Arbor Organic Technologies

Organic Emulsifier Blend 60

Product Number: A60014
INCI Name: Oryza Sativa (Rice) Bran Extract & Cyamopsis Tetragonoloba (Guar) Gum

Technical Dossier

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Table of Contents

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Technical Data Sheet

Arbor Organic Technologies

Code Number: A60014

INCI Name: Oryza Sativa (Rice) Bran Extract & Cyamopsis Tetragonoloba (Guar) Gum
INCI Status: Conforms
REACH Status: Complies
CAS Number: 90106-37-9 & 9000-30-0
EINECS Number: N/A & 232-536-8

Origin: Botanical
Processing:
GMO Free
No Ethoxylation
No Irradiation
No Sulphonation
Additives:
Preservatives: None
Antioxidants: None
Other additives: None
Solvents Used: N/A
Appearance: Light Beige to Tan Free Flowing Powder
Soluble/ Miscible: Water Dispersible
Microbial Count: <100 CFU/g, No Pathogens

Suggested Use Levels: 1.0 - 4.0%
Suggested Applications:
Primary Emulsification, Improves Sensory Attributes, Pigment Dispersion

Background

Today's consumer is concerned about synthetic ingredients in cosmetic products they use every day. Due to growing consumer awareness of sustainability and vegan lifestyle, all natural and plant-based products are on demand, while most functional ingredients in cosmetic formulations are synthetically derived. At Arbor Organic Technologies, we have developed a USDA-NOP certified organic emulsifier to deliver the solution from nature to the personal care industry. **Organic Emulsifier Blend 60** combines the natural emulsifying properties of rice bran with guar gum to stabilize emulsions for elegant formulations.

Traditionally, organic emulsifiers lack the ability to deliver elegant formulations. Due to the fact that emulsification is a natural process, nature has the solution for cosmetic formulators. Nature produces its own emulsifiers, which allow both oil and water-soluble biomolecules to coexist as complex systems in plants. Specifically, the combination of organic rice bran and guar gum form a natural emulsifier ideal for oil-in-water systems. **Organic Emulsifier Blend 60** is developed through a proprietary manufacturing process to bring elegance in organic formulations.

Science

Rice bran is a byproduct of rice milling, and is rich in protein, carbohydrates, and fatty acid content. Rice bran naturally contains lipase, an enzyme that hydrolyzes lipids into free fatty acids (FFA) leading to rancidity in final products.[1] If rice bran is stabilized, immediately after milling, rancidity is prevented. Some of the common methods used for lipase deactivation in rice bran are high temperature treatments and chemical processing. These methods are known to improve the shelf life of rice bran products but also they can significantly lower the nutrient content and purity of ingredients.[2]

Arbor Organic Technologies has developed a proprietary method of three-dimensional particle assembly designed with stabilized, pure rice bran proteins and lipids combined in guar gum, which help create an exceptional certified organic emulsifier ideal for a variety of oil-in-water systems.

Benefits of **Organic Emulsifier Blend 60:**

- Stabilizes Emulsions
- Enhances Aesthetics of Formulations
- Great for Oil-in-Water Systems
- USDA-NOP Certified

This method combines only physical processes including wet extrusion and cryogenic milling to avoid the use of harsh chemicals or denaturing high temperatures preventing the loss of natural emulsifying properties of rice bran.

Organic rice bran is stabilized using a wet extrusion process, carried out in high moisture, a low-temperature system in the presence of amylase, an enzyme capable of digesting starches. The high moisture environment helps gelatinize carbohydrates of rice bran allowing the amylase enzymes to selectively digest the extra starch particles leaving out a high content of lipids and proteins. Then, protein and lipid content is determined prior to the addition of organic guar gum powder in the micro-milling process. Derived from *Cyamopsis tetragonolbles*, guar gum mainly functions in the water phase of an emulsion by naturally binding with water molecules to improve viscosity and texture of formulations. The cryogenic miller allows us to design the ideal microparticles composed of a balanced amount of dehydrated lipophilic and hydrophilic components necessary for stable emulsification. As a result, the three-dimensional design of rice bran and guar gum microparticles encourages a stable and balanced affinity to both oil and water molecules in systems with a 20% lipid load. Dehydrated components of microparticles hydrate immediately once mixed in oil and water phases of emulsions creating homogenous, smooth creams and lotions. **Organic Emulsifier Blend 60 i**s a USDA certified organic emulsifier composed of selectively assembled microparticles that exhibits a natural affinity to both oil and water molecules allowing formulators to create stable emulsions.

Benefits

The physical texture of a formula offers the immediate perception of a cosmetic product. A formulation that glides smoothly on the skin elicits positive emotions for the product. However, organic cosmetic regulations challenge formulators when creating organic finished products. Arbor Organic Technologies changes the perspectives on organic cosmetic formulations. Designed with the latest technology and quality ingredients, **Organic Emulsifier Blend 60** is able to establish purely luxurious, stable emulsions that deliver multifunctional benefits all in one. We combine the efficacy from nature with our green and clean technology. Forming an easy to use, all-in-one emulsifier that breaks the challenges in the organic cosmetic marketplace. **Organic Emulsifier Blend 60** is ideal for oil-in-water emulsions to help form a variety of textures involving gels, creams, and lotions. This product not only elevates the sensory properties of formulations but also adds pigment dispersion and moisturization benefits to organic cosmetic products.

Efficacy

Emulsion Study

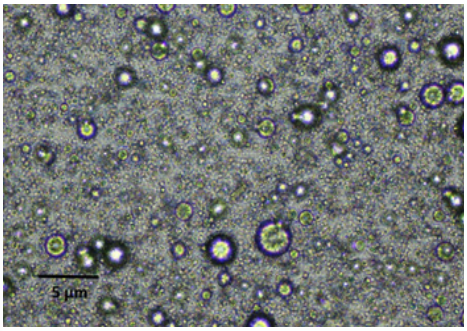

Figure 1. Emulsion with **Organic Emulsifier Blend 60** (magnification 400x)

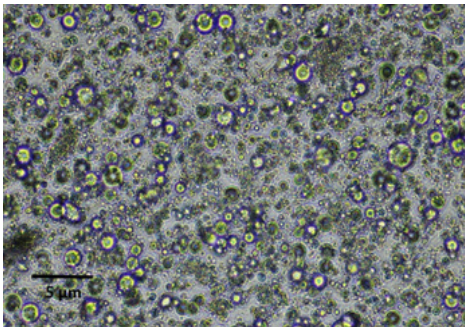

Figure 2. Emulsion with Rice Emulsifier and Guar Gum (magnification 400x)

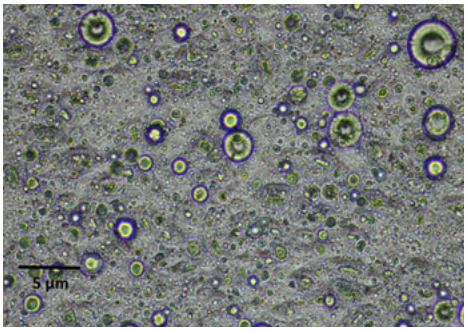

Figure 3. Emulsion with Cosmowax P (magnification 400x)

An emulsification study was performed to determine the ability of **Organic Emulsifier Blend 60** to form stable emulsions compared to a generic non-organic emulsifier and organic rice emulsifier blend with guar gum. Generally, a good emulsion lacks clumps or crystals and contains small and evenly dispersed micelles. Clumps, crystals, and agglomerates are signs of unstable emulsions that are prone to phase separation. As shown in Figure 1, the emulsion created with **Organic Emulsifier Blend 60** contains various small size micelles evenly dispersed throughout the emulsion. Figure 2, represents an emulsion made with a standard organic rice emulsifier and guar gum, which displays a good amount of small size micelles, however, crystals and agglomerates are also present.

