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July 15, 2020

Via FDA Industry Systems Electronic Submission Portal

Steven Casper, Ph.D.
Ethnobotanist
Office of Dietary Supplement Programs (HFS-810)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Drive
College Park, MD 20740-3835

Re: Submission of New Dietary Ingredient Notification

Dear Dr. Casper:

On behalf of Innophos, LLC, pursuant to Section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act, we are submitting, via the CFSAN Online Submission Module (COSM) electronic portal, a New Dietary Ingredient Notification (NDIN) for Advantra Z® Bitter Orange (*Citrus aurantium* L) Extract. This submission addresses questions raised by FDA in its December 9, 2019 letter regarding the previous NDIN submission (No. 1123) for this same ingredient on August 5, 2019. We will be following up with the Agency within fifteen (15) days of the FDA's receipt of this NDIN.

Most importantly, Innophos intends for Advantra Z® standardized to 50 % p-synephrine to be used in dietary supplements under the following conditions: the maximum recommended daily dose of Advantra Z® is 50 mg (25 mg of p-synephrine), with a duration of thirty (30) to sixty (60) days; users of the dietary supplement are healthy adults, and it should not be used by children, during pregnancy or lactation; and the dietary supplement should be avoided or only used in consultation with a physician if the person has a medical condition, or is taking prescription medications.

In addition, information has been provided regarding additional human studies that demonstrate safety, particularly related to lack of cardiovascular effects. Also incorporated into the document are a series of studies that relate to mechanisms of action of p-synephrine and in-depth discussion regarding mild, well-tolerated cardiovascular effects and limited poor binding to α-1, α-2, β-1 or β-2 adrenergic receptors. A recent study affirming that p-synephrine and bitter orange extract are non-mutagenic, non-teratogenic and non-cytotoxic is also provided.

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With respect to the issues raised by FDA in its December 9, 2019 letter, Innophos acknowledges and addresses each of FDA's concerns in this revised NDIN submission. FDA indicated that it was unable to identify the dietary ingredient based on the information provided. In the revised submission, we have provided detailed information regarding the analytical methods used to characterize and standardize the extract. Provided are HPLC standard curves and spectra of p-synephrine standards, various HPLC spectra of extracts standardized to p-synephrine performed by external analytical laboratories, data sheets from the past three years performed by external analytical laboratories, spectra and analytical data sheets of the same three lots of the extract over a two year period of time, and TLC and IR spectra confirming the identity of the product (Appendices 6.1-6.3).

FDA stated that it was not able to establish safety based on the history of use that was provided when used under the conditions recommended or suggested. Use of standardized bitter orange (Citrus *aurantium* L.) extracts (Advantra Z®) is based on the totality of available evidence, including exposure to the active constituent p-synephrine, in the form of human studies, animal studies, *in vitro* safety and mechanistic studies, over a 1000 years history of human use, and recommendations of other administrative bodies and organizations.

From a historical perspective, we expanded on the decisions of other global regulatory bodies on the safe use of *p*-synephrine, some at up to 50 mg a day. The Natural Health Products Directorate of Health Canada defined guidelines for the use of bitter orange extract and *p*-synephrine (Marles, 2011) and concluded that "*At doses up to 50 mg/day in healthy adults, p-synephrine is classified as Type III, meaning that the use of, or exposure to, a single-ingredient p-synephrine product under these conditions is not likely to cause any adverse health consequences*". The European Food Safety Authority (EFSA) (2009) in evaluating exposure to p-synephrine to determine a safe level stated that "*A theoretical calculation of an exposure to p-synephrine from food for a person with an individual preference for a Citrus-rich diet could be estimated based upon the values given above as high as 54 mg of p-synephrine (2 litres of orange juice)/day/person or even 280 mg p-synephrine (more than 1 kg of mandarins containing 1 litre of juice)/day/person*", with no known or demonstrated adverse effects. The German Federal Institute for Risk Assessment (FIRA) concluded that the intake of *p*-synephrine was 25.7 mg/day for high consumers without adverse effects (Bakhiya et al., 2017). The daily amount of *p*-synephrine suggested for use in our submission is well supported by the evaluations of global regulatory bodies.

FDA also stated that it was unable to establish the safety of our dietary ingredient Advantra Z® in the previous NDIN because the ninety (90)-day rat study lacked heart rate and blood pressure end points. These parameters are not among the standard recommended test elements as per OECD Test Guideline 408 because approximately 15 parameters that are assessed provide information regarding potential cardiovascular effects. Lack of significant effects of the test agent (Advantra Z®) with respect to all of these parameters is a clear indication of a lack of cardiovascular effects.

FDA's letter of December 9, 2019 raised questions regarding the calculation of the no observed adverse effect level (NOAEL) for *p*-synephrine based on the 90-day rat study. Several effects believed to be incidental were small, not dose dependent, did not occur in both sexes, were believed to be incidental, were within the historical control range with no correlating toxicological effects, and therefore of no toxicological significance. (Deshmukh et al. 2017b). There were no associated clinically remarkable, significant adverse alterations. Therefore, based on standard toxicological data interpretation, incidental effects are not considered when determining values for the NOAEL.

FDA indicated that a safety study in second animal species was needed. In a study in mice, the animals were treated daily for twenty-eight (28) days with bitter orange extract (7.5% *p*-synephrine) at doses of 400, 2,000 or 4000 mg/kg (corresponding to 30, 150 and 300 mg *p*-synephrine/kg, respectively) or with 30 mg or 300 mg *p*-synephrine/kg (Arbo et al., 2009). No adverse effects were observed regarding blood pressures or heart rates, organ weights, or biochemical parameters in the treated mice at any of the doses up to and including 300 mg/kg *p*-synephrine as compared to the controls**.**

It was noted that FDA was unable to establish safety of Advantra Z® based on the 60-day clinical study in which subjects received 98 mg *p*-synephrine per day. Other studies that are discussed have shown that a daily dose of 50 mg *p*-synephrine for fifteen (15) days and 103 mg *p*-synephrine for 15 and 30 days were without adverse effects. In the 60-day study, no significant changes were noted in systolic or diastolic blood pressures, or 44 individual blood chemistry and hematological parameters in control or *p*-synephrine treated groups. No adverse events were reported by any of the clinical subjects or observed by the investigators, for the standardized bitter orange extract (Advantra Z®). All of these considerations combined, animal data, human data and history of use/regulatory agency evaluations, at the proposed dose of 25 mg *p*-synephrine (50 mg Advantra Z) for 30-60 days provide a reasonable expectation of safety consistent with the statute and regulations on NDINs.

Innophos is proposing that at a daily intake of 25 mg *p*-synephrine in the form of standardized bitter orange extract (Advantra Z®), the duration of use should not exceed sixty (60) days. As a consequence, the adjusted *p*-synephrine estimated daily intake (EDI) from the usage of 8 months out of 12 months per year is: 25 mg ÷ 70 kg bw x 8 months/12 months = 0.2380 mg/kg bw/day. Given the conservatism of the estimation and patterns of use, the margins of safety are adequate to conclude that *p*-synephrine and Advantra Z® (bitter orange extract) are reasonably expected to be safe under its intended conditions of use in dietary supplements.

As noted, representatives of Innophos will be following up with the Agency within fifteen (15) days of the FDA's receipt of this NDIN.

* * *

Innophos, LLC appreciates FDA's attention to this submission and looks forward to a favorable response. Please direct any communications about this NDIN to:

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For your reference, the contact person at Innophos, LLC for this NDIN is:

Anthony J. Kiefner, Esq.
Assistant General Counsel
Innophos Holdings, Inc.
259 Prospect Plains Road, Building A
Cranbury, NJ 08512
Phone: 609-366-1274
Email: anthony.kiefner@innophos.com

In addition, the following individual is authorized to communicate with FDA on Innophos, LLC's behalf:

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Please let me know if you have any questions.

Sincerely yours,

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NEW DIETARY INGREDIENT NOTIFICATION

Advantra Z® - Bitter Orange (*Citrus aurantium L.*) Extract

Prepared by:

Innophos, LLC
259 Prospect Plains Road, Building A
Cranbury, NJ 08512

Submitted by:

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July 15, 2020

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List of Tables and Figures

1. New Dietary Ingredient Identity Information

1.1 Description of the identity of the NDI

The new dietary ingredient (NDI) is Advantra Z®, a standardized bitter orange extract obtained from carefully selected, dried immature fruits (bitter oranges) from the plant *C. aurantium* L. The extract is light brown to dark brown color powder with earthy characteristics, slightly pungent odor, and bitter taste.

C. aurantium contains the protoalkaloid *p*-synephrine, which is considered as the "active constituent," and small amounts of other protoalkaloids. Flavonoids, including limonene, hesperidin, neohesperidin, naringin and tangaretin, are found in bitter orange peel, flowers, and leaves, but are present only in very small amounts in bitter orange extract of dried immature fruits.

Innophos intends for Advantra Z® to be used in dietary supplements under the following conditions:

- Users of the dietary supplement should be healthy adults. Should not be used by children, during pregnancy or lactation.
- Avoid using the dietary supplement and/or consult with a physician if you have a medical condition, or taking prescription medications.
- The maximum recommended daily dose of Advantra Z® is 50 mg (25 mg of *p*-synephrine). Manufacturers of finished dietary supplements may choose to deliver this amount in a single serving or divide this total into correspondingly-reduced serving amounts taken twice a day (spaced out by 6 to 8 hours) or three times per day (spaced out by 4 to 6 hours).
- Duration of use not to exceed 30-60 days with a 15-day gap before again using the product.

Based on FDA's draft guidance for industry entitled "Dietary Supplements: New Dietary Ingredient Notifications [NDINs] and Related Issues" (August 2016) (FDA 2016), the intake pattern should be considered to be "intermittent." Per FDA 2016 (page 70): "Intermittent use, for purposes of this guidance, means less than daily chronic use and can be either daily and finite in duration or non-daily and lifetime in duration."

1.2 Description of the evidence verifying the identity of the NDI

Bitter orange extract is derived from bitter oranges from the plant *C. aurantium* L. It can be verified based on the plant source, its color, odor, taste, and appearance. Additionally, the product identity and quality is standardized by parameters such as identification (FTIR, TLC, HPLC), levels of *p*-synephrine content (*e.g.*, 50%) and loss on drying with details provided below.

Standardized bitter orange reference materials have been prepared and characterized by the National Institute of Standards and Technology (Putzbach et al., 2007; Sander et al., 2008).

1.3 NDI Manufacture

1.3.1 Raw materials

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Table 1. Taxonomic Classification of *Citrus aurantium* L.

Rank	Scientific Name and Common Name
Kingdom	Plantae- Plants
Subkingdom	Tracheobionta - Vascular plants
Superdivision	Spermatophyta - Seed plants
Division	Magnoliophyta - Flowering plants
Class	Magnoliopsida - Dicotyledons
Subclass	Rosidae
Order	Sapindales
Family	Ratuceae- Rue family
Genus	*Citrus* L – citrus
Species	*Citrus* × *aurantium* L. (pro sp.) [*maxima* × *reticulata*] – sour orange
Subspecies	*Citrus* × *aurantium* L. ssp. *aurantium* – sour orange

1.3.2 Formulation ingredients

N/A

1.3.3 Manufacturing process (Confidential Commercial Information)

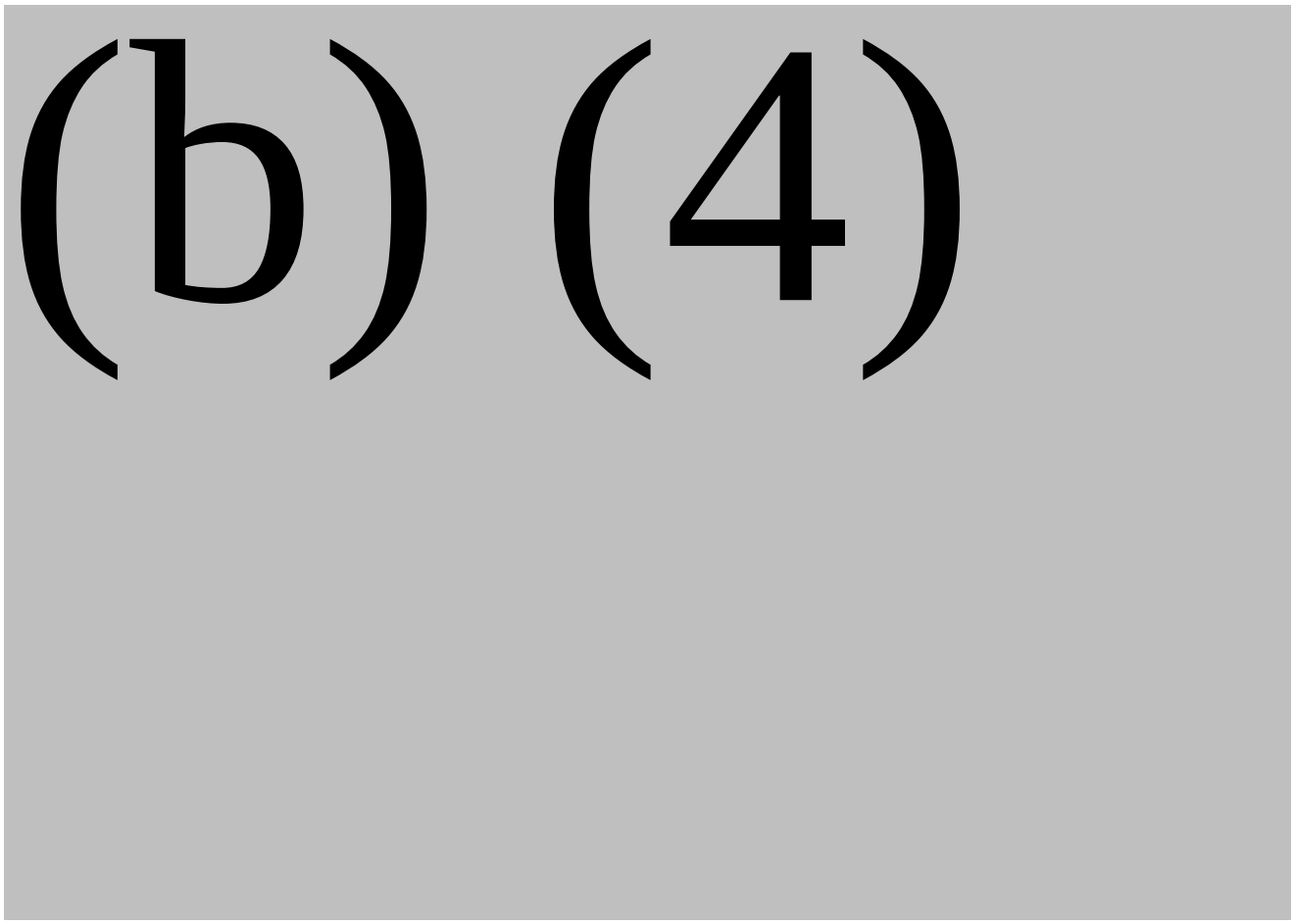
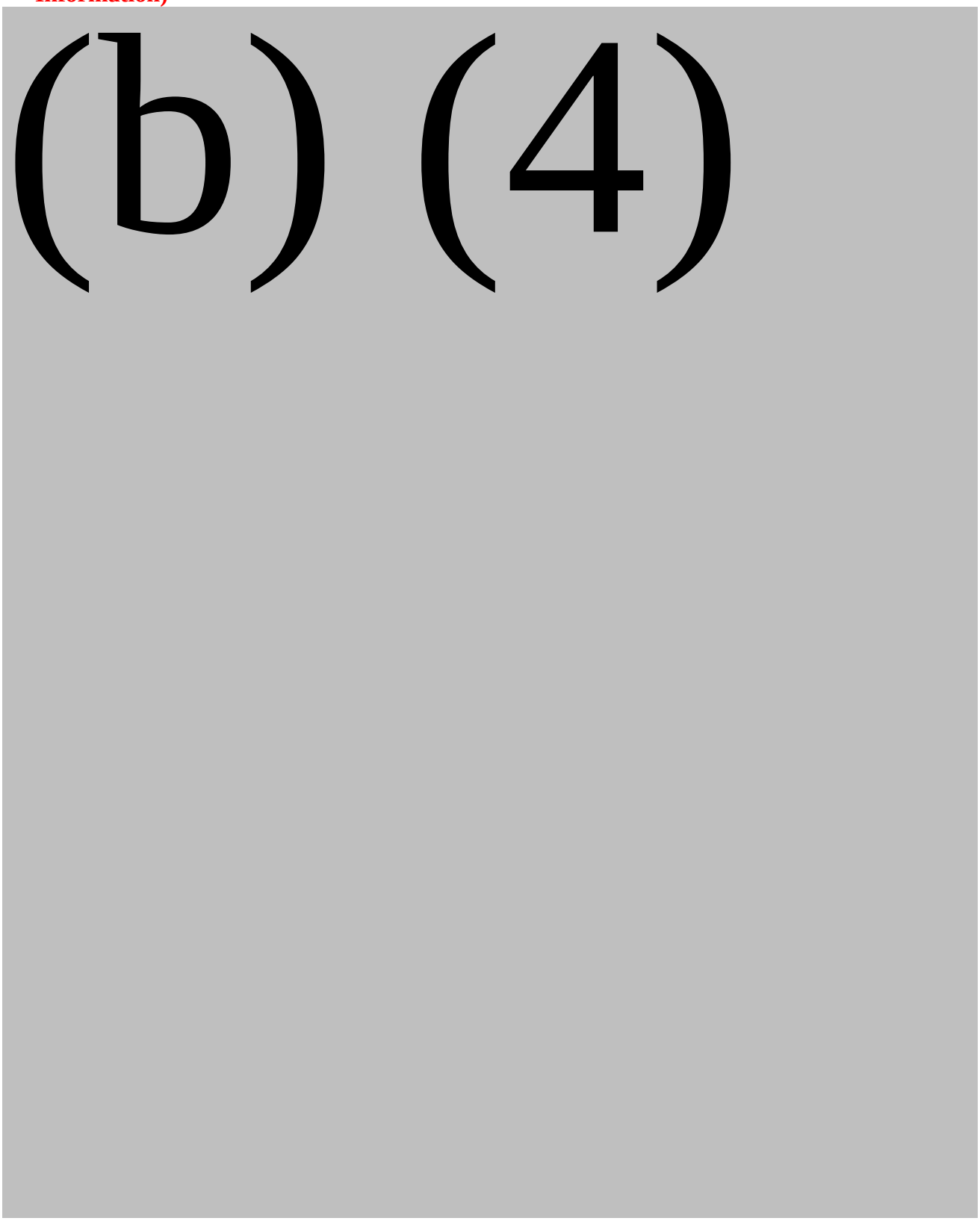


