AIBMR Life Sciences, Inc.
Natural and Medicinal Products Research

EXPERT PANEL REPORT

The Generally Recognized as Safe (GRAS) Status

of

Beta-hydroxybutyrate (BHB) Salts

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Introduction

The subject of this GRAS determination is beta-hydroxybutyrate (BHB) salts (sodium, calcium, magnesium, and potassium) sold by Ketone Labs. (hereinafter called "Ketone Labs") with its principal offices at 8688 Ruffian Lane, Suite C Newburgh, IN 47630. BHB is an endogenous compound produced in the liver during periods of fasting or starvation that acts as an energy substrate. This report includes a detailed description of BHB and its salts, including their manufacturing processes, quality control, and history of exposure, as well as a discussion of toxicological studies performed on BHB and related compounds, establishing the safety of this ingredient.

A panel of experts ("Expert Panel") who are qualified by training and experience to evaluate the safety of food ingredients has determined through scientific procedures, and corroborated by a history of safe use (exposure), that BHB salts are Generally Recognized as Safe (GRAS) for their intended use and, therefore, exempt from pre-market approval requirements of the Federal Food, Drug, and Cosmetic Act.

The members of the Expert Panel are Judith Hauswirth, PhD (Chair), John R. Endres, ND, and Amy Clewell, ND, DABT. The curricula vitae (CVs) for these experts can be found at the end of this summary document. The Expert Panel, independently and collectively, critically evaluated materials submitted by Ketone Labs, as well as other documents, following a thorough review of the literature and other information available in the public domain. The aforementioned are cited in the references section of this document or are included in the appendices to this report. This report is a summary of the Expert Panel's evaluation and provides the basis for the unanimous conclusion by the Expert Panel that the intended use of BHB is GRAS based on scientific procedures.

Description and Characterization

BHB (also known as 3-hydroxybutanoic acid; 3-hydroxybutyric acid) is an endogenous compound commonly referred to as a "ketone body" (**see Figure 1**). The term "ketone bodies" refers to three compounds, BHB, acetoacetate and acetone. Chemically, acetone is the only compound that is a simple ketone, while acetoacetate is a ketoacid. BHB, a hydroxyacid, does not contain a ketone functional group, but is referred to as a ketone body due to its structural relationship to acetoacetate (**see Figure 2**). BHB and acetoacetate are the most predominate of the three compounds in the body, are freely interconverted, and are usually present in a 1:1 BHB:acetoacetate ratio; however, with prolonged fasting, this ratio can rise to 6:1.[1] Ketone bodies act as oxidative substrates to provide energy for the body and brain in times of glucose deprivation.[2]

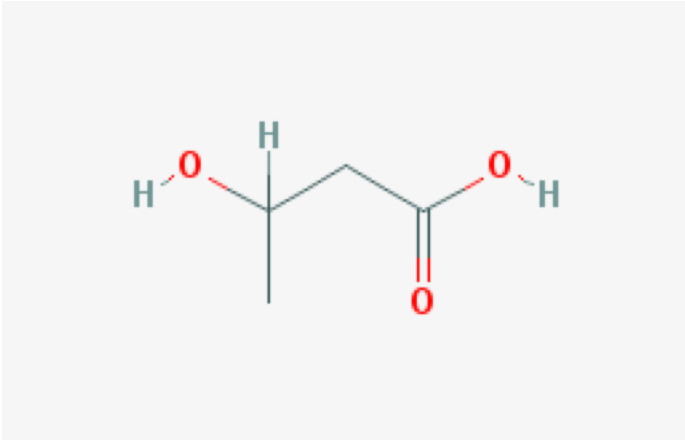

Figure 1. Chemical Structure of BHB

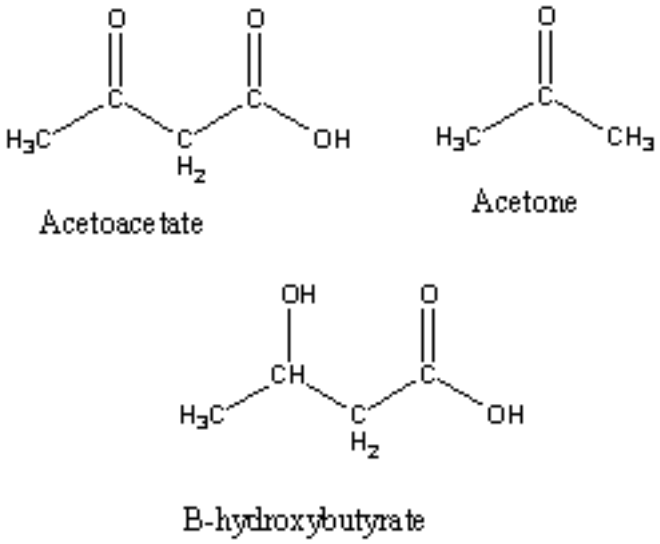

Figure 2. Chemical Structures of Ketone Bodies

BHB is produced in the liver from acetyl-CoA following the beta-oxidation of fatty acids, a metabolic process called ketogenesis. The ketogenic process involves the following steps: 1) beta-oxidation of fatty acids to acetyl CoA, 2) formation of acetoacetyl CoA, 3) conversion of acetoacetyl CoA to 3-hydroxy-3-methylglutaryl CoA (HMG-CoA), 4) conversion of HMG-CoA to acetoacetate, and 5) the

reduction of acetoacetate to BHB.[1] BHB is produced in low amounts during normal metabolism and higher amounts during periods of fasting or starvation, ketogenic diets, prolonged intense exercise or in disease states (e.g. inborn errors of metabolism, inadequately treated type I diabetes). A typical range of ketone bodies in the blood is 0.2–0.5 mM, with limited food intake levels this range can increase to approximately 5–7 mM, and in pathological states such as diabetic ketoacidosis, ketone body levels can reach >25mM.[3] In times of glucose deprivation, it is estimated that the body can produce 150–185 g of ketone bodies per day (achieving a blood level of ~5–8mM), the majority of which is BHB, without negative effects.[4, 5, 6] The stabilization of plasma ketone bodies over time in fasting states suggests that there are negative feedback mechanisms that exist in healthy individuals to inhibit the production of unsafe ketone levels in the body, such as those seen in diabetic ketoacidosis (>25mM).[5, 7, 8]

Once produced, BHB is transported to non-hepatic tissues, where it is oxidized into acetoacetate in the mitochondria of cells. Acetoacetate is then metabolized to acetoacetyl-CoA and subsequently back into acetyl-CoA, a process called ketolysis.[1] Acetyl-CoA is then utilized in the citric acid cycle for the production of energy. Ketogenesis and ketolysis are highly regulated biochemical processes, intricately related to energy homeostasis and associated hormones.[8]

Pharmacokinetics

Ketone bodies have a very rapid clearance from the plasma, and are quickly interconverted and distributed throughout the body within minutes, as demonstrated in various animal and human trials.

In a human pharmacokinetic study conducted on (R)-3-hydroxybutyl (R)-3-hydroxybutyrate, a ketone ester that is hydrolyzed to D-beta-hydroxybutyrate and (R)-1,3-butanediol upon ingestion (the latter of which is further metabolized to D-beta-hydroxybutyrate and acetoacetate in the liver), healthy participants (n=18) consumed a single dose of 140, 357 or 714 mg/kg bw of the ketone ester as part of a meal replacement milkshake drink following an overnight fast.[9] Blood samples were taken for pharmacokinetic analysis at baseline (prior to dosing) and at 1, 2, 3, 4, 6, 8, 12 and 24 hours post ingestion. The intact ketone ester was not detected in the plasma of any of the participants, however, both D-beta-hydroxybutyrate and acetoacetate levels increased proportionately with the increasing doses of the ketone ester. The C_{max} of D-beta-hydroxybutyrate was 0.28, 1.00 and 3.30 mM for the 140, 357 and 714 mg/kg bw groups, respectively, and the time to reach maximum peak concentrations ranged from 1.5–2.5 hours. The $t_{1/2}$ of D-beta-hydroxybutyrate ranged from 0.77–3.06 hours. The apparent total plasma clearance of D-beta-hydroxybutyrate was nearly three-fold higher in those consuming the lowest dose compared to the highest dose, a phenomenon seen in

other studies.[7] An increased rate of utilization of ketone bodies coupled with a decrease in clearance appears to be a normal regulatory mechanism, whether the ketone bodies are endogenously produced or exogenously provided.[10] At the highest single dose (approx. 150 g in a 70 kg person), blood beta-hydroxybutyrate did not exceed "normal" levels (5.5 mM) and the participants' glucose levels remained above 65 mg/dL (2.5 mM) and below 400 mg/dL (22.2 mM).

The pharmacokinetics of single and multiple intravenous (IV) doses of KTX 0101 (sodium BHB) up to 2000 mg/kg/day were investigated in several species (rats, rabbits and dogs).[11] BHB plasma levels were proportional to the administered dose, BHB was rapidly cleared from the plasma in all three species, and there was no evidence of gender differences in the pharmacokinetics of KTX 0101. In rats, levels returned to endogenous levels within 30–60 minutes; with the exception of the 2000 mg/kg dose, for which BHB was still detectable after 1 hour, but not after 24 hours. In dogs, this dose was not detectable after 3 hours. There was also no evidence of accumulation after repeated administration of KTX 0101 for ≤1 month in rats or dogs and ≤13 days in female rabbits.

Using radiolabelling, the tissue distribution of KTX 0101 (sodium BHB) was also investigated. In male rats, plasma levels of radioactivity declined rapidly in a biphasic manner with a half-life of 32 min for <2 hours and 37 hours from 2–120 hours after administration of single doses of 10, 30 or 100 mg/kg or an infusion of 30 mg/kg/h for 1 hour. In rats, high levels of radioactivity were found in blood, skeletal muscle, skin, urine, liver, Harderian gland, parotid salivary gland, tongue and kidneys after 5 minutes. Low levels of radioactivity were found in the brain, eyes, thyroid gland, epididymis and prostate gland. Concentrations decreased over a period of 24–120 hours, when radioactivity was still detected in the nasal cavity, Harderian gland, adrenal gland, spinal cord, skeletal muscle and skin. Radioactivity increased in fat during this time, which the authors attributed to *de novo* synthetic incorporation of the radiolabel into fat constituents. There was no evidence of radioactive KTX 0101 binding to plasma proteins in rat, dog or human plasma in vitro.

Seven days after the IV administration of KTX 0101 in male rats (10, 30 or 100 mg/kg bolus doses or 30 mg/kg continuous infusion for 1 hour), the radioactive BHB had been excreted in the urine (2.7–6.0%), the feces (1.2–1.8%) and the overwhelming majority as carbon dioxide (81.0–89.2%), which is consistent with the mitochondrial metabolism of BHB into carbon dioxide and water.

Ketogenic Diets and History of Consumption

Pierre Marie, a French neurologist, first proposed fasting as a treatment for epilepsy in the late 19th century.[4] This was followed by the use of ketogenic diets (high fat, low carbohydrate diets that induce ketosis), a more acceptable anti-convulsion treatment that was promoted by scientists of prestigious institutions such as the Mayo Clinic and Johns Hopkins University.[12] More recently, ketogenic diets, used to increase blood ketone levels to "therapeutic ranges" (~2–7 mmol/L) have been implicated as a potential treatment for a wide variety of neurological disorders including Amyotrophic Lateral Sclerosis (ALS), and Parkinson's and Alzheimer's diseases.[13, 4] These diets have also become popular for weight loss and other conditions such as sleep disorders, migraines, autism and pain.[14, 15] The extensive history of the use of ketogenic diets for these various health benefits suggests that mild ketosis in humans does not have adverse effects, and instead, may provide health benefits.

Although BHB is not commonly found in foodstuff, it is known to be present in cow's milk at low levels, and the milking process is known to increase levels in raw milk due to the large requirement for lactose in milk production that can induce ketosis.[16, 17] Ketosis occurs because the Kreb's cycle and gluconeogenesis compete for oxaloacetate; the shortage of oxaloacetate prevents acetyl-CoA in the liver from entering the Krebs cycle, which results in an increase in ketone body production.[16, 17] One study analyzed 2500 milk samples and found that BHB levels ranged from 10–631 μM (mean levels were 49 μM or 1.2 mg/8 fl oz.), and in ketotic cows, these levels ranged from 10–684 μM (mean level was 141 μM or 3.5 mg/8 fl oz.).[18]

BHB Salts

Ketone Labs produces BHB salts (racemic 50:50 mixtures of D:L-BHB) , in which BHB is chemically (ionically) bonded to sodium, calcium, magnesium, or potassium cations (**see Figures 3–6**). Magnesium BHB is a trihydrate, containing three water molecules. In general, salts of chemical compounds do not alter the safety profile of the compound itself, due to the rapid hydrolysis of such compounds upon ingestion, and the well-known safety of such ions.

