AIBMR Life Sciences, Inc.
Natural and Medicinal Products Research

The Basis for the Conclusion that the Intended Use of D-Beta-hydroxybutyrate (D-BHB) Salts are Generally Recognized as Safe (GRAS)

Prepared on behalf of:
Ketone Labs

Prepared by:
AIBMR Life Sciences, Inc.
2800 E. Madison, Suite 202
Seattle, WA 98112

November 6, 2018

Table of Contents

Figures and Tables

Appendices

Part 1: Statements and Certification

1.1 Expert Evaluation & Independent Conclusion of GRAS Status

A panel of experts ("GRAS Panel") who are qualified by training and experience to evaluate the safety of food ingredients was convened by *Ketone Labs* (hereinafter called "Ketone Labs"), the proponent of this GRAS conclusion, for the purpose of evaluating whether the available scientific data, information, and methods establish that D-beta-hydroxybutyrate salts are safe under the intended conditions of use in food. Ketone Labs has considered the view of the GRAS Panel and all factors pertaining to eligibility criteria and has concluded that D-beta-hydroxybutyrate salts are Generally Recognized as Safe (GRAS) for their intended use, consistent with section 201(s) of the Federal Food, Drug and Cosmetic Act.

1.2 Responsible Individual and Principal Company Address

Rob Rogers
Founder
Ketone Labs
5140 A Old Boonville Hwy
Evansville, IN 47715
Tel: (385)-218-8634
robrogers@axcess-global.com

1.3 Names of the Substances

The names of the substances are D-beta-hydroxybutyrate salts (D-BHB) of calcium, magnesium, potassium, and sodium.

1.4 Intended Conditions of Use

D-BHB salts are intended to be used as ingredients in the following food categories: bars, dry beverage concentrates, nutrition powders, energy drinks, sports drinks, fluid replacements, and nutrition drinks at the addition levels ranging from 3.5 to 200 mg/g, as specified in Part 3. D-BHB salts are not intended for use in foods where standards of identity would preclude such use, infant formula, or any products that would require additional regulatory review by USDA.

1.5 Statutory Basis for GRAS Conclusion

The conclusion of GRAS status of D-BHB salts for their intended conditions of use, stated in Part 1.4 of this report, has been made based on scientific procedures.

1.6 Not Subject to Premarket approval

Ketone Labs has concluded that D-BHB salts are GRAS for their intended conditions of use, stated in Part 1.4 of this report, and, therefore, such use of D-BHB salts is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act.

1.7 Data and Information Availability Statement

The data and the information that serve as the basis for this GRAS conclusion are available in the appendices of this report.

1.8 Certification of Completion

Ketone Labs herby certifies that, to the best of our knowledge, this independent conclusion of GRAS status is a complete, representative, and balanced dossier that includes unfavorable information, as well as favorable information, known to us and pertinent to the evaluation of the safety and GRAS status of the use of D-BHB salts.

_____ _____

Rob Rogers Date

Founder

Ketone Labs

1.9 GRAS Panel

The members of the GRAS 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 report. The GRAS Panel, independently and collectively, critically evaluated materials submitted by

Ketone Labs, as well as other documents, following a thorough search of the peer-reviewed literature and other information available in the public domain. The aforementioned are cited in Parts 2 through 6 and listed in Part 8 of this report and/or are included in the appendices to this GRAS dossier. This report is a summary of the GRAS Panel's evaluation and provides an expert opinion (Part 7) regarding the safety of D-BHB salts under the conditions of their intended use.

Part 2: Identity, Manufacture, Specifications, and Physical or Technical Effect

2.1 Identification

Ketone Labs produces D-BHB salts, in which BHB is chemically (ionically) bonded to calcium (Ca), magnesium (Mg), potassium (K), or sodium (Na) cations (see Figures 1–4). 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. Molecular formulas and weights of each salt are listed in Table 1.

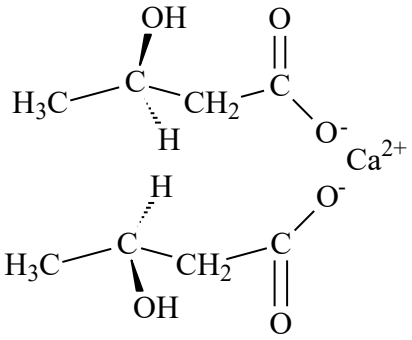


Figure 1. Structural formula of D-BHB calcium salt

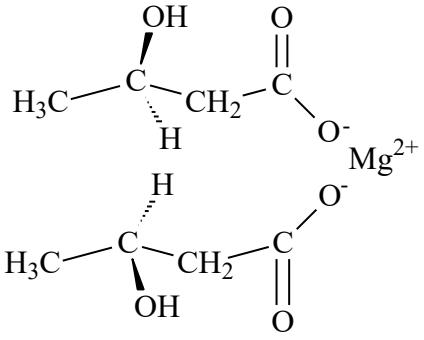


Figure 2. Structural formula of D-BHB magnesium salt

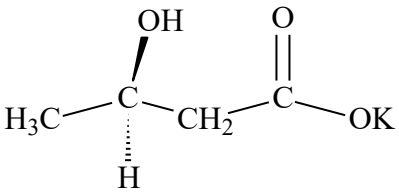


Figure 3. Structural formula of D-BHB potassium salt

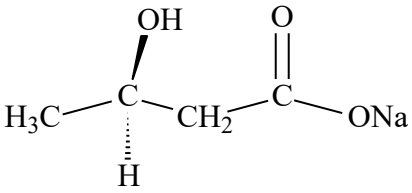


Figure 4. Structural formula of D-BHB sodium salt

Table 1. Molecular formulas and weights of D-BHB salts

D-BHB Salt	Molecular formula	Molecular weight
Ca-D-BHB	$C_8H_{14}O_6Ca$	246.27
Mg-D-BHB	$C_8H_{14}O_6Mg$	230.48
K-D-BHB	$C_4H_7O_3K$	142.20
Na-D-BHB	$C_4H_7O_3Na$	126.09

2.2 Manufacturing

2.2.1 Manufacturing Overview

Manufacturing flowcharts for Ketone Labs' D-BHB salts are provided in Figures 5–8 and in Appendix A.

