BenfoPure® - New Dietary Ingredient Premarket Notification

BenfoPure® brand of benfotiamine (*S*-benzoylthiamine O-monophosphate)

This NDI is made pursuant to the requirements of "Nutritional Supplements, New Dietary Ingredients, Requirements for Pre-Marketing Notification" (21CFR 190.6).

Submitted by: (b) (4)

Submitted on behalf of: Hamari Chemicals, Ltd.

1-4-9 Kunijima, Higashi-Yodogawa-ku

Osaka, Japan 533-0024

Submission Date:

15 October 2010

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Cover Letter – BenfoPure® New Dietary Ingredient Submission

15 October 2010

Food and Drug Administration

Division of Standards and Labeling Regulations

Office of Nutritional Products, Labeling and Dietary Supplements (HFS-820)

Center for Food Safety and Applied Nutrition

Food and Drug Administration

5100 Paint Branch Parkway

College Park, MD, 20740-3835

Dear CFSAN:

Hamari Chemicals, Ltd hereby submits this **New Dietary Ingredient Premarket Notification** pursuant to the requirements of 21CFR 190.6 which specifies that at least 75 days prior to marketing a dietary supplement containing a new dietary ingredient, that the manufacturer submit information providing the basis upon which we have concluded that the new dietary ingredient (NDI) is reasonably expected to be safe.

Thank you in advance for your consideration of our notification.

Sincerely,

(b) (4)

1.0 Preface to the New Dietary Ingredient Submission:

Pursuant to 21CFR 190.6(b)(3)(ii), Benfotiamine is submitted as a new dietary source of thiamine (vitamin B_1) as a nutritional supplement. As will be discussed further, Benfotiamine is a lipid-soluble thiamine vitamin derivative, is biologically equivalent to vitamin B1 (thiamine) and is an efficient way to supplement thiamine in the human diet.

DSHEA defines dietary ingredients to include one or any combination of the following:

- *vitamins*
- *minerals*
- *herbs or other botanicals*
- *amino acids*
- *dietary substance used by man to supplement the diet by increasing the total dietary intake (e.g. enzymes or tissue from organs or glands)*
- *concentrates, metabolites or extracts.*

Benfotiamine is a member of a family of compounds known as allithiamines, naturally occurring forms of the vitamin B1 present in garlic, onions, leeks, shallots and other similar vegetables. Chemically known as S-Benzoylthiamine O-Monophosphate, its discovery was originally described by Wada et al in the journal *Science* in 1961[1].

While the term allithiamine originally referred to one particular thiamine derivative, first discovered by Fujiwara and his colleagues in 1954[2], the term has since come to refer to the entire class of high-bioavailability thiamine derivatives sharing a common set of biochemical features – a class which includes benfotiamine.

The primary literature supports this fact with investigators specifically describing benfotiamine as "an allithiamine." Benfotiamine has been widely studied; Stracke et al. describe benfotiamine as "an Allithiamine," which they explicate to be "a lipid-soluble derivative of vitamin B1 with high bioavailability"[3]. Loew, in reviewing the pharmacokinetics of various thiamine derivatives, describes benfotiamine as "an allithiamine," and discusses the "lipophilic thiamine analogues, the allithiamines (acetiamine, benfotiamine, or fursultiamine [TTFD])" [4]. Again, in their pharmacokinetic study of tissue distribution of thiamine vs. benfotiamine, Hilbig and Rahmann describe "benfotiamine, CAS 22457-89-2" as a "lipid-soluble allithiamine"[5]. Ziems and colleagues, in their recent study on the metabolism of benfotiamine, introduce their study with the statement that "Lipid-soluble thiamine derivatives belonging to the allithiamine group were discovered nearly 40 years ago"[6]. Among these agents, they include S-benzoylthiamine-O-monophosphate (BTMP) – i.e., benfotiamine.

Thiamine hydrochloride and thiamine mononitrate are widely distributed in the US as dietary supplements for vitamin B_1. In Japan and Europe, Benfotiamine has been an approved source of vitamin B_1 for decades, providing a strong argument to the ultimate safety of the compound.

2.0 Regulatory foundation and content of this submission

This premarket notification is being submitted pursuant to the requirements of 21CFR 190.6 which specifies that at least 75 days prior to marketing a dietary supplement containing a new dietary ingredient, that the manufacturer provide information providing the basis upon which we have concluded that the new dietary ingredient (NDI) is reasonably expected to be safe. The following information is provided in accordance with this requirement:

- Name and complete address for the manufacturer (sponsor) of the NDI
- Name and contact information for the regulatory correspondent
- Name and complete address of the contract manufacturer who manufactures the BenfoPure® product
- Complete description of the dietary supplement including:
    - Name of the NDI
    - Chemical characteristics of the NDI
    - Level of the NDI in the dietary supplement
    - Conditions of use for the dietary supplement
    - History of use or other evidence of safety demonstrating that the dietary supplement can reasonably be expected to be safe.
    - Product specifications for the NDI and the dietary supplement
    - A brief description of the method of manufacture of the NDI and dietary supplement
    - Product stability data for the NDI and the dietary supplement

