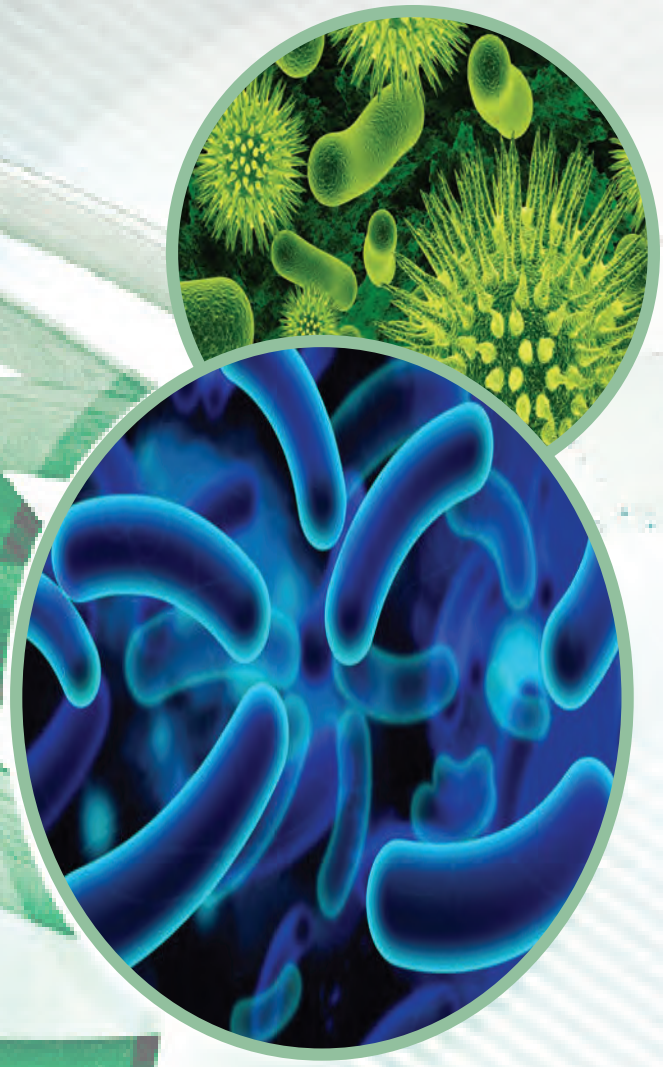
ACTIVE MICRO TECHNOLOGIES

Technical Dossier



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Improving solar choice antimicrobial

SynerCide Herbal Fusion

Code Number: M17002

INCI Name: Hexylene Glycol & Caprylyl Glycol & Origanum vulgare Leaf Extract & Thymus vulgaris (Thyme) Leaf Extract & Rosmarinus officinalis (Rosemary) Leaf Extract & Water

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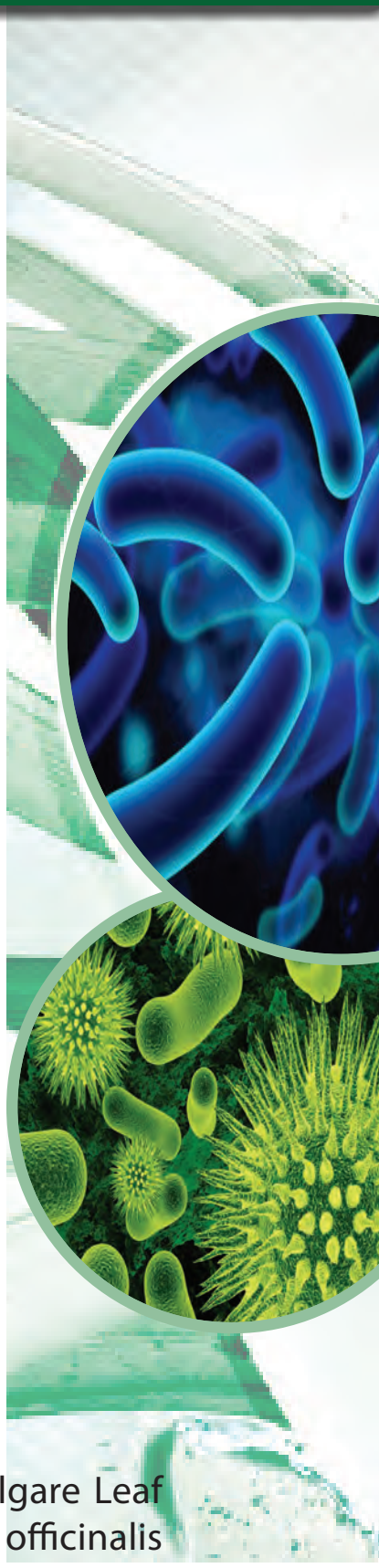

SynerCide Herbal Fusion Code Number: M17002
INCI Name: Hexylene Glycol & Caprylyl Glycol & Origanum vulgare Leaf Extract & Thymus vulgaris (Thyme) Leaf Extract & Rosmarinus officinalis (Rosemary) Leaf Extract & Water

SynerCide Herbal Fusion

Technical Data Sheet

BACKGROUND

Advances in technology have allowed for the evolution of preservation techniques throughout the centuries. Some of the original approaches included dehydration and fermentation. Other traditional methods used for preservation have involved creating herbal tisanes. Of course today there is a need to preserve more than just food. Although some of the aforementioned techniques are still utilized, until recently, more popular methods for the preservation of cosmetics have been the use of ingredients such as parabens and phenoxyethanol. However as these ingredients have fallen out of favor with consumers, chemists have been striving to find effective alternatives.

SCIENCE

Active Micro Technologies has taken a unique approach by developing the SynerCide Fusion line of products, which are a fusion of traditional and more modern preservation techniques. These actives are intended to provide skin conditioning and hydrating benefits as well as inhibit microbial growth.

SynerCide Herbal Fusion consists of oregano, thyme and rosemary extracted in caprylyl and hexylene glycols. In this system, the glycols act as more than just a solvent as they are understood to provide emollient and humectant properties.

BENEFITS

Oregano is a widely recognized herb that is frequently used as an accent in a variety of Mediterranean cuisines. Traditionally used as an antiseptic, the botanical contains phytocompounds such as oleanolic and caffeic acids. These are capable of providing protection against oxidative stress which contributes to aging. In addition to its culinary uses, thyme has been used medicinally to treat both infection and inflammation. Thymol, an essential oil in thyme was historically used on bandages before the advent of antibiotics. Long credited with improving one's memory, research has shown that Rosemary may actually enhance cognitive function while providing antioxidant protection. The herb is rich in carnosic, rosmarinic and caffeic acids. In addition to their health benefits, all three herbs possess potent antimicrobial benefits.

Code Number: M17002
INCI Nomenclature:
Hexylene Glycol & Caprylyl Glycol & *Origanum vulgare* Leaf Extract & *Thymus vulgaris* (Thyme) Leaf Extract & *Rosmarinus officinalis* (Rosemary) Leaf Extract & Water
INCI Status: Approved
REACH Status: Fully Compliant
CAS Number: 107-41-5 & 1117-86-8 & 84012-24-8 & 84929-51-1 & 84604-14-8 & 7732-18-5
EINECS Number: 203-489-0 & 214-254-7 & 281-670-3 & 284-535-7 & 283-291-9 & 231-791-2
Origin: Botanical:
Origanum vulgare & *Thymus vulgaris* & *Rosmarinus officinalis*
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: Hexylene Glycol & Caprylyl Glycol & Water
Appearance: Clear to Slightly Hazy Liquid
Soluble/Miscible: Fully Water Soluble
Suggested Use Levels: 1.0 - 2.0%
Suggested Applications:
Skin Conditioner, Antimicrobial

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Version 4 - 02.20.14

SynerCide Herbal Fusion

EFFICACY TESTING

SynerCide Herbal Fusion is useful in a variety of cosmetic and personal care applications as testing has revealed that it is a broad spectrum antimicrobial. Minimum inhibitory concentration (MIC) testing and a double challenge study confirm that **SynerCide Herbal Fusion** is effective against gram positive and gram negative bacteria, as well as yeast and mold.

