Where\ R\ is\ fluorescence\ reading$$

$$Net\ AUC = AUC_{sample} - AUC_{blank}$$

The standard curve was obtained by plotting the Net AUC of different Trolox® concentrations against their concentration. ORAC values of samples were then calculated automatically using the Gen5 software to interpolate the sample's Net AUC values against the Trolox® standard curve. ORAC measurements for the test material were expressed in micro moles Trolox® equivalents (µMTE), where 1 ORAC unit is equal to 1 µMTE.

ORAC values are also calculated in Units/milliliter (U/mL). The equation used for the calculation is shown below:

$$ORAC\ (U/mL) = (50 \times Dilution\ Factor) \times \left(\frac{AUC_{Sample} - AUC_{Blank}}{AUC_{Trolox} - AUC_{Blank}}\right)$$

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Results

AMTicide® VAF exhibited potent antioxidant activity at a 2.0% concentration.

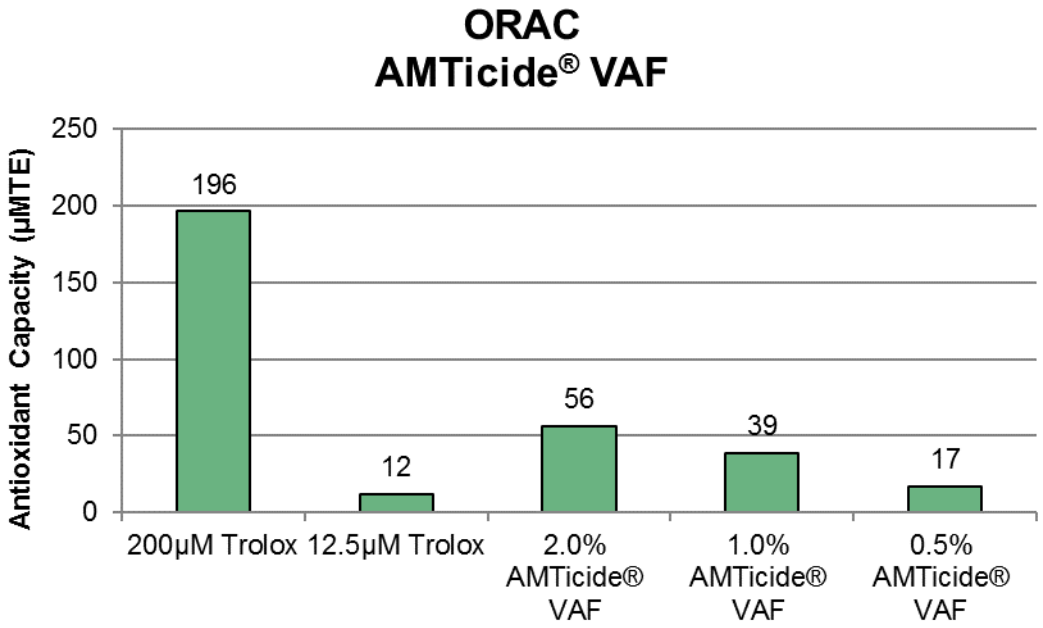

Figure 1: Antioxidant capacities

Discussion

As shown in figure 1, **AMTicide® VAF (M14004)** exhibited antioxidant activity comparable to 50 µM Trolox®. The antioxidant capacity of **AMTicide® VAF** increased as the concentration increased, as a result we can assure that its ability to minimize oxidative stress is dose dependent.

AMTicide® VAF was designed as a multifunctional antimicrobial, however with the present study we can confirm that this unique ingredient is not only capable of providing functional benefits but it is also capable of providing potent antioxidant benefits when added to cosmetic applications.

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Inhibition Activity Data

Product Name: AMTicide® VAF
Code Number: M14004
Lot Number: N200731B
Test Request Number: 6818
CAS #'s: 92128-81-9 (or) 68582-99-0 & 8013-01-2
EINECS #'s: 295-779-9 (or) N/A & 232-387-9
INCI Name: *Bacillus* Ferment & *Saccharomyces* Ferment Filtrate

Organism (ATCC #)	Minimum Inhibitory Concentration (%)
E.coli #8739	4.0
S. aureus #6538	2.0
P. aeruginosa #9027	2.0
C. albicans #10231	0.5
A. brasiliensis #16404	0.5

QA Signature Monica Beltran

Date 08-19-2020

This is an Electronically Generated Document

Headspace Preservation Test

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Test Article: AMTicide® VAF
Code Number: M14004

Test Performed: Headspace Preservation Test
Challenge Test

Sponsor:
Active Micro Technologies, LLC
107 Technology Drive
Lincolnton, NC 28092

Test Request Number: 3561

Study Director: *Maureen Danaher*
Principle Investigator: *Monica Beltran*

Purpose:

This study focuses on the risk of microbial growth within the headspace of a cosmetic and/or personal care final product container. Cosmetic companies are required to control the optimal preservation of their commercial products, since microbial contamination in cosmetics represents an important risk for consumer health.[1] Headspace preservation can be an issue when packaging does not protect a product adequately from mold contamination obtained from the manufacturing environments, poor storage conditions or consumer use (such as dipping fingers into the containers). Phenoxyethanol has been used as the effective industry standard due to its volatile nature, but having a synthetic nature highly regulated worldwide, a natural alternative solution is needed. Thus, the purpose of this study was to determine the preservation adequacy of a natural 1.0% AMTicide® VAF vs. synthetic Phenoxyethanol and an Unpreserved Generic Cream involving the headspace and the final cosmetic product itself.

Test Organisms:

Isolated environmental mold by Sedimentation method (settle plates):

- *Penicillium brevicompactum.*
- *Fusarium* sp.

*These molds were isolated from the environment to obtain a 'real-life' example of contamination from manufacturing areas, storage conditions or consumer use.

Container Specifications:

- 1 oz. White polypropylene double wall radius jars from SKS Bottle and Packaging, Inc.

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Inoculum Suspension Preparation:

- Using a sterile pipette, 9 ml of Buffered Sodium Chloride Peptone Solution pH 7 was added to enough test tubes and autoclaved at 121^{0}C for 20 minutes. After sterilization, using a sterile cotton enough *Penicillium brevicompactum* and *Fusarium* sp. were swabbed and added to a test tube with sterile Buffered Sodium Chloride Peptone Solution pH 7 separately.
- The inoculum suspension was prepared to achieve a minimum of 10^6 CFU/ml microbial population. 1:10 serial dilutions were made to achieve the microbial concentration using sterile Buffered Sodium Chloride Peptone Solution pH 7.
- Using Sabouraud Dextrose Agar (SDA), the initial inoculum suspensions were plated per duplicate to quantify the population after exposure in the test material for a specific time.

Products Tested:

1.	AMTicide® VAF in Generic Cream Base	Test Sample
2.	Phenoxyethanol in Generic Cream Base	Positive Control
3.	Unpreserved Generic Cream Base	Negative Control

Test Method:

1. The base cream formula used to perform the test was poured into individual cosmetic containers at 45^{o}C in order to create the best environment for mold to grow under the test conditions. Each plastic jar was filled to approximately 75% of its capacity (~25ml) leaving 25% capacity of each jar empty as headspace. AMTicide® VAF 1.0% and Phenoxyethanol 1.0% was subsequently added separately.
2. Each cap of the cosmetic containers was inoculated separately with 10^6 CFU/ml of *Penicillium brevicompactum* and *Fusarium* sp.
3. The caps of each container were closed and stored at room temperature (23 – 25^{o}C).
4. Using a pre-moisture Buffered Sodium Chloride Peptone Solution pH 7 sterile swab, a sample was taken from each cap.
5. Each cap was tested at 7, 14, 21 and 28 days after the initial inoculation. Table 1. represents the preservation efficacy of AMTicide® VAF compared with the control.
6. Over the surface of SDA the samples were spread plated and incubated at 25±2°C for 3 to 5 days.
7. Serial dilutions from each container were evaluated 0, 7, 14, 21, and 28 days after the initial inoculation to determine quantitatively the number of viable microorganisms remaining in the base cream formula. Table 2. represents the percent reduction of viable organisms after being introduced into the base cream formula.

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Test Product	Testing Day	*Penicillium brevicompactum*	*Fusarium* sp.
AMTicide® VAF	Day 7	No Growth	No Growth
	Day 14	No Growth	No Growth
	Day 21	No Growth	No Growth
	Day 28	No Growth	No Growth
Phenoxyethanol	Day 7	Growth	Growth
	Day 14	Growth	No Growth
	Day 21	Growth	No Growth
	Day 28	No Growth	No Growth
Unpreserved Generic Cream	Day 7	Growth	Growth
	Day 14	Growth	Growth
	Day 21	Growth	Growth
	Day 28	Growth	Growth

Table 1. Headspace Preservation Test results for AMTicide® VAF 1.0% and Phenoxyethanol 1.0% in Generic Cream Formula Vs Unpreserved Generic Cream

Test Product	Testing Day	*Penicillium brevicompactum* 2.4×10^6 CFU/ml	*Fusarium* sp. 3.5×10^6 CFU/ml
AMTicide® VAF	Day 7	>99.999%	>99.999%
	Day 14	>99.999%	>99.999%
	Day 21	>99.999%	>99.999%
	Day 28	>99.999%	>99.999%
Phenoxyethanol	Day 7	68.785%	88.963%
	Day 14	75.263%	>99.999%
	Day 21	88.123%	>99.999%
	Day 28	>99.999%	>99.999%
Unpreserved Generic Cream	Day 7	2.631%	2.879%
	Day 14	3.820%	5.684%
	Day 21	4.631%	6.196%
	Day 28	5.287%	7.177%

Table 2. Challenge Test results for AMTicide® VAF 1.0% and Phenoxyethanol 1.0% in Generic Cream Formula Vs Unpreserved Generic Cream

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Results and Discussion:

Under the conditions of this test, AMTicide® VAF exhibited the best preservation efficacy of headspace in a cosmetic and/or personal care product container compared to Phenoxyethanol, a synthetic known volatile preservative available in the industry. The results of this study indicate AMTicide® VAF is capable of effectively protecting products exposed to an open environment of mold contamination.

AMTicide® VAF and Phenoxyethanol are being incorporated throughout the generic cream base instead of adding to the top. The results of this second part of the study demonstrates how effectively AMTicide® VAF works for headspace vs. general preservation in final cosmetic formulations.

References:

1. Alvares, G. Trinidad, M. Llompart, M. Garcia, C. Gonzalez, T. Lores, M. A novel outlook on detecting microbial contamination in cosmetic products: analysis of biomarker volatile compounds by solid-phase microextraction gas chromatography-mass spectrometry. Analytical Methods, Issue 2, 2013.

Headspace Preservation Test

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(704) 276-7100 • Fax (704) 276-7101

Test Article: AMTicide® VAF
Code Number: M14004

Test Performed: Headspace Preservation Test

Test Request Number: 3561

Sponsor:
Active Micro Technologies, LLC
107 Technology Drive
Lincolnton, NC 28092

Study Director: *Maureen Danaher*
Principle Investigator: *Monica Beltran*

Purpose:

This study focuses on the risk of microbial growth within the headspace of a cosmetic and/or personal care final product container. Cosmetic companies are required to control the optimal preservation of their commercial products, since microbial contamination in cosmetics represents an important risk for consumer health.[1] Headspace preservation can be an issue when packaging does not protect a product adequately from mold contamination obtained from the manufacturing environments, poor storage conditions or consumer use (such as dipping fingers into the containers). Phenoxyethanol has been used as the effective industry standard due to its volatile nature, but having a synthetic nature highly regulated worldwide. Its effectiveness was tested in Generic Cream formula in a previous study demonstrating the poor capability of Phenoxyethanol in the preservation efficacy of headspace in Generic Cream Formula compared with AMTicide® VAF, a natural alternative solution. The purpose of this study was to determine the headspace preservation adequacy of 1.0% AMTicide® VAF of different cosmetic formulations as a complementary information for the previous study performed in Generic Cream Formula.

Test Organisms:

Isolated environmental mold by Sedimentation method (settle plates):

- *Penicillium brevicompactum.*
- *Fusarium* sp.

*These molds were isolated from the environment to obtain a 'real-life' example of contamination from manufacturing areas, storage conditions or consumer use.

Container Specifications:

- 1 oz. White polypropylene double wall radius jars from SKS Bottle and Packaging, Inc.

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Inoculum Suspension Preparation:

- Using a sterile pipette, 9 ml of Buffered Sodium Chloride Peptone Solution pH 7 was added to enough test tubes and autoclaved at 121^0C for 20 minutes. After sterilization, using a sterile cotton enough *Penicillium brevicompactum* and *Fusarium* sp. were swabbed and added to a test tube with sterile Buffered Sodium Chloride Peptone Solution pH 7 separately.
- The inoculum suspension was prepared to achieve a minimum of 10^6 CFU/ml microbial population. 1:10 serial dilutions were made to achieve the microbial concentration using sterile Buffered Sodium Chloride Peptone Solution pH 7.
- Using Sabouraud Dextrose Agar (SDA), the initial inoculum suspensions were plated per duplicate to quantify the population after exposure in the test material for a specific time.

Products Tested:

1.	AMTicide® VAF in Split End Masque Formula	Test Sample
2.	AMTicide® VAF in Renewing Body Scrub Formula	Test Sample
3.	AMTicide® VAF in Exfoliating Face Mask Formula	Test Sample
4.	AMTicide® VAF in Beards Balm Formula	Test Sample
5.	Unpreserved Split End Masque Formula	Negative Control
6.	Unpreserved Renewing Body Scrub Formula	Negative Control
7.	Unpreserved Exfoliating Face Mask Formula	Negative Control
8.	Unpreserved Bear Balm Formula	Negative Control

Test Method:

1. AMTicide® VAF 1.0% was added to each of the following formulations: Split End Masque, Renewing Body Scrub, Exfoliating Face Mask and Beard Balm formulas, at the end of the manufacturing process. To perform the test each formula were poured into individual cosmetic containers at 45°C in order to create the best environment for mold to grow under the test conditions. Each plastic jar was filled to approximately 75% of its capacity (~25ml) leaving 25% capacity of each jar empty as headspace.
2. Each cap of the cosmetic containers was inoculated separately with 10^6 CFU/ml of *Penicillium brevicompactum* and *Fusarium* sp.
3. The caps of each container were closed and stored at room temperature (23 – 25°C).
4. Using a pre-moisture Buffered Sodium Chloride Peptone Solution pH 7 sterile swab, a sample was taken from each cap.
5. Each cap was tested at 7, 14, 21 and 28 days after the initial inoculation. Table 1, 2, 3 and 4 represents the preservation efficacy of AMTicide® VAF compared with the control for each cosmetic formulation.
6. Over the surface of SDA the samples were spread plated and incubated at 25±2°C for 3 to 5 days.

