Abstract

Creatyl-Leucine (CLL) is a synthetic derivative of creatine and Leucine joined by an amide bond between the carbonyl group of creatine and the amino group of Leucine for the purpose of protecting creatine from spontaneous degradation in solution. Because of interest in this novel compound for use in beverages, we investigated its toxicological potential, according to internationally accepted guidelines in [1] a bacterial reverse mutation test, [2] an in vitro mammalian chromosomal aberration test, [3] an in vivo mammalian micronucleus test, and [4] a 90-day repeated-dose oral toxicity study in rats. No evidence of mutagenicity, up to the recommended concentrations in the in vitro tests, or genotoxic activity, up to the limit dose in the in vivo study, was observed. No mortality, general toxicity, or toxic effects on organs or tissues was observed in Hud.Han:Wistar rats in the 90-day gastric study at doses of 1250, 2500, and 5000 mg/kg bw/d, and the NOAEL was determined as the highest dose level.

Introduction

The studies were recently published in the international Journal of Toxicology,1-4 Creatine and Leucine occur naturally in foods such as meats, seafood and dairy products with Leucine also being found in some grains, soybeans, nuts, beans, seaweed and sesame.5,6

Creatine and Leucine are not known to naturally form bonds with one another endogenously in humans or in nature. CLL is synthesized by subjecting Leucine to a series of chemical processes, resulting in creatine and Leucine connected by an amide bond, thus creating a novel compound.

Bacterial Reverse Mutation Test

S. typhimurium strains: TA100, TA100c, TA1537 and E. coli strain: WP2 uvrA.

OECD (471), EC (440/2008 B13/14), EPA (OPPTS 870.5100), ICH (S2b) and GLP compliant.

No concentration-related or biologically relevant increases in revertant colonies were observed in any faster strain up to the highest level tested with or without metabolic activation (5000 µg/plate).

In Vivo Mammalian Chromosomal Aberration Test

Test Article, creatyl-Leucine

- Soluble in water
- Test article provided by the sponsor of the studies, VFX Sports Nutrition.
- Purity >98%

Formulated in water for the bacterial reverse mutation test, mouse micronucleus test, and in Ouboecc’s modified Eagle’s medium for the chromosomal aberration test.

In Vivo Mammalian Erythrocyte Micronucleus Test

Male SPF Wistar mice were used (Hsd.Han:Wistar). OECD (474), EPA (OPPTS 870.5395) and GLP compliant.

Performed with permission of the laboratory’s Institutional Animal Care and Use committee, and in accordance with Institutional guidelines for Care and Use of Laboratory Animals.

No statistically significant increases in the frequency of micronucleated polychromatophilic erythrocytes compared to control were detected up to the highest level tested (limit dose of 2000 mg/kg bw).

Ninety-Day Repeated Oral Toxicity Study

S. typhimurium strains: TA100, TA100c, TA1537 and E. coli strain: WP2 uvrA.

OECD (471), EC (440/2008 B13/14), EPA (OPPTS 870.5100), ICH (S2b) and GLP compliant.

No concentration-related or biologically relevant differences in revertant colonies were observed in any faster strain up to the highest level tested with or without metabolic activation (5000 µg/plate).

No target organs or treatment-related toxicological effects were identified. The NOAEL of the 90-day repeated-dose oral toxicity study was 5000 mg/kg bw/d—the highest dose tested.

Conclusions

- No evidence of in vitro mutagenicity or clastogenicity or in vivo genotoxicity was detected in the bacterial reverse mutation, in vitro mammalian chromosomal aberration and in vivo mouse micronucleus tests.
- No target organs or treatment-related toxicological effects were identified. The NOAEL of the 90-day repeated-dose oral toxicity study was 5000 mg/kg bw/d—the highest dose tested.

References:

Abstract/Poster

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A Toxicological Assessment of Creatyl-L-Leucine

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- Weight/weight gain: There were no toxicologically relevant differences in body weight, weight gain, food consumption, or feed efficiency in any of the test groups compared to controls.
- Blood/serum: Slight sporadic differences in several laboratory parameters were statistically significantly different compared to controls; however, they were not considered toxicologically relevant. Findings were as follows:
  - Decreased % bioslpipol (male low- and high-dose groups), effect not dose-related, not consistent across sexes, were within historical control ranges and were without corresponding histopathological findings.
  - Decreased hemoglobin and hematocrit (females high-dose group) effect not consistent across sexes, results were within historical control ranges and were without corresponding histopathological findings.

- Decreased liver weights (males high-dose group) were within historical control ranges and were without corresponding histopathological findings.

- Increased activated (male middle- and high-dose groups) effect not consistent across sexes, results were within historical control ranges and were without corresponding histopathological findings.

- Increased creatinine (female intermediate- and high-dose groups) effect not consistent across sexes, results were within historical control ranges and were without corresponding histopathological findings.

- Increased liver weight (male high-dose group) effect not consistent across sexes, results were within historical control ranges and were without corresponding histopathological findings.

- Increased calcium (male high-dose group) effect not dose-related, not consistent across sexes, results were within historical control ranges and were without corresponding histopathological findings.

- Increased chloride (male high-dose group) effect not consistent across sexes, results were within historical control ranges and were without corresponding histopathological findings.

- Increased sodium (all male test groups) effect not dose-related, not consistent across sexes, were within historical control ranges and were without corresponding histopathological findings.

- Increased protein (male high-dose group) effect not consistent across sexes, results were within historical control ranges and were without corresponding histopathological findings.

- Increased total protein (male middle- and high-dose groups) effect not dose-related, not consistent across sexes, results were within historical control ranges and were without corresponding histopathological findings.