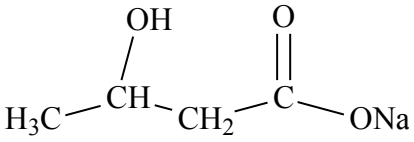

Figure 3. Chemical Structure of BHB Sodium Salt

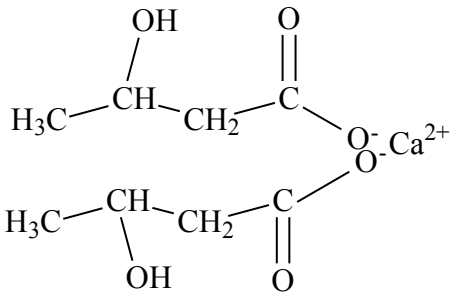

Figure 4. Chemical Structure of BHB Calcium Salt

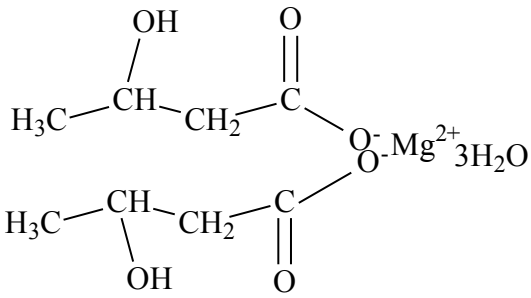

Figure 5. Chemical Structure of BHB Magnesium Salt Trihydrate

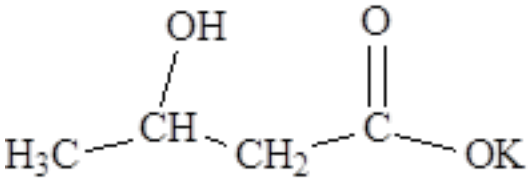

Figure 6. Chemical Structure of BHB Potassium Salt

Sodium

Ketone Labs' sodium salt of BHB contains 18.0–19.0% sodium. Sodium is the most abundant cation in the human body and the principal cation of extracellular fluid. Sodium is required for maintaining the osmotic balance of extracellular fluid, and therefore, is the determinant of extracellular and plasma volume. Sodium is also vital in maintaining the membrane potential of cells and the active transport of molecules across cell membranes.[19] The major source of

sodium in the diet is added salt (sodium chloride) and it is estimated that processed foods account for 75% of total sodium consumption.

The sodium dietary reference intakes (DRI) for various age groups, based on adequate intake levels, are 1.0–1.5 g/day; however, 115 mg/day is likely sufficient to provide for growth and replace daily sodium losses. It is estimated that sodium consumption is much greater than the DRI, typically 1.8–5.0 g per day. In 2016, the FDA released a draft guidance to encourage the voluntary reduction of sodium in processed, packaged and prepared foods intended to address the "excessive intake of sodium in the current population". The FDA encourages sodium intake of less than 2.3 g/day for most individuals. The tolerable upper intake levels (UL) for sodium based on the scientific rationale of the adverse effects of sodium intake on blood pressure, are currently set at 1.5–2.3 g/day for various life stages; the UL for those over the age of 18 is 2.3g/day.[19]

Calcium

Ketone Labs' calcium salt of BHB contains 16.0–17.0% calcium. Calcium is the most common mineral in the body, the majority of which is found in bones and teeth (~99%). It is also ubiquitous in the diet. Among the richest dietary sources of calcium are dairy products (milk, yogurt and cheese), calcium-fortified orange juice and certain leafy green vegetables, including broccoli and kale.

Calcium is considered GRAS for human consumption as a food ingredient; various forms of calcium are listed as "specific substances affirmed as GRAS" in 21 CFR 182 and 184; the majority of these regulations have no limitation of use other than current good manufacturing practice. The current DV for calcium is 1000 mg/day. The tolerable UL for calcium, based on the toxicity states of hypercalcemia and hypercalciuria (although the Food and Nutrition Board states that there is no clear basis for a dose-response relationship in these conditions and various confounding variables exist) is 2500 mg/day for males and females ages 19–50 and 2000 mg/day for males and females aged 51–70.[20]

Magnesium

Ketone Labs' magnesium trihydrate salt of BHB contains 8.3–9.0% magnesium and ~19% water. Magnesium is only second to potassium as the most abundant intracellular cation in the body. Ninety-nine percent of total body magnesium is found within human cells or deposited in bone, and bone contains 50–60% of the total content of magnesium in the body.[21] Magnesium is found in a variety of foods including nuts, legumes, whole grains, spices, leafy green vegetables, chocolate and seafood. [22]

Magnesium is considered GRAS for human consumption as a food ingredient; various forms of magnesium are listed as "specific substances affirmed as GRAS"

in 21 CFR 184, with no limitation of use other than current good manufacturing practice. The current daily value (DV) for magnesium is 400 mg; however an UL for magnesium is 350 mg, which is derived from the concern that excessive magnesium supplementation can cause osmotic diarrhea, and is based on nonfood sources only.[23]

Potassium

Ketone Labs' potassium salt of BHB contains 25.5–29.5% potassium.

Potassium is the most abundant intracellular cation in the body and has a role in muscle contraction, and cardiovascular and genitourinary function.[19, 24] Potassium assists in the regulation of the body's acid-base balance and contributes to establishing a membrane potential in nerve fibers.[25] Total body potassium is about 55 mmol/kg of body weight, 98% of which is distributed in the intracellular fluid (primarily in the muscle, liver, and erythrocytes) and 2% in the extracellular fluid.[26] Potassium is found in all natural foods, including dairy foods, fish, fruit (e.g., apricots, avocados, bananas), legumes, meat, nuts, vegetables (e.g., carrots, onions, spinach, potatoes, winter squash), and coffee.[24, 25, 27]

Potassium is considered GRAS for human consumption as a food ingredient in various forms, most of which are acceptable at levels not to exceed current good manufacturing practice (21 CFR sections 184.1610–184.1643). EFSA and WHO recommend potassium intakes of at least 3,500 or 3,510 mg/day, respectively, for adults and less than that for children aged 2–15 years old, based on the energy requirements of children relative to those of adults.[27] The current FDA adequate intake (AI) recommendation for potassium is 4,700 mg/day for adults and teens aged 14–18 years; 4,500 mg/day for aged 9–13; 3,800 mg/day for children aged 4–8 years; 3,000 mg/d for children aged 1–3 years; and 700 and 400 mg/day respectively for those 7–12 and 0–6 months old. The AIs are used for potassium since a DRI could not be established due to insufficient data from dose response trials.[19] A UL for potassium is not established because in a healthy population, potassium intake from foods in amounts greater than the AI poses no potential for increased risk as excess potassium is easily excreted in the urine; in individuals with urinary potassium excretion impairment (e.g., people with kidney failure, who are usually under the care of a physician), potassium intake below the AI would be appropriate.[19, 25] According to dietary surveys, the median intake of dietary potassium in the US is about 2,200–2,400 mg/day for adult women and 2,800–3,300 mg/day for adult men.[19, 28] EFSA notes that while there is no upper limit for potassium, there have been a few case reports in which supplemental potassium doses of 5,000–7,000 mg have caused adverse effects on heart function in healthy adults.[25] Thus, EFSA considers the risk of adverse effects from potassium intake from food sources (up to 5,000–6,000 mg/day) in adults to be low for the general healthy population.[25]

Several potassium chloride supplement studies have reported gastrointestinal (GI) side effects, such as discomfort, esophageal injury and small-bowel ulceration, specifically and most often with slow-release, wax-matrix, potassium chloride (KCl) tablets.[29, 30] Doses in those studies ranged from 0.8 to 3.6 g KCl/day (0.42 to 1.9 g of potassium). While in some cases, there were co-occurring medical conditions in the subjects such as cardiac enlargement and multiple medications, some adverse effects were also observed in healthy volunteers who ingested the wax-matrix form of KCl.[29-31] Authors concluded that the supplemental wax-matrix slow-release potassium played a role in the undesirable GI effects, potentially due to the tablet not moving past the esophagus in supine patients or in those with esophageal compression from other causes.[30] To further investigate, McMahon et al. (1982) performed a randomized clinical trial to compare the effect of microencapsulated KCl with that of the wax-matrix formulation on the esophageal and duodenal mucosa in 48 healthy male volunteers.[31] Participants underwent upper GI endoscopy before and after seven days of ingesting their respective form of KCl, 2.4 g, three times daily. Results showed that those taking the wax-matrix preparation had dramatically higher occurrences of mucosal pathology than those taking the microencapsulated form. Over the three phases of the study, the wax-matrix group's gastroscopy rating was 97 (the scoring represents instances of hyperemia, erosions, and ulcerations), whereas the microencapsulated group's score was 4. In another trial where participants (n=175) ingested 2.3 g/day of microencapsulated potassium chloride for 6 months, no occurrences of gastrointestinal upset were reported.[32] The McMahon et al. study results also showed that the presence or absence of symptoms were not a reliable indicator of GI mucosa status; 18 of the 24 subjects receiving the wax-matrix KCl had endoscopic lesions, but only 5 of the 18 experienced epigastric discomfort. Additionally, slower intestinal transit time increases the likelihood of GI lesion development. The Food and Nutrition Board Panel on DRI for potassium concluded that the specific product and vehicle was a critical element in the increased number of side effects from supplemental potassium.[19]

Manufacturing, Production, and Quality Management

Manufacturing Overview

The manufacturing flowcharts of Ketone Labs' BHB salts can be found below and in **Appendix A.**