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Test Product	Testing Day	*Penicillium brevicompactum*	*Fusarium* sp.
AMTicide® VAF	Day 7	No Growth	No Growth
	Day 14	No Growth	No Growth
	Day 21	No Growth	No Growth
	Day 28	No Growth	No Growth
Unpreserved Split End Masque	Day 7	Growth	Growth
	Day 14	Growth	Growth
	Day 21	Growth	Growth
	Day 28	Growth	Growth

Table 1. Headspace Preservation Test results for AMTicide® VAF 1.0% vs. Unpreserved Split End Masque

Test Product	Testing Day	*Penicillium brevicompactum*	*Fusarium* sp.
AMTicide® VAF	Day 7	No Growth	No Growth
	Day 14	No Growth	No Growth
	Day 21	No Growth	No Growth
	Day 28	No Growth	No Growth
Unpreserved Split End Masque	Day 7	Growth	Growth
	Day 14	Growth	Growth
	Day 21	Growth	Growth
	Day 28	Growth	Growth

Table 2. Headspace Preservation Test results for AMTicide® VAF 1.0% vs. Unpreserved Renewing Body Scrub Formula

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Test Product	Testing Day	*Penicillium brevicompactum*	*Fusarium* sp.
AMTicide® VAF	Day 7	No Growth	No Growth
	Day 14	No Growth	No Growth
	Day 21	No Growth	No Growth
	Day 28	No Growth	No Growth
Unpreserved Split End Masque	Day 7	Growth	Growth
	Day 14	Growth	Growth
	Day 21	Growth	Growth
	Day 28	Growth	Growth

Table 3. Headspace Preservation Test results for AMTicide® VAF 1.0% vs. Unpreserved Exfoliating Face Mask Formula

Test Product	Testing Day	*Penicillium brevicompactum*	*Fusarium* sp.
AMTicide® VAF	Day 7	No Growth	No Growth
	Day 14	No Growth	No Growth
	Day 21	No Growth	No Growth
	Day 28	No Growth	No Growth
Unpreserved Split End Masque	Day 7	Growth	Growth
	Day 14	Growth	Growth
	Day 21	Growth	Growth
	Day 28	Growth	Growth

Table 4. Headspace Preservation Test results for AMTicide® VAF 1.0% vs. Unpreserved Beard Balm Formula

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Results and Discussion:

Under the conditions of this test, AMTicide® VAF exhibited the best preservation efficacy of headspace in a cosmetic and/or personal care product container compared with the unpreserved version of each cosmetic formulation with no volatile properties. The results of this study indicate AMTicide® VAF is capable of effectively protecting products exposed to an open environment of mold contamination.

References:

1. Alvares, G. Trinidad, M. Llompart, M. Garcia, C. Gonzalez, T. Lores, M. A novel outlook on detecting microbial contamination in cosmetic products: analysis of biomarker volatile compounds by solid-phase microextraction gas chromatography-mass spectrometry. Analytical Methods, Issue 2, 2013.

ACTIVE MICRO TECHNOLOGIES

Dermal and Ocular Irritation Tests

107 Technology Drive • Lincolnton, NC 28092
(704) 276-7100 • Fax (704) 276-7101

Tradename: AMTicide® VAF

Code: M14004

CAS #: 92128-81-9 (or) 68582-99-0 & 8013-01-2

Test Request Form #: 1683

Lot #: NC171207-C

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Danaher*
Principle Investigator: *Jennifer Goodman*

Test Performed:
In Vitro EpiDerm™ Dermal Irritation Test (EPI-200-SIT)
EpiOcular™ Eye Irritation Test (OCL-200-EIT)

SUMMARY

In vitro dermal and ocular irritation studies were conducted to evaluate whether **AMTicide® VAF** would induce dermal or ocular irritation in the EpiDerm™ and EpiOcular™ model assays.

The product was tested according to the manufacture's protocol. The test article solution was found to be a **non-irritant at 1.0% in base formulation**. Reconstructed human epidermis and cornea epithelial model were incubated in growth media overnight to allow for tissue equilibration after shipping from MatTek Corporation, Ashland, MA. Test substances were applied to the tissue inserts and incubated for 60 minutes for liquid and solid substances in the EpiDerm™ assay and 30 minutes for liquid substances and 90 minutes for solid substances in the EpiOcular™ assay at 37°C, 5% CO_2, and 95% relative humidity (RH). Tissue inserts were thoroughly washed and transferred to fresh plates with growth media. After post substance dosing incubation is complete, the cell viability test begins. Cell viability is measured by dehydrogenase conversion of MTT [(*3-4,5-dimethyl thiazole 2-yl*)], present in the cell mitochondria, into blue formazan salt that is measured after extraction from the tissue. The irritation potential of the test chemical is dictated by the reduction in tissue viability of exposed tissues compared to the negative control.

Under the conditions of this assay, the test article was considered to be **non-irritating at 1.0% in base formulation**. The negative and positive controls performed as anticipated.

I. Introduction

A. Purpose

In vitro dermal and ocular irritation studies were conducted to evaluate whether a test article would induce dermal or ocular irritation in the EpiDerm™ and EpiOcular™ model assays. MatTek Corporation's reconstructed human epidermal and human ocular models are becoming a standard in determining the irritancy potential of test substances. They are able to discriminate between irritants and non-irritants. The EpiDerm™ assay has accuracy for the prediction of UN GHS R38 skin irritating and no-label (non-skin irritating) test substances. The EpiOcular™ assay can differentiate chemicals that have been classified as R36 or R41 from the EU classifications based on Dangerous Substances Directive (DSD) or between the UN GHS Cat 1 and Cat 2 classifications.

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II. Materials

A. Incubation Conditions: 37°C at 5% CO_2 and 95% relative humidity

B. Equipment: Forma humidified incubator, ESCO biosafety laminar flow hood, Synergy HT Microplate reader; Pipettes

C. Media/Buffers: DMEM based medium; DPBS; sterile deionized H_2O

D. Preparation: Pre-incubate (37°C) tissue inserts in assay medium; Place assay medium and MTT diluent at 4°C, MTT concentrate at -20°C, and record lot numbers of kit components

E. Tissue Culture Plates: Falcon flat bottom 96-well, 24-well, 12-well, and 6-well tissue culture plates

F. Reagents: MTT (1.0mg/mL); Extraction Solution (Isopropanol); SDS (5%); Methyl Acetate

G. Other: Nylon Mesh Circles (EPI-MESH); Cotton tip swabs; 1mL tuberculin syringes; Ted Pella micro-spatula; 220mL specimen containers; sterile disposable pipette tips; Parafilm

III. Test Assay

A. Test System

The reconstructed human epidermal model, EpiDerm™, and cornea epithelial model, EpiOcular™, consist of normal human-derived epidermal keratinocytes which have been cultured to form a multilayer, highly differentiated model of the human epidermis and cornea epithelium. These models consist of organized basal, spinous, and granular layers, and the EpiDerm™ systems also contains a multilayer stratum corneum containing intercellular lamellar lipid layers that the EpiOcular™ system is lacking. Both the EpiDerm™ and EpiOcular™ tissues are cultured on specially prepared cell culture inserts.

B. Negative Control

Sterile DPBS and sterile deionized water are used as negative controls for the EpiDerm™ and EpiOcular™ assays, respectfully.

C. Positive Control

Known dermal and eye irritants, 5% SDS solution and Methyl Acetate, were used as positive controls for the EpiDerm™ and EpiOcular™ assays, respectfully.

D. Data Interpretation Procedure

a. EpiDerm™

An irritant is predicted if the mean relative tissue viability of the 3 tissues exposed to the test substance is reduced by 50% of the mean viability of the negative controls and a non-irritant's viability is > 50%.

b. EpiOcular™

An irritant is predicted if the mean relative tissue viability of the 2 tissues exposed to the test substance is reduced by 60% of the mean viability of the negative controls and a non-irritant's viability is > 40%.

IV. Method

A. Tissue Conditioning

Upon MatTek kit arrival at Active Micro Technologies, LLC the tissue inserts are removed from their shipping medium and transferred into fresh media and tissue culture plates and incubated at 37°C at 5% CO_2 and 95% relative humidity for 60 minutes. After those 60 minutes the inserts are transferred into fresh media and tissue culture plates and incubated at 37°C at 5% CO_2 and 95% relative humidity for an additional 18 to 21 hours.

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B. Test Substance Exposure

a. EpiDerm™

30µL (liquid) or 25mg (solid) of the undiluted test substance is applied to 3 tissue inserts and allowed to incubate for 60 minutes in a humidified incubator (37°C, 5% CO_2, 95% RH).

b. EpiOcular™

Each tissue is dosed with 20µL DPBS prior to test substance dosing. 50µL (liquid) or 50mg (solid) of the undiluted test substance is applied to 2 tissue inserts and allowed to incubate for 90 minutes in a humidified incubator (37°C, 5% CO_2, 95% RH).

C. Tissue Washing and Post Incubation

a. EpiDerm™

All tissue inserts are washed with DPBS, dried with cotton tipped swab, and transferred to fresh media and culture plates. After 24 hours the inserts are again transferred into fresh media and culture plates for an additional 18 to 20 hours.

b. EpiOcular™

Tissue inserts are washed with DPBS and immediately transferred into 5mL of assay medium for 12 to 14 minutes. After this soak the inserts are transferred into fresh media and tissue culture plates for 120 minutes for liquid substances and 18 hours for solid substances.

D. MTT Assay

Tissue inserts are transferred into 300µL MTT media in pre-filled plates and incubated for 3 hours at 37°C, 5% CO_2, and 95% RH. Inserts are then removed from the MTT medium and placed in 2mL of the extraction solution. The plate is sealed and incubated at room temperature in the dark for 24 hours. After extraction is complete the tissue inserts are pierced with forceps and 2 x 200µL aliquots of the blue formazan solution is transferred into a 96 well plate for Optical Density reading. The spectrophotometer reads the 96-well plate using a wavelength of 570 nm.

V. Acceptance Criterion

A. Negative Control

The results of this assay are acceptable if the mean negative control Optical Density (OD_{570}) is ≥ 1.0 and ≤ 2.5 (EpiDerm™) or ≥ 1.0 and ≤ 2.3 (EpiOcular™).

B. Positive Control

a. EpiDerm™

The assay meets the acceptance criterion if the mean viability of positive control tissues expressed as a % of the negative control is ≤ 20%.

b. EpiOcular™

The assay meets the acceptance criterion if the mean viability of positive control tissues is < 60% of control viability.

C. Standard Deviation

Since each irritancy potential is predicted from the mean viability of 3 tissues for EpiDerm™ and 2 tissues for EpiOcular™, the variability of the replicates should be < 18% for EpiDerm™ and < 20% EpiOcular™.

VI. Results

A. Tissue Characteristics

The tissue inserts included in the MatTek EpiDerm™ and EpiOcular™ assay kits were in good condition, intact, and viable.

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B. Tissue Viability Assay
The results are summarized in Figures 1 and 2. In no case was the tissue viability ≤ 50% for EpiDerm™ or ≤ 60% for EpiOcular™ in the presence of the test substance. The negative control mean exhibited acceptable relative tissue viability while the positive control exhibited substantial loss of tissue viability and cell death.

C. Test Validity
The data obtained from this study met criteria for a valid assay.

VII. Conclusion
Under the conditions of this assay, the test article substance was considered to be **non-irritating at 1.0% in base formulation**. The negative and positive controls performed as anticipated.

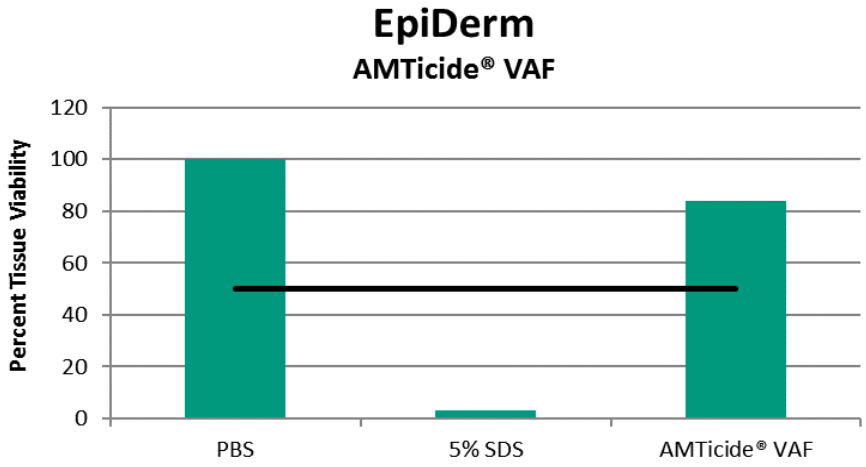

Figure 1: EpiDerm tissue viability

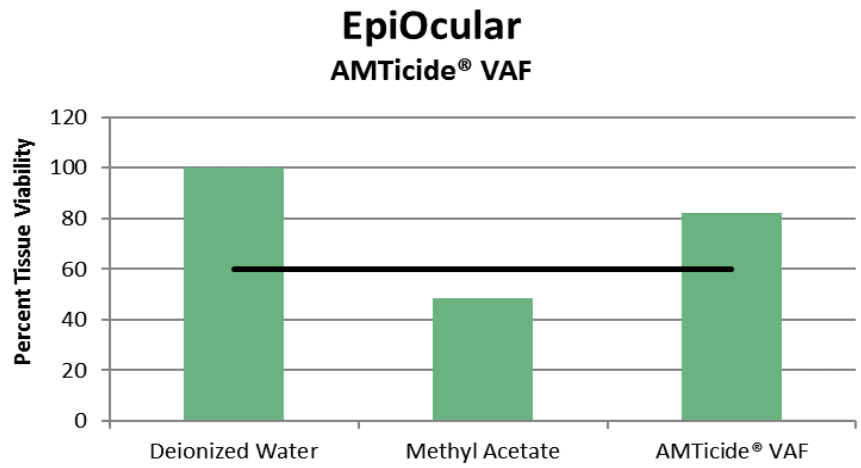

Figure 2: EpiOcular tissue viability

OECD TG 442C: *In Chemico* Skin Sensitization

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(704) 276-7100 • Fax (704) 276-7101

Tradename: AMTicide® VAF

Code: M14004

CAS #: 92128-81-9 (or) 68582-99-0 & 8013-01-2

Test Request Form #: 4038

Lot #: NC171207-C

Sponsor: *Active Micro Technologies, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Danaher*
Principle Investigator: *Jennifer Goodman*

Test Performed:
OECD TG 442C: *In Chemico* Skin Sensitization
Direct Peptide Reactivity Assay (DPRA)

Introduction

A skin sensitizer is a substance that will lead to an allergic response following skin contact[1]. Haptenation is the covalent binding of a hapten, or low-molecular weight substance or chemical, to proteins in the skin. This is considered the prominent mechanism which defines a chemical as a sensitizer. Haptenation is described as a "molecular initiating event" in the OECD Adverse Outcome Pathway (AOP) for skin sensitization which summarizes the key events known to be involved in chemically-induced allergic contact dermatitis[2]. The direct peptide reactivity assay (DPRA) is designed to mimic the covalent binding of electrophilic chemicals to nucleophilic centers in skin proteins by quantifying the reactivity of chemicals towards the model synthetic peptides containing cysteine and lysine. The DPRA is able to distinguish sensitizers from non-sensitizer with 82% accuracy (sensitivity of 76%; specificity of 92%)[3].

This assay was conducted to determine skin sensitization hazard of **AMTicide® VAF** in accordance with European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) and OECD Test Guideline 442C.

Assay Principle

The DPRA is an *in chemico* method which addresses peptide reactivity by measuring depletion of synthetic heptapeptides containing either cysteine or lysine following 24 hours incubation with the test substance. The peptide is a custom material containing phenylalanine to aid in detection. Depletion of the peptide in the reaction mixture is measured by HPLC with gradient elution and UV detection at 220 nm. Cysteine and lysine peptide percent depletion values are then calculated and used in a prediction model which allows assigning the test chemical to one of four reactivity classes used to support the discrimination between sensitizers and non-sensitizers.

1. United Nations Economic Commission (UNECE) (2013) Global Harmonized System of Classification and Labelling of Chemicals (GHS) 5th Revised Edition
2. OECD (2012). The Adverse Outcome Pathway for Skin Sensitization Initiated by Covalent Binding to Proteins. Part 1: Scientific Evidence. Series on Testing and Assessment No. 168
3. EC EURL ECVAM (2012) Direct peptide reactivity assay (DPRA) validation study report; pp 1 -74.

