

Notification Report

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Pre-Market Notification For New Dietary Supplement-Creatine Ethyl Ester (Cre-Ester™)

1.0 Name and address of the distributor and manufacturer

The distributor of the creatine ethyl ester shall be:

Pro-Nutrient Technologies, Inc.
11515 North 84th Street
Omaha, NE 68122
Phone: 402 573 6500
Fax: 402 573 7646

Contact person:
Sam Augustine, Pharm. D.
President
saugusti@unmc.edu
Phone: (402) 559-5774
Fax: (402) 559-9543

Creatine ethyl ester is manufactured for Pro-Nutrient Technologies by:

Mr. Mark Faulkner
Vireo Systems, Inc.
305 Williams Avenue
Madison, TN 37115-2626
Toll Free (800) 251-4166
Local (615) 865-8310
Fax (615) 865-8327

2.0 Name of New Dietary Ingredient

The new dietary ingredient shall be:

Creatine Ethyl Ester HCl (Cre-Ester™), CAS Registry Number 15366-32-2; Creatine Ethyl Ester bisulfate (Cre-Ester™), CAS Registry Number pending.

3.0 Description of the dietary supplement or dietary supplements that contain the new dietary ingredient

Cre-Ester™ will be marketed by Pro-Nutrient Technologies in 250 and 500 mg capsules or tablets. The product will be packaged in units of thirty and ninety. Pro-Nutrient Technologies will also distribute Cre-Ester™ as a bulk raw material for incorporation into other nutritional supplement products. The bulk powder form of Cre-Ester™ is suitable for encapsulation, compression into tablets, or formulation in drink mixes, nutritional bars, and sports drinks.

4.0 Level of the new dietary ingredient in the product

Recommended daily dosing of Cre-Ester™ shall be 500 milligrams to 5 grams per day, taken in a single or divided daily dose. Maximum daily exposure should not exceed 30 grams per day.

5.0 Conditions of use of the product

The following information pertaining to the use and contraindications of Cre-Ester™ will be supplied on the packaging information included in both the bulk-powder product and any capsule or tablet formulations produced by Pro-Nutrient Technologies.

The intended use for Cre-ester™ is as a dietary supplement for maintaining muscular health. This statement has not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure, mitigate or prevent any disease.

Cre-Ester™ should not be used by pregnant or lactating women; individuals diagnosed with, or at risk for, renal or hepatic dysfunction; individuals taking Antabuse™ (Disulfiram); or individuals with a known hypersensitivity to any component of the product. Cre-Ester should not be used by children under 18 years of age, unless directed by a physician or qualified health care professional.

6.0 Safety of Creatine Ethyl Ester

Discussion of the safety concerns relating to Cre-Ester™ are divided into three separate categories: (1) historical perspective based on safety data in the scientific literature, (2) in vitro toxicity data, and (3) in vivo animal toxicity data.

6.1 Historical Perspective

Creatine ethyl ester is a structurally related chemical analog of creatine. The difference between creatine and creatine ethyl ester is that the carboxylic acid group of creatine has been masked through the formation of an ester linkage. The masking of the carboxylic acid, results in a creatine-based compound with both increased aqueous solubility and enhanced membrane partitioning compared to the standard creatine monohydrate.¹

Creatine ethyl ester has not previously been introduced into the American diet. However, the constituent components and proposed metabolic end products (i.e. creatine and ethanol) have long been present in the American diet. Below is a brief summary of the scientific literature supporting the safety of creatine and ethanol.

Numerous studies have been published, evaluating the relative safety of creatine salts supplemented to healthy adults, specifically creatine monohydrate.²⁻⁵ These human studies include both short term and long term studies, and have determined that supplementation with creatine monohydrate is not associated with any adverse

health effects. No difference was noted in serum markers of liver or kidney function between creatine-supplemented groups as compared to placebo.^{3,6,7,8} Two case reports of kidney dysfunction following creatine use exist within the medical literature, however, neither was able to demonstrate a causative relationship with supplement use.⁹⁻¹⁰ Creatine is an accepted ergogenic supplement in all major athletic organizations, including: IOC,¹¹ NCAA,¹² and other major sports organizations.

Ethyl alcohol is a metabolite formed from the breakdown of the Cre-Ester™ pronutrient.¹ The complete metabolic conversion of a three gram daily dose of Cre-Ester™ would yield less than one gram (0.707 g) of ethyl alcohol, a level recognized as safe for other food and health products, such as vanilla extract.¹³ A single serving of an alcoholic beverage contains over ten times that amount of ethyl alcohol.¹⁴ Ethyl alcohol meets the “Food Chemicals Codex”, 4th ed. (1996, p. 136)¹⁵ and is affirmed as GRAS in 21 CFR 184.1293.¹⁶

6.2 In Vitro Toxicity Data Concerning Creatine Ethyl Ester

As skeletal muscle is a major target tissue for creatine supplements, and has the highest stores of creatine in the body, the toxicity of creatine ethyl ester hydrochloride was examined in vitro using a mouse skeletal muscle cell line (C2C12). For these studies, cultured C2C12 cells were exposed to various concentrations of creatine ethyl ester hydrochloride (0-100 mM) for a period of 4 hours. After 4 hours of exposure, the cells were washed and provided fresh culture media and incubated, under aseptic conditions, for an additional 72 hours. Toxicity was assessed using the MTT assay¹⁷. The data from these studies are provided in Appendix 1. Within the concentration range examined, there were no statistically significant effects of Cre-Ester™ on C2C12 cell viability. The effects of Cre-Ester™ on cell viability were similar to that observed with creatine monohydrate, a commonly used form of creatine supplementation (Appendix A).