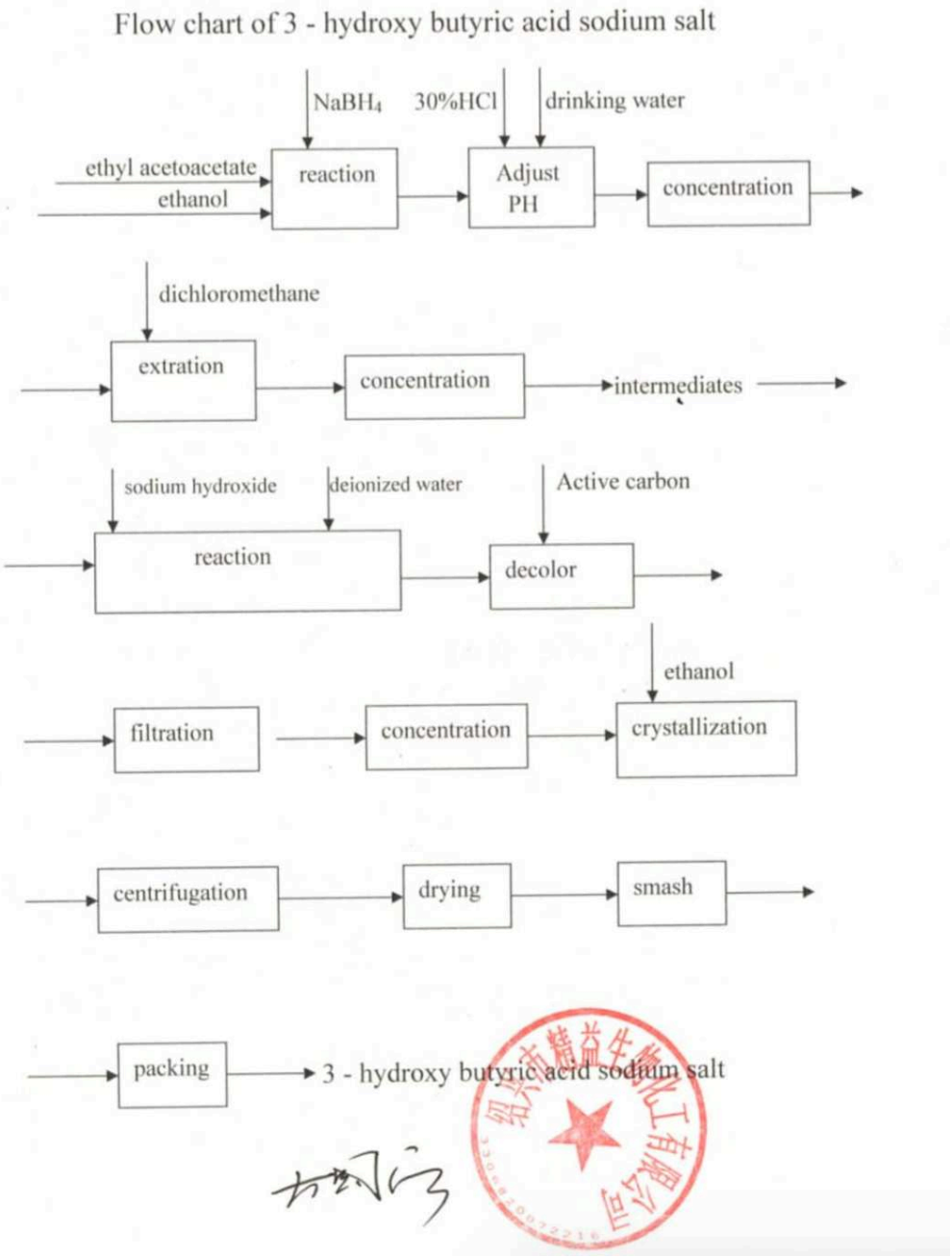

Figure 7. Manufacturing Flowchart for Sodium BHB

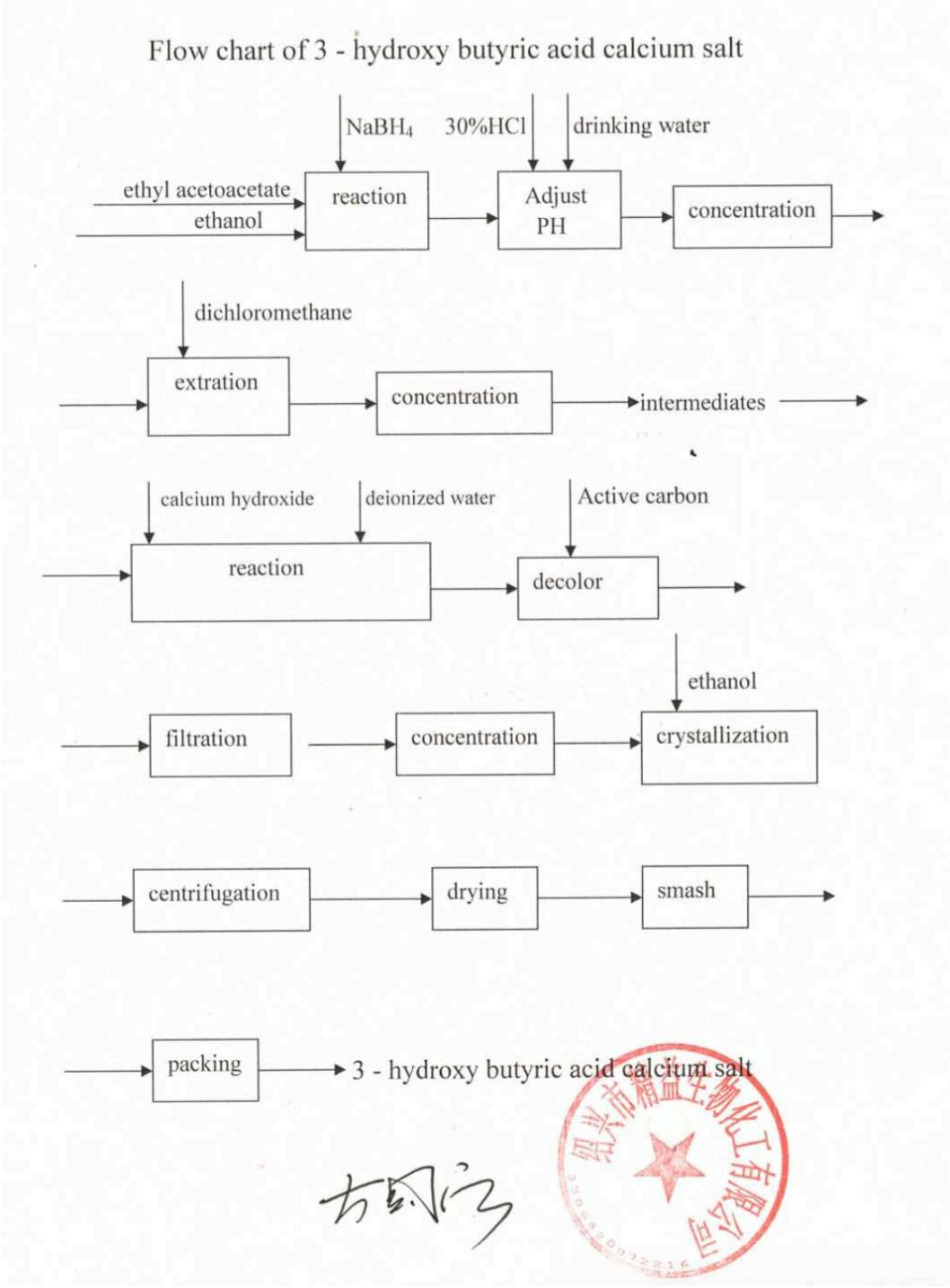

Figure 8. Manufacturing Flowchart for Calcium BHB

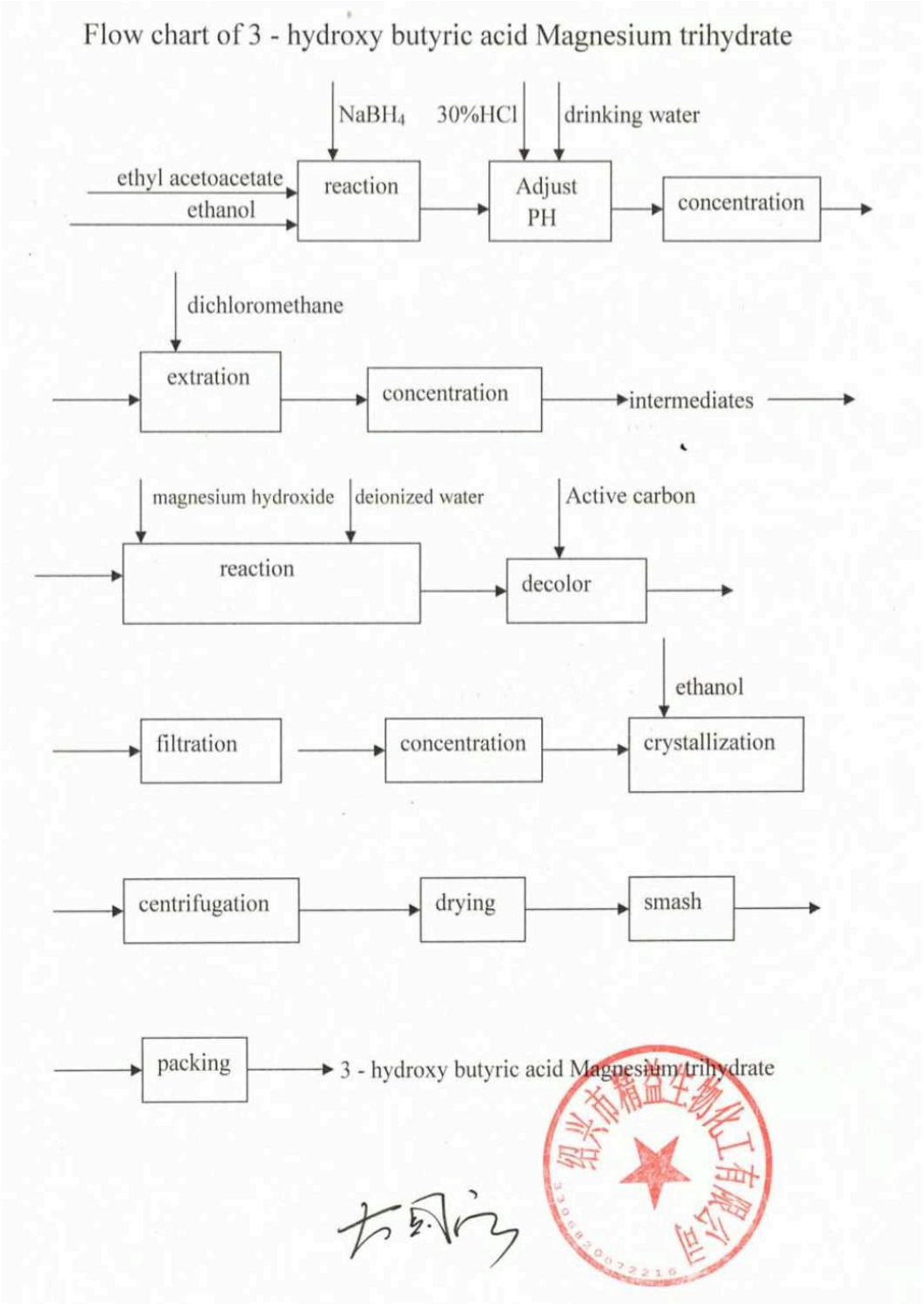

Figure 9. Manufacturing Flowchart for Magnesium BHB

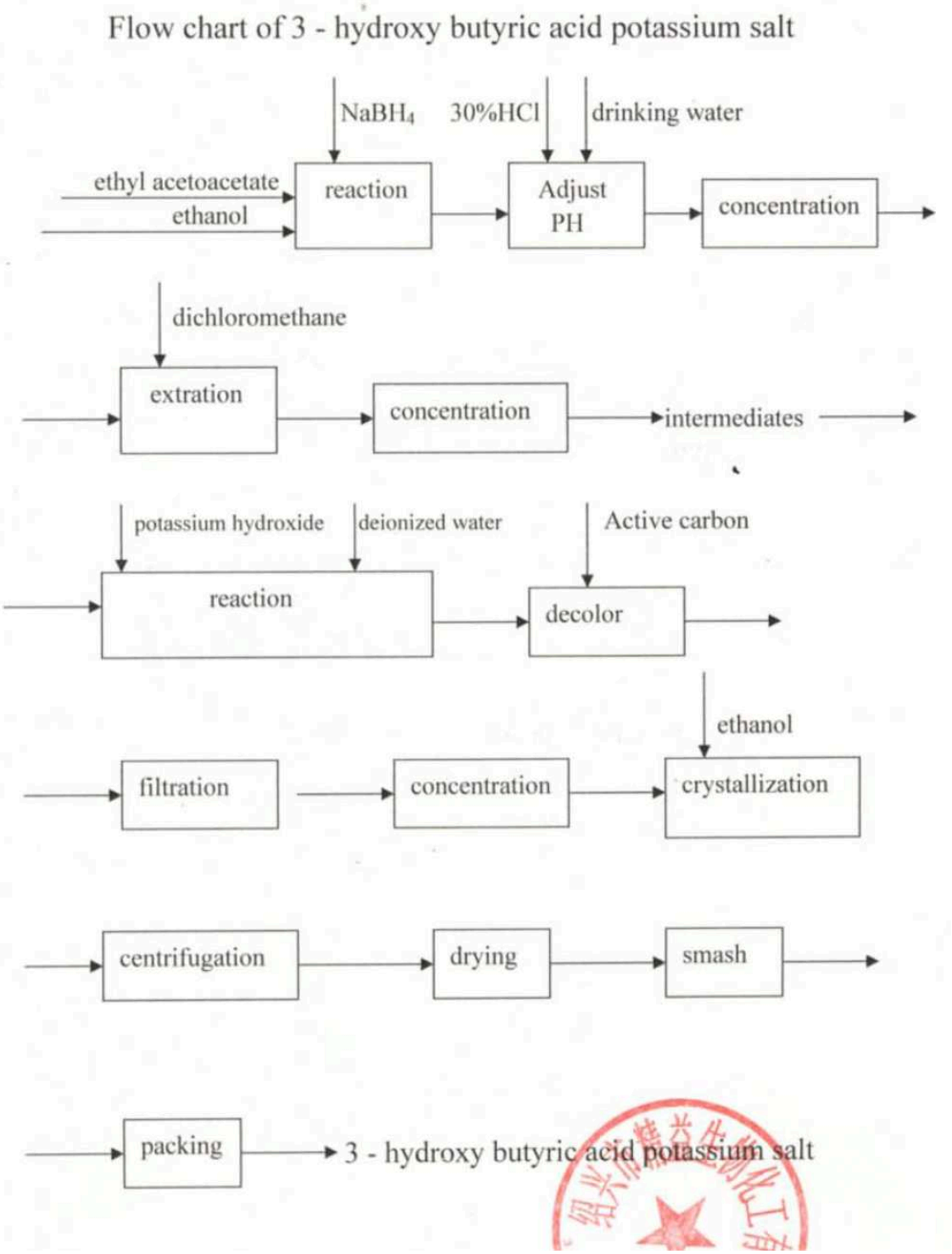

Figure 10. Manufacturing Flowchart for Potassium BHB

Good Manufacturing Practice

BHB salts from Ketone Labs are produced under strict adherence to current GMP standards set to comply with the U.S. Code of Federal Regulations, 21 CFR part 110 (see **Appendix B**).

Specifications

The product specifications for BHB salts along with the specification methods are listed in **Tables 1–4** below (see **Appendix C**).

Ketone Labs tests for residual solvents via a periodic testing schedule, therefore, these are not found on the specifications below, which indicate batch-by-batch analysis. The specification for dichloromethane is set at "non detectable", with a detection limit of 0.05 ppm. Other residual solvents have specifications of no more than (NMT) 0.5%. Ketone Labs tested six batches of BHB salts from October 1, 2016–December 1, 2016 and found that they all met these residual solvent specifications. Due to the reliability of the manufacturing and quality control processes, Ketone Labs now tests every fifth batch to ensure compliance with residual solvent specifications and adheres to standard operating procedures (SOPs) to reject any batch that does not meet these specifications. An example of residual solvent analyses on BHB salts can be found in **Appendix D.**

Table 1. Sodium BHB Specifications

Test Items	Specification	Method
Chemical Tests		
Assay	NLT 98.0%	Internal
Sodium	18.0–19.0%	CP2015
BHB (free)	NLT 80%	Internal
Physical Characteristics		
Appearance	White crystalline powder	Visual
Loss on drying	NMT 1.0%	CP2015
Bulk density (g/mL)	NLT 0.35	Internal
Tapped density (g/mL)	NLT 0.60	Internal
Heavy Metals		
Total Heavy Metals	NMT 10 ppm	CP2015
Lead	NMT 3 ppm	GB5009. 12-2010, GFAAS
Arsenic	NMT 1 ppm	GB5009. 11-2003, AFS
Cadmium	NMT 1 ppm	GB5009. 15-2014, GFAAS
Mercury	NMT 0.1 ppm	GB5009. 17-2003, AFS
Microbiological Tests		
Total Plate Count	NMT 1000 cfu/g	GB4789.15-2010
Yeast & Mold	NMT 100 cfu/g	GB4789.15-2010
E. coli	Negative/25g	GB4789.3-2010
Salmonella	Negative/25g	GB4789.4–2010

*NLT=not less than; NMT=not more than; CP=Chinese Pharmacopeia; GB=Chinese National Food Safety Standard

Table 2. Calcium BHB Specifications

Test Items	Specification	Method
Chemical Tests		
Assay	NLT 98.0%	Internal
Calcium	16.0–17.0%	Internal
BHB (free)	NLT 82.0%	Internal
Physical Characteristics		
Appearance	White crystalline powder	Visual
Loss on drying	NMT 4.0%	CP2015
Bulk density (g/mL)	NLT 0.30	Internal
Tapped density (g/mL)	NLT 0.55	Internal
Heavy Metals		
Total Heavy Metals	NMT 10 ppm	CP2015
Lead	NMT 3 ppm	GB5009. 12-2010, GFAAS
Arsenic	NMT 1 ppm	GB5009. 11-2003, AFS
Cadmium	NMT 1 ppm	GB5009. 15-2014, GFAAS
Mercury	NMT 0.1 ppm	GB5009. 17-2003, AFS
Microbiological Tests		
Total Plate Count	NMT 1000 cfu/g	GB4789.2-2010
Yeast & Mold	NMT 100 cfu/g	GB4789.15-2010
E. coli	Negative/25g	GB4789.3-2010
Salmonella	Negative/25g	GB4789.4-2010

*NLT=not less than; NMT=not more than; CP=Chinese Pharmacopeia; GB=Chinese National Food Safety Standard

