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September 24, 2019

Via Email (Fred.Hines@fda.hhs.gov and Steven.Casper@fda.hhs.gov)

Fred Hines, D.V.M. (Consumer Safety Officer)
Steven Casper, Ph.D. (Evaluation and Research Staff)

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: Amendment to New Dietary Ingredient Notification (NDIN) #1123 for Advantra Z® Bitter Orange (*Citrus aurantium* L.) Extract (Innophos, LLC)

Dear Drs. Hines and Casper:

On behalf of Innophos, LLC, we are submitting this amendment to Innophos' NDIN #1123 for Advantra Z® Bitter Orange (*Citrus aurantium* L.) Extract (dated August 5, 2019). In email messages dated September 19 - 20, 2019, Dr. Casper requested that Innophos provide the information requested in the two sections presented below.

I. Clarify Recommended Dosing and Maximum Daily Ingestion of Advantra Z®

In Section 1.1 of the NDIN (and with similar wording in Section 3, item 5a of the NDIN cover sheet), Innophos described the intended dosing regimen for Advantra Z® as follows: "A typical dose of *p*-synephrine is 50 mg (100 mg Advantra Z®). Doses should be spaced out by 6 to 8 hours for twice daily dosing and 4 to 6 hours for thrice daily dosing." Innophos' intention is for a maximum of 100 mg Advantra Z® (50 mg *p*-synephrine) to be ingested per day. This is consistent with the statement in Section 4.2 of the NDIN that "the dosage recommendation for Advantra Z® containing 50% p-synephrine is 100 mg per day for dietary supplements."

Innophos expects that companies making finished dietary supplements incorporating Advantra Z® as a dietary ingredient may wish to divide the 100 mg (50 mg) total into separate

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doses taken two, or possibly three, times per day. In that case, the amount of Advantra Z® per dose would be adjusted so that no more than 100 mg (50 mg) are consumed per day.

To clarify this intent, Innophos amends the discussion in Section 1.1 of the NDIN to read as follows: **"The maximum recommended daily dose of Advantra Z® is 100 mg (50 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)."**

Innophos believes this amended discussion clarifies the dosing recommendation and confirms that the maximum daily ingestion of Advantra Z® is intended to be 100 mg (50 mg of *p*-synephrine).

II. Copies of Original Study Reports for Four Safety Studies Cited in the NDIN

In connection with (b) (4) non-clinical studies discussed in the NDIN, Innophos noted that a study report with raw data was available from(b) (4)

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Innophos, LLC appreciates FDA's attention to this amendment and looks forward to a favorable response to the NDIN. Please let us know if you have any questions or need further information.

Sincerely yours,

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Attachments (b) (4) non-clinical study reports

View Notification

NDI Number	Filing Date	Category of Compound
2019001123	08/05/2019	Botanical (including algae and fungi)

Section 1: Contact Information

Contacts List

Type	Name	Address
Submitter	(b) (4)	
Owner	Innophos, L.L.C.	259 Prospect Plains Rd Bldg A, Cranbury, NJ 08512-3706 United States
Primary	(b) (4)	ted

Submitter

Type of Submitter
Agent / Attorney / Consultant

Company Name *(if applicable)*
(b) (4)

Owner

Type of Submitter
Manufacturer of NDI

Company Name *(if applicable)*
Innophos, L.L.C.

Corrected Company Name

Mailing Address Line 1
259 Prospect Plains Rd Bldg A

Mailing Address Line 2

Country
United States

Z P or Postal Code
08512-3706

City
Cranbury

State or Province
NJ

Primary

Type of Contact
Agent / Attorney / Consultant

First Name of Contact Person
(b) (4)

Mailing Address
(b) (4)

Section 2: General Administrative Information

1. Name of the New Dietary Ingredient
Advantra Z® - Bitter Orange (Citrus aurantium L.) Extract

2. Have you designated information in your notification that you view as a trade secret or as confidential commercial information?
Yes, information is designated at the place where it occurs in the notification