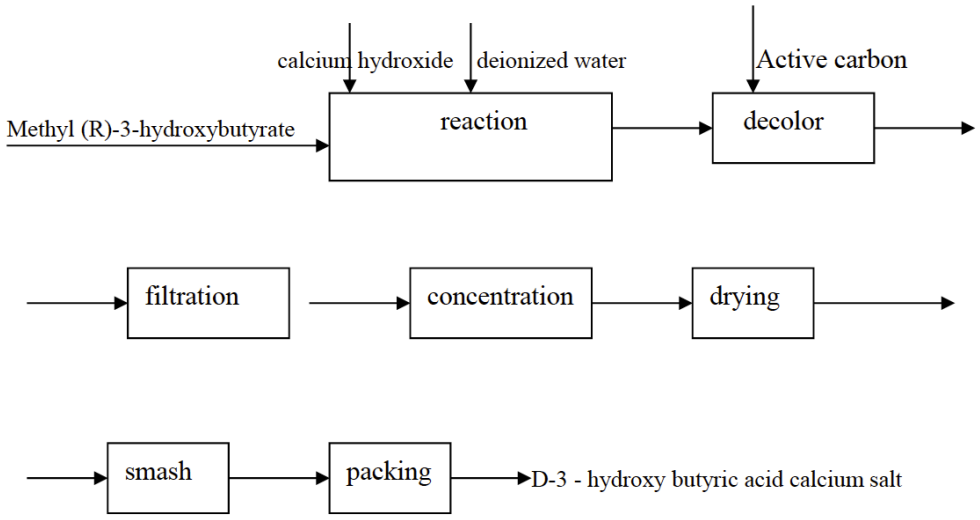


Figure 5. Manufacturing flowchart for D-BHB calcium salt

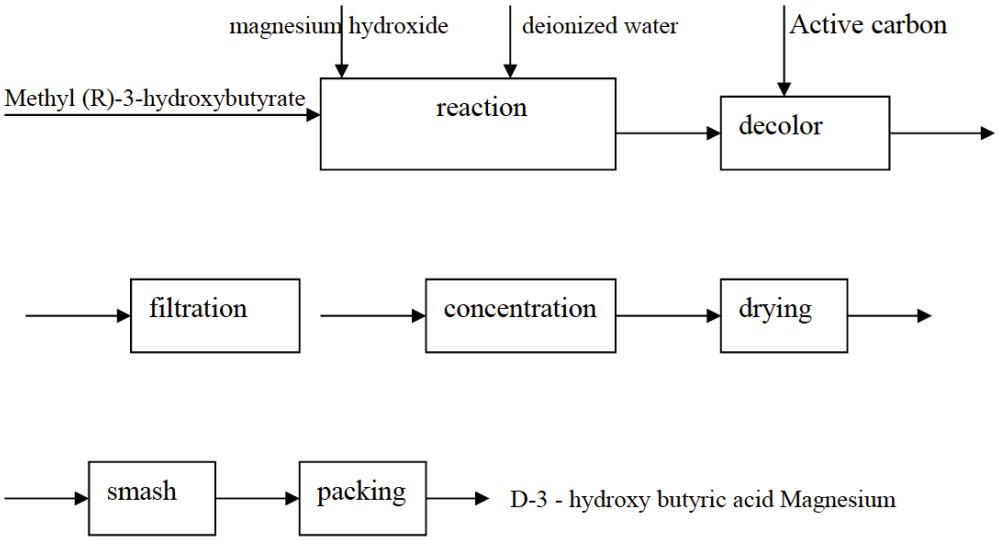


Figure 6. Manufacturing flowchart for D-BHB magnesium salt

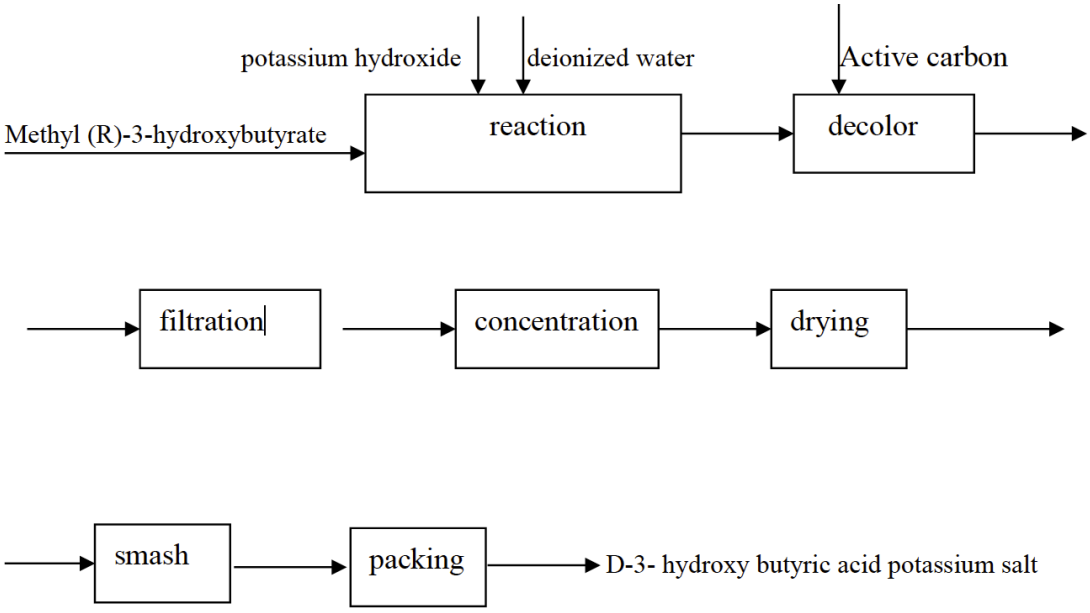


Figure 7. Manufacturing flowchart for D-BHB potassium salt

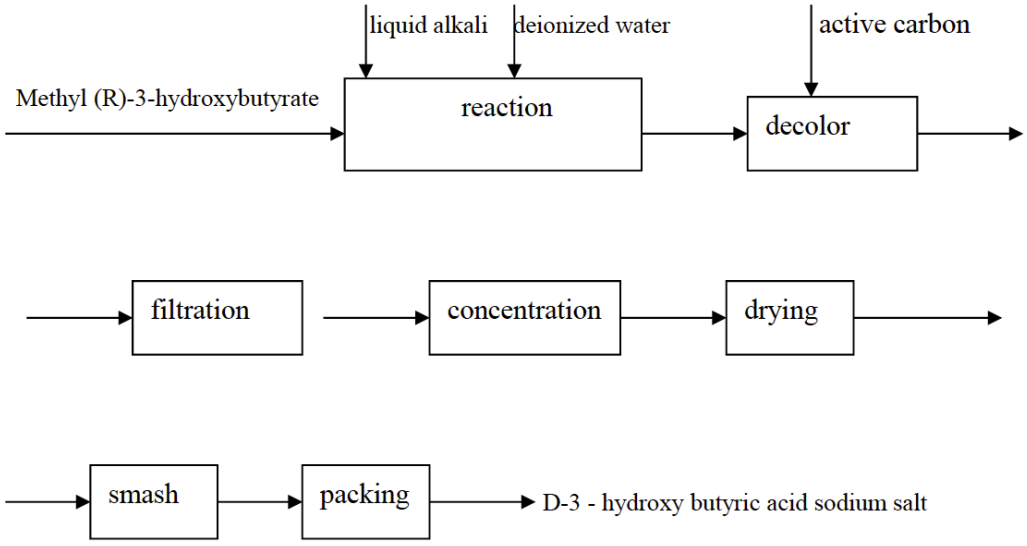


Figure 8. Manufacturing flowchart for D-BHB sodium salt

2.2.2 Good Manufacturing Practice

D-BHB from Ketone Labs is produced under strict adherence to current GMP standards set to comply with the U.S. Code of Federal Regulations (see Appendix B).

2.3 Specifications

The specifications for the food-grade D-BHB salts, along with the specification methods, are listed in Tables 2–5 below (see Appendix C).

Table 2. D-BHB calcium salt specifications

Test Items	Specification	Method
Marker Compounds		
Assay (%)	≥95.0	Internal
BHB (Free) (%)	78.0–83.0	Internal HPLC*
Ca^{2+} (%)	13.5–18.5	Internal ICP-MS
Physical Characteristics		
Appearance	White crystalline powder	By visual
Specific rotation (º) [alpha]D^{20}	-12.5 – -17.5 (c=6, H_20)	cCP(0621)
Loss on drying (%)	≤2.0	cCP(0831)
Mesh	95% pass 30 mesh	cCP(0982)
Bulk density	Report only	cUSP(616)
Tapped density	Report only	cUSP(616)
Heavy Metals		
Total Heavy Metals (ppm)	≤10	cCP(0821)**
Arsenic (ppm)	≤1.0	GB5009.11-2003, AFS**
Cadmium (ppm)	≤1.0	GB5009.15-2014, GFAAS**
Lead (ppm)	≤3.0	GB5009.12-2010, GFAAS**
Mercury (ppm)	≤0.1	GB5009.17-2003, AFS**
Microbiological Tests		
Total Aerobic Microbial	≤1000 cfu/g	GB4789.2-2016
Total Yeast & Mold	≤100 cfu/g	GB4789.15-2016
E. coli	Negative/25 g	GB4789.3-2016
Salmonella	Negative/25 g	GB4789.4-2016

Abbreviations: AFS, atomic fluorescence spectrometry; cCP, current Chinese Pharmacopeia; cUSP, current United States Pharmacopeia; GFAAS, atomic absorption spectrometry

*See Appendix C.1

**GB refers to Chinese Pharmacopeia, test instrument is ICP-MS

Table 3. D-BHB magnesium salt specifications

Test Items	Specification	Method
Marker Compounds		
Assay (%)	≥95.0	Internal
BHB (Free) (%)	78.0–83.0	Internal HPLC*
Mg^{2+} (%)	7.0–11.5	Internal ICP-MS
Physical Characteristics		
Appearance	White crystalline powder	By visual
Specific rotation (º) [alpha]D^{20}	-13.0 – -18.0 (c=6, H_20)	cCP(0621)
Loss on drying (%)	≤7.0	cCP(0831)
Mesh	95% pass 30 mesh	cCP(0982)
Bulk density	Report only	cUSP(616)
Tapped density	Report only	cUSP(616)
Heavy Metals		
Total Heavy Metals (ppm)	≤10	cCP(0821)**
Arsenic (ppm)	≤1.0	GB5009.11-2003, AFS**
Cadmium (ppm)	≤1.0	GB5009.15-2014, GFAAS**
Lead (ppm)	≤3.0	GB5009.12-2010, GFAAS**
Mercury (ppm)	≤0.1	GB5009.17-2003, AFS**
Microbiological Tests		
Total Aerobic Microbial	≤1000 cfu/g	GB4789.2-2016
Total Yeast & Mold	≤100 cfu/g	GB4789.15-2016
E. coli	Negative/25 g	GB4789.3-2016
Salmonella	Negative/25 g	GB4789.4-2016