In-Vivo Sensory Analysis

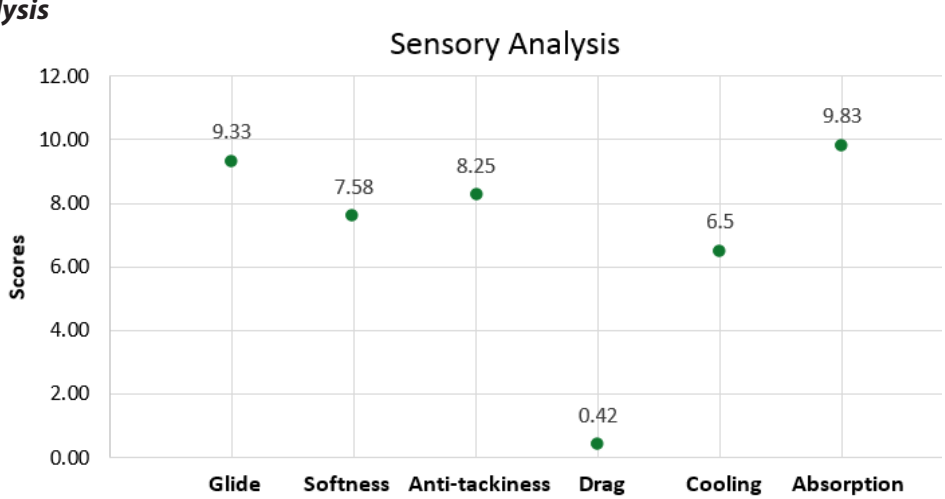

Figure 4. Average Skin Sensory Properties of 3% **Organic Emulsifier Blend 60** Lotion.

A sensory analysis study was performed with **Organic Emulsifier Blend 60** in an organic lotion to determine its physical properties in skin care applications. 12 panelists were asked to apply a small amount of lotion containing 3% **Organic Emulsifier Blend 60** onto the volar arm area. This analysis ranked the glide, cooling, and dragging properties on a scale of 0-10. After 60 seconds, the panelists assessed softness, anti-tackiness, and absorption. As shown by Figure 4, the average scores for immediate skin properties are 9.33, 6.5, and 0.42 for glide, cooling, and drag. 3% **Organic Emulsifier Blend 60** in the organic lotion delivered a gliding and cooling sensation with minimal to no drag when tested on the volar arms. After 60 seconds, average scores for softness, anti-tackiness, and absorption are 7.58, 8.25, and 9.83. These results show that lotion containing **Organic Emulsifier Blend 60** is able to absorb quickly while enhancing the softness and smoothness of the skin.

Compatibilities/Incompatibilities Data

Compatibilities of Organic Emulsifier Blend 60
Ethanol (up to 15%)
Glycols
Anionic/Amphoteric Surfactants
Electrolytes (monovalent and divalent)
Salicylates
Cationic Surfactants

Table 1. Compatibilities

As shown in Table 1, **Organic Emulsifier Blend 60** is compatible with ethanol, glycols, surfactants, electrolytes, salicylates, and even cationic surfactants. No incompatibilities concerning organic formulations have been determined.

References
1. Mian N. Riaz, et al. Comparison of Different Methods for Rice Bran Stabilization and Their Impact on Oil Extraction and Nutrient Destruction, Cereal Foods World, 2010 AACC International, Inc.
2. Jeon, H., Lee, I., Han, Y., Jeong, H., Park, H., Jung, J., & Rhee, J. (2017). Physicochemical Characteristics of Powder from Cryogenic Grinding of Aronia, Grapefruit, Black Bean, and Germinated Brown Rice. Microbiology and Biotechnology Letters,45(4), 291-298. doi:10.4014/mbl.1712.12014

Arbor Organic Technologies

www.arborорganictechnologies.com

SPECIFICATION

Product Name: Organic Emulsifier Blend 60
Code Number: A60014
CAS #'s: 90106-37-9 & 9000-30-0
EINECS #'s: N/A & 232-536-8
INCI Name: Oryza Sativa (Rice) Bran Extract(+) & Cyamopsis Tetragonoloba (Guar) Gum(+)
Status: Conforms

Certified as "Organic" as outlined by the USDA National Organic Program by Where Food Comes From Organic

Specification	Parameter
Appearance	Free Flowing Powder
Color	Light Beige to Tan
Odor	Characteristic
pH (5% in Water)	4.5 – 7.5
Loss on Drying (1g-1hr-105°C)	15.00% Maximum
Microbial Content Yeast & Mold Gram Negative Bacteria	< 200 CFU/g; No pathogens < 200 CFU/g 0 CFU/g

(+) Organic Plant Matter

- Product may not meet Microbiological specification after initial use.

Lot to lot variation may exist beyond our control due to seasonal weather and resulting crop variations in our supply of the natural plant materials.

Compositional Breakdown

Organic Emulsifier Blend 60
Code: A60014

Compositional Breakdown:

Ingredient	%
Oryza Sativa (Rice) Bran Extract	60.00
Cyamopsis Tetragonoloba (Guar) Gum	40.00

Arbor Organic Technologies hereby confirms that to the best of our knowledge, none of the potential 26 fragrance allergens listed below are present in our finished product or as an intentional component in the raw materials used to manufacture this product. We do not routinely analyze our product for the substances listed below:

ALLERGENS listed in Annex III of EU Cosmetic Regulation(EC) No. 1223/2009	
INCI NAME	CAS NUMBER
Alpha-Isomethyl Ionone	127-51-5
Amyl Cinnamal	122-40-7
Amylcinnamyl Alcohol	101-85-9
Anise Alcohol	105-13-5
Benzyl Alcohol	100-51-6
Benzyl Benzoate	120-51-4
Benzyl Cinnamate	103-41-3
Benzyl Salicylate	118-58-1
Butylphenyl Methylpropional	80-54-6
Cinnamal	104-55-2
Cinnamyl Alcohol	104-54-1
Citral	5392-40-5
Citronellol	106-22-9
Coumarin	91-64-5
Eugenol	97-53-0
Evernia Furfuracea (Treemoss) Extract	90028-67-4
Evernia Prunastri (Oakmoss) Extract	90028-68-5
Farnesol	4602-84-0
Geraniol	106-24-1
Hexyl Cinnamal	101-86-0
Hydroxycitronellal	107-75-5
Hydroxyisohexyl 3-Cyclohexene Carboxaldehyde (Lyral)	31906-04-4
Isoeugenol	97-54-1
Limonene (sum of d, l and dl)	5989-27-5
Linalool	78-70-6
Methyl 2-Octynoate	111-12-6

BACTERIAL REVERSE MUTALTION TEST (AMES)

Test Article: Organic Emulsifier Blend 60
Code Number: A60014
CAS #: 90106-37-9 & 9000-30-0

Sponsor:
Active Concepts, LLC
107 Technology Drive
Lincolnton, NC 28092

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

Test Performed:
Genotoxicity: Bacterial Reverse Mutation Test

Reference:
OECD471/ISO10993.Part 3

Test Request Number: 6967

SUMMARY

A *Salmonella typhimurium/Escherichia coli* reverse mutation standard plate incorporation study described by Ames *et al.* (1975) was conducted to evaluate whether a test article solution **Organic Emulsifier Blend 60** would cause mutagenic changes in the average number of reveratants for histidine-dependent *Salmonella typhimurium* strains TA98, TA100, TA1537, TA1535 and tryptophan-dependent *Escherichia coli* strain WP2*uvr*A in the presence and absence of Aroclor-induced rat liver S9. This study was conducted to satisfy, in part, the Genotoxicity requirement of the International Organization for Standardization: Biological Evaluation of Medical Devices, Part 3: Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity.