Figure 1. Manufacturing Process for Bitter Orange Extract (Confidential Commercial Information)



1.3.4 NDI specifications (Confidential Commercial Information)

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Table 2. Specifications of Advantra Z® (Bitter Orange Extract) (Confidential Commercial Information)

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Table 3. Typical compositional analysis of Advantra Z® (Bitter Orange Extract)
(Confidential Commercial Information)

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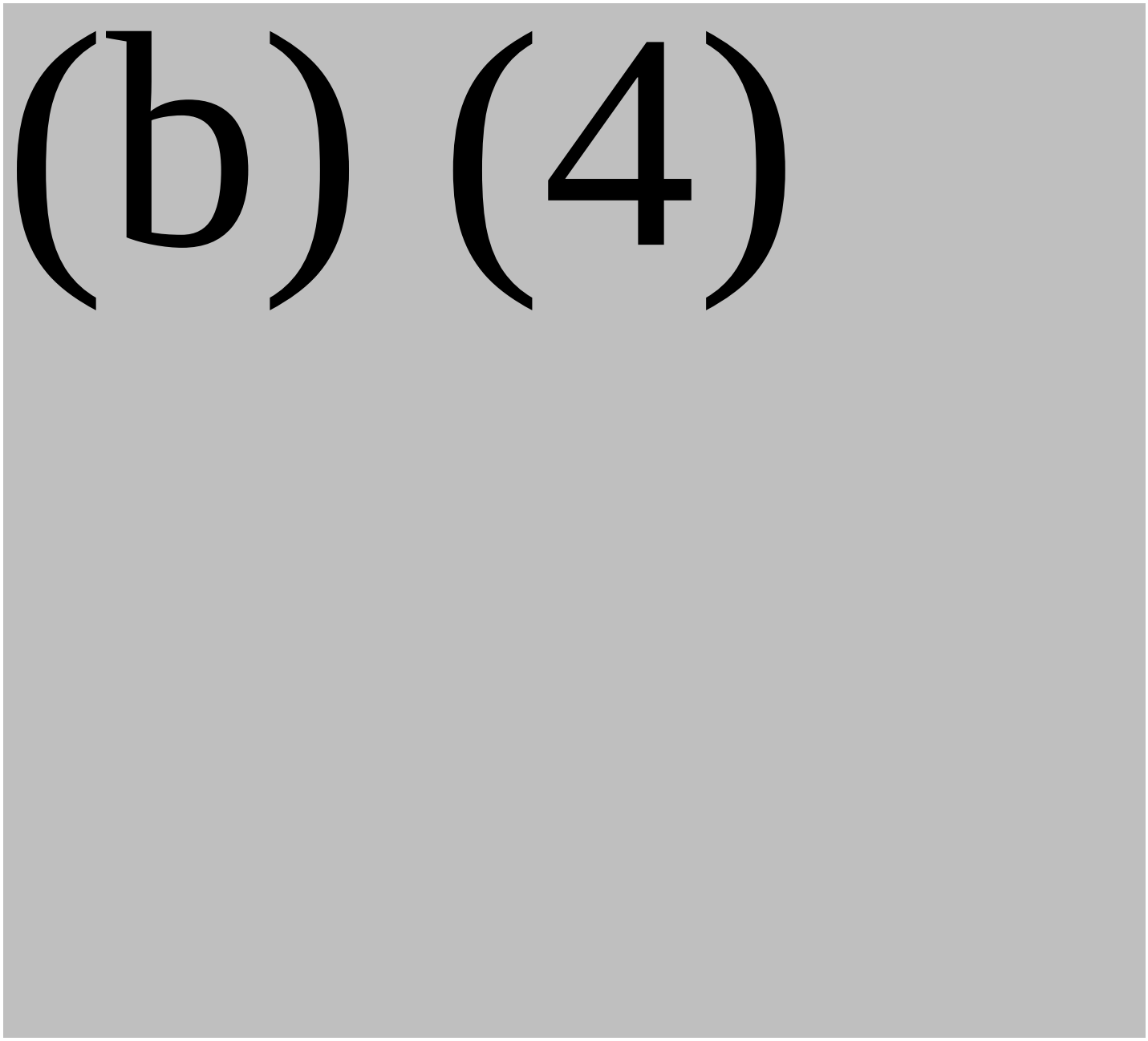
1.3.5 Methods of analysis (Confidential Commercial Information)



1.3.6 Analysis of potentially toxic processes

N/A

1.3.7 Disintegration and dissolution profile

N/A

1.3.8 Shelf-life and conditions of storage (Confidential Commercial Information)

Bitter orange extract should be stored in a well-closed, air-tight container that is protected from light and moisture, in a dry place at room temperature. The shelf-life of bitter orange extract is two years. (b) (4)

[redacted]

Table 4. Two Year Stability Studies Of Advantra® Z 50 % (Confidential Commercial Information)

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2. Dietary Supplement Manufacture

This section is not applicable (N/A) because the subject of this NDI, the bitter orange extract, contains no other ingredients. Therefore, the safety of the NDI is the same as the safety of the dietary supplement.

2.1 Raw materials

N/A

2.2 Formulation ingredients other than the NDI

N/A

2.3 Manufacturing process

N/A

2.4 Product specifications

N/A

2.5 Methods of analysis

N/A

2.6 Analysis of potentially toxic processes

N/A

2.7 Disintegration and dissolution profile

N/A

2.8 Shelf-life and conditions of storage

N/A

3. History of Use or Other Evidence of Safety

3.1 History of use

The history of use of bitter orange (*Citrus aurantium* L.*)* and bitter orange extracts are not the primary basis for determining the safety of Advantra Z® (standardized bitter orange extract). The historical use does corroborate the fact that humans have been exposed to *p*-synephrine, the primary active constituent, for over 1000 years and are widely exposed on a daily basis without adverse effects. The safety of Advantra Z® is based on the totality of available evidence, including exposure to the active constituent *p*-synephrine in the form of human studies, animal studies, *in vitro* studies and history of human use.

The EFSA Scientific Opinion on a Qualified Presumption of Safety (QPS) approach (2014) stated that "dried hydro-alcoholic extracts of dried immature fruits and dried peel of the immature and mature fruits of *Citrus x aurantium* ssp. *amara* Engl. are suitable for QPS status, provided that the use of extracts is restricted to levels where (-)-synephrine intakes in the form of food supplement(s) do not exceed significantly historical intake levels from traditional foods."

The EFSA (2009) opined that "A theoretical calculation of an exposure to *p*-synephrine from food for a person with an individual preference for a Citrus-rich diet could be estimated based upon the values given above as high as 54 mg of *p*-synephrine (2 litres of orange juice)/day/person or even 280 mg *p*-synephrine (more than 1 kg of mandarins containing 1 litre of juice)/day/person. The intake of orange marmalade adds only about 1 mg of *p*-synephrine per serving to these values."

The EFSA (2009) further noted that "It could be hypothesized that a dose of e.g. 30 mg of *p*-synephrine/day/person that occurs from the intake of 500 mg of bitter orange extract (standardized to 6% *p*-synephrine) in weight loss pills equals to the daily intake of 1.1-2 litres of orange juice (containing 15-27 mg *p*-synephrine per litre depending on the variety), or of 0.1-0.4 litres of mandarin juice, or of 30 servings of marmalade, or of decoctions from 8-12 grams of dried bitter orange peel (as used for traditional medicinal purposes, e.g. indigestion)."

It should also be noted that the Natural Health Products Directorate of Health Canada defined guidelines for the use of bitter orange extract and *p*-synephrine (Marles, 2011). The Health Canada report concluded that "At doses up to 50 mg/day in healthy adults, *p*-synephrine is classified as Type III, meaning that the use of, or exposure to, a single-ingredient *p*-synephrine product under these conditions is not likely to cause any adverse health consequences."

p-Synephrine is present in other *Citrus* species in addition to *C. aurantium* such as Marrs sweet oranges, clementines, tangerines and mandarin oranges, with juices containing from 20-40 mg *p*-synephrine/8 oz. glass (Dragull et al., 2008; Uckoo et al., 2010). A study determined the *p*-synephrine content in 33 dried immature fruit samples and 27 dried ripe fruit samples of bitter oranges collected from different geographic regions of China, the primary commercial source of bitter orange extracts (Li et al., 2016). The authors used HPLC and variable UV detection. The immature fruits were collected in May to June and the ripe fruits in July and thereafter. The average *p*-synephrine content of the dried immature fruit was 5.44 % (1.0-12.3%) while the *p*-synephrine content of the dried ripe fruits averaged 0.85% (0.0-2.4%) with variations based on different geographic origins.

Vieira et al. (2007) reported that the average amount of *p*-synephrine in seven orange juice products averaged 16.0 mg/liter, while levels as high as 2.3 mg/liter were found in orange soft drinks. Bader et al. (2017) developed a highly sensitive method to quantitate *p*-synephrine in citrus juices and reported that the screening revealed high *p*-synephrine concentrations of 150-420 nmol/mL (25-70 µg/mL) in orange (n=11) and tangerine (n=2) juices, whereas 20-100 times lower levels were found in juice from grapefruit (n=14), lemon (n=5), pomelo (n=2), and lime (n=4). It has been also reported that in humans, *p*-synephrine is found in trace amounts in the adrenal gland and may be a trace biogenic amine.

The following discussion describes the historical exposure to the primary active ingredient *p*-synephrine that is present in bitter oranges and extracts derived from the immature fruits, as well as other citrus species. Again, the history of use of bitter orange (*Citrus aurantium* L.*)* and bitter

orange extracts is not the primary basis for determining the safety of Advantra Z® (standardized bitter orange extract).

Bitter orange is native to southern China and northeastern India. Also known as bitter, bigarade or Seville orange, it was brought to Arabia in the 9th Century and first reported growing in Europe in Sicily by 1000 A.D. The commercial growing of bitter orange for human consumption began around Seville, Spain in the 12th Century and bitter orange was the only citrus grown in Europe for 500 years. Exported to the New World in the 16th Century, it was soon found growing wild in Bermuda, Jamaica, Puerto Rico and Barbados and was cultivated in Brazil and Mexico. In the 18th Century, bitter oranges were exported from Florida to England. Bitter orange trees are still found in the Everglades at sites of former Indian villages (Morton, 1987).

Although native to the Far East, bitter orange is also cultivated extensively throughout the Mediterranean, North Africa, the Middle East, India, West Tropical Africa, Guinea, the West Indies and Brazil. Known to volunteer in the wild from self-sown seeds, it is found growing wild from Georgia to Argentina. In a textbook chapter on pharmacognosy, Trease and Evans (1966) summarized the history of bitter orange trees, indicating that they were first brought to Europe about AD 1200 from Northern India via South America. The sweet orange was not known in Europe until about the 15th century.

The available information suggests that bitter orange and its preparations and extracts have been used for centuries. In China, the immature fruits of bitter orange are known as *Fructus aurantii immaturis*, and have been used for centuries for therapeutic purposes, primarily for gastrointestinal disorders (Chen and Chen, 2004; Fang et al., 2009; Xutian et al., 2014). According to Traditional Chinese Medicine (Chen & Chen, 2004), Zhi shi (immature bitter orange) "is one of the best herbs to treat gastrointestinal disorders characterized by stagnation and accumulation" and "is one of the best herbs to relieve distention and hardness of the epigastric area caused by cholecystitis." Pharmacologically, this compendium reports that Zhi shi does not affect heart rate or respiration and has minimal toxicity.

Youngken (1950) described the collection and preparation of bitter orange peel USP and its uses as an aromatic bitter and flavoring agent with an average dose of 1 g for the dried material and 4 mL for bitter orange peel tincture USP. Bitter orange peel has been used in South America in folk remedies for anxiety, insomnia, and epilepsy (Carvalho-Freitas and Costa, 2002). Bitter orange peel also has an official monograph in the German Expanded Commission E compendia of medicinal herbs (American Botanical Council, 2000). Bitter orange peel is occasionally used as an ingredient in stomachic and laxative preparations. Africans apply the bitter orange juice on ulcers, yaws and areas afflicted with rheumatism. In Italy, Mexico and Latin America, decoctions of leaves are given for their antispasmodic, stimulant, tonic and stomachic action. Flower extracts are used as sedatives and bark extracts are taken as a tonic, stimulant and vermifuge (Leung and Foster, 1996; Morton, 1987). The natives of Guam use the macerated leaves and crushed peel as a washing soap and shampoo. Pectin from bitter orange has also exhibited antibacterial and antifungal activity.

The oil derived from bitter oranges has been used as a food and fragrance ingredient since the early 1900s. Bitter oranges also are used as a food in the production of marmalade in the United Kingdom and South Africa. Marmalade is made with both the peel and pulp of bitter orange. The

juice is used as a flavoring for fish and on meat during cooking in Spain. In Egypt, it has been fermented into wine. In Mexico, the pulp is salted and covered with hot chili pepper paste prior to consumption (Morton, 1987). As of May 2019, according to USDA Food Composition Databases, 10 food products containing the term bitter orange are marketed in the US. Thus, this data indicate that people are exposed to bitter orange or products prepared from it.

The active constituent of bitter orange, *p*-synephrine is also present in other plants and fruits. The available information suggests that exposure to *p*-synephrine is very common. People are exposed to *p*-synephrine on a daily basis from various juices, and food and beverage (orange flavored liqueurs) products such as marmalade prepared from Citrus species as Seville orange, Marrs sweet oranges, grapefruit, mandarin, clementines and other orange-related species that contain *p*-synephrine. A wide variety of citrus juices contain about 5 mg *p*-synephrine per 8 oz glass (Blumenthal, 2004), while juice from mandarin oranges may contain more than 20 mg and as much as 40 mg *p*-synephrine per 8 oz glass (Blumenthal, 2004; Dragull et al., 2008; Uckoo et al., 2010). It has been reported that a typical sweet orange contains about 6 mg *p*-synephrine (Mattoli et al., 2005).

In addition to the historical consumption of bitter orange products discussed above, tens of millions of doses of bitter orange extract (*p*-synephrine)-containing products have been consumed in the United States as well as internationally over the past 20 years by hundreds of thousands if not millions of individuals. As the literature shows, there are no reports of any serious, adverse incidents directly attributable to this ingredient (Stohs et al., 2011a; Stohs & Shara, 2013; Stohs, 2017).

In summary, the available information demonstrates that human beings are regularly exposed to bitter orange preparations including bitter orange extracts, and consequently their active constituent, *p*-synephrine without relevant adverse effects, and most specifically, absence of reports of cardiovascular effects. The historical use is relevant to our proposal in that it demonstrates a very long exposure of humans to bitter orange products and *p*-synephrine.

From a regulatory perspective, bitter orange flowers and peel are considered as Generally Recognized as Safe (GRAS) by FDA (21 CFR 182.20 ("Essential oils, oleoresins (solvent-free), and natural extractives (including distillates)")). Around the world, bitter oranges are considered as a common food commodity for human consumption, as is exemplified by the fact that standard of identity for frozen concentrated orange juice includes optional addition of juice from *C. aurantium* (21 CFR 146.146). According to this regulation, bitter orange (*C. aurantium*) may be used in the preparation of concentrated orange juice at levels not to exceed 5% of the unconcentrated volumes. FDA's "Substances Added to Food (formerly EAFUS)" database lists bitter orange flowers, bitter orange peel extract, and bitter orange peel oil each as a "flavoring agents or adjuvant" under 21 CFR 182.20.

The Flavor and Extract Manufacturers' Association (FEMA) has determined orange peel bitter oil (*C. aurantium* L. *lba* L.) (No. 2823) as GRAS for use in food (such as beverages, ice cream, candy, baked goods, gelatin and pudding and chewing gum) as a flavoring ingredient (Hall and Oser, 1968). Bitter orange is also listed in the National Institutes of Health's (NIH's) Office of Dietary

Supplement Label Database (DSLD), indicating that it is currently used in products marketed in the U.S. As of May 2020, this database included over 300 products that contain "bitter orange" or "*Citrus aurantium*" variations as ingredients listed somewhere on the label.

3.1.1 Description of the relationship between the historically consumed material and the NDI or dietary supplement containing the NDI

As noted above, the historical use demonstrates the fact that human beings have been exposed to *p*-synephrine, the primary active constituent, for over 1000 years and that humans are widely exposed on a daily basis without adverse effects. As also noted, the history of use of bitter orange and bitter orange extracts are not the primary basis for determining the safety of Advantra Z® (standardized bitter orange extract). The Advantra Z® bitter orange extract is a standardized aqueous/ethanol extract obtained from immature fruits (bitter oranges) from the plant *C. aurantium* L. The *p*-synephrine content is adjusted to be 50%. Advantra Z® is also the test article for many of the toxicological and human studies described below.

3.1.2 Describe identity information verifying the relationship between the historically consumed material and the NDI or dietary supplement containing the NDI

Food grade specifications of Advantra Z® have been established and are presented in Table 2. The product's identity and quality are standardized by parameters such as identification (FTIR, TLC, HPLC), levels of *p*-synephrine content (targeting 50%) and loss on drying which are described in more detail above. The final product contains 50% *p*-synephrine. All lots are subjected to FTIR-TLC to further ensure batch to batch consistency. *p*-Synephrine content of the finished extract and that which is stated on each Certificate of Analysis is determined by HPLC as described above. The compositional and nutritional analysis of bitter orange extract revealed the presence of several components that are summarized in Table 3 (above).