Table 3. Magnesium BHB Specifications

Test Items	Specification	Method
Chemical Tests		
Assay	NLT 98.0%	Internal
Magnesium	8.3–9.0%	Internal
BHB (free)	NLT 71.0%†	Internal
Physical Characteristics		
Appearance	White crystalline powder	Visual
Loss on drying	NMT 19.0%	CP2015
Bulk density (g/mL)	NLT 0.20	Internal
Tapped density (g/mL)	NLT 0.50	Internal
Heavy Metals		
Total Heavy Metals	NMT 10 ppm	CP2015
Lead	NMT 3 ppm	GB5009. 12-2010, GFAAS
Arsenic	NMT 1 ppm	GB5009. 11-2003, AFS
Cadmium	NMT 1 ppm	GB5009. 15-2014, GFAAS
Mercury	NMT 0.1 ppm	GB5009. 17-2003, AFS
Microbiological Tests		
Total Plate Count	NMT 1000 cfu/g	GB4789.2-2010

Yeast & Mold	NMT 100 cfu/g	GB4789.15-2010
E. coli	Negative/25g	GB4789.3-2010
Salmonella	Negative/25g	GB4789.4-2010

*NLT=not less than; NMT=not more than; CP=Chinese Pharmacopeia; GB=Chinese National Food Safety Standard

† The remaining % of BHB Magnesium Trihydrate is water (~19%)

Table 4. Potassium BHB Specifications

Test Items	Specification	Method
Chemical Tests		
Assay	NLT 98.0%	Internal
Potassium (ICP-MS)	25.5–29.5%	cCP(0412)
BHB (free)	NLT 70.0 %	Internal (HPLC)
Physical Characteristics		
Appearance	White crystalline powder	Visual
Loss on drying	NMT 1.0%	cCP(0831)
Mesh	100% pass 30 mesh	cCP(0982)
Bulk density	NLT 0.35 g/mL	cUSP(616)
Tapped density	NLT 0.55 g/mL	cUSP(616)
Heavy Metals		
Total Heavy Metals	NMT 10 ppm	cCP(0821)
Lead	NMT 3.0 ppm	GB5009. 12-2010, GFAAS
Arsenic	NMT 1.0 ppm	GB5009. 11-2003, AFS
Cadmium	NMT 1.0 ppm	GB5009. 15-2014, GFAAS
Mercury	NMT 0.1 ppm	GB5009. 17-2003, AFS
Microbiological Tests		
Total Plate Count	NMT 1000 cfu/g	GB4789.2-2016
Yeast & Mold	NMT 100 cfu/g	GB4789.15-2016
E. coli	Negative/25g	GB4789.3-2016
Salmonella	Negative/25g	GB4789.4-2016

*NLT=not less than; NMT=not more than; CP=Chinese Pharmacopeia; GB=Chinese National Food Safety Standard

Batch Analysis

Production conformity and consistency of Ketone Labs' BHB salts is tested in production lots (**see Tables 5–8**). Three non-consecutive batch analyses for each salt were reasonably consistent and met the product specifications for physical/chemical composition, heavy metals, and microbial analyses (see **Appendix E**).

Table 5. Sodium BHB Batch Analyses

Test Items	Specification	28A-KY20161001	28C-KY20160502	28A-KY20161002
Chemical Tests				
Assay (%)	NLT 98.0	98.3	98.4	98.2
BHB (%)	NLT 80	80.1	80.2	80.1
Sodium (%)	18.0–19.0	18.2	18.2	18.1
Physical Characteristics				
Appearance	White crystalline powder	White crystalline powder	White crystalline powder	White crystalline powder
Loss on drying (%)	NMT 1.0	0.12	0.09	0.11
Bulk density (g/mL)	NLT 0.35	0.43	0.48	0.45
Tapped density (g/mL)	NLT 0.60	0.72	0.71	0.74
Heavy Metals				
Total Heavy Metals	NMT 10 ppm	≤10 ppm	Conforms	Conforms
Lead	NMT 3 ppm	≤3 ppm	Conforms	Conforms
Arsenic	NMT 1 ppm	≤1 ppm	Conforms	Conforms
Cadmium	NMT 1 ppm	≤1 ppm	Conforms	Conforms
Mercury	NMT 0.1 ppm	≤0.1 ppm	Conforms	Conforms
Microbiological Tests				
Total Plate Count	NMT 1000 cfu/g	≤1000 cfu/g	Conforms	Conforms
Yeast & Mold	NMT 100 cfu/g	≤100 cfu/g	Conforms	Conforms
E. coli	Negative/25g	Negative	Negative	Negative
Salmonella	Negative/25g	Negative	Negative	Negative

*NLT=not less than; NMT=not more than

Table 6. Calcium BHB Batch Analyses

Test Items	Specification	28C-LY20161001	28C-KY20161002	28C-KY20160501
Chemical Tests				
Assay (%)	NLT 98.0	98.4	98.3	98.5
BHB (%)	NLT 82	82.2	82.1	82.2
Calcium (%)	16.0–17.0	16.2	16.2	16.3
Physical Characteristics				
Appearance	White crystalline powder	White crystalline powder	White crystalline powder	White crystalline powder
Loss on drying (%)	NMT 4.0	0.28	0.29	0.15
Bulk density (g/mL)	NLT 0.30	0.34	0.33	0.40
Tapped density (g/mL)	NLT 0.55	0.65	0.64	0.68
Heavy Metals				
Total Heavy Metals	NMT 10 ppm	Conforms	Conforms	Conforms
Lead	NMT 3 ppm	Conforms	Conforms	Conforms
Arsenic	NMT 1 ppm	Conforms	Conforms	Conforms
Cadmium	NMT 1 ppm	Conforms	Conforms	Conforms
Mercury	NMT 0.1 ppm	Conforms	Conforms	Conforms
Microbiological Tests				

Total Plate Count	NMT 1000 cfu/g	Conforms	Conforms	Conforms
Yeast & Mold	NMT 100 cfu/g	Conforms	Conforms	Conforms
E. coli	Negative/25g	Negative	Negative	Negative
Salmonella	Negative/25g	Negative	Negative	Negative

*NLT=not less than; NMT=not more than

Table 7. Magnesium BHB Trihydrate Batch Analyses

Test Items	**Specification**	**28D-KY20160501**	**28D-KY20160101**	**28D-KY20161001**
Chemical Tests				
Assay (%)	NLT 98.0	98.5	98.7	98.7
BHB (%)	NLT 71.0	71.5	71.5	72.4
Magnesium (%)	8.3–9.0†	8.6	8.7	8.7
Physical Characteristics				
Appearance	White crystalline powder	White crystalline powder	White crystalline powder	White crystalline powder
Loss on drying (%)	NMT 19.0	18.4	18.5	17.6
Bulk density (g/mL)	NLT 0.20	0.28	0.28	0.29
Tapped density (g/mL)	NLT 0.50	0.59	0.60	0.61
Heavy Metals				
Total Heavy Metals	NMT 10 ppm	Conforms	Conforms	Conforms
Lead	NMT 3 ppm	Conforms	Conforms	Conforms
Arsenic	NMT 1 ppm	Conforms	Conforms	Conforms
Cadmium	NMT 1 ppm	Conforms	Conforms	Conforms
Mercury	NMT 0.1 ppm	Conforms	Conforms	Conforms
Microbiological Tests				
Total Plate Count	NMT 1000 cfu/g	Conforms	Conforms	Conforms
Yeast & Mold	NMT 100 cfu/g	Conforms	Conforms	Conforms
E. coli	Negative/25g	Negative	Negative	Negative
Salmonella	Negative/25g	Negative	Negative	Negative

*NLT=not less than; NMT=not more than

† The remaining % of BHB Magnesium Trihydrate is water (~19%)

Table 8. Potassium BHB Batch Analysis

Test Items	**Specification**	**28B-KY20170403**	**28B-KY20170602**	**28B-KY20170604**
Chemical Tests				
Assay	NLT 98.0%	98.4	98.6	98.5
BHB	NLT 70.0%	71.3	71.5	71.4
Potassium	25.5–29.5%	27.1	27.1	27.1
Physical Characteristics				
Appearance	White crystalline powder	White crystalline powder	White crystalline powder	White crystalline powder

Loss on drying	NMT 1.0%	0.16	0.14	0.23
Mesh	100% pass 30 mesh	Conforms	Conforms	Conforms
Bulk density	NLT 0.35 g/mL	0.40	0.61	0.51
Tapped density	NLT 0.55 g/mL	0.59	0.83	0.71
Heavy Metals				
Total Heavy Metals	NMT 10 ppm	Conforms	Conforms	Conforms
Lead	NMT 3.0 ppm	Conforms	Conforms	Conforms
Arsenic	NMT 1.0 ppm	Conforms	Conforms	Conforms
Cadmium	NMT 1.0 ppm	Conforms	Conforms	Conforms
Mercury	NMT 0.1 ppm	Conforms	Conforms	Conforms
Microbiological Tests				
Total Plate Count	NMT 1000 cfu/g	Conforms	Conforms	Conforms
Yeast & Mold	NMT 100 cfu/g	Conforms	Conforms	Conforms
E. coli	Negative/25g	Negative	Negative	Negative
Salmonella	Negative/25g	Negative	Negative	Negative

*NLT=not less than; NMT=not more than

Shelf–Life Stability

Shelf life stability studies were conducted on Ketone Labs' sodium BHB, lot number 28A-KY20160501 under conditions of 40 °C and 75% relative humidity (see **Appendix F**). Sodium BHB was tested at the study initiation and at 1, 3, 4, and 5 months thereafter. At the 5-month point, BHB was still present at 79.65–84.2%, with only one of the four data points below its specification of NLT 80% BHB.

Safety Assessment

Toxicology Studies

Genotoxicity, 28-day repeated-dose intravenous and reproductive toxicity studies on the sodium salt of D-beta-hydroxybutyrate (KTX 0101)

A published toxicological evaluation of KTX 0101 (97.9–100% sodium BHB) included genotoxicity studies, 4-week repeated dose intravenous (IV) studies, and reproductive toxicity studies (also IV dosing) that are outlined below. Many details of these studies were not adequately reported; however, overall they demonstrate a lack of toxic effects of IV-administered sodium BHB in various species.[11] While studies utilizing IV dosing tend not to be extremely relevant when evaluating oral dosing safety, we discuss IV dosing here because of normal human endogenous BHB production, which places BHB directly into the circulation. The authors reported that the genotoxicity studies indicated the substance had no

mutagenic potential in bacterial reverse mutation, in vitro mammalian chromosomal aberration or in vivo mouse micronucleus assays, although the data were not shown in the publication.

In single dose IV tolerability studies, KTX 0101 was administered at levels of ≤3800 mg/kg in rats and ≤4000 mg/kg in dogs (dose groups not clear in report). In rats, 1/5 females died in both the 2400 and 3000 mg/kg dose groups. At 3800 mg/kg, 2/5 rats died in both the male and female groups. The females that died were found lying prone and tachycardic, while the males experienced decreases in motor activity and respiratory rate, clonic convulsions, prostate posture and self-soiling. In all surviving animals, all symptoms rapidly reversed within 6 hours. Cerebral hemorrhage was noted post mortem in 2/2 females and 1/2 males that died, and at the highest doses of KTX 0101, lesions of the kidneys were also observed "in some animals" (specifics not given). These findings were considered to be a direct consequence of the volume and nature of the solution (not defined in the report other than to say it was mildly to markedly hypertonic) used to inject KTX 0101 and not a toxic effect of the test article itself. Authors reported no other significant findings and reported that no overt signs of toxicity were observed in rats at doses of ≤2000 mg/kg in females and ≤3000 mg/kg in males. In dogs, no deaths occurred at any dose. Increased micturition and drinking were observed at 2000 mg/kg and at 3000 mg/kg, and these effects were combined with dryness of the nasal septa (report misuses the term "nasal speculum" instead of nasal septa), attenuated pupillary reflex and salivation. At 4000 mg/kg, staggering, tachypnea, tachycardia, hyperthermia and hind limb extension were observed. These symptoms reversed within 24 hours. No abnormalities were observed at necropsy, with the exception of reduced spleen weight at the highest dose (significance not reported), the toxicological relevance of which was considered equivocal (no explanation was provided). The maximum tolerated intravenous dose (MTD) for repeated administration was concluded to be 2000 mg/kg bw/day for rats and dogs.

Four-week repeated-dose IV studies were also performed in rats and dogs at levels of 500, 1000 and 2000 mg/kg bw/day. In rats, decreases in RBC, Hg and Hct were observed at 2000 mg/kg bw/day. Spleen weights were also increased at this dose and histopathological examination revealed extramedullary, erythrocytic hematopoiesis, which was reversed upon cessation of test article administration. No signs of toxicity were observed in rats at doses ≤1000 mg/kg bw/day. In dogs, 2000 mg/kg bw/day induced vomiting, salivation, decreased motor activity and reddish urine, which was transient and observed during and immediately after dosing. No changes were observed in body weight, food consumption, or hematological and blood chemistry parameters. Further, upon necropsy, no changes were noted in organ weights or upon histopathological examinations. No

signs of toxicity were observed at doses ≤1000 mg/kg bw/day in dogs. This study identified a NOAEL of 1000 mg/kg bw/day (~810 mg/kg bw/day BHB) (IV dose) of KTX 0101 in both rats and dogs.

Lastly, reproductive toxicity studies were conducted in female rabbits and both sexes of rats at IV doses of 500, 1000 and 2000 mg/kg bw/day (length of study not reported). At 2000 mg/kg bw/day, 2/6 rabbits died, indicating that this intravenous dose is close to the lethal limit in this species. In three female rats, a prolonged diestrus occurred in the 2000 mg/kg bw/day group, but the toxicological relevance of this finding was considered equivocal. No effects on reproductive function, fertility (in either sex) or fetal development were found in either species. It was concluded that IV administration of 2000 mg/kg bw/day of KTX 0101 had no toxic effects on reproductive function or fetal development in either species.

These studies indicate a low toxicity of KTX 0101 and of the sodium salt of BHB by the IV route of administration. The osmotic load, sodium levels, volume load and tonicity of the solution (solution not defined in the report other than to say it was mildly to markedly hypertonic) were all considered to have contributed to the findings in the toxicological assessments.