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Materials

A.	**Equipment:**	HPLC-UV (Waters Alliance 2695 - Waters 996 Photodiode Array); Pipettes; Analytical balance
B.	**HPLC/Guard Columns:**	Agilent Zorbax SB-C18 2.1mm x 100mm x 3.5µm; Phenomenex Security Guard C18 4mm x 2mm
C.	**Chemicals:**	Trifluoroacetic acid; Ammonium acetate; Ammonium hydroxide; Acetonitrile; Cysteine peptide (Ac-RFAA**C**AA-COOH); Lysine peptide (Ac-RFAA**K**AA-COOH); Cinnamic aldehyde
D.	**Reagents/Buffers:**	Sodium phosphate buffer (100mM); Ammonium acetate buffer (100mM)
E.	**Other:**	Sterile disposable pipette tips

Methods

Solution Preparation:

- 0.667mM Cysteine Peptide in 100mM Phosphate Buffer (pH 7.5)
- 0.667mM Lysine Peptide in 100mM Ammonium Acetate Buffer (pH 10.2)
- 100mM Cinnamic Aldehyde in Acetonitrile
- 100mM* **AMTicide® VAF** in Acetonitrile

*For mixtures and multi-constituent substances of known composition such as **AMTicide® VAF**, a single purity should be determined by the sum of the proportion of its constituents (excluding water), and a single apparent molecular weight determined by considering the individual molecular weights of each component in the mixture (excluding water) and their individual proportions. The resulting purity and apparent molecular weight can then be used to calculate the weight of test chemical necessary to prepare a 100 mM solution.

Reference Controls:

- Reference Control A: For calibration curve accuracy
- Reference Control B: For peptide stability over analysis time of experiment
- Reference Control C: For verification that the solvent does not impact percent peptide depletion

Sample, Reference Control, and Co-Elution Control Preparation:

- Once these solutions have been made they should be incubated at room temperature, protected from light, for 24±2 hours before running HPLC analysis.
- Each chemical should be analyzed in triplicate.

1:10 Ratio, Cysteine Peptide 0.5mM Peptide, 5mM Test Chemical	1:50 Ratio, Lysine Peptide 0.5mM Peptide, 25mM Test Chemical
• 750µL Cysteine Peptide Solution (or 100mM Phosphate Buffer, pH 7.5, for Co-Elution Controls) • 200µL Acetonitrile • 50µL Test Chemical Solution (or Acetonitrile for Reference Controls)	• 750µL Lysine Peptide Solution (or 100mM Ammonium Acetate Buffer, pH 10.2, for Co-Elution Controls) • 250µL Test Chemical Solution (or Acetonitrile for Reference Controls)

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Calibration Curve:

- Standards are prepared in a solution of 20% Acetonitrile:Buffer
  - For the Cysteine peptide using the phosphate buffer, pH 7.5
  - For the Lysine peptide using the ammonium acetate buffer, pH 10.2

	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6	Standard 7
mM Peptide	0.534	0.267	0.1335	0.0667	0.0334	0.0167	0.000

HPLC Analysis:

- HPLC-UV system should be equilibrated at 30°C with 50% Mobile Phase A (0.1% (v/v) trifluoroacetic acid in water) and 50% Mobile Phase B (0.085% (v/v) trifluoroacetic acid in acetonitrile) for 2 hours
- Absorbance is measured at 220nm
- Flow Conditions:

Time	Flow	%A	%B
0 minutes	0.35 mL/min	90	10
10 minutes	0.35 mL/min	75	25
11 minutes	0.35 mL/min	10	90
13 minutes	0.35 mL/min	10	90
13.5 minutes	0.35 mL/min	90	10
20 minutes	End Run		

Data and Reporting

Acceptance Criteria:

1. The following criteria must be met for a run to be considered valid:
   a. Standard calibration curve should have an $r^2 > 0.99$.
   b. Mean percent peptide depletion values of three replicates for the positive control cinnamic aldehyde should be between 60.8% and 100% for the cysteine peptide and between 40.2% and 69% for the lysine peptide and the maximum standard deviation should be <14.9 for the percent cysteine depletion and <11.6 for the percent lysine depletion.
   c. Mean peptide concentration of reference controls A should be 0.50±0.05mM and the coefficient of variable of the peptide peak areas for reference B and C in acetonitrile should be <15.0%.

2. The following criteria must be met for a test chemical's results to be considered valid:
   a. Maximum standard deviation should be <14.9 for percent cysteine depletion and <11.6 for percent lysine depletion.
   b. Mean peptide concentration of the three reference control C should be 0.50±0.05mM.

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Prediction Model:

Cysteine 1:10/Lysine 1:50 Prediction Model		
Mean of Cysteine and Lysine % Depletion	**Reactivity Class**	**Prediction**
0% < Mean % Depletion < 6.38%	Minimal Reactivity	Non-sensitizer
6.38% < Mean % Depletion < 22.62%	Low Reactivity	Sensitizer
22.62% < Mean % Depletion < 42.47%	Moderate Reactivity	Sensitizer
42.47% < Mean % Depletion < 100%	High Reactivity	Sensitizer

If co-elution occurs with the lysine peptide, than the cysteine 1:10 prediction model can be used:

Cysteine 1:10 Prediction Model		
Mean of Cysteine and Lysine % Depletion	**Reactivity Class**	**Prediction**
0% < Cys % Depletion < 13.89%	Minimal Reactivity	Non-sensitizer
13.89% < Cys % Depletion < 23.09%	Low Reactivity	Sensitizer
23.09% < Cys % Depletion < 98.24%	Moderate Reactivity	Sensitizer
98.24% < Cys % Depletion < 100%	High Reactivity	Sensitizer

Therefore the measured values of % depletion in the three separated runs for each peptide depletion assay include:

Cysteine 1:10/Lysine 1:50 Prediction Model		
Mean of Cysteine and Lysine % Depletion	**Reactivity Class**	**Prediction**
3.25	Minimal Reactivity	Non-sensitizer
3.21	Minimal Reactivity	Sensitizer
3.26	Minimal Reactivity	Sensitizer

Cysteine 1:10 Prediction Model		
Mean of Cysteine and Lysine % Depletion	**Reactivity Class**	**Prediction**
3.18	Minimal Reactivity	Non-sensitizer
3.22	Minimal Reactivity	Sensitizer
3.21	Minimal Reactivity	Sensitizer

Results and Discussion

The data obtained from this study met criteria for a valid assay and the controls performed as anticipated.

Percent peptide depletion is determined by the following equation:

$$Percent\ Peptide\ Depletion = \left[1 - \left(\frac{Peptide\ Peak\ Area\ in\ Replicate\ Injection}{Mean\ Peptide\ Peak\ Area\ in\ Reference\ Controls\ C}\right)\right] \times 100$$

Based on HPLC-UV analysis of **AMTicide® VAF** we can determine that this product is not a sensitizer and will not cause allergic contact dermatitis. The Mean Percent Depletion of Cysteine and Lysine was 2.89% causing minimal reactivity in the assay giving us the prediction of a non-sensitizer.

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OECD TG 442D: *In Vitro* Skin Sensitization

Tradename: AMTicide® VAF

Code: M14004

CAS #: 92128-81-9 (or) 68582-99-0 & 8013-01-2

Test Request Form #: 4037

Lot #: NC171207-C

Sponsor: *Active Micro Technologies, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Danaher*
Principle Investigator: *Jennifer Goodman*

Test Performed:
OECD TG 442D: In Vitro Skin Sensitization
ARE-Nrf2 Luciferase Test Method

Introduction

Skin sensitization refers to an allergic response following skin contact with the tested chemical, as defined by the United Nations Globally Harmonized System of Classification and Labelling of Chemicals[1]. Substances are classified as skin sensitizers if there is evidence in humans that the substance can lead to sensitization by skin contact or positive results from appropriate tests, both *in vivo* and *in vitro*. Utilization of the KeratinoSens™ cell line allows for valid *in vitro* testing for skin sensitization.

This assay was conducted to determine skin sensitization potential of **AMTicide® VAF** in accordance with the UN GHS.

Assay Principle

The ARE-Nrf2 luciferase test method addresses the induction of genes that are regulated by antioxidant response elements (ARE) by skin sensitizers. The Keap1-Nrf2-ARE pathways have been shown to be major regulator of cytoprotective responses to oxidative stress or electrophilic compounds. These pathways are also known to be involved in the cellular processes in skin sensitization. Small electrophilic substances such as skin sensitizers can act on the sensor protein Keap1 (Kelch-like ECH-associated protein 1), by covalent modification of its cysteine residue, resulting in its dissociation from the transcription factor Nrf2 (nuclear factor-erythroid 2-related factor 2). The dissociated Nrf2 can then activate ARE-dependent genes such as those coding for phase II detoxifying enzymes.

The skin sensitization assay utilizes the KeratinoSens™ method which uses an immortalized adherent human keratinocyte cell line (HaCaT cell line) that has been transfected with a selectable plasmid to quantify luciferase gene induction as a measure of activation of Keap1-Nrf2-antioxidant/electrophile response element (ARE). This test method has been validated by independent peer review by the EURL-ECVAM. The addition of a luciferin containing reagent to the cells will react with the luciferase produced in the cell resulting in luminescence which can be quantified with a luminometer.

1. United Nations (UN) (2013). Globally Harmonized System of Classification and Labelling of Chemicals (GHS), Fifth revised edition, UN New York and Geneva, 2013

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Materials

A. Incubation Conditions:	37°C at 5% CO_2 and 95% relative humidity (RH)
B. Equipment:	Humidified incubator; Biosafety laminar flow hood; Microplate Reader; Pipettes
C. Cell Line:	KeratinoSens™ by Givaudan Schweiz AG
D. Media/Buffers:	Dulbecco's Modified Eagle Medium (DMEM); Fetal Bovine Serum (FBS); Phosphate Buffered Saline (PBS); Geneticin
E. Culture Plate:	Flat bottom 96-well tissue culture treated plates
F. Reagents:	Dimethyl Sulfoxide (DMSO); Cinnamic Aldehyde; ONE-Glo Reagent; 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT); sodium lauryl sulfate (SLS)
G. Other:	Sterile disposable pipette tips; wash bottles

Methods

KeratinoSens™ were into seeded four 96-well tissue culture plates and allowed to grow to 80 – 90% confluency in DMEM containing 10% FBS and 500µg/mL G418 geneticin. Twelve test concentrations of **AMTicide® VAF** were prepared in DMSO with a concentration range from 0.98 - 2000 µM. These 12 concentrations were assayed in triplicate in 2 independently performed experiments. The positive control was cinnamic aldehyde for which a series of 5 concentrations prepared in DMSO had final test concentrations of 4 – 64µM. The negative control was a 1% test concentration of DMSO.

24 hour post KeratinoSens™ seeding, the culture media was removed and replaced with fresh media containing 10% FBS without G418 geneticin. 50 µL of the above described test concentrations was added to the appropriate wells. The treated plates were then incubated for 48 hours at 37°C in the presence of 5% CO_2 and 95% relative humidity. After treatment incubation was complete the media was removed and the wells were washed with PBS 3 times.

One of the four plates was used for a cytotoxicity endpoint, where MTT was added to the wells and incubated for 4 hours at 37°C in the presence of 5% CO_2. SLS was then added to the wells and incubated overnight at room temperature. A spectrometer measured the absorbance at 570 nm. The absorbance values (optical density) were then used to determine the viability of each well by comparing the optical density of each test material treated well to that of the solvent control wells to determine the IC_{50} and IC_{30} values.

The remaining 3 plates were used in the luciferase induction endpoint of the assay. 100 µL of Promega's ONE-Glo Reagent was added to 100 µL of fresh media containing 10% FBS without geneticin. Cells were incubated for 5 minutes to induce cell lysis and release luciferin into the media. Plates were read with a luminometer and $EC_{1.5}$ and maximum response (I_{max}) values were obtained.

Data and Reporting

Acceptance Criteria:

1. Gene induction obtained with the positive control, cinnamic aldehyde, should be statistically significant above the threshold of 1.5 in at least one of the tested concentrations (from 4 to 64 µM).
2. The EC1.5 value should be within two standard deviations of the historical mean and the average induction in the three replicates for cinnamic aldehyde at 64 µM should be between 2 and 8.
3. The average coefficient of variability of the luminescence reading for the negative (solvent) control DMSO should be below 20% in each experiment.

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A KeratinoSens™ prediction is considered positive if the following conditions are met:

1. The Imax is higher than 1.5-fold and statistically significantly higher as compared to the solvent (negative) control
2. The cellular viability is higher than 70% at the lowest concentration with a gene induction above 1.5 fold (i.e., at the EC1.5 determining concentration)
3. The $EC_{1.5}$ value is less than 1000 µM (or < 200 µg/ml for test chemicals with no defined MW)
4. There is an apparent overall dose-response for luciferase induction

Results

Compound	Classification	$EC_{1.5}$ (µM)	IC_{50}	I_{max}
Cinnamic aldehyde	Sensitizer	19	289.19 µM	31.4
DMSO	Non-Sensitizer	No Induction	243.24 µM	0.19
AMTicide® VAF	Non-Sensitizer	No Induction	> 1000 µM	0.38

Table 1: Overview of KeratinoSens™ Assay Results

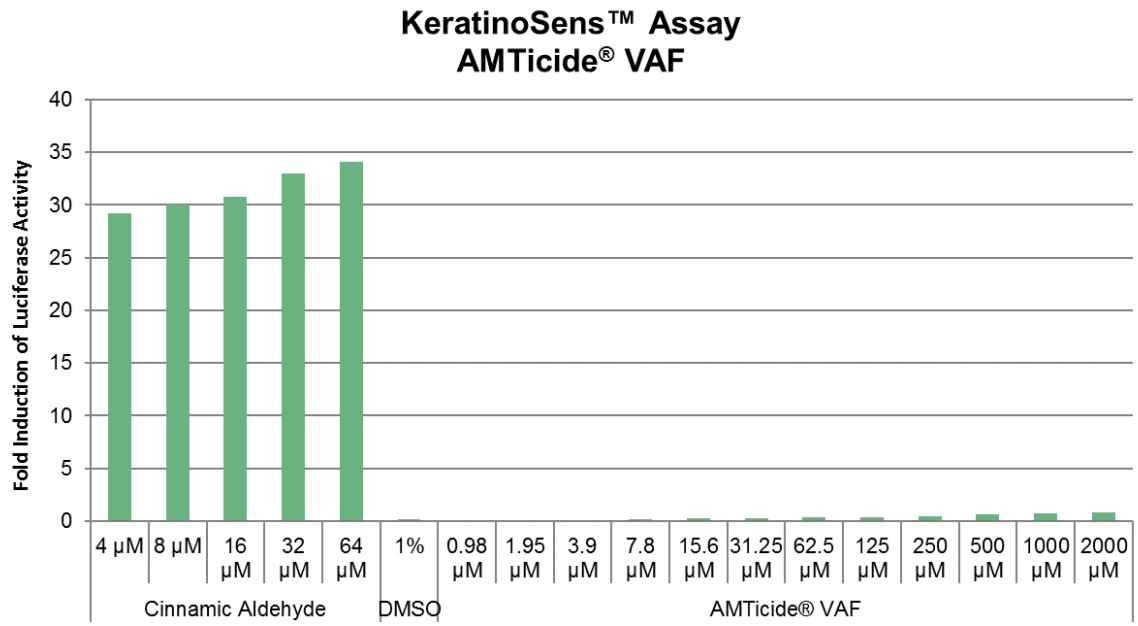

Figure 1: Fold Induction of Luciferase

Discussion

As shown in the results, **AMTicide® VAF (M14004)** was not predicted to be a skin sensitizer based on the KeratinoSens™ ARE-Nrf2 Luciferase Test Method as there was not a significant increase in luciferase expression. It can be concluded that **AMTicide® VAF** can be safely used in cosmetics and personal care products at typical use levels.