3.1.3 Historical conditions of use and cumulative exposure estimate for the historically consumed material

Bitter orange is extensively used in the production of marmalade that is routinely consumed around the world. Additionally, bitter orange or its juice is also used in flavoring food. The available information suggests that people are also exposed to the active constituent of bitter orange (*i.e.*, *p*-synephrine) from various sources. In addition to bitter orange, *p*-synephrine is also found in other citrus foods that are more commonly consumed. Thus, human beings are exposed to *p*-synephrine on a daily basis from juices and food. For the German population, the total daily intake of *p*-synephrine via conventional food, estimated under considerations of maximum concentrations of *p*-synephrine, amounts to 6.7 mg/day for average consumers and to 25.7 mg/day for high consumers (Bakhiya et al., 2017). Estimations for the French population considering the maximum levels in citrus fruits yielded an average *p*-synephrine intake of 4.3 mg/day and 17.7 mg/day at the 95th percentile (Bakhiya et al., 2017).

The dosage recommendation for *p*-synephrine is 25 mg/day for dietary supplements which may be taken in divided doses. The exposure to *p*-synephrine is near the background intake of 95th

percentile for German and French populations. As will be discussed below, doses of 49 mg *p*-synephrine orally in the form of Advantra Z® (standardized to 50% p-synephrine) twice a day (98 mg *p*-synephrine per day) for 60 days had no cardiovascular, hematological, or clinical biochemical (blood chemistries) effects, demonstrating the safety of the recommended dose (Kaats et al., 2013).

3.1.4 Adverse events associated with historically consumed material

Bitter orange fruits, peels, and the oil from peel are all used to make orange marmalade. The average consumption of culinary products containing bitter orange peel (mostly marmalade, liqueurs, beer and sweets) may be considered safe on the historical level. It can be expected that the amount of *p*-synephrine from such a dose does not affect physiological functions significantly. The exposure to *p*-synephrine from the average consumption of such products is comparable to the exposure that occurs from the consumption of other Citrus products as orange fruits or juices that have become a normal part of a common European and American diets (*e.g*. the amount of 1 mg of *p*-synephrine is being consumed in 1 serving of Citrus marmalade or in 37 – 67 mL of orange juice).

3.1.5 Alternative rationale for reasonable expectation of safety based on history of use

N/A

3.2 Other evidence of safety

The intake of Advantra Z® would be considered to be "intermittent" based on the information in Section 1.1 of this NDIN. Under FDA 2016 Section VI.B ("History of Use or Other Evidence of Safety"), question 19 recommends the following studies to assess the safety of an ingredient (like Advantra Z®) where the dietary supplement containing the NDI is intended for intermittent use, the NDI has a documented history of safe daily chronic use, and the proposed use of the NDI leads to intake levels that are greater than the levels consumed historically:

(1) A two-study genotox battery

(2) A 14-day range-finding oral study to establish an MTD in an appropriate animal model;

(3) A 90-day subchronic oral study (same species as the range-finding study) to establish an MTD and a NOAEL for use in calculating the margin of safety;

(4) A single-dose or repeat-dose tolerability study in humans and/or an ADME study in animals, humans, or both; and

(5) A teratology study (rodent or non-rodent) (see note at end of list). Note: The teratology study is not needed if the product is labeled as not for use by women of childbearing age, pregnant or lactating women, or children 13 and younger.

As users of the dietary supplement should be healthy adults and the dietary supplement should not be used by children, during pregnancy or lactation, a teratology study would not be applicable. Nevertheless, as detailed subsequently, a series of studies were conducted on Advantra Z® or other bitter orange extract products including the recommended studies and more.

A number of experimental *in vitro,* animal and human studies with bitter orange or its extracts have been conducted to evaluate its safety, effects and mechanisms of action. Predecessor companies of Innophos, LLC (including Novel Ingredients and Nutratech) and other independent researchers conducted a series of safety studies, such as acute toxicity, short-term toxicity, subchronic toxicity, and mutagenicity. These studies were conducted as per OECD guidelines and following Good Laboratory Practices (GLP) recommendations in accordance with FDA 2016. The findings from these toxicity studies were published in two separate peer-reviewed journals that have high impact factors (Deshmukh et al., 2017a; 2017b) or in other peer-reviewed and published articles. In addition to the specific toxicity studies, reproductive and developmental studies including a teratology study as well as numerous human clinical efficacy and tolerability studies of bitter orange extract were investigated. Furthermore, numerous mechanistic studies have been conducted *in vitro* and in animals.

3.2.1 Absorption, distribution, metabolism and excretion (ADME) studies

Hengtmann and Aulepp (1978) studied the pharmacokinetics and metabolism of tritiated *p*-synephrine in human subjects (article published in German language). Following short intravenous infusion in six patients about 80% of the administered radioactivity was recovered in urine. Two-thirds of the urinary tritium activity consisted of the deaminated *p*-hydroxymandelic acid. Only 10% were excreted as unchanged *p*-synephrine. Following oral ingestion in ten volunteers, the total urinary radioactivity was quite comparable to the intravenous experiments. Therefore, complete enteric absorption has to be stated. The amount of unchanged *p*-synephrine amounted, however, only to 2.5% of the dose in urine. The resulting bioavailability was calculated to be 22% only. The half-life of *p*-synephrine was determined to be about two hours. Following oral ingestion, absorption was fast and the peak concentrations were observed between 1 and 2 hours after administration.

Bader et al. (2017) analyzed *p*-synephrine levels in human urine before and after the ingestion of orange juice. Application of the stable isotope dilution analysis method to quantitate *p*-synephrine in sulfatase/glucuronidase-treated urine samples (n = 10) after orange juice consumption showed an increase of *p*-synephrine from trace levels (0.1 ± 0.1 nmol/mL) in the 2-day washout phase to a maximum concentration of 8.9 (±5.5) nmol/mL found four hours after ingestion of orange juice. This shows that *p*-synephrine present in orange juice is absorbed and excreted in urine.

Suzuki et al. (1979) reported that *p*-synephrine is a substrate for monoamine oxidase (MAO) in rat brain mitochondria. The Km and Vmax values were determined as 250 µM and 32.6 nmoles/mg of protein/30 min, respectively. The inhibition studies showed that the *p*-synephrine oxidation was carried out by both type A and type B monoamine oxidase and a major part of the activity was due to type A monoamine oxidase.

The biosynthesis of *p*-synephrine is believed to involve L-phenylalanine, L-tyramine, and N-methyltyramine (Wheaton & Stewart, 1969). *p*-Octopamine, the N-demethylated derivative of *p*-synephrine, may not play a major role in the biosynthesis of *p*-synephrine (Thevis et al., 2012; Medana et al., 2013; Stohs, 2015). Synthesis of *p*-synephrine has been detected in the rat brain. The presence of *p*-synephrine has been detected in human urine independent of supplementation or oral ingestion, and is considered by some scholars to be a trace amine (Khan & Nawaz, 2016; D'Andrea et al., 2019; Stohs et al., 2020).

Contrary to popular belief, available information indicates that little or no *p*-octopamine is present in bitter orange extracts (Pellati and Benvenuti, 2007; Stohs, 2015; see **Appendix 6.2**). Although *p*-synephrine undergoes rapid N-demethylation to *p*-octopamine, no *p*-octopamine is detected in the urine at doses of up to 150 mg *p*-synephrine orally due to rapid oxidative deamination of the *p*-octopamine (Thevis et al., 2012; Medana et al., 2013).

p-Synephrine has been shown to be rapidly taken up from the blood by the liver. The single pass extraction of *p*-synephrine was shown to be higher than 90% at a portal concentration of 10 µM in isolated perfused rat liver (da Silva-Pereira et al., 2016), indicating rapid removal and metabolism. The half-life of *p*-synephrine has been estimated to be in the range of 2-3 hours (Hengstmann & Aulepp, 1978; Haller et al., 2005; 2008) which is in agreement with the observations of Shara et al. (2016; 2017).

From the plasma concentration data, it appears that *p*-synephrine has a low bioavailability when taken orally. After dosing of ten healthy subjects with 46.9 mg *p*-synephrine, the measured Cmax was about 2.85 ng/ml, the Tmax about 75 min and the half-life about 2-3 hours (Haller al., 2008). *p*-Synephrine plasma levels of 10.3 ng/mL at two hours (Shara et al., 2016) and approximately 2.6 ng/mL at four hours (Shara et al., 2018) after oral consumption of 49 mg *p*-synephrine (Advantra Z®) have been reported. After ingestion of 21 mg p-synephrine by adults engaging in moderate physical activity, the measured plasma *p*-synephrine levels were below 2 ng/ml. Likewise, a pharmacokinetic study of the Sympatol® (synephrine tartrate) showed that the time to peak plasma concentration for orally taken synephrine was 1 to 2 hours, and the elimination half-life was about two hours (Bakhyia et al., 2017), although one cannot directly compare pharmacokinetic properties of the tartrate salt of a synthetic product with the free base present in a bitter orange extract.

In summary, the available information indicates that following oral ingestion, *p*-synephrine, the active constituent of bitter orange, is rapidly absorbed and eliminated. The half-life of *p*-synephrine has been estimated to be in the range of 2-3 hours. Cmax and Tmax of *p*-synephrine have been reported as 2.85 ng/ml and about 75 min, respectively. The available information suggests that *p*-synephrine is unlikely to accumulate in the body. Given the rapid metabolism and elimination of *p*-synephrine, it is important to consider whether adequate blood levels are achieved to produce potential cardiovascular effects. These studies indicate low blood concentrations of *p*-synephrine ranging from 2.6 to 10 ng/mL. At these low blood levels and the rapid elimination, as well as poor beta-adrenergic receptor binding, *p*-synephrine is unlikely to

produce cardiovascular effects, which is supported by over 30 human clinical studies (Stohs, 2017; Suntar et al., 2018; Stohs et al., 2018; 2020).

3.2.2 Acute toxicity studies

An acute oral toxicity study was conducted on bitter orange extract (Advantra Z®) in female Sprague-Dawley rats per OECD Test Guideline 425 (Deshmukh et al., 2017a) according to FDA 2016. These results are summarized in Table 6. In this study, three female rats, fasted overnight, were dosed in a step-wise manner with 5000 mg/kg body weight of bitter orange extract which contained 50% *p*-synephrine, and observed for 24 hours. Initially, a single animal was dosed with 5000 mg/kg body weight of the 50% *p*-synephrine-containing bitter orange extract. When the dosed animal survived, two additional animals were dosed sequentially so that the three animals were tested at a minimum of 48-hour intervals, and all animals survived.

Following the acute administration of the extract at 5000 mg/kg, the animals were observed for death or abnormal clinical signs over a period of 14 days and were then terminated. Their body weights were recorded at one day prior to dosing (day 0), on the day of dosing (day 1, fasting body weight), on day 7, and at termination on day 15 when they were subjected to complete necropsy. Administration of single dose of 5000 mg/kg body weight of the bitter orange extract did not result in the death of any of the animals and no overt signs of toxicity were evident. Therefore, the acute oral LD_{50} value of the test item was found to be greater than 5000 mg/kg body weight in rats.

The raw data for this acute oral toxicity study are provided in the **Appendix 6.4** in a report from Intox Pvt Ltd. dated May 13, 2017. The statistically analyzed and summarized data were published by Deshmukh et al., 2017a.

The bitter orange extract used in this study contained small amounts of the protoalkaloids N-methyltyramine, tyramine, *p*-octopamine and hordenine with the sum total of these compounds being less than 4% of the total protoalkaloids in the extract or in other words less than 2 mg per 100 mg of the extract standardized to 50% *p*-synephrine. In most cases, the sum of these minor protoalkaloids is 2% or less (see Eurofins Scientific analyses in **Appendix 6.2**). Therefore, the minor protoalkaloids did not negatively impact the LD_{50} of the extract which was greater than 5000 mg/kg.

At a dose of 1000 mg/kg of the extract in rats, this would represent a dose of less than 20 mg/kg of the total minor protoalkaloids without evident adverse effects. For a 70 kg human this would translate into a dose of 1400 mg or less of the minor protoalkaloids, an amount approximately 2800 times that ingested at commonly used doses of the extract. It can therefore be concluded that the minor protoalkaloids do not contribute adverse effects to the standardized bitter orange extract (Advantra Z®).

3.2.3 Dose range-finding studies

In a subsequently conducted exploratory repeated dose experiment, three female Sprague Dawley rats were daily given the bitter orange extract (containing 50% *p*-synephrine) at a dose of 2000 mg/kg body weight by oral gavage for 14 consecutive days. These results are summarized in Table

5. The treatment was well tolerated, and the rats did not demonstrate any overt signs of toxicity. (See report in **Appendix 6.5**; Deshmukh et al., 2017a).

Based on the findings from the above-mentioned studies, groups of rats (5/sex/group) were administered bitter orange extract containing 50% *p*-synephrine by oral gavage for 14 days at doses of 0, 250, 500, 1000, and 2000 mg/kg/day (Deshmukh et al., 2017a). All animals were observed twice daily for mortality or morbidity. All rats that were found dead in the cage were subjected to detailed necropsy examination and tissue samples were preserved in 10% neutral buffered formalin. During the course of the study and at termination, all standard toxicity related parameters were measured.

The results of this 14-day study demonstrated that the extract at and up to the dose of 1000 mg/kg body weight/day did not have any effect on the survival of the male and female rats in the study. Daily treatment with the extract at a dose of 2000 mg/kg body weight/day resulted in the deaths of two male rats and one female rat (Deshmukh et al., 2017a). One female rat treated with the 2000 mg/kg dose of the extract daily was found dead in the cage on day 5. A male rat treated daily with 2000 mg/kg of the extract was found dead in the cage on day-9 of the study, while another male rat was found dead in the cage on day-12 of the study. All other male and female rats survived the 14-day treatment until their termination on day-15. No remarkable necropsy findings were noted. Necropsy findings indicated impaction of the stomach with ingesta and the test item, or the presence of excess residues of extract in the intestine.

Because rats died at the 2000 mg/kg bw/day dose, additional groups of 5 male and 5 female rats were also included in the study and were treated at the lower daily dose of 250 mg/kg for 14 days. At higher doses (1000 and 2000 mg/kg bw/day) transient signs of discomfort in rats immediately after gavage administration were noted. Feed intake values were comparable to the control groups during the first week of the study, while during the second week of the study male rats treated with 2000 mg/kg bw/day of the bitter orange extract consumed approximately 8% less chow than the control group, while the female rats treated with 500 mg and 1000 mg/kg bw/day consumed approximately 13% less chow than the corresponding control group.

No significant changes in hematological parameters were noted in any treatment groups. Similarly, no remarkable alterations in the clinical chemistry parameters were noted. Some of the changes noted were considered incidental in nature (Deshmukh et al., 2017a). Necropsy findings, organ weights, hematology and chemistry suggested an absence of systemic or organ specific toxicity. No clinical abnormalities were observed in the male and female rats treated at and up to 500 mg/kg/day, and therefore 500 mg/kg of the bitter orange extract can be considered the no-observed-effect-level (NOEL).

No clinically significant effects were observed at any dose with respect to body weights, absolute and relative organ weights, gross pathological findings, and hematological or clinical chemistry parameters in rats of either sex. Small decreases were observed with respect to food consumption in both male and female rats at doses of 500 mg/kg and above during the second week of the study. At 1000 and 2000 mg/kg bw/day, the rats exhibited transient and fully reversible abnormal clinical signs from 6th day to the end of the study which involved burrowing of their heads in the bedding

material and staying hypoactive for about 15-45 minutes following oral administration of the extract. The reason for the burrowing activity is not known. Previous studies in mice have demonstrated a reduction in locomotor activity (hypoactivity) (Arbo et al., 2008). These authors demonstrated this decrease in activity using a spontaneous locomotor test with doses of bitter orange extract of 5,000 and 10,000 mg/kg bw and *p*-synephrine at 300 mg/kg bw in mice. The transient hypoactivity observed at a dose of 1000 mg/kg of the extract is considered a pharmacological response and not as a symptom of a toxic effect.

The maximum tolerated dose (MTD) or the no-observed-adverse-effect–level (NOAEL) for the extract can be considered 1000 mg/kg bw/day. The no-observed-effect-level (NOEL) was determined to be 500 mg/kg/day.

The raw data for this 14-day oral dose range finding study is presented in **Appendix 6.5** in a report from Intox Pvt Ltd. dated September 6, 2017. The statistically analyzed and summarized data were published by Deshmukh et al., 2017a.

Table 5. Animal Safety LD50, and 14 Day Dose Range Finding Studies:

Test article	Species	Number/group, sex	Exposure regime, duration	Dose (mg/kg bw/day)	Result	Reference
Acute oral toxicity study						
Advantra Z® (50 % synephrine)	Sprague-Dawley rats	3 females	Single dose, observe for 14 days	5000	LD50 > 5000 mg/kg bw/day	Deshmukh et al., 2017a; Appendix 6.4
14-day dose range-finding study						
Advantra Z® (50 % synephrine)	Sprague-Dawley rats	5/sex/group	14 days	0,250,500, 1000, 2000 mg/kg/day	No deaths up to 1000 mg/kg. No clinical chemistry, hematological, necropsy or organ weight changes at any dose. Transient piloerection and burying of heads in bedding at two highest doses. 3 rats died at 2000 mg/kg due to GI impaction. NOEL = 500 mg/kg	Deshmukh et al., 2017a; Appendix 6.5

3.2.4 Subchronic (90 day) or chronic studies

A 90-day subchronic study (Deshmukh et al., 2017b; **Appendix 6.6)** was conducted for bitter orange extract (Advantra Z®) in rats per OECD Test Guideline 408, as recommended in FDA

2016. This Guideline is the procedure that is widely used and the generally accepted standard for the determination of subchronic toxicity, and included all prescribed assessments and endpoints. These results are summarized in Table 6. Sprague Dawley rats (10/sex/group; 6-8 weeks old) were gavaged daily with bitter orange extract standardized to 50% *p*-synephrine at dose levels of 0 (control- G1), 100 (low dose- G2), 300 (mid dose- G3), or 1000 (high dose- G4) mg/kg bw/day for 90 consecutive days. Two additional groups of animals (5/sex/group) for the recovery study received 0 (control- G1R) and 1000 (high dose-G4R) mg/kg bw/day of the extract for 90 days, followed by no additional treatment for 28 days.