The stock test article was tested at eight doses levels along with appropriate vehicle control and positive controls with overnight cultures of tester strains. The test article solution was found to be noninhibitory to growth of tester strain TA98, TA100, TA1537, TA1535 and WP2*uvr*A after Sport Inhibition Screen.

Separate tubes containing 2 ml of molten top agar at 45^0C supplemented with histidine-biotin solution for the *Salmonella typhimurium* strains and supplemented with tryptophan for *Escherichia coli* strain were inoculated with 100 µl of tester strains, 100 µl of vehicle or test article dilution were added and 500 µl aliquot of S9 homogenate, simulating metabolic activation, was added when necessary. After vortexing, the mixture was poured across the Minimal Glucose Agar (GMA) plates. Parallel testing was also conducted with positive control correspond to each strain, replacing the test article aliquot with 50µl aliquot of appropriate positive control. After the overlay had solidified, the plates were inverted and incubated for 48 hours at 37^0C. The mean numbers of revertants of the test plates were compared to the mean number of revertants of the negative control plates for each of the strains tested. The means obtained for the positive controls were used as points of reference.

Under the conditions of this assay, the test article solution was considered to be Non-Mutagenic to *Salmonella typhimurium* tester strains TA98, TA100, TA1537, TA1535 and *Escherichia coli* tester strain WP2*uvr*A. The negative and positive controls performed as anticipated. The results of this study should be evaluated in conjunction with other required tests as listed in ISO 100993, Part 3: Tests for Genotoxicity, Carcinogenicity, and Reproductive Toxicology.

All *Salmonella* tester strain cultures demonstrated the presence of the deep rough mutation (*rfa*) and the deletion in the *uvr*B gene. Cultures of tester strains TA98 and TA100 demonstrated the presence of the Pkm101 plasmid R-factor. All WP2 *uvr*A cultures demonstrated the deletion in the *uvr*A gene. All cultures demonstrated the characteristic mean number of spontaneous revertants in the vehicle controls as follows: TA98, 10-50; TA100, 80-240; TA1535, 5-45; TA1537, 3-21, WP2*uvr*A, 10-60.

I. Introduction

A. **Purpose**
A *Salmonella typhimurium/Escherichia coli* reverse mutation standard plate incorporation study was conducted to evaluate whether a test article solution would cause mutagenic changes in the average number of revertants for *Salmonella typhimurium* tester strains TA98, TA100, TA1537, TA1535 and *Escherichia coli* WP2*uvr*A in the presence and absences of the S9 metabolic activation. Bacterial reverse mutation tests have been widely used as rapid screening procedures for the determination of mutagenic and potential carcinogenic hazards.

II. Materials

A. **Storage Conditions:** Room temperature (23-25C).
B. **Vehicle:** Sterile DI Water.
C. **Preparation:** Eight different doses level were prepared immediately before use with sterile DI water.
D. **Solubility/Stability:** 100% Soluble and Stable.
E. **Toxicity:** No significant inhibition was observed.

III. Test System

A. Test System
Each *Salmonella typhimurium* and *Escherichia coli* tester strain contains a specific deep rough mutation (*rfa)*, the deletion of *uvr*B gene and the deletion in the *uvr*A gene that increase their ability to detect mutagens, respectively. These genetically altered *Salmonella typhimurium* strains (TA98, TA100, TA1537 and TA1535) and *Escherichia coli* strain (WP2*uvr*A) cannot grow in the absence of histidine and tryptophan, respectively. When placed in a histidine-tryptophan free medium, only those cells which mutate spontaneously back to their wild type states are able to form colonies. The spontaneous mutation rate (or reversion rate) for any one strain is relatively constant, but if a mutagen is added to the test system, the mutation rate is significantly increased.

Tester strain	Mutations/Genotypic Relevance
TA98	hisD3052, Dgal chlD bio *uvr*B *rfa* pKM101
TA100	hisG46, Dgal chlD BIO *uvr*B *rfa* pKM101
TA1537	hisC3076, *rfa*, Dgal chlD bio *uvrB*
TA 1535	hisG46, Dgal chlD bio *uvr*B *rfa*
WP2*uvr*A	trpE, *uvr*A

rfa	=	causes partial loss of the lip polysaccharide wall which increases permeability of the cell to large molecules.
uvrB	=	deficient DNA excision-repair system (i.e., ultraviolet sensitivity)
pKM101	=	plasmid confers ampicillin resistance (R-factor) and enhances sensitivity to mutagens.
*uvr*A	=	All possible transitions and transversions, small deletions.

B. Metabolic Activation
Aroclor induced rat liver (S9) homogenate was used as metabolic activation. The S9 homogenate is prepared from male Sprague Dawley rats. Material is supplied by MOLTOX, Molecular Toxicology, Inc.

C. Preparation of Tester strains
Cultures of *Salmonella typhimurium* TA98, TA100,TA1537, TA1535 and *Escherichia coli* WP2uvrA were inoculated to individual flasks containing Oxoid broth No.2. The inoculated broth cultures were incubated at 37°C in an incubator shaker operating at 140-150 rpm for 12-16 hours.

D. Negative Control
Sterile DI water (vehicle without test material) was tested with each tester strain to determine the spontaneous reversion rate. Each strain was tested with and without S9 activation. These data represented a base rate to which the number of reveratants colonies that developed in each test plate were compared to determine whether the test material had significant mutagenic properties.

E. Positive Control
A known mutagen for each strain was used as a positive control to demonstrate that tester strains were sensitive to mutation to the wild type state. The positive controls are tested with and without the presence of S9 homogenate.

F. Titer of the Strain Cultures:
Fresh cultures of bacteria were grown up to the late exponential or early stationary phase of growth; to confirm this, serial dilutions from each strain were conducted, indicating that the initial population was in the range of 1 to $2x10^9$/ml.

IV. Method

A. Standard Plate Incorporation Assay:
Separate tubes containing 2 ml of molten top agar supplemented with histidine-biotin solution for the *Salmonella typhimurium* and tryptophan for *Escherichia coli* were inoculated with 100 µl of culture for each strain and 100 µl of testing solution or vehicle without test material. A 500 µl aliquot of S9 homogenate, simulating metabolic activation, was added when necessary. The mixture was poured across Minimal Glucose Agar plates labeled with strain number and S9 activation (+/-). When plating the positive controls, the test article aliquot was replaced by 50µl aliquot of appropriate positive control. The test was conducted per duplicate. The plates were incubated for 37°C for 2 days. Following the incubation period, the revertant colonies on each plate were recorded. The mean number of reveratnts was determined. The mean numbers of revertants of the test plates were compared to the mean number of reverants of the negative control of each strain used.

V. Criteria for a Valid Test

For the test solution to be evaluated as a test failure or “potential mutagen” there must have been a 2-fold or greater increase in the number of mean revertants over the means obtained from the negative control for any or all strains. Each positive control mean must have exhibited at least a 3-fold increase over the respective negative control mean of the *Salmonella* and *Escherichia coli* tester strains used.

All *Salmonella* tester strain cultures must demonstrate the presence of the deep rough mutation (*rfa*) and the deletion in the *uvr*B gene. Cultures of tester strains TA98 and TA100 must demonstrate the presence of the pKM101 plasmid R-factor. All WP2 *uvr*A cultures must demonstrate the deletion in the *uvr*A gene. All cultures must demonstrate the characteristic mean number of spontaneous revertants in the vehicle controls as follows: TA98, 10-50; TA100, 80-240; TA1535, 5-45; TA1537, 3-21, WP2*uvr*A, 10-60. To ensure that appropriate numbers of bacteria are plated, tester strain culture titers must be greater than or equal to $0.3x10^9$ cells/ml. The mean of each positive control must exhibit at least 3.0-fold increase in the number of revertants over the mean value of the respective vehicle control. A minimum of three non-toxicdose levels is required to evaluate assay data. A dose level is considered toxic if one of both of the following criteria are met: (1). A >50% reduction in the mean number of revertants per plate as compared to the mean vehicle control value. This reduction must be accompanied by an abrupt dose-dependent drop in the revertant count. (2). At least a moderate reduction in the background lawn.