6.3 In vivo Toxicity Data Concerning Creatine Ethyl Ester

In vivo studies were also performed in adult male Sprague-Dawley rats to evaluate the toxicity of creatine ethyl ester. Rats were dosed for seven consecutive days with Cre-Ester™ hydrochloride via oral gavage. The dosage administered was calculated based on a 30 g equivalent in a 70 kg human (i.e. 430 mg/kg). This dose was chosen as it represents the highest daily dose of Cre-Ester to be taken by humans (as reported in Section 4.0 Level of New Dietary Ingredient). A total of eight rats were randomly selected for the treatment group (Cre-Ester™). The control group consisted of an additional eight rats that were given physiological saline solution (2 ml/kg) via oral gavage for seven consecutive days. The parameters assessed included weight gain as well as a complete metabolic panel for blood chemistry at the conclusion of the study. In addition, 2 rats from the treatment and control groups were chosen at random for comprehensive necropsy. The data from these studies are found in Appendix B. These studies show no

significant changes in weight gain between the control and treatment groups over the seven-day course of the study. Furthermore, no significant differences in blood chemistries were observed between the Cre-Ester™ treated and vehicle control groups at the conclusion of the study. The various blood tests performed included protein, albumin, glucose, calcium, potassium, sodium, and chloride levels; liver enzymes (ALT, and AST levels); and renal function (BUN, creatinine and BUN/creatinine ratio). Comprehensive necropsy of selected Cre-Ester™ treated and control rats showed no gross lesions. Histopathological examination of selected tissues, including liver, kidney, bladder, stomach, heart, brain, and spleen showed no abnormalities (necropsy reports from Research Animal Diagnostic Laboratory, University of Missouri, are included in material in Appendix B). Based on the animal studies shown, there is no evidence of toxicity with the oral consumption of Cre-Ester™ at a dose equivalent of 30g in humans.

6.4 Human In Vivo Data Concerning Creatine Ethyl Ester

Human experience with Cre-Ester™ involves five adult males ranging in age from 39 years to 64 years. The daily dosage and length of exposure to the supplement ranged from 1.0 to 3.0 grams and 238 to 414 days respectively. No significant abnormalities in blood or urine chemistries were noted. There was one slightly elevated measurement for serum creatinine of 1.7 mg/dL (normal 0.8-1.5 mg/dL) in subject number 4, however, the use of the albumin/creatinine ratio, a more reliable index of kidney function was well within the normal range. All blood and urine chemistries were performed at the clinical laboratory of the Nebraska Health Systems, Omaha, Nebraska. The complete laboratory findings are given in Appendix C.

7.0 Quality Assurance Issues Regarding Creatine Ethyl Ester

Cre-Ester™ will be manufactured under cGMP conditions by Vireo Systems, Inc. and supplied, enclosed and contained, in vacuum-sealed plastic lined PVC 20-kilo drums for use as a bulk raw material. All lots of Cre-Ester™ will undergo quality assurance testing prior to packaging and shipment. The raw bulk product will consist of at least 95% Cre-Ester™, with creatine and creatinine constituting the remaining material. The purity of the raw bulk product will be determined using both high pressure liquid chromatography (HPLC) and nuclear magnetic resonance (NMR) spectroscopy methods. As further validation of the synthetic process used, samples from a recent batch synthesis of Cre-Ester™ were recently submitted to an independent analytical laboratory for analysis of potential metal and organic contaminants. The content of a variety of metals in the Cre-Ester™ sample was analyzed using toxicity characteristic leaching procedure (TCLP) and the results of this analysis are reported in Appendix D. Of the metals examined arsenic, mercury, selenium, silver, chromium, lead, cadmium, nickel, and copper were below the limits of detection of the assay. The batch sample contained a small amount of barium (0.32 mg/ml) and zinc (0.17 mg/ml). However, these amounts were well below the maximum permissible level for the metals examined. The Cre-

Ester™ sample was also shown to be free of all chemical carcinogens examined. These included benzene, carbon tetrachloride, chlorobenzene, chloroform, 1,4-dichlorobenzene, 1,2-dichloroethane, 1,1-dichloroethene, methyl ethyl ketone, tetrachlorethene, trichloroethene, and vinyl chloride (see report in Appendix D). Based on this information, we are confident of the synthetic process to be used for production of Cre-Ester™ and the resulting purity and safety of the product to the consumer.

8.0 Summary

Dietary supplements containing creatine have been available to consumers for over two decades. Over this time, no significant health concerns have been identified in either controlled human studies, or acute and subacute toxicity studies in laboratory animals. Cre-Ester™ is a new analog of creatine with improved solubility and permeability properties. Based on both our in vitro cell culture studies examining acute Cre-Ester™ toxicity and in vivo rat studies examining 7-day exposure to Cre-Ester™, there were no apparent indicators of toxicity with this compound. In addition, blood and urine profiles of five human males on daily supplementation with Cre-Ester™ that ranged from 1 to 3 grams taken from 238 to 414 days at the time of testing showed no significant changes in their blood or urine chemistries. Thus, this data, along with the historical experience with creatine based products, indicates that this new creatine derivative is safe when used in accordance with the packaging instructions.

References for Cre-ester™ Pre-Market Notification:

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