Recovery groups were not included for the 100 (low dose- G2), and 300 (mid dose- G3) based on the fact that the NOEL from the 14-day study was 500 mg/kg. Furthermore, the lack of effects at these two doses was confirmed in the 90-day study, therefore obviating the need for the recovery groups. The Report on this 90-day subchronic toxicity study found in the **Appendix 6.6**. The results of this Report inadvertently made reference to 28-day recovery groups for the 100 mg/kg and 300 mg/kg doses which did not occur.

In a previous review of this 90-day study, FDA raised questions about the absence of heart rate and blood pressure endpoints. These parameters are not among the standard recommended test elements as per OECD Test Guideline 408. They are not included for several reasons: (i) it is not possible to satisfactorily and directly assess heart rate and blood pressure without compromising the integrity of the entire toxicity study; (ii) the accurate methods of measuring these parameters are invasive and require cannulation of the animals; and (iii) the non-invasive method (tail-cuff) is not accurate, and requires extensive handling and immobilization of the animals for extended periods of time.

Another reason why blood pressure and heart rate are not included directly in the 90-day subchronic toxicity study is the fact that an extensive number of parameters that are assessed provide information regarding potential cardiovascular effects. These factors include the following:

--general and detailed clinical examinations; --neurological/functional observations;

--clinical hematology; -- urinalysis;

--clinical biochemistry including AST, sodium, calcium, potassium, total cholesterol, creatinine, BUN, and triglycerides;

--organ weights including heart weights;

--and gross and microscopic pathology of the heart and other organs.

Lack of significant effects of the test agent (Advantra Z®) with respect to all of these parameters is a clear indication of a lack of cardiovascular effects.

Throughout the study period the animals were observed for clinical signs of toxicity and mortality/morbidity (twice daily). Detailed clinical examinations (including neurotoxicity), body weight, and feed consumption (weekly) were recorded. Once during the 12th week of treatment,

an evaluation of sensory reactivity, assessments of grip strength and motor activity were performed. Using a video camera, the following locomotor assessments (quantitative parameters) were recorded: total distance travelled (m), average speed (m/s), absolute turn angle (º), rotations of the animal's body and absolute head turn angle (º). These assessments were performed on both sexes with 15 animals (10 + 5 recovery) per control and 1000 mg/kg groups and 10 animals (no recovery animals) for the 100 mg/kg and 300 mg/kg groups.

Urinalyses were performed on day-88 of the treatment period on animals from the control and high dose groups. During week 13, blood samples were collected. During week 4 of the recovery period blood samples were also taken. All standard hematological and clinical chemistry parameters were investigated. Organ weights were recorded and over 40 tissues and organs were harvested at necropsy and fixed in 10% buffered neutral formalin. Histopathological examination was carried out on full sets of tissues collected from the high dose (G4) and control groups (G1). As treatment related adverse effects were not observed in any tissues/organs at the high dose level, the low dose (100 mg/kg; G2) group and mid-dose (300 mg/kg; G3) group as well as the recovery groups were not examined (Deshmukh et al., 2017b).

There were no clinical signs of adverse effects and no mortality noted in any animals during the course of the study. The daily general clinical examinations and the weekly detailed clinical examinations of rats conducted during the 90-day treatment period and the 28-day recovery period revealed that except for the mild and transient signs of discomfort and piloerection exhibited by rats treated at the highest dose of 1000 mg/kg bw/day and to which the rats eventually adapted. No clinical signs of adverse effects were observed. Thus, bitter orange extract administration did not induce any remarkable and abnormal clinical signs indicative of systemic toxicity in rats of either sex. Treatment of male and female rats with the extract at doses of 100 mg/kg (G2) and 300 mg/kg bw (G3) did not induce any abnormal clinical signs throughout the 90-day treatment period of the study.

With respect to functional observations, treatment of male and female rats with the 50 % *p*-synephrine-containing *C. aurantium* extract at and up to the dose of 1000 mg/kg did not induce any remarkable and abnormal alterations in qualitative and quantitative parameters of their sensory reactivity, grip strength and motor activity. This 'functional observational battery' was carried out during the 12th week of the study. No differences were noted for animals of either sex at any dose of the extract relative to the control groups with respect to posture, movement, respiration, lacrimation, salivation, skin and hair coat, and gait.

The values of quantitative parameters (frequencies of urination, defecation and rearing, the landing foot splay and the grip strength) of the treatment group rats of both sexes did not differ significantly ($P>0.05$) from those of the vehicle control group rats during the 12th week of the study.

Furthermore, treatment of male and female rats with the extract at and up to the dose of 1000 mg/kg did not induce any remarkable and abnormal alterations in quantitative parameters of their locomotor activity as were assessed in week 12 of treatment by placing them in an open field and tracking their movements individually for a period of 10 minutes (600 seconds) per rat by means of a video camera connected to a validated software system. The values of quantitative parameters,

including total distance travelled (m), average speed (m/s), absolute turn angle (°), rotations of the animal's body, and absolute head turn angle (°) of the treatment group rats of both sexes did not differ significantly (P>0.05) from those of the vehicle control group rats.

Table 6. 90-day Subchronic Oral Safety Study:

Test article	Species	Number/group, sex	Duration	Dose (mg/kg bw/day) orally	Result	Reference
Advantra Z® (50 % synephrine)	Sprague-Dawley rats	10/sex/group. 5/sex/group at 0 and 1000 mg/kg for 28- day post-treatment observation.	90 days	0,100,300, 1000 mg/kg/day	No adverse effects up to 1000 mg/kg/day. No changes in clinical chemistry, urinalysis, hematology, organ or body weights, histopathology, cardiovascular*, sensory reactivity, locomotion ophthalmic, or feed consumption. Transcient piloerection and burying of heads at 1000 mg/kg/day to which rats adapted. Noel = 300 mg/kg. NOAEL = >1000 mg/kg	Deshmukh et al., 2017b

***Absence of cardiovascular effects as demonstrated by a lack of effect on: clinical hematology; clinical biochemistry, including AST, sodium, calcium, potassium, total cholesterol, creatinine, BUN, and triglycerides; urinalysis; organ weights including heart weights; gross and microscopic pathology of hearts and other organs; general and detailed clinical examinations; and neurological and functional observations.**

The ophthalmological examinations did not reveal any treatment-related lesions. Administration of bitter orange extract containing 50% *p*-synephrine at and up to the dose of 1000 mg/kg bw/day did not induce any remarkable and abnormal alterations in qualitative and quantitative parameters of their sensory reactivity, grip strength and motor activity as was evident during assessment of the 'functional observational battery' carried out during the 12th week of the study. The values of quantitative parameters did not reveal any treatment related alterations (Deshmukh et al., 2017b).

No changes in body weight were observed during the study in treated animals, compared to controls. The mean feed consumption was comparable in all the dose groups of both the sexes. Except for a slight to mild increase in relative but not absolute heart weights in male and female rats treated at 1000 mg/kg, no changes in organ weights were noted. The increase in relative heart weight was not considered as an adverse alteration in the absence of sharp dose dependence, small magnitude, comparability to historical control data, and absence of any correlated histopathology.

A small decrease noted in the absolute thymus weight of male rats treated with the highest dose (1000 mg/kg bw/day) at the end of 90 days was not considered as treatment related, as it was not dose dependent, only found in males, not in relative weight of thymus and lacked any histopathological correlations (Deshmukh et al., 2017b).

Oral administration of the bitter orange extract to male and female rats for 90 days at and up to the dose of 1000 mg/kg did not induce any treatment-related alterations in hematological parameters. Except for a slight and reversible (non-adverse) elevation of the BUN and urea levels in the high dose group (G4) of male rats, oral administration of the bitter orange extract at levels up to 1000 mg/kg bw/day did not induce alterations in clinical chemistry parameters. The values of BUN, and consequently the calculated urea levels, of high dose (G4) male rats were found to be slightly higher (statistically significant) than those of the respective vehicle control group values. The alteration was not dose-dependent, and the apparently significant levels were well within historical control ranges for BUN and urea for Sprague Dawley rats in the test facility, as well as in other laboratories (see for example, Manne et al., 2015), and similar to the values for the female rats.

Although as per histopathology findings in G4 male rats, there was an incidence of minimal lymphocytic infiltration (2/10) and of solitary cortical cyst (1/10) in kidneys, and one of these rats had significantly increased kidney weight, no clear correlation could be established. However, lymphocyte infiltration in kidneys is a common occurrence in kidneys of control SD rats (Romero et al., 1999). Cortical cysts are also known to occur in control SD rats (McMartin et al., 1992). Therefore, although considered to be treatment induced, this finding was considered to be non-adverse in nature. Additionally, small but statistically significant but clinically insignificant differences were noted for calcium in male (G4) and female rats (G3), and albumin in female rats (G3).

These effects were small, not dose dependent, did not occur in both sexes, were believed to be incidental, were within the historical control range with no correlating toxicological effects, and therefore of no toxicological significance (Deshmukh et al., 2017b). It must be emphasized that there have to be clinically remarkable, significant alterations before such findings can be called adverse. Such was not the case. Dependence only on statistical significance is very misleading and inappropriate. Based on standard toxicological data interpretation, these incidental events cannot be considered adverse or clinically significant. Therefore, they are not considered when determining values for NOAEL.

The urinalysis parameters, including microscopic appearance of the centrifuged deposits, did not reveal any significant changes as compared to control (Deshmukh et al., 2017b).

There were no treatment-related macroscopic or histopathological findings in any of the groups. All the gross and histopathological changes observed were considered as spontaneous and incidental to rats of this particular strain and age. A few isolated instances of macroscopic and microscopic findings were considered to be incidental and unrelated to treatment.

Based on the findings of this 90-day subchronic study, the investigators concluded that the extract induced a few non-adverse alterations at the highest tested dose of 1000 mg/kg body weight, while

no such alterations were induced at and up to the dose level of 300 mg/kg body weight. Hence the no-observed-effect level (NOEL) for bitter orange extract is determined as 300 mg/kg bw/day. and by considering the observed findings as non-adverse, the no-observed-adverse-effect-level (NOAEL) is determined to be greater than 1000 mg/kg bw/day (Deshmukh et al., 2017b). As noted above, the NOEL for the 14-day study was 500 mg/kg while the NOAEL was 1000 mg/kg.

The raw data for this 90-day subchronic toxicity study are presented in **Appendix 6.6** in a report from Intox Pvt Ltd. dated September 6, 2017. The statistically analyzed and summarized data were published by Deshmukh et al., 2017b.

3.2.5 Reproductive or developmental studies

To study whether relatively pure *p*-synephrine or *p*-synephrine present as a constituent of bitter orange extract produced developmental toxicity, Sprague-Dawley rats were dosed daily by gavage with one of several different doses of synephrine from one of two different extracts (Hansen et al., 2011). These results are summarized in Table 7. Caffeine was added to some doses, however, we focused our review only on the groups that were not dosed with caffeine. Animals were sacrificed on GD 21, and fetuses were examined for the presence of various developmental toxic endpoints. Extracts contained either 6% synephrine (also referred to as bitter orange or BO) or 90% synephrine (also referred to as synephrine or SE). The analytical test demonstrated that the BO extract contained 7.25% synephrine, 0.63% hordenine, 0.10% octopamine and 0.09% tyramine by weight. The SE extract contained 95.0% synephrine, 0.05% hordenine, 0.39% octopamine, and 0.02% tyramine by weight.

A dose-finding study was done with groups of seven rats. Animals were bred overnight, and the morning that a vaginal sperm plug was found was considered GD 0. Rats were dosed daily by gavage from GD 3 until GD 20 and were sacrificed on GD 21. Eight dose groups were examined: vehicle (0.25% methyl cellulose); 1.0, 2.5, 5.0, 10.0 and 25.0 mg synephrine/kg body weight using the SE extract; 10.0 and 25.0 mg synephrine/kg body weight using the BO extract. The results show that at least five rats were pregnant in each group; one animal died during dosing (gavage error), and one animal delivered early (probable missed sperm plug). There were no differences in maternal weight gain, gravid uterine weight, food consumption or in any maternal organ weight. The corrected weight gain was significantly decreased for the 10.0 mg/kg BO group only (Hansen et al., 2011).

The only significant differences in fetal outcome were increased fetal weight, female weight, male weight and the weight of the heart, kidneys and thymus from the pups of the 1.0 mg/kg BO groups. There were no differences in the average numbers of implants, live fetuses or non-live fetuses per litter among the treatment groups. Although the differences were not statistically significant, there were decreases in the numbers of implants/litter and live fetuses/litter and an increase in the number of non-live fetuses/litter at the highest dose of BO (25 mg synephrine/kg body weight). A similar effect was observed at the highest dose of SE (25 mg synephrine/kg body weight), although the differences from control values were less pronounced. These data suggest that higher doses of synephrine might induce more significant embryotoxic effects. No malformations or skeletal anomalies were identified in the dose-finding study (Hansen et al., 2011).

Based on the results of the dose-finding experiment, a full teratology study was then conducted. Because none of the doses in the dose-finding study produced developmental toxicity, higher doses were used in the full study. The day that the sperm plug was found was considered GD 0; rats were gavage dosed daily from GD 3 to GD 20. Groups of 25 rats were treated with one of nine doses. These doses were vehicle (0.25% methyl cellulose), 10, 25, 50, or 100 mg of synephrine/kg body weight (using the BO extract), 50 and 100 mg synephrine/kg body weight (using the SE extract); 50 mg synephrine/kg body weight + 25 mg caffeine/kg body weight (using the SE extract), and 25 mg caffeine/kg body weight. The results show that 16-22 of the animals in each group (25-26 females) were pregnant. There were no differences among the groups in the number of implants/litter (Hansen et al., 2011).

Table 7. Reproductive/Developmental Study:

Test article	Species	Number/group, sex	Duration	Dose (mg/kg bw/day)	Result	Reference
6% synephrine (SE) 95% synephrine 6% synephrine (SE) 95% synephrine	Pregnant female Sprague-Dawley rats	7 rats/group 7 rats/group 25 rats/group 25 rats/group	21 days	10, 25 mg/kg SE 1,2.5,5,10, 25 mg/kg SE 50,100 mg kg SE 50,100 mg kg SE	Up to 100 mg/kg SE, no adverse effects of embryo lethality, or fetal weights. No effect on incidence of gross, visceral, or skeletal abnormalities. At up to 100 mg/kg SE, "did not produce developmental toxicity in Sprague Dawley rats".	Hansen et al., 2011

When compared to the vehicle control group, there was a significant decrease in the number of live implants/litter in the 100 mg/kg SE group. There were no differences between treatment and control groups in the mean fetal weight or in the percentage of live pups that were males. There were three fetuses with malformations and numerous skeletal anomalies among all groups. None of these malformations and anomalies appeared to be due to the treatment (Hansen et al., 2011).

The highest dose of synephrine (100 mg/kg SE) significantly decreased the number of live fetuses/litter, but the same synephrine dose (100 mg/kg) using the BO extract did not alter this endpoint. Since the same synephrine dose from the second extract did not alter the number of live fetuses/litter, this suggests that the significant finding with the SE extract may have been a spurious observation (Hansen et al., 2011).

The results obtained in this study suggest that *p*-synephrine present either as a relatively pure compound or in an extract with octopamine, hordenine and tyramine does not produce

maternal or developmental toxicity at doses as high as 100 mg synephrine/kg body weight (Hansen et al., 2011).

3.2.6 Other animal safety studies

Several animal studies have assessed the potential cardiovascular effects of bitter orange extract and *p*-synephrine. These studies are summarized in Table 8. In a safety study, mice were treated daily for 28 days with bitter orange extract (7.5% *p*-synephrine) at doses of 400, 2,000 or 4000 mg/kg (corresponding to 30, 150 and 300 mg *p*-synephrine/kg, respectively) or with 30 mg or 300 mg *p*-synephrine/kg (Arbo et al., 2009). The 300 mg/kg dose is 420 times a typical 50 mg dose of *p*-synephrine for a 70 kg human. No adverse effects were observed regarding blood pressures or heart rates, organ weights, or biochemical parameters in the treated mice at any of the doses up to and including 300 mg/kg *p*-synephrine as compared to the controls. A reduction in body weight gain was observed at all doses of *p*-synephrine and the bitter orange extract relative to controls.

In addition, 30 mg/kg and 300 mg/kg doses of *p*-synephrine and the high (4000 mg/kg) dose of the bitter orange extract resulted in increases in the antioxidant and tissue protectant glutathione (GSH) and inhibition of glutathione peroxidase, while the bitter orange extract decreased malondialdehyde content (an indicator of lipid peroxidation and lipid damage). *p*-Synephrine increased catalase which neutralizes hydrogen peroxide (Arbo et al., 2009). Taken together, the results indicated a beneficial effect for both the bitter orange extract and *p*-synephrine with respect to weight loss without adverse cardiovascular effects at very high doses while also providing antioxidant and tissue protective effects.

The physiological effects of administering *p*-synephrine in the form of 6% *p*-synephrine-containing bitter orange extract (corresponding to doses of 10 mg/kg and 50 mg/kg *p*-synephrine) and as well as 10 mg/kg and 50 mg/kg isolated *p*-synephrine to rats for 28 days was determined (Hansen et al., 2012). A review of the data indicates that minimal, statistically significant but clinically insignificant effects were produced by these high doses of *p*-synephrine with respect to heart rate and blood pressure. More specifically, the half-life of *p*-synephrine is about two hours and at this time point the 50 mg dose of *p*-synephrine resulted in a 3 mm Hg increase in systolic blood pressure and less than a 2 mm Hg increase in diastolic blood pressure, while the heart rate increased by only 2 BPM. These effects are easily produced by walking fast or ascending several steps.