3. Are you providing a redacted copy of some or all of the notification?
Yes, redacted copy of complete notification

4. Are all citations to published information accompanied by reprints or full photostatic copies of the publications?
Yes

5. Are the notification and all publications submitted in English or accompanied by a complete and accurate English translation?
Yes

Section 3: Description of NDI and Dietary Supplement Containing the NDI

1. New Dietary Ingredient Type
Herb or other botanical

Concentrate, metabolite, constituent, extract, or combination of any ingredient described above

2. Name of the new dietary ingredient and related information
Maximum level of new dietary ingredient in each serving of dietary supplement (include units)

100 mg/day

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

Corrected NDI Name

Synonyms and Trade Name
Advantra Z®

Corrected Synonyms and Trade Name

Latin Binomial Name (LBN)
Citrus aurantium L.

Correcetd Latin Binomial Name (LBN)

Author of LBN
N/A

Corrected Author of LBN

Plant Part and Strain
Immature fruits of bitter orange plant

3. Dietary supplement serving form
Tablet

Capsule

Powder

Softgel

Liquid

Gelcap

Sachet

Other - Bulk ingredient supplier

4. Description of dietary supplement (Include the level of NDI and all other ingredients in one unit of the dietary supplement. If the notification concerns an NDI that is a combination of two or more other NDIs, you should provide the following information for each component NDI: Synonyms, Trade Name, Plant Part, Strain, Latin Binomial Name, Author of Latin Binomial Name, and NDI type. Where relevant, also include the following additional information: CAS registry number, Unusual form (e.g., malted barley or immature apples), Type of manufacture (e.g., greater than 99% purity, 50:1 dry leaf extract, or fermentation product)).
Advantra Z® is a standardized bitter orange extract obtained from (b) (4)

5. Conditions of Use of the Dietary Supplement

5a. Serving instructions (e.g., "take with food", "take before bed", "dissolve in a glass of water", etc).
Innophos intends for Advantra Z® to be used in dietary supplements under the following conditions Avoid using the dietary supplement and/or consult with a physician if have a medical condition, or taking prescription medications (b) (4) Doses should be spaced out by 6 to 8 hours for twice daily dosing and 4 to 6 hours for thrice daily dosing Duration of use not to exceed two months with a one-month gap before again using the product.

5b. Dietary supplement serving size (weight or volumetric measure), serving frequency (# of servings/day, interval between servings), duration of use and maximum total daily intake level
A typical dose of Advantra Z® is 100 mg (b) (4)). Doses should be spaced out by 6 to 8 hours for twice daily dosing and 4 to 6 hours for thrice daily dosing. Duration of use not to exceed two months with a one-month gap before again using the product.

5c. Target populations / excluded populations / other restrictions
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 have a medical condition, or taking prescription medications.

Section 4: Safety Information Attachment

Attachment

Name of Attachment
NDIN Advantra Z Bitter Orange Extract 08-05-19.pdf (fileDownload.htm?module=NDI&fileIdEnc=L3UwNC9mdXJsc1VwbG9hZC8vNjI3NTg1ZmItODZmOC00YjY5LThkNGUtNjJiZTI2M2M4NjViL05ESU4gQWR2YW50cmEgW 4126-497b-8994-e53d23ebc477)

Section 5: Additional Attachments

Attachment(s)