VI. Results and Discussion

A. Solubility:
Water was used as a solvent. Solutions from the test article were made from 0.015 to 50mg/ml.

B. Dose levels tested:
The maximum dose tested was 5000 µg per plate. The dose levels tested were 1.5, 5.0, 15, 50, 150, 500, 1500 and 5000 µg per plate.

C. Titer (Organisms/ml):
5×10^8 UFC/ml plate count indicates that the initial population was in the range of 1 to 2×10^9 UFC/ml.

D. Standard Plate Incorporation Assay

In no case was there a 2-fold or greater increase in the mean number of revertant testing strains TA98, TA100, TA1537, TA1535 and WP2*uvr*A in the presence of the test solution compared with the mean of vehicle control value. The positive controls mean exhibited at least a 3-fold increase over the respective mean of the *Salmonella typhimurium* and *Escherichia coli* tester strains used. The results are summarized in Appendix 2.

All *Salmonella* tester strain cultures demonstrated the presence of the deep rough mutation (*rfa*) and the deletion in the *uvr*B gene. Cultures of tester strains TA98 and TA100 demonstrated the presence of the Pkm101 plasmid R-factor. All WP2 *uvr*A cultures demonstrated the deletion in the *uvr*A gene. All cultures demonstrated the characteristic mean number of spontaneous revertants in the vehicle controls as follows: TA98, 10-50; TA100, 80-240; TA1535, 5-45; TA1537, 3-21, WP2*uvr*A, 10-60.

VII. Conclusion

All criteria for a valid study were mete as described in the protocol. The results of the Bacterial Reverse Mutation Assay indicate that under the conditions of this assay, the test article solution was considered to be Non-Mutagenic to *Salmonella typhimurium* tester strains TA98, TA100, TA1537, TA1535 and *Escherichia coli* WP2*uvr*A.The negative and positive controls performed as anticipated. The results of this study should be evaluated in conjunction with other required tests as listed in ISO 100993, Part 3: Tests for Genotoxicity, Carcinogenicity, and Reproductive Toxicology.

Appendix 2:

Bacterial Mutation Assay
Plate Incorporation Assay Results

	Concentration µg per Plate	*TA98*		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	28	25	27
	1500	23	21	22
	500	33	32	33
	150	28	20	24
	50	22	24	23
	15	27	29	28
	5.0	20	23	22
	1.5	14	16	15
Test Solution w/o S9	5000	20	21	21
	1500	33	33	33
	500	37	36	37
	150	28	29	29
	50	31	30	31
	15	23	22	23
	5.0	24	26	25
	1.5	19	23	21
DI Water w/S9		20	35	28
DI Water w/o S9		32	36	34
2-aminoanthracen w/ S9		375	382	379
2-nitrofluorene w/o S9		229	261	245
Historical Count Positive w/S9				
Historical Count Positive w/o S9				
Historical Count Negative w/S9				
Historical Count Negative w/o S9				

*CFU = Colony Forming Units
*Mean = Average of duplicate plates

	Concentration µg per Plate	***TA100***		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	105	102	104
	1500	111	114	113
	500	110	122	116
	150	109	103	106
	50	103	108	106
	15	122	123	123
	5.0	135	132	134
	1.5	124	123	124
Test Solution w/o S9	5000	133	130	128
	1500	129	126	128
	500	100	120	110
	150	102	110	106
	50	112	132	122
	15	123	125	124
	5.0	102	112	107
	1.5	132	110	121
DI Water w/S9		188	165	177
DI Water w/o S9		133	145	139
2-aminoanthracen w/ S9		482	432	457
Sodium azide w/o S9		410	454	432
Historical Count Positive w/S9		**224-3206**		
Historical Count Positive w/o S9		**226-1837**		
Historical Count Negative w/S9		**55-268**		
Historical Count Negative w/o S9		**47-250**		

*CFU = Colony Forming Units
*Mean = Average of duplicate plates

	Concentration µg per Plate	*TA1537*		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	10	11	11
	1500	12	16	14
	500	17	16	17
	150	18	13	16
	50	17	17	17
	15	16	18	17
	5.0	12	13	13
	1.5	10	11	11
Test Solution w/o S9	5000	16	15	16
	1500	10	11	11
	500	12	15	14
	150	13	12	13
	50	19	18	19
	15	16	17	17
	5.0	14	11	13
	1.5	13	13	13
DI Water w/S9		10	12	11
DI Water w/o S9		15	13	14
2-aminoanthracen w/ S9		362	388	375
2-aminoacridine w/o S9		325	310	318
Historical Count Positive w/S9		**13-1934**		
Historical Count Positive w/o S9		**17-4814**		
Historical Count Negative w/S9		**0-41**		
Historical Count Negative w/o S9		**0-29**		

*CFU = Colony Forming Units
*Mean = Average of duplicate plates

	Concentration µg per Plate	***TA1535***		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	15	14	15
	1500	22	23	23
	500	25	26	26
	150	21	25	23
	50	23	20	22
	15	26	28	27
	5.0	20	21	21
	1.5	22	24	23
Test Solution w/o S9	5000	23	26	25
	1500	28	29	29
	500	31	33	32
	150	20	19	20
	50	22	23	23
	15	25	24	25
	5.0	22	23	23
	1.5	27	23	25
DI Water w/S9		21	22	22
DI Water w/o S9		29	31	30
2-aminoanthracen w/ S9		283	222	253
Sodium azide w/o S9		475	463	469
Historical Count Positive w/S9		**22-1216**		
Historical Count Positive w/o S9		**47-1409**		
Historical Count Negative w/S9		**1-50**		
Historical Count Negative w/o S9		**1-45**		

*CFU = Colony Forming Units
*Mean = Average of duplicate plates

	Concentration µg per Plate	*WP2uvrA*		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	22	23	23
	1500	20	21	21
	500	17	19	18
	150	22	25	24
	50	35	33	34
	15	28	25	27
	5.0	21	23	22
	1.5	30	33	32
Test Solution w/o S9	5000	44	41	43
	1500	46	43	45
	500	40	35	38
	150	32	33	33
	50	31	34	33
	15	39	37	38
	5.0	34	35	35
	1.5	32	33	33
DI Water w/S9		50	41	41
DI Water w/o S9		52	57	55
2-aminoanthracen w/ S9		492	475	484
Methylmethanesulfonate w/o S9		411	405	408
Historical Count Positive w/S9		**44-1118**		
Historical Count Positive w/o S9		**42-1796**		
Historical Count Negative w/S9		**8-80**		
Historical Count Negative w/o S9		**8-84**		

*CFU = Colony Forming Units
*Mean = Average of duplicate plates

Arbor Organic Technologies

Dermal and Ocular Irritation Tests

107 Technology Drive, Lincolnton, NC 28092 • info@arbor-organics.com
Phone: +1-704-276-7100 • Fax: +1-704-276-7101

Sample: Organic Emulsifier Blend 60

Code: A60014

CAS #: 90106-37-9 & 9000-30-0

Test Request Form/Submission #: 4948

Lot #: NC190121-E

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

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

SUMMARY

In vitro dermal and ocular irritation studies were conducted to evaluate whether **Organic Emulsifier Blend 60** 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 **non-irritating**. 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.

Arbor Organic Technologies

Dermal and Ocular Irritation Tests

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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.

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.

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

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.

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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.

B. Tissue Viability Assay

The results are summarized in Figure 1. 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.