The abstract from this study is confusing and contradictory (Hansen et al., 2012). The authors state that “synephrine either as the bitter orange extract or as pure synephrine increased heart rate and blood pressure”. However, in the next sentence they note that “Animals [rats] treated with 95 % *p*-synephrine showed minimal effects on heart rate and blood pressure”. Thus, the cardiovascular increases initially indicated for *p*-synephrine were not of a significant nature. *p*-Synephrine had no effect on the uncorrected QT interval.

The bitter orange product used in this study contained only 6 % p-synephrine (Hansen et al., 2012), which produced somewhat greater effects on heart rate and blood pressure than p-synephrine alone. The authors did not determine or speculate what may have been responsible for these effects in the bitter orange extract. It should be kept in mind that the Advantra Z® product, which is the

subject of this NDIN, contains 50 % *p*-synephrine, and generally less than 2 % of other protoalkaloids, as previously noted. Due to the unknown nature of the other constituents present in the product used by Hansen et al. (2012), these results cannot be extrapolated to Advantra Z®.

Table 8. Cardiovascular Animal Studies:

Test article	Species	Total number, number/group, sex	Exposure regime, duration	Dose (mg/kg bw/day)	Results	Reference
7.5% synephrine (SE) 95% synephrine	CF1 Albino mice	9-10 CF1 male mice/group	28 days	30, 150, 300 mg SE/kg/day 30,300 mg SE/kg/day	No effects on blood pressures, heart rate, blood chemistries, or organ weights with extract or 95 % SE. Decreased body weights. Antioxidant activities at 300 mg synephrine/kg/day as extract or 95 % SE.	Arbo et al. 2009
6% synephrine (SE) 95% synephrine	Sprague-Dawley rats Female	Replicates of 16 animals per group	daily by gavage for 28 days	0, 10, 50 mg/kg SE with and without CAF	Clinically insignificant increases** in HR and BP with 95 % SE. AT 2 hrs with 50 mg/kg SE, 2 mm Hg ↑ in SBP and DBP, and 2 BPM ↑ HR.	Hansen et al. 2012
6% synephrine (SE) 95% synephrine	Sprague-Dawley rats Female	13--14 animals per group-exercised treadmill 3 days/week	daily by gavage for 28 days	0, 10, 50 mg/kg SE	Clinically insignificant increases** in HR and BP. AT 2 hrs with 50 mg/kg SE, 6 mm Hg ↑ in SBP, 5 mm Hg ↑ in DBP, and 4 BPM ↓HR. Greater effects with 50 mg SE/kg as extract. QT interval prolonged with extract.	Hansen et al. 2013

** Adrenergic receptor binding of *p*-synephrine in rodents is at least 10-fold greater than in humans (Carpéné et al., 1999; 2014; Mercader et al., 2011), and therefore effects in rodents cannot be extrapolated directly to humans.

The potential cardiovascular effects of bitter orange extract and *p*-synephrine were also examined in exercised rats given 10 and 50 mg/kg *p*-synephrine from either purified *p*-synephrine or a 7.25 % *p*-synephrine-containing bitter orange extract for 28 days (Hansen et al., 2013). The rats ran on a treadmill 3 days/week for 30 min/day. Cardiovascular effects were monitored for up to 8 hours after dosing. *p*-Synephrine exhibited small statistically significant, but clinically insignificant effects on blood pressure and inconclusive effects on heart rate at the high dose.

Exceedingly high doses of *p*-synephrine were required to produce these effects in rats, doses unrelated to those commonly used in humans (Hansen et al., 2012; 2013). It must be emphasized that the adrenergic receptor binding of *p*-synephrine in rodents is at least 10-fold greater than in humans (Carpéné et al., 1999; 2014; Mercader et al., 2011) which can readily account for the observed, small cardiovascular effects in rodents (Hansen et al., 2012; 2013) when no such effects are observed in humans (Stohs et al., 2012, 2019, 2020; Stohs & Shara 2013; Kaats et al., 2013, 2017; Shara et al., 2018; Stohs 2017; Suntar et al., 2018; Ratamess et al., 2015; 2016; 2018), and

therefore the small but clinically insignificant effects in rodents cannot be extrapolated directly to humans.

Assume that a 50 mg/kg dose of *p*-synephrine in rats (equivalent to a 3500 mg dose in a 70 kg human) results in a 3-6 mm Hg increase in systolic blood pressure (Hansen et al., 2012; 2013). The adrenergic receptor binding of *p*-synephrine in rats is least 10-times greater than in humans (Carpéné et al., 1999; 2014; Mercader et al., 2011). Assuming the effect on heart rate and blood pressure is directly proportional to the degree of adrenergic receptor binding for an agonist as well as indirect effects, then the response in a human would be at least 10-times less than in rats. Therefore, a 3-6 mm Hg increase in systolic blood pressure in rats would be expected to be less than 1 mm Hg increase in humans, which is clinically insignificant. This is borne out by numerous studies in humans where no statistically significant or clinically significant effects on blood pressures or heart rates have been observed (Kaats et al., 2013; 2017; Shara et al., 2016; 2018; Gutiérrez-Hellin, et al., 2016; Gutiérrez-Hellin & Del Coso, 2016; 2018a; 2018b; Ratamess et al., 2015; 2016; 2018; Stohs, 2017; 2018; Suntar et al., 2018).

The effects of orally administering 50 and 100 mg/kg of *p*-synephrine to rats and rabbits for up to 20 days on the pharmacokinetics and pharmacodynamics of gliclazide has been reported (Vatsavai and Kilari, 2018). *p*-Synephrine had no effect on the pharmacokinetics of gliclazide either as a single dose or daily multiple doses. *p*-Synephrine exhibited small changes in blood sugar levels in response to gliclazide. No adverse effects were reported in response to these daily high doses of *p*-synephrine for up to 20 days in either rats or rabbits. A daily dose of 100 mg/kg of *p*-synephrine would equate to a daily dose of 7000 mg for a 70 kg human as compared to a commonly used daily dose of 25-50 mg.

Ventura et al. (2018) evaluated the effects of a bitter orange extract on the pharmacokinetics of lamotrigine in rats following a single oral dose (164 mg/kg) and after daily dosing of the extract for 14 days. The most significant finding was a decrease in time of the lamotrigine to reach peak drug concentration. There was no effect on the area under the curve (AUC), indicating that the extract had no effect on the blood levels of lamotrigine. The authors concluded that there were no relevant interactions that would be expected to occur in clinical practice. No adverse effects were reported in response to these daily high doses of the bitter orange extract for up to 14 days in the rats, consistent with previous studies.

3.2.7 Genetic toxicology studies

Mutagenic potential of bitter orange extract containing 50% *p*-synephrine (Advantra Z®) were investigated in the bacterial *Salmonella typhimurium* reverse mutation assay (Ames Test) performed by the pre-incubation method using the tester strains TA1535, TA97a, TA98, TA100 and TA102 (Deshmukh et al., 2017a; **Appendix 6.7**). These studies are summarized in Table 9. The test was conducted in duplicate in the presence and absence of an S9 metabolic activation system. The assay was conducted as per OECD 471 Guidance on Genotoxicity Testing. The bacterial strains were grown in Oxoid Nutrition Broth No. 2 and exposed to the bitter orange extract (Advantra Z®) in triplicate cultures at the concentrations of 50, 150, 500, 1500, or 5000 µg/plate. Liver post-mitochondrial S9 fractions, induced in rats by phenobarbital and β-naphthoflavone, were used as the metabolic activation systems. The exposed bacterial strains were plated onto minimal glucose agar medium supplemented with L-histidine. The plates were

incubated at 37ºC for about 68 hours after which the histidine revertant colonies were counted and their frequencies were compared with that in vehicle control groups. Sodium azide, 3-methylmethane sulfonate, ICR 191, and 4-nitroquinoline-N-oxide were used as positive mutagenic controls without metabolic activation while 2-aminofluorene, 2-aminoanthracene and danthrone were used as positive mutagenic controls with metabolic activation. In order to confirm the reproducibility of the results, the entire study was carried out twice.

The mean number of histidine revertant colonies for all the treatment groups was compared with the number of revertants in the respective vehicle control group. The mutagenic activity of the bitter orange extract containing 50% *p*-synephrine was assessed by applying the following criteria. The bitter orange extract was considered to be positive (mutagenic) if it induced a concentration dependent increase and/or an increase at one or more concentrations in revertant frequency which was at least 2-fold (3-fold for TA1535) of that observed in the corresponding concurrent vehicle control. If the results for the bitter orange extract did not meet the above criteria, it was considered non-mutagenic in this test. The results indicated that the bitter orange extract did not induce cytotoxic effects in the tester strains at and up to a concentration of 5000 µg/plate in the presence and absence of metabolic activation. Therefore, it can be concluded that bitter orange extract containing 50% *p*-synephrine is non-mutagenic in the *S. typhimurium* reverse mutation assay (Ames Test). The reproducibility of the negative control results was confirmed by repeating the experiment (Deshmukh et al., 2017a).

The raw data for this mutagenicity study are presented in **Appendix 6.7** in a report from Intox Pvt Ltd. dated April 4, 2017. The statistically analyzed and summarized data were published by Deshmukh et al., 2017a.

More recently, potential cytotoxic, genotoxic, mutagenic and pro-oxidant effects of *p*-synephrine were assessed in human hepatic (HepG2) cells in culture (Ribeiro et al., 2019). The HepG2 cells were treated with 2, 20 and 200 µM *p*-synephrine for up to 24 hours. *p*-Synephrine was not cytotoxic, genotoxic or mutagenic at up to and including 200 µM (33.4 µg/mL), and exhibited antioxidant activity at 20 and 200 µM as indicated by an increase in reduced glutathione (GSH) and glutathione peroxidase (GPx).

Several other studies also suggest that bitter orange extract is non-mutagenic. A mutagenicity screening of the aqueous and methanolic extracts of 104 crude preparations, including *C. aurantium* (bitter orange) was conducted by Morimoto et al. (1982). (See Table 9). The assays involved the *Salmonella typhimurium* microsomal reversion assay using strains TA98 and TA100 and the *Bacillus subtilis* rec-assay using *Bacillus subtilis* strains H17 REC+ and M45 REC. Bitter orange extracts were found to be negative in both assays, while various other crude drugs were positive in both assays.

Table 9. Mutagenicity Studies:

Test article	Species	Total number, number/group, sex	Exposure regime, duration	Dose (mg/kg bw/day)	Results	Reference
Advantra Z® (50 % synephrine)	Ames Test - *Salmonella typhimurium* tester strains TA1535, TA97a, TA98, TA100 and TA102	Triplicate cultures in duplicate with and without metabolic activation of S9 fraction	plates incubated at 37ºC for about 68 hours	0, 50, 150, 500, 1500, or 5000 µg/plate	Bitter orange extract containing 50% *p*-synephrine was non-mutagenic in the *S. typhimurium* reverse mutation assay (Ames Test). The reproducibility of the negative control results was confirmed by repeating the experiment	Deshmukh et al., 2017a; Appendix 6.7
Bitter orange extract	*Ames test-Salmonella typhimurium* strains TA98 and TA100; Rec-assay *Bacillus subtilis* strains H17 REC+ and M45 REC.	Cultures in duplicate with and without metabolic activation of S9 fraction	Plates incubated at 37ºC for about 48 hours Overnight incubation	0.1 ml each solution/plate	Negative in both assays, while various other crude drugs were positive in both assays.	Morimoto et al. 1982
p-synephrine	HepG2 Human liver cells	Cells in culture	24-hour exposure, 37ºC	0, 2, 20, 200 µM	Not cytotoxic, genotoxic or mutagenic. Some antioxidant activity	Ribeiro et al., 2019

3.2.8 Allergenicity studies

A search in the literature did not yield any allergenicity reports or studies on bitter orange or its extract.

3.2.9 Human clinical studies conducted with Bitter Orange Extracts and Synephrine

In a series of specifically designed human clinical trials, effects of bitter orange extract (Advantra Z®) were investigated. These specific human studies are summarized in Table 10. Of the 18 specific trials, in nine studies effects of bitter orange extract alone were studied, while in the remaining studies effects of bitter orange and caffeine were studied. In these specifically designed studies, no adverse effects of the bitter orange extract were noted. In these studies, a total of over 350 subjects received Advantra Z®. In a specific human study, daily ingestion of bitter orange extract containing 98 mg synephrine for 60 days did not reveal any adverse effects (Kaats et al.,

2013). All these specifically designed studies with bitter orange extract support the safety of Advantra Z® at proposed use levels. These studies are further described along with other human clinical studies of bitter orange preparations and *p*-synephrine below.

Table 10. Prominent Human Clinical Studies Conducted with Bitter Orange Extracts:

Test article	Human	Total number, number/group, sex	Exposure regime, duration	Dose (mg/kg bw/day) orally	Results	Reference
Advantra Z® (50 % synephrine)	Healthy, males and females	67 total 22 placebo 22 p-synephrine + naringin + hesperidin 23 Advantra Z®.	Double-blinded, placebo-controlled; twice a day, 60 days	0, 49 mg synephrine twice daily (98 mg/day) capsule.	No significant changes. No effects on heart rates, blood pressure, or 44 blood chemistry or blood cell parameters. Well tolerated.	Kaats et. al. 2013
Advantra Z® (50 % synephrine)	Healthy males and females	40 total: 10-placebo 10-30 days, 20-15 days cross over.	Double-blinded, placebo-controlled; twice a day, 15 or 30 days	0, 51.5 mg synephrine twice daily (103 mg/day) chew.	No adverse effects. No changes in heart rate or blood pressure. Increases in energy and appetite control.	Kaats et. al., 2017
Advantra Z® (50 % synephrine)	Healthy males and females	16 total cross-over	Double-blinded, placebo-controlled cross- over. 15 days total. Assessments at 5, 10 and 15 days.	49 mg synephrine/day capsule	No significant changes. No effects on heart rates, ECGs, blood pressure, or blood chemistry or blood cell parameters.	Shara et al. 2018
Advantra Z® (50 % synephrine, SE)	Healthy males and females	8 healthy subjects	Double-blinded, placebo-controlled 6 way cross-over single dose with week washout between treatments.	0; 103 mg SE;.	No effects on ECG or heart rate 103 mg SE ↓ diastolic BP.	Ratamess et al., 2018; Bush et al., 2018
Advantra Z® (50 % synephrine, SE)	Healthy males and females	12 healthy subjects during resistance exercise	Double-blinded, placebo-controlled cross-over with week washout between treatments.	0 or 100 mg SE chew/day for 3 days.	No heart rate of blood pressure effects due to SE. ↑ in fat oxidation and energy expenditure.	Ratamess et al., 2015,2016

Test article	Human	Total number, number/group, sex	Exposure regime, duration	Dose (mg/kg bw/day) orally	Results	Reference
Synephrine HCl (SE)	healthy subjects with exercise	13 subjects 18 subjects 17 subjects 13 subjects	Double-blinded, placebo-controlled cross-over	0, 3 mg/kg SE 0, 3 mg/kg SE 0,1,2,3 mg/kg SE	No adverse cardiovascular or other effects under any conditions. Maximum fat oxidation at 2 & 3 mg/kg SE.	Gutierrez-Hellin et al., 2016; Gutierrez-Hellin & Del Coso, 2016; 2018a; 2018b
Advantra Z (46.9 mg synephrine, SE) Xenadrine EFX (Complex) with 5.5 mg SE	Healthy subjects	10 subjects cross over	Double-blinded, placebo-controlled 3-arm cross-over	*0, 46.9 mg synephrine (SE)* *5.5 mg SE*	No effect of SE on BP. ↑ HR at 6 hrs but not at peak blood levels at 2 hrs. Meal at 3 hrs associated with ↑ HR. EFX ↑ in HR and BP	Haller et al., 2005
Complex polyherbal with 21 mg synephrine	Healthy subjects	10 subjects moderately intense (30 min) cycle exercise	Double-blinded, placebo-controlled 3-arm cross-over	0, 21 mg SE under resting conditions and 1 hr prior to exercise	Non-significant effect on heart rate and SBP post-exercise.	Haller et al., 2008
6% Synephrine	Young healthy subjects	15 subjects	Double-blinded, placebo-controlled cross-over	0, 54 mg SE	Clinically insignificant cardiovascular changes. As compared to baseline, 2 mm Hg ↑SBP, 2 mm Hg ↓DBP, 2 BPM ↑HR	Bui et al., 2006
6% Synephrine	Healthy subjects	18 subjects	Double-blinded, placebo-controlled cross-over	0, 27 mg SE	No effect of SE on SBP, DBP, QT interval, cardiac index or systemic vascular resistance index	Min et al., 2005

Clinical studies with longer exposure period are summarized below. In a double-blinded, placebo-controlled clinical trial, Kaats et al. (2013) assessed the safety of bitter orange extract alone or in combination with the Citrus polyphenols naringin and hesperidin. In this study, 23 healthy human subjects were given a standardized bitter orange extract (Advantra Z®) in capsule form which contained 49 mg *p*-synephrine twice a day (total of 98 mg *p*-synephrine/day) for 60 days, while another 22 subjects received 49 mg *p*-synephrine plus 300 mg naringin and 50 mg hesperidin (Kinetiq™) twice daily. A group of 22 subjects received the placebo (Kaats et al., 2013).

The subjects were evaluated for several parameters at baseline, day 30 and day 60. No significant changes were noted in systolic or diastolic blood pressures, blood chemistries or blood cell counts in control or *p*-synephrine treated groups. Small, clinically insignificant differences in heart rates were observed between the *p*-synephrine plus naringin and hesperidin group and the *p*-synephrine alone as well as the placebo group. Data were provided for base line and ending values for each of the study groups for 44 individual blood chemistry and hematological tests, none of which showed statistically significant differences between the placebo-controlled group and the treated groups. Kaats et al., 2013).

Specifically, twice daily dosing with 49 mg *p*-synephrine as Advantra Z® relative to the placebo-controlled group had no effect on any of the following blood test parameters:

--Enzymes: ALP, AST, ALT

--High sensitivity C-reactive protein (hs-CRP)—inflammatory biomarker

--Lipids: cholesterol (total, HDL and LDL), triglycerides

--Electrolytes: sodium, potassium, chloride, calcium, CO_2

--Proteins: total protein, albumin, globulin, albumin/globulin ratio

--BUN, creatinine, BUN/creatinine ratio

--Other: glucose, TSH, total bilirubin, eCGF

--Red blood cell count, hemoglobin, hematocrit, MCV, MCH, MCHC, RDW, and platelet counts.