Abbreviations: AFS, atomic fluorescence spectrometry; cCP, current Chinese Pharmacopeia; cUSP, current United States Pharmacopeia; GFAAS, atomic absorption spectrometry

*See Appendix C.1

**GB refers to Chinese Pharmacopeia, test instrument is ICP-MS

Table 4. D-BHB potassium salt specifications

Test Items	Specification	Method
Marker Compounds		
Assay (%)	≥95.0	Internal
BHB (Free) (%)	66–71	Internal HPLC*
K^+ (%)	24.0–29.5	cCP(0412) ICP-MS
Physical Characteristics		
Appearance	White crystalline powder	By visual
Specific rotation (º) [alpha]D^{20}	-11.0 – -15.0 (c=6, H_20)	cCP(0621)
Loss on drying (%)	≤2.0	cCP(0831)
Mesh	95% pass 30 mesh	cCP(0982)
Bulk density	Report only	cUSP(616)
Tapped density	Report only	cUSP(616)
Heavy Metals		

Total Heavy Metals (ppm)	≤10	cCP(0821)**
Arsenic (ppm)	≤0.5	GB5009.11-2003, AFS**
Cadmium (ppm)	≤0.5	GB5009.15-2014, GFAAS**
Lead (ppm)	≤0.5	GB5009.12-2010, GFAAS**
Mercury (ppm)	≤0.1	GB5009.17-2003, AFS**
Microbiological Tests		
Total Aerobic Microbial	≤1000 cfu/g	GB4789.2-2016
Total Yeast & Mold	≤100 cfu/g	GB4789.15-2016
E. coli	Negative/25 g	GB4789.3-2016
Salmonella	Negative/25 g	GB4789.4-2016

Abbreviations: AFS, atomic fluorescence spectrometry; cCP, current Chinese Pharmacopeia; cUSP, current United States Pharmacopeia; GFAAS, atomic absorption spectrometry

*See Appendix C.1

**GB refers to Chinese Pharmacopeia, test instrument is ICP-MS

Table 5. D-BHB sodium salt specifications

Test Items	Specification	Method
Marker Compounds		
Assay (%)	≥94.0	Internal
BHB (Free) (%)	76.0–81	Internal HPLC*
Na^+ (%)	15.5–20.5	cCP(0412) (ICP-MS)
Calcium silicate (%)	1	Internal, by addition
Physical Characteristics		
Appearance	White crystalline powder	By visual
Specific rotation (º) [alpha]D^{20}	-12.0 – -17.0 (c=6, H_20)	cCP(0621)
Loss on drying (%)	≤2.0	cCP(0831)
Mesh	95% pass 30 mesh	cCP(0982)
Bulk density	Report only	cUSP(616)
Tapped density	Report only	cUSP(616)
Heavy Metals		
Total Heavy Metals	≤10	cCP(0821)**
Arsenic	≤1.0	GB5009.11-2003, AFS**
Cadmium	≤1.0	GB5009.15-2014, GFAAS**
Lead	≤3.0	GB5009.12-2010, GFAAS**
Mercury	≤0.1	GB5009.17-2003, AFS**
Microbiological Tests		
Total Aerobic Microbial	≤1000 cfu/g	GB4789.2-2016
Total Yeast & Mold	≤100 cfu/g	GB4789.15-2016
E. coli	Negative/25 g	GB4789.3-2016
Salmonella	Negative/25 g	GB4789.4-2016

Abbreviations: AFS, atomic fluorescence spectrometry; cCP, current Chinese Pharmacopeia; cUSP, current United States Pharmacopeia; GFAAS, atomic absorption spectrometry

*See Appendix C.1

**GB refers to Chinese Pharmacopeia, test instrument is ICP-MS

2.3.1 Batch Analysis

Production conformity and consistency of Ketone Labs' D-BHB salts are tested in production lots. Batch analyses of three non-consecutive lots are shown below and are reasonably consistent and met the product specifications for marker compounds, physical characteristics, D-BHB and the respective salt, heavy metals, and microbial analyses (see Appendix D).

Table 6. D-BHB calcium salt batch analyses

Test Items	Specification	Lot No./Date of Manufacture		
		28C(R)-20180901 Sept 11, 2018	28C(R)-20180903 Sept 13, 2018	28C(R)-20180906 Sept 15, 2018
Marker Compounds				
Assay	≥95.0	95.3	95.2	95.1
BHB (Free) (%)	78.0–83.0	79.8	79.7	79.6
Ca^{2+} (%)	13.5–18.5	15.5	15.5	15.5
Physical Characteristics				
Appearance	White crystalline powder	White crystalline powder	White crystalline powder	White crystalline powder
Specific rotation (º) [alpha]D^{20}	-12.5 – -17.5 (c=6, H_20)	-16.7	-16.5	-16.4
Loss on drying (%)	≤2.0	0.21	.028	0.32
Mesh	95% pass 30 mesh	Conforms	Conforms	Conforms
Bulk density	Report only	0.35	0.36	0.34
Tapped density	Report only	0.54	0.55	0.53
Heavy Metals				
Total Heavy Metals (ppm)	≤10	Conforms	Conforms	Conforms
Arsenic (ppm)	≤1.0	Conforms	Conforms	Conforms
Cadmium (ppm)	≤1.0	Conforms	Conforms	Conforms
Lead (ppm)	≤3.0	Conforms	Conforms	Conforms
Mercury (ppm)	≤0.1	Conforms	Conforms	Conforms
Microbiological Tests				
Total Aerobic Microbial	≤1000 cfu/g	Conforms	Conforms	Conforms
Total Yeast & Mold	≤100 cfu/g	Conforms	Conforms	Conforms
E. coli	Negative/25 g	Negative	Negative	Negative
Salmonella	Negative/25 g	Negative	Negative	Negative

Table 7. D-BHB magnesium salt batch analyses

Test Items	Specification	Lot No./Date of Manufacture		
		28D(R)-20180902 Sept 8, 2018	28D(R)-20180904 Sept 10, 2018	28D(R)-20180906 Sept 12, 2018
Marker Compounds				
Assay (%)	≥95.0	95.7	95.4	95.3
BHB (Free) (%)	78.0–83.0	81.0	80.2	80.1
Mg^{2+} (%)	7.0~11.5	9.2	9.1	9.0
Physical Characteristics				
Appearance	White crystalline powder	White crystalline powder	White crystalline powder	White crystalline powder
Specific rotation (º) [alpha]D^{20}	-13.0 – -18.0 (c=6, H_20)	-15.6	-15.4	-15.1
Loss on drying (%)	≤7.0	5.5	6.1	6.2
Mesh	95% pass 30 mesh	Conforms	Conforms	Conforms
Bulk density	Report only	0.36	0.38	0.39
Tapped density	Report only	0.56	0.58	0.61
Heavy Metals				
Total Heavy Metals (ppm)	≤10	Conforms	Conforms	Conforms
Arsenic (ppm)	≤1.0	Conforms	Conforms	Conforms
Cadmium (ppm)	≤1.0	Conforms	Conforms	Conforms
Lead (ppm)	≤3.0	Conforms	Conforms	Conforms
Mercury (ppm)	≤0.1	Conforms	Conforms	Conforms
Microbiological Tests				
Total Aerobic Microbial	≤1000 cfu/g	Conforms	Conforms	Conforms
Total Yeast & Mold	≤100 cfu/g	Conforms	Conforms	Conforms
E. coli	Negative/25 g	Negative	Negative	Negative
Salmonella	Negative/25 g	Negative	Negative	Negative

Table 8. D-BHB potassium salt batch analyses

Test Items	Specification	Lot No./Date of Manufacture		
		28B(R)-20180802 Aug 19, 2018	28B(R)-20180804 Aug 21, 2018	28B(R)-20180806 Aug 23, 2018
Marker Compounds				
Assay (%)	≥95.0	95.2	95.3	95.4
BHB (Free) (%)	66–71	69.1	69.1	69.2
K^+ (%)	24.0–29.5	26.1	26.2	26.2
Physical Characteristics				
Appearance	White crystalline powder	White crystalline powder	White crystalline powder	White crystalline powder
Specific rotation (º) [alpha]D^{20}	-11.0 – -15.0 (c=6, H_20)	-12.7	-12.6	-12.8
Loss on drying (%)	≤2.0	1.7	1.8	1.9
Mesh	95% pass 30 mesh	Conforms	Conforms	Conforms
Bulk density	Report only	0.49	0.48	0.48
Tapped density	Report only	0.70	0.69	0.68
Heavy Metals				
Total Heavy Metals (ppm)	≤10	Conforms	Conforms	Conforms
Arsenic (ppm)	≤0.5	Conforms	Conforms	Conforms
Cadmium (ppm)	≤0.5	Conforms	Conforms	Conforms
Lead (ppm)	≤0.5	Conforms	Conforms	Conforms
Mercury (ppm)	≤0.1	Conforms	Conforms	Conforms
Microbiological Tests				
Total Aerobic Microbial	≤1000 cfu/g	Conforms	Conforms	Conforms
Total Yeast & Mold	≤100 cfu/g	Conforms	Conforms	Conforms
E. coli	Negative/25 g	Negative	Negative	Negative
Salmonella	Negative/25 g	Negative	Negative	Negative