OECD 201 Freshwater Alga Growth Inhibition Test

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(704) 276-7100 • Fax (704) 276-7101

Tradename: AMTicide® VAF

Code: M14004

CAS #: 92128-81-9 (or) 68582-99-0 & 8013-01-2

Test Request Form #: 6090

Lot #: S190711C

Sponsor: *Active Micro Technologies, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Danaher*
Principle Investigator: *Jennifer Goodman*

Test Performed:
OECD 201
Freshwater Alga Growth Inhibition Test

Introduction

The purpose of the present study is to determine the toxicity of **AMTicide® VAF** by exposing the exponentially growing test organism *Pseudokirchneriella subcapitata* to the test substance for 72 hours and measuring the growth and growth inhibition through cell counting against the control. The response is evaluated as a function of the exposure concentration in comparison with the average growth of replicate, unexposed control cultures.

OECD Guideline 201 on "Fresh Alga and Cyanobacteria, Growth Inhibition Test", adopted in 1984, extended the guideline to include additional species and update it to meet the requirements for hazard assessment and classification of chemicals in 2006.

Assay Principle

Pseudokirchneriella subcapitata, are exposed to the test substance at a range of concentrations for a period of 72 hours. The cultures are allowed unrestricted exponential growth under nutrient sufficient conditions and continuous light for a sufficient period of time to measure reduction of the specific growth rate. Growth and growth inhibition are quantified from measurements of the algal biomass as a function of time. The test endpoint is inhibition of growth, expressed as the logarithmic increase in biomass during the 72 hour exposure period. The results are analyzed in order to calculate the EC_{10} and EC_{20} at 72 hours. The response is evaluated as a function of the exposure concentration in comparison with the average growth of replicate, unexposed control cultures.

A reliable analytical method for the quantification of the substance in the test solutions with reported recovery efficiency and limit of determination should be available. A reference substance may be tested for EC_{50} as a means of assuring that the test conditions are reliable.

Analysis of the concentration of the test substance at the start and end of the test of a low and high test concentration around the expected EC_{50} may be sufficient where it is likely that exposures concentrations will vary less than 20% from the nominal values during the test.

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Materials

- Glass Flasks with air-permeable stopper
- Automated Pipette
- pH Meter
- Temperature Control Apparatus
- Microscope with counting chamber
- *Pseudokirchneriella subcapitata* (ATCC 22662)
- Gorham's medium for algae (ATCC MD-0625)

Methods

Test Conditions

- Incoculum Culture
  - Inoculum culture is incubated under the same conditions as the test cultures for 2-4 days allowing for exponential growth to prevail before the start of the test. This is done to ensure that growth is within the normal range for the test strain under the culturing conditions.
- Initial Biomass
  - The initial biomass in the test cultures must be the same in all test cultures and sufficiently low to allow exponential growth throughout the incubation period without risk of nutrient depletion. The initial biomass should not exceed 0.5 mg/L as dry weight.
- Exposure Period
  - 72 hours
- Number of Test Organisms
  - *Pseudokirchneriella subcapitata* 5 x 10^{3-4} cells/ml
- Test Concentration
  - Adopt a concentration range of at least 5 concentrations, causing a range of 5-75% inhibition of algal growth rate expressed as E_rC_x
- Culture Method
  - Illumination: Continuous uniform fluorescent illumination
  - Temperature: The temperature is between 21°C to 24°C
  - pH: pH of the control medium should not increase be more than 1.5 units during test

Measurement of Test Substance Concentrations

- Measurement of biomass is done by manual cell counting by microscope.
- Algal biomass in each flask is determined daily during test period.
- At the beginning and end of exposure, measure test substance concentrations at the lowest and highest test concentration groups.
  - For volatile or adsorptive substances, additional measurements are recommended at 24 hours intervals during exposure period.

Observation

- Microscopic observation should be performed to verify a normal and healthy appearance of the inoculum culture and to observe any abnormal appearance of the algae at the end of the test.

Test Condition Measurements

- Measure pH in the control and at the highest test concentration at the beginning and end of the exposure period.
- Water temperature should be measured at the beginning and end of the exposure period.

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Data and Reporting

I. Data
   a. Tabulate the estimated biomass concentration in test cultures and controls together with the concentrations of test materials and the times of measurement, recorded with a resolution of at least whole hours, to produce plots of growth curves.
   b. For each response variable to be analyzed, use the concentration-response relationship to calculate point estimates of EC_x values. Recent scientific developments have led to a recommendation of abandoning the concept of NOEC and replacing it with regression based point estimates EC_x, specifically EC_{10} and EC_{20}.

II. Test Report
   a. The test report must include the following:
      i. Test substance:
         1. Physical nature and relevant physical-chemical properties
         2. Chemical identification data, including purity
      ii. Test species:
         1. Source and species of *Pseudokirchneriella subcapitata*, supplier of source (if known), and the culture conditions (including source, kind and amount of food, feeding frequency)
      iii. Test conditions:
         1. Description of test vessels: type and volume of vessels, volume of solution, density of *Pseudokirchneriella subcapitata* per test vessel, number of test vessels (replicates) per concentration
         2. Methods of preparation of stock and test solutions including the use of any solvent or dispersants, concentrations used
         3. Details of dilution water: source and water quality characteristics (pH, hardness, Ca/Mg ratio, Na/K ratio, alkalinity, conductivity, etc); composition of reconstituted water if used
         4. Incubation conditions: temperature, light intensity and periodicity, pH, etc.
      iv. Results:
         1. The nominal test concentrations and the result of all analyses to determine the concentration of the test substance in the test vessels; the recovery efficiency of the method and the limit of determination should also be reported
         2. All physical-chemical measurements of temperature and pH made during the test
         3. The EC_{10} and EC_{20} at 72 hours for percent inhibition with confidence intervals and graphs of the fitted model used for calculation, the slopes of the dose-response curves and their standard error; statistical procedures used for determination of EC_{10} and EC_{20}.

$$Percent\ (\%)Inhibition = \frac{\mu c - \mu T}{\mu c} \times 100$$

μ_c: mean value for average specific growth rate (μ) in the control group
μ_T: average specific growth rate for the treatment replicate

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Results

General Information:

Name of new chemical substance	AMTicide® VAF		
INCI Nomenclature	Bacillus Ferment & Saccharomyces Ferment Filtrate		
CAS number	92128-81-9 (or) 68582-99-0 & 8013-01-2		
Formulation Method	Fermentation		
Molecular weight	559.21 Da		
Purity of the new chemical substance used for the test (%)	100%		
Lot number of the new chemical substance used for the test	S190711C		
Names and contents of impurities	N/A		
Solubility in water	Soluble		
Properties at room temperature	Clear to Slightly Hazy Gardner 3 Maximum Liquid, Characteristic Odor		
Stability	Stable Under Normal Conditions		
Solubility in solvents, etc.	**Solvent**	**Solubility**	**Stability in solvent**
	N/A	N/A	N/A

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OECD 201 Freshwater Alga Growth Inhibition Test

Test Materials and Methods:

Items			**Contents**
Test Organisms	Species		*Pseudokirchneriella subcapitata*
	Source		ATCC
	Reference substance (EC_{50})		3,5-dichlorophenol
Culture	Kind of Medium		Gorham's Medium for Algae
	Conditions (Temperature)		22°C ± 2°C
Test Conditions	Test Vessel		Glass
	Material Water	Kind	Deionized
		Hardness	250 mg/L
		pH	7.4
	Date of Exposure		08/05/2019
	Test Concentrations		200, 89.4, 42.3, 19.2, 7.8 mg/L
	Number of organisms		5 x 103-4 cells/ml
	Number of Replicates	Exposure Group	4
		Control Group	4
	Test Solution Volume		5 mL
	Vehicle	Use or Not	N/A
		Kind	N/A
		Concentration	N/A
		Number of Replicates	N/A
	Photoperiod		Continuous

Test Results:

Items		**Contents**
Toxicity Value	Percent Inhibition EC_{10} and EC_{20}	271.22 mg/L and 406.19 mg/L
Exposure Concentrations Used for Calculation	Nominal Values	200, 89.4, 42.3, 19.2, 7.8 mg/L
Remarks		Not harmful to aquatic organisms

Discussion

After 72 hours, the percent inhibition for **AMTicide® VAF** was determined to be 271.22 mg/L EC_{10} and 406.19 mg/L EC_{20}. The conditions of OECD guideline 201 for the validity of the test were adhered to, this product is not classified and therefore not harmful to aquatic organisms.

OECD 301B Ready Biodegradability Assay

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Tradename: AMTicide® VAF

Code: M14004

CAS #: 92128-81-9 (or) 68582-99-0 & 8013-01-2

Test Request Form #:

Lot #: NC171207-C

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Danaher*
Principle Investigator: *Jennifer Goodman*

Test Performed:
OECD 301 B
Ready Biodegradability: CO_2 Evolution (Modified Sturm Test)

Introduction

A study was conducted to assess the ready biodegradability of **AMTicide® VAF** in an aerobic aqueous medium. In the OECD guideline 301 for ready biodegradability, six methods are provided as options. This report uses method B, CO_2 Evolution, also known as a Modified Sturm Test. This method was chosen based on the solubility, volatility, and adsorbing capabilities of the test sample.

Assay Principle

A solution or suspension of the test substance in a mineral medium is inoculated and incubated under aerobic conditions in the dark or in diffuse light. The amount of DOC (Dissolved Organic Carbon) in the test solution due to the inoculum should be kept as low as possible compared to the amount of organic carbon due to the test substance. Allowance is made for the endogenous activity of the inoculum by running parallel blanks with inoculum but without test substance. A reference compound is run in parallel to check the procedures' operation.

In general, degradation is followed by the determination of parameters such as DOC, carbon dioxide production, and oxygen uptake. Measurements are taken at sufficiently frequent intervals to allow the identification of the beginning and end of biodegradation.

Normally this test lasts for 28 days, but it may be ended before that time if the biodegradation curve reaches a plateau for at least three determinations. Tests may also be prolonged beyond 28 days when the curve shows that biodegradation has started but the plateau has not yet been reached. In such cases the test substance would not be classified as readily biodegradable.

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The pass levels for ready biodegradability are 70% removal of DOC and 60% of ThOD (Theoretical Oxygen Demand) or $ThCO_2$ (Theoretical Carbon Dioxide) production for respirometric methods. They are lower in the respirometric methods since, as some of the carbon from the test chemical is incorporated into new cells, the percentage of CO_2 produced is lower than the percentage of carbon being used. These pass values have to be reached in a 10-day window within the 28-day period of the test. The 10-day window begins when the degree of biodegradation has reached 10% DOC, ThOD, or $ThCO_2$ and must end before day 28 of the test. Test substances which reach the pass levels after the 28-day period are not deemed to be readily biodegradable.

In order to check the procedure, reference compounds which meet the criteria for ready biodegradability are tested by setting up an appropriate vessel in parallel as part of normal test runs. Suitable compounds are freshly distilled aniline, sodium acetate, and sodium benzoate. These compounds all degrade in this method even when no inoculum is deliberately added.

Because of the nature of biodegradation and of the mixed bacterial populations used as inocula, determinations should be carried out at least in duplicate. It is usually found that the larger the concentration of microorganisms initially added to the test medium, the smaller the variation between replicates.

Materials

- Water
  - Deionized or distilled, free from inhibitory concentrations of toxic substances
  - Must contain no more than 10% of the organic carbon content introduced by the test material
  - Use only one batch of water for each series of tests
- Mineral media
  - To prepare the mineral medium, mix 10 mL of solution A with 800 mL water. Then add 1 mL each of solutions B, C, and D and make up to 1 liter with water.
  - Solution A (Dissolve in water and make up to 1 liter; pH 7.4)
    - Potassium dihydrogen orthophosphate, KH_2PO.....8.5g
    - Dipotassium hydrogen orthophosphate, K_2HPO_4.....21.8g
    - Disodium hydrogen orthophosphate dehydrate, $Na_2HPO_4.2H_2O$.....33.4g
    - Ammonium chloride, NH_4Cl.....0.5g
  - Solution B (Dissolve in water and make up to 1 liter)
    - Calcium chloride, anhydrous, $CaCl_2$.....27.50g
      Or
    - Calcium chloride dehydrate, $CaCl_2.2H_2O$.....36.40g
  - Solution C (Dissolve in water and make up to 1 liter)
    - Magnesium sulphate heptahydrate, $MgSO_4.7H_2O$.....22.50g
  - Solution D (Dissolve in water and make up to 1 liter.)
    - Iron (III) chloride hexahydrate, $FeCl_3.6H_2O$.....0.25g
  - Flasks, 2-5 liters each, fitted with aeration tubes reaching nearly to the bottoms of the vessels and an outlet
  - Magnetic stirrers
  - Gas absorption bottles
  - Device for controlling and measuring air flow
  - Apparatus for carbon dioxide scrubbing, for preparation of air which is free from carbon dioxide; alternatively, a mixture of CO_2-free oxygen and CO_2-free nitrogen from gas cylinders in the correct proportions (20% O_2 : 80% N_2)
  - Device for determination of carbon dioxide, either titrimetrically or by some form of inorganic carbon analyzer

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- Stock solutions of test substances
  - When solubility of the substance exceeds 1 g/L, dissolve 1-10 g, as appropriate, of test or reference substance in water and make up to 1 liter. Otherwise, prepare stock solutions in mineral medium or add the chemical directly to the mineral medium, making sure it
- Inoculum
  - The inoculum may be derived from the following sources
    - Activated sludge
    - Sewage effluents
    - Surface waters
    - Soils
    - Or from a mixture of these.
  - Inoculum may be pre-conditioned to the experimental conditions, but not pre-adapted to the test substance. Pre-conditioning consists of aerating activated sludge in mineral medium or secondary effluent for 5-7 days at the test temperature. Pre-conditioning sometimes improves the precision of the test method by reducing blank values.

Methods

I. Preparation of flasks: As an example, the following volumes and weights indicate the values for 5-liter flasks containing 3 liters of suspension. If smaller volumes are used, modify the values accordingly.

a. To each 5-liter flask, add 2,400 mL mineral medium.
b. Add an appropriate volume of the prepared activated sludge to give a concentration of suspended solids of not more than 30 mg/L in the final 3 liters of inoculated mixture. Alternatively, first dilute the prepared sludge to give a suspension of 500-1000 mg/L in the mineral medium before adding an aliquot to the contents of the 5-liter flask to attain a concentration of 30 mg/L.
c. Aerate these inoculated mixtures with CO_2-free air overnight to purge the system of carbon dioxide.
d. Add the test material and reference compound, separately, as known volumes of stock solutions, to replicate flasks to yield concentrations, contributed by the added chemicals, of 10 – 20 mg DOC or TOC per liter. Leave some flasks without addition of chemicals as inoculum controls. Add poorly soluble test substances directly to the flasks on a weight or volume basis. Make up the volumes of suspensions in all flasks to 3 liters by the addition of mineral medium previously aerated with CO_2-free air.
e. If required, use one flask to check the possible inhibitory effect of the test substance by adding both the test and reference substances at the same concentrations as present in the other flasks.

f. If required, check whether the test substance is degraded abiotically by using a sterilized uninoculated solution of the chemical. Sterilize by the addition of a toxic substance at an appropriate concentration.
g. If barium hydroxide is used, connect three absorption bottles, each containing 100 mL of 0.0125M barium hydroxide solution, in series to each 5-liter flask. The solution must be free of precipitated sulfate and carbonate and its strength must be determined immediately before use.
h. If sodium hydroxide is used, connect two traps, the second acting as a control to demonstrate that all the carbon dioxide was absorbed in the first. Absorption bottles fitted with serum bottle closures are suitable. Add 200 mL 0.05M sodium hydroxide to each bottle. This is sufficient to absorb the total quantity of carbon dioxide evolved when the test substance is completely degraded.
i. In a typical run, the following flasks are used:
   i. Flasks 1 & 2: containing test substance and inoculum (test suspension)
   ii. Flasks 3 & 4: containing only inoculum (inoculum blank)
   iii. Flask 5: containing reference compound and inoculum (procedure control)
   iv. Flask 6: containing test substance and sterilizing agent (abiotic sterile control)
   v. Flask 7: containing test substance, reference compound and inoculum (toxicity control)

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II. Start the test by bubbling CO_2-free air through the suspensions at a rate of 30-100 mL/minute.