Microorganism Tested	MIC (%)
E. coli	0.50
S. aureus	1.00
P. aeruginosa	0.50
C. albicans	0.50
A. brasiliensis	2.00

Figure 1. MIC data for **SynerCide Herbal Fusion**

	S. aureus	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. brasiliensis*	*K. pneumoniae*	*B. cepacia*
Inoculum Level (initial)	2.17E+06	2.46E+06	2.25E+06	8.93E+05	2.86E+05	3.02E+05	2.04E+06
Day 0	49.44%	51.88%	107.25%	93.14%	89.96%	47.32%	>99.999%
Day 1	>99.999%	>99.999%	>99.999%	>99.999%	93.65%	>99.999%	>99.999%
Day 2	>99.999%	>99.999%	>99.999%	>99.999%	97.71%	>99.999%	>99.999%
Day 3	>99.999%	>99.999%	>99.999%	>99.999%	98.44%	>99.999%	>99.999%
Day 7	>99.999%	>99.999%	>99.999%	>99.999%	99.63%	>99.999%	>99.999%
Day 14	>99.999%	>99.999%	>99.999%	>99.999%	99.999%	>99.999%	>99.999%
Day 21	>99.999%	>99.999%	>99.999%	>99.999%	99.999%	>99.999%	>99.999%
Day 28	>99.999%	>99.999%	>99.999%	>99.999%	99.999%	>99.999%	>99.999%
Inoculum level (re-inoculated)	1.28E+06	2.69E+06	2.18E+06	7.19E+06	2.56E+05	1.34E+06	2.13E+06
Day 0	31.42%	45.38%	53.13%	34.89%	14.22%	27.37%	>99.999%
Day 1	>99.999%	>99.999%	>99.999%	>99.999%	98.37%	>99.999%	>99.999%
Day 2	>99.999%	>99.999%	>99.999%	>99.999%	99.61%	>99.999%	>99.999%
Day 3	>99.999%	>99.999%	>99.999%	>99.999%	98.74%	>99.999%	>99.999%
Day 7	>99.999%	>99.999%	>99.999%	>99.999%	99.90%	>99.999%	>99.999%
Day 14	>99.999%	>99.999%	>99.999%	>99.999%	99.999%	>99.999%	>99.999%
Day 21	>99.999%	>99.999%	>99.999%	>99.999%	99.999%	>99.999%	>99.999%
Day 28	>99.999%	>99.999%	>99.999%	>99.999%	99.999%	>99.999%	>99.999%

Table 2. Percent change in microorganism concentration for 2% **SynerCide Herbal Fusion** in an O/W emulsion

USE RECOMMENDATIONS

Optimal conditions for formulating with **SynerCide Herbal Fusion** include maintaining a pH between 3 and 8 and temperatures below 70˚C. If formulating at higher temperatures, it is recommended to add the ingredient on cooling, even after an emulsion has been formed. Whether interested in enhancing epidermal hydration or replacing more conventional preservatives, using **SynerCide Herbal Fusion** allows formulators to develop contemporary products for the discerning consumer.

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Specification

Product Name: SynerCide Herbal Fusion
Code Number: M17002
CAS #'s: 107-41-5 & 1117-86-8 & 84012-24-8 & 84929-51-1 & 84604-14-8 & 7732-18-5
EINECS #'s: 203-489-0 & 214-254-7 & 281-670-3 & 284-535-7 & 283-291-9 & 231-791-2
INCI Name: Hexylene Glycol & Caprylyl Glycol & *Origanum vulgare* Leaf Extract & *Thymus vulgaris* (Thyme) Leaf Extract & *Rosmarinus officinalis* (Rosemary) Leaf Extract & Water

Specification	Parameter
Appearance	Clear to Hazy Liquid
Color	Amber to Brown
Odor	Characteristic
Specific Gravity (25°C)	0.970 – 1.020
Refractive Index (25°C)	1.3960 – 1.4040
Heavy Metals	< 20 ppm
Arsenic	< 2 ppm
Minimum Inhibitory Concentration[1]	
Organism (ATCC#)	
E. coli (#8739)	0.25 – 2.00%
S. aureus (#6538)	0.25 – 1.00%
P. aeruginosa (#9027)	0.25 – 1.00%
C. albicans (#10231)	0.25 – 2.00%
A. brasiliensis (#16404)	0.25 – 2.00%

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

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Note:

1) Refer to Inhibition Activity Data

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Compositional Breakdown

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SynerCide Herbal Fusion
Code: M17002

Compositional Breakdown:

Ingredient	%
Hexylene Glycol	30.00
Capyrlyl Glycol	15.00
Origanum Vulgare Leaf Extract	15.00
Thymus Vulgaris (Thyme) Leaf Extract	15.00
Rosmarinus Officinalis (Rosemary) Leaf Extract	15.00
Water	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 SynerCide Herbal Fusion does not contain allergen levels exceeding the following (Gas Chromatography-Mass Spectrometer Coupled):

ALLERGENS Dir 2003 15 CEE		
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	100-51-69	< 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	< 0.30
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	90028-68-5	< 0.02
Evernia furfuracea	90028-67-4	< 0.00
Amylcinnamyl Alcohol	101-85-9	< 1.00

Compositional Breakdown

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This is to certify that SynerCide Herbal Fusion 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

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Compositional Breakdown

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Parathion	< 0.50
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

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Cellular Viability Assay Analysis

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Tradename: SynerCide Herbal Fusion

Code: M17002

CAS #: 107-41-5 & 1117-86-8 & 84012-24-8 & 84929-51-1 & 84604-14-8 & 7732-18-5

Test Request Form #: 1516

Lot #: 41591P

Sponsor: *Active Micro Technologies, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

Test Performed:
Cellular Viability Assay

Introduction

The cellular viability assay is useful for quantitatively measuring cell-mediated cytotoxicity, cell proliferation and mitochondrial metabolic activity. Increased metabolism in a cell indicates ample cellular respiration and adenosine triphosphate (ATP) production. ATP is the molecular energy of cells and is required in basic cell function and signal transduction. A decrease in ATP levels indicates cytotoxicity and decreased cell function while an increase in ATP levels indicates healthy cells.

The cellular viability assay was conducted to assess the ability of **SynerCide Herbal Fusion** to increase cellular metabolic activity in cultured dermal fibroblasts.

Assay Principle

The assay utilizes a nonfluorescent dye, resazurin, which is converted to a fluorescent dye, resorufin, in response to chemical reduction of growth medium from cell growth and by respiring mitochondria. Healthy cells that are in a proliferative state will be able to easily convert resazurin into resorufin without harming the cells. This method is a more sensitive assay than other commonly used mitochondrial reductase dyes such as MTT. An increase in the signal generated by resazurin-conversion is indicative of a proliferative cellular state.

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Materials

A.	**Kit:**	PrestoBlue™ Cell Viability Reagent (Invitrogen, A13261)
B.	**Incubation Conditions:**	37°C at 5% CO_2 and 95% relative humidity (RH)
C.	**Equipment:**	Forma humidified incubator; ESCO biosafety laminar flow hood; Light microscope; Pipettes
D.	**Cell Line:**	Normal Human Dermal Fibroblasts (NHDF) (Lonza; CC-2511)
E.	**Media/Buffers:**	Basal Medium (Fibrolife; LM-0001), 500µg/mL Human Serum Albumins (Fibrolife; LS-1001), 0.6µM Linoleic Acid (Fibrolife; LS-1001), 0.6µg/mL (Fibrolife; LS-1001), 5ng/mL Fibroblast Growth Factor (Fibrolife; LS-1002), 5mg/mL Epidermal Growth Factor (Fibrolife; LS-1003), 30pg/mL Transforming Growth Factor β-1 (Fibrolife; LS-2003), 7.5mM L-Glutamine (Fibrolife; LS-1006), 1µg/mL Hydrocortisone Hemisuccinate (Fibrolife; LS-1007), 50µg/mL Ascorbic Acid (Fibrolife; LS-1005), 5µg/mL Insulin (Fibrolife; LS-1004)
F.	**Culture Plate:**	Falcon flat bottom 96-well tissue culture treated plates
G.	**Reagents:**	PrestoBlue™ reagent (10X)
H.	**Other:**	Sterile disposable pipette tips

Methods

Human dermal fibroblasts were seeded into 96-well tissue culture plates and allowed to grow to confluency in complete serum-free media. A 10-fold serial dilution was performed resulting in **SynerCide Herbal Fusion** concentrations of 0.1% and 0.01% in complete serum-free media and incubated with fibroblasts for 24 hours.

Ten microliters of viability reagent was added to 90µL of cell culture media in culture wells and a fluorometric measurement was taken at 560nm for excitation and 590nm for emission.

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Results

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

SynerCide Herbal Fusion did not exhibit negative effects on cell metabolism.