Table 9. D-BHB sodium salt batch analyses

Test Items	Specification	Lot No./Date of Manufacture		
		28A(R)-20180801 Aug 11, 2018	28A(R)-20180803 Aug 13, 2018	28A(R)-20180805 Aug 14, 2018
Marker Compounds				
Assay (%)	≥94.0	94.6	94.7	94.5
BHB (Free) (%)	76.0–81	77.3	77.4	77.3
Na^+ (ICP-MS)(%)	15.5–20.5	17.3	17.3	17.2
Calcium silicate (%)	1.0	1.0	1.0	1.0
Physical Characteristics				
Appearance	White crystalline powder	White crystalline powder	White crystalline powder	White crystalline powder
Specific rotation (º) [alpha]D^{20}	-12.0 – -17.0 (c=6, H_20)	-14.3	-14.4	-14.2
Loss on drying (%)	≤2.0	0.6	1.7	1.8
Mesh	95% pass 30 mesh	Conforms	Conforms	Conforms
Bulk density	Report only	0.39	0.40	0.41
Tapped density	Report only	0.50	0.51	0.52
Heavy Metals				
Total Heavy Metals	≤10	Conforms	Conforms	Conforms
Arsenic	≤1.0	Conforms	Conforms	Conforms
Cadmium	≤1.0	Conforms	Conforms	Conforms
Lead	≤3.0	Conforms	Conforms	Conforms
Mercury	≤0.1	Conforms	Conforms	Conforms
Microbiological Tests				
Total Aerobic Microbial	≤1000 cfu/g	Conforms	Conforms	Conforms
Total Yeast & Mold	≤100 cfu/g	Conforms	Conforms	Conforms
E. coli	Negative/25 g	Negative	Negative	Negative
Salmonella	Negative/25 g	Negative	Negative	Negative

2.3.2 Residual Solvent Analysis

Ketone Labs tests all D-BHB salts for residual solvents on a periodic testing schedule (skip lot, every 5 lots); therefore, the solvents are not included in the specifications or certificates of analysis. Although solvents are not utilized in the manufacture of calcium, magnesium, and sodium D-BHB, solvent testing is performed to test for solvents that could be present in trace amounts in the raw materials used to make those salts.

Results for solvent testing are available in Appendix B. The allowable limit for dichloromethane is set at “non-detectable” with a detection limit of 0.05 ppm. The allowable limit for other residual solvents is no more than 0.05%.

2.4 Physical or Technical Effect

D-BHB is not intended to produce any physical or other technical effects that are relevant to the safety of the ingredient.

Part 3: Dietary Exposure

3.1 Intended Use

D-BHB salts, manufactured in accordance with current GMP, are intended to be used as ingredients in the food categories and at the addition levels shown in Table 10. D-BHB salts are not intended for use in foods where standards of identity would preclude such use, infant formula, or any products that would require additional regulatory review by USDA.

Table 10. Intended uses for D-BHB Salts

Food Category	Maximum addition levels (mg/g)							
	Ca D-BHB	Ca / D-BHB equivalents (Ca is ~18.5% of Ca D-BHB)	Mg D-BHB	Mg / D-BHB equivalents (Mg is ~11.5% of Mg BHB)	K D-BHB	K / D-BHB equivalents (K is ~29.5% of K BHB)	Na D-BHB	Na / D-BHB equivalents (Na is ~20.5% of Na D-BHB)
Bars	25	4.63 / 20.75	18	2.07 / 14.94	100	29.5 / 71	25	5.13 / 20.25
Beverage concentrates, dry, not reconstituted	40	7.4 / 33.2	50	5.75 / 41.5	200	59 / 142	40	8.2 / 32.4
Nutrition powders	40	7.4 / 33.2	50	5.75 / 41.5	200	59 / 142	40	8.2 / 32.4
Energy drinks	3.5	0.65 / 2.9	5	0.58 / 4.15	20	0.58 / 4.15	3.5	0.72 / 2.84
Sports drinks	3.5	0.65 / 2.9	5	0.58 / 4.15	20	0.58 / 4.15	3.5	0.72 / 2.84
Fluid replacements	3.5	0.65 / 2.9	5	0.58 / 4.15	20	0.58 / 4.15	3.5	0.72 / 2.84
Nutrition drinks	3.5	0.65 / 2.9	5	0.58 / 4.15	20	0.58 / 4.15	3.5	0.72 / 2.84

3.2 Exposure Estimates

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' D-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.[1]

Estimates derived from Creme of the total aggregate exposures to D-BHB salts at both the mean and 90th percentiles are shown in Tables 11, 13, 15, and 17 (absolute consumption as mg/day) and 12, 14, 16, and 18 (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 estimated aggregate D-BHB salts consumption data 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 F 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.[2] RSE values of greater than 25–30% are often considered reasonable cut-offs by which to consider a value unreliable.[2, 3] 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 11. Estimated absolute exposure to D-BHB calcium salt (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 12. Estimated exposure to D-BHB calcium salt relative 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 D-BHB, the highest absolute exposure estimate was that for males 20 years and older at the 90th percentile, at 2540 mg Ca D-BHB/day (highlighted in Table 11). This is equivalent to 469.9 mg of calcium and 2108.2 mg D-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 Ca D-BHB/kg bw/day (highlighted in Table 12).

Table 13. Estimated absolute exposure to D-BHB magnesium salt (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 14. Estimated exposure to D-BHB magnesium salt relative 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 D-BHB, the highest absolute exposure estimate was that for males 20 years and older at the 90th percentile, at 3357.2 mg Mg D-BHB/day (highlighted in Table 13). This is equivalent to 386.1 mg of magnesium and 2786.5 mg D-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 Mg D-BHB/kg bw/day (highlighted in Table 14).

Table 15. Estimated Exposure to D-BHB potassium salt (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 16. Estimated Exposure to D-BHB potassium salt relative 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 D-BHB potassium salt, the highest absolute exposure estimate was that for males 20 years and older at the 90th percentile, at 13950.0 mg K D-BHB/day (highlighted in Table 15). This is equivalent to 4115.3 mg of potassium and 9904.5 mg D-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 K D-BHB/kg bw/day (highlighted in Table 16). This is equivalent to 82.3 mg/kg bw/day of potassium and 241 mg/kg bw/day of BHB.

Table 17. Estimated absolute exposure to D-BHB sodium salt (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 18. Estimated exposure to D-BHB sodium salt relative 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 D-BHB sodium salt, the highest absolute exposure estimate was that for males 20 years and older at the 90th percentile, at 2540 mg Na D-BHB/day (highlighted in Table 17). This is equivalent to 520.7 mg of sodium and 2057.4 mg D-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 Na D-BHB/kg bw/day (highlighted in Table 18).

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

- Ca D-BHB: 2163.9 mg/day (absolute) and 35.8 mg/kg bw/day (relative to body weight)
- Mg D-BHB: 2865.5 mg/day (absolute) and 45.7 mg/kg bw/day (relative to body weight)
- K D-BHB: 11714.4 mg/day (absolute) and 185.2 mg/kg bw/day (relative to body weight)
- Na D-BHB: 2163.9 mg/day (absolute) and 35.8 mg/kg bw/day (relative 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 D-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.

3.2.1 Calcium

Ketone Labs' calcium salt of D-BHB contains 13.5–18.5% calcium (469.9 mg/day calcium at the 90th percentile). 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 daily value (DV) for calcium is 1000 mg/day. The tolerable upper limit (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.[4] Thus, the very conservative estimated intake of calcium from calcium D-BHB at the 90th percentile falls well below the UL for calcium intake.

3.2.2 Magnesium

Ketone Labs' magnesium salt of BHB contains 7.0–11.5% magnesium (386.1 mg/day magnesium at the 90th percentile). 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.[5] Magnesium is found in a variety of foods including nuts, legumes, whole grains, spices, leafy green vegetables, chocolate and seafood. [6]

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 DV for magnesium is 400 mg; however the UL for nonfood sources of magnesium is 350 mg/day, which is derived from the concern that excessive magnesium supplementation (bolus dosing) can cause osmotic diarrhea.[7] While the 90th percentile estimated daily intake of magnesium from magnesium D-BHB is slightly above the UL for nonfood sources of magnesium, it is unlikely that a consumer would consume that amount as a bolus dose because the exposure estimation is based on addition of the ingredient to various foods that would likely be consumed throughout the day. Therefore, it is unlikely that the estimated exposure to magnesium D-BHB would cause undesirable effects.