Name of Attachment
047-Kaats GR Miller H 2013 60 day double blind.pdf (fileDownload.htm?module=NDI&fileIdEnc=L3UwNC9mdXJsc1VwbG9hZC8vNjI3NTg1ZmItODZmOC00YjY5LThkNGUtNjJiZTI2M2M4NjViLzA0Ny1LYWF0cyBHUiBNaWk
105-USDA 2019.pdf (fileDownload.htm?module=NDI&fileIdEnc=L3UwNC9mdXJsc1VwbG9hZC8vNjI3NTg1ZmItODZmOC00YjY5LThkNGUtNjJiZTI2I
020-Coman OA Palinescu H 2009 beta 3 adrenergic receptors.pdf (fileDownload.htm?module=NDI&fileIdEnc=L3UwNC9mdXJsc1VwbG9hZC8vNjI3NTg1ZmItODZmOC00YjY5LThkNGUtNjJiZTI2M2M4NjViLzAyMC1Db21hbiBPQSBQYW 7a9170729b8a)
015-Carpene MA Tester X 2014 High doses Synephrine.pdf (fileDownload.htm?module=NDI&fileIdEnc=L3UwNC9mdXJsc1VwbG9hZC8vNjI3NTg1ZmItODZmOC00YjY5LThkNGUtNjJiZTI2M2M4NjViLzAxNS1DYXJwZW5lIE1BIFRI
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051-Li P Zeng SL 2016 comparison chromatographic.pdf (fileDownload.htm?module=NDI&fileIdEnc=L3UwNC9mdXJsc1VwbG9hZC8vNjI3NTg1ZmItODZmOC00YjY5LThkNGUtNjJiZTI2M2M4NjViLzA1MS1MaSBQIFplbmcgU0w
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024-Doctorian T Do B 2017 aortic dissection.pdf (fileDownload.htm?module=NDI&fileIdEnc=L3UwNC9mdXJsc1VwbG9hZC8vNjI3NTg1ZmItODZmOC00YjY5LThkNGUtNjJiZTI2M2M4NjViLzAyNC1Eb2N0b3JpYW4gVCI

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091-Stohs SJ Preuss HG 2011a primary protoalkaloid.pdf (fileDownload.htm?module=NDI&fileIdEnc=L3UwNC9mdXJsc1VwbG9hZC8vNjI3NTg1ZmItODZmOC00YjY5LThkNGUtNjJiZTI2M2M4NjViLzA5MS1TdG9ocyBTSiBQcm\65ab76d8bcb4)
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037-Haller CA Duan M 2008 human pharmacology.pdf (fileDownload.htm?module=NDI&fileIdEnc=L3UwNC9mdXJsc1VwbG9hZC8vNjI3NTg1ZmItODZmOC00YjY5LThkNGUtNjJiZTI2M2M4NjViLzAzNy1fYWxsZXlqQ0EqRHVI
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018-Chung H Kwon SW 2013 apical ballooning.pdf (fileDownload.htm?module=NDI&fileIdEnc=L3UwNC9mdXJsc1VwbG9hZC8vNjI3NTg1ZmItODZmOC00YjY5LThkNGUtNjJiZTI2M2M4NjViLzAxOC1DaHVuZyBIIEt3b24g

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Section 6: Certification

Name of Submitter
(b) (4)

Title of Submitter
Agent

I certify that the information in the notification is true and accurate and that I am authorized to submit the notification on behalf of the notification owner.

☑ I Agree.

(b) (4)

(b) (4)

August 5, 2019

Via FDA Industry Systems Electronic Submission Portal

Fred Hines
Consumer Safety Officer
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. Hines:

On behalf of Innophos, LLC, pursuant to Section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act, we are submitting, via the FDA Industry Systems electronic portal, a New Dietary Ingredient Notification (NDIN) for Advantra Z®, a standardized bitter orange (*Citrus aurantium* L) extract.

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

(b) (4)

Please let me know if you have any questions.

Sincerely yours,

(b) (4)

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:

(b) (4)

August 5, 2019

Table of Contents

List of Tables and Figures

(b) (4)

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 (b) (4)

(b) (4)

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 have a medical condition, or taking prescription medications.
- A typical dose of *p*-synephrine is 50 mg (100 mg Advantra Z®).
- Doses should be spaced out by 6 to 8 hours for twice daily dosing and 4 to 6 hours for thrice daily dosing.
- Duration of use not to exceed two months with a one-month gap before again using the product.

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.