III. CO_2 Determination
   a. It is mandatory to follow the CO_2 evolution from the test suspensions and inoculum blanks in parallel and it is advisable to do the same for the other test vessels.
   b. During the first ten days it is recommended that analyses of CO_2 should be made every second or third day and then at least every fifth day until the 28th day so that the 10-day window period can be identified. On the days of CO_2 measurement, disconnect the barium hydroxide absorber closest to the test vessel and titrate the hydroxide solution with 0.05M HCl using phenolphthalein as the indicator. Move the remaining absorbers one place closer to the test vessel and place a new absorber containing 100 mL fresh 0.0125M barium hydroxide at the far end of the series. Make titrations are needed (for example, when substantial precipitation is seen in the first trap and before any is evident in the second, or at least weekly). Alternatively, with NaOH as absorbent, withdraw a sample of the sodium hydroxide solution from the absorber nearest to the test vessel using a syringe. The sample volume needed will depend on the carbon analyzer used, but sampling should not significantly change the absorbent volume over the test period. Inject the sample into the IC part of the carbon analyzer for analysis of evolved carbon dioxide directly. Analyze the contents of the second trap only at the end of the test in order to correct for any carry-over of carbon dioxide.
   c. On the 28th day withdraw samples, optionally, for DOC and/or specific chemical analysis. Add 1 mL of concentrated hydrochloric acid to each test vessel and aerate them overnight to drive off the carbon dioxide present in the test suspensions. On day 29 make the last analysis of evolved carbon dioxide.

Data and Reporting

I. Treatment of Results
   a. Data from the test should be entered onto the attached data sheet.
   b. The amount of CO_2 produced is calculated from the amount of base remaining in the absorption bottle. When 0.0125M $Ba(OH)_2$ is used as the absorbent, the amount remaining is assessed by titrating with 0.05M HCl.
   c. Since 1 mmol of CO_2 is produced for every mol of $Ba(OH)_2$ reacted to $BaCl_2$ and 2 mmol of HCl are needed for the titration of the remaining $Ba(OH)_2$ and given that the molecular weight of CO_2 is 44 g, the weight of CO_2 produced (in mg) is calculated by:

$$\frac{0.05 \times (50 - mL\ HCl\ Titrated) \times 44}{2} = 1.1 \times (50 - mL\ HCl\ Titrated)$$

   Therefore, the factor to convert volume of HCl titrated to mg CO_2 produced is 1.1 in this case. Calculate the weights of CO_2 produced from the inoculum alone and from the inoculum plus test substance using the respective titration values. The difference is the weight of CO_2 produced from the test substance alone.

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d. The percentage biodegradation is calculated from:

$$\%\ Degradation = \frac{mg\ CO_2\ Produced}{ThCO_2 \times mg\ Test\ Substance\ Added} \times 100$$

Or

$$\%\ Degradation = \frac{mg\ CO_2\ Produced}{mg\ TOC\ Added\ in\ Test\ \times 3.67} \times 100$$

$$Where\ 3.67\ is\ the\ conversion\ factor\ \left(\frac{44}{12}\right)\ for\ carbon\ to\ carbon\ dioxide$$

e. When NaOH is used as the absorbent, calculate the amount of CO_2 produced after any time interval from the concentration of inorganic carbon and the volume of absorbent used. Calculate the percentage degradation from:

$$\%\ ThCO_2 = \frac{mg\ IC\ from\ Test\ Flask - mg\ IC\ from\ Blank}{mg\ TOC\ Added\ as\ Test\ Substances} \times 100$$

f. Display the course of degradation graphically and indicate the 10-day window. Calculate and report the percentage removal achieved at the plateau, at the end of the test, and/or at the end of the 10-day window, whichever is appropriate.
g. When appropriate, calculate DOC removals using the equation given in 301 A paragraph 27.
h. When an abiotic control is used, calculate the percentage abiotic degradation by:

$$\%\ Abiotic\ Degradation = \frac{CO_2\ Produced\ by\ Sterile\ Flask\ After\ 28\ Days\ (mg)}{ThCO_2\ (mg)} \times 100$$

Validity of Tests

The IC content of the test substance suspension in the mineral medium at the beginning of the test must be less than 5% of the TC, and the total CO_2 evolution in the inoculum blank at the end of the test should not normally exceed 40 mg/L medium. If values greater than 70 mg CO_2/L are obtained, the data and experimental technique should be examined critically.

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OECD 301B Ready Biodegradability Assay

Data Sheet

Laboratory	Active Concepts Tissue Culture Laboratory		
Test Start Date	02/05/2018		
Test Substance	**Name**	**AMTicide® VAF**	
	Stock Solution Concentration	2 g/L	
	Initial Concentration in Medium	20 mg/L	
Inoculum	**Source**	Activated Sludge	
	Treatment Given	Centrifugation	
	Pre-conditioning	N/A	
	Suspended Solids Concentration in Reaction Mixture	4 mg/L	
Reference Material	Sodium Benzoate	**Concentration**	20 mg/L
CO_2 Production and Degradability	**Method**	**$Ba(OH)_2$**	0.0125M
		NaOH	N/A
		Other	N/A
Total Contact Time	28 Days		
Total CO_2 Evolved Measurements	**Days**	2, 4, 11, 17, 23, 28	
Degradation Over Time	87.6% and 89.2% after 28 days		
Remarks	Test material was readily biodegradable		
Conclusion	This test met the criteria for a valid assay		

Discussion

Based on the testing conducted in accordance with the specified test method, **AMTicide® VAF** achieved 88.4% biodegradation after 28 days of testing. The product met method requirements for the Readily Biodegradable classification.

Phototoxicity Assay Analysis

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Tradename: AMTicide® VAF

Code: M14004

CAS #: 92128-81-9 (or) 68582-99-0 & 8013-01-2

Test Request Form #: 4041

Lot #: NC171207-C

Sponsor: Active Micro Technologies, LLC*; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Danaher*
Principle Investigator: *Jennifer Goodman*

Test Performed:
In Vitro EpiDerm™ Model (EPI-200-SIT) Phototoxicity

SUMMARY

In vitro phototoxicity irritation studies were conducted to evaluate whether **AMTicide® VAF** would induce phototoxic irritation in the EpiDerm™ model assay.

The product was tested according to the manufacturer's protocol. The test article solution was found to be a **non-photoirritant** at concentrations of 0.01%, 0.001%, and 0.0001%. Reconstructed human epidermis was incubated in growth media for one hour to allow for tissue equilibration after shipping from MatTek Corporation, Ashland, MA. Test substance was applied to the tissue inserts in **four varying** concentrations and incubated overnight at 37°C, 5% CO_2, and 95% relative humidity (RH). The following day, the appropriate tissue inserts were irradiated (UVA) for 60 minutes with 1.7 mW/cm^2 (=6 J/cm^2). After substance incubation, irradiation, and washing was completed, the cell viability test was conducted. Cell viability was measured by dehydrogenase conversion of MTT [(*3-4,5-dimethyl thiazole 2-yl*)], present in the cell mitochondria, into blue formazan salt that was measured after extraction from the tissue. The photoirritation potential of the test chemical was dictated by the reduction in tissue viability of UVA exposed tissues compared to non-UVA exposed tissues.

Under the conditions of this assay, the test article was considered to be **non-phototoxic** at concentrations of 0.01%, 0.001%, and 0.0001%. The negative and positive controls performed as anticipated.

I. Introduction

A. Purpose

In vitro dermal phototoxicity study was conducted to evaluate whether a test article would induce photoirritation in the EpiDerm™ model assay. MatTek Corporation's reconstructed human epidermal model is becoming a standard in determining the phototoxicity potential of a test substance. This assay is able to discriminate between photoirritants and non-photoirritants at varying concentrations.

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II. Materials

A. Incubation Conditions:	37°C at 5% CO_2 and 95% relative humidity
B. Equipment:	Forma humidified incubator, ESCO biosafety laminar flow hood, Synergy HT Microplate reader; UVA-vis Irradiation Equipment; UVA meter; Pipettes
C. Media/Buffers:	Dulbecco's Modified Eagle Medium (DMEM) based medium; Dulbecco's Phosphate-Buffered Saline (DPBS); sterile deionized H_2O
D. Preparation:	Pre-incubate (37°C) tissue inserts in assay medium; Place assay medium and MTT diluent at 4°C, MTT concentrate at -20°C, and record lot numbers of kit components
E. Tissue Culture Plates:	Falcon flat bottom 96-well, 24-well, and 6-well tissue culture plates
F. Reagents:	MTT (*3-4,5-dimethyl thiazole 2-yl*) (1.0mg/mL); Extraction Solution (Isopropanol); Chlorpromazine; Triton X-100 (1%)
G. Other:	Wash bottle; sterile disposable pipette tips; Parafilm; forceps

III. Test Assay

A. Test System

The reconstructed human epidermal model, EpiDerm™ consists of normal human-derived epidermal keratinocytes which have been cultured to form a multilayer, highly differentiated model of the human epidermis. This model consists of organized basal, spinous, and granular layers, and contains a multilayer stratum corneum containing intercellular lamellar lipid layers. The EpiDerm™ tissues are cultured on specially prepared cell culture inserts.

B. Negative Control

Sterile deionized water is used as the negative controls for the EpiDerm™ Phototoxicity assay.

C. Positive Control

Concentrations of chloropromazine, ranging from 0.001% to 0.1%, were used as positive controls for the EpiDerm™ Phototoxicity assay.

D. Data Interpretation Procedure

A photoirritant is predicted if the mean relative tissue viability of the 2 tissues exposed to the test substance and 60 minutes of 6 J/cm^2 is reduced by 20% compared to the non-irradiated control tissues.

IV. Method

A. Tissue Conditioning

Upon MatTek kit arrival at Active Micro Technologies, LLC the tissue inserts are removed from their shipping medium and transferred into fresh media and tissue culture plates and incubated at 37°C at 5% CO_2 and 95% relative humidity for 60 minutes. After those 60 minutes the inserts are transferred into fresh media and tissue culture plates and tissue insert dosing begins.

B. Test Substance Exposure

50µL of the diluted test substance in their respective concentrations are applied to 2 tissue inserts and allowed to incubate for overnight in a humidified incubator (37°C, 5% CO_2, 95% RH).

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C. Tissue Irradiation

Tissue inserts in their 6-well plates are UVA-irradiated for 60 minutes with 6 J/cm^2 at room temperature. The non-irradiated tissue inserts are incubated at room temperature in the dark.

D. Tissue Washing and Post Incubation

After UVA-irradiation and dark incubation is complete the tissue inserts are washed using sterile DPBS and transferred to fresh 6-well plates and media for overnight incubation at 37°C, 5% CO_2, 95% RH.

E. MTT Assay

Tissue inserts are transferred into 300µL MTT media in pre-filled plates and incubated for 3 hours at 37°C, 5% CO_2, and 95% RH. Inserts are then removed from the MTT medium and placed in 2mL of the extraction solution. The plate is sealed and incubated at room temperature in the dark for 24 hours. After extraction is complete the tissue inserts are pierced with forceps and 2 x 200µL aliquots of the blue formazan solution is transferred into a 96 well plate for Optical Density reading. The spectrophotometer reads the 96-well plate using a wavelength of 570 nm.

V. Acceptance Criterion

A. Negative Control

The results of this assay are acceptable if the mean negative control Optical Density (OD_{570}) is ≥ 0.8.

B. Positive Control

The assay meets the acceptance criterion if a dose dependent reduction in cell viability in the UVA-irradiated tissues is between 0.00316% and 0.0316%.

C. Standard Deviation

Since the phototoxicity potential is predicted from the mean viability of 2 tissues for the EpiDerm™ Phototoxicity Protocol, the variability of the replicates should not exceed 30%.

VI. Results

A. Tissue Characteristics

The tissue inserts included in the MatTek EpiDerm™ assay kit were in good condition, intact, and viable.

B. Tissue Viability Assay

The results are summarized in Figure 1. Cell viability is calculated for each tissue as a percentage of the corresponding vehicle control either irradiated or non-irradiated. Tissue viability was not reduced by 20% in the presence of the test substance and UVA-irradiation at concentrations 0.01%, 0.001%, and 0.0001%. The negative control mean exhibited acceptable relative tissue viability while the positive control exhibited dose dependent loss of tissue viability and cell death.

C. Test Validity

The data obtained from this study met criteria for a valid assay. The negative and positive controls performed as anticipated.

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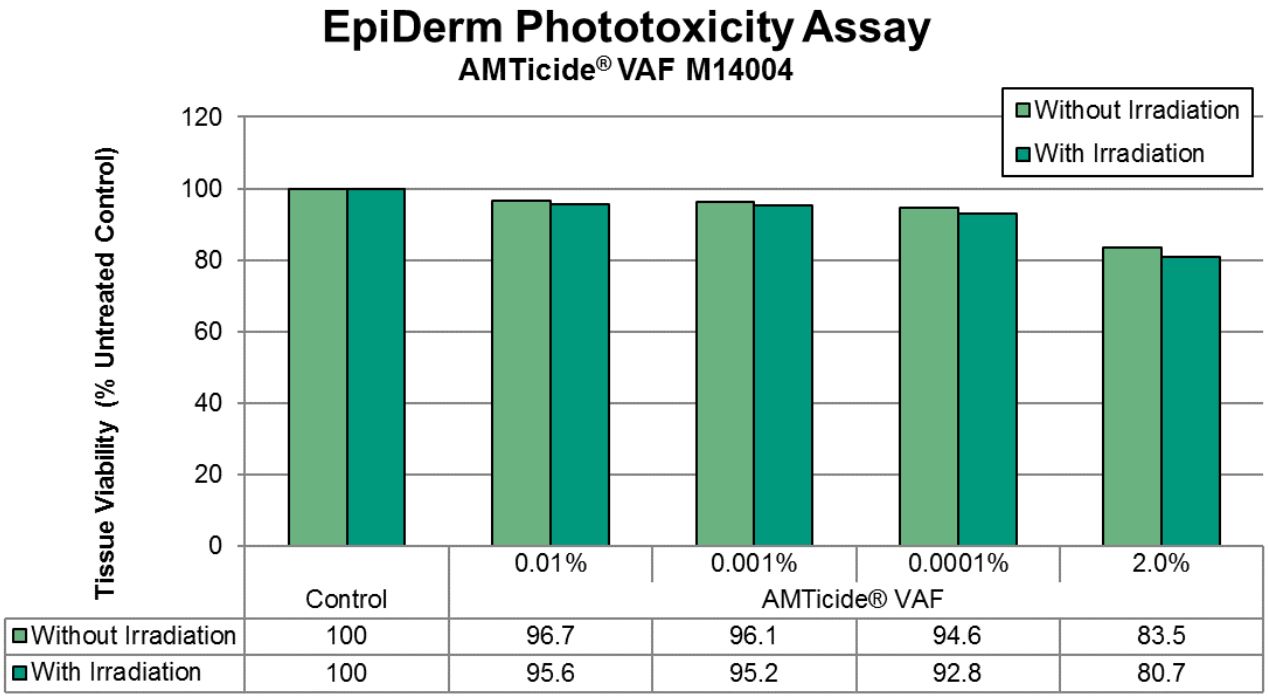
VII. Conclusion

Phototoxicity (photoirritation) is defined as an acute toxic response that is elicited after exposure of the skin to certain chemicals and subsequent exposure to light. Under the conditions of this assay, the test article substance was considered to be **non-phototoxic** at concentrations of 0.01%, 0.001%, and 0.0001%. The negative and positive controls performed as anticipated

There is a decrease in viability at the 2.0% test concentration with and without irradiation. Using any test substance at this high of a concentration will have noticeable effects on cellular viability due to the fact that that substance is replacing the cell's nutrients. We can safely say that **AMTicide® VAF** is not a photoirritant when used at the suggested use level of 1.0% maximum.