--Absolute counts or percentages of neutrophils, lymphocytes, monocytes, eosinophils and basophils or white blood cell counts.

No adverse events were reported by any of the subjects or observed by the investigators, thus demonstrating the tolerability of twice daily dosing of 49 mg p-synephrine in the form of the standardized bitter orange extract (Advantra Z®) The results of this study indicate that bitter orange extract and *p*-synephrine appear to be without adverse effects at a dose of up to 98 mg/day for 60 days when consumed either alone or in combination with naringin and hesperidin, based on the parameters measured (Kaats et al., 2013), and is therefore reasonably expected to be safe.

Lower doses of p-synephrine (Advantra Z®) were not used since earlier studies indicated a lack of adverse effect of *p*-synephrine at doses up to 50 mg (Min et al., 2005; Haller et al., 2005; Bui et al., 2006; Stohs et al. 2011c), and higher doses than 49 mg twice daily were not used because no preliminary studies existed involving higher doses. Subsequent studies used doses as high as 104 mg *p*-synephrine, and doses as high as 3 mg/kg of synephrine HCl (210 mg for a 70 kg individual) have been used (Gutiérrez-Hellin & Coso 2016; 2018a; 2018b; Gutiérrez-Hellin et al., 2016) without adverse effects including cardiovascular effects.

In a randomized, placebo-controlled cross-over study specifically designed to address potential cardiovascular effects, bitter orange extract (Advantra Z®) was administered at a dose of 49 mg *p*-synephrine daily in capsule form for 15 days to 16 male and female subjects (8 each) (Shara et

al., 2018). No significant effects on ECGs, heart rates, systolic and diastolic blood pressures, serum chemistries or blood cell counts occurred on days 5, 10 or 15 relative to baseline and placebo control. No adverse effects were reported or observed by any of the subjects. Bitter orange extract (*p*-synephrine) exhibited no effects relative to the placebo at any of the time points regarding serum electrolytes, glucose, lipids, proteins, and liver and kidney function indicators, or on blood cell counts with differentials and platelets.

In this study, *p*-synephrine blood levels were determined at 7 and 14 days following the initiation of the study for control and *p*-synephrine-treated groups at approximately four hours after ingestion of the *p*-synephrine (Advantra Z®) to ensure that all subjects had ingested the bitter orange extract. Serum levels indicated that all subjects complied regarding ingestion of the bitter orange extract. No significant differences were observed in *p*-synephrine levels between days 7 and 14 which were approximately and 2.6 ng/mL (Shara et al., 2018).

In a placebo-controlled, double-blind study that assessed safety, energy and appetite control, chocolate-flavored chews containing 51.5 mg *p*-synephrine (Advantra Z®) or placebo were consumed 15-30 min before the two largest meals of the day for 15 or 30 days (Kaats et al., 2017). Thus, the subjects consumed 103 mg *p*-synephrine or placebo daily. Forty subjects were involved in the study and all subjects completed the study. No changes in heart rate or blood pressure were noted, and no adverse effects were reported for either the *p*-synephrine-treated group (103 mg *p*-synephrine per day) or the placebo control group. Therefore, the product was well tolerated. Statistically significant increases in energy and appetite/eating control were reported by the subjects with respect to the *p*-synephrine chew as compared to the placebo control chew.

Colker et al. (1999) investigated the effects of a bitter orange extract, caffeine and St. John's Wort on body fat loss, lipid levels and mood states in normal weight and obese individuals (n=20). The product which was consumed on a daily basis for 42 days contained 975 mg *C. aurantium* extract (6% synephrine alkaloids), 528 mg caffeine, and 900 mg St. John's Wort. The total daily intake of phenylethylamine-related protoalkaloids was approximately 58.5 mg. All subjects in the study followed an 1800 kcal/day American Heart Association Step One diet, and performed a circuit training exercise program three days per week. No significant changes in blood pressure, heart rate, electrocardiographic findings, serum chemistry or urinalysis were noted and no significant changes were observed in the results of the Profile of Mood States Questionnaire for fatigue or vigor.

In a double-blind cross-over study by Kalman et al. (2000), six healthy human subjects received two capsules of the complex product Xenadrine EFX® (12 mg *p*-synephrine) for 56 days. No effects on heart rate or blood pressure were observed, and no subjective complaints or adverse events were reported.

Gurley et al. (2004) conducted a study in 12 human subjects that received bitter orange extract for 28 days. The daily consumption of *p*-synephrine was 30.6 mg. The authors concluded that a supplement containing *C. aurantium* extract did not appear to significantly modulate cytochrome P450 enzyme activities in human subjects, and therefore posed minimal risk for cytochrome P450-mediated, herb-drug interactions. The bitter orange extract had no significant effect on CYP1A2,

CYP2D6, CYP2E1 or CYP3A4, the major drug-metabolizing cytochrome enzymes. No adverse effects were observed.

In a randomized, double-blind placebo-controlled study involving healthy, overweight adults, Zenk et al. (2005) investigated the effects of a product containing bitter orange extract (Advantra Z®) on metabolic rate and body composition. A total of 35 subjects completed the 8-week study. Each adult received three capsules of the product twice daily or a placebo in conjunction with a calorie-restricted diet and an exercise program. The product contained 6 mg *p*-synephrine/capsule (36 mg *p*-synephrine/day). The product also contained 3-acetyl-7-oxo-dehydroepiandrosterone (17 mg), *Coleus forskohlli* extract (50 mg extract, 10 mg forskolin), yerba mate extract (167 mg), guarana extract (233 mg extract, 51 mg caffeine), piperine (1.67 mg from *Piper nigrum*) and dandelion leaf and root powder (83 mg). No changes in heart rate or blood pressure were observed and no serious adverse events were reported. The relative role of each of the ingredients cannot be determined.

Jung et al. (2017a; 2017b) investigated the effects of ingesting a multi-component pre-workout dietary supplement with or without 20 mg *p*-synephrine (Advantra Z®) once per day for 8-weeks during training. The group receiving the product with *p*-synephrine contained 27 male subjects. After 8 weeks, no statistically significant effects were observed between groups with time regarding blood pressures, heart rates, or blood chemistry panels. Pre-workout dietary supplementation with and without *p*-synephrine did not increase the incidence of reported side effects or significantly affect the number of blood values above clinical norms compared to placebo group.

3.2.10 Other Human Safety Studies Conducted with Bitter Orange Extracts and Synephrine

In addition to the above studies, a number of other studies have addressed the safety and mechanisms associated with bitter orange extract and *p*-synephrine (Advantra Z®) for shorter periods of time. Various authors have assumed without evidence that *p*-synephrine exhibits cardiovascular activity because of its structural similarity to ephedrine (See for example: Penzak et al., 2001; Bent et al., 2004; Fugh-Berman and Myers, 2004; Inchiosa, 2010; Rasmussen et al., 2012; Natural Medicines Comprehensive Database, 2016; Bakhyia et al., 2017).

The study by Bui et al. (2006) is frequently cited as evidence for the exertion of cardiovascular effects by *p*-synephrine and bitter orange extracts without a careful examination of the methods and results. They conducted a randomized, double-blind, placebo-controlled crossover study involving 13 healthy subjects who received a single dose of 900 mg bitter orange extract (Nature's Way) standardized to 6% *p*-synephrine (54 mg *p*-synephrine) or the placebo. Heart rate and blood pressure were measured every hour for six hours. These investigators concluded that the *p*-synephrine increased heart rate and blood pressure.

Various problems exist with regard to the study. The results are misleading in that the authors only discussed data relative to placebo which represented small but larger differences than with respect to baseline (zero time, within subjects). As compared to baseline, small but clinically insignificant increases in heart rate (2.0 BPM) and systolic blood pressure (3.9 mm Hg) with a decrease in

diastolic blood pressure (-1.5 mm Hg) occurred at the 2-hour timepoint, a time point at which greatest effect would be expected to occur based on half-life and peak blood levels. A confounding factor is that the subjects were fed a meal one hour after receiving the study product. The thermic effects of food are well known, would have impacted heart rate and blood pressure, and may explain effects lasting five hours.

In order to show statistical significance, the authors resorted to the use of non-traditional statistical analyses, including least square means and difference in least square means, and a multivariable mixed model. It is doubtful that any statistical significance would have been shown using a standard two-tailed t-test. The effects on heart rate and blood pressure were small, clinically insignificant and have not been observed in numerous other studies, several of which are described below.

A placebo-controlled, randomized cross-over study was designed to examine the cardiovascular effects of a standardized bitter orange extract (Advantra Z®) at a dose of 49 mg *p*-synephrine in capsule form in 9 male and 9 female healthy subjects (Shara et al., 2016. Heart rates, blood pressures, and electrocardiograms (ECGs) were determined at baseline, 30, 60, 90 min, 2, 4, 6 and 8 hours, while blood samples were drawn for analysis at 2 and 8 hours). The ECGs were continuously monitored over the 8 hours of the study and read blindly by a cardiologist. Data at each timepoint were analyzed using a two-tailed t-test and expressed as mean values with the standard deviation and standard error of the mean.

No significant changes occurred in ECGs, heart rates, blood pressures, 40-item serum chemistries or blood cell counts, and no adverse effects were reported or observed. A small decrease in diastolic blood pressure (-4.5 mm Hg) occurred at the 60-minute time point in the *p*-synephrine-treated group. The authors concluded that *p*-synephrine does not act as a stimulant at the 49 mg dose used in the study (Shara et al., 2016).

A study addressed the cardiovascular effects of a high (103 mg) dose of *p*-synephrine (Advantra Z®) in 16 healthy subjects (Ratamess et al., 2018). The study was double-blind, placebo-controlled cross-over in design. The subjects received single doses of placebo *or p*-synephrine. A washout period of at least one week was employed between the arms of the study.

Cardiovascular effects including diastolic and systolic blood pressures, heart rates and ECGs were determined over a three-hour time period. Blood pressures were assessed every 15 minutes with an automated blood pressure cuff. Heart rate data were collected via a continuously recording ECG. Heart rate data were also collected using a Polar heart rate monitor as well as via the blood pressure cuff, thus assessing heart rates via three technologies. A two-way (time and treatment) analysis of variance with repeated measures was used, as well as Tukey's post hoc tests, and partial eta square size effects.

In this study by Ratamess et al. (2018), *p*-synephrine consumption (103 mg) did not significantly affect systolic blood pressure or heart rate at any time point over the three hours of the study. Compared to placebo controls, *p*-synephrine alone (103 mg) resulted in small (-2-4 mm Hg) but significantly lower diastolic blood pressure at 60- and 120-minutes post-consumption. These findings agree with the previous observations of Shara et al. (2016). These data support previous studies and indicate that *p*-synephrine does not induce cardiovascular stress at a dose of 103 mg (Ratamess et al., 2018).

In addition to assessing the cardiovascular effects with respect to *p*-synephrine, blood samples were drawn at baseline and three hours post-ingestion of the products to determine immune, lipid and chemistry panels (Bush et al., 2018). Two questionnaires were completed by the subjects, one concerned with possible side effects and the other with general wellness and mental state. The data in the above studies (Ratamess et al., 2018; Bush et al., 2018) demonstrated that consumption of *p*-synephrine did not negatively affect acute blood parameters, did not produce stimulant-like perceptual mood effects, and did not induce cardiovascular effects at a dose of 103 mg *p*-synephrine.

Haller et al. (2005) studied the effects of a multi-component product (Xenadrine EFX) and Advantra Z® (46.9 mg *p*-synephrine) in 10 subjects in a single dose, randomized, placebo-controlled cross-over study. Advantra Z® had no effect on blood pressure. However, an increase in heart rate was observed that can be attributed to the thermic effect of food and occurred at a time point that does not coincide with peak blood levels of *p*-synephrine. In yet another study, Haller et al. (2008) reported an increase in diastolic blood pressure with a complex product (Ripped Fuel Extreme Cut) that contained 21 mg *p*-synephrine. The increase in diastolic blood pressure was not attributed to the *p*-synephrine.

Stohs et al. (2011c) studied the effect of 50 mg *p*-synephrine (Advantra Z®, 60% *p*-synephrine) alone or in combination with the flavonoids naringin and hesperidin in 40 human subjects on resting metabolic rate. The study was a randomized, placebo-controlled, double blind design with the vehicle for the *p*-synephrine being one ounce of tomato juice. Measurements were taken at baseline prior to consuming the product and at 75 min. At this time point, a 6.9% increase in resting metabolic rate was observed in response to the *p*-synephrine alone relative to the placebo-control group. The consumption of 600 mg naringin and 100 mg hesperidin in combination with 50 mg *p*-synephrine resulted in a 17.7% increase in RMR relative to placebo. No significant effects were observed with respect to blood pressure or heart rate for any of the treatment groups, nor were there any significant differences in responses to a 10 item self-report questionnaire which addressed nervousness, tension, anxiety, hunger, energy, headache, general discomfort, and sleepiness.

In a randomized, placebo-controlled crossover designed study involving 12 male athletes, each subject was randomly assigned (in double-blind manner) a supplement containing100 mg of *p*-synephrine (Advantra Z®) or placebo (Ratamess et al., 2015; Ratamess et al., 2016). The supplements or placebo were consumed for three days. The subjects performed a controlled resistance exercise protocol. Supplement treatments were separated by a one-week washout period. No adverse effects were observed or reported with respect to *p*-synephrine consumption.

The effects of a bitter orange extract containing 58.5 mg p-synephrine on heart rate variability after strength training in 10 male subjects was investigated (de Oliveira Sant'Ana et al., 2019). The study was randomized, double-blind and cross over in design with a one-week washout period between arms. Heart rate variability was determined before and after supplementation and again 60 minutes after exercise. The results of the study demonstrated no significant heart rate variability after consumption of the *p*-synephrine product.

In summary, the effects of bitter orange extract (Advantra Z®) have been investigated in over 30 human clinical studies (Stohs, 2017; Stohs & Ratamess, 2017; Suntar et al., 2018; Stohs et al., 2019). The majority of these studies were designed to investigate the efficacy and safety of bitter orange extract or a mixture of ingredients. In these studies, over 700 subjects participated, 45% of the subjects were overweight/obese. These studies lasted for up to 12 weeks. In general, the findings from these studies indicate that bitter orange extract alone (*p*-synephrine) did not produce significant adverse events such as an increase in heart rate or blood pressure, or alter electrocardiographic data, serum chemistry, blood cell counts or urinalysis. Approximately 43% of the subjects consumed a bitter orange extract (*p*-synephrine) alone with the vast majority being the product Advantra Z®.

In addition to the above studies that have specifically used Advantra Z® as the standardized bitter orange extract, a number of human studies have employed *p*-synephrine (the primary active component in bitter orange extract) as *p*-synephrine HCl with a purity of >95%. Gutiérrez-Hellin et al. (2016) investigated the ingestion of *p*-synephrine HCl 3mg/kg or placebo on performance of 13 sprint athletes in a cross-over designed study. The *p*-synephrine did not produce any adverse effects and it had no effect on headaches, gastrointestinal discomforts, muscle pain or insomnia. The dose of 3 mg/kg is an oral dose of 210 mg *p*-synephrine HCl for a 70 kg (154 lb) individual or 240 mg for an 80 kg (176 lb) individual.

Gutiérrez-Hellin & Coso (2016) assessed the effects of ingesting *p*-synephrine HCl 3 mg/kg or placebo in 18 healthy subjects on increased fat oxidation which was determined at rest and during a cycle ergometer range exercise. The authors reported *p*-synephrine increased maximal fatty acid oxidation. No adverse effects were reported.

The dose response effects of *p*-synephrine on fat oxidation rate during exercise of increasing intensity were determined (Gutiérrez-Hellin & Coso, 2018a). Seventeen subjects received either 0, 1, 2, or 3 mg/kg of p-synephrine HCl in four separate trials with a 72-hour washout time between trials. The authors reported that none of the doses affected heart rates during the cycle ergometer exercise tests. Maximum fat oxidation during the exercise trials occurred with the 2 and 3 mg/kg of *p*-synephrine.

These same authors examined the effects of *p*-synephrine ingestion on substrate oxidation during a cycle ergometer ramp exercise (Gutiérrez-Hellin & Coso, 2018b). Thirteen subjects were involved in a cross over study that consisted of placebo or 3 mg/kg of *p*-synephrine. In comparison with the placebo, *p*-synephrine did not alter heart rates during the exercise test, and increased maximal fat oxidation.

Various studies indicate that the lipolytic activity of *p*-synephrine is due to binding to β-3 adrenergic receptors in adipose tissues (Stohs et al., 2011b). These same β-3 adrenergic receptors are also associated with cardiovascular tissues, and their activation results in a down-regulation of cardiovascular stimulation (Rozec et al., 2006; Moens et al., 2010). Thus, *p*-synephrine stimulation of β-3 adrenoreceptors in the cardiovascular system may not result in an increase in blood pressure or heart rate but exhibit a modulating rather than a stimulatory effect. A down-regulation of

cardiovascular effects via this mechanism may also explain the small decrease in diastolic blood pressure observed in several studies (Shara et al., 2016; Ratamess et al., 2018).

3.2.11 Summary of Review Articles

Various peer reviewed and published articles have summarized the published and unpublished clinical studies involving bitter orange extract (Marles, 2011; Stohs et al., 2012; Stohs & Shara, 2013; Lynch, 2018; Stohs, 2017; Suntar et al., 2018; Stohs et al., 2019; 2020).

An extensive review and health risk assessment of *p*-synephrine, *p*-octopamine and caffeine was conducted by the Natural Health Products Directorate of Health Canada, which also defined guidelines for use of these ingredients (Marles, 2011). Unfortunately, this report has not been widely distributed because it was not concurrently translated into French. The report concluded that consumption of up to 50 mg per day of *p*-synephrine alone in healthy adults "is not likely to cause any adverse health consequences."