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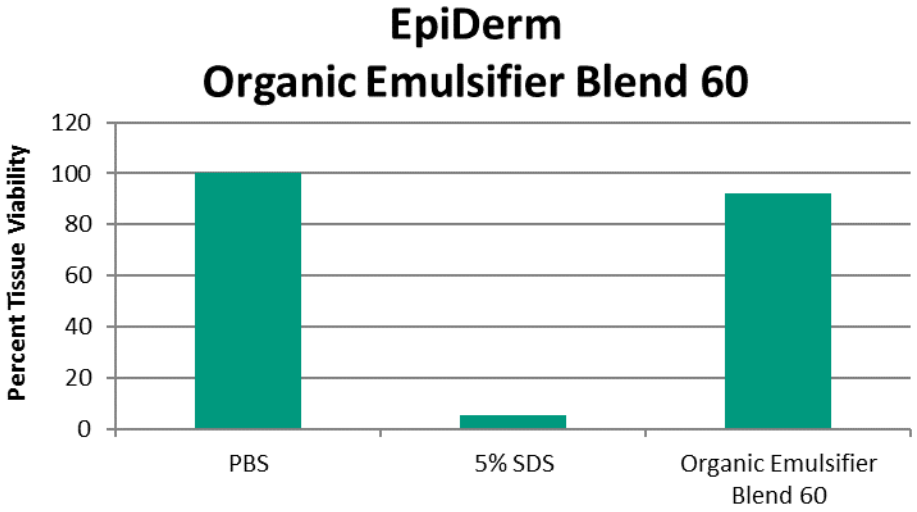


Figure 1: EpiDerm tissue viability

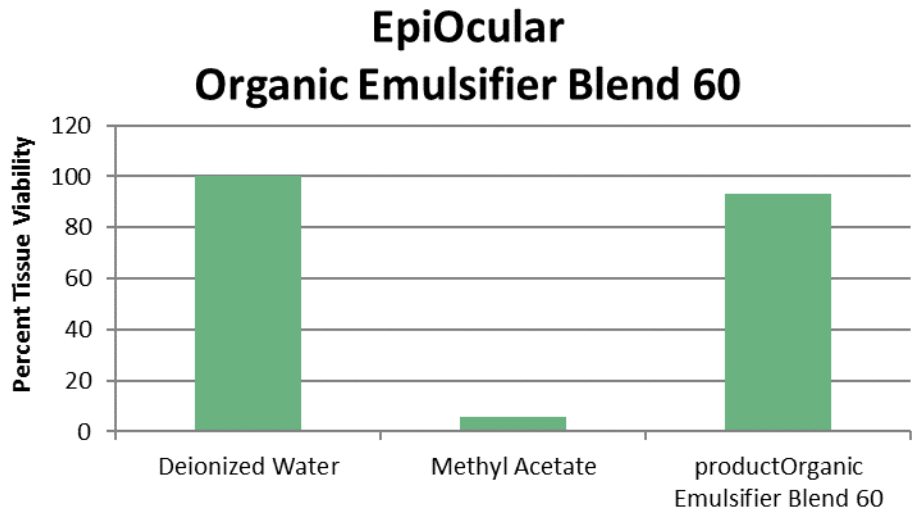


Figure 2: EpiOcular tissue viability

OECD 201 Freshwater Alga Growth Inhibition Test

Arbor Organic Technologies www.arborganictechnologies.com

Tradename: Organic Emulsifier Blend 60

Code: A60014

CAS #: 90106-37-9 & 9000-30-0

Test Request Form #: 6975

Lot #: S200102E

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

Test Performed:
OECD 201
Freshwater Alga Growth Inhibition Test

Introduction

The purpose of the present study is to determine the toxicity of **Organic Emulsifier Blend 60** by exposing the exponentially growing test organism *Pseudokirchneriella subcapitata* to the test substance for 72 hours and measuring the growth and growth inhibition through cell counting against the control. The response is evaluated as a function of the exposure concentration in comparison with the average growth of replicate, unexposed control cultures.

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

Assay Principle

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

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

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

Materials

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

Methods

Test Conditions

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

Measurement of Test Substance Concentrations

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

Observation

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

Test Condition MeasurementsMeasure 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.

Data and Reporting

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

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

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

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

Results

General Information:

Name of new chemical substance	Organic Emulsifier Blend 60		
INCI Nomenclature	Oryza Sativa (Rice) Bran Extract(+) & Cyamopsis Tetragonoloba (Guar) Gum(+)		
CAS number	90106-37-9 & 9000-30-0		
Formulation Method	Extraction		
Molecular weight	573.54 Da		
Purity of the new chemical substance used for the test (%)	100%		
Lot number of the new chemical substance used for the test	S200102E		
Names and contents of impurities	N/A		
Solubility in water	Water Dispersible		
Properties at room temperature	Free Flowing Light Beige to Tan Powder, Characteristic Odor		
Stability	Stable Under Normal Conditions		
Solubility in solvents, etc.	**Solvent**	**Solubility**	**Stability in solvent**
	N/A	N/A	N/A

OECD 201 Freshwater Alga Growth Inhibition Test

Test Materials and Methods:

Items			Contents
Test Organisms	Species		*Pseudokirchneriella subcapitata*
	Source		ATCC
	Reference substance (EC_{50})		3,5-dichlorophenol
Culture	Kind of Medium		Gorham's Medium for Algae
	Conditions (Temperature)		22°C ± 2°C
Test Conditions	Test Vessel		Glass
	Material Water	Kind	Deionized
		Hardness	250 mg/L
		pH	7.4
	Date of Exposure		02/03/2020
	Test Concentrations		200, 89.4, 42.3, 19.2, 7.8 mg/L
	Number of organisms		5 x 103-4 cells/ml
	Number of Replicates	Exposure Group	4
		Control Group	4
	Test Solution Volume		5 mL
	Vehicle	Use or Not	N/A
		Kind	N/A
		Concentration	N/A
		Number of Replicates	N/A
	Photoperiod		Continuous

Test Results:

Items		Contents
Toxicity Value	Percent Inhibition EC_{10} and EC_{20}	123.99 mg/L and 198.41 mg/L
Exposure Concentrations Used for Calculation	Nominal Values	200, 89.4, 42.3, 19.2, 7.8 mg/L
Remarks		Not harmful to aquatic organisms

Discussion

After 72 hours, the percent inhibition for **Organic Emulsifier Blend 60** was determined to be 123.99 mg/L EC_{10} and 198.41 mg/L EC_{20}. The conditions of OECD guideline 201 for the validity of the test were adhered to, this product is not classified and therefore not harmful to aquatic organisms.

OECD 301B Ready Biodegradability Assay

Arbor Organic Technologies

www.arborорganictechnologies.com

Tradename: Organic Emulsifier Blend 60

Code: A60014

CAS #: 90106-37-9 & 9000-30-0

Test Request Form #: 6979

Lot #: S200102E

Sponsor: *Arbor Organic Technologies, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Danaher*
Principle Investigator: *Michael Hovis*

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

Introduction

A study was conducted to assess the ready biodegradability of **Organic Emulsifier Blend 60** 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.