3.0 Contact Information

NDI Owner: Hamari Chemicals, Ltd

1-4-9 Kunijima, Higashi-Yodogawa-ku

Osaka, Japan 533-0024

Manufacturer: (b) (4)

The regulatory correspondent is:

(b) (4)

4.0 Name, Identity and Chemical Characterization of the NDI

1. BenfoPure® meets the definition of a dietary ingredient under the FD&C Act, Section 201 [21 U.S.C 321(ff)(1)]: *"a vitamin, a mineral, a herb or other botanical, an amino acid, a dietary substance for use by man to supplement the diet by increasing total dietary intake; or a concentrate, metabolite, constituent, extract or combination of any of the above dietary ingredients***."** Benfotiamine fits the definition of an NDI under the meaning of (section 201(ff) of the act (21 U.S.C. 321(ff)) because it is widely recognized as a form of thiamine and therefore a vitamin.

2. Additionally, Benfotiamine (CAS 22457-89-2) is a member of class of compounds known as "allithiamines" which are forms of thiamine that occur naturally in crushed garlic, onions, leeks, shallots, and other members of the *Allium* genus of plants.

3. Furthermore, this new dietary ingredient meets the requirement that the product containing the dietary ingredient must be a dietary supplement (section 201(ff) of the act (21 U.S.C. 321(ff)). A dietary supplement is defined as a *"product (other than tobacco) intended to supplement the diet that contains one or more dietary ingredients. A dietary supplement is limited to products that are intended for ingestion in tablet, capsule, powder, softgel, gelcap and liquid form, that are not represented as conventional food or as the sole item of a meal or of the diet, and that are labeled as dietary supplements."*

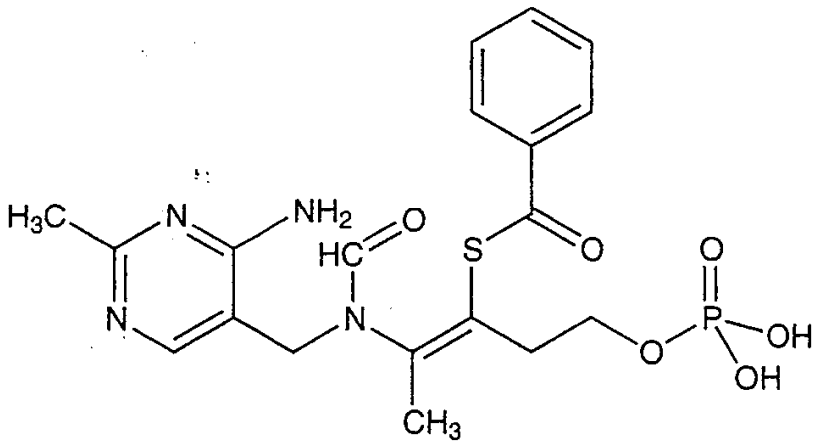
4. BenfoPure® is a lipid-soluble thiamin derivative where the thiazole ring, essential for vitamin activity, is open but closes upon passive absorption through the intestinal mucosa, leading to higher blood and tissue concentrations of thiamine at relatively low doses as compared to thiamine salt derivatives and other lipid-soluble forms of thiamine. Benfotiamine exhibits rapid absorption kinetics, and increased thiamine concentration and activity in numerous tissues.

5. BenfoPure® is intended to be orally ingested in tablet, capsule, powder, softgel, gelcap or liquid form, is not represented as a conventional food or sole item of a meal or diet, and is labeled as a dietary supplement, therefore the governing definitions for both a new dietary ingredient and a dietary supplement apply to BenfoPure®.

6. BenfoPure® (BENFOTIAMINE) is S-benzoylthiamine-O-monophosphate, common name **Benfotiamine**, CAS Number 22457-89-2, chemical formula, molecular formula and structure depicted below:

Benfotiamine is found in the Merck Index 14th Edition: monograph #1038, Benfotiamine. Merck & Co Inc. NJ, USA. (2006)[7]:

1038. Benfotiamine. [22457-89-2] Benzenecarbothioic acid *S*-[2-[[(4-amino-2-methyl-5-pyrimidinyl)methyl]formylamino]-1-[2-(phosphonooxy)ethyl]-1-propenyl] ester; thiobenzoic acid *S*-ester with *N*-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-*N*-(4-hydroxy-2-mercapto-1-methyl-1-butenyl)formamide *O*-phosphate; *S*-benzoylthiamine monophosphate; BTMP; Biotamin; Vitanevril. $C_{19}H_{23}N_4O_6PS$; mol wt 466.45. C 48.92%, H 4.97%, N 12.01%, O 20.58%, P 6.64%, S 6.87%. Vitamin B_1 source. Prepn: A. Ito *et al.*, **DE 1130811** (1962), *C.A.* **57,** 13764h (1962). Exists as 3 temperature dependent crystalline forms, α, γ, δ: A. Ito *et al., Takamine Kenkyusho Nempo* **14,** 64 (1962); *C.A.* **59,** 3920a (1963). Pharmacokinetics: H. Nogami *et al., Chem. Pharm. Bull. (Tokyo)* **18,** 1937 (1970). Clinical bioequivalence to thiamine: R. Bitsch *et al., Ann. Nutr. Metab.* **35,** 292 (1991).



(Z)-form

Crystals, dec 165° (δ-form).
THERAP CAT: Vitamin (enzyme cofactor).

As will be further discussed, this lipid-soluble form of thiamine is more rapidly absorbed and has an improved toxicity profile relative to other forms of vitamin B_1 currently in use in the United States.

Product Specifications:

(b) (4)

Manufacturing Process for the NDI

Benfotiamine is manufactured by (b) (4)

4.0 BenfoPure® Dietary Supplement

The formulation of the supplement is as shown above. Benfotiamine is typically filled into tablets, capsules, gelcaps, or in other forms intended for oral supplementation.

Dietary Supplement Usage:

The recommended intake of BenfoPure® is up to 600 mg/day. For a 50Kg person, this would be equivalent to 2.5 mg/kg up to 10 mg/kg. Note that the oral LD_{50} in mice was 15,000 mg/kg.

Conditions of Use:

The BenfoPure® dietary supplement is labeled for supplementation of vitamin B_1 under the following conditions of use:

- The recommended dose is up to 600 mg/day.
- The dietary supplement is specifically designed for use by healthy, non-pregnant, non-lactating adults.
- The dietary supplement is not intended for use by children, pregnant women and lactating mothers.

Product Stability

Stability data for the Hamari BenfoPure product can be found in Attachment C. Stability data is presented for (b) (4) lots at room temperature thru (b) (4) and another (b) (4) lots through (b) (4) . Data is also provided for (b) (4) lots at (b) (4) All tests are within specifications.

6.0 Evidence of Safety

History of Use:

While thiamine hydrochloride and thiamine mononitrate are widely distributed in the US as dietary supplements, in Japan and Europe, Benfotiamine has also been an approved source of vitamin B_1. Pertinent safety information is discussed below.

Biochemistry and Pharmacokinetics

Benfotiamine's structure contains an open thiazole ring that closes upon absorption, producing biologically active thiamine. Several clinical trials in healthy adults have demonstrated the superior absorption kinetics of lipid-soluble thiamine analogues, including benfotiamine, compared to water-soluble thiamine salts. Higher plasma thiamine levels are achieved with oral benfotiamine administration, and blood and tissue thiamine concentrations are maintained longer. Oral benfotiamine dosages in these studies ranged from 40-600 mg daily.

Benfotiamine is absorbed via passive diffusion through the intestinal mucosa and is rapidly converted to biologically active thiamine. Peak plasma concentrations of thiamine after oral benfotiamine administration are at least four times greater than those observed after oral administration of water-

soluble thiamine salts[1]. Half-life of benfotiamine is similar to thiamine salts, but bioavailability of thiamine eight days after administration is roughly 25 percent of the original dose, about 3.6 times greater than after an oral dose of thiamine hydrochloride salt[14].

Selected References: Safety, Mechanism and Clinical Experience

The following references speak to the safety, mechanism and clinical experience associated with oral supplementation with Benfotiamine.

Safety

Following the original discovery of Benfotiamine, Tadao Wada and his colleagues performed safety and efficacy testing of the vitamin in animals. The LD_{50} following oral administration in hybrid mice was 15,000 mg/kg with a 95% confidence limit compared to 9000 mg/kg for thiamine hydrochloride. They saw similar results with intravenous and intraperitoneal injection of Benfotiamine (LD_{50} 2200 mg/kg, and 1810 mg/kg respectively) compared to thiamine hydrochloride at 100 mg/kg. Additionally, following oral administration of Benfotiamine in dogs, total blood thiamine levels maintained approximately a 4-fold increase versus administration of a comparable dose of thiamine hydrochloride. They discovered a similar effect in humans, finding approximately a 10-fold increase in the urinary excretion of thiamine following oral administration of benfotiamine versus thiamine hydrochloride at a dose of 100mg[1].

Masaya Araki and his colleagues further studied the toxicity of benfotiamine, undertaking a chronic toxicity study in rats. In this study, rats were continuously fed food supplemented with 1000-ppm benfotiamine and sacrificed after one-year of this regimen. Pathology demonstrated no significant histological changes as compared to thiamine hydrochloride causing the authors to conclude that chronic toxicity of benfotiamine is as low as thiamin hydrochloride[8].