Oral 28-day and developmental toxicity studies of (*R*)-3-hydroxybutyl (*R*)-3-hydroxybutyrate, published by Clarke et al. (2012).[33]

Available data indicates that (R)-3-Hydroxybutyl (R)-3-hydroxybutyrate is rapidly hydrolyzed to BHB and 1,3-butanediol in the gut; the latter is then further metabolized to BHB and acetoacetate in the liver.[33] Therefore, the subchronic and developmental studies described below on this BHB ketone ester are considered relevant to the toxicity of BHB.

A 28-day feeding study was conducted according to GLP and the study design and sample sizes were "based on" the US FDA Redbook 2000:IV.C.3a in Crl:WI (Wistar) rats. Males (337–357 g) and females (200–225 g), 9 weeks of age, were randomized into groups (10 animals/sex/group) to receive a test diet containing the test article (30% of calories from ketone ester), or one of two control diets, carbohydrate (CHO) (69% of calories from CHO of which 14.5% was added corn starch) or fat (34% of calories from fat of which 5.8% was added palm oil). The diets were matched for protein content. During the test period, the ketone ester diet was offered *ad libitum.* The control animals received an amount of food approximately equal to the amount consumed by ketone ester-fed rats that consumed most of their daily rations. The rats received a mean of 12 and 15.1 g/kg bw/day of the ketone ester for male and female rats, respectively. Animals were observed twice daily for changes in skin, fur, eyes and mucous membranes and respiratory, circulatory, autonomic, central nervous system, somatomotor activity and behavioral patters. Clinical signs were recorded once daily (afternoon

observations were also recorded if they differed from the morning observations) and all animals received a detailed clinical examination once a week. Each animal was weighed prior to randomization, and before dosing on days 1, 8, 15, 22, 28, and prior to necropsy on day 29. Hematology, clinical chemistry and urinalysis parameters were evaluated. Gross examinations were conducted on all external surfaces of the body; all orifices; cranial cavity; external surfaces of the brain and spinal cord; nasal cavity and paranasal sinuses; joints; thoracic, abdominal and pelvic cavities; and viscera. Organ weights (adrenals, brain, pituitary gland, prostate, heart, spleen, kidneys, liver, thymus, testes, lungs, ovaries, uterus, and seminal vesicles) were recorded and relative weights calculated. Histopathological examinations were performed on the liver, kidneys, stomach, duodenum, jejunum, ileum, colon, brain, heart and skeletal muscles.

All animals survived to necropsy. No treatment-related clinical signs of toxicity were observed in daily cage side observations or weekly physical examinations, with the exception of one male that had piloerection, decreased food consumption and weight loss. The piloerection ceased after three weeks, and although the animal did not fully regain the initial body weight, by the end of the study the rat was gaining weight and appeared normal. Animals from the male and female ketone ester groups and both male control groups lost weight during the first 8–12 days of the study, but regained weight thereafter. The ketone ester-fed rats consumed significantly less food and gained significantly less weight than the animals fed control diets. These findings were attributed to the change and palatability of the diets. Additionally, reduced food consumption and weight gain are consistent with reports of reduced energy intake and reduced body weight in humans consuming ketogenic diets.[34, 35]

Various hematological parameters and clinical chemistry parameters were significantly different in the ketone-fed male and female rats compared to controls, and although historical data was not presented, the authors reported that all of the findings remained within normal historical control ranges (**see Tables 9 and 10**). The only exception was serum LDH, which was significantly increased in ketone ester-fed males compared to animals fed either of the control diets. Levels were slightly above the upper limit of the historical range of the testing facility. However, these increases were small in magnitude and were not associated with toxicological changes in hemolytic or histological findings in the heart, liver, or kidneys. No significant differences were noted in urinalysis parameters (data not shown).

Table 9. Summary of Selected Hematological Findings in the 28-day Repeated Dose Toxicity Study

Group	RBC	HGB	HCT	MCV	MCH	MCHC	PLT	RET	APTT	Fibrinogen

	$x10^{12}$/L	g/L	%	fL	pg	g/L	$x10^{9}$/L	x109/L	Sec	g/L
Males										
CHO Diet	7.62 ± 0.44	146 ± 8	39.3 ± 2.1	51.5 ± 1.2	19.1 ± 0.5	371 ± 2	1303 ± 145	194 ± 30	16.1 ± 1.7	2.6 ± 0.29
Fat Diet	7.80 ± 0.32	146 ± 7	39.2 ± 1.8	50.3 ± 1.0	18.7 ± 0.4	371 ± 5	1376 ± 149	193 ± 33	16.7 ± 2.2	2.41 ± 0.19
Ketone Ester Diet	8.41 ± 0.28*†	158 ± 8*†	42.1 ± 1.9*†	50.0 ±1.1*	18.8 ± 0.5	375 ± 6	1387 ± 218	229 ± 36*†	13.8 ± 2.2*†	2.57 ± 0.30
Females										
CHO Diet	7.73 ± 0.25	153 ± 4	40.0 ± 1.4	51.7 ± 1.1	19.8 ± 0.5	382 ± 5	1316 ± 125	141 ± 25	15.1 ± 1.9	2.18 ± 0.2
Fat Diet	7.47 ± 0.20	143 ± 3	38.1 ± 1.0	51.1 ± 1.4	19.2 ± 0.5	375 ± 6	1482 ± 85	158 ± 34	15.3 ± 1.6	1.98 ± 0.15
Ketone Ester Diet	8.36 ± 0.40*†	160 ± 6*†	41.8 ±1.6*†	50.1 ± 1.5*	19.1 ± 0.5*	382 ± 4†	1146 ± 252†	169 ± 42	14.6 ± 1.9	2.21 ± 0.19†

Data represent the mean values and the standard deviation.

Only parameters with statistically significant findings are shown in table.

*=significantly different compared to the CHO diet; †=significantly different compared to the fat diet

Table 10. Summary of Selected Clinical Chemistry Findings in the 28-day Repeated Dose Toxicity Study

Group (mg/kg bw/d)	A/G	ALP u/L	ALB g/L	BIL µmol/L	LDH u/L	K mmol/L	ALT u/L	CK u/L	CHOL mmol/L	LDH u/L	Na mmol/L	TRI mmol/L
Male (n=10 each)												
CHO Diet	1.2 ± 0.1	140 ± 32	29 ± 1	3.4 ± 0.8	751 ± 381	4.5 ± 0.4	39 ± 5	109 ± 50	1.56 ± 0.23	751 ± 381	142 ± 1	0.62 ± 0.17
Fat Diet	1.2 ± 0.1	160 ± 41	29 ± 2	3.2 ± 0.9	636 ± 447	4.4 ± 0.4	41 ± 6	92 ± 63	1.95 ± 0.38	636 ± 447	141 ± 2	0.51 ± 0.08
Ketone Ester Diet	1.3 ± 0.1†	145 ± 23	32 ± 2*†	5.0 ± 1.7*†	1205 ± 676†	4.9 ± 0.3†	47 ± 8*	175 ± 96†	2.29 ± 0.49*	1205 ± 676†	140 ± 1*	0.67 ± 0.13†
Female (n=10 each)												
CHO Diet	1.5 ± 0.2	105 ± 24	36 ± 3	4.4 ± 0.4	1230 ± 581	4.0 ± 0.2	32 ± 5	179 ± 92	1.59 ± 0.23	1230 ± 581	142 ± 1	0.72 ± 0.09
Fat Diet	1.4 ± 0.1	148 ± 44	35 ± 3	6.2 ± 6.4	1278 ± 376	4.1 ± 0.5	34 ± 11	167 ± 42	1.96 ± 0.36	1278 ± 376	141 ± 2	0.68 ± 0.10
Ketone Ester Diet	1.4 ± 0.1	106 ± 23†	34 ± 2	4.9 ± 0.8	1876 ± 662*†	4.2 ± 0.4	38 ± 7	265 ± 115	1.99 ± 0.35**	1876 ± 662*†	140 ± 1*	0.75 ± 0.15

Data represent the mean values and the standard deviation.

Only parameters with statistically significant findings are shown in table.

*=significantly different compared to the CHO diet; †=significantly different compared to the fat diet

No statistically significant differences were found in organ weights (absolute or relative to body/brain weights) with the exception of absolute weights of the uterus. The absolute uterine weights in the ketone-fed rats were significantly lower compared to controls, but remained within historical controls. No differences were found in relative uterine weights; therefore, it was considered that the lower uterine weights were a result of the lower body weight of the female rats fed the ketone diet and not an adverse effect (microscopic examination was not performed on uterine tissues). Two male rats and four female rats that received the fat diet, as

well as one female ketone-fed rat had slight yellow discoloration of the livers, which were presumed to be fat accumulation.

Upon histopathological examination, several hepatocellular findings were also noted (see **Table 11**). An increase in the round, clear, sharply demarcated cytoplasmic vacuoles (interpreted as lipid) were observed in females of all three groups and in two males of the fat diet. In the females, small microvesicles were distributed throughout the cytoplasm, some distended by single or multiple large vacuoles and some cells were presumed to be perisinusoidal stellate cells. This pattern of vacuolation is consistent with a mild form of steatosis. Additionally, necroinflammatory foci were observed in all groups and in both sexes. These findings were not considered test article-related due to the fact that they occurred in all test groups and liver function enzymes were within normal ranges. Several animals in all three groups also had findings of myocyte necrosis and repair and focal histiocytosis. These muscle changes were graded as minimal with the exception of one male and one female in the ketone ester group, in which these findings were graded as mild. In heart tissue, microfocal myocardial fibrosis was found in two animals in the fat diet group and one in the ketone ester group (sex not specified). These lesions are known to be detected in the early stages of murine progressive cardiomyopathy, a spontaneous background condition in rats, and therefore, were not considered to be toxicologically relevant. No other histopathological findings were attributed to administration of the test article.

Table 11. Summary of Reported Histopathology Findings in a 28-day Repeated Dose Toxicity Study

	Group	Fat diet control	Carb diet control	30% ketone ester BHB diet
Organs	Observations	N=10	N=10	N=10
Males				
Liver	Slight yellow discoloration	2/10	0/10	0/10
	Minimal vacuolation	2/10	0/10	0/10
	Minor necroinflammatory changes-multiple microfocal non-hematopoeitic clusters of macrophages and undifferentiated mononuclear cells	1/10	3/10	1/10
Muscle	See description in the text above	Data not provided		
Heart	See description in the text above	Data not provided		
Kidney	Tubular basophilia or interstitial inflammation	"Occurred in the kidneys in animals of all three groups" sex not specified		
Females				
Liver	Slight yellow discoloration	4/10	0/10	1/10
	Cytoplasmic vacuoles	Present	Present	Present
	Small microvesicles in otherwise normal cytoplasm	Study authors report that this finding was present in females—specific data was not provided. Authors		

		report that the pattern of vacuolation was consistent with a mild form of fatty liver.		
	Minor necroinflammatory changes-multiple microfocal non-hematopoeitic clusters of macrophages and undifferentiated mononuclear cells	5/10 (mild in 4/10)	10/10 (mild in 0/10)	7/10 (mild in 5/10)
	Lipid vacuolation, minimal or higher	10/10	10/10	10/10
	Lipid vacuolation, mild or greater	10/10	6/10	8/10
	Lipid vacuolation, moderate or greater	3/10	1/10	3/10
Muscle	See description in the text above	Data not provided		
Heart	See description in the text above	Data not provided		
Jejunum	Focal necrosis and mineralization of the germinal center of a Peyer's patch	0/10	0/10	2/10 graded as minimal
Kidneys	Mild nephrocalcinosis	"Observed mainly in females"		
	Tubular basophilia or interstitial inflammation	"Occurred in kidneys of some animals in all three groups" (sex not specified)		

Conclusion: The ketone ester in the diet did not cause adverse effects at 12 and 15.1 g/kg bw/day in male and female Wistar rats, respectively.

The developmental toxicity study was conducted according to GLPs and "based on" US FDA Redbook IV.C.9.b guidelines. Female Crl:WI (Han) rats were mated and those with confirmed pregnancy (either by observation of spermatozoa in a vaginal smear and/or a copulatory plug in situ) were considered to be at day 0 of gestation and randomized (25/group) to the test article or control groups. The test article group was administered BHB ketone ester (described above) at 2000 mg/kg bw/day and the control group was administered water that had been filtered via reverse osmosis at 2 mL/kg bw/day via gavage on gestation days (GD) 6–20. Rats were observed for their general appearance on GD 0, daily before test article administration, hourly after administration for the first four hours and at the end of the normal working day for the first four days of the administration period. Observations were made between 1–2 hours after administration the subsequent days. Body weights and food consumption were recorded daily. On GD 21, blood was collected for hematological and clinical chemistry examinations and rats were sacrificed and examined for gross lesions. Caesarean sections were conducted and the reproductive tract was dissected and examined. The gravid uterus was examined and fetuses were removed. The number and distribution of corpora lutea, implantation sites, placentas, live and dead fetuses and early and late resorptions were recorded and fetuses were examined for external, visceral and skeletal abnormalities.

No toxicologically relevant clinical observations were noted. Maternal body weights were comparable between groups throughout the test period. Significantly decreased body weight gain, body weight corrected for gravid uterine weight, and food consumption were observed in the test article-fed dams compared to control animals. No test-related effects on hematological parameters were noted. Significant reductions in alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were found in the test group compared to controls; however, these decreases are typically not considered toxicologically significant. No gross lesions were noted in the liver at necropsy, therefore, these findings were not considered toxicologically relevant. Pregnancy occurred at similar rates in both groups; in 22 rats in the control group and in 24 rats in the test group. There were no dead fetuses and all measured litter parameters were comparable between groups, with the exception of significantly lower fetal body weights in males of the test group. The differences in body weights were slight (4% decrease) and the average value was stated by the study authors to be within historical control ranges of the test facility. Female fetal body weights were decreased but not statistically. No significant differences were noted in gross external, soft tissue or skeletal malformations. Although the incidence of any fetal abnormality was significantly higher in the test group (alterations/litter = 9 ± 11; fetuses with any alteration = 23 (8%)) compared to controls (alterations/litter = 3 ± 6; fetuses with any alteration = 9 (4%)), this was driven by skeletal variations (data not provided), and the incidence of the variations between groups did not significantly differ for each specific abnormality and therefore, this overall difference was considered to be of no toxicological concern.