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

(b) (4)

(b) (4)

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)**

(b) (4)

(b) (4)

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

(b) (4)

1.3.4 NDI specifications (Confidential Commercial Information)

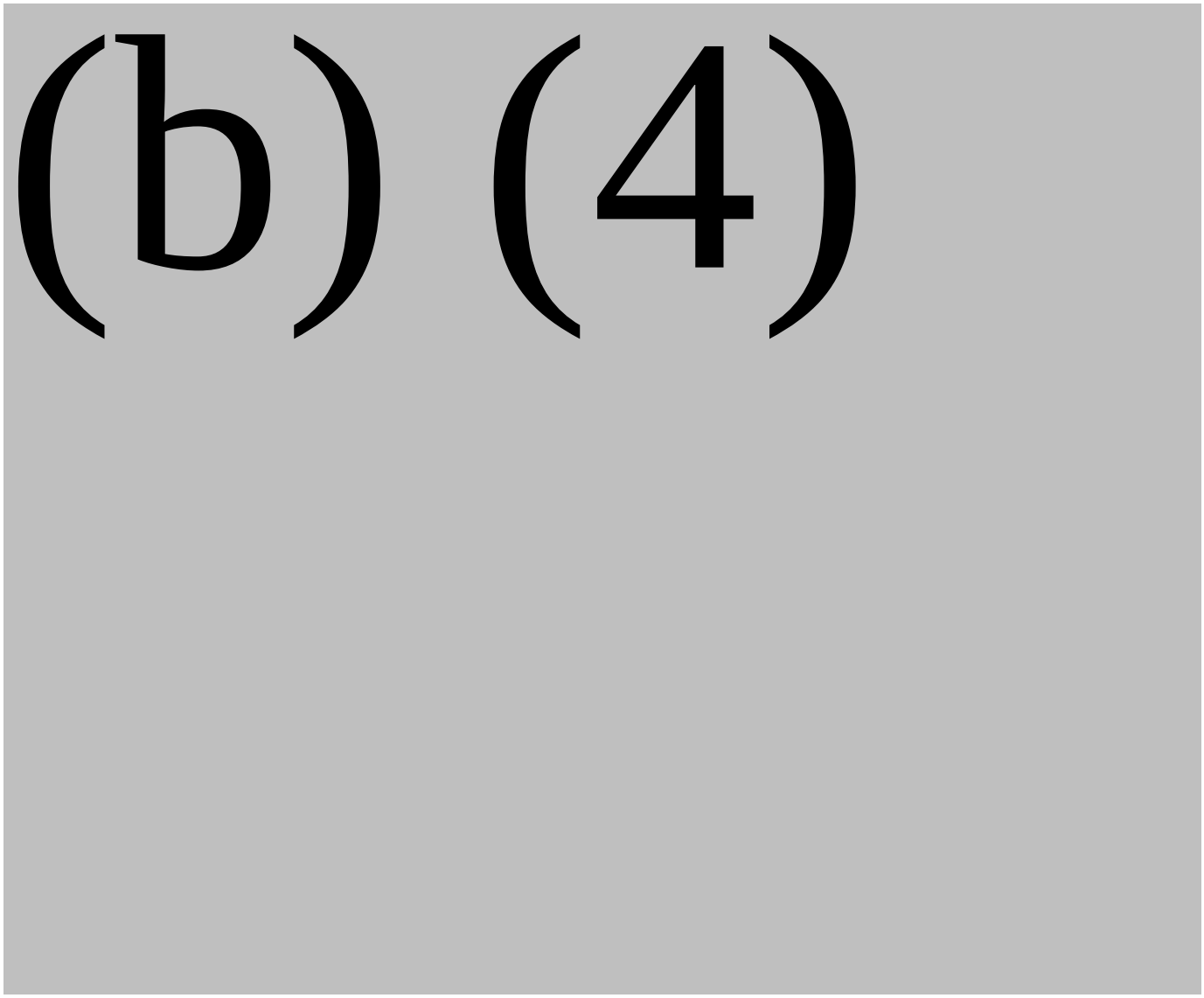
(b) (4)

(b) (4)

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

(b) (4)

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



1.3.5 Methods of analysis

The specification methods of analysis include (b) (4)

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

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.