3.2.3 Potassium

Ketone Labs' potassium salt of D-BHB contains 24.0–29.5% potassium (4115.3 mg/day potassium at the 90th percentile). Potassium is the most abundant intracellular cation in the body and has a role in muscle contraction, and cardiovascular and genitourinary function.[8, 9] Potassium assists in the regulation of the body's acid-base balance and contributes to establishing a membrane potential in nerve fibers.[10] 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.[11] 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.[8, 10, 12]

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.[12] 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 dietary reference intake (DRI) could not be established due to insufficient data from dose response trials.[9] 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.[9, 10] 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.[9, 13] 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.[10] 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.[10]

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.[14, 15] 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.[14-16] 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.[15] 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.[16] 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.[17] 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.[9]

The form of potassium found in potassium D-BHB is not that used in the studies in which adverse effects were found. Furthermore, the estimated exposure from potassium in potassium D-BHB at the 90th percentile is below the AI level and not expected to be problematic in a healthy population.

3.2.4 Sodium

Ketone Labs' sodium salt of D-BHB contains 15.0–20.5% sodium (520.7 mg/day sodium at the 90th percentile). 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.[9] 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 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 UL levels 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.[9] Thus, the potential sodium exposure from sodium D-BHB is well below the UL.

Part 4: Self-limiting Levels of Use

While there are no known specific self-limiting levels of use, D-BHB salts do have a sharp, bitter taste when consumed individually and may have subjective palatability limits in various foods.

Part 5: Experience Based on Common Use in Food Prior to 1958

The GRAS conclusion for D-BHB salts is based on scientific procedures, and thus, experience based on common use in food prior to 1958 is not considered pivotal information. To the best of our knowledge, D-BHB salts were not commonly used in foods prior to 1958.

Part 6: Narrative

6.1 Overview

BHB (also known as 3-hydroxybutanoic acid; 3-hydroxybutyric acid) in the D form, is an endogenous compound commonly referred to as a "ketone body" (see Figure 9).[18] 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. D-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.[19] Ketone bodies act as oxidative substrates to provide energy for the body and brain in times of glucose deprivation.[20]

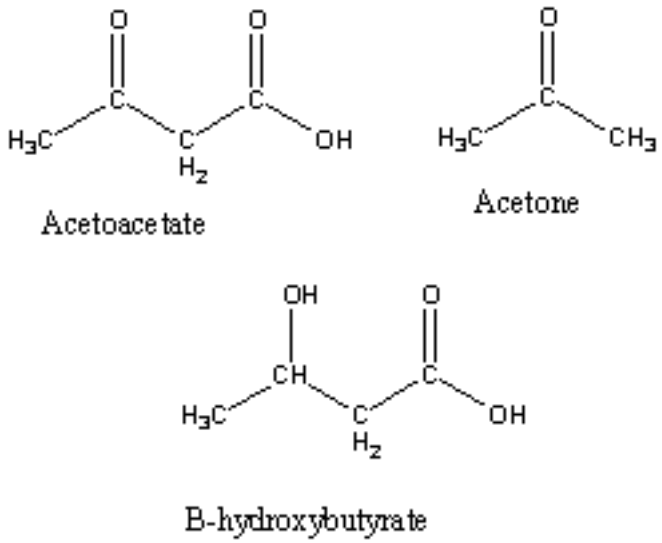


Figure 9. Chemical structures of ketone bodies

In humans, D-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.[19] D-BHB is produced in low amounts during normal metabolism and higher amounts during periods of fasting or starvation, during intake of ketogenic diets, prolonged intense exercise or in disease states (e.g. inborn errors of metabolism and 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 >25 mM.[21] 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 ~5–8 mM), the majority of which is BHB, without negative effects.[22] [23, 24] 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 (>25 mM).[23, 25, 26]

Once produced, D-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.[19] 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.[26]

6.2 Pharmacokinetics

Ketone bodies rapidly clear 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 (see Figure 10), a ketone ester that is hydrolyzed to D-BHB 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.[27] 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-BHB and acetoacetate levels increased proportionately with the increasing doses of the ketone ester. The C_{max} of D-BHB 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-BHB ranged from 0.77–3.06 hours. The apparent total plasma clearance of D-BHB was nearly three-fold higher in those consuming the

lowest dose compared to the highest dose, a phenomenon seen in other studies.[25] 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.[28] At the highest single dose (approx. 150 g in a 70 kg person), blood BHB did not exceed "normal" levels (5.5 mM).

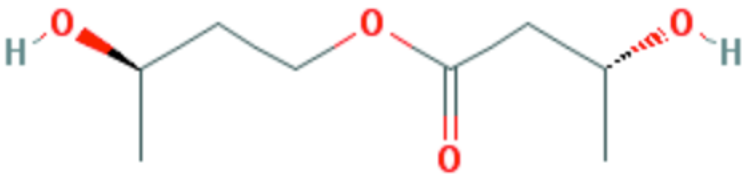


Figure 10. Chemical structure of (R)-3-hydroxybutyl (R)-3-hydroxybutyrate[29]

The pharmacokinetics of single and multiple intravenous (IV) doses of KTX 0101 (sodium D-BHB) up to 2000 mg/kg/day were investigated in several species (rats, rabbits and dogs).[30] While studies utilizing IV dosing tend not to be extremely relevant when evaluating oral pharmacokinetics, we discuss IV dosing here because of normal human endogenous BHB production, which places BHB directly into the circulation. 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 radiolabeling, the tissue distribution of intravenous doses of KTX 0101 (sodium D-BHB) were 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 D-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.

In a recent three-part study on human metabolism of exogenous ketones in healthy volunteers, Stubbs et al., (2017) compared the pharmacokinetics of ingestion of a ketone ester drink (KE) ((R)-3-hydroxybutyl (R)-3-hydroxybutyrate, (1 mol of KE delivered 2 mol of D-BHB equivalent)) with that of a ketone salt drink (KS) (sodium and potassium BHB, 50:50 D:L BHB)[31]. Available data indicates that (R)-3-hydroxybutyl (R)-3-hydroxybutyrate is rapidly hydrolyzed to D-BHB and 1,3-butanediol in the gut; the latter is then further metabolized to D-BHB and acetoacetate in the liver.[32] Study 1 consisted of a randomized four-arm cross-over study on healthy volunteers (n=15) in which blood D-BHB concentrations were compared following ingestion of equimolar amounts of BHB as KE or KS at two doses (1.6 and 3.2 mmol/kg) over four visits. Blinding was not possible because of unmaskable differences in taste (bitter vs salty).[31] It is important to note that the D-BHB content of KE is approximately double that of KS. The D-BHB C_{max} for KE and KS were 2.8 ± 0.2 mM and 1.0 ± 0.1 mM, respectively. After the peak, blood D-BHB disappearance was non-linear, following first order elimination kinetics. D-BHB T_{max} was approximately 2-fold longer after KS (racemic) consumption versus KE (D-BHB) consumption ($p<0.01$). AUC for KS D-BHB was approximately 30–60% lower than that for the KE drink ($p<0.01$). L-BHB plasma levels in high dose KS (racemic) drink group were higher than D-BHB levels; total BHB C_{max} was 3.4 ± 0.2 mM and total AUC of 549 ± 19 mmol.min. After 4 h, plasma L-BHB was 1.9 ± 0.2 mM. Both D- and L-BHB were excreted in proportion to their blood AUCs (KS group).

A follow up experiment was conducted in order to evaluate L-BHB elimination.[31] Five subjects consumed 3.2 mmol/kg of BHB as KE and KS followed by hourly blood and breath sample collection for 4 hours and then samples at 8 h and 24 h post-ingestion. The concentration of L-BHB in the KS group was 1.1 ± 0.1 mM at

4h, 0.7 ± 0.2 mM at 8 h, and undetectable at 24 h post-ingestion. The low amounts of D-BHB (0.3 ± 0.1 mM) detected at 24 h were attributed by the authors to endogenous production. Breath acetone test results suggest that D-BHB is readily converted to acetone, but L-BHB is not.

The amount of D-BHB excreted via the urine (the exact amount was not provided in the paper) increased with D-BHB intake; the volume of excreted D-BHB was <1.5% of the total ingested BHB and that value did not differ between matched doses of KE and KS.