Conclusion: The study authors concluded that (*R*)-3-hydroxybutyl (*R*)-3-hydroxybutyrate did not adversely affect the development of rats exposed to the ingredient *in utero* at a level of 2 g/kg bw/day.

Self-Limiting Levels of Use

BHB is known to have a sharp, bitter taste, which will likely restrict its use in common conventional foods.

Past Sales and Reported Adverse Events

No FDA letters regarding concern for safety to companies that market products containing BHB or BHB salts were located. A search of MedWatch, FDA's adverse event reporting program, and FDA's Recalls, Market Withdrawals, & Safety Alerts search engine did not uncover any mention of BHB products. After

supplying more than 6,000 kilos over more than 12 months, there have been no serious adverse events have been reported to Ketone Labs.

Additional Scientific Studies

A recent 28-day study in Sprague-Dawley rats investigated the effects of a sodium/potassium BHB mineral salt (BMS) and other "ketones" (1,3-butanediol, medium chain triglyceride oil, BMS + medium chain triglyceride oil and 1,3 butanediol acetoacetate diester) on blood ketone, triglyceride and lipoprotein levels, as well as on body and organ weights.[36] Although multiple related test articles were investigated, for the dossier at hand, we report the findings specific to BMS. Rats received oral BMS doses of 5 g/kg bw/day (days 1–14) and 10 g/kg bw/day (days 15–28) via gavage for 28 days. Interestingly, BMS did not elicit a significant elevation of blood beta-hydroxybutyrate at any time point. BMS significantly reduced body weight gain in the rats during weeks 2–4, although authors report that the animals' weight stayed within the "healthy weight range" for their age. BMS significantly reduced blood glucose 12 hours post-administration in week 4 of the study. No significant changes were found in total, LDL or HDL cholesterol, or triglycerides compared to control. A significant reduction in spleen weight was noted in the co-administration of BMS and medium-chain triglycerides, but not with BMS alone. Similarly, the co-administration BMS and medium-chain triglycerides resulted in significantly increased liver weight relative to body weight compared to controls; however, this was not seen with BMS administration alone. BMS alone was not associated with any adverse effects in the 28-day repeated dose oral administration in rats.

An unpublished study conducted on Wistar rats (n=50) to examine the effect of (*R*)-3-hydroxybutyl (*R*)-3-hydroxybutyrate on the physical performance and cognitive function of the rat, as well as any effects on the general health of the animals was briefly discussed in Clarke et al. 2014 and in GRN 515. The rats were randomized to receive a Western diet (n=20), a high-CHO diet (n=10), or a ketone ester diet (n=20) (30% of the energy as ketone ester) for 66 days. The average amount of ketone ester consumed was 13.7 g/kg bw/day and plasma BHB levels were found to be approximately 2-fold higher in the ketone ester group than the other groups. After 66 days, body weights and heart weights did not differ between the ketone ester diet and control diets. Plasma cholesterol and triglyceride levels were significantly lower in the ketone ester-fed rats compared to the Western diet-fed rats and plasma glucose was significantly lower in those rats consuming the ketone ester. No adverse effects were noted.

A number of additional animal trials have demonstrated a lack of safety concern with oral and intravenous administration of BHB, as various salts or ketone esters

that are metabolized into BHB, including long-term studies and studies in animals with impaired insulin sensitivity and glucose dysregulation. [37-40] [41, 42]

Human Studies

High dose intravenous (up to 1,463 mg/kg/day) and oral (3,500 mg/kg day) administration of sodium BHB have also been evaluated in a vast number of human clinical trials without serious side effects and appear to have beneficial outcomes.[43-48] [3, 7, 10, 49-51] Several of these studies were conducted in infants and children with metabolic disorders and demonstrated the safety and efficacy of such treatment.[52-54] Side effects from the administration of sodium BHB appear to be mainly gastrointestinal and are mild to moderate in nature.[9, 11]

A human clinical study focused on safety was recently conducted on the ketone ester, (R)-3-hydroxybutyl (R)-3-hydroxybutyrate.[9] Participants (n=36) consumed 140, 357 or 714 mg/kg bw of the ketone ester three times daily (a total of up to 2142 mg/kg bw/day) for five consecutive days. The participants remained within the research center for the five days and were closely monitored for adverse events. The participants returned to the research center seven days following discharge for a follow-up examination. Blood levels of BHB and blood glucose were assessed throughout the five days to ensure hyperketonemia, hypoglycemia or hyperglycemia did not develop. Clinical chemistry, hematology and urinalysis parameters were measured at screening, admission (prior to first dose), discharge (morning of day 6) and at the 7-day follow-up. Adverse event monitoring, physical examination and vital sign measurements were conducted at screening, on each day of dosing (prior to administration), 1–2 hours post administration, at discharge, and at the 7-day follow-up. Possible treatment-related adverse events occurred in 4 out of 12 participants in the low dose group, 1 out of 12 participants in the mid-dose group, and in 12 out of 12 participants in the high dose group. The ketone ester was considered “well tolerated” in the low and mid-dose groups; the adverse events were mild and only “possibly” related to the test article. The noted adverse events in all groups were mainly gastrointestinal in nature, including flatulence, nausea, diarrhea, constipation, vomiting and abdominal pain that were mild to moderate in severity, resulting in the discontinuation of two participants. It was noted that the gastrointestinal side effects may also have been due to the consumption of large volumes of the milk-based drink that was the carrier material for the test article (3.3 liters of drink per day in the high dose group). Other adverse events included headaches, dizziness, lethargy and somnolence, deemed “probable” in relation to the test article. These events were considered mild in severity and all adverse events were resolved by the end of the study, with the exception of one positive fecal occult test in the lowest dose group (420 mg/kg bw/day). In all dose groups, vital signs were stable, no treatment-related abnormalities were noted on physical exam, and no abnormal changes were found

in hematology, clinical chemistry and urinalysis parameters.

Johns Hopkins Hospital recently established an Adult Epilepsy Diet Center (AEDC) due to an increasing demand to provide ketogenic diets to adults with epilepsy. In a prospective, open-label, observational five-year study, the hospital enrolled 168 adults (ages 18–86) who began or continued on a ketogenic diet after the initial clinic visit from August 2010 to September 2015.[55] The median diet duration for those already on or naïve to diet therapy was 32 months and 25 months, respectively, with 78% and 37% achieving ≥50% seizure reduction. Hyperlipidemia (39%) and weight loss (19%) were the most common side effects associated with the ketogenic diet. The authors reported that hyperlipidemia has been shown in previous studies to reverse spontaneously in the majority of patients within the first year of treatment. Weight loss was often intended or a welcomed effect. The only other side effects with greater than 5% incidence were worsening seizures and gastrointestinal discomfort. It was concluded that ketogenic diets were a safe and effective long-term treatment for adult epilepsy.

Regulatory Opinions

JECFA concluded (based on studies performed in the 1970's) that short-term metabolic studies indicated that 1,3-butanediol, a metabolic precursor to BHB, was without toxic effects in humans at levels up to 10% of total dietary energy, despite its hypoglycemic effects.[56] Additionally, the administration of 1,3-butanediol for two years had no toxic effects in rats (10% of the diet) or dogs (3% of the diet). JECFA determined an acceptable daily intake for humans to be 0–4 mg/kg bw/day. It has been demonstrated that ~100% and 30% of the R and S enantiomers, respectively, of 1,3-butanediol are metabolized to ketones (BHB and acetoacetate).

A GRAS notification (GRN 515) was submitted to the FDA by TdeltaS (Oxfordshire, UK) for their ≥97.5% D-BHB ester ((R)-3-hydroxybutyl (R)-3-hydroxybutyrate) on May 8, 2014. D-BHB ester was determined GRAS for use as an ingredient in sports beverages (liquid or powder form), gels and bars, with a maximum expected intended intake of 1.1 g/kg bw/day. The safety determination was based on a 10-fold margin of safety (MOS) from the 28-day repeated dose feeding toxicity study on ((R)-3-hydroxybutyl (R)-3-hydroxybutyrate) conducted by Clarke et al. described above. A 10-fold MOS was considered acceptable due to the fact that in the 28-day study, the rats consumed a very high level of the ester without toxic effects (12 and 15.1 g/kg bw/day in male and female rats, respectively), the ester is metabolized to endogenous ketone bodies, and a human clinical trial demonstrated that an intake level of the ketone ester of ~150 g/day produced physiological plasma ketone body levels. TdeltaS indicated that the ingredient would be specifically targeted to "high performance athletes rather than

for use in conventional foods for the general population". Because of this, the notifier did not perform a detailed exposure assessment; however, FDA did perform such an analysis and determined that the mean and 90th percentile average daily exposure to the ingredient for persons aged 2 and older was 17 g/person/day and 35 g/person/day, respectively. Because this ingredient is quickly hydrolyzed into D-BHB and 1,3-butanediol upon ingestion, the safety data presented in this notification indirectly supports the safety of BHB salts. A no objection letter from the Agency was received on March 5, 2015.

Intended Use and Estimated Daily Intake

For the purpose of this GRAS self-determination, Ketone Labs BHB salts, manufactured in accordance with GMP, are intended to be used as ingredients in the food categories and at the maximum addition levels shown in **Table 12.** Ketone Labs BHB salts are not intended for use in infant formula, meat, poultry, egg products, catfish, or any products that would require additional regulatory review by USDA. They will generally be marketed toward adult athletes, and are not intended to be intentionally marketed to children. USDA Food Composition Database and nutritionvalue.org databases were searched and no data was found reporting the presence of BHB in foods. Several nutrition websites state that BHB is present in eggs and milk; however, no references were provided. One study located in the literature found the equivalent of approximately 1.2–3.5 mg of BHB per 8 fl oz. of milk.[18]

Table 12. Intended uses for BHB Salts

Food Category	Maximum addition levels (mg/g)							
	Na-BHB	Na / BHB equivalents (Na~18% of NaBHB)	Ca-BHB	Ca / BHB equivalents (Ca~16% of CaBHB)	Mg-BHB	Mg / BHB equivalents (Mg~8.4% of MgBHB)	K-BHB	K / BHB equivalents (K ~27.5% of KBHB)
Bars	25	4.5 / 20.5	25	4.0 / 21.0	18	1.5 / 16.5	100	27.5/72.5
Beverage concentrates, dry, not reconstituted	40	7.2 / 32.8	40	6.4 / 33.6	50	4.2 / 45.8	200	55/145
Nutrition powders	40	7.2 / 32.8	40	6.4 / 33.6	50	4.2 / 45.8	200	55/145
Energy drinks	3.5	0.6 / 2.9	3.5	0.6 / 2.9	5	0.4 / 4.6	20	5.5/14.5
Sports drinks	3.5	0.6 / 2.9	3.5	0.6 / 2.9	5	0.4 / 4.6	20	5.5/14.5
Fluid replacements	3.5	0.6 / 2.9	3.5	0.6 / 2.9	5	0.4 / 4.6	20	5.5/14.5
Nutrition drinks	3.5	0.6 / 2.9	3.5	0.6 / 2.9	5	0.4 / 4.6	20	5.5/14.5

Exposure estimates combine data on the quantity of a particular food category that is consumed with the intended concentration level of an ingredient to be added to that food category. Creme Food Safety software 3.6 (www.cremeglobal.com) was used for the statistical analysis related to estimated consumption levels of Ketone Labs BHB salts. Creme software is a probabilistic modeling tool that uses high-performance computing to predict intake (including total aggregate exposure) of food groups and/or individual ingredients. Creme Food Safety performs calculations using large scale food consumption data sets; in this case, the U.S. National Health and Nutrition Examination Surveys' (NHANES) What We Eat in America (WWEIA) data sets, which are released every two years. NHANES uses a non-consecutive two-day 24-hour dietary-recall protocol for data

collection. In the current assessment, data from individual dietary records from Day 1 and Day 2 of NHANES 2011–2012 survey were utilized within the Creme software.

It should also be noted that this type of intake methodology is generally considered to be a 'worst case' approach as a result of several conservative assumptions made in the consumption estimates. In addition, it is well established that the length of a dietary survey affects the estimated consumption of individual users. Short-term surveys tend to overestimate the level of the average daily intake among consumers, especially at the extremes of distribution.[57]

Estimates derived from Creme of the total aggregate exposures to BHB salts at both the mean and 90th percentiles are shown in **Tables 13, 15, 17, and 19** (absolute consumption as mg/day) and **14, 16, 18, and 20** (consumption relevant to body weight as mg/kg bw/day). The latter estimates were based on each individual's body weight from the survey, as opposed to averaged body weights. Calculations also incorporated the NHANES assigned "sample weights" for each individual in the survey, which measure the number of people in the population represented by that specific person, and helps ensure that the results are representative of the entire U.S. population. Sample weights for NHANES participants incorporate adjustments for unequal selection probabilities and certain types of non-response, as well as an adjustment to independent estimates of population sizes for specific age, sex, and race/ethnicity categories.

The tables below show the aggregate consumption data of BHB salts from all food categories for "Food Consumers", which includes only data from individuals who reported consuming one or more of the food categories over the two-day survey period (see **Appendix G** for full Creme Global data report).

The relative standard error (RSE, calculated by dividing the standard error of the estimate by the estimate itself and multiplying by 100) is a statistical criterion that can be used to determine the reliability of estimates as pertains to the population; the larger the RSE, the less reliable estimate.[58] RSE values of greater than 25–30% are often considered reasonable cut-offs by which to consider a value unreliable.[58, 59] For the purpose of this GRAS determination, an RSE value of greater than 25% was used to indicate that the estimated value was unreliable with regard to representing the population. RSE values are shown in the tables below for the 90th percentile values only, as the 90th percentile values are the most pertinent for the exposure estimates. All values, except those for males aged 0–2, were considered reasonably reliable using the 25% cut-off.