	Control	0.01%	0.001%	0.0001%	2.0%
Without Irradiation	100	96.7	96.1	94.6	83.5
With Irradiation	100	95.6	95.2	92.8	80.7

Figure 1: EpiDerm Phototoxicity Graph

Safety Statement

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Product Name: AMTicide® VAF

Code: M14004

INCI Name: Bacillus Ferment & Saccharomyces Ferment Filtrate

AMTicide® VAF is manufactured by co-fermenting *Bacillus subtilis* and *Saccharomyces boulardii* in a defined media under controlled conditions of pH, temperature, and time. The material is then filtered to isolate and extract the volatile organic metabolites.

AMTicide® VAF was tested using *in vitro* dermal and ocular irritation models, including phototoxicity irritation (EpiDerm™ EPI-200-SIT). This product was found to be non-irritating in all models, including non-phototoxic for the *in vitro* dermal model.

A *Salmonella typhimurium* reverse mutation standard plate incorporation study was conducted to evaluate whether AMTicide® VAF would cause mutagenic changes in the average number of revertants for histidine-dependent *Salmonella typhimurium* strains in the presence and absence of S9 metabolic activation. This study was conducted to satisfy, in part, the Genotoxicity requirement of the International Organization for Standardization: Biological Evaluation of Medical Devices, Part 3: Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity. AMTicide® VAF was considered to be nonmutagenic to the *Salmonella typhimurium* tester strains under the conditions of this assay.

AMTicide® VAF was also tested via the OECD TG 442C Direct Peptide Reactivity and OECD TG 442D In Vitro Skin Sensitization Assays in accordance with the EURL ECVAM and UN GHS guidelines. This product was determined to be a non-skin sensitizer in both *in chemico* and *in vitro* models.

A Freshwater Alga Growth Inhibition test via OECD 201 was subsequently performed to determine the potential toxicity of AMTicide® VAF. In this assay, *Psuedokirchneriella subcatpitata* are exposed to the test substance for 72 hours and growth and growth inhibition through cell count against control is performed. The response is evaluated as a function of the exposure concentration in comparison with the average growth of replicate, unexposed control cultures. After 72 hours, the percent inhibition for AMTicide® VAF was determined to be 271.22 mg/L EC_{10} and 406.19 mg/L EC_{20}. The results of this assay indicate that the product is not classified and therefore not harmful to aquatic organisms.

AMTicide® VAF was also assessed for ready biodegradability in an aerobic aqueous medium via the OECD 301 B Ready Biodegradability: CO2 Evolution (Modified Sturm Test). AMTicide® VAF achieved 88.4% biodegradation after 28 days of testing, indicating that the product meets method requirements for the Ready Biodegradable classifications.

The full reports for each safety study analyzing AMTicide® VAF are attached for reference.

Due to the restriction placed on animal testing of cosmetic raw materials, and Active Concepts, LLC's internal non-animal testing policy, this product was not tested for NOAEL. However, there is substantial amounts of published data for each material used to manufacture AMTicide® VAF that provide useful information to calculate approximate NOAEL and demonstrate the non-cytotoxic effects of this product. Investigation of the following data along with US Food and Drug Administration guidelines[1] have allowed us to estimate AMTicide® VAF exposure based off dosage in topical form, with an approximate NOAEL of 326.76 mg/kg/day.

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For example, at an average 2% use level in 1 oz (or 28 g) finished product per day on a person averaging a 65 kg body weight, a daily exposure of 8.62 mg/kg is expected (28 g x 2% = 0.56 g or 560 mg; 560 mg / 65 kg). Although there is not one established standard to convert LD_{50}, to NOAEL, using a conservative conversion factor such as the ISO 10993-17 standard[1,2] and published LD_{50} data, it is expected that AMTicide® VAF has an estimated aforementioned NOAEL of 326.76 mg/kg/day: [(published LD_{50} x BW)/CF] + published NOAEL, so [(1040 mg/kg x 65 kg)/10,000] + 320 mg/kg/day.

When AMTicide® VAF is used at approximately 2% in a finished formula sample we do not expect exposure to exceed 8.62 mg/kg daily, which is well under the NOAEL estimate of 326.76 mg/kg/day.

Bacillus subtilis is a diverse bacterial species used as a natural component of food for humans, namely known for its probiotic dietary benefits. As a starting material for AMTicide® VAF, *Bacillus subtilis* undergoes co-fermentation in order to collect by-products of the metabolic process. Published NOAEL for *Bacillus subtilis* has been reported as 2,000 mg/kg/day in male and female rats.[3] When converting from animal to human equivalent doses (i.e. from rats to humans) a general conversion factor of 0.16 can be used.[4] Therefore the human equivalent NOAEL for *Bacillus subtilis* is expected to be 320 mg/kg/day.

Saccharomyces boulardii is a tropical species of yeast first isolated in 1923. Since then, the microorganismfamously known as brewer's yeast and baker's yeast has been used in a variety of applications ranging from baking to dietary supplements. Some studies indicate that *Saccharomyces boulardii* can play a key role in gastrointestinal health and does not appear to be toxic when consumed orally by rats, with an LD_{50} of 6500 mg/kg.[5] The human equivalent is thus expected to be 1040 mg/kg.

As previously mentioned, *Bacillus subtilis* and *Saccharomyces boulardii* are commonly used in the food and nutraceutical industries. Since these microorganisms are intentionally used in food and dietary supplements, their ferments may be classified as Generally Recognized as Safe (GRAS) according to the FDA's Federal Food, Drug and Cosmetic Act.[6]

The act states:

> Any substance that is intentionally added to food is a food additive, that is subject to premarket review and approval by FDA, unless the substance is generally recognized, among qualified experts, as having been adequately shown to be safe under the conditions of its intended use, or unless the use of the substance is otherwise excluded from the definition of a food additive.[6]

The Cosmetic Ingredient Review (CIR) has also published reports assessing the safety of various ferments. These reports conclude that ferments and their filtrates are safe for use in cosmetic formulations in the present practices of use and concentration.[7] While these reports do not specify the exact INCIs listed in AMTicide® VAF, it supports the safety and use of our fermented *Bacillus subtilis* and *Saccharomyces boulardii* within the product.

Several published data sets exist to support the safety of AMTicide® VAF. Additionally, the molecular weight of this product (approximately 559.21 Da) is larger than what is required to penetrate skin. Therefore, hazards that may otherwise occur via this route are not an issue. Toxicological, irritation, and sensitization assays have all been performed with favorable results for each. This knowledge combined with the tested and published toxicity assays allows us to support the safety of AMTicide® VAF in cosmetic applications.

It is logically concluded that AMTicide® VAF is safe in cosmetic applications at a 1.0% maximum use level. No further testing is required at this time.

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1. U.s. Food and Druf Administration (FDA) – Assessing Safety When Toxicity Data are Limited. https://pdfs.semanticscholar.org/presentation/a8c3/4a1ed34f929156bbc3d8db6693b6f22c8f9b.pdf
2. APIC 2014, Gudance on Aspects of Cleaning Validation in Active Pharmaceutical Ingredient Plants.https://apic.cefic.org/pub/APICCleaningValidationGuide-updateSeptember2016-final.pdf
3. BIO-CAT Microbials, PRISM Clinical Research - Safety Assessment of Bacillus subtilis MB40 for Probiotic Use in Foods and Dietary Supplements. https://bcmicrobials.com/
4. Guidance for Industry Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers – https://www.fda.gov/regulatory-information/search-fda-guidance-documents/estimating-maximum-safe-starting-dose-initial-clinical-trials-therapeutics-adult-healthy-volunteers
5. Centre for Research & Development, Unique Biotech Limited, SP Biotech Park - Safety assessment studies of probiotic Saccharomyces boulardii strain Unique 28 in Sprague-Dawley rats. https://www.ncbi.nlm.nih.gov/pubmed/21986361
6. Federal Food, Drug and Cosmetic Act. U.S Food and Drug Administration. www.fda.gov.
7. Cosmetic Ingredient Review (CIR) https://cir-safety.org/

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Certificate of Origin

AMTicide® VAF
Code: M14004

Active Micro Technologies, LLC certifies that all raw material(s) used to manufacture the above listed ingredient originate in the United States of America.

Active Micro Technologies, LLC certifies that all raw material(s) used to manufacture the above listed ingredient are prepared from non-GMO organisms and are BSE/TSE Free.

Active Micro Technologies, LLC certifies the below sources for each item listed in our INCI Name:

INCI Name	Source
Propanediol	Plant (*Zea mays*)
Bacillus Ferment	Bacteria (*Bacillus*)
Saccharomyces Ferment Filtrate	Yeast (*Saccharomyces cerevisiae*)

Active Micro Technologies, LLC certifies that the above listed ingredient can be classified as Vegan Compliant.

Active Micro Technologies, LLC certifies that the above listed ingredient has never been tested on animals.

SECTION 1. IDENTIFICATION

Product Name/Identifier	AMTicide® VAF
Product Code	M14004
Recommended Use	Topical Cosmetic Use; Antimicrobial
Restrictions on Use	None
Supplier/Manufacturing Site	Active Micro Technologies, LLC
Address	107 Technology Drive Lincolnton, NC 28092, USA
Telephone No. (24hrs)	1-704-276-7100
Fax No.	1-704-276-7101
Emergency Telephone #	1-704-276-7100 (Mon-Fri: 8:00AM – 5:00PM EST)

SECTION 2. HAZARD(S) IDENTIFICATION

Classification:

GHS / CLP
Basis for Classification: Based on present data no classification and labeling is required according to GHS, taking into account the national implementation (United Nations version 2011)

USA
OSHA Regulatory Status: This material is non-hazardous as defined by the American OSHA Hazard Communication Standard (29 CFR 1910.1200).

Europe
Basis for Classification:
-According to present data no classification and labeling is required according to Directives 67/548/EEC or 1999/45/EC.
-This product is not classified as hazardous to health or environment according to the CLP regulation.

Labeling Elements:

Pictograph: No hazard symbol expected

Hazard statements/Signal Word: Not applicable

Precautionary statements:
P233: Keep container tightly closed
P281: Use personal protective equipment as required
P402: Store in a dry place
P404: Store in a closed container
P410: Protect from sunlight
P411: Store at temperatures not exceeding 32°C

Other hazards which do not result in classification:

No particular fire or explosion hazard.
By mechanical effect: No particular hazards.
By hydroscopic effect: No particular hazards.

US NFPA 704 (National Fire Protection Association) Hazard Rating System:

Health hazard: Rating 0; Normal Material
Flammability: Rating 0, Will Not Burn
Reactivity: Rating 0, Stable
Other Hazard Information: None

Results of PBT and vPvB assessment:

-PBT: Not applicable
-vPvB: Not applicable

SECTION 3. COMPOSITION / INFORMATION ON INGREDIENTS

Common Chemical Name: Bacillus Ferment & Saccharomyces Ferment Filtrate

Generic name:

Chemical Family: Ferment

Description: Mixture: consisting of the following components. This section describes all components of the mixture

Substance	CAS Numbers	EC Numbers	Percentage
Propanediol	504-63-2	207-997-3	80.00%
Bacillus Ferment	92128-81-9 (or) 68582-99-0	295-779-9 (or) N/A	10.00%
Saccharomyces Ferment Filtrate	8013-01-2	232-387-9	10.00%

Formula: Not applicable

SECTION 4. FIRST-AID MEASURES

General: In all cases of doubt, or when symptoms persist, seek medical attention.

Inhalation: Move to fresh air from exposure area. Get medical attention for any breathing difficulty.

Skin contact: Rinse with soap and water. Get medical advice if irritation develops.

Eye contact: Immediately rinse with water for at least 15 minutes, while keeping the eyes wide open. Consult with a physician.

Ingestion:	Consult with a physician.
Protection of first-aiders:	No special protection required.

SECTION 5. FIRE-FIGHTING MEASURES

Fire and explosion hazards:	Not considered to be a fire and explosion hazard
Extinguishing media:	
Suitable:	Water, dry chemicals, foam & carbon dioxide.
Not suitable:	None known
Fire fighting:	Move container from fire area if it can be done without risk. Avoid inhalation of material or combustion by-products. Stay upwind and keep out of low area
Protection for fire-fighters:	Boots, gloves, goggles.

SECTION 6. ACCIDENTAL RELEASE MEASURES

Personal precautions:	Avoid contact with eyes. Personal Protective Equipment: -Protective goggles
Environmental precautions:	Prevent entry into sewers and waterways. Do not allow material to contaminate ground water system
Methods for cleaning up:	
Recovery:	Pick up free liquid for recycling or disposal. Residual liquid can be absorbed on an inert material.
Cleaning/Decontamination:	Wash non-recoverable remainder with water.
Disposal:	For disposal of residues refer to sections 8 & 13.

SECTION 7. HANDLING AND STORAGE

Handling

Technical measures:	Labeling: Keep out of the reach of children.
Measures:	For industrial use, only as directed.
Safe handling advice:	Wash hands after use. Avoid storage near feed or food stuff.

Storage

Technical measures:	Keep container closed.
Recommended Storage Conditions:	Store in a dry place at temperatures not exceeding 32°C. Based on stability studies, the optimum storage temperature for maximization of shelf life is 23 - 25°C. However, it may be stored at temperatures between 16 and 32°C if such specific temperature control is not available. Do not freeze. Please refer to stability data for effects heat or cold may have on the specifications of the product.
Incompatible products:	Avoid contact with strong oxidizers. Refer to the detailed list of incompatible materials (Section 10 Stability/Reactivity)
Packaging:	Product may be packaged in normal commercial packaging.
Packaging materials:	Recommended - Polypropylene & High Density Polyethylene

SECTION 8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Precautionary statements: Ensure adequate ventilation

Control parameters

Occupational exposure Limits:

France:	Not Determined
ACGIH:	Not Determined
Korea:	Not Determined
UK:	Not Determined
Surveillance procedures:	Not Determined
Engineering measures:	Not Determined

Personal Protective Equipment:

Respiratory protection:	Local exhaust
Hand protection:	Protective gloves made of rubber or neoprene.
Eye protection:	Safety glasses.
Collective emergency equipment:	Eye fountain.
Skin and Body Protection:	Suitable protective clothing
Hygiene measures:	Handle in accordance with good industrial hygiene and safety practice.
Measures related to the Environment:	No particular measures.

SECTION 9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance:	Clear to slightly hazy liquid
Color (Gardner):	3 Maximum
Odor:	Characteristic

pH (25°C):	4.0 – 6.0
Refractive Index (25°C):	1.400 – 1.500
Heavy Metals:	< 20 ppm
Lead:	< 10 ppm
Arsenic:	< 2 ppm
Cadmium:	< 1 ppm
Specific Gravity:	Not determined
Vapor density:	Not applicable
Boiling Point:	Not determined
Freezing Point:	Not determined
Melting point:	Not applicable
Flash point:	> 200°F
Oxidizing properties:	Non oxidizing material according to EC criteria.
Solubility:	
In water:	Soluble
In organic solvents:	Not determined
Log P:	Not determined

SECTION 10. STABILITY AND REACTIVITY

Stability: Stable under ordinary conditions of use and storage up to one year then re-test to full product specifications to extend shelf life

Hazardous reactions: None known

Conditions to avoid: No dangerous reactions known under use of normal conditions. Avoid extreme heat.