Cellular metabolism results are shown as mean fluorescence units (MFU) and expressed as percentage change, calculated by the below equation:

$$Percent\ (\%)Change = \frac{MFU_{Control} - MFU_{Sample}}{MFU_{Control}} \times 100$$

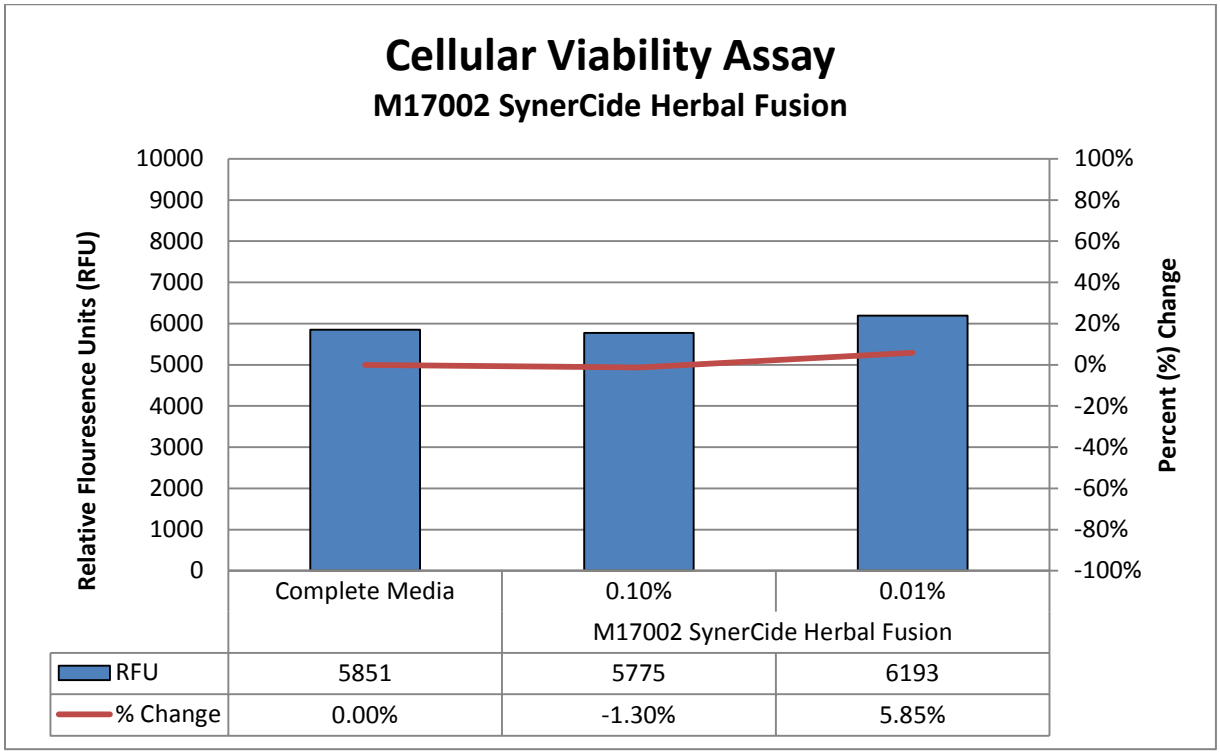

Figure 1: Cellular Metabolism of **SynerCide Herbal Fusion**-treated fibroblasts

Discussion

In this study, **SynerCide Herbal Fusion** (code M17001) was tested to evaluate its effects on the viability of normal human dermal fibroblasts (NDHF). At concentrations of 0.1% and 0.01%, **SynerCide Herbal Fusion**, nor the preservatives contained therein exhibited any inhibition of cell viability. It can therefore be concluded that at normal use concentrations **SynerCide Herbal Fusion** is not cytotoxic.

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Inhibition Activity Data

Product Name: SynerCide Herbal Fusion
Code Number: M17002
Lot Number: 41591P
Test Request Number: 1501
CAS #'s: 107-41-5 & 1117-86-8 & 84012-24-8 & 84929-51-1 & 84604-14-8 & 7732-18-5
EINECS #'s: 203-489-0 & 214-254-7 & 281-670-3 & 284-535-7 & 283-291-9 & 231-791-2
INCI Name: Hexylene Glycol & Caprylyl Glycol & *Origanum vulgare* (Oregano) Leaf Extract & *Thymus vulgaris* (Thyme) Leaf Extract & *Rosmarinus officinalis* (Rosemary) Leaf Extract & Water

Organism (ATCC #)	Minimum Inhibitory Concentration (%)
E.coli #8739	1.0
S. aureus #6538	1.0
P. aeruginosa #9027	0.5
C. albicans #10231	0.5
A. brasiliensis #16404	1.0

QA Signature Monica Beltran

Date 09-11-2015

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Antimicrobial Efficacy Test
PCPC Section 20
Method 3

Determination of Preservation Adequacy of Water- Miscible Personal Care Products

Test Product

SynerCide Herbal Fusion
Code: M17002

Test Request #:

1502

Purpose

This study was initiated to determine the efficacy of a cosmetic ingredient with antimicrobial properties in a cream formulation against bioburden as a function of time.

Study Dates

The study was started on July 20th, 2011 and was completed on September 20th, 2011.

Test Organisms

1. *Escherichia coli:* ATCC #8739
2. *Pseudomonas aeruginosa:* ATCC #9027
3. *Staphylococcus aureus:* ATCC #6538
4. *Aspergillus brasiliensis:* ATCC #16404
5. *Candida albicans:* ATCC #10231

Neutralization:

Verification of neutralization of the antimicrobial properties of the product was demonstrated prior to performing the test for microbial content by inoculating the product dilution with a low level of challenge microorganisms (100 CFU) and verifying recovery of this viable inoculum. This provides evidence that the antimicrobial has been neutralized and there are no false positive results during the Challenge Test.

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Test Method

Fifty grams of Generic Cream pH 5 with 2% SynerCide Herbal Fusion was weighed into five individual containers. Each container was inoculated with one of the five test organisms. The inoculum concentration for each organism was standardized using the 0.5 McFarland turbidity standard and further diluted to yield approximately 10^6 to 10^8 microorganisms/ml. The amount of each inoculum added to each sample was no more than 1% of the product weight, as to not alter the product composition.

The inoculated samples were evaluated 0, 7, 14, 21, and 28 days after the initial inoculation to determine quantitatively the number of viable microorganisms remaining. On the 28th day of testing the samples were re-inoculated and evaluated 7, 14, 21, and 28 days after the second exposure to determine the number of viable microorganisms. The table below represents the percent reduction of viable organisms after being introduced into the test formulation.

	Organisms				
Inoculum (initial) CFU/ml	*E. coli*	*P. aeruginosa*	*S. aureus*	*A. brasiliensis*	*C. albicans*
	2.46×10^6	2.25×10^6	2.17×10^6	2.86×10^5	8.93×10^5
Day 0*	51.878%	107.247%	49.436%	89.963%	93.140%
Day 7	>99.999%	>99.999%	>99.999%	99.631%	>99.999%
Day 14	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 21	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 28	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Inoculum (re-inoculated) CFU/ml	2.69×10^6	2.18×10^6	1.28×10^6	2.56×10^5	7.19×10^5
Day 7	>99.999%	>99.999%	>99.999%	99.901%	>99.999%
Day 14	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 21	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%
Day 28	>99.999%	>99.999%	>99.999%	>99.999%	>99.999%

Table 1. Challenge Test results for Generic Cream pH 5 with 2% SynerCide Herbal Fusion inoculated on Day 0 and re-inoculated on Day 28. Results show % reduction in viable organisms.

* The days listed in the first column refer to the inoculum/plating day. Bacteria results are read 2 days after plating day, and mold and yeast results are read 5 days after plating day.

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Results & Discussion

The results obtained from the Neutralization Test of each product using Dey/Engley (D/E) broth, indicate that the neutralization steps conducted prior to performing the Challenge Test are indeed effective for avoiding false positive Challenge Test results.

The results of this Challenge Test demonstrate the effectiveness of the preservation system used in Generic Cream pH 5 with 2% SynerCide Herbal Fusion. The recommendations stated in Section 13, Determination of Preservative Adequacy in Cosmetic Formulations, in the PCPC Microbiology Guidelines are as follows:

Bacteria – There should be at least a 99.9% (3 log) reduction of vegetative bacteria within 7 days following each challenge and no increase for the duration of the test period.

Yeasts and Molds – There should be at least a 90% (1 log) reduction of yeasts and molds within 7 days following each challenge and no increase for the duration of the test period.

The Gram positive and Gram negative bacteria were reduced by 99.9% within 7 days of each challenge, mold and yeast were reduced by greater than 90% within 7 days of each challenge. By the end of each 28-day test period Gram positive and Gram negative bacteria were reduced by 99.999% or greater, mold and yeast were reduced 99.0% or greater.