Table 13. Estimated Exposure to NaBHB (mg/day)

Population Group	Age (yrs)	M/F	Food Consumers						90th % RSE
			n	% Total	mg/day				
					Mean	Mean SE	90th	90th SE	
Infants/ Toddlers	0–2	M	35	15.3	696.9	150.9	993.4	619.4	62.4
		F	32	10.9	575.6	54.4	822.2	140.7	17.1
Children	3–11	M	167	25.5	831.1	74.5	1550.1	169.9	11.0
		F	131	23.9	607.8	52.7	1197.4	174.5	14.6
Teenagers	12–19	M	131	30.9	1250.4	98.8	2315.8	242.5	10.5
		F	117	24.5	982.8	86.3	1977.7	267.4	13.5
Adults	20+	M	402	22.0	1441.0	107.8	2540.0	411.9	16.2
		F	355	18.3	892.9	61.4	1718.5	238.2	13.9
Total M/F	All ages	M	735	23.3	1309.0	76.9	2388.9	174.8	7.3
		F	635	19.3	859.9	48.2	1662.5	159.7	9.6
Total population	All ages	Both genders	1370	21.2	1098.7	47.0	2163.9	99.0	4.6

SE = standard error; RSE = relative standard error (<25% is considered reliable).

Table 14. Estimated Exposure to NaBHB relevant to body weight (mg/kg bw/day)

Population Group	Age (yrs)	M/F	Food Consumers						90th % RSE
			n	% Total	mg/kg bw/day				
					Mean	Mean SE	90th	90th SE	
Infants/ Toddlers	0–2	M	35	15.3	53.1	9.3	85.3	39.7	46.5
		F	32	10.9	48.2	5.4	76.6	10.3	13.4
Children	3–11	M	167	25.5	31.9	2.8	64.3	7.6	11.8
		F	131	23.9	21.8	2.1	39.9	6.1	15.3
Teenagers	12–19	M	131	30.9	19.2	1.9	39.7	6.9	17.4
		F	117	24.5	16.8	1.5	29.8	4.4	14.8
Adults	20+	M	402	22.0	17.0	1.4	33.3	3.6	10.8
		F	355	18.3	12.6	1.1	27.8	3.4	12.2
Total M/F	All ages	M	735	23.3	20.3	1.1	41.6	4.7	11.3
		F	635	19.3	15.1	0.9	32.4	2.1	6.5
Total population	All ages	Both genders	1370	21.2	17.9	0.7	35.8	2.3	6.4

For sodium BHB, the highest absolute exposure estimate was that for males 20 years and older at the 90th percentile, at 2540 mg NaBHB/day (highlighted in **Table 13**). This is equivalent to 457.2 mg of sodium and 2082.8 mg BHB. The highest exposure estimate relative to body weight at the 90th percentile (with an RSE of <25%) was that for females 0–2, at 76.6 mg NaBHB/kg bw/day (highlighted in **Table 14**).

Table 15. Estimated Exposure to CaBHB (mg/day)

Population Group	Age (yrs)	M/F	Food Consumers						90th % RSE
			n	% Total	mg/day				
					Mean	Mean SE	90th	90th SE	
Infants/ Toddlers	0–2	M	35	15.3	696.9	150.9	993.4	619.4	62.4
		F	32	10.9	575.6	54.4	822.2	140.7	17.1
Children	3–11	M	167	25.5	831.1	74.5	1550.1	169.9	11.0
		F	131	23.9	607.8	52.7	1197.4	174.5	14.6
Teenagers	12–19	M	131	30.9	1250.4	98.8	2315.8	242.5	10.5
		F	117	24.5	982.8	86.3	1977.7	267.4	13.5
Adults	20+	M	402	22.0	1441.0	107.8	2540.0	411.9	16.2
		F	355	18.3	892.9	61.4	1718.5	238.2	13.9
Total M/F	All ages	M	735	23.3	1309.0	76.9	2388.9	174.8	7.3
		F	635	19.3	859.9	48.2	1662.5	159.7	9.6
Total population	All ages	Both genders	1370	21.2	1098.7	47.0	2163.9	99.0	4.6

SE = standard error; RSE = relative standard error (<25% is considered reliable).

Table 16. Estimated Exposure to CaBHB relevant to body weight (mg/kg bw/day)

Population Group	Age (yrs)	M/F	Food Consumers						90th % RSE
			n	% Total	mg/kg bw/day				
					Mean	Mean SE	90th	90th SE	
Infants/ Toddlers	0–2	M	35	15.3	53.1	9.3	85.3	39.7	46.5
		F	32	10.9	48.2	5.4	76.6	10.3	13.4
Children	3–11	M	167	25.5	31.9	2.8	64.3	7.6	11.8
		F	131	23.9	21.8	2.1	39.9	6.1	15.3
Teenagers	12–19	M	131	30.9	19.2	1.9	39.7	6.9	17.4
		F	117	24.5	16.8	1.5	29.8	4.4	14.8
Adults	20+	M	402	22.0	17.0	1.4	33.3	3.6	10.8
		F	355	18.3	12.6	1.1	27.8	3.4	12.2
Total M/F	All ages	M	735	23.3	20.3	1.1	41.6	4.7	11.3
		F	635	19.3	15.1	0.9	32.4	2.1	6.5
Total population	All ages	Both genders	1370	21.2	17.9	0.7	35.8	2.3	6.4

For calcium BHB, the highest absolute exposure estimate was that for males 20 years and older at the 90th percentile, at 2540 mg CaBHB/day (highlighted in **Table 15**). This is equivalent to 406.4 mg of calcium and 2133.6 mg BHB. The highest exposure estimate relative to body weight at the 90th percentile (with an RSE of <25%) was that for females 0–2, at 76.6 mg CaBHB/kg bw/day (highlighted in **Table 16**).

Table 17. Estimated Exposure to MgBHB (mg/day)

Population Group	Age (yrs)	M/F	Food Consumers						90th % RSE
			n	% Total	mg/day				
					Mean	Mean SE	90th	90th SE	
Infants/ Toddlers	0–2	M	35	15.3	743.6	227.5	1017.4	1057.2	103.9
		F	32	10.9	558.3	72.2	781.3	202.8	26.0
Children	3–11	M	167	25.5	965.4	111.1	1941.7	316.5	16.3
		F	131	23.9	626.7	72.5	1494.1	285.6	19.1
Teenagers	12–19	M	131	30.9	1605.9	138.1	3170.3	283.4	8.9
		F	117	24.5	1170.3	132.7	2666.8	398.1	14.9
Adults	20+	M	402	22.0	1778.0	146.4	3357.2	502.8	15.0
		F	355	18.3	999.0	84.3	2296.1	250.4	10.9
Total M/F	All ages	M	735	23.3	1613.7	104.6	3151.5	220.3	7.0
		F	635	19.3	962.8	67.2	2211.3	193.2	8.7
Total population	All ages	Both genders	1370	21.2	1308.9	64.1	2865.5	134.3	4.7

SE = standard error; RSE = relative standard error (<25% is considered reliable).

Table 18. Estimated Exposure to MgBHB relevant to body weight (mg/kg bw/day)

Population Group	Age (yrs)	M/F	Food Consumers						90th % RSE
			n	% Total	mg/kg bw/day				
					Mean	Mean SE	90th	90th SE	
Infants/ Toddlers	0–2	M	35	15.3	56.0	14.3	95.8	69.0	72.0
		F	32	10.9	47.2	6.8	63.6	23.3	36.6
Children	3–11	M	167	25.5	36.0	4.1	73.7	10.9	14.8
		F	131	23.9	21.9	2.3	53.0	6.2	11.7
Teenagers	12–19	M	131	30.9	24.4	2.5	52.8	8.4	15.9
		F	117	24.5	19.6	2.1	41.1	6.6	16.1
Adults	20+	M	402	22.0	21.1	1.8	43.3	5.4	12.5
		F	355	18.3	14.3	1.5	33.6	5.1	15.2
Total M/F	All ages	M	735	23.3	24.5	1.5	53.3	5.8	10.9
		F	635	19.3	16.7	1.2	39.8	3.9	9.8
Total population	All ages	Both genders	1370	21.2	20.9	1.0	45.7	2.6	5.7

For magnesium BHB, the highest absolute exposure estimate was that for males 20 years and older at the 90th percentile, at 3357.2 mg MgBHB/day (highlighted in **Table 17**). This is equivalent to 282 mg of magnesium and 3075.2 mg BHB. The highest exposure estimate relative to body weight at the 90th percentile (with an RSE of <25%) was that for males 3–11, at 73.7 mg MgBHB/kg bw/day (highlighted in **Table 18**).

Table 19. Estimated Exposure to KBHB (mg/day)

Population Group	Age (yrs)	M/F	Food Consumers						90th % RSE
			n	% Total	mg/day				
					Mean	Mean SE	90th	90th SE	
Infants/ Toddlers	0–2	M	35	15.3	3360.7	885.9	4376.3	4016.1	91.8
		F	32	10.9	2646.8	276.1	3680.2	721.1	19.6
Children	3–11	M	167	25.5	4190.4	431.6	8169.5	1225.7	15.0
		F	131	23.9	2881.7	289.0	6090.8	1033.5	17.0
Teenagers	12–19	M	131	30.9	6689.7	550.6	13089.5	1205.4	9.2
		F	117	24.5	5049.9	502	10865.4	1579.7	14.5
Adults	20+	M	402	22.0	7494.2	589.8	13950.0	1954.1	14.0
		F	355	18.3	4389.9	338.1	9277.8	1097.7	11.8
Total M/F	All ages	M	735	23.3	6811.8	421.0	13049.2	912.2	7.0
		F	635	19.3	4239.5	267.8	8845	789.4	8.9
Total population	All ages	Both genders	1370	21.2	5607.4	257.5	11714.4	536.3	4.6

SE = standard error; RSE = relative standard error (<25% is considered reliable).

Table 20. Estimated Exposure to KBHB relevant to body weight (mg/kg bw/day)

Population Group	Age (yrs)	M/F	Food Consumers						90th % RSE
			n	% Total	mg/kg bw/day				
					Mean	Mean SE	90th	90th SE	
Infants/ Toddlers	0–2	M	35	15.3	254.5	55.2	383.2	262.0	68.4
		F	32	10.9	222.7	27.0	316.4	73.9	23.4
Children	3–11	M	167	25.5	158.3	15.9	323.3	41.8	12.9
		F	131	23.9	102.1	10.1	223.4	32.5	14.5
Teenagers	12–19	M	131	30.9	101.9	10.2	211.6	33.3	15.7
		F	117	24.5	85.2	7.9	170.0	26.3	15.5
Adults	20+	M	402	22.0	88.7	7.4	174.7	20.9	12.0
		F	355	18.3	62.6	6.0	138.6	21.4	15.4
Total M/F	All ages	M	735	23.3	104.4	6.0	215.8	25.5	11.8
		F	635	19.3	74.2	4.9	169.3	13.6	8.0
Total population	All ages	Both genders	1370	21.2	90.3	4.0	185.2	11.8	6.4

For potassium BHB, the highest absolute exposure estimate was that for males 20 years and older at the 90th percentile, at 13950.0 mg KBHB/day (highlighted in **Table 19**). This is equivalent to 3836.25 mg of potassium and 10113.75 mg BHB. The highest exposure estimate relative to body weight at the 90th percentile (with an RSE of <25%) was that for males 3–11, at 323.3 mg KBHB/kg bw/day (highlighted in **Table 20**). This is equivalent to 82.3 mg/kg bw/day of potassium and 241 mg/kg bw/day of BHB.

In summary, according to the estimates above, approximately 21.2% of the U.S. total population was identified as potential consumers of BHB salts from the proposed food uses. The 90th percentile aggregate estimated exposure levels for the total population were as follows:

- NaBHB: 2163.9 mg/day (absolute) and 35.8 mg/kg bw/day (related to body weight)
- CaBHB: 2163.9 mg/day (absolute) and 35.8 mg/kg bw/day (related to body weight)
- MgBHB: 2865.5 mg/day (absolute) and 45.7 mg/kg bw/day (related to body weight)
- KBHB: 11714.4 mg/day (absolute) and 185.2 mg/kg bw/day (related to body weight)

It should be noted that these estimates are extremely conservative, as they assume that 100% of the intended use bars and beverages in the marketplace will contain BHB salts. A 100% market share in all of these product categories is obviously not realistic, and hence these estimates are likely much higher than what the exposures will be in reality.

Basis for the GRAS Determination

The scientific procedures forming the pivotal and corroborative data of the safety assessment comprise the technical element of the GRAS standard. The common knowledge element is comprised of the general availability of the pivotal data establishing the technical element and the consensuses of the Expert Panel. Together, the technical element and the common knowledge element form the basis for the GRAS self-determination of BHB salts.

Technical Element

BHB and its salts have been the subjects of thorough safety assessments as described above. The safety of these ingredients is supported by toxicological studies in animals, clinical studies without occurrence of serious adverse events, and their history of use.