In an additional study performed by Haruhiko Minakami and colleagues, *Studies on Thiamine Phosphates: Pharmacological Actions of S-Benzoylthiamine-o-monophosphate*, it was again demonstrated that acute toxicity in mice was lower than that seen with thiamine hydrochloride. In contrast to other thiamine derivatives, the pharmacological activity of S-benzoylthiamine O-monophosphate on the heart, circulation, and intestine was weaker following benfotiamine administration[9].

Absorption & Pharmacokinetics

Further studies by Shindo and colleagues were undertaken to assess the mechanism of absorption and kinetic properties of Benfotiamine following oral dosage. Absorption of Benfotiamine was immediate, and the amount of free thiamine gradually increased with time, with no Benfotiamine detected in the blood. Most of the thiamine found was present in red blood cells[10]. As discovered in a follow-up study by the same group, S-benzoylthiamine (SBT) was a main component of the thiamine absorbed and that this moiety was rapidly converted to thiamine through exchange of the SH group with glutathione in the cell[11].

A number of other groups have assessed the bioavailability of thiamine following Benfotiamine administration. Using autoradiography techniques following administration of 3H-labeled benfotiamine or thiamine hydrochloride to mice, Hilbig and Rahmann demonstrated that thiamine from benfotiamine exhibited far greater incorporation in tissues versus the thiamine hydrochloride salt[5]. Geyer and his colleagues also demonstrated a significant increase in the bioavailability of thiamine from benfotiamine than from either thiamine hydrochloride or thiamine mononitrate in broiler chickens[12].

Bitsch *et al* assessed the bioavailability and pharmacokinetics of benfotiamine in comparison to thiamine mononitrate in humans. They found a 55% increase ($p<0.0001$) in plasma AUC levels of thiamine following a benfotiamine dose that was 60% less than the dosage of thiamine mononitrate provided to an alternate group, with no significant changes in C_{max} or t_{max} between the two groups. However, there was almost a three-fold increase in the hemolysate C_{max}, correlated with a two-fold increase in thiamine concentration (AUC)[13]. These results indicate the improved bioavailability of thiamine following benfotiamine administration in both plasma and red blood cells when compared to the water-soluble thiamine mononitrate.

In another randomized human trial, published by Schreeb *et al* in 1997 comparing the pharmacokinetics of benfotiamine and thiamine mononitrate, the mean AUC 1-10 hours following benfotiamine administration was five times higher than that of thiamine mononitrate, with a C_{max} value 6.7 times larger and t_{max} reached within 35-40 minutes compared to 70-75 minutes following administration of thiamine mononitrate. There were also notable differences in red blood cell thiamine concentration (3.5 to 14.8-fold increase) and urinary excretion of thiamine (10 times higher) following administration of benfotiamine. Thus indicating a significantly higher bioavailability of thiamine following benfotiamine administration when compared to thiamine mononitrate[14]. Greb and Bitsch found similar results in a follow-up clinical study wherein benfotiamine demonstrated a more rapid and earlier increase in thiamine levels following oral administration of benfotiamine when compared to fursultiamin and thiamine disulfide[15]. Ziems and his laboratory reported similar results with a dramatic increase in serum thiamine concentration following oral ingestion of benfotiamine in patients[6].

Animal and In Vitro Studies

Animal studies have noted the beneficial effects of benfotiamine on the circulatory system. In particular, Katare and his colleagues at the Bristol Heart Institute recently discovered that administration of Benfotiamine to streptozotocin-induced diabetic mice prevented diabetes-induced diastolic dysfunction and cardiac failure via the well-described Akt-Pim-1 pathway[16]. An additional study by Katare also demonstrated the ability of benfotiamine to improve functional cardiac recovery following infarction via the Akt pathway[17]. This correlates with a prior study indicating benfotiamine's ability to rescue cardiomyocyte contractility in diabetic mice[18]. As further evidence of benfotiamine's effect on the circulatory system, Balakumar demonstrated that benfotiamine decreases nicotine and uric-acid induced vascular endothelial dysfunction in a rat model and these results were similar to those obtained using atorvastatin[19].

Powerful effects have also been noted in the brain, where it has been observed that benfotiamine significantly decreases β-amyloid deposition, while increasing cognitive abilities in transgenic animals susceptible to Alzheimer's in a dose-dependent manner[20]. Additionally, the vitamin mitigated cerebral oxidative damage in diabetic mice at a dose of 100mg/kg/day[21].