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Phase	Ingredient	Supplier	%
I	Water	-	85.5
	Glycerin	PT. Musim Mas	5.0
	Stearic Acid	Acme Hardesty Oleochemicals	2.5
II	Mineral Oil	RITA	5.0
	Lanolin	RITA	0.5
	Petrolatum	RITA	0.5
	Sepigel 305	Seppic	1.0

Manufacturing Process:

1. Phase I:
Charge water into main beaker and begin propeller mixing. A vortex should form. Begin heating to 80^0C while adding the ingredients.

2. Phase II:
In a separate beaker, combine ingredients and heat to 80^0C while mixing. Mix until homogenous. Then add to the main beaker with high-speed mixing. Maintain temperature at 80^0C and mix for 15 minutes. Begin force cooling to 25^0C.

3. Check the pH and adjust it if necessary.

Specifications:

Appearance: White to Off-White Emulsion
pH: 5.0 – 8.0
*If a different pH is desired, adjust using Citric Acid (50%) or NaOH (25%). Formula is stable in the 3.0 – 7.0 pH range.

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Antimicrobial Efficacy (Challenge) Testing

The intent of performing an Antimicrobial Efficacy or Challenge test is to evaluate whether an antimicrobial agent or preservation system in a given cosmetic formulation has the ability to prevent the growth of test microorganisms. The test methodology employed by Active Micro Technologies (AMT) is based on the methods published in the CTFA Microbiology Guidelines. AMT's goal is to assist our customers by providing a screening test of a product formulation that is approaching finalization. It is expected that the formulation(s) submitted for Challenge testing contain AMT antimicrobials and have already passed the customer's internal stability tests. It is also anticipated that formal challenge testing of the final formulation will subsequently be performed by the customer at an outside lab of their choosing.

The information contained in this report is provided by Active Micro Technologies after the exercise of all reasonable care and skill in its compilation, preparation, and issue. It is provided without liability regarding its subsequent application and use. This type of screening test will be conducted only for validation of the efficacy of the antimicrobial agent or preservative system in the specific formulation tested. It does not address the suitability of the overall formula, nor does it address the regulatory status of any component therein. This testing does not account for the possibility of environmental microorganisms and cannot be relied upon as sufficient to justify commercialization of the product tested. By submitting samples for testing, the customer acknowledges that they will not hold Active Micro Technologies responsible for products launched based solely on the support of these studies.

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Time Kill Test
E2315
Assessment of Antimicrobial Activity Using a Time Kill Procedure

Product

SynerCide Herbal Fusion

Test Request #:

1916

Purpose

This study was initiated to measure the change in population of aerobic microorganisms within a specified sampling time when tested against a cosmetic ingredient.

Study Dates

The study was started on April 19th, 2016 and was completed on April 25th, 2016.

Test Organisms

1. *Escherichia coli:* ATCC #8739
2. *Pseudomonas aeruginosa:* ATCC #9027
3. *Staphylococcus aureus:* ATCC #6538
4. *Bacillus subtilis* ATCC #6051
5. *Aspergillus brasiliensis:* ATCC #16404
6. *Candida albicans:* ATCC #10231

Neutralization:

Inactivation of the antimicrobial activity of the test material is achieved through the dilution of the test material during the sampling time at specified sampling intervals.

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Test Method

Ten grams of 4% SynerCide Herbal Fusion solution was weighed into six individual containers. Each container was inoculated with one of the six test organisms. The inoculum concentration for each organism was standardized using the 0.5 McFarland turbidity standard and further diluted to yield approximately 10^6 microorganisms/ml. The amount of each inoculum added to each sample was no more than 1% of the product weight, as to not alter the product composition. Serial dilutions from each container were performed to enumerate the surviving microorganisms using the Plate Count Technique.

The activity of the test material inoculated was evaluated at determine time intervals of 30 seconds, 1, 5, 10 and 30 minutes after the inoculation to determine quantitatively the number of viable microorganisms remaining after the incubation time.

Organisms	Inoculum Concentration CFU/ml	Percentage of Reduction				
		30 seconds	1 minute	5 minute	10 minute	30 minutes
E.coli * ATCC# 8739	7.3×10^6	99.9%	99.9%	99.9%	99.9%	99.9%
S.aureus ATCC# 6538	5.1×10^6	99.9%	99.9%	99.9%	99.9%	99.9%
P.aeruginosa ATCC# 9027	3.7×10^5	99.9%	99.9%	99.9%	99.9%	99.9%
B.subtilis ATCC# 6051	1.1×10^6	99.9%	99.9%	99.9%	99.9%	99.9%
A.brasiliensis ATCC# 16404	1.0×10^6	99.9%	99.9%	99.9%	99.9%	99.9%
C.albicans ATCC# 10231	3.6×10^6	99.9%	99.9%	99.9%	99.9%	99.9%

Table 1. Time Kill Test results for 4% SynerCide Herbal Fusion inoculated with 10^6 microorganisms' population. Results show % reduction in viable organisms after inoculation and sampling time intervals.

*Bacteria results are read 2 days after plating day, and mold and yeast results are read 5 days after plating day.

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Results & Discussion

The results of this Time Kill Test determine the changes in population of aerobic microorganisms within a specified sampling time when tested against 4% SynerCide Herbal Fusion.

The Gram positive and Gram negative bacteria as well as the yeast and mold were reduced by 99.9% within 30 seconds interval of the test after the inoculation.

Safety Statement

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Product Name: SynerCide Herbal Fusion

Product Code: M17002

INCI Name: Hexylene Glycol & Caprylyl Glycol & *Origanum vulgare* Leaf Extract & *Thymus vulgaris* (Thyme) Leaf Extract & *Rosmarinus officinalis* (Rosemary) Leaf Extract & Water

INCI Status: Approved

SynerCide Herbal Fusion is a blend of naturally occurring herbs that provide antimicrobial benefits. Extracted in water and blended with caprylyl and hexylene glycols, SynerCide Herbal Fusion provides emollient and humectant properties.

The Cosmetic Ingredient Review (CIR) states that Caprylyl Glycol is safe for use at 5.0% and Hexylene Glycol is safe for use at 6.0%. SynerCide Herbal Fusion contains 30% Hexylene Glycol and 15% Caprylyl Glycol. Given our recommended use level of 1 – 2%, the finished product will contain a maximum of 0.6% Hexylene Glycol and 0.3% Caprylyl Glycol both of which are well within safe levels of use.

The CIR was established in 1976 with the support of the U.S. Food and Drug Administration and the Consumer Federation of America. The Cosmetic Ingredient Review thoroughly reviews and assesses the safety of ingredients used in cosmetics in an open, unbiased, and expert manner, and publishes the results in peer-reviewed literature.

SynerCide Herbal Fusion contains herbal extracts of oregano, thyme and rosemary. The FDA (Food and Drug Administration) states in sections 201 and 409 of the Federal Food, Drug and Cosmetic Act that "any substance that is intentionally added to food is a food additive, that is subject to review and approval by FDA, unless the substance is generally recognized, among qualified experts, as having been adequately shown to be safe under conditions of its use or unless the use of the substance is otherwise excluded for the definition of a food additive."

SynerCide Herbal Fusion contains natural herbal extracts of oregano, thyme and rosemary, which can be generally recognized as safe (GRAS). As a result, this knowledge combined with the statement from the CIR, allows us to support the safety of SynerCide Herbal Fusion in cosmetic applications at use levels of 1% to 2% and requires no further testing.

Due to the restriction placed on the animal testing of cosmetic raw materials, and our internal non-animal testing policy, Active Micro Technologies, LLC does not test for NOAEL.

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Dermal and Ocular Irritation Tests

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(704) 276-7100 • Fax (704) 276-7101

Tradename: SynerCide Herbal Fusion

Code: M17002

CAS #: 107-41-5 & 1117-86-8 & 84012-24-8 & 84929-51-1 & 84604-14-8 & 7732-18-5

Test Request Form #: 1443

Lot #: 41591P

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

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 **SynerCide Herbal Fusion** 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**. 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**. 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 Concepts, 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**. The negative and positive controls performed as anticipated.

