Safety Statement

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Product Name: Mycofuse® Protect

Code: 16916

INCI Name: Water & Lentinus Edodes Mycelium Extract & Lactobacillus Ferment

Mycofuse® Protect is manufactured by growing *Lentinus edodes* (Shiitake) in cell culture, followed by Mycelium extraction and filtration. Lactobacillus Ferment is added as a processing aid that helps reduce susceptibility of microbial contamination.

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

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

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

A Freshwater Alga Growth Inhibition test via OECD 201 was subsequently performed to determine the potential toxicity of Mycofuse® Protect. In this assay, *Psuedokirchneriella subcatpitata* are exposed to the test substance for 72 hours and growth and growth inhibition through cell count against control is performed. The response is evaluated as a function of the exposure concentration in comparison with the average growth of replicate, unexposed control cultures. After 72 hours, the percent inhibition for Mycofuse® Protect was determined to be 120.48 mg/L EC_{10} and 200.73 mg/L EC_{20}. The results of this assay indicate that the product is not classified and therefore not harmful to aquatic organisms.

Mycofuse® Protect was also assessed for ready biodegradability in an aerobic aqueous medium via the OECD 301 B Ready Biodegradability: CO2 Evolution (Modified Sturm Test). Mycofuse® Protect achieved 91.3% biodegradation after 28 days of testing, indicating that the product meets method requirements for the Ready Biodegradable classifications.

The full reports for each safety study analyzing Mycofuse® Protect are attached for reference

Due to the restriction placed on animal testing of cosmetic raw materials, and Active Concepts, LLC's internal non-animal testing policy, this product was not tested for NOAEL. However, there is substantial amounts of published data for *Lentinus edodes* that provides useful information to calculate approximate NOAEL and demonstrate the non-cytotoxic effects of Mycofuse® Protect. Investigation of the following data for *Lentinus edodes* along with US Food and Drug Administration guidelines[1] has allowed us to estimate Mycofuse® Protect exposure based off dosage in topical form, with an approximate NOAEL of 560 mg/kg/day.

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For example, at an average 2% use level in 1 oz (or 28 g) finished product per day on a person averaging a 65 kg body weight, a daily exposure of 8.62 mg/kg Mycofuse® Protect is expected (28 g x 2% = 0.56 g or 560 mg; 560 mg / 65 kg). Using the FDA guidelines as well as the following published NOAEL data[2,3] , it is expected that Mycofuse® Protect has an estimated aforementioned NOAEL of 560 mg/kg/day [(average of human equivalent, published NOAELs for each component), so (800 mg/kg/day + 320 mg/kg/day)/2].

When Mycofuse® Protect is used at approximately 2% in a finished formula sample we do not expect exposure to exceed 8.62 mg/kg daily, which is well under the NOAEL estimate of 560 mg/kg/day.

Published NOAEL for *Lentinus edodes* has been reported as more than 2,000 mg/kg/day in male and female rats.[2] Using the aforementioned conversion factor, the human equivalent NOAEL is expected to be 320 mg/kg/day. *Lentinus edodes* is widely used in food industries with minimal toxicity.[4]

Lactobacillus is a genus of microorganisms used to produce a variety of food products. It is a type of lactic acid bacteria (LAB) that converts various sugars into lactic acid. Any existing LAB in Mycofuse® Protect is removed by filtration. The FDA has published a number of regulations that detail the allowed uses of LAB, often as sources of enzymes used to produce food.[5] Microbes have been used in food for millennia, such as fermented vegetable, yogurt, bread, meat and more.

Published NOAEL for LAB such as Lactobacillus fermentum has been reported as 5,000 mg/kg/day in male and female rats.[3] When converting from animal to human equivalent doses (i.e. from rats to humans) a general conversion factor of 0.16 can be used.[6] Therefore the human equivalent NOAEL is expected to be 800 mg/kg/day.

Both *Lentinus edodes* and LAB products are commonly used in the food and nutraceutical industries. Since *Lentinus edodes* and Lactobacillus are intentionally used in food, their extracts/ferments may be classified as Generally Recognized as Safe (GRAS) according to the FDA's Federal Food, Drug and Cosmetic Act.[3]

The act states:

> Any substance that is intentionally added to food is a food additive, that is subject to premarket review and approval by FDA, unless the substance is generally recognized, among qualified experts, as having been adequately shown to be safe under the conditions of its intended use, or unless the use of the substance is otherwise excluded from the definition of a food additive.[3]

Several, published data sets exist to support the safety of Mycofuse® Protect. Additionally, the molecular weight of this product (approximately 3,109 Da) is larger than what is required to penetrate skin. Therefore, hazards that may otherwise occur via this route are not an issue. It is presented in an aqueous carrier, all but eliminating its risk for inhalation. Toxicological, irritation, and sensitization assays have all been performed with favorable results for each. This knowledge combined with the tested and published toxicity assays allows us to support the safety of Mycofuse® Protect in cosmetic applications.

It is logically concluded that Mycofuse® Protect is safe in cosmetic applications at use levels of 1.00 – 10.00 %. No further testing is required at this time.

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1. U.S. Food and Drug Administration (FDA) – Assessing Safety When Toxicity Data are Limited. https://pdfs.semanticscholar.org/presentation/a8c3/4a1ed34f929156bbc3d8db6693b6f22c8f9b.pdf
2. Yoshioka Y, Tamesada M, Tomi H. A repeated dose 28-day oral toxicity study of extract from cultured Lentinula edodes mycelia in Wistar rats. J Toxicol Sci. 2010 Oct;35(5):785-91. doi: 10.2131/jts.35.785. PMID: 20930474.
3. Jia Xu Dong, et al. A Subchronic Toxicitiy Study on Lactobacillus Fermentum GM 090 in Rat. Biomedical and Environmental Sciences. 2013: 680 –683 http://www.besjournal.com/Articles/Archive/2013/No8/201308/t20130827_87151.html
4. VanderMolen, Karen M., et al. "Safety Assessment of Mushrooms in Dietary Supplements by Combining Analytical Data with in Silico Toxicology Evaluation." Food and Chemical Toxicology, Pergamon, 4 Mar. 2017, www.sciencedirect.com/science/article/pii/S0278691517300984#bib16.
5. Federal Food, Drug and Cosmetic Act. U.S Food and Drug Administration. www.fda.gov.
6. Guidance for Industry Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers. https://www.fda.gov/downloads/Drugs/Guidances/UCM078932.pdf%23search=%27guidekines+for+industry+sfe+starting%27