Materials to avoid: No dangerous reaction known with common products.

Hazardous decomposition products: None known

SECTION 11. TOXICOLOGICAL INFORMATION

Ingestion:	Not Determined
Dermal:	Non-Irritant (Dermal Irritection Model)
Ocular:	Non-Irritant (Ocular Irritection Model)
Inhalation:	Not Determined
Acute toxicity data:	Non-Irritant, Non-Primary Sensitizer & Non-Photo Irritant
Sensitization:	Non-Primary Irritant & Non-Primary Sensitizers; Will not cause allergic contact dermatitis (In Chemico Skin Sensitization Direct Peptide Reactivity Assay & In Vitro Skin Sensitization ARE-Nrf2 Luciferase Test Method)

Repeated dose toxicity: No known effects
Subacute to chronic toxicity: Not Determined

Mutagenicity: Non-Mutagenic (OECD471/ISO10993.Part 3 – Genotoxicity: Bacterial Reverse Mutation Test)

Additional Toxicological Information: This product is not subject to classification according to the calculation method of the General EU Classification Guidelines for Preparations as issued in the latest version.

Specific effects:

Carcinogenicity: No known effects
Mutagenicity: No known effects
Reproductive toxicity: No known effects
Neuro-toxicity: No known effects

For more information: Does not present any particular risk on handling under normal conditions of good occupational hygiene practice.

This product has not been tested for the following:
-Primary cutaneous and corrosive irritation
-Acute oral toxicity

SECTION 12. ECOLOGICAL INFORMATION

Ecotoxicity
Effects on the aquatic environment: EC_{10} (Freshwater Alga): 271.22 mg/L - Not harmful to aquatic organisms
EC_{20} (Freshwater Alga): 406.19 mg/L - Not harmful to aquatic organisms

Biodegradability:
Persistence: Readily Biodegradable (88.4% biodegradation after 28 days of testing)

Bioaccumulation:
Octanol / water partition coefficient: Not Determined

Mobility:
Precipitation:
Expected behavior of the product: Ultimate destination of the product: Soil & sediment.

Other Adverse Effects: None known

SECTION 13. DISPOSAL CONSIDERATIONS

Residues from product

Prohibition: Do not allow the product to be released into the Environment.
Destruction/Disposal: Dispose of in accordance with relevant local regulations

Contaminated packaging

Decontamination/cleaning: Cleaning is not required prior to disposal.
Destruction/Disposal:

Note: Take all necessary precautions when disposing of this product according to local regulations.

SECTION 14. TRANSPORT INFORMATION

UN Number: None
UN Shipping Name: None

Transport Hazard Class: Not classified as dangerous for transport

Land (rail/road): Material is not restrictive for land transport and is not regulated by ADR/RID
Sea: Material is not restrictive for sea transport and is not regulated by IMO/IMDG
Air: Material is not restrictive for land transport and is not regulated by ICA/IATA

Marine Pollutant: No

Transport/Additional Information: Not regulated for US DOT Transport in non-bulk containers
This material is not dangerous or hazardous

Special Precautions for User: None known

The above regulatory prescriptions are those valid on the date of publication of this sheet. However, given the possible evolution of transport regulations for hazardous materials and in the event of the MSDS in your possession dating back more than 12 months, it is advisable to check their validity with your sales office.

SECTION 15. REGULATORY INFORMATION

Labeling:
EC regulations: This product does not need to be labeled in accordance with EC Directives or respective national laws

Further regulations

United Kingdom: Handle in accordance with relevant British regulation: control of substance Hazardous to Health Regulations Environmental Hygiene Guidance: EH40
Workplace Exposure Limits (revised annually)

Korea regulations:
Industrial safety and hygiene regulation: No
Hazardous material control regulation: No
Fire prevention regulation: No

Other regulations:

EINECS inventory status:	Propanediol:	207-997-3
	Bacillus Ferment:	295-779-9 (or) N/A
	Saccharomyces Ferment Filtrate:	232-387-9
TSCA inventory status:	Exempt	
AICS inventory status:	Not Listed: 92128-81-9 Listed: 504-63-2 & 68582-99-0 & 8013-01-2	
Canadian (CEPA DSL) inventory status:	Not Listed: Bacillus Ferment (92128-81-9) Listed as 1,3-Propanediol & Bacillus, bacterium genus (NDSL) & Yeast, ext. (DSL)	
Japan (MITI list):	Propanediol & Bacillus Ferment & Saccharomyces Ferment Filtrate	
Korea:	Propanediol & Bacillus Ferment & Saccharomyces Ferment Filtrate	
China inventory status:	Propanediol & Bacillus Ferment & Saccharomyces Ferment Filtrate	
Philippines inventory status:	Not Listed: Bacillus Ferment (92128-81-9) Listed: 1,3-Propanediol & Bacillus (bacterium genus) & Yeast, ext.	

Note: The regulatory information given above only indicates the principal regulations specifically applicable to the products described in this sheet. The user's attention is drawn to the possible existence of additional provision which complete these regulations. Please refer to all applicable international, national and local regulations and provisions

SECTION 16. OTHER INFORMATION

Prohibited uses:	For specific uses, food industry, ask the manufacturer for more information.
Last Revision Date:	10/28/2019
Preparation Date:	02/25/2020
MSDS summary of changes	- Added Mutagenicity Data – Section 11 (Toxicological Information) - Added Irritation & Sensitization Data – Section 11 (Toxicological Information) & Updated Biodegradability Data – Section 12 (Ecological Information) - Updated Precautionary Statement – Section 2 (Hazards Information), Updated Recommend Storage Conditions – Section 7 (Handling & Storage), Updated Appearance – Section 9 (Physical & Chemical Properties) & Added Acute Toxicity Data – Section 11 (Toxicological Information) - Updated Description – Section 3 (Composition / Information on Ingredients) & Added Ecotoxicity Data – Section 12 (Ecological Information)

The information given is based on our knowledge of this product, at the time of publication in good faith. The attention of the user is drawn to the possible risks incurred by using the product for any other purpose other than which it was intended. This is not in any way excuse the user from knowing and applying all the regulations governing their activity. It is sole responsibility of the user to take all precautions required in handling the product. The purpose of mandatory regulation mentioned is to help the user to fulfill his obligations regarding the use of products. This information is not exhaustive, this is not exonerate the user from ensuring that legal obligations other than those mentioned, relating to the use and storage.

AMTicide® VAF
Formulation Guidelines

Code: M14004
INCI Name: Bacillus Ferment & Saccharomyces Ferment Filtrate
CAS #: 92128-81-9 (or) 68582-99-0 & 8013-01-2
EINECS #: 295-779-9 (or) N/A & 232-387-9

Temperature Stability	Stable up to 70° Celsius
pH Stability	3 to 8
Suggested Use Level	1.0% Maximum
Solubility	Water Soluble
Formulation Guidelines	Incorporate **AMTicide® VAF** into formulations at a pH between 3 and 8, during the cooling phase of the process at temperatures lower than 70°C. .

Version#1/05.24.18

AMTicide® VAF Manufacturing Flow Chart

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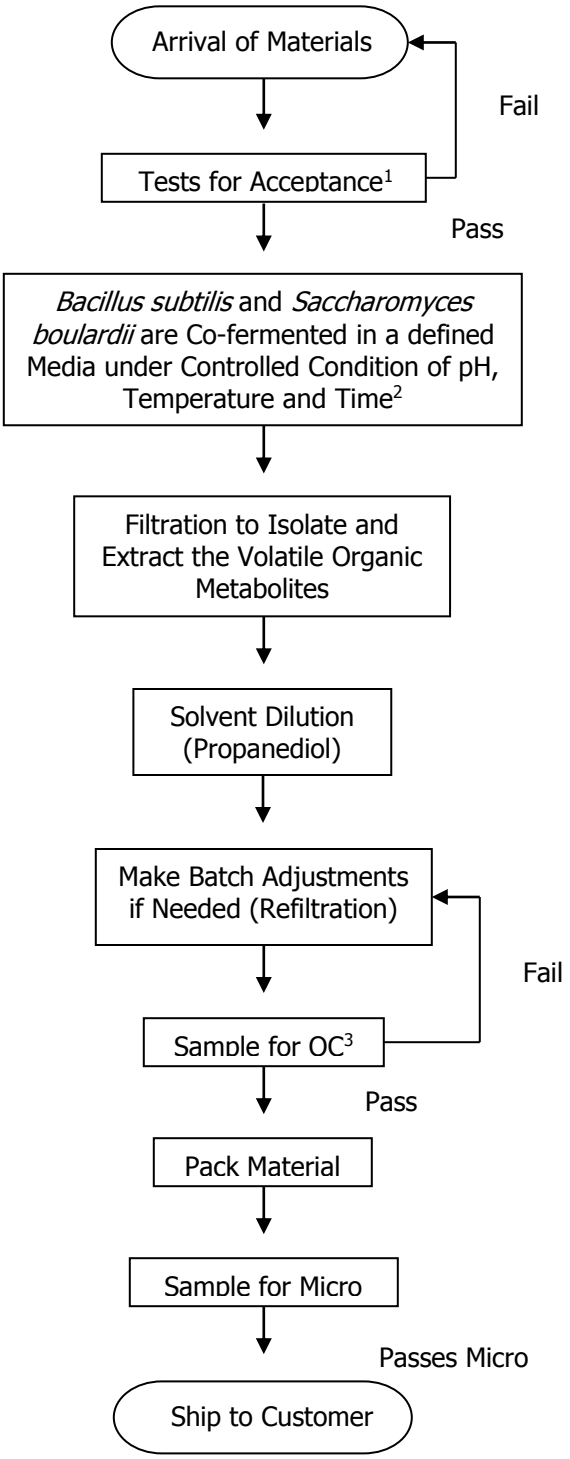

1. Incoming raw materials are checked to see that their Certificates of Analysis match previous batches. Appearance, consistency and odor are compared against retain samples. Liquids are compared on an IR spectrophotometer against previous batches. All of the materials are checked for microbial contamination upon receipt.

2. Defined medium consisting of Ammonium Sulfate, Magnesium Sulfate, Disodium Phosphate & Yeast Autolysate. The temperature of the fermentation is maintained at 20ºC ± 2ºC, and the pH is controlled to 6.0 ± 0.2ºC. The fermentation is run to ensure the culture has achieved early stationary phase, typically 12 to 18 hours.

3. When the presence of microorganisms has been assured under a microscope, the batch is checked against the criteria on the Specification sheet. Appearance, color, odor, pH and refractive index are checked immediately. If they confirm the production run meets the standard specifications, a sample is sent to an outside lab to confirm the Heavy metal. Lead, Arsenic and Cadmium content.

Version#1/03-07-18

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AMTicide® VAF
Certificate of Compliance

Code: M14004
INCI Name: Bacillus Ferment & Saccharomyces Ferment Filtrate
INCI Status: Conforms
CAS #: 92128-81-9 (or) 68582-99-0 & 8013-01-2
EINECS #: 295-779-9 (or) N/A & 232-387-9

Below is a list of processing aids used, but not declared on the ingredient label:

INCI Name	CAS#	EINECS#	Percentage (%)	Function
Propanediol	504-63-2	207-997-3	80.00%	Solvent

The following information on regulatory clearances is believed to be accurate and is given in good faith as a guide to a global use of our ingredients in cosmetic applications. No representation or warranty as to its competences or accuracy is made. Information is offered for use in general cosmetic applications and may vary in particular applications. Users are responsible for determining the suitability of these products for their own particular use. All regulatory decisions should be made on the advice of your regulatory group or legal counsel.

Country / Regulatory Body	Status of Product
EU (CosIng)	Compliant
USA (TSCA)	Exempt
Australia (AICS)	Compliant
Japan (METI)	Compliant
Canada (DSL)	Contact Us
China (IECIC)	Compliant
Brazil (ANVISA)	Compliant
Korea (KECI)	Compliant
Philippines (PICCS)	Compliant
Mexico (COFEPRIS)	Compliant

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AMTicide® VAF
Code: M14004

Attention must be paid to the use of AMTicide® VAF in the equivalent of OTC formulations (eg. quasi-drugs in Japan, or therapeutic goods in Australia). Some countries maintain restricted inventories of raw materials that can be used in those applications so more detailed guidance may be required.

AMTicide® VAF and any components or impurities are in compliance with the rules governing cosmetic products in the European Union (Directive 76/768/ECC & Regulation No. 1223/2009). The recommended use levels for AMTicide® VAF is 1.00% Maximum.

AMTicide® VAF is in compliance with the standardized set of rules developed and approved by the NPA (Natural Products Association).

AMTicide® VAF is considered a non-hazardous material. All significant toxicological routes of absorption have been considered as well as the systemic effects and margin of safety (MoS) based on a no observed adverse effects level (NOAEL). Due to the restriction placed on animal testing of cosmetic raw materials, and Active Micro Technologies, LLC's internal non-animal testing policy, this product was not tested for NOAEL.

AMTicide® VAF was tested using *in vitro* dermal and ocular irritation models. This product was found to be non-irritating in both models.

To our knowledge the above material is free of CMR (*) substances, as defined according to Regulation (EC) No 1272/2008 and Cosmetic Regulation (EC) No 1223/2009 as amended. Products supported for Personal Care applications will not be classified as CMR (*), as defined by (EC) 1272/2008 on the Classification, Labelling and Packaging of Substances and Mixtures, unless supported by a positive SCCS opinion.

(*) Carcinogenic, Mutagenic, toxic for Reproduction

Active Micro Technologies, LLC certifies that to the best of our knowledge our product does not contain any material listed on California Proposition 65.

Active Micro Technologies, LLC certifies that AMTicide® VAF does not contain any materials prohibited by Halal laws.

AMTicide® VAF is REACH Compliant and free of the following:

- 1-4-Dioxane
- Butylated hydroxyanisole (BHA)
- Butylated hydroxytoluene (BHT)
- Camphor
- Decamethylcyclopentasiloxane (D5)
- Dodecamethylcyclohexasiloxane (D6)
- Ethylene oxide (EO)
- Eucalyptol
- Formaldehyde or formaldehyde donors
- Furocoumarins
- Glycol ethers
- Gluten
- Irradiation
- Lactose
- Menthol
- Mycotoxin
- Nanoparticles
- Nitrosamines
- Octamethylcyclotetrasiloxane (D4)
- Palm oil/palm kernel oil (or derivatives)
- Parabens
- Paraffin/petroleum products
- Phthalates
- Polyethylene glycol (PEG)
- Residual solvents
- Sulfates
- Volatile organic compounds

Split End Masque
Formulation Code: FNHP02-08

Ingredient	Trade Name/Vendor	%
Phase I		
Punica Granatum Sterols	ABS Pomegranate Sterols/Active Concepts	**42.40**
Cyclopentasiloxane & Dimethicone/Bis-Vinyldimethicone/ Silsesquioxane Crosspolymer & Silk	SilDerm® Softening/Active Concepts	**1.00**
Stearamidopropyl Dimethylamine	Tego® Amid S18/Evonik	**0.30**
Cetyl Esters	Cetyl Esters Wax /Spectrum Chemical	**1.50**
Cetearyl Alcohol & Glyceryl Stearate & Almondeth-20	Phytomulse® Almond/Active Concepts	**5.00**
PPG-3 Benzyl Ether Myristate	CRODAMOL STS/Croda	**4.00**
Olea Europea (Olive) Fruit Oil	AC Olive Oil Clear/Active Concepts	**1.00**
Prunus Amygdalus (Sweet Almond) Dulcis Oil	AC Almond Oil/Active Concepts	**1.00**
Limnanthes Alba (Meadowfoam) Seed Oil	AC Meadowfoam Oil/Active Concepts	**1.00**
Phase II		
Water	Water/Local	**68.61**
Citric Acid	Citric Acid/Sigma-Aldrich	**0.04**
Hydroxypropyltrimonium Hydrolyzed Rice Protein/Siloxysilicate & Oryza Sativa (Rice) Extract	AC Split End Complex/Active Concepts	**5.00**
Phase III		
Glycerin & Mentha Piperita (Peppermint) Extract & Rosmarinus Officinalis (Rosemary) Leaf Extract & Vitis Vinifera (Grape) Seed Extract	ABS Mint Blend G PF/Active Concepts	**1.00**
Butylene Glycol & Water & Chamomilla Recutita (Matricaria) Flower Extract	ABS Chamomile Extract PF/Active Concepts	**0.05**
Water & Hydrolyzed Vegetable Protein PG-Propyl Silanetriol	AC Keratin Hydrolysate SILOX /Active Concepts	**1.00**
Phase IV		
Leuconostoc/Radish Root Ferment Filtrate	Leucidal® Liquid/Active Micro Technologies	**4.00**
Lactobacillus & Cocos Nucifera (Coconut) Fruit Extract	AMTicide® Coconut/Active MicroTechnologies	**4.00**

Process Instructions:

1. Combine ingredients of Parts I and II separately with mixing and heat to 80°C.
2. Add Part II to Part I, mixing well.
3. Cool to 50°C and add ingredients of Part III with mixing.
4. Cool to 35°C and add Part IV.
5. Cool to room temperature and adjust pH to 4.5, if necessary.