Study 2 consisted of a randomized five-arm cross-over study (n=16) in which inter- and intra-participant repeatability of ketosis was examined following ingestion of identical KE drinks. Over five visits, participants consumed one of the following, twice while fasted and twice following a standardized meal: KE, 4.4 mmol/kg or 2.2 mmol/kg or an isocaloric control. The high carb and calorie meal significantly decreased peak D-BHB by approximately 1 mM and reduced the D-BHB AUC by 27%. D-BHB T_{max} was not significantly changed by a meal nor was urinary ketone excretion affected (results were repeatable). D-BHB C_{max} ranged from 1.3–3.5 mM in a fed state and 2.3–4.7 mM when fasting.

In Study 3, blood D-BHB (n=14) was measured after equal amounts of KE were given as three drinks (3 drinks of 4.4 mmol/kg at 3 h intervals (n=12)) or a constant nasogastric infusion (an initial bolus of 4.4 mmol/kg of BHB via nasogastric tube followed by an infusion of 1.1 mmol.kg.h^{-1} beginning after the initial bolus, for 8 h (n=4)). Each administration amounted to 13.2 mmol/kg of BHB (6.6 mmol/kg or 1161 mg/kg of KE) over a total of 9 hours. Serial drinks or NG infusion of KE maintained blood ketone concentrations >1mM for 9 h. For participants consuming drinks every 3 h, blood D-BHB rose but did not return to baseline by consumption of the next drink. The difference in C_{max} between drinks 2 (3.4 ± 0.2 mM) and 3 (3.8 ± 0.2 mM) was not statistically significant; the rate of D-BHB appearance fell slightly with successive drinks. D-BHB elimination was not statistically significantly changed after each bolus.

6.3 Toxicology Studies

6.3.1 Genotoxicity, 28-day repeated-dose intravenous, and reproductive toxicity studies on the sodium salt of D-beta-hydroxybutyrate (KTX0101)

A published toxicological evaluation of KTX 0101 (97.9–100% sodium D-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.[30] 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 clearly defined 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 D-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.

6.3.2 Oral 28-day and developmental toxicity studies of (R)-3-hydroxybutyl (R)-3-hydroxybutyrate)

As previously mentioned, available data indicates that (R)-3-Hydroxybutyl (R)-3-hydroxybutyrate is rapidly hydrolyzed to D-BHB and 1,3-butanediol in the gut; the latter is then further metabolized to D-BHB and acetoacetate in the liver (1 mol of the test article is equal to 2 mol BHB).[31, 32] Therefore, the subchronic and developmental studies described below on this BHB ketone ester are considered relevant to the toxicity of D-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.[32] 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 patterns. 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.[33, 34]

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 19 and 20). 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 19. Summary of selected hematological findings in the 28-day repeated dose oral toxicity study[32]

Group	RBC $x10^{12}$/L	HGB g/L	HCT %	MCV fL	MCH pg	MCHC g/L	PLT $x10^9$/L	RET x109/L	APTT Sec	Fibrinogen 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 20. Summary of selected clinical chemistry findings in the 28-day repeated dose oral toxicity study[32]

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 21). 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 21. Summary of reported histopathology findings in a 28-day repeated dose oral toxicity study[32]

	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 ((R)-3-Hydroxybutyl (R)-3-hydroxybutyrate, which is rapidly hydrolyzed/metabolized to D-BHB) 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.[32] 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 ((R)-3-Hydroxybutyl (R)-3-hydroxybutyrate, as 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.[32]

6.4 Additional Scientific Studies

6.4.1 Animal Studies

Kesl, et al., (2016) conducted a 28-day study in Sprague-Dawley rats in which they 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.[35] 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 whole blood BHB 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. [36-39] [40, 41]

6.4.2 Human Studies

High dose intravenous (up to 1,463 mg/kg bw/day) and oral (up to 2142 mg/kg bw/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.[42-47] [21, 25, 28, 48-50] Several of these studies were conducted in infants and children with metabolic disorders and demonstrated the safety and efficacy of such treatment.[51-53] Side effects from the administration of sodium BHB appear to be mainly gastrointestinal and are mild to moderate in nature.[27]

A human clinical study focused on safety was recently conducted on the ketone ester, (R)-3-hydroxybutyl (R)-3-hydroxybutyrate.[27] 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 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.[54] 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.

6.5 Authoritative Safety Opinions

6.5.1 World Health Organization

JECFA concluded (based on studies performed in the 1970s) 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.[55] Additionally, oral 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.

6.6 Current U. S. Regulatory Status

Searched entities included: beta-hydroxybutyrate, 3-hydroxybutyrate. A summary of the pertinent search results is shown below:

A GRAS notification (GRN 515) was submitted to the FDA by TdeltaS (Oxfordshire, UK) for their ≥97.5% ketone ester ((R)-3-hydroxybutyl (R)-3-hydroxybutyrate) on May 8, 2014. The 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.

6.7 Allergenicity

Salts of BHB do not contain or have added, and are manufactured in a facility free of, all eight major allergens (milk, egg, fish, Crustacean shellfish, tree nuts, wheat, peanuts, and soybeans) identified, and required to be disclosed in labeling, in the Food Allergen Labeling and Consumer Protection Act (FALCPA). Additionally, Salts of D-BHB do not contain gluten, oats, celery, mustard, sesame seeds, sulfur dioxide and sulfites or any derivatives or products of the aforementioned.

No reports of allergic reactions to D-BHB salts were found in our investigation of the literature.

6.8 History of Consumption and Ketogenic Diets

Pierre Marie, a French neurologist, first proposed fasting as a treatment for epilepsy in the late 19th century.[22] 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.[56] 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.[57, 22] These diets have also become popular for weight loss and other conditions such as sleep disorders, migraines, autism and pain.[58, 59] 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.[60, 61] 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.[60, 61] 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.).[62]

6.9 Past Sales and Reported Adverse Events

According to Ketone Labs approximately 2000 kg (equivalent to 350,000 servings as defined in this report) of the company's D-BHB have been sold within the U.S. since its market introduction in 2018. Ketone Labs states that no serious adverse event reports with the consumption of this ingredient to date have been received by the company.

No FDA letters regarding concern for safety to companies that market products containing D-BHB were located. A search of FDA's Substances Added to Food (formerly EAFUS), MedWatch (FDA's adverse event reporting program), FDA's Recalls, Market Withdrawals, & Safety Alerts search engine, and FDA's Center for Food Safety and Applied Nutrition Adverse Event Reporting System did not uncover any mention of BHB products. All databases were accessed on August 23, 2018.

6.10 Similar Product in the Marketplace

A general Internet search and searches of several large distributors of dietary supplements resulted in a number of findings of BHB-containing products, illustrating this ingredient is widely available in the U.S. Despite this prevalence, we are unaware of any serious adverse events attributed to BHB (racemers were not defined on the labels). Examples of products containing BHB are listed in Table 22.

Table 22. U.S. products containing BHB

Company	Product Name	Serving Size(s)
PrimaForce	BHB	6 g BHB salts (calcium, sodium, and magnesium salts)
Nutricost	Magnesium Ketone Salt	6500 mg BHB magnesium salt
Giant Sports International	Keto Beta Hydroxybutyrate	14.6 g BHB salts (calcium, sodium, and magnesium salts)

Kiss my Keto	Exogenous Ketones with electrolytes	11.7 g BHB salts (calcium, sodium, and magnesium salts)
Perfect Keto	Exogenous Ketones, chocolate flavor	11.38 g BHB salts (calcium, sodium, and magnesium salts)

6.11 Basis for the GRAS Conclusion

The scientific procedures establishing the safety of D-BHB comprise the technical element of the GRAS standard. The common knowledge element is comprised of the general availability and general acceptance, throughout the scientific community of qualified experts, of the technical element. Together, the technical element and the common knowledge element form the basis for Ketone Labs' conclusion conclusion of GRAS status of D-BHB for its intended uses.

6.11.1 Technical Element

D-BHB has been the subject of a thorough safety assessment as described above. The totality of evidence supporting the safety of D-BHB is comprised of pivotal data and information that establish the safety of D-BHB under the conditions of its intended use (the technical element) and data and information that are corroborative of safety.

The method of manufacture and specifications describe the safe production, the high-quality control standards, and identity of D-BHB.

Pivotal data for this independent conclusion is the 28-day repeated-dose 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 D-BHB and 1,3-butanediol in the gut. The latter is then further metabolized to D-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, 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.