Intertek Scientific and Regulatory Consultancy provides scientific, toxicology and regulatory services with respect to dietary supplements. Its reports are used as a basis for making recommendations regarding the use and safety of supplements. Intertek conducted an in-depth scientific literature review and issued a report on the safety of bitter orange extracts (Advantra Z®) standardized to *p*-synephrine alone, and provided recommended guidelines for its use (Lynch, 2018). The report concluded that "the available data indicate that *p*-synephrine does not act as a cardiovascular stimulant." (Lynch, 2018).

The Intertek report further indicated that "[a]s a single one-time dose, up to 100 mg *p*-synephrine would unlikely to be associated with adverse effects in healthy populations, and that "a maximum daily dose of 125 mg/day, as 2 divided 62.5 mg, or 3 divided 42 mg doses, is supported based on pharmacokinetic data assuming that the doses are spaced out during the day." The Intertek report also recommended "that doses should be spaced out by 6 to 8 hours for twice daily dosing and 4 to 6 hours for thrice daily dosing." It recommended "that the product be labeled to indicate that: prior consultation with a physician is recommended; duration of use not to exceed 8 weeks; users of the product should be healthy; avoid using the product and/or consult a physician if they have a medical condition, taking prescription medications, or other dietary supplements." (Lynch, 2018).

Detailed reviews have assessed extant peer reviewed literature regarding the safety, efficacy, and mechanisms of action of *Citrus aurantium* (bitter orange) extracts and *p*-synephrine (Stohs, 2017; Stohs et al., 2019). Approximately 30 human studies were reviewed which indicate that *p*-synephrine and bitter orange extracts do not result in cardiovascular effects and do not act as stimulants at commonly used doses. Mechanistic studies indicate that *p*-synephrine exerts its effects through multiple actions which have been reviewed and are discussed below (Stohs et al., 2020). Because *p*-synephrine exhibits greater adrenergic receptor binding in rodents than humans, data from animals cannot be directly extrapolated to humans. These reviews, as well as several other assessments published in recent years, has concluded that bitter orange extract and *p*-synephrine are safe for use in dietary supplements and foods at the commonly used doses (25-50 mg).

Another overview of bitter orange was published which concluded that "both the extract and isolated compounds have no unwanted effects in humans at therapeutic doses" and therefore, can confidently be used in various dietary formulations (Suntar et al., 2018). These peer-reviewed and published reviews all concluded that, at commonly used doses, bitter orange extracts standardized to *p*-synephrine are unlikely to produce cardiovascular or other adverse effects, and the effects of ephedrine and other adrenergic agonists cannot be extrapolated to *p*-synephrine.

Confusion and mis-understanding exist regarding the lack of cardiovascular and other adverse health effects of *p*-synephrine relative to ephedrine and *m*-synephrine (phenylephrine) which are known for their effects on the cardiovascular system. As a consequence, a recent review has summarized and compared the pharmacological/toxicological mechanisms associated with these compounds (Stohs et al., 2020). These molecules have some structural similarities. However, the structural and stereochemical differences of *p*-synephrine as related to ephedrine and *m*-synephrine result in markedly different adrenergic receptor binding characteristics as well as other mechanistic differences which are reviewed in Stohs et al. (2020).

p-Synephrine exhibits little binding to α-1, α-2, β-1 and β-2 adrenergic receptors, nor is it known to exhibit indirect actions leading to an increase in available levels of endogenous norepinephrine and epinephrine at commonly used doses (25-50 mg). The relative absence of these mechanistic actions provides an explanation for their lack of production of cardiovascular effects at commonly used oral doses as compared to ephedrine and *m*-synephrine. As a consequence, the effects of ephedrine and *m*-synephrine cannot be directly extrapolated to *p*-synephrine which exhibit significantly different pharmacokinetic, and physiological/pharmacological properties. These conclusions are supported by human, animal and *in vitro* studies that are discussed (Stohs et al., 2020). A more detailed discussion of these mechanisms is provided below.

3.2.12 Adverse event reports and monitoring

Several clinical case reports and FDA adverse event reports (AERs) associated with bitter orange (*C. aurantium*)-containing products were reported. The cases and reports related to possible involvement of bitter orange-containing products with cardiovascular incidents and other adverse events. In all these reports, bitter orange extract and/or *p*-synephrine were implicated as the possible causative agent. However, in all reported AERs and case reports, the products involved were complex, containing as many as 12 herbal ingredients, were poly-alkaloidal and poly-protoalkaloidal, and included other ingredients with known cardiovascular effects as yohimbine, guarana, and caffeine. In no case was a direct link provided between bitter orange extract and the described event (Stohs, 2010; Stohs & Ray, 2019).

The published clinical adverse case reports included acute lateral-wall myocardial infarction (Nykamp et al., 2004), exercise induced syncope associated with QT prolongation (Nasir et al., 2004), ischemic stroke (Bouchard et al., 2005), variant angina (Gange et al., 2006), ischemic colitis (Sultan et al., 2006), rhabdomyolysis (Burke et al., 2007), coronary spasm and thrombosis (Smedema & Muller, 2008), vasospasm and stroke (Holmes & Tavee, 2008), ST segment-elevation myocardial infarction (Thomas et al., 2009), and ventricular fibrillation (Stephensen & Sarlay, 2009). Furthermore, in one case report it was suggested that a bitter orange-containing dietary supplement may have masked bradycardia and hypotension while exacerbating weight loss

in an individual with anorexia nervosa (Gray & Woolf, 2005), although no evidence was provided that an actual adverse event had occurred.

The products consumed in all cases were multi-ingredient. However, in each case reference was specifically made to *C. aurantium,* bitter orange or *p*-synephrine as the most likely causative agent. Critical reviews of these published case reports indicate a wide range of confounding factors including: heart murmur, pre-existing heart disease, hypertriglyceridemia, obesity, a history of smoking, gastroesophageal disease, physical inactivity, sickle cell trait, dehydration, pneumonia, possible use of anabolic steroids and/or performance enhancing drugs, high caffeine intake, and high alcohol consumption. These factors may have been responsible for the observed response/condition and/or made the subject more susceptible to the ingredients in the complex product in question. Furthermore, products were not always being taken as recommended, and it was not always clear if the subjects were using other unreported dietary supplements and/or drugs (Stohs, 2010; Stohs & Ray, 2019). A more probable culprit for at least some of these effects may have been the high caffeine intake associated with the products in question.

Firenzuoli et al. (2005) presented a case study in which the subject was reported to have consumed a product that contained 500 mg of a bitter orange extract (6% *p*-synephrine), and subsequently experienced tachycardia. A repeat cardiovascular experience occurred a month later after again consuming the dietary supplement. The authors did not indicate the composition of the dietary supplement and what other ingredients were present. No analysis was conducted on the product in an attempt to identify ingredients that may have been responsible. As a consequence, it is not clear whether the response was due to the bitter orange extract or some other ingredient or adulterant. It is also not clear whether this was a pharmacological/toxicological response or an allergic or hypersensitivity response to some constituent present in the product. Due to a lack of adequate information, it is not possible to conclude that the causative agent was the bitter orange extract and *p*-synephrine.

A case report was published involving a 52-year old woman that was consuming several weight management dietary supplements and experienced severe psychosis (Retamero et al., 2011). She admitted to consuming two products above the recommended doses. One “fat burner” product was reported to contain bitter orange extract and at least four ingredients that contained caffeine and related xanthines. The second calorie control product contained five ingredients with caffeine and related xanthines. Her urinary drug screen was positive for amphetamines which the authors concluded may have been due to synephrine, although a study has shown that synephrine does not result in a positive test for amphetamines, although false positives are not uncommon (Nguyen et al., 2006). The subject was also taking various medications at the time of admission to the emergency room. The authors did not consider the possibility that the subject may have been consuming amphetamines in addition to the dietary supplements or that the product may have been adulterated with an amphetamine, or that the medications she was taking may have interacted with the amphetamine and/or various supplements, resulting in the psychosis.

The subject had a history of anxiety, depression and hypothyroidism as well as abuse of various substances (Retamero et al., 2011). The authors did not report or had no knowledge of how much *p*-synephrine, caffeine or other ingredients were being consumed on a daily basis or whether

amphetamine or other drugs may have been involved. They did not review the current literature or discuss the fact that the subject may have been taking as much as 800 mg caffeine per day. However, they concluded that the subject was "susceptible to synephrine and caffeine adverse neuropsychiatric effects" (Retamero et al., 2011).

A case study was published which reported that apical ballooning syndrome occurred in a young woman who consumed a dietary supplement that contained *p*-synephrine (Chung et al., 2013). No evidence or information was provided substantiating the claim, other than reference to several other case studies. The authors did not review the current scientific literature. Furthermore, the authors did not indicate the names and composition of the products being taken, the amounts of the various ingredients, how many different dietary supplements were being taken, the amounts of the products being taken by the subject, and the conditions under which the supplements were being taken. No chemical analysis of the products in question was undertaken. No evidence directly linking the supplement use to the observed syndrome was provided. As a consequence, it is not possible to establish a cause and effect relationship.

Nykamp et al. (2004) reported a case of possible association of acute lateral-wall myocardial infarction (MI) and bitter orange supplement. This report was reviewed by Stohs (2010), and is typical of the case reports that were reviewed. The subject, a woman with a history of smoking tobacco, was reportedly inactive (did not exercise) and had a heart murmur – any or all of which could have contributed to her MI. For one year, she had been using a product called "Edita's Skinny Pill" containing 300 mg bitter orange. It was concluded, "the acute lateral-wall MI was possibly associated with bitter orange." The authors speculate that the patient's use of the bitter orange dietary supplement may have precipitated an MI based on her underlying but previously undetected cardiovascular disease. They employed the Naranjo probability scale to confirm the possible association with bitter orange. The amount of *p*-synephrine in the product was not reported but it may have contained 18 mg. In an extensive review, Blumenthal (2005) also summarized this report, as well as other available literature on adverse effects related to the intake of bitter orange extract and *p*-synephrine and the confusion arising from the available adverse events reports.

In another case report, Doctorian and Do (2017) published a case of ascending aortic dissection in a male who consumed two doses of a pre-workout supplement of unidentified composition which was reported to contain *p*-synephrine. The authors did not indicate how much *p*-synephrine was in the product. In actuality, the product contained 135 mg caffeine, 10 mg *p*-synephrine, 1.5 grams beta-alanine, *Mucuna pruriens* extract standardized for L-DOPA, and about a dozen other ingredients. The authors did not provide a review of current literature, cited other case reports and speculative review articles. It is impossible to show a cause and effect relationship between the ingredients in the product including *p*-synephrine and the ascending aortic dissection.

Another case report was published involving a case of ST-segment elevation myocardial infarction (STEMI) in a 22-year old male who consumed a sports performance product (Unnikrishnan et al., 2018). The authors indicate that the patient took three scoops of the product in question, but failed to note that the recommended dose is one scoop. The authors indicated that the product contained synephrine, caffeine, yohimbine, huperzine, *Mucuna pruriens*, ashwagandha extract, and other

ingredients. No information was provided on how much of these ingredients were in the product although the amount of caffeine is clearly stated on labels. The patient consumed between 600-750 mg of caffeine depending on the product, since several forms of the product exist. This amount of caffeine constitutes at least twice the amount known to cause cardiovascular effects (Ratamess et al., 2018), and the equivalent of 5-6 cups of coffee consumed at one time. The cardiovascular effects of yohimbine combined with caffeine are not known. Regardless, the case report cannot be attributed solely to synephrine, but is more likely to be associated with the combination of ingredients, including the levels of caffeine that are associated with adverse effects (alone).

In this case study, as in other case reports, the authors did not perform a thorough review of the literature. Instead, the authors cited another case study but did not reference any current literature. The authors stated that "the patient's condition could be attributed to his use of synephrine-containing weight loss and performance–enhancing supplements." The authors further cited an article involving a product that contained ephedrine. No link or direct association with *p*-synephrine was demonstrated.

Although these case reports raise the level of awareness with regard to the use of complex products, it is not possible to extrapolate the cause of these adverse effects to bitter orange extract or *p*-synephrine which may have been present in the products. "Due to the lack of detailed reporting, few numbers of subjects, and concomitant exposure to other products, causal relationships of *p*-synephrine exposure with cardiovascular effects cannot be ascertained" (Rossato et al., 2011). However, "the present knowledge of the pharmacology of *p*-synephrine, and the results of the most recent clinical trials, at least as present in the form of Advantra Z® (*i.e.*, ~50% as *p*-synephrine), is inconsistent with it having a causal role in the case reports of cardiovascular incidents" (Lynch, 2018).

No evidence showing a direct link between bitter orange extract and the adverse events is provided by any of the case studies. In some cases, the actual composition of the product was not completely identified. Per the product labels, the products all contained multiple alkaloid and polyalkaloidal ingredients, including several with known cardiovascular effects. In only one case was a challenge test conducted to determine if the identified product was in fact associated with the described adverse event. However, the responsible ingredient(s) was not determined. In no case were any of the products analyzed to determine the actual composition of the products or the amount of *p*-synephrine present, if at all. In no case were a wide range of other ingredients present with potential cardiovascular effects considered by the authors (Stohs & Ray, 2019).

In summary, among the available published case studies and FDA AERs, no reports conclusively demonstrating an adverse reaction to bitter orange extract or *p*-synephrine have been published. Given the structural similarity between ephedrine and synephrine, there is confusion over adverse events associated with ephedrine. Some of the adverse events associated with ephedra/ephedrine have been also claimed to be associated with bitter orange because of mere presence of bitter orange on the label in these products. Finally, it should be noted that the available "case reports are incomplete, uncontrolled, retrospective, lack operational criteria for identifying when an adverse event has actually occurred, and resemble nothing so much as hearsay evidence, a type of evidence that is prohibited in all courts of industrialized societies." (Karch, 2007).

3.2.13 Mechanistic Studies

As previously noted, the mechanisms of action of *p*-synephrine, ephedrine, *p*-octopamine and *m*-synephrine (phenylephrine) have been reviewed and contrasted (Stohs et al., 2020). This discussion will primarily focus on the actions of *p*-synephrine, relative to ephedrine and *m*-synephrine.

Cardiovascular effects of ligands are associated with direct adrenergic receptor binding and/or through indirect effects as the release of norepinephrine and epinephrine. In general, vasoconstriction occurs when ligands bind to α-adrenergic receptors, while binding to β-1 adrenergic receptors result in myocardial contractility and increased heart rate. Ligand binding to β-2 adrenergic receptors is associated with bronchodilation (Inchiosa, 2011). β-3 adrenoreceptors are located in white and brown adipose tissues and muscles as well as other tissues, and their activation results in various metabolic effects such as increases in lipolysis, and improvements in insulin resistance, glycemic control and lipid profiles (Coman et al., 2009).

Brown et al. (1988) observed that [R-(-)] stereoisomer (l-form) of *p*-synephrine was approximately 1000-fold less active in binding to rat aorta α-1 adrenergic receptors and α-2 adrenergic receptors from rabbit saphenous vein than norepinephrine. *m*-Synephrine (phenylephrine) binding was 150- and 6-fold less, respectively, to these two receptors than norepinephrine. The [S-(+)] stereoisomer (*d*-forms) of *p*-synephrine exhibited over 100-fold lower binding active than the [R-(-)] stereoisomer (*l*-forms) to α-1 and α-2 adrenergic receptors.

Ma et al. (2010) concluded that *p*-synephrine acts as an antagonist rather than an agonist with respect to human α-2a- and α-2c adrenergic receptors. Furthermore, *p*-synephrine was approximately 50-fold less potent in activating human α-1a adrenergic receptors. Several studies have concluded that the hydroxyl group in the para position of the ring as occurs in *p*-synephrine decreases adrenergic receptor binding and the subsequent cardiovascular effects (Mukherjee et al., 1976; Ma et al., 2010). Jordan et al. (1987) concluded that *p*-synephrine bound to the β-1 and β-2 adrenergic receptor about 10,000-fold or less actively than norepinephrine in guinea pig atria and trachea.

Carpene' et al. (1999) examined the lipolytic activity of a number of potential β-3 adrenergic receptor agonists including *p*-synephrine, *p*-octopamine and noradrenaline (norepinephrine) in white fat cells from hamsters, rats, dogs, humans and guinea pigs. *p*-Synephrine was partially active in stimulating lipolysis in all species while tyramine, dopamine, and *β*-phenylethylamine exhibited no activity. This study demonstrated marked differences in adrenergic receptor binding among the various biogenic amines that were assessed, with binding to rodent adrenergic receptors being much greater than adrenergic receptors from humans.

In a subsequent study, the lipolytic activity of *p*-synephrine, *p*-octopamine, tyramine and *N*-methyltyramine were compared in rat and human adipocytes based on β-3 adrenergic receptor binding (Mercader et al., 2011). In rat fat cells, at a concentration of 10 µg/mL both *p*-synephrine exhibited approximately 60 % of the lipolytic activity of 1 nM/mL of isoprenaline while tyramine

and N-methyltyramine exhibited no effect or were weakly antagonistic. In human adipocytes, 10 µg/mL of *p*-synephrine exhibited approximately 10 % of the lipolytic activity of 1 µM/mL of isoprenaline. Various studies indicate that *N*-methyltyramine acts as an α-adrenergic receptor antagonist while promoting appetite and inhibiting lipolysis, effects counter to those of ephedrine, *p*-synephrine and *p*-octopamine (Stohs and Hartman, 2015).

An extension of previous studies affirmed that the adrenergic receptor binding of *p*-synephrine in rodents was at least 10-fold greater than in humans while tyramine and *N*-methyltyramine exhibited no binding activity (Carpene' et al., 2014). These results support previous observations that effects produced in rodents at specific doses cannot be directly extrapolated to humans (Mercader et al., 2011). In this study, high concentrations *p*-synephrine were shown to activate glucose transport in human fat cells.