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

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 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 orange also is 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 summary, the available information demonstrates that human beings are regularly exposed to bitter orange preparations and consequently its active constituent, *p*-synephrine. Bitter orange is widely used in the preparation of marmalade that is commonly consumed as a food, primarily as a bread spread. The juice derived from bitter orange is also used in some food preparations as a flavoring. The presence of *p*-synephrine has been reported in several other varieties of citrus fruits, thus indicating that human beings are routinely exposed to *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). The American Herbal Products Association (AHPA) has on file historical records regarding bitter orange availability as a wholesale ingredient in the form of "solid and powdered extract as well as fluid extract and tincture forms," its offering as a "concentrated extract for syrups," and its use in an herbal formula sold as a "combination fresh herb extract." 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 2019, this database included two finished dietary supplement products with "bitter orange" in the product name and over 350 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

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 (<50%) and loss on drying. The final product contains 50% *p*-synephrine. The Fourier-transform infrared spectroscopy (FTIR) finger printing for the extract has been developed to test the consistency of the production. All lots are subjected to FTIR-TLC to further ensure batch to batch consistency. The compositional and nutritional analysis of bitter orange extract revealed the presence of several components that are summarized in Table 3.

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 95^{th} percentile (Bakhiya et al., 2017).

The dosage recommendation for *p*-synephrine is 50 mg/day for dietary supplements. The exposure to *p*-synephrine is around twice the background intake of 95^{th} percentile for German and French populations.

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 like 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

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. The findings from these toxicity studies were published in two separate peer-reviewed journals that have high impact factors (Deshmukh et al., 2017a; 2017b). In addition to the specific toxicity studies, in human clinical studies, efficacy and tolerability of bitter orange extract were investigated

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 microM 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 monamine 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, is not thought to play a major role in the biosynthesis of *p*-synephrine (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. 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). 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 (Hengtmann & 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 approximately 30 human clinical studies (Stohs, 2017).

3.2.2 Acute toxicity studies

An acute oral toxicity study was conducted on bitter orange extract (Advantra Z®) in male and female Sprague-Dawley rats per OECD Test Guideline 425 (Deshmukh et al., 2017a). 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 toxicity study are available upon request 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 7% of the total protoalkaloids in the extract or in other words less than 4 mg per 100 mg of the extract standardized to 50% *p*-synephrine. 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 40 mg of the minor protoalkaloids without evident adverse effects. For a 70 kg human this would translate into a dose of 2800 mg or less of the minor protoalkaloids, an amount approximately 56 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 four consecutive days. The treatment was well tolerated, and the rats did not demonstrate any overt signs of toxicity. (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 were 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 may be considered a pharmacological response and not a toxic adverse 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 are available upon request in a report from Intox Pvt Ltd. dated September 6, 2017. The statistically analyzed and summarized data were published by Deshmukh et al., 2017a.

3.2.4 Subchronic (90 day) or chronic studies

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

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, an assessment of grip strength, and motor activity were performed. Using a video camera 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). 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 or the 28-day recovery period.

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. The microscopic evaluation of stained blood smears did not reveal any abnormal and immature cells in animals of either sex. 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. 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. Therefore, although considered to be treatment induced, this finding was considered to be non-adverse in nature. Additionally, small but statistically significant 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, were believed to be incidental, and of no toxicological significance. 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 unrelated to treatment. Based on the findings of this 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).

The raw data for this 90-day subchronic toxicity study are available upon request in a report form 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). Caffeine was added to some doses. 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. 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.

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. 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. Body weights of the maternal animals were significantly lower in the 50 mg/kg BO + caffeine group as well as in the 25 mg/kg caffeine only group relative to the vehicle control group. The lower weights may have been due to decreased food consumption; the 50 mg/kg BO + caffeine group ate significantly less overall than did the control group. The caffeine only group had the second lowest overall food consumption, but this was not statistically different from the control 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.

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. Body weight was significantly decreased for both the caffeine only and the 50 mg/kg BO + caffeine groups. This decrease appeared to be due to a decrease in food consumption, since these