Figure 1: EpiDerm tissue viability

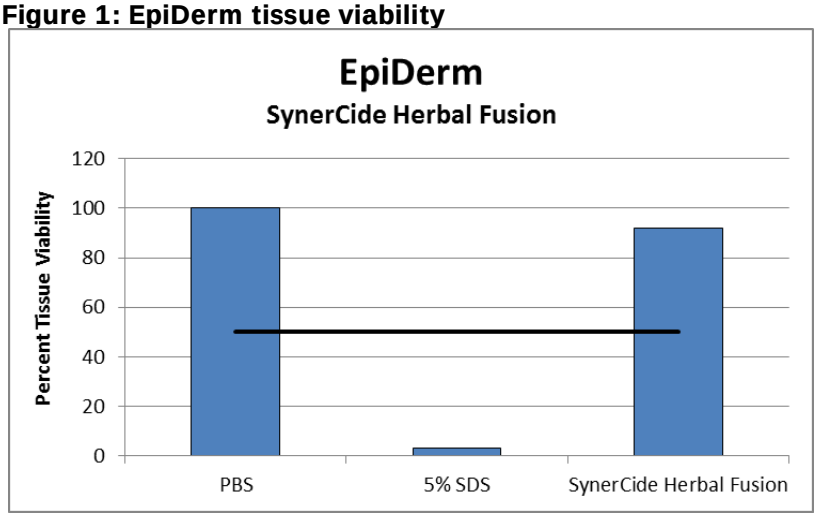
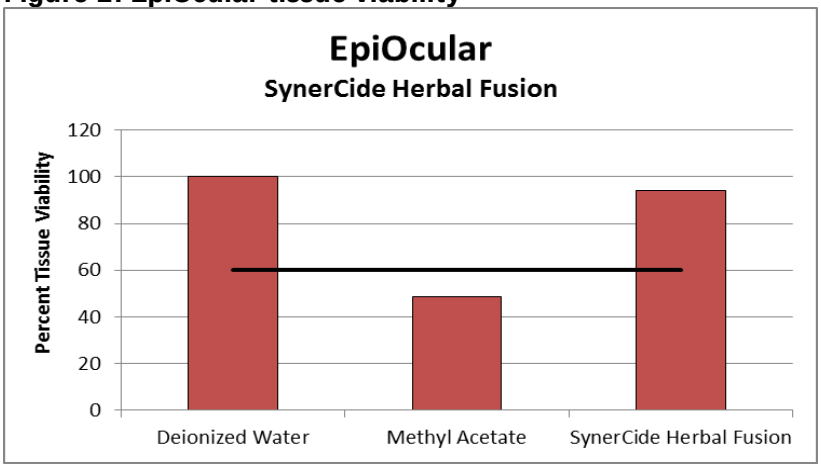


Figure 2: EpiOcular tissue viability



Moisturization / Hydration Assay

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(704) 276-7100 • Fax (704) 276-7101

Tradename: SynerCide Herbal Fusion

Code: M17002

CAS #: 107-41-5 & 1117-86-8 & 84012-24-8 & 84929-51-1 & 84604-14-8 & 7732-18-5

Test Request Form #: 1532

Lot #: 41591P

Sponsor: *Active Micro Technologies, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

Test Performed:
Moisturization/Hydration Assay

Introduction

An *in-vivo* study was conducted over a period of four hours to evaluate the moisturization benefits **SynerCide Herbal Fusion**. 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 **SynerCide Herbal Fusion.**

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 after 4 hours. The test material consisted of 0.5% **SynerCide Herbal Fusion** in a base lotion.

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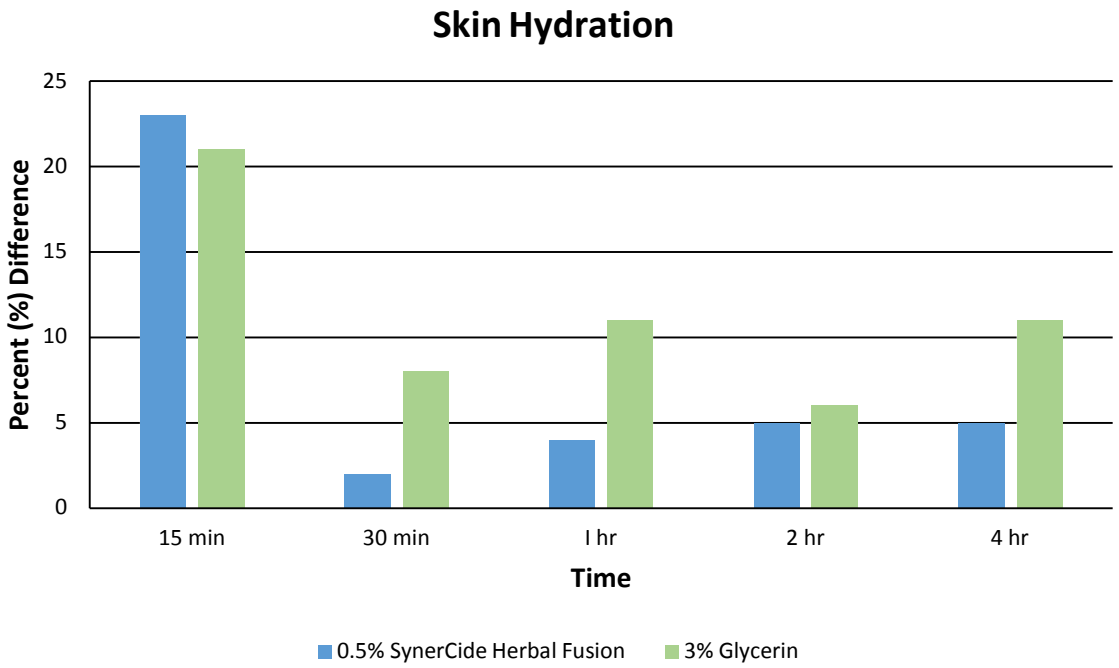
For added perspective, measurements of an untreated test site and a site treated with a base lotion (Cetaphil Moisturizing for All Skin Types) were recorded.

Results

SynerCide Herbal Fusion showed high moisturizing capabilities at a 0.5% concentration.



Discussion

As evidenced in a four hour efficacy study of **SynerCide Herbal Fusion** on skin, hydration improved. The present study confirms that **SynerCide Herbal Fusion** is capable of providing strong moisturizing and skin hydrating benefits when added to cosmetic applications.

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OECD TG 442C: *In Chemico* Skin Sensitization

Tradename: SynerCide Herbal Fusion

Code: M17002

CAS #: 107-41-5 & 1117-86-8 & 84012-24-8 & 84929-51-1 & 84604-14-8 & 7732-18-5

Test Request Form #: 1429

Lot #: 41591P

Sponsor: *Active Micro Technologies, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

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 **SynerCide Herbal Fusion** 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 Breeze - Waters 2998 Photodiode Array Detector); 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 **SynerCide Herbal Fusion** in Acetonitrile

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

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 **SynerCide Herbal Fusion (code M17002)** 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 1.74% 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: SynerCide Herbal Fusion

Code: M17002

CAS #: 107-41-5 & 1117-86-8 & 84012-24-8 & 84929-51-1 & 84604-14-8 & 7732-18-5

Test Request Form #: 1430

Lot #: 41591P

Sponsor: *Active Micro Technologies, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

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 **SynerCide Herbal Fusion** 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 **SynerCide Herbal Fusion** 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	31.6
DMSO	Non-Sensitizer	No Induction	243.24 µM	1.2
SynerCide Herbal Fusion	Non-Sensitizer	No Induction	> 1000 µM	0.3

Table 1: Overview of KeratinoSens™ Assay Results

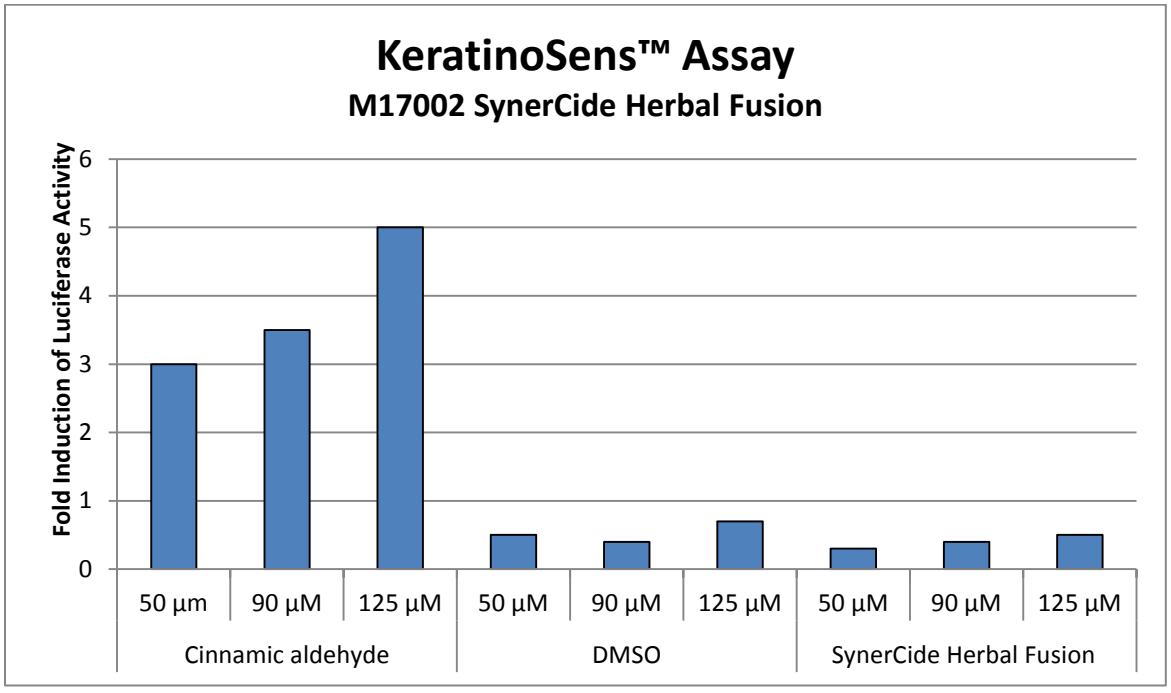

Figure 1: Fold Induction of Luciferase

Discussion

As shown in the results, **SynerCide Herbal Fusion (code M17002)** 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 **SynerCide Herbal Fusion** can be safely used in cosmetics and personal care products at typical use levels.