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
- 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)

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

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

Data and Reporting

I. Treatment of Results

a. Data from the test should be entered onto the attached data sheet.

b. The amount of CO_2 produced is calculated from the amount of base remaining in the absorption bottle. When 0.0125M $Ba(OH)_2$ is used as the absorbent, the amount remaining is assessed by titrating with 0.05M HCl.

c. Since 1 mmol of CO_2 is produced for every mol of $Ba(OH)_2$ reacted to $BaCl_2$ and 2 mmol of HCl are needed for the titration of the remaining $Ba(OH)_2$ and given that the molecular weight of CO_2 is 44 g, the weight of CO_2 produced (in mg) is calculated by:

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

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

d. The percentage biodegradation is calculated from:

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

Or

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

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

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

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

f. Display the course of degradation graphically and indicate the 10-day window. Calculate and report the percentage removal achieved at the plateau, at the end of the test, and/or at the end of the 10-day window, whichever is appropriate.

g. When appropriate, calculate DOC removals using the equation given in 301 A paragraph 27.

h. When an abiotic control is used, calculate the percentage abiotic degradation by:

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

Validity of Tests

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

Data Sheet

Laboratory	Active Concepts Tissue Culture Laboratory		
Test Start Date	01/27/2020		
Test Substance	**Name**	Organic Emulsifier Blend 60	
	Stock Solution Concentration	2 g/L	
	Initial Concentration in Medium	20 mg/L	
Inoculum	**Source**	Activated Sludge	
	Treatment Given	Centrifugation	
	Pre-conditioning	N/A	
	Suspended Solids Concentration in Reaction Mixture	4 mg/L	
Reference Material	Sodium Benzoate	**Concentration**	20 mg/L
CO_2 Production and Degradability	**Method**	**$Ba(OH)_2$**	0.0125M
		NaOH	N/A
		Other	N/A
Total Contact Time	28 Days		
Total CO_2 Evolved Measurements	**Days**	2, 4, 11, 17, 23, 28	
Degradation Over Time	87.4% and 89.1% 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, **Organic Emulsifier Blend 60** achieved 88.3% biodegradation after 28 days of testing. The product met method requirements for the Readily Biodegradable classification.

Tradename: Organic Emulsifier Blend 60

Code: A60014

CAS #: 90106-37-9 & 9000-30-0

Test Request Form #: 6980

Lot #: S200102E

Sponsor: *Arbor Organic Technologies, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Danaher*
Principle Investigator: *Michael Hovis*

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 **Organic Emulsifier Blend 60** 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.

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* **Organic Emulsifier Blend 60** in Acetonitrile

  *For mixtures and multi-constituent substances of known composition such as **Organic Emulsifier Blend 60,** a single purity should be determined by the sum of the proportion of its constituents (excluding water), and a single apparent molecular weight determined by considering the individual molecular weights of each component in the mixture (excluding water) and their individual proportions. The resulting purity and apparent molecular weight can then be used to calculate the weight of test chemical necessary to prepare a 100 mM solution.

Reference Controls:

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

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

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

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

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.

Prediction Model:

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

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

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

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

Cysteine 1:10/Lysine 1:50 Prediction Model		
Mean of Cysteine and Lysine % Depletion	**Reactivity Class**	**Prediction**
2.95	Minimal Reactivity	Non-sensitizer
2.99	Minimal Reactivity	Non-sensitizer
3.01	Minimal Reactivity	Non-sensitizer

Cysteine 1:10 Prediction Model		
Mean of Cysteine and Lysine % Depletion	**Reactivity Class**	**Prediction**
3.00	Minimal Reactivity	Non-sensitizer
3.02	Minimal Reactivity	Non-sensitizer
3.05	Minimal Reactivity	Non-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 **Organic Emulsifier Blend 60 (A60014)** we can determine this product is not classified as a sensitizer and is not predicted to cause allergic contact dermatitis. The Mean Percent Depletion of Cysteine and Lysine was 3.00% causing minimal reactivity in the assay giving us the prediction of a non-sensitizer.

Tradename: Organic Emulsifier Blend 60

Code: A60014

CAS #: 90106-37-9 & 9000-30-0

Test Request Form #: 6976

Lot #: S200102E

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

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

Introduction

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

This assay was conducted to determine skin sensitization potential of **Organic Emulsifier Blend 60** 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

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 **Organic Emulsifier Blend 60** 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 – 64 µM. 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.

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	31.88
DMSO	Non-Sensitizer	No Induction	243.24 µM	0.19
Organic Emulsifier Blend 60	Non-Sensitizer	No Induction	> 1000 µM	0.38

Table 1: Overview of KeratinoSens™ Assay Results (I_{max} equals the average induction values Fg.1)

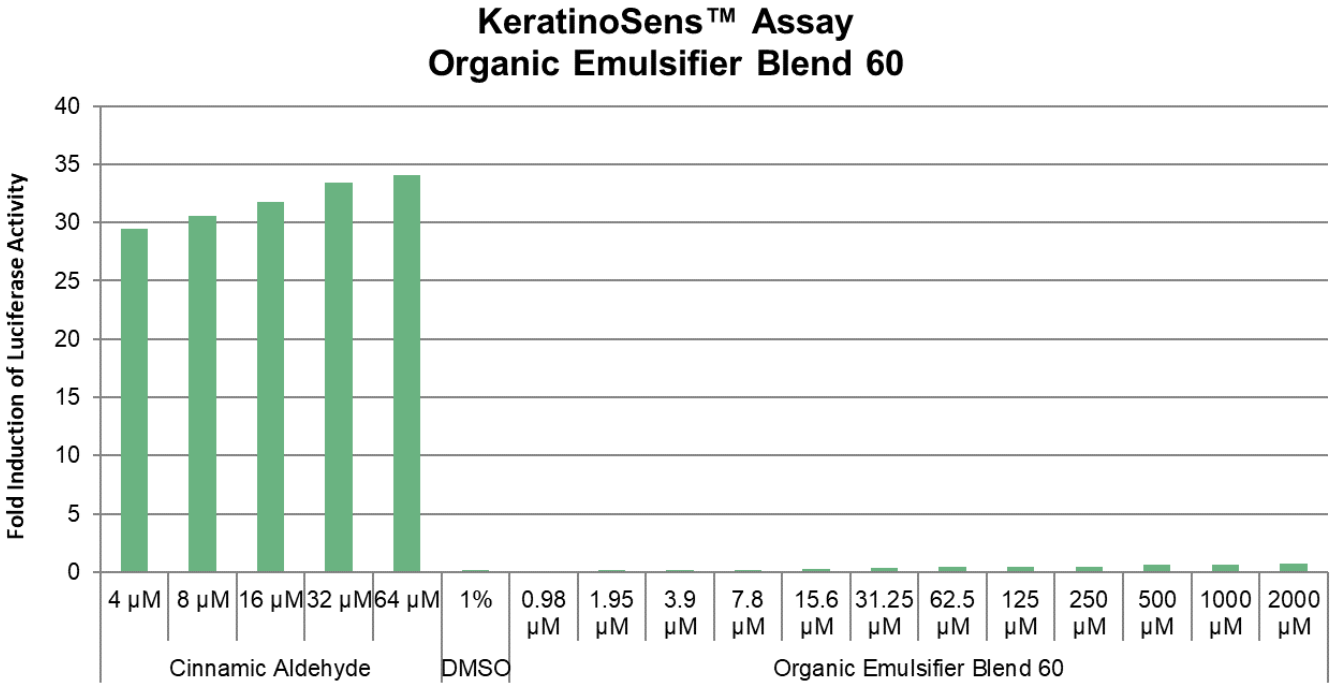


Figure 1: Fold Induction of Luciferase

Discussion

As shown in the results, **Organic Emulsifier Blend 60 (A60014)** 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 **Organic Emulsifier Blend 60** can be safely used in cosmetics and personal care products at typical use levels.