Effects of benfotiamine on diabetic retinopathy published in *Nature Medicine* by Dr. Brownlee's laboratory at Albert Einstein Medical College indicate that benfotiamine inhibits the advanced glycation end-product (AGE), hexosamine, and DAG-PKC pathways in addition to hyperglycemia-induced NFκB activation, activating transketolase-dependent conversion of glyceraldehyde-3-phosphate and fructose-6-phosphate to pentose sugars and preventing diabetic retinopathy in diabetic rats[22]. Karachalias *et al*, initiated a separate study to investigate benfotiamine's effects on protein damage in the retina, renal glomeruli, nerve, plasma, and urine using isotopic dilution analysis LC-MS/MS to quantify these various adducts in tissues. They demonstrated that benfotiamine ameliorated AGE content, and urinary excretion of glycation, oxidation, and nitration free adducts in diabetic rats in a manner similar to thiamine at a moderate dose (70mg/kg) [23]. Another study of the effects on the eye conducted by the Ramana laboratory at University of Texas, investigated the effects of benfotiamine on ocular inflammation following endotoxin administration. Significant decreases in inflammatory cytokines, inflammatory infiltrates, NFκB activation, Cox-2 and iNOS expression were exhibited in infected rats following oral administration of benfotiamine[24].

Chronic neuropathy is a common symptom of diabetes, typically treated with tricyclic antidepressants, or anticonvulsants; drugs with severe side effects. The Granados-Soto lab undertook a study to examine the effect of benfotiamine on chronic neuropathic pain, and allodynia. The results of their study clearly demonstrated that oral administration of benfotiamine at doses of 100-300mg/kg significantly reversed the nociceptive response to formalin injection of the hind paw and significantly reduced streptozotocin-induced tactile allodynia with the maximal antiallodynic effect at 2.5 hours post-dose. In a surgical model of allodynia, the antiallodynic effect was observed within 1-2 hours after benfotiamine dosage[25].

Chronic alcohol ingestion decreases levels of thiamine. In rats subjected to a long-term study, benfotiamine reversed alcohol-induced thiamine depletion and increased blood and tissue total thiamine levels more than four-fold over controls, and two-fold more than animals treated with an equimolar amount of thiamine hydrochloride[26].

Benfotiamine exhibits demonstrable effects on numerous cell types cultured *in vitro*. It has been shown to ameliorate the toxicity of cells cultured in high concentrations of glucose, most probably through its interaction with the Akt pathway and decreased matrix metalloproteinase activity[27-29]. It has also exhibited direct anti-oxidant effects and prevents Angiotensin II induced DNA damage in addition to enhancing insulin synthesis, glucose metabolism, and inhibiting apoptosis[30-31].

Clinical Reports

Several clinical studies also allude to the beneficial effects of benfotiamine in human pathologies, most notably in diabetes. Note that these studies are discussed only to further demonstrate the safety of benfotiamine; no therapeutic or drug claims are intended.

Dr. Brownlee's laboratory recently discovered benfotiamine alone increased transketolase activity in circulating monocytes by 2-3-fold following a 600mg daily oral dose of benfotiamine in type I diabetic patients. Combining this treatment with α-lipoic acid normalized angiopoeitin II levels, and 6-keto-$PGF_{1\alpha}$ (a critical anti-arthrogenic enzyme) activity while significantly decreasing N-acetylglucosamine modified protein levels by 40% (a marker of the hexosamine pathway)[32]. Additionally, an investigation by the Tschoepe laboratory discovered benfotiamine plays a critical role in adiponectin activity following a meal high in AGEs. Adiponectin regulates insulin sensitivity and is decreased in patients with type II diabetes. Benfotiamine significantly reduced postprandial hyperglycemia in patients with type II diabetes[33]. In a previous study, the same group found that pre-treatment with benfotiamine (1,050 mg/day for 3 days) prevented microvascular endothelial dysfunction and oxidative stress in type II diabetic patients following a meal high in AGEs[34].

Diabetic patients often experience a painful polyneuropathy as a result of their constant hyperglycemic condition. In a clinical study by Stracke *et al* of 24 diabetic patients over 12 months given benfotiamine (120mg/day) in combination with vitamins B_6 and B_{12} hemoglobin A1 concentrations decreased significantly while peroneal nerve conduction velocity significantly increased[3]. Similar results were obtained following high-dose administration of benfotiamine (320 mg/day) in diabetic patients with polyneuropathy with positive results within 3 weeks of initiating therapy[35]. More recent studies in 2003, indicated a more pronounced response following oral dosage with 400mg/day of benfotiamine as indicated by an increase in patient and physician pain assessment measurements[36]. Stracke and his colleagues undertook a much larger study of 165 patients with diabetic polyneuropathy in 2008 undergoing 6 weeks of daily treatment with 300mg or 600mg of benfotiamine. Following 6 weeks of treatment there was a noticeable pain reduction in the 600mg group[37]. In a study of alcohol induced polyneuropathy in 104 patients, Woelk and colleagues observed a significant decrease in paralysis and considerably more patients were pain free following administration of oral benfotiamine than placebo or other B vitamin supplementation[38].