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 use of the BHB salts as shown in Table 10, 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 relative to body weight (with an RSE value of <25%, making it reasonably reliable) were highest for females ages 0–2 for sodium D-BHB and calcium D-BHB. The 90th percentile exposures relative to body weight were highest for males ages 3–11 for magnesium D-BHB and potassium D-BHB. The NOAEL from the above 28-day repeated dose oral toxicity study allows for adequate margins of safety (MOS) for D-BHB as D-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 D-BHB MOS for exposure to the calcium, magnesium, and sodium salt forms was greater than 100, which is considered reasonable for ingredients added to foods. The calculations are shown below:

- MOS for calcium D-BHB: The highest EDI based on body weight was 76.6 mg/kg bw/day (Table 12). At most, D-BHB makes up 83% of the calcium D-BHB product, so the highest EDI for D-BHB alone can be calculated as 64 mg/kg bw/day. The MOS (7000 mg/kg bw/day divided by 64 mg/kg bw/day) is equal to 109.
- MOS for magnesium D-BHB trihydrate: The highest EDI based on body weight was 73.7 mg/kg bw/day (Table 14). At most, D-BHB makes up 83% of the magnesium D-BHB product, so the highest EDI for D-BHB alone can be calculated as 61 mg/kg bw/day. The MOS (7000 mg/kg bw/day divided by 61 mg/kg bw/day) is equal to 115.
- MOS for potassium D-BHB: The highest EDI based on body weight was 323.3 mg/kg bw/day (Table 16). At most, D-BHB makes up NLT 71% of the potassium D-BHB product, so the highest EDI for D-BHB alone can be calculated as 230 mg/kg bw/day. The MOS (7000 mg/kg bw/day divided by 230 mg/kg bw/day) is equal to 30. 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 D-BHB as described herein and in GRN 515.

- MOS for sodium D-BHB: The highest EDI based on body weight was 76.6 mg/kg bw/day (Table 18). At most, D-BHB makes up 81% of the sodium D-BHB product, so the highest EDI for D-BHB alone can be calculated as 62 mg/kg bw/day. The MOS (7000 mg/kg bw/day divided by 62 mg/kg bw/day) is equal to 113.
- If all four D-BHB salts were consumed at their respective 90th percentile exposure levels, the total exposure to D-BHB would be 417 mg/kg bw/day of D-BHB (64 + 61 + 230 + 62 = 417). In this case, the MOS for D-BHB (7000 mg/kg bw/day divided by 417 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 D-BHB, or 88.56 g/day) resulted in D-BHB plasma levels that are known to occur physiologically and are generally considered as safe.[27] The current absolute maximum anticipated D-BHB exposure level (417 mg/kg bw/day x average 70 kg adult = 29.2 g/day) is approximately 33% of the BHB contained in the ketone ester study (88.56 g/day).

With regard to exposure to the calcium, magnesium, potassium, and sodium 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 calcium, magnesium, and sodium are 1000 mg, 400 mg, and 2400 mg, respectively and the AI for potassium is 4700 mg/day. The addition levels of calcium, magnesium, and sodium are similar to levels that are found in a serving of other commonly consumed foods in the same categories or the 90th percentile exposure estimates are near or below the DV, AI, or UL (see Appendix G). Thus, the exposure levels, and hence the addition levels, are considered within safe parameters for a healthy population.

6.11.2 Common Knowledge Element

The scientific data, information, and methods herein reported, that provide the basis of this GRAS conclusion by scientific procedures are published and available in the public domain. Part 8 of this GRAS notice contains the citations for the published

studies. This publicly available data and information fulfills the requirement for general availability of the scientific data, information, and methods relied on to establish the technical element of the GRAS standard. The peer-review of the published studies and lack of Letters to the Editor or other dissenting opinions, together with the opinion of the GRAS Panel convened to review and analyze the data herein reported provides ample evidence of consensus among qualified experts that there is reasonable certainty that consumption of D-BHB for its intended use is not harmful. The general availability and acceptance of this scientific data, information, and methods satisfies the common knowledge element of this GRAS conclusion.

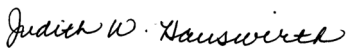
6.12 Data and Information that is Inconsistent with the GRAS Conclusion

We have reviewed the available data and information and are not aware of any data and information that are, or may appear to be, inconsistent with our conclusion of GRAS status.

Part 7: Opinion of the GRAS Panel

The GRAS Panel has, independently and collectively, critically evaluated this safety assessment of Ketone Labs' D-BHB Salts and unanimously opine that the scientific data, information, and methods herein described establish that D-BHB salts, produced in accordance with Good Manufacturing Practice and meeting the specifications presented in the document, are reasonably certain to be safe under the conditions of their intended uses as food ingredients. In addition, the scientific data, information, and methods that form the basis of this opinion are generally available, and the GRAS Panel believes, as evidenced in Part 6.11.2 of the report, that other experts qualified by training and experience to evaluate the safety of food ingredients would concur with this opinion. Therefore, the GRAS Panel further opines that this dossier and the opinion of the GRAS Panel support the conclusion by Ketone Labs that D-BHB salts are generally recognized as safe for their intended conditions of use.

Panel Members: ***Date:***



November 6, 2018

Judith Hauswirth, PhD
Chair of Expert Panel

November 6, 2018

John R. Endres, ND

Panel Member

November 6, 2018

Amy Clewell, ND, DABT
Panel Member

7.1 *Curricula vitae* of the GRAS 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 conclusion 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 Seattle, 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 conclusions. 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 Publicly 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.

Part 8: Supporting Data and Information

Literature searches for the safety assessment described in Part 6 of this GRAS notice were conducted from July 2018 through August 2018.

8.1 Data and Information that are *not* Generally Available

All of the information described in this report is generally available.

8.2 References that *are* Generally Available

1. Lambe J, Kearney J, et al. The influence of survey duration on estimates of food intakes and its relevance for public health nutrition and food safety issues. *Eur J Clin Nutr*. 2000;54(2):166-73
2. Wright JD, Wang CY, et al. Dietary intake of ten key nutrients for public health, United States: 1999-2000. *Adv Data*. 2003(334):1-4
3. Klein R, Proctor S, et al. Healthy People 2010 criteria for data suppression. *Statistical Notes*. 2002;24
4. Food and Nutrition Board and Institute of Medicine. Report Brief. Dietary Reference Intakes for calcium and Vitamin D. National Academy of Sciences. 2011. 1-4.
5. Musso CG. Magnesium metabolism in health and disease. *Int Urol Nephrol*. 2009;41(2):357-62
6. Groff J and Gropper S. Magnesium. Advanced Nutrition and Human Metabolism: Third Edition. Belmont: Wadsworth/Thomson: 2000. 389-392.
7. Food and Nutrition Board and IOM. 6. Magnesium. Dietary Reference Intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride. www.nap.edu, National Academy of Sciences; 1997: 190-249.
8. Balch JF and Balch PA. Minerals. Prescription for Nutritional Healing. Garden City Park: Avery Publishing Group: 1997. 22-29.
9. Food and Nutrition Board. Dietary Reference Intakes for water, potassium, sodium, chloride, and sulfate. Panel on Dietary Reference Intakes for Electrolytes and Water, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Institute of Medicine, National Academy of Sciences. 2005. 1-638.
10. EFSA Panel on Dietetic Products Nutrition and Allergies (NDA). Dietary reference values for potassium. *EFSA Journal*. 2016;14(10)

11. Greenlee M, Wingo CS, et al. Narrative review: evolving concepts in potassium homeostasis and hypokalemia. *Ann Intern Med*. 2009;150(9):619-25
12. WHO. Guideline: Potassium intake for adults. World Health Organization. 2012.
13. Hajjar IM, Grim CE, et al. Impact of diet on blood pressure and age-related changes in blood pressure in the US population: analysis of NHANES III. *Arch Intern Med*. 2001;161(4):589-93
14. Leijonmarck CE and Raf L. Gastrointestinal lesions and potassium chloride supplements. *Lancet*. 1985;1(8419):56-7
15. Lambert JR and Newman A. Ulceration and stricture of the esophagus due to oral potassium chloride (slow release tablet) therapy. *Am J Gastroenterol*. 1980;73(6):508-11
16. McMahon FG, Ryan JR, et al. Upper gastrointestinal lesions after potassium chloride supplements: a controlled clinical trial. *Lancet*. 1982;2(8307):1059-61
17. Whelton PK, Buring J, et al. The effect of potassium supplementation in persons with a high-normal blood pressure. Results from phase I of the Trials of Hypertension Prevention (TOHP). Trials of Hypertension Prevention (TOHP) Collaborative Research Group. *Ann Epidemiol*. 1995;5(2):85-95
18. Mathews C, van Holde K, et al., Eds. Biochemistry. 4th Edition. Pearson; 2013.
19. Laffel L. Ketone bodies: a review of physiology, pathophysiology and application of monitoring to diabetes. *Diabetes Metab Res Rev*. 1999;15(6):412-26
20. Owen OE, Morgan AP, et al. Brain metabolism during fasting. *J Clin Invest*. 1967;46(10):1589-95
21. Shivva V, Cox PJ, et al. The Population Pharmacokinetics of D-beta-hydroxybutyrate Following Administration of (R)-3-Hydroxybutyl (R)-3-Hydroxybutyrate. *AAPS J*. 2016
22. Cahill GF, Jr. and Veech RL. Ketoacids? Good medicine? *Trans Am Clin Climatol Assoc*. 2003;114:149-61; discussion 162-3
23. Hashim SA and VanItallie TB. Ketone body therapy: from the ketogenic diet to the oral administration of ketone ester. *J Lipid Res*. 2014;55(9):1818-26
24. Reichard GA, Jr., Owen OE, et al. Ketone-body production and oxidation in fasting obese humans. *J Clin Invest*. 1974;53(2):508-15
25. Balasse EO. Kinetics of ketone body metabolism in fasting humans. *Metabolism*. 1979;28(1):41-50
26. Fukao T, Lopaschuk GD, et al. Pathways and control of ketone body metabolism: on the fringe of lipid biochemistry. *Prostaglandins Leukot Essent Fatty Acids*. 2004;70(3):243-51