Phototoxicity Assay Analysis

Arbor Organic Technologies

www.arborganictechnologies.com

Tradename: Organic Emulsifier Blend 60

Code: A60014

CAS #: 90106-37-9 & 9000-30-0

Test Request Form #: 6977

Lot #: S200102E

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

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

SUMMARY

In vitro phototoxicity irritation studies were conducted to evaluate whether **Organic Emulsifier Blend 60** 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%, 1.5%, 5.0% and 10.0%. Reconstructed human epidermis was incubated in growth media for one hour to allow for tissue equilibration after shipping from MatTek Corporation, Ashland, MA. Test substance was applied to the tissue inserts in four varying concentrations and incubated overnight at 37°C, 5% CO_2, and 95% relative humidity (RH). The following day, the appropriate tissue inserts were irradiated (UVA) for 60 minutes with 1.7 mW/cm^2 (=6 J/cm^2). After substance incubation, irradiation, and washing was completed, the cell viability test was conducted. Cell viability was measured by dehydrogenase conversion of MTT [(*3-4,5-dimethyl thiazole 2-yl*)], present in the cell mitochondria, into blue formazan salt that was measured after extraction from the tissue. The photoirritation potential of the test chemical was dictated by the reduction in tissue viability of UVA exposed tissues compared to non-UVA exposed tissues.

Under the conditions of this assay, the test article was considered to be non-phototoxic at concentrations of 0.5%, 1.5%, 5.0%, and 10.0%. The negative and positive controls performed as anticipated.

Phototoxicity Assay Analysis

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.

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 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 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).

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%.

Phototoxicity Assay Analysis

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%, 1.5%, and 5.0%. The negative control mean exhibited acceptable relative tissue viability while the positive control exhibited dose dependent loss of tissue viability and cell death.

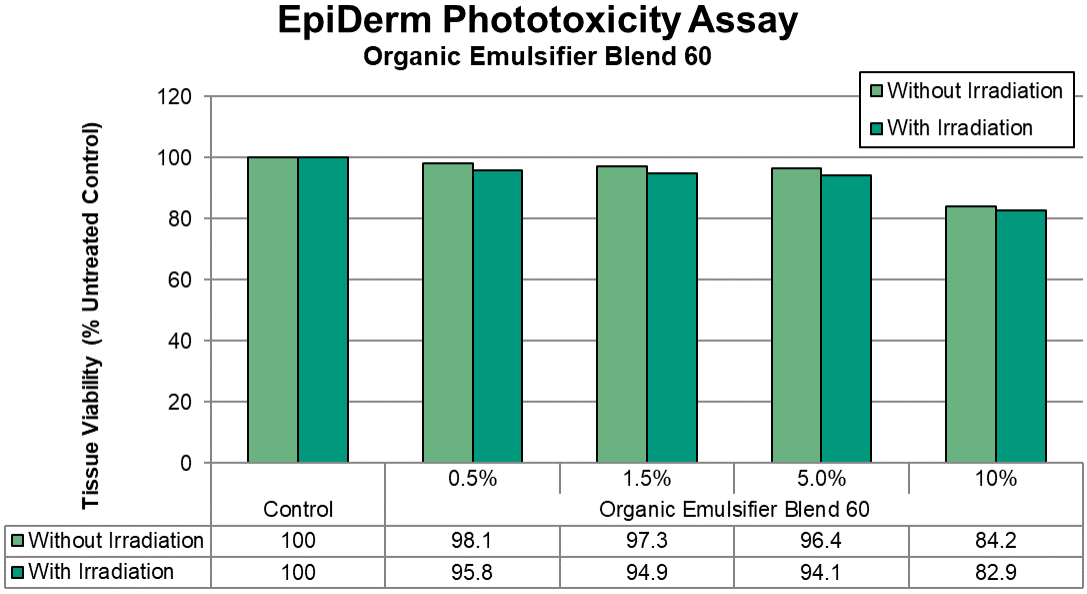
C. Test Validity

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

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%, 1.5%, 5.0%, and 10.0%.The negative and positive controls performed as anticipated.

There is a slight decrease in viability at the 10% concentration but viability does not decrease more than the acceptable 20%. We can safely say that **Organic Emulsifier Blend 60** is not a photoirritant when used at the suggested use levels of 1.0% - 4.0%.



	Control	0.5%	1.5%	5.0%	10%
Without Irradiation	100	98.1	97.3	96.4	84.2
With Irradiation	100	95.8	94.9	94.1	82.9

Figure 1: EpiDerm Phototoxicity Graph

Arbor Organic Technologies

www.arborорganictechnologies.com

Certificate of Origin

Organic Emulsifier Blend 60
Code: A60014

Certified as "Organic" as outlined by the USDA National Organic Program by Where Food Comes From Organic

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

Arbor Organic 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.

Arbor Organic Technologies, LLC. certifies the below sources for each item listed in our INCI Name:

INCI Name	Source
Oryza Sativa (Rice) Bran Extract	Plant (*Oryza sativa*)
Cyamopsis Tetragonoloba (Guar) Gum	Plant (*Cyamopsis tetragonoloba*)

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

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

Arbor Organic Technologies, LLC. certifies that the above listed ingredient is manufactured to adhere to the USDA National Organic Program's guidelines and therefore is free of residual pesticides and heavy metals.

Arbor Organic Technologies, LLC. certifies that the above listed ingredient has the following ISO 16128 value, based on the Compositional Breakdown:

Natural Index (NI)	Natural Origin Index (NOI)
1	1

SAFETY DATA SHEET

SECTION 1. IDENTIFICATION

Product Name/Identifier	Organic Emulsifier Blend 60
Product Code	A60014
Recommended Use	Topical Cosmetic Use
Restrictions on Use	None
Supplier/Manufacturing Site	Arbor Organic 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 Reg. (EC) No 1272/2008.
-This product is not classified as hazardous to health or environment according to the CLP regulation.

Labeling Elements:

Pictograph: No hazard symbol expected

Hazard statements/Signal Word: Not applicable

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

Other hazards which do not result in classification:

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

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

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

Results of PBT and vPvB assessment:

-PBT: Not applicable
-vPvB: Not applicable

SECTION 3. COMPOSITION / INFORMATION ON INGREDIENTS

Common Chemical Name: Oryza Sativa (Rice) Bran Extract & Cyamopsis Tetragonoloba (Guar) Gum

Generic name:

Chemical Family: Blend

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

Substance	CAS Numbers	EC Numbers	Percentage
Oryza Sativa (Rice) Bran Extract	90106-37-9	N/A	60.00%
Cyamopsis Tetragonoloba (Guar) Gum	9000-30-0	232-536-8	40.00%

Formula: Not applicable

SECTION 4. FIRST-AID MEASURES

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

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

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

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

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

SECTION 5. FIRE-FIGHTING MEASURES

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

SECTION 6. ACCIDENTAL RELEASE MEASURES

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

SECTION 7. HANDLING AND STORAGE

Handling

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

Storage

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

SECTION 8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Precautionary statements: Ensure adequate ventilation

Control parameters

Occupational exposure Limits:

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

Personal Protective Equipment:

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

SECTION 9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance:	Free flowing powder
Color:	Light beige to tan
Odor:	Characteristic

pH (5% in Water):	4.5 – 7.5
Loss on Drying (1g-1hr-105°C):	15.00% Maximum
Microbial Content:	< 200 CFU/g; No pathogens
Yeast & Mold:	< 200 CFU/g
Gram Negative Bacteria:	0 CFU/g
Vapor density:	Not applicable
Boiling Point:	Not applicable
Freezing Point:	Not applicable
Melting point:	Not determined
Flash point:	Not applicable
Oxidizing properties:	Non oxidizing material according to EC criteria.
Solubility:	
In water:	Dispersible
In organic solvents:	Not determined
Log P:	Not determined

SECTION 10. STABILITY AND REACTIVITY

Stability:	Stable under ordinary conditions of use and storage up to one year then re-test to full product specifications to extend shelf life
Hazardous reactions:	None known
Conditions to avoid:	No dangerous reactions known under use of normal conditions. Avoid extreme heat.
Materials to avoid:	No dangerous reaction known with common products.