Kidney malfunction and failure are often the result of diabetes. Several clinical studies have evaluated the effects of benfotiamine on kidney disorders. In 1999, the Stein laboratory reported altered thiamine kinetics in patients with end-stage renal disease. Specifically, thiamine blood levels in patients administered 100mg thiamine were significantly less than those given 100 mg of benfotiamine. Additionally, high intracellular concentrations of thiamine diphosphate were found in those patients given benfotiamine versus those administered thiamine mononitrate which could increase protection against the adverse effects of uremia often observed in these patients[39]. The same laboratory obtained further confirmation of these results in a separate study the following year. In their follow-up study

they report a 4.3-fold increase in blood TDP levels of patients with end-stage renal disease, with a peak plasma concentration exceeding that of normal subjects by 51% following a 100mg oral dose of benfotiamine[40]. In 2008, the Stopper laboratory reported that benfotiamine reduced the genomic damage in peripheral lymphocytes of hemodialysis patients following 600mg of benfotiamine daily. Patients with and without diabetes experienced a significant decrease in lymphocytic micronuclei frequency, and plasma AGE concentration following the benfotiamine treatment[41]. As further clinical evidence of its safety, Dr. Di Rocco published a case report in the *Lancet* in 1997 wherein a 6-month old child presenting with dilated cardiomyopathy of unknown origin was treated with thiamine with limited results. At the age of 20-months therapy with benfotiamine at a dose of 600mg/day by mouth was initiated. The child now exhibits normal physiological measurements, and no signs of heart failure[42].

7.0 Conclusion

While not an exhaustive review of the research pertaining to oral supplementation with Benfotiamine, the citations reviewed here are representative and clearly demonstrate the safety and improved utilization of thiamine with the lipid-soluble benfotiamine vitamin compared to the water-soluble forms of the vitamin (thiamine HCL and thiamine mononitrate). Combined with this, decades of human use in Europe and Japan provide additional evidence regarding the safety of benfotiamine. Finally, benfotiamine is a member of the allithiamine family which is comprised of thiamine derivatives found in garlic, shallots and several other vegetables. Benfotiamine increases the bioavailability of thiamine in man, BenfoPure® meets the regulatory definition of a dietary ingredient and is an appropriate candidate for the nutritional supplementation of thiamine in the diet of man in the United States.

Signature:

[Please see the signature page attached to the hardcopy and copied (PDF) to the electronic copy]

M. Kawatsuji

Director

Hamari Chemicals USA, Inc.
12725 SW Millikan Way, Suite 300
Beaverton, OR 97005

APPENDIX A - REFERENCES:

1. Wada T, Takagi H, Minakami H, Hamanaka W, Okamoto K, Ito A, Sahashi Y. A New Thiamine Derivative, S-Benzoylthiamine O-Monophosphate. *Science*. 1961; 134:195-196.
2. Fujiwara, M, Watanabe, H, Matsui, K. "Allithiamine" a Newly Found Derivative of Vitamin B_1. *The Journal of Biochemistry*. 1954; 41(1): 29-39.
3. Stracke H, Lindemann A, Federlin K. A benfotiamine-vitamin B combination in treatment of diabetic polyneuropathy. *Exp Clin Endocrinol Diabetes*. 1996; 104(4): 311-316.
4. Loew D. Pharmacokinetics of thiamine derivatives especially of benfotiamine. *Int J Clin Pharmacol Ther.* 1996; 34:47-50.
5. Hilbig R, Rahmann H. Comparative autoradiographic investigations on the tissue distribution of benfotiamine versus thiamine in mice. *Arzneimittelforschung*. 1998 May; 48(5): 461-8.
6. Ziems M., Netzel M. Bitsch I. Biokinetic parametyers and metabolism of S-benzoyl-O-monophosphate. *Biofactors*. 2000; 11:109-110.
7. Merck Index 14th Edition: monograph #1038, Benfotiamine. Merck & Co Inc. NJ USA. (2006).
8. Araki M, Takao H, Yukio S, Tadao W. *S Benzoylthiamine monophosphate, Pathological studies on chronic toxicity of S Benzoylthiamine monophosphate.*
9. Minakami H, Hiromu T, Tadao W. *Studies on Thiamine Phosphates: Pharmacological Actions of S-Benzoylthiamine-o-monophosphate.*
10. Shindo H, Okamato K, Wada T, Koike H, Kumakura S. Studies of the Absorption of S-Benzoylthiamine O-Monophosphate: (III) Mechanism of Intestinal Absorption. *Vitamins*. 1968; 38(1): 30-37.
11. Shindo H, Okamato K, Jun-ichi T, Takahashi I. Studies of the Absorption of S-Benzoylthiamine O-Monophosphate: (II) Permeability to Red Cell Membranes. *Vitamins*. 1968; 38(1): 21-29.
12. Geyer J, Netzel M, Bitsch I, Frank T, Bitsch R, Kramer K, Hoppe P. Bioavailability of Water- and Lipid-soluble Thiamin Compounds in Broiler Chickens. *Int J Vitam Nutr Res*. 2000; 70(6):311-316.
13. Bitsch R, Wolf M, Moller J, Heuzeroth L, Dieter G. Bioavailability Assessment of the Lipophilic Benfotiamine as Compared to Water-Soluble Thiamin Derivative. *Ann Nutr Metabolism*. *1991; 35:292-296.*
14. Schreeb KH, Freudenthaler S, Vormfelde SV, Gundert-Remy S, Gleiter CH. Comparative Bioavailability of Two Vitamin B1 Preparations (Benfotiamine and Thiamine mononitrate). *European Journal of Clinical Pharmacology*. (1997), 52(4), 319-320.
15. Greb A, Bitsch R. Comparative bioavailability of various thiamine derivatives after oral administration. *Int J Clin Pharmacol Ther*. 1998; 36:216-221.
16. Katare R, Caprolae A, Oikawa A, Meloni M, Emanueli C, Madeddu P. Vitamin B_1 Analog Benfotiamine Prevents Diabetes-Induced Diastolic Dysfunction and Heart Failure Through Akt/Pim-1-Mediated Survival Pathway. *Circ Heart Fail*. 2010 March; 3(2): 294-305.
17. Katare R, Caporali A, Emanueli C, Madeddu P. Benfotiamine Improves Functional Recovery of the Infarcted Heart via Activation of Pro-Survival G6PD/Akt Signaling Pathway and Modulation of Neurohormonal Response. *Journal of Molecular and Cellular Cardiology*. 2010; 49: 625-638.