The totality of evidence for the safety of BHB salts is comprised of pivotal and corroborative data. Pivotal data for this determination is the 28-day repeated oral toxicity study in rats on the ketone ester, (R)-3-Hydroxybutyl (R)-3-hydroxybutyrate. The available data indicates that (R)-3-Hydroxybutyl (R)-3-hydroxybutyrate is rapidly hydrolyzed to BHB and 1,3-butanediol in the gut. The latter is then further metabolized to BHB and acetoacetate in the liver. In the 28-

day study on (R)-3-Hydroxybutyl (R)-3-hydroxybutyrate, no toxicological effects were noted at 15.1 and 12 g/kg bw/day in female and male Wistar rats, respectively. (R)-3-Hydroxybutyl (R)-3-hydroxybutyrate has a molecular weight of 176.21 g/mol and BHB has a molecular weight of 104.10453 g/mol; therefore, it is reasonable to assume it comprises approximately 59% of the ketone ester (104.10453/176.21 = 0.59). Therefore, using the ketone ester NOAEL of 12 g/kg bw/day from the 28-day repeated dose toxicity study, we can calculate a NOAEL of 7 g/kg bw/day for BHB. This calculation is very conservative, considering that 1,3-butanediol is also rapidly converted into BHB and acetoacetate upon consumption.

The pivotal data is corroborated by the developmental toxicity study conducted on (R)-3-Hydroxybutyl (R)-3-hydroxybutyrate, the endogenous nature of BHB and the high physiological levels of BHB that occur without adverse effects during certain physiological conditions, the extensive history of the use of ketogenic diets, and the lack of serious adverse events reported in clinical trials using various BHB in various forms. The totality of evidence also relates to the high-quality control standards for this ingredient.

In addition to what is outlined above, the safety profile of BHB is unique in that it is supported by the fact that it is an endogenous compound that acts as an energy substrate to the body, and normal physiological processes control its metabolism and homeostasis within the body.

Based on the intended uses of the BHB salts as shown in **Table 12**, the 90th percentile absolute exposures were highest for males 20 years of age or older. This was true for all four salt forms. The 90th percentile exposures based on body weight (with an RSE value of <25%, making it reasonably reliable) were highest for females ages 0–2 for sodium BHB and calcium BHB. The 90th percentile exposures based on body weight were highest for males ages 3–11 for magnesium BHB and potassium BHB. The NOAEL from the above 28-day repeated dose oral toxicity study allows for adequate margins of safety (MOS) for BHB as BHB salts under the conditions of their intended use, as can be determined by dividing the NOAEL by the estimated daily exposure values (EDI). As detailed below, the BHB MOS for exposure to the sodium, calcium and magnesium salt forms was greater than 100, which is considered reasonable for ingredients added to foods. The MOS for the potassium salt form was 29, which while less than the usual 100-fold safety factor, is considered reasonably safe given the safety profiles for potassium and for BHB (see more details below). The calculations are shown below:

- MOS for sodium BHB: The highest EDI based on body weight was 76.6 mg/kg bw/day (**Table 14**). BHB makes up 82% of the sodium BHB product, so the highest EDI for BHB alone can be calculated as 63 mg/kg

bw/day. The MOS (NOAEL divided by the EDI: 7000 mg/kg bw/day divided by 63 mg/kg bw/day) is equal to 111.

- MOS for calcium BHB: The highest EDI based on body weight was 76.6 mg/kg bw/day (**Table 16**). BHB makes up 84% of the magnesium BHB product, so the highest EDI for BHB alone can be calculated as 64 mg/kg bw/day. The MOS (NOAEL divided by the EDI: 7000 mg/kg bw/day divided by 64 mg/kg bw/day) is equal to 109.
- MOS for magnesium BHB trihydrate: The highest EDI based on body weight was 73.7 mg/kg bw/day (**Table 18**). BHB makes up 73% of the magnesium BHB product, so the highest EDI for BHB alone can be calculated as 54 mg/kg bw/day. The MOS (NOAEL divided by the EDI: 7000 mg/kg bw/day divided by 54 mg/kg bw/day) is equal to 130.
- MOS for potassium BHB: The highest EDI based on body weight was 323.3 mg/kg bw/day (**Table 20**). BHB makes up NLT 70% (and at most, 74.5%) of the potassium BHB product, so the highest EDI for BHB alone can be calculated as 241 mg/kg bw/day. The MOS (NOAEL divided by the EDI: 7000 mg/kg bw/day divided by 241 mg/kg bw/day) is equal to 29. While this MOS is less than the typical 100-fold safety factor, we consider it reasonable due to the safety profiles for both potassium (described above) and BHB as described herein and in GRN 515.
- If all four BHB salts were consumed at their respective 90th percentile exposure levels, the total exposure to BHB would be 422 mg/kg bw/day of BHB (63 + 64 + 54 + 241= 422). In this case, the MOS for BHB (NOAEL divided by the EDI: 7000 mg/kg bw/day divided by 422 mg/kg bw/day) is equal to 17. It is reasonable to consider less than a 100-fold MOS in this circumstance, similar to what was done in GRN 515 (which used an MOS of 10 and received the no objection letter from FDA), due to the fact that these compounds are rapidly hydrolyzed into endogenous ketone bodies; additionally, Clarke et al. (2012, also cited in GRN 515) demonstrated that ingestion of ~150 g/day of a ketone ester (59% of which was BHB, or 88.56 g/day) resulted in BHB plasma levels that are known to occur physiologically and are generally considered as safe.[9] The current absolute maximum anticipated BHB exposure level (422 mg/kg bw/day x average 70 kg adult = 29.5 g/day) is approximately one-third of the BHB contained in the ketone ester study (88.56 g/day).

It should be also noted that while EDI data from children aged 0–2 was utilized for the calculations, BHB products are not intended to be used in infant formula or to be marketed to this children of this age group. As is shown in **Tables 13, 15,**

17, and 19 the EDIs for other population groups are significantly lower in many cases, making the margin of safety for those populations significantly higher.

With regard to exposure to the sodium, magnesium, calcium, and potassium minerals in the salts, the exposures are considered reasonable with regard to daily values (based on a 2000 calorie diet) and adequate intakes for these minerals. As previously discussed, the daily values for sodium, magnesium, and calcium are 2400 mg, 400 mg, and 1000 mg, respectively, and the AI for potassium is 4,700 mg/day. The addition levels of sodium, calcium, and magnesium are similar to levels that are found in a serving of other commonly consumed foods in the same categories while addition levels for potassium are generally higher than those found in foods of the same categories (**see Appendix H**). However, given the potassium AI of 4700 mg/day and the absence of UL for potassium, the exposure levels, and hence the addition levels, are considered within safe parameters for a healthy population.

Common Knowledge Element

The scientific studies, performed in laboratory animals and humans and herein reported that provide the basis of this GRAS determination by scientific procedures are published and available in the public domain. The reference section of this notification contains the citations for the published studies. This published data fulfills the requirement for general availability of the pivotal scientific data contributing to the technical element of the GRAS standard. The opinion of the Expert Panel convened to review and analyze the data herein reported provide ample evidence of consensus among qualified experts that there is reasonable certainty that consumption of BHB salts for their intended use is not harmful. The general availability of the pivotal safe data discussed herein together with the opinion of the Expert Panel satisfies the common knowledge element of this GRAS self-determination.

Conclusion

The Expert Panel has, independently and collectively, critically evaluated this safety assessment of Ketone Labs' BHB salts and unanimously conclude that the intended use of Ketone Labs' BHB salts as food ingredients, produced in accordance with Good Manufacturing Practice and meeting the specifications presented in the document that is the basis for the GRAS determination, is generally recognized as safe. The Expert Panel further concludes that the intended use is GRAS based on scientific procedures and corroborated by a history of safe use (exposure). The Expert Panel believes that other experts qualified by training and experience to evaluate the safety of food ingredients would concur with this GRAS conclusion.

Panel Members: ***Date:***

January 10, 2018

Judith Hauswirth, PhD

Chair of Expert Panel

January 10, 2018

John R. Endres, ND

Panel Member

January 10, 2018

Amy Clewell, ND, DABT

Panel Member

EXPERT PANEL

Judith Hauswirth PhD—Panel Chair

Dr. Hauswirth has a PhD in biochemistry from Oregon State University, Corvallis, Oregon 1969 and a BS in chemistry, University of California, Davis, California, 1965. She also received a National Institutes of Health postdoctoral fellowship in pharmacology at Yale University, New Haven, Connecticut and a National Cancer Institute Career Development Award and research grant. She is currently the sole proprietor of her own consulting firm where she provides expert consultation to private clients on toxicology issues related to toxicity testing, risk assessment, and hazard evaluation. She also provides regulatory advice, serves as an expert in data compensation cases, evaluates laboratory reports, and assists in designing atypical toxicology studies and monitors toxicology studies of all types. She has served as an expert on GRAS self-determination panels and made presentation to the EPA Human Science Review Board and the Scientific Advisory Panel. She has over 38 years of experience in toxicology, biochemistry, and drug metabolism, including basic research and regulatory toxicology.

She is a member of the American Chemistry Society and a past member of the American College of Toxicology, the New York Academy of Sciences, and the Association of Government Toxicologists. She was councilor for the American College of Toxicology from 1997 to 2000. She was, also, an advisor to the National Academy of Sciences Committee on Pesticides in the Diets of Infants and Children. She received the Food and Drug Commendable Service Award for management and quality of output, the FDA Group Recognition Award as a member of the Nitrofuran Hearing Team, the EPA Bronze Medal for Commendable Service for formulation of the inerts policy, and the EPA Bronze Medal for Commendable Service for performance on the Toxicology Branch Peer Review Committee.

She has worked for several consulting firms as a toxicologist, including van Gemert and Hauswirth, LLC, Charles, Conn, and van Gemert, LLC, ChemReg International, LLC, and Jellinek, Schwartz, and Connolly where she became the Vice President of Toxicology and Chemistry. Prior to her consulting career, she was a Branch Chief at the Environmental Protection Agency in the Office of Pesticides Program, Health Effects Division and acted as Director of the Division of Drugs and Environmental Toxicology at the Food and Drug Administration. While at FDA, she was part of the Center for Veterinary Medicine and the Bureau of Foods and did basic research in the area of genotoxicity and mutagenicity as well as her roles as manager and expert in toxicology testing and regulation of food animal drugs. At the Biochemistry Research Division of Sinai Hospital of Baltimore, where she became the assistant director, she conducted basic research

on the role of nutrition in the metabolism of carcinogens. She has published book chapters in the areas of plant biochemistry, vitamin E, and pesticide toxicology. She has published in journals such as Cancer Research, Archives of Biochemistry and Biophysics, and Environmental Mutagens.

John R. Endres, ND—Panel Member

Dr. Endres is the chief scientific officer for AIBMR Life Sciences, Inc. in Puyallup, Washington. Dr. Endres earned a degree in naturopathic medicine at Bastyr University in Kenmore, Washington and is licensed by Washington State Department of Health as a physician. He is a full member of the Society of Toxicology (SOT). Dr. Endres has been a member of numerous expert panels assembled for the evaluation of GRAS Self-determinations. He meets frequently with FDA Office of Food Additive Safety (OFAS) in College Park, MD for FDA GRAS pre-notification meetings. Dr. Endres has been a contributing author on many safety assessments published in academic journals specializing in toxicology. He is frequently the monitoring scientist for toxicology studies designed to study the safety of ingredients to be added to foods and dietary supplements. Dr. Endres is on the Editorial Advisory Boards for Nutritional Outlook, Functional Ingredients, and is on the Executive Advisory Board for Vitafoods Europe. Most recently he became one of 33 voting members on the NSF International Joint Committee to develop Publically Available Standards (PAS) for GRAS on behalf of the Grocery Manufacturers Association (GMA). At AIBMR, he manages a team of scientific and regulatory consultants specializing in the natural products and functional foods industries.

Prior to his work at AIBMR, Dr. Endres was involved in cancer research conducted at the Bastyr University Research Institute (BURI) and Fred Hutchinson Cancer Research Center, both located in Seattle, Washington. He screened botanical extracts for their inhibitory effect on the growth of various cancer cell lines. A particular area of interest was garlic plant parts in various breast cancer cell lines as well as the anti–proliferative effects of *Curcuma longa* (turmeric) on various colon cancer cell lines. He has also been the recipient of grants to present research in the United Kingdom at Westminster University, Middlesex University, and Oxford Natural Products. He has also presented research at other venues, including American Medical Association sponsored conferences where, in 2001, he received an Award of Excellence in Research. Dr. Endres was a teaching assistant in laboratory chemistry and a research assistant in natural products research, with a focus on production, purification, and analytical chemistry of whole plant extracts while attending Bastyr University.

Amy Clewell, ND, DABT—Panel Member

Dr. Amy Clewell is the Vice President of Scientific and Regulatory Affairs at AIBMR Life Sciences. Dr. Clewell earned a Bachelor of Science degree in biology from Indiana University in Bloomington, Indiana and a doctoral degree in Naturopathic Medicine from Bastyr University in Kenmore, Washington. She maintains her physician's license in the State of Washington. She is a diplomat of the American Board of Toxicology, a full member of the Society of Toxicology (SOT), and has been a member of numerous expert panels assembled for the evaluation of GRAS Self-determinations. Dr. Clewell is an author on many peer–reviewed journal publications, especially related to the toxicological evaluation of food ingredients. Her authorship also includes book chapters and trade articles. She has over 10 years of experience in natural products regulatory consulting, and specializes in the preparation of generally recognized as safe (GRAS) self-determination dossiers, as well as FDA GRAS and New Dietary Ingredient (NDI) notifications. She is also involved in the evaluation and compilation scientific research on the efficacy of ingredients and regulatory compliance for natural products. She plays a strong role in the management of projects at AIBMR Life Sciences.

In addition to work at AIBMR, Dr. Clewell has clinical experience as a licensed physician in Washington State, as well as extensive research experience. Her work in research began as a student and laboratory technician as an undergraduate at Indiana University where she spent three years working in the area of translational initiation using *Saccharomyces cerevisiae* as a model system. She continued her research pursuits for another five years as a research technician and laboratory manager in Dr. Karla Kirkegaard's laboratories at both the University of Colorado and Stanford University, studying the biochemistry of polio and hepatitis C virus propagation using an *S. cerevisiae* model. She remained active in research in various capacities while attending Bastyr University for her doctorate.

She is the past-president of the Indiana Association of Naturopathic Physicians and a current member of the American Association of Naturopathic Physicians.

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