Hazardous decomposition products: None known

SECTION 11. TOXICOLOGICAL INFORMATION

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

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

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

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

Specific effects:

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

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

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

SECTION 12. ECOLOGICAL INFORMATION

Ecotoxicity
Effects on the aquatic environment: EC_{10} (Freshwater Alga): 123.99 mg/L - Not harmful to aquatic organisms
EC_{20} (Freshwater Alga): 198.41 mg/L - Not harmful to aquatic organisms

Biodegradability:
Persistence: Readily Biodegradable (88.3% biodegradation after 28 days of testing)

Bioaccumulation:
Octanol / water partition coefficient: Not Determined

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

Other Adverse Effects: None known

SECTION 13. DISPOSAL CONSIDERATIONS

Residues from product

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

Contaminated packaging

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

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

SECTION 14. TRANSPORT INFORMATION

UN Number: None
UN Shipping Name: None

Transport Hazard Class: Not classified as dangerous for transport

Land (rail/road): Material is not restrictive for land transport and is not regulated by ADR/RID
Sea: Material is not restrictive for sea transport and is not regulated by IMO/IMDG
Air: Material is not restrictive for air transport and is not regulated by ICAO/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:	Oryza Sativa Bran Extract:	N/A
	Cyamopsis Tetragonoloba Gum:	232-536-8
TSCA inventory status:	Exempt	
AICS inventory status:	Exempt: 90106-37-9 Listed: 9000-30-0	
Canadian (CEPA DSL) inventory status:	Exempt: Oryza Sativa (Rice) Bran Extract (90106-37-9) Listed: Guar gum (DSL)	
Japan (MITI list):	Oryza Sativa (Rice) Bran Extract & Cyamopsis Tetragonoloba (Guar) Gum	
Korea:	Oryza Sativa (Rice) Bran Extract & Cyamopsis Tetragonoloba (Guar) Gum	
China inventory status:	Oryza Sativa (Rice) Bran Extract & Cyamopsis Tetragonoloba (Guar) Gum	
Philippines inventory status:	Exempt: Oryza Sativa (Rice) Bran Extract (90106-37-9) Listed: Guar gum	

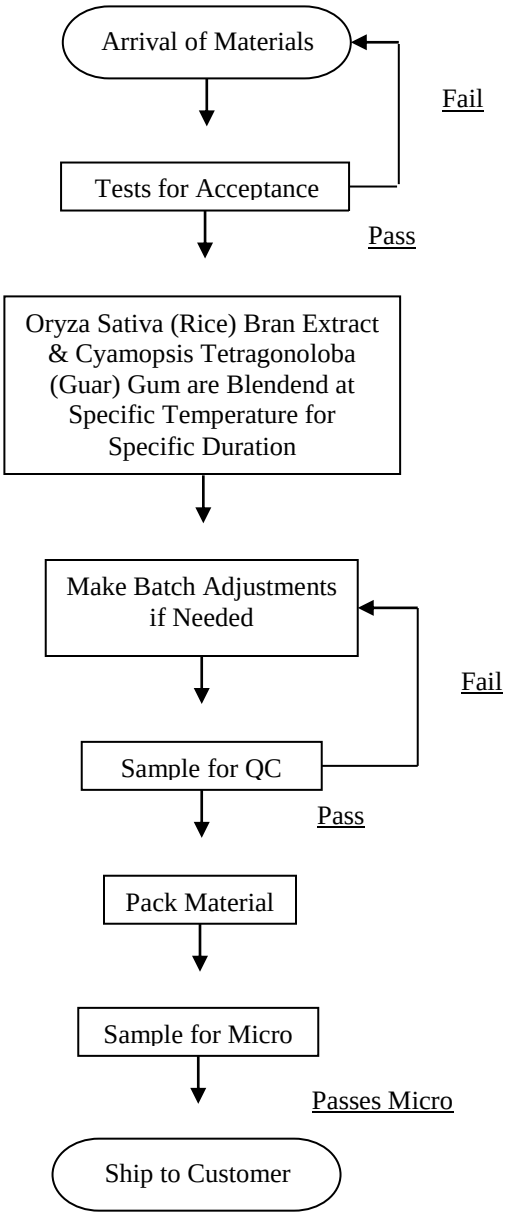
Note: The regulatory information given above only indicates the principal regulations specifically applicable to the products described in this sheet. The user's attention is drawn to the possible existence of additional provision which complete these regulations. Please refer to all applicable international, 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:	03/10/2020
Preparation Date:	09/17/2020
MSDS summary of changes	- Added Irritation Data – Section 11 (Toxicological Information) - Updated Solubility – Section 9 (Physical & Chemical Properties) - Updated Microbial & Yeast & Mold Content – Section 9 (Physical & Chemical Properties) - Updated Precautionary Statement – Section 2 (Hazards Information), Updated Recommend Storage Conditions – Section 7 (Handling & Storage) & Added Acute Toxicity Data – Section 11 (Toxicological Information) - Updated Europe Basis for Classification – Section 2 (Hazards Information), Updated Acute Toxicity Data & Added Sensitization & Mutagenicity Data – Section 11 (Toxicological Information) & Added Ecotoxicity & Biodegradability Data – Section 12 (Ecological Information)

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

MANUFACTURING FLOW CHART-ORGANIC EMULSIFIER BLEND 60-A60014



Arbor Organic Technologies

Organic Emulsifier Blend 60
Certificate of Compliance

Code: A60014
INCI Name: Oryza Sativa (Rice) Bran Extract & Cyamopsis Tetragonoloba (Guar) Gum
INCI Status: Conforms
CAS #: 90106-37-9 & 9000-30-0
EINECS #: N/A & 232-536-8

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

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

info@arbor-organics.com • +1 (704)-276-7100 • Fax: +1 (704)-276-7101

Arbor Organic Technologies

Organic Emulsifier Blend 60
Code: A60014

Attention must be paid to the use of Organic Emulsifier Blend 60 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.

Organic Emulsifier Blend 60 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 Organic Emulsifier Blend 60 is 1.00 – 4.00%.

Organic Emulsifier Blend 60 is in compliance with the standardized set of rules developed and approved by the NPA (Natural Products Association).

Organic Emulsifier Blend 60 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 Arbor Organic Technologies, LLC's internal non-animal testing policy, this product was not tested for NOAEL.

Organic Emulsifier Blend 60 was tested using *in vitro* dermal and ocular irritation models. This product was found to be non-irritating in both models.

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

(*) Carcinogenic, Mutagenic, toxic for Reproduction

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

Organic Emulsifier Blend 60 is REACH Compliant and free of the following:

- Formaldehyde or formaldehyde donors
- Gluten
- Glycol ethers
- Lactose
- Nanoparticles
- Nitrosamines
- Palm oil/palm kernel oil (or derivatives)
- Parabens
- Paraffin/petroleum products
- Pesticide residues
- Phthalates
- Polyethylene glycol (PEG)
- Residual solvents
- Sulfates
- Volatile organic compounds

info@arbor-organics.com • +1 (704)-276-7100 • Fax: +1 (704)-276-7101

Organic Emulsifier Blend 60 Formulation Guidelines

Code: A60014
INCI Name: Oryza Sativa (Rice) Bran Extract & Cyamopsis Tetragonoloba (Guar) Gum
CAS #: 90106-37-9 & 9000-30-0
EINECS #: N/A & 232-536-8

Temperature Stability	4.0 – 80.0°C
pH Stability	4 to 7 pH
Suggested Use Levels	1.00 – 4.00%
Solubility	Water Dispersible
Formulation Guidelines	Recommended to add to the aqueous phase. Sprinkle **Organic Emulsifier Blend 60** slowly in aqueous phase. Avoid formation of clumps to speed up dispersion process. High shear mixing or homogenization works best for optimal dispersion. Allow to hydrate.

Version#3/08-21-19