18. Ceylan-Isik AF, Wu S, Li A, Shi-Yan L, Ren J. High-Dose Benfotiamine Rescues cardiomyocyte Contractile Dysfunction in Streptozotocin-Induced Diabetes Mellitus. *Journal of Applied Physiology*. 2006; 100: 150-156.
19. Balakumar P, Sharma R, Singh M. Bnefotiamine Attenuates Nicotine and Uric-Acid Induced Vascular Endothelial Dysfunction in the Rat. *Pharmacological Research* 2008; 58:356-363.
20. Pan X, Gong N, Zhao J, Yu Z, Gu F, Chen J, Sun X, Zhao L, Yu M, Zhiru X, Dong W, Qin Y, Fei G, Zhong C, Xu TL. Powerful Beneficial Effects of Benfotiamine on Cognitive Impairment and β-amyloid Deposition in Amyloid Precursor Protein/Presenillin-1 Transgenic Mice. *Brain*. 2010; 133: 1342-1351.
21. Wu S, and Ren J. Benfotiamine Alleviates Diabetes-Induced Cerebral Oxidative Damage Independent of Advanced Glycation End-Product, Tissue Factor and TNF-α. *Neuroscience Letters*. 2006; 394:158-162.
22. Hammes HP, Du X, Edelstein D, Taguchi T, Matsumura T, Ju Q, Lin J, Bierhaus A, Nawroth P, Hannak D, Neumaier M, Bergfeld R, Giardino I, Brownlee M. Benfotiamine Blocks Three Major Pathways of Hyperglycemic Damage and Prevents Experimental Diabetic Retinopathy. *Nature Medicine*. 2003; 9: 294-299.
23. Karachalias N, Babachi-Jajidi R, Rabbani N, Thornalley PJ. Increased Protein Damage in Renal Glomeruli, Retina, Nerve, Plasma, and Urine and its Prevention by Thiamine and Benfotiamine Therapy in a Rat Model of Diabetes. *Diabetologica*. 2010; 53:1506-1516.
24. Yadav UCS, Subramanyam S, Ramana KV. Prevention of Endotoxin-Induced Uveitis in Rats by Benfotiamine, a Lipophillic Analogue of Vitamin B_1. *Invest Opthalmol Vis Sci.* 2009; 50(5): 2276-2282.
25. Sanchez-Ramirez GM, Caram-Salas N, Rocha-Gonzalez HI, Vidal-Cantu GC, Medina-Santillan R, Reyes-Garcia G, Granados-Soto V. Benfotiamine Relieves Inflammatory and Neuropathic Pain in Rats. *European Journal of Pharmacology*. 2006; 530: 48-53.
26. Netzel M, Ziems M, Jung KH, Noll E, Borsch C, Bitsch I. Effect of High-Dosed Thiamine Hydrochloride and S-Benzoyl-Thiamine-O-Monophosphate on Thiamin Status After Chronic Ethanol Administration. *Biofactors*. 2000; 11: 111-113.
27. Beltramo E, Berrone E, Buttiglieri S, Porta M. Thiamine and Benfotiamine Prevent Increased Apoptosis in Endothelial Cells and Pericytes Cultured in High Glucose. *Diabetes/Metabolism Research and Reviews*. 2004; 20: 330-336.
28. Marchetti V, Menghini R, Stefano R, Vivanti A, Feccia T, Lauro D, Fukamizu A, Lauro R, Federici M. Benfotiamine Counteracts Glucose Toxicity Effects on Endothelial Progenitor Cell Differentiation via Akt/FoxO Signaling. *Diabetes*. 2006; 55:2231-2237.
29. Tarallo S, Beltramo E, Berrone E, Dentelli P, Porta M. Effects of High Glucose and Thiamine on the Balance Between Metalloproteinases and Their Tissue Inhibitors in Vascular Cells. *Acta Diabetologica*. 2010; 47: 105-111.
30. Schmid U, Stopper H, Heidland A, Schupp N. Benfotiamine Exhibits Direct Antioxidative Capacity and Prevents Induction of DNA Damage *In Vitro*. *Diabetes/Metabolism Research and Reviews*. 2008; 24: 371-377.

