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Genotoxicity and sub-chronic toxicity of MYOLUTION® (branched chain keto acids)

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ABSTRACT

MYOLUTION®, which consists of a mixture of the branched chain keto acids, keto-leucine, keto-isoleucine and keto-valine, as their calcium salts, may potentially be used as a food ingredient based on the reported contributions of these compounds to muscle health and exercise performance. Tests on genotoxicity and sub-chronic toxicity were performed to evaluate the safety of branched chain keto acids. No genotoxic effects were observed in the bacterial mutation assay or the in vitro micronucleus assay in human lymphocytes. In the 28 day and 90 day repeated dose toxicity studies no test item related mortality or toxicological effects on clinical signs, body weight, food consumption, urine parameters, hematology, clinical biochemistry parameters, organ weight, gross pathological findings and histopathology were observed. Based on the studies described here, MYOLUTION® does not exert a genotoxic effect, and a no-observed-adverse-effect-level of 3318.38 mg/kg bw/day in males and 3733.28 mg/kg bw/day in females was determined in the 90 day repeated dose toxicity study.

1. Introduction

The purpose of this article is to provide safety information for MYOLUTION®, which is a mixture of the branched chain keto acid calcium salts. Up till now, the main application of the BCKA calcium salts has been as components of a commercial clinical nutrition preparation used in cases of kidney disease. In order to expand the application of the BCKA salts into the area of dietary supplements, especially for elderly nutrition and sports nutrition, toxicological studies supporting the safety of MYOLUTION® have been conducted.

The mixture of branched chain keto acid calcium salts marketed as MYOLUTION® consists of the calcium salts of the three acids, keto-leucine, (3*RS*)-keto-isoleucine (as a racemic mixture) and keto-valine in the approximate ratio of 2:1:1. These branched chain keto acids (BCKA) are the analogs of the respective branched chain amino acids leucine, isoleucine and valine (BCAA). Their structures are shown in Fig. 1.

The BCKA can be manufactured by chemical synthesis or alternatively by a fermentation process (Karau et al., 2010). For this work, keto-leucine and keto-valine were produced via fermentation, while keto-isoleucine was produced chemically. They are prepared as the sodium salts and acidified with a mineral acid. The free acids are extracted, and a calcium salt is added to precipitate the purified product MYOLUTION® as a mixture of the BCKA calcium salts. The content of the individual branched chain keto acids in MYOLUTION® is 40–60% keto-leucine calcium salt, 20–30% keto-isoleucine calcium salt and 20–30% keto-valine calcium salt for all production batches. A food GMP concept including HACCP has been established for the MYOLUTION® production process.

Orally administered BCKA are predominantly metabolized by absorption from the gut, followed by incorporation into muscle protein or catabolic metabolism via the citrate cycle. The three BCKA are converted in the body to the corresponding BCAA, catalyzed by the action of branched chain aminotransferase (BCAT). This reaction is reversible and employs the glutamate – α-keto-glutarate cycle as an ammonia source. Unlike other aminotransferase enzymes, the BCAT are found principally in skeletal muscle. This means that dietary BCKA and BCAA are mainly transported to

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Abbreviations

BCKA	Branched Chain Keto Acids
BCAA	Branched Chain Amino Acids
DMSO	Dimethyl sulfoxide
NOAEL	No-Observed-Adverse-Effect Level

the muscle to be metabolized or incorporated into muscle protein, rather than being metabolized in the liver (Fig. 2). It has been shown in animal experiments using radiolabeling, that dietary BCAA and dietary BCKA are both incorporated into muscle (Imura and Walser, 1990; Swain et al., 1990). The metabolic products of BCKA are the same as for BCAA, namely incorporation into the citric acid cycle (tricarboxylic acid cycle) as a source of energy (Cole, 2015). As BCKA are either metabolized or transaminated to BCAA, there is no accumulation of BCKA in the body. Skeletal muscle protein is constantly being renewed at the rate of 1–2% per day, as a result of catabolism of BCAA and rebuilding of muscle protein from dietary BCKA and/or BCAA after exercise (Wall and van Loon, 2013). It can thus be concluded that BCKA and BCAA behave in the same manner with respect to their metabolism.

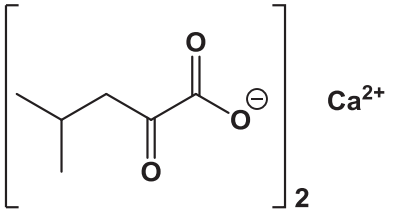
The nutritional effects of BCAA have been extensively reviewed (Rajendram et al., 2015), and this may also be carried across to the BCKA. BCKA had been applied in the field of sports nutrition to prevent the build-up of ammonia in the body which could have an effect on fatigue and athletic performance (Liu et al., 2012; Prado et al., 2011). The use of MYOLUTION® is likely to expand further, particularly in the areas of supplements for elderly nutrition and sports nutrition.

2. Materials and methods

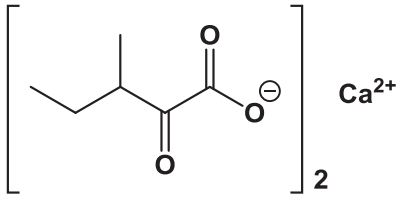
All toxicology studies have been performed in the years 2015–2016 in compliance with the standards in place at the time of their conduct with regard to animal welfare and methodology. Details of the methodology used have been provided for each of the studies conducted in accordance with recognized standard test methods such as the OECD test guidelines. All tests have been performed in compliance with European Commission Regulation (EC) No. 440 (EC, 2008). All in-vivo studies were performed at BSL BIOSERVICE, Planegg, Germany being fully accredited by AAALAC (Association for Assessment and Accreditation of Laboratory Animal Care International) and GLP certified. Furthermore, these studies were approved by the Institutional Animal Ethics Committee and they were performed in compliance with the German Animal Welfare Act (BGBl, 1972). The in-vitro studies were performed at Envigo CRS GmbH, Rossdorf, Germany being GLP certified in the year. Unless described otherwise below, all studies were performed in fully compliance with “Chemikaliengesetzt” (Chemical Act of the Federal Republic of Germany) in its currently valid version being in accordance with the GLP standards published as OECD Principles of Good Laboratory Practices (OECD, 1998a). MYOLUTION® specified as above has been applied as the test material.

2.1. In vitro gene mutation assay in bacteria

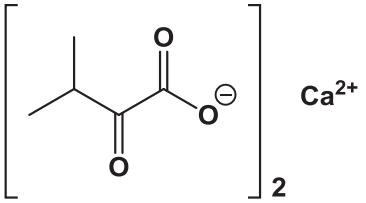
The in vitro gene mutation assay in bacteria utilized four strains of *Salmonella typhimurium* - TA98, TA100, TA1535 and TA1537 –



Keto-leucine calcium
KIC calcium
α-oxo-isocaproic acid, calcium salt
4-Methyl-2-oxopentanoic acid, calcium salt (2:1)
51828-95-6



Keto-isoleucine calcium
KMV calcium
α-oxo-β-methylvaleric acid, calcium salt
3-Methyl-2-oxopentanoic acid, calcium salt (2:1)
66872-75-1



Keto-valine calcium
KIV calcium
α-oxo-isovaleric acid, calcium salt
3-Methyl-2-oxobutanoic acid, calcium salt (2:1)
51828-94-5

Fig. 1. Chemical structures of branched chain keto acids present in MYOLUTION®.