ACTIVE MICRO TECHNOLOGIES

Phototoxicity Assay Analysis

107 Technology Drive • Lincolnton, NC 28092
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Tradename: SynerCide Herbal Fusion

Code: M17002

CAS #: 107-41-5 & 1117-86-8 & 84012-24-8 & 84929-51-1 & 84604-14-8 & 7732-18-5

Test Request Form #: 1140

Lot #: 41591P

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

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

SUMMARY

In vitro phototoxicity irritation studies were conducted to evaluate whether **SynerCide Herbal Fusion** 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 0.5%, 1.3%, and 4.5%. 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 five 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 0.5%, 1.3%, and 4.5%. 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 of 0.5%, 1.3%, and 4.5%. 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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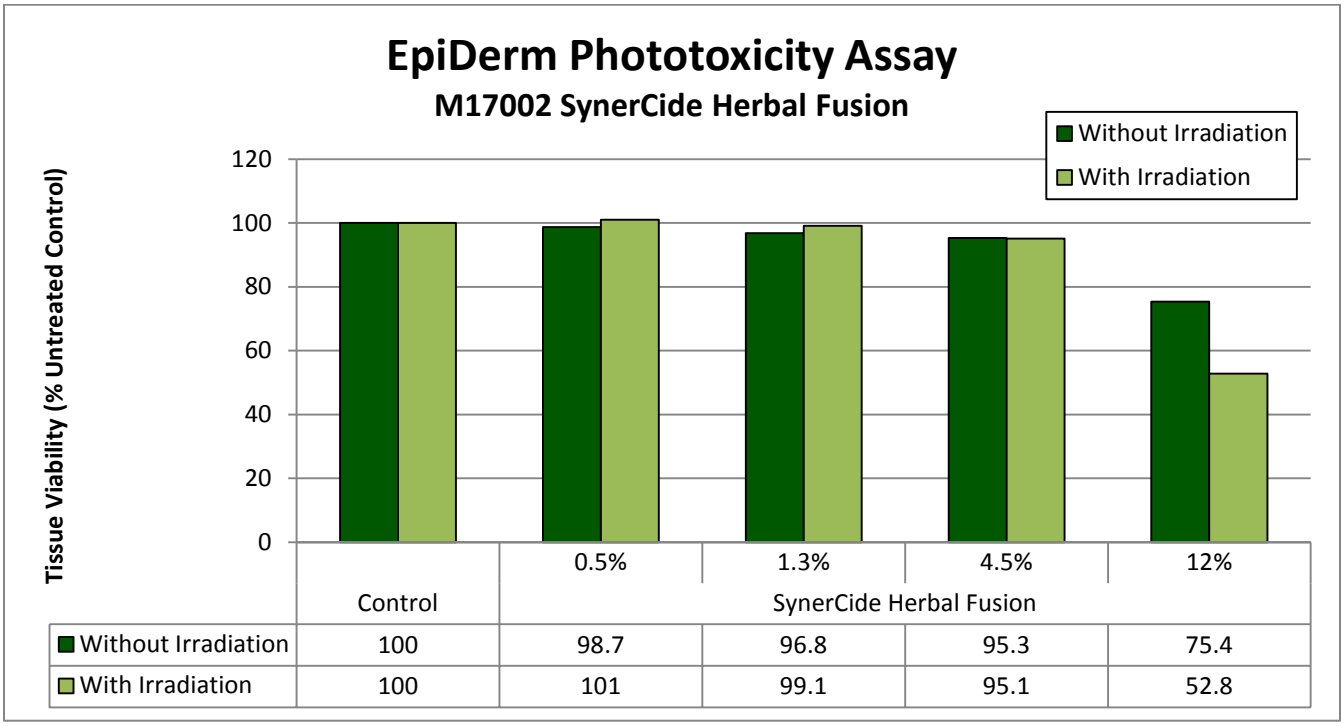
VII. Conclusion

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 0.5%, 1.3%, and 4.5%. There is a decrease in viability at the 12% 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 **SynerCide Herbal Fusion** is not a photoirritant when used at the suggested use levels of 1 – 2%.



	Control	SynerCide Herbal Fusion 0.5%	1.3%	4.5%	12%
Without Irradiation	100	98.7	96.8	95.3	75.4
With Irradiation	100	101	99.1	95.1	52.8

Figure 1: EpiDerm Phototoxicity Graph

ACTIVE MICRO TECHNOLOGIES

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OECD 202 Acute *Daphnia* Assay

Tradename: SynerCide Herbal Fusion

Code: M17002

CAS #: 107-41-5 & 1117-86-8 & 84012-24-8 & 84929-51-1 & 84604-14-8 & 7732-18-5

Test Request Form #: 1411

Lot #: 41591P

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

Test Performed:
OECD 202
Daphnia spp. Acute Immobilization Test

Introduction

The purpose of the present study is to determine the toxicity of **SynerCide Herbal Fusion** by exposing Daphnia spp. to the test substance for 48 hours and measuring the immobilization rate against the control. The present study defines an organism as being immobilized when it does not move for 15 seconds after the test vessel is gently shaken.

OECD Guideline 202 on "*Daphnia* spp., Acute Immobilization Test and Reproduction Test", adopted in 1984, included two parts: Part I – the 24 hour EC_{50} acute immobilization test and Part II – the reproduction test (at least 14 days). Revision of the reproduction test resulted in the adoption and publication of Test Guideline 211 on "*Daphnia magna* Reproduction Test" in September 1998. Consequently, the new version of Guideline 202 is restricted to the acute immobilization test.

Assay Principle

Young daphnids, aged less than 24 hours at the start of the test, are exposed to the test substance at a range of concentrations for a period of 48 hours. Immobilization is recorded at 24 hours and 48 hours and compared with control values. The results are analyzed in order to calculate the EC_{50} at 48 hours. EC_{50} is the concentration estimated to immobilize 50% of the daphnids within a stated exposure period. Immobilization refers to those animals that are not able to swim within 15 seconds after gentle agitation of the test vessel, even if they can still move their antennae.

The water solubility and vapor pressure of the test substance should be known. A reliable analytical method for the quantification of the substance in the test solutions with reported recovery efficiency and limit of determination should also be available.

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A reference substance may be tested for EC_{50} as a means of assuring that the test conditions are reliable.

For this assay to be valid, the following performance criteria apply:

- In the control, not more than 10% of the daphnids should have been immobilized.
- The dissolved oxygen concentration at the end of the test should be at least 3 mg/L in control and test vessels.

Materials

- Glass Test Tubes and/or Beakers
- Dissolved Oxygen Meter
- pH Meter
- Temperature Control Apparatus
- Total Organic Carbon (TOC) Analyzer
- Chemical Oxygen Demand (COD) Analyzer
- *Daphnia magna* Straus
    - Use organisms less than 24 hours old. Do not use first offspring of parents.
- Water
    - Use water suitable for culturing and testing Daphnia spp. It can be natural water (surface water or groundwater), dechlorinated tap water, or artificially prepared water (Table 1), but must satisfy the conditions listed in Table 2. Do not use Elendt M4 or M7 media or water containing chelating agents for testing metal-containing substances.