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Exfoliating Face Mask
Formulation Code: FNHP02-27

Ingredient	Trade Name/Vendor	%
Phase I		
Water	Water/Local	**12.75**
Ribes nigrum (Black Currant) Fruit Extract	PhytoCide Black Currant Powder/ Active Micro Technologies	**2.00**
Glycerin	Glycerin/Spectrum Chemical	**7.00**
Phase II		
Caprylic/Capric Triglycerides	Neobee® M-5/Stepan	**3.00**
Cetyl Alcohol	Cetyl Alcohol/Rita Corp.	**3.00**
Cetearyl Alcohol (and) Ceteareth 20	AC Emulsifying Wax C20/Active Concepts	**5.00**
Glyceryl Stearate	Cerasynt™ GMS/ISP	**4.00**
Behentrimonium Chloride	Varisoft® BT 85/Evonik	**3.00**
Phormium Tenax Seed Oil	AC Flax Seed Oil CLA/Active Concepts	**6.00**
Persea Gratissima (Avocado) Oil	Avocado Oil/Arista	**3.00**
Euterpe Oleracea Sterols	ABS Acai Sterols EFA/Active Concepts	**5.00**
Phase III		
Lactobacillus/Punica Granatum Fruit Ferment Extract	ACB Modified Pomegranate Enzyme PF/ Active Concepts	**3.00**
Lactobacillus/Arundinaria gigantea Ferment Filtrate	ACB Bio-Water Bamboo/Active Concepts	**6.00**
Water & Vaccinium Myrtillus Fruit/Leaf Extract & Saccharum Officinarum (Sugar Cane) Extract & Citrus Aurantium Dulcis (Orange) Fruit Extract & Citrus Limon (Lemon) Fruit Extract & Acer Saccharum (Sugar Maple) Extract	ACB Fruit Mix/Active Concepts	**6.00**
Water & Phospholipids & Tocopheryl Acetate & Retinyl Palmitate & Ascorbyl Palmitate	AC Vitamin Liposome ACE PF/ Active Concepts	**3.00**
Water & Saccharomyces/Zinc Ferment & Saccharomyces/Copper Ferment & Saccharomyces/Magnesium Ferment & Saccharomyces/Iron Ferment & Saccharomyces/Silicon Ferment	ACB Bio-Chelate PF/Active Concepts	**3.00**
Lactobacillus/Arundinaria Gigantea Ferment Extract	ACB Bamboo Bioferment PF/ Active Concepts	**10.00**
Caolinitic Mineral Clay	Amazonian White Clay/Jarchem	**10.00**
Phase IV		
Sodium Hydroxide (25%)	Sodium Hydroxide/Spectrum Chemical	**0.25**
Phase V		
Lactobacillus Ferment Filtrate	Leucidal® Liquid SF/Active Micro Technologies	**4.00**
Fragrance	Optional	**1.00**

Version#1/04.06.18

Exfoliating Face Mask
Formulation Code: FNHP02-27

Process Instructions:

Phase I: Charge water into main beaker and begin propeller mixing. A vortex should form. Charge remaining ingredients and begin heating to 75°C.

Phase II: Combine ingredients in a separate container and heat to 80°C. Once temperatures have been reached add Phase II to main and maintain temperature of 78°C.

Phase III: Begin force cooling. Charge each ingredient individually at 50°C.

Phase IV: Add at 45°C.

Phase V: Add at 45°C.

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Beard Balm
Formulation Code: FNTA01-10

Ingredient	Trade Name/Vendor	%
Phase I		
Water	Water	**75.90**
Selaginella Lepidophylla Extract	Phytofuse® Renew/Active Concepts	**3.00**
Carbomer	Carbopol® Ultrez 10 Polymer/Lubrizol	**0.40**
Glycerin	Glycerin/Spectrum Chemical	**2.00**
Propanediol	Zemea® Propanediol/Dupont Tate & Lyle	**2.00**
Phase II		
Acrylates/C10-30 Alkyl Acrylate	Pemulen™ TR-1/Lubrizol	**0.20**
Cocos Nucifera (coconut) Fruit Extract	AcquaSeal® Coconut/Active Concepts	**3.00**
Euterpe Oleracea Sterols & Linoleic Acid & Oleic Acid & Linolenic Acid	ABS Acai Sterols EFA/Active Concepts	**3.00**
Phase III		
Acrylates Crossspolymer-3	Fixate™ Freestye Polymer/Lubrizol	**3.00**
Acrylates/Dimethicone Copolymer	SilDerm® Acrylate/Active Concepts	**3.00**
Phase IV		
Sodium Hydroxide (18%)	Sodium Hydroxide/Spectrum Chemical	**0.50**
Phase V		
Lactobacillus Ferment & Lactobacillus & Cocos Nucifera (Coconut) Fruit Extract	Leucidal® SF Complete/Active Micro Technologies	**4.00**

Process Instructions:

1. Add Phytofuse® Renew to water while mixing. Allow to mix thoroughly.
2. Sprinkle Carbopol® Ultrez 10 polymer on the surface while mixing and wait until the polymer is self-wetted.
3. Add remaining Phase I ingredients ensuring fully dispersed before adding the next.
4. Combine Phase II and mix until the polymer is finely dispersed.
5. Add Part II to Part I under rapid mixing. Allow mixing for at least 20-30 minutes.
6. Homogenize product.
7. Add Phase III with gentle mixing
8. Neutralize with Sodium Hydroxide to pH 5.0-5.5 while mixing. Add Phase V while mixing.

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ACTIVE MICRO TECHNOLOGIES

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Date Issued: April 20, 2018

ALLERGEN DECLARATION

RE: *AMTicide® VAF (M14004)*

Please be advised that this form is to certify that the above referenced product, manufactured at Active Micro Technologies, LLC, does not contain any of the allergens listed below:

Eggs – or egg products

Milk – or milk products (includes whey, lactose, casein, milk, cream)

Peanuts – or peanut products

Fish – (includes fish: surimi, cod, pollack, whitefish)

Shellfish – (shrimp, lobster, crab, clams, etc.)

Soybeans – or soybean products (includes soya powder, protein, oil, lecithin, tofu)

Wheat – or wheat products (includes Gluten)

Tree nuts – (almond, brazil nut, cashew, chestnut, hazelnut, filbert, pine nuts (pinyon, pinon), pistachio, pecan, macadamia, walnut).

Palm Oil – or palm kernel oil

If you have any further questions or concerns, please contact us at: 1-704-276-7100

Heavy Metals Statement

107 Technology Drive • Lincolnton, NC 28092
(704) 276-7100 • Fax (704) 276-7101

June 15, 2020

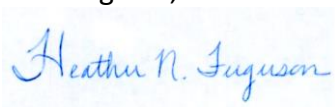
To Whom It May Concern,

This letter is to certify that AMTicide® VAF (M14004) has the following heavy metals profile:

Heavy Metals:	Less than 20 ppm
Chromium:	Less than 20 ppm
Lead:	Less than 10 ppm
Nickel:	Less than 10 ppm
Cobalt:	Less than 10 ppm
Antimony:	Less than 5 ppm
Arsenic:	Less than 2 ppm
Mercury:	Less than 1 ppm
Cadmium:	Less than 1 ppm

**Please note: The above levels illustrate the Maximum Limits. Values for Chromium, Nickel, Cobalt, Antimony and Mercury do not appear on the Specification for AMTicide® VAF.

Best Regards,



Tomorrow's Vision... *Today!* ®

Heather Ferguson |R&D Coordinator

107 Technology Drive | Lincolnton, NC 28092

Direct: 704.276.7083|Main: 704.276.7100|Fax: 704.276.7101

Email: hferguson@activeconceptsllc.com

www.activeconceptsllc.com

ATTESTATION OF CONFORMITY
- RAW MATERIALS -
COSMOS

This attestation has been granted by ECOCERT Greenlife to the company:

ACTIVE MICRO TECHNOLOGIES LLC

107 Technology Drive
LINCOLNTON, NC 28092
UNITED STATES OF AMERICA

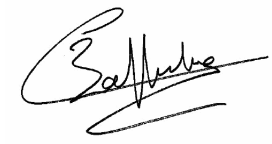
whose non-organic raw materials (listed hereafter) have been assessed as compliant to the standard:

COSMOS (v3.0 – September 2018)

This attestation of conformity has been issued on the basis of the terms and conditions for the verification of raw materials according to the COSMOS standard available on the COSMOS association website: https://cosmos-standard.org/ and the conformity has been established according to the requirements related to the raw materials contained in this standard.

Issued in : L'Isle Jourdain,
the: 23/05/2019,

by: Matthieu Bouffartigue
Raw materials service manager



Valid until: 31/12/2019

ECOCERT Greenlife S.A.S. - Capital 50 000 € - BP 47 - 32600 L'ISLE JOURDAIN - FRANCE
TVA Intracommunautaire n° FR 55 509 534 095
Phone: +33 5 62 07 50 16 - Fax: +33 5 62 07 74 96 - Mail: mpgreenlife@ecocert.com

ATTESTATION OF CONFORMITY - COSMOS

List of the approved raw materials of: **ACTIVE MICRO TECHNOLOGIES LLC**

PPAI: Physically Processed Agro-Ingredients
CPAI: Chemically Processed Agro-Ingredients
NNI: Non Natural Ingredients (Petrochemical origin)
PeMo: Petrochemical Moiety
CSPO: Raw material proceeding from certified sustainable palm/palm kernel oil

The asterisk * is used to identify the commercial name of the raw materials concerned by the appendices II and/or V of the Cosmos-standard.
Unless an exception, the following references are published on the ECOCERT raw materials online database for approved raw materials available at the following link: http://ap.ecocert.com/ecoproduits.

Commercial name / INCI / Function	%PPAI	%CPAI	%NNI	%PeMo	Restriction	Approved since
AMTicide Coconut (M14003) *Lactobacillus (and) Cocos Nucifera (Coconut) Fruit Extract* Skin conditioning, Hair conditioning	0	100	0	0		01/01/2019
AMTicide® VAF (M14004) *Bacillus Ferment (and) Saccharomyces Ferment Filtrate* Skin Conditioning, Antifungal	0	100	0	0		23/05/2019
Arborcide OC (M15010) *Leuconostoc Ferment Filtrate* Skin conditioning, Antimicrobial	0	50	0	0		01/01/2019
Leucidal Advanced - Aloe (M15015) *Water (and) Leuconostoc/Aloe Barbadensis Leaf/Sorbus Aucuparia Fruit Ferment Filtrate* Moisturizing, Skin conditioning, Antimicrobial	0	18	0	0		01/01/2019
Leucidal Advanced - Rowan (M15018) *Water (and) Leuconostoc/Sorbus Aucuparia Fruit Ferment Filtrate* Emollient, Skin conditioning, Antimicrobial	0	16	0	0		01/01/2019

Valid until: 31/12/2019

WARNING: The sole purpose of the present attestation is to allow the raw material(s) to be used in finished products to be certified as compliant to the standard specified in the first page. In no event this attestation should constitute proof of the actual certification of the conformity of the raw material(s) to this standard. In that context, the raw material(s) listed in this attestation must not be qualified and / or marketed as «organic» raw material(s) certified in accordance with the abovementioned standard.
The approval of the raw material (s) listed in the present attestation is personally addressed to the above-mentioned beneficiary. It is the beneficiary' s liability to ensure that its own customers are aware of the requirements and prohibitions defined in the terms and conditions and governing any reference to and use of the approval of the raw material(s) and that they abide by it.

ECOCERT Greenlife S.A.S. - Capital 50 000 € - BP 47 - 32600 L'ISLE JOURDAIN - FRANCE
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ATTESTATION OF CONFORMITY - COSMOS

List of the approved raw materials of: **ACTIVE MICRO TECHNOLOGIES LLC**

Commercial name / INCI / Function	%PPAI	%CPAI	%NNI	%PeMo	Restriction	Approved since
Leucidal Liquid (M15008) *Leuconostoc/Radish Root Ferment Filtrate* Moisturizing, Skin conditioning, Antimicrobial	0	52	0	0		01/01/2019
Leucidal Liquid AE LFHC (M15008LFHC) *Lactobacillus/Radish Root Ferment Filtrate* Skin conditioning, Antimicrobial	0	100	0	0		01/01/2019
Leucidal Liquid Complete (M15024) *Leuconostoc/Radish Root Ferment Filtrate (and) Lactobacillus (and) Cocos Nucifera (Coconut) Fruit Extract* Moisturizing, Skin conditioning, Antimicrobial	0	64	0	0		01/01/2019
Leucidal Liquid PT (M15021) *Lactobacillus Ferment* Skin conditioning, Antimicrobial	0	18	0	0		01/01/2019
Leucidal Liquid SF (M15019) *Lactobacillus Ferment* Moisturizing, Skin conditioning, Antimicrobial	0	10	0	0		01/01/2019
Leucidal Liquid SF (M15019CHI) *Leuconostoc/Radish Root Ferment Filtrate* Skin conditioning, Antimicrobial	0	10	0	0		01/01/2019
Leucidal SF Complete (M15025) *Lactobacillus Ferment (and) Lactobacillus (and) Cocos Nucifera (Coconut) Fruit Extract* Moisturizing, Skin conditioning, Antimicrobial	0	32,5	0	0		01/01/2019
Leucidal® SF Max (M15019MAX) *Lactobacillus Ferment* Ferment / Skin Conditioning, Antimicrobial	0	25	0	0		01/01/2019

Valid until: 31/12/2019

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Commercial name / INCI / Function	%PPAI	%CPAI	%NNI	%PeMo	Restriction	Approved since
Myavert (M13001) *Lactoperoxidase (and) Glucose Oxidase* Skin conditioning, Hair conditioning, Antimicrobial	0,95	0,95	0	0		01/01/2019
PhytoCide Aspen Bark Extract Powder (M16002) *Populus Tremuloides Bark Extract* Skin conditioning, Antimicrobial	100	0	0	0		01/01/2019
PhytoCide Black Currant Powder (M16001) *Ribes Nigrum (Black Currant) Fruit Extract* Soothing, Skin conditioning, Antimicrobial	100	0	0	0		01/01/2019
PhytoCide Elderberry OS (M16003) *Sambucus Nigra Fruit Extract* Skin conditioning, Antimicrobial	100	0	0	0		01/01/2019

Valid until: 31/12/2019

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