Several studies have examined the effects of *p*-synephrine on carbohydrate metabolism in perfused rat liver (Peixoto et al. 2012; de Oliveira et al., 2014). *p*-Synephrine increased glycogenolysis, glycolysis, oxygen uptake, glucose output and perfusion pressure. These effects were shown to be at least in part mediated by α- and β-adrenergic signaling, while requiring the simultaneous participation of both cAMP and Ca^{2+} (de Oliveira et al., 2014). The authors concluded that most of the actions *p*-synephrine were catabolic.

Neuromedin U2 receptor (NMUR2) is present in the hypothalamic regions of the brain and is involved in the regulation of energy balance, food intake, nociception and stress (Zheng et al., 2014). As was demonstrated in NMUR2 negative and short hairpin RNA knockdown HEK293 cell lines, *p*-synephrine binds to this receptor with high efficacy and potency. The ability of *p*-synephrine to suppress appetite and enhance eating control has been affirmed in humans (Kaats et al., 2017) and animals (Arbo et al., 2009). How well *p*-synephrine can cross the blood brain barrier to achieve functional concentrations and bind to NMUR2 has not been specifically determined.

In an *in vitro* study, the effect of *p*-synephrine on glucose consumption and its mechanism of action were determined in L6 skeletal muscle cells in culture (Hong et al., 2012). *p*-Synephrine dose-dependently increased basal glucose consumption by over 50 % relative to controls, and had no effect on cell viability. The increased glucose consumption by *p*-synephrine involved Glut4-dependent glucose uptake that in turn was dependent upon *p*-synephrine stimulation of AMP-activated protein kinase (AMPK) phosphorylation.

The effects of *p*-synephrine on lipid accumulation and glucose production have been assessed in H411E rat liver cells (Cui et al., 2014). *p*-Synephrine dose-dependently decreased glucose production, and α- and β-adrenergic receptor antagonists did not alter this effect. These results indicated that the effects of *p*-synephrine on gluconeogenesis did not require involvement of adrenergic receptors.

Several studies have demonstrated the anti-inflammatory activity of *p*-synephrine. *p*-Synephrine suppressed lipopolysaccharide (LPS)-induced acute lung injury in mice by reducing the number of inflammatory cells in the lungs, decreasing the levels of reactive species, enhancing superoxide dismutase activity, decreasing tumor necrosis alpha (TNF-α) and interleukin-6 (IL-6), and

increasing IL-10 (Wu et al., 2014). In normal human fibroblasts and NIH/3T3 mouse fibroblasts in culture, *p*-synephrine inhibited IL-4-induced eotaxin-1 expression through the inhibition of signal transducer and activator of transcription (STAT6) phosphorylation which acts as a signal transducer immediately downstream from IL-4 (Roh et al., 2014).

Eotaxin-1 is a potent chemoattractant and mediator for eosinophils which are associated with inflammation. STAT6 is critical in activating cytokine gene expression and cytokine signaling in immune and target tissue cells. *p*-Synephrine also inhibited eosinophil recruitment induced by eotaxin-1 overexpression. *m*-Synephrine had little effect on eotaxin-1 induction and therefore little anti-inflammatory activity. These results indicated that *p*-synephrine exerts anti-inflammatory effects at least in part by inhibiting eotaxin-1 expression (Roh et al., 2014). Arbo et al. (2009) reported that in mouse livers *p*-synephrine exhibited antioxidant and tissue protective activities by enhancing reduced glutathione content, decreasing glutathione peroxidase activity and increasing catalase activity.

Ephedrine exhibits multiple mechanisms of action consisting of an indirect effect which involves the release of norepinephrine and epinephrine as well as a direct effect on adrenergic receptors to produce cardiovascular effects (Haller and Benowitz, 2000; Inchiosa, 2011; Mund & Frishman, 2013). Through the indirect effect of ephedrine, norepinephrine and epinephrine act on α-1, β-1, and β-2 adrenergic receptors to produce cardiovascular affects, while interacting with β-3 adrenergic receptors to promote thermogenesis (Mund & Frishman, 2013).

The immunochemical identification of β-3 adrenergic receptors in various tissues of obese human subjects treated with ephedrine was determined by De Matteis et al. (2002). Ephedrine administration increased the expression of β-3 adrenergic receptors in obese subjects, with the detection of these receptors in adipocytes and ventricular myocardium as well as smooth muscle of the gall bladder, colon, ileum and prostate. The authors also concluded that the "expression in ventricular myocardium is consistent with the evidence that the β-3 adrenergic receptor mediates a negative inotropic effect on this tissue". These results are consistent with the well-known ability of the ability of ephedrine to suppress appetite and facilitate weight management and weight loss

A group of G protein-coupled receptors known as trace amine-associated receptors (TAAR) have been identified in recent years in various human and animal tissues, and serve as neuromodulators ((see for example Khan & Nawaz, 2016;). Because they are present in much smaller amounts than the predominant neurotransmitters, the amines which interact with these receptors are referred to as "trace amines".

The TAARs constitute another mechanism whereby *p*-synephrine may exert various physiological and pharmacological effects either by acting as neurotransmitter precursors or neuromodulators, and serve as biomarkers. For example, the circulating levels of *p*-synephrine are increased in Parkinson's disease patients while norepinephrine levels are decreased as compared to normal healthy individuals (D'Andrea et al., 2019). However, no significant neurological effects have been demonstrated by the oral ingestion of *p*-synephrine at commonly consumed doses.

The above described *in vitro* studies demonstrate that *p*-synephrine exhibits effects involving a variety of mechanisms in addition to selective binding to some adrenergic receptors with limited involvement of α- and β-1 and β-2 adrenergic receptors. These mechanistic observations provide the rationale and understanding for the lack of cardiovascular effects and why effects of ephedrine and *m*-synephrine cannot be extrapolated to *p*-synephrine.

3.2.14 Other relevant studies for safety assessment

As previously noted, bitter orange extracts standardized to 50% *p*-synephrine may contain small amounts of the minor protoalkaloids *p*-octopamine, N-methyltyramine, tyramine and hordenine in amounts of approximately 0-1%, 2-4%, 0-1% and 0-1%, respectively, of the total protoalkaloidal content (Stohs, 2015; see Certificates of Analysis in Appendix 6.2). The sum of these minor alkaloids represents typically 2 % or less of the total protoalkaloidal content of standardized extracts.

p-Octopamine exhibits adrenergic receptor binding characteristics similar to *p*-synephrine, with very poor binding to α-1, α-2, β-1 and β-2 while exhibiting greater binding to β-3 adrenergic receptors (Brown et al., 1988; Carpéné et al., 1999; Stohs, 2015). As a consequence, the effects of *p*-octopamine are similar to *p*-synephrine, and Health Canada has recommended identical dosing considerations (Marles, 2011). Because *p*-octopamine is present is either absent or present in very small amounts, its contribution to the effects of standardized bitter orange extracts is negligible.

The presence of N-methyltyramine and hordenine in germinated barley is well known, and they have been shown to occur in various beers in the ranges of 0.6-4.6 and 1.0-6.3 mg/L, respectively (Sommer et al., 2019). Therefore, consumption of one or two common 12 oz beers can result in the ingestion of greater amounts of N-methyltyramine than in a typical dose of a standardized extract of bitter orange. Hordenine showed no changes in heart rate, respiratory rate, body temperature or behavior when given orally at a dose of 2 mg/kg to horses (a dose of 1000 mg for an average 500 kg horse). Therefore, no effect would be projected in a human that consumed 0.5 mg or less of hordenine from a typical dose of a standardized bitter orange extract, an amount that is less than present in an average beer.

N-Methyltyramine is rapidly absorbed and undergoes N-demethylation to tyramine followed by rapid oxidative deamination. N-Methyltyramine and tyramine are both weak adrenergic antagonists (inhibitors) with respect to fat metabolism and as compared to *p*-synephrine and *p*-octopamine (Mercader et al., 2011). Tyramine has an LD_{50} in rats greater than 2000 mg/kg, indicating a very high degree of safety (Til et al., 1997). Because the antagonistic effects of N-methyltyramine and tyramine are weak, no adverse effects are observed or predicted, although in the presence of much higher amounts of either substance the antagonism and inhibition of the effects of *p*-synephrine might occur.

Finally, the lack of effects due to the minor protoalkaloids present in standardized bitter orange extract is borne out by the facts that they were present in the extract when $LD_{50,}$ dose ranging finding and 90-day safety studies demonstrated no adverse effects (Deshmukh et al., 2017a, 2017b). Furthermore, these minor protoalkaloids have been present in approximately 20 human studies conducted with Advantra Z®, the standardized bitter orange extract, without the production

of cardiovascular or other adverse effects (Stohs, 2017; Stohs et al., 2019: 2020). Thus, it can be concluded that they do not significantly contribute to the overall effects of the bitter orange extract.

Bitter orange extracts standardized to 50% *p*-synephrine also contain 0.5-1.0% hesperidin and other bioflavonoids. Thus, 100 mg of the extract would contain 1 mg or less of bioflavonoids. Acute and chronic toxicity studies indicate that the LD_{50} for hesperidin exceeds 4800 mg/kg while the low-observed-adverse-event-level (LOAEL) was 1000 mg/kg in both male and female Sprague-Dawley rats (Li et al., 2019). These results demonstrate a very good safety profile for hesperidin, and the small amount present in standardized bitter orange extract would not be expected to exert adverse effects at this concentration. In addition, hesperidin is a well-known antioxidant and anti-inflammatory with various health benefits, and the presence of small amounts of hesperidin may contribute to the antioxidant and tissue-protective effects that have been observed in animal and *in vitro* studies with bitter orange extracts (Arbo et al., 2009; Stohs, 2017).

4. Basis for Concluding That the New Dietary Ingredient Will Reasonably Be Expected to be Safe for Use in the Dietary Supplement

Human and animal studies as well as *in vitro* studies have been conducted on Advantra Z®, other *Citrus aurantium* (bitter orange) extracts, and its primary active constituent *p*-synephrine. A high degree of safety has been demonstrated, and no significant adverse events have been directly attributed to bitter orange extract or *p*-synephrine. Key points are summarized below.

- Studies in rats have shown that the LD50 of a *Citrus aurantium* extract containing 50% *p*-synephrine [Advantra Z®] is greater than 5000 mg/kg. The no-observed-adverse-effect-level [NOAEL] in a subchronic 90-day study in rats was 1000 mg/kg for a *Citrus aurantium* extract containing 50% *p*-synephrine [Advantra Z®].

- A 90-day subchronic toxicity study in rats at a dose of up to and including 1000 mg/kg *Citrus aurantium* extract containing 50% *p*-synephrine [Advantra Z®] did not produce cardiovascular effects based on the lack of significant effects on the following parameters: neurological/functional observations; clinical hematology; clinical biochemistry including AST, sodium, calcium, potassium, total cholesterol, creatinine, BUN, and triglycerides; urinalysis; organ weights including heart weights; gross and microscopic pathology of the heart and other organs, and general and detailed clinical examinations.

- Studies conducted in rats at NCTR in conjunction with the FDA have shown that "Animals [rats] treated with 95 % *p*-synephrine showed minimal effects on heart rate and blood pressure".

- Studies in mice have shown that doses of up to 300 mg/kg did not increase heart rate or blood pressure.

- *p*-Synephrine does not act as a cardiovascular stimulant in humans at commonly used doses (25-50 mg) because it exhibits little or no α-1, α-2, β-1 or β-2 adrenergic receptor agonist either directly or indirectly.

- Cardiovascular effects observed in rodents cannot be directly extrapolated to humans because adrenergic receptors in rodents bind at least 10 time more readily to *p*-synephrine than adrenergic receptors from humans.

- *p*-Synephrine and *Citrus aurantium* (bitter orange) extract are non-mutagenic, non-teratogenic and non-cytotoxic.

- Over 30 peer reviewed studies involving over 700 human subjects have demonstrated the safety and efficacy of bitter orange extract and *p-synephrine* at commonly used doses (25-50 mg). No adverse effects have been reported.

- As a single one-time dose, up to 100 mg *p*-synephrine is unlikely to be associated with adverse effects in healthy populations. No adverse effects have been seen or reported in human subjects at this dose.

- Extensive *in vitro* studies have demonstrated that *p*-synephrine functions as a metabolic enhancer without cardiovascular stimulant effects.

- *p*-Synephrine exerts a number of beneficial effects through various mechanisms not involving adrenergic receptors and that are not associated with adverse events.

- Bitter orange and products containing *p*-synephrine have been consumed for over 1000 years without adverse effects.

- Millions of doses of bitter orange extracts containing *p*-synephrine have been consumed over the past 20 years without any adverse effects being directly attributed.

- At least six major review articles published in the past seven years have concluded that bitter orange extract and its primary active constituent *p*-synephrine at commonly used doses are reasonably expected to be safe and free of adverse effects, including cardiovascular and neurological effects, when used as a dietary supplement.

4.1 Determination of the No-Observed-Adverse-Effect-Level (NOAEL) or Lowest-Observed-Adverse-Effect-Level (LOAEL)

The NOAEL in a 90-day study in rats was determined to be greater than 1000 mg/kg body weight for bitter orange extract containing 50% *p*-synephrine (Advantra Z®). Therefore, the NOAEL for *p*-synephrine is greater than 500 mg/kg. The NOEL for Advantra Z® was determined to be 300 mg/kg bw/day in the same study (meaning the NOEL for *p*-synephrine would be 150 mg/kg bw/day). The NOEL in a 14-day study was determined to be 500 mg/kg. The determination of these values was in accordance with FDA Guidance 2016.

4.2 Determination of the Estimated Daily Intake (EDI)

The dosage recommendation for *p*-synephrine (Advantra Z® containing 50% *p*-synephrine) is 25 mg per day for dietary supplements. FDA assumes an average of 70 kg body weight for an adult (FDA 2016, page 94), and therefore the EDI for Advantra Z® containing 25 mg *p*-synephrine would be:

p-synephrine EDI = 25 mg/day ÷ 70 kg = 0.3571 mg/kg bw/day.

As the dietary supplement containing Advantra Z® is intended for intermittent use (FDA 2016, page 70), namely, the duration of use not to exceed 30 days with a 15 day gap or 60 days with a 30 day gap before again using the product, the adjusted *p*-synephrine EDI from the usage of 8 months out of 12 months per year for 50 mg of the extract that contains 25 mg *p*-synephrine would be:

Adjusted *p*-synephrine EDI = 25 mg/day ÷ 70 kg bw x 8 months/12 months = **0.2380 mg/kg bw/day**

4.3 Determination of the margin of safety (MOS)

Per FDA 2016 (pages 92, 93), the margin of safety (MOS) for a dietary ingredient is calculated by dividing the NOAEL in animal or human studies by the EDI of the dietary ingredient. So a margin of safety of 100-fold means the doses shown to be without adverse effects in animals or humans are 100 times greater than the levels that would be consumed from the use of the dietary supplement.

The *p*-synephrine NOAEL is 500 mg/kg bw/day and NOEL is 150 mg/kg bw/day based on the 90-day rat safety/toxicity study. The margin of safety between the NOAEL and the adjusted EDI for *p*-synephrine (Advantra Z® containing 50% *p*-synephrine) is:

***p*-Synephrine MOS** = 500 mg/kg bw/day ÷ 0.2380 mg/kg bw/day = **2100**

The margin of safety between the NOEL and the adjusted EDI for *p*-synephrine (Advantra Z® containing 50% *p*-synephrine) is:

150 mg/kg bw/day ÷ 0.2380 mg/kg bw/day = **630**

4.4 Safety narrative and conclusion

The margin of safety between the NOAEL and the adjusted EDI for *p*-synephrine is **2100,** and **630** between the NOEL and the adjusted EDI for *p*-synephrine. Importantly, the dietary supplement containing *p*-synephrine (Advantra Z®) is intended for intermittent use and bitter orange extract has a documented history of safe daily chronic use. These margins of safety are adequate to conclude that *p*-synephrine and bitter orange extract (Advantra Z®) are reasonably expected to be safe under its intended conditions of use in dietary supplements.

The above estimates are very conservative because it assumes that a person consumes dietary supplement containing Advantra Z® at the maximum recommended daily dose of 50 mg (25 mg of *p*-synephrine) at once. In reality, the person may consume less than the maximum recommended daily dose, and manufacturers of finished dietary supplements may choose to deliver this amount in a single serving or divide this total into correspondingly-reduced serving amounts taken twice a day (spaced out by 6 to 8 hours) or three times per day (spaced out by 4 to 6 hours). As previously discussed, the half-life of *p*-synephrine has been shown to be between 2-3 hours (Hengstmann & Aulepp, 1978; Haller e al., 2005; 2008; Shara et al., 2016) thus, substantially decreasing blood levels.

In addition, the duration of use of the dietary supplement is not to exceed 30 days with a 15-day gap or 60 days with a 30-day gap before again using the product. During the gap, metabolism is normalized, and overall exposure to the product is decreased.

In conclusion, given the conservatism of the estimation and the use pattern of the dietary supplement, the margins of safety are adequate to conclude that *p*-synephrine and Advantra Z® (standardized bitter orange extract) are reasonably expected to be safe under its intended conditions of use in dietary supplements.

4.5 Alternative basis for reasonable expectation of safety

N/A

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6. Comments – Appendices (All Confidential Commercial Information)

6.1. HPLC, TLC and IR Analytical Spectra of 50 % *p*-Synephrine-containing Bitter Orange Extracts

6.2. Assorted Analytical Data Sheets for 50 % *p*-Synephrine-containing Bitter Orange Extracts from Intertek and Eurofins Independent Analytical Laboratories

6.3. Stability Study Analytical Data Sheets from Three Lots of 50 % *p*-Synephrine-containing Bitter Orange Extracts

6.4. *Citrus aurantium* 50 %-Acute Oral toxicity in Rat Final Report, May 13, 2017

6.5. Repeated Dose (14-Day) Oral Toxicity Study of Advantra Z® 50 % in Rats Final Report, September 6, 2017

6.6. *Citrus aurantiun* 50 % *p*-Synephrine (Advantra Z®) Repeated Dose (90-Day) Oral Toxicity Study in Rat Final Report, September 6, 2017

6.7. *Citrus aurantium* 50 % *p*-synephrine *Salmonella typhimurium* Reverse Mutation Assay (Ames Test) Final Report, April 4, 2017