31. Oh SH, Witek R, Bae SH, Darwiche H, Jung Y, Pi L, Brown A, Peterson BE. Detection of Transketolase in Bone Marrow-Derived Insulin-Producing Cells: Benfotiamine Enhances Insulin Synthesis and Glucose Metabolism. *Stem Cells and Development*. 2009; 16: 37-45.
32. Du X, Edelstein D, Brownlee M. Oral Benfotiamine Plus α-lipoic Acid Normalises Complication-Causing Pathways in Type I Diabetes. *Diabetologica*. 2008; 51: 1930-1932.
33. Stirban A, Negrean M, Stratmann B, Gotting C, Salomon J, Kleesiek K, Tschoepe D. Adiponectin Decreases Postprandially Following a Heat-Processed Meal in Individuals with Type 2 Diabetes. *Diabetes Care*. 2007; 30(10): 2514-2516.
34. Stirban A, Negrean M, Stratmann B, Gawlowski T, Horstmann T, Gotting C, Kleesiek K, Mueller-Roesel M, Koschinsky T, Uribarri J, Vlassara H, Tschoepe D. Benfotiamine Prevents Macro- and Microvascular Endothelial Dysfunction and Oxidative Stress Following a Meal Rich in Advanced Glycation End Products in Individuals with Type 2 Diabetes. *Diabetes Care*. 2006; 29(9): 2064-2070.
35. Winkler G, Pal B, Nagybeganyi E, Porochnavec M, Kempler P. Effectiveness of Different Benfotiamine of Painful Diabetic Neuropathy. *Arzneimittelforschung*. 1999 Mar; 49(3): 220-224.
36. Haupt E, Ledermann H, Kopcke W. Benfotiamine in the Treatment of Diabetic Polyneuropathy—a Three-week Randomized, Controlled Pilot Study (BEDIP Study). *Int Journal of Clinical Pharmacology and Therapeutics*. 2005; 43(2): 71-77.
37. Stracke H, Gaus W, Achenbach U, Federlin K, Bretzel RG. Benfotiamine in Diabetic Polyneuropathy (BENDIP): Results of a Randomised, Double-Blind, Placebo-controlled Clinical Study. *Exp Clin Endocrinol Diabetes*. 2008; 116: 600-605.
38. Woelk H, Lehrl S, Bitsch R, Kopcke W. Benfotiamine in Treatment of Alcoholic Polyneuropathy. An 8-Week Randomized Controlled Study (BAP1 Study). *Alcohol & Alcoholism*. 1998; 33(6): 631-638.
39. Frank T, Bitsch R, Maiwald J, Stein G. Alteration of thiamine pharmacokinetics by end-stage renal disease (ESRD). *Int J Clin Pharmacol Ther*. 1999 Sep; 37(9):449-55.
40. Frank T, Bitsch R, Maiwald J, Stein G. High thiamine diphosphate concentrations in erythrocytes can be achieved in dialysis patients by oral administration of benfotiamine. *Eur J Clin Pharmacol*. 2000 Jun; 56(3):251-7.
41. Schupp N, Dette ME, Schmid U, Bahner U, Winkler M, Heidland A, Stopper H. Benfotiamine Reduces Genomic Damage in Peripheral Lymphocytes of Hemodialysis Patients. *Naunyn-Schmiedeberg's Arch Pharmacol*. 2008; 378: 283-291.
42. Di Rocco M, Patrini C, Rimini A, Rindi G. A 6-month-old Girl with Cardiomyopathy who Nearly Died. *Lancet*. 1997; 349:616.

APPENDIX B – - BENFOPURE® CERTIFICATE OF ANALYSIS

(b) (4)

APPENDIX C - BENFOPURE® STABILITY DATA