Substance	Concentration
Particulate Matter	<20 mg/L
Total Organic Carbon	<2 mg/L
Unionized Ammonia	<1 ug/L
Residual Chlorine	<10 ug/L
Total Organophosphorus Pesticides	<50 ng/L
Total Organochlorine Pesticides plus Polychlorinated Biphenyls	<50 ng/L
Total Organic Chlorine	<25 ng/L

Table 1: Chemical Characteristics of Suitable Water

Substance	Amount Added to 1 Liter Water	To prepare the reconstituted water, add the following volumes of stock solutions to 1 liter water
Calcium Chloride	11.76 grams	25 mL
Magnesium Sulfate	4.93 grams	25 mL
Sodium Bicarbonate	2.59 grams	25 mL
Potassium Chloride	0.23 grams	25 mL

Table 2: Examples of Suitable Reconstituted Test Water

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Methods

Test Conditions

- Test Method
  - Test is performed under a static, semi-static, or flow-through condition. If test substance is unstable, a semi-static or flow-through test is recommended.
- Exposure Period
  - 48 hours
- Test Volume
  - At least 2 milliliters
- Number of Test Organisms
  - At least 20 organisms for each test concentration and the control.
- Test Concentration
  - Adopt a concentration range of at least 5 concentrations, with the highest concentration inducing 100% immobilization and no effect at the lowest concentration.
- Culture Method
  - Illumination: The photoperiod is set to 16 hours light and 8 hours dark
  - Temperature: The temperature is between 18°C to 22°C
  - Dissolved Oxygen Concentration: Must be kept at 3mg/L or higher
  - Feeding: Do not feed test organisms

Observation

- Observe mobility of the organisms at least twice (i.e., at 24 and 48 hours after exposure).
- The organisms are considered immobilized when they do not move for 15 seconds after test vessel is gently shaken.

Measurement of Test Substance Concentrations

- 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.

Test Condition Measurements

- Measure dissolved oxygen in the control and at the highest test concentration at the beginning and end of the exposure period.
- 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. Data should be summarized in tabular form, showing for each treatment group and control, the number of daphnids used, and immobilization at each observation. The percentages immobilized at 24 and 48 hours are plotted against test concentrations. Data are analyzed by appropriate statistical methods (e.g. probit analysis, etc.) to calculate the slopes of the curves and the EC_{50} with 95% confidence limits (p = 0.95).
   b. Where the standard methods of calculating the EC_{50} are not applicable to the data obtained, the highest concentration causing no immobility and the lowest concentration producing 100% immobility should be used as an approximation for the EC_{50} (this being considered the geometric mean of these two concentrations).

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 *Daphnia*, 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, number of daphnids 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, dissolved oxygen, 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, pH and dissolved oxygen made during the test
         3. The EC_{50} at 48 hours for immobilization 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_{50}.

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Results

General Information:

Name of new chemical substance	SynerCide Herbal Fusion		
INCI Nomenclature	*Hexylene Glycol & Caprylyl Glycol & Origanum vulgare (Oregano) Leaf Extract & Thymus vulgaris (Thyme) Leaf Extract & Rosmarinus officinalis (Rosemary) Leaf Extract & Water*		
CAS number	107-41-5 & 1117-86-8 & 84012-24-8 & 84929-5-1 & 84604-14-8 & 7732-18-5		
Structural or rational formula (if neither is available, summarize its formulation method)	Botanical: Origanum vulgare & Thymus vulgaris & Rosmarinus officinalis		
Molecular weight	194.1 Daltons		
Purity of the new chemical substance used for the test (%)	100%		
Lot number of the new chemical substance used for the test	41591P		
Names and contents of impurities	n/a		
Solubility in water	100%		
Properties at room temperature	Clear to Slightly Hazy Liquid		
Stability	Stable		
Solubility in solvents, etc.	**Solvent**	**Solubility**	**Stability in solvent**
	n/a	n/a	n/a

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Test Materials and Methods:

Items			Contents
Test Organisms	Species		*Daphnia magna*
	Source		Carolina Biological Supply Company
	Reference substance (EC_{50})		Potassium dichromate (0.94 mg/L)
Culture	Kind of Medium		Elendt Medium M4
	Conditions (Temperature/Photoperiod)		20°C/16 Hour Light-8 Hour Dark
Test Conditions	Test Vessel		Glass
	Material Water	Kind	Elendt Medium M4
		Hardness	250 mg/L
		pH	7.4
	Date of Exposure		07/06/2015
	Test Concentrations		200, 90.9, 41.3, 18.8, 8.5 mg/L
	Number of organisms		120
	Number of Replicates	Exposure Group	4
		Control Group	4
	Test Solution Volume		2 mL
	Vehicle	Use or Not	N/A
		Kind	N/A
		Concentration	N/A
		Number of Replicates	N/A
	Culture Method (Static, Semi-Static, Flow-Through)		Static
	Water Temperature		20°C ± 2°C
	Dissolved Oxygen Concentration (DO)		3 mg/L
	Photoperiod		16 Hour Light-8 Hour Dark
	Statistical Method		Probit Analysis

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Test Results:

Items		**Contents**
Toxicity Value	48hr EC50	931.8 mg/L
Exposure Concentrations Used for Calculation	Nominal Values	200, 90.9, 41.3, 18.8, 8.5 mg/L
Remarks		Not harmful to aquatic organisms

Discussion

After 48 hours, the EC50 value for **SynerCide Herbal Fusion** was determined to be 931.8 mg/L. The conditions of OECD guideline 202 for the validity of the test were adhered to: The immobility of controls in purified drinking water (dilution water) did not exceed 10%. According to the EU Directive 93/67/EEC, this product is not classified and therefore not harmful to aquatic organisms.

ACTIVE MICRO TECHNOLOGIES

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OECD 301B
Ready Biodegradability Assay

Tradename: SynerCide Herbal Fusion

Code: M17002

CAS #: 107-41-5 & 1117-86-8 & 84012-24-8 & 84929-51-1 & 84604-14-8 & 7732-18-5

Test Request Form #: 1410

Lot #: 41591P

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

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

Introduction

A study was conducted to assess the readily biodegradability of **SynerCide Herbal Fusion** 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 data sheet below.
   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

I. 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.

ACTIVE MICRO TECHNOLOGIES

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OECD 301B
Ready Biodegradability Assay

Data Sheet

Laboratory	Active Concepts Tissue Culture Laboratory		
Test Start Date	7/6/2015		
Test Substance	**Name**	SynerCide Herbal Fusion	
	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	83.9% and 84.5% 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, **Synercide Herbal Fusion** achieved 84.2% biodegradation after 28 days of testing. The product met method requirements for the Readily Biodegradable classification.

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Date Issued: September 1, 2015

ALLERGEN DECLARATION

RE: *SynerCide Herbal Fusion (M17002)*

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

Corn – or corn products

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

Heavy Metals Statement

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May 10, 2016

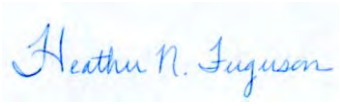
To Whom It May Concern,

This letter is to certify that SynerCide Herbal Fusion (M17002) has the following heavy metals profile:

Heavy Metals:	Less than 20 ppm
Lead:	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 Lead, Antimony, Mercury and Cadmium do not appear on the Specification for SynerCide Herbal Fusion.

Best Regards,



Tomorrow's Vision... *Today!*®

Heather Ferguson |R&D Coordinator

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Email: hferguson@activeconceptsllc.com

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Certificate of Origin

SynerCide Herbal Fusion
Code: M17002

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-Free.

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

INCI Name	Source
Hexylene Glycol	Synthetic
Caprylyl Glycol	Synthetic
Origanum Vulgare Leaf Extract	Plant (*Origanum vulgare*)
Thymus Vulgaris (Thyme) Leaf Extract	Plant (*Thymus vulgaris*)
Rosmarinus Officinalis (Rosemary) Leaf Extract	Plant (*Rosmarinus officinalis*)
Water	Water

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	SynerCide Herbal Fusion
Product Code	M17002
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 25°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: Hexylene Glycol & Caprylyl Glycol & Origanum Vulgare Leaf Extract & Thymus Vulgaris (Thyme) Leaf Extract & Rosmarinus Officinalis (Rosemary) Leaf Extract & Water

Generic name:

Chemical Family: Plant Extract

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

Substance	CAS Numbers	EC Numbers	Percentage
Hexylene Glycol	107-41-5	203-489-0	30.00%
Caprylyl Glycol	1117-86-8	214-254-7	15.00%
Origanum Vulgare Leaf Extract	84012-24-8	281-670-3	15.00%
Thymus Vulgaris (Thyme) Leaf Extract	84929-51-1	284-353-7	15.00%
Rosmarinus Officinalis (Rosemary) Leaf Extract	84604-14-8	283-291-9	15.00%
Water	7732-18-5	231-791-2	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 cool, dry place. This product should be stored at room temperature (23 - 25°C). It should not be exposed to excessive heat or cold. Do not freeze.
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 hazy liquid – product may settle upon standing
Color:	Amber to brown
Odor:	Characteristic
Specific Gravity (25°C):	0.970 – 1.020
Refractive Index (25°C):	1.3960 – 1.4040
Heavy Metals:	< 20 ppm
Arsenic:	< 2 ppm
Minimum Inhibitory Concentration Organism (ATCC#):	
E. coli (#8739):	0.25 – 2.00%
S. aureus (#6538):	0.25 – 1.00%
P. aeruginosa (#9027):	0.25 – 1.00%
C. albicans (#10231):	0.25 – 2.00%
A. brasiliensis (#16404):	0.25 – 2.00%
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: EC50 (Acute Daphnia): 931.8 mg/L - Not harmful to aquatic organisms

Sensitization: Non-Primary Sensitizer (In-Vitro Skin Sensitization Report & Direct Peptide Reactivity Assay)

Repeated dose toxicity: No known effects

Subacute to chronic toxicity: Not Determined

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
-Mutagenicity/genotoxicity

SECTION 12. ECOLOGICAL INFORMATION

Ecotoxicity
Effects on the aquatic environment: Not Determined

Biodegradability:
Persistence: Readily Biodegradable

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:	Hexylene Glycol:	203-489-0
	Caprylyl Glycol:	214-254-7
	Origanum Vulgare Leaf Extract:	281-670-3
	Thymus Vulgaris Leaf Extract:	284-535-7
	Rosmarinus Officinalis Leaf Extract:	283-291-9
	Aqua:	231-791-2
TSCA inventory status:	Exempt	
AICS inventory status:	107-41-5 & 1117-86-8 & 84012-24-8 & 84929-51-1 & 84604-14-8 & 7732-18-5	
Canadian (CEPA DSL) inventory status:	Exempt: Origanum Vulgare Leaf Extract (84012-24-8) & Thymus Vulgaris (Thyme) Leaf Extract (84929-51-1) Listed as 2,4-Pentanediol, 2-methyl- (DSL) & Octane-1,2-diol (DSL) & Rosemary, ext. (DSL) & Water (DSL)	
Japan (MITI list):	Hexylene Glycol & Caprylyl Glycol & Origanum Vulgare Leaf Extract & Thymus Vulgaris (Thyme) Leaf Extract & Rosmarinus Officinalis (Rosemary) Leaf Extract & Water	
Korea:	Hexylene Glycol & Caprylyl Glycol & Origanum Vulgare Leaf Extract & Thymus Vulgaris (Thyme) Leaf Extract & Rosmarinus Officinalis (Rosemary) Leaf Extract & Water	
China inventory status:	Hexylene Glycol & Caprylyl Glycol & Origanum Vulgare Leaf Extract & Thymus Vulgaris (Thyme) Leaf Extract & Rosmarinus Officinalis (Rosemary) Leaf Extract & Water	
Philippines inventory status:	Exempt: Origanum Vulgare Leaf Extract (84012-24-8) Not Listed: Caprylyl Glycol (1117-86-8) Listed as 2,4-Pentanediol, 2-methyl- & Thyme, Thymus vulgaris, ext. & Rosemary, ext. & Water	

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:	08/13/2015
Preparation Date:	05/11/2016
MSDS summary of changes	- Corrected Tradename – Section 1 (Identification) & Added Precautionary Statements – Section 2 (Hazards Identification) - Added Heavy Metals & Arsenic – Section 9 (Physical & Chemical Properties) & Updated Transport Information – Section 14 (Transport Information) - Added Acute Toxicity Data & Sensitization Data – Section 1 (Toxicological Information) & Added Biodegradability Data – Section 12 (Ecological Information) - Added Minimum Inhibitory Concentration – Section 9 (Physical & Chemical Properties)

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.

SynerCide Herbal Fusion Manufacturing Flow Chart

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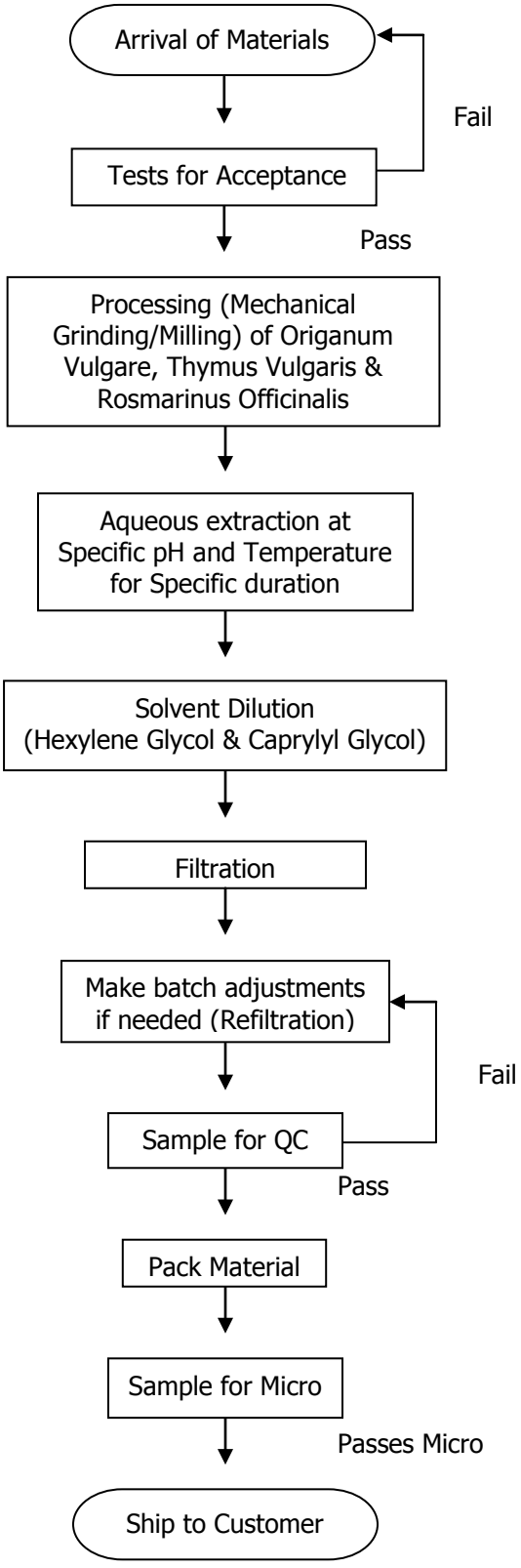

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SynerCide Herbal Fusion
Certificate of Compliance

Code: M17002
INCI Name: Hexylene Glycol & Caprylyl Glycol & Origanum Vulgare Leaf Extract & Thymus Vulgaris (Thyme) Leaf Extract & Rosmarinus Officinalis (Rosemary) Leaf Extract & Water
INCI Status: Approved
CAS #: 107-41-5 & 1117-86-8 & 84012-24-8 & 84929-51-1 & 84604-14-8 & 7732-18-5
EINECS #: 203-489-0 & 214-254-7 & 281-670-3 & 284-535-7 & 283-291-9 & 231-791-2

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 (REACH)	Compliant
USA (TSCA)	Exempt
Australia (AICS)	Compliant
Japan (METI)	Compliant
Canada (DSL)	Compliant
China (IECSC)	Compliant
Brazil	Compliant
Korea (KECI)	Compliant
Philippines (PICCS)	Contact Us

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SynerCide Herbal Fusion
Code: M17002

Attention must be paid to the use of SynerCide Herbal Fusion 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.

SynerCide Herbal Fusion and its components and 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 SynerCide Herbal Fusion is 1.00 – 2.00%.

SynerCide Herbal Fusion 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.

SynerCide Herbal Fusion 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.

(*) 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 SynerCide Herbal Fusion does not contain any materials prohibited by Halal laws.

SynerCide Herbal Fusion is REACH Compliant and free of the following:

- Formaldehyde or formaldehyde donors
- Gluten
- Lactose
- Nanoparticles
- Nitrosamines
- Palm oil/palm kernel oil (or derivatives)
- Parabens
- Paraffin
- Phthalates
- Residual solvents
- Sulfates
- Volatile organic compounds

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Raw Component Regulations

Please note that the below are global regulations for the raw materials used to manufacture SynerCide Herbal Fusion and are not for the product itself.

SynerCide Herbal Fusion contains 30.00% Hexylene Glycol. See below for a list of regulations:

Hexylene Glycol:

- **USA: Safe up to 6.00%**
  *Journal Citation: JACT 4(5):223-48, 1985 confirmed 02/04 IJT 25(S2), 2006