27. Clarke K, Tchabanenko K, et al. Kinetics, safety and tolerability of (R)-3-hydroxybutyl (R)-3-hydroxybutyrate in healthy adult subjects. *Regul Toxicol Pharmacol*. 2012;63(3):401-8
28. Hall SE, Wastney ME, et al. Ketone body kinetics in humans: the effects of insulin-dependent diabetes, obesity, and starvation. *J Lipid Res*. 1984;25(11):1184-94
29. National Center for Biotechnology Information. PubChem Compound Database; CID=44631890. Ketone ester. [Retrieved October 29, 2018] from https://pubchem.ncbi.nlm.nih.gov/compound/44631890#section=Top.
30. Smith SL, Heal DJ, et al. KTX 0101: a potential metabolic approach to cytoprotection in major surgery and neurological disorders. *CNS Drug Rev*. 2005;11(2):113-40
31. Stubbs BJ, Cox PJ, et al. On the Metabolism of Exogenous Ketones in Humans. *Front Physiol*. 2017;8:848
32. Clarke K, Tchabanenko K, et al. Oral 28-day and developmental toxicity studies of (R)-3-hydroxybutyl (R)-3-hydroxybutyrate. *Regul Toxicol Pharmacol*. 2012;63(2):196-208
33. Johnstone AM, Horgan GW, et al. Effects of a high-protein ketogenic diet on hunger, appetite, and weight loss in obese men feeding ad libitum. *Am J Clin Nutr*. 2008;87(1):44-55
34. McClernon FJ, Yancy WS, Jr., et al. The effects of a low-carbohydrate ketogenic diet and a low-fat diet on mood, hunger, and other self-reported symptoms. *Obesity (Silver Spring)*. 2007;15(1):182-7
35. Kesl SL, Poff AM, et al. Effects of exogenous ketone supplementation on blood ketone, glucose, triglyceride, and lipoprotein levels in Sprague-Dawley rats. *Nutr Metab (Lond)*. 2016;13:9
36. Kashiwaya Y, Bergman C, et al. A ketone ester diet exhibits anxiolytic and cognition-sparing properties, and lessens amyloid and tau pathologies in a mouse model of Alzheimer's disease. *Neurobiol Aging*. 2013;34(6):1530-9
37. Park S, Kim da S, et al. Central infusion of ketone bodies modulates body weight and hepatic insulin sensitivity by modifying hypothalamic leptin and insulin signaling pathways in type 2 diabetic rats. *Brain Res*. 2011;1401:95-103
38. Umpleby AM, Chubb D, et al. The effect of ketone bodies on leucine and alanine metabolism in dogs. *Clin Sci (Lond)*. 1988;74(1):41-8
39. Muller MJ, Paschen U, et al. Effect of ketone bodies on glucose production and utilization in the miniature pig. *J Clin Invest*. 1984;74(1):249-61
40. Heitmann RN and Fernandez JM. Autoregulation of alimentary and hepatic ketogenesis in sheep. *J Dairy Sci*. 1986;69(5):1270-81

41. Puchowicz MA, Smith CL, et al. Dog model of therapeutic ketosis induced by oral administration of R,S-1,3-butanediol diacetoacetate. *J Nutr Biochem*. 2000;11(5):281-7
42. Chiolero R, Mavrocordatos P, et al. Effects of infused sodium acetate, sodium lactate, and sodium beta-hydroxybutyrate on energy expenditure and substrate oxidation rates in lean humans. *Am J Clin Nutr*. 1993;58(5):608-13
43. Frolund L, Kehlet H, et al. Effect of ketone body infusion on plasma catecholamine and substrate concentrations during acute hypoglycemia in man. *J Clin Endocrinol Metab*. 1980;50(3):557-9
44. Sherwin RS, Hendler RG, et al. Effect of ketone infusions on amino acid and nitrogen metabolism in man. *J Clin Invest*. 1975;55(6):1382-90
45. Pan JW, de Graaf RA, et al. [2,4-13 C2]-beta-Hydroxybutyrate metabolism in human brain. *J Cereb Blood Flow Metab*. 2002;22(7):890-8
46. Pan JW, Telang FW, et al. Measurement of beta-hydroxybutyrate in acute hyperketonemia in human brain. *J Neurochem*. 2001;79(3):539-44
47. Nair KS, Welle SL, et al. Effect of beta-hydroxybutyrate on whole-body leucine kinetics and fractional mixed skeletal muscle protein synthesis in humans. *J Clin Invest*. 1988;82(1):198-205
48. Mikkelsen KH, Seifert T, et al. Systemic, cerebral and skeletal muscle ketone body and energy metabolism during acute hyper-D-beta-hydroxybutyratemia in post-absorptive healthy males. *J Clin Endocrinol Metab*. 2015;100(2):636-43
49. Amiel SA, Archibald HR, et al. Ketone infusion lowers hormonal responses to hypoglycaemia: evidence for acute cerebral utilization of a non-glucose fuel. *Clin Sci (Lond)*. 1991;81(2):189-94
50. Lecocq FR and McPhaul JJ, Jr. The Effects of Starvation, High Fat Diets, and Ketone Infusions on Uric Acid Balance. *Metabolism*. 1965;14:186-97
51. Plecko B, Stoeckler-Ipsiroglu S, et al. Oral beta-hydroxybutyrate supplementation in two patients with hyperinsulinemic hypoglycemia: monitoring of beta-hydroxybutyrate levels in blood and cerebrospinal fluid, and in the brain by in vivo magnetic resonance spectroscopy. *Pediatr Res*. 2002;52(2):301-6
52. Van Hove JL, Grunewald S, et al. D,L-3-hydroxybutyrate treatment of multiple acyl-CoA dehydrogenase deficiency (MADD). *Lancet*. 2003;361(9367):1433-5
53. Bougneres PF, Ferre P, et al. Glucose metabolism in hyperinsulinemic infants: the effects of fasting and sodium DL-beta-hydroxybutyrate on glucose production and utilization rates. *J Clin Endocrinol Metab*. 1983;57(5):1054-60
54. Cervenka MC, Henry BJ, et al. Establishing an Adult Epilepsy Diet Center: Experience, efficacy and challenges. *Epilepsy Behav*. 2016;58:61-8

55. Joint FAO/WHO Expert Committee on Food Additives. Butane-1,3-diol.
56. Veech RL. The therapeutic implications of ketone bodies: the effects of ketone bodies in pathological conditions: ketosis, ketogenic diet, redox states, insulin resistance, and mitochondrial metabolism. *Prostaglandins Leukot Essent Fatty Acids*. 2004;70(3):309-19
57. Vanitallie TB, Nonas C, et al. Treatment of Parkinson disease with diet-induced hyperketonemia: a feasibility study. *Neurology*. 2005;64(4):728-30
58. Stafstrom CE and Rho JM. The ketogenic diet as a treatment paradigm for diverse neurological disorders. *Front Pharmacol*. 2012;3:59
59. Hall KD, Chen KY, et al. Energy expenditure and body composition changes after an isocaloric ketogenic diet in overweight and obese men. *Am J Clin Nutr*. 2016;104(2):324-33
60. Veech RL. Ketone ester effects on metabolism and transcription. *J Lipid Res*. 2014;55(10):2004-6
61. Nielsen NI, Larsen T, et al. Quarter health, milking interval, and sampling time during milking affect the concentration of milk constituents. *J Dairy Sci*. 2005;88(9):3186-200
62. Larsen T and Nielsen NI. Fluorometric determination of beta-hydroxybutyrate in milk and blood plasma. *J Dairy Sci*. 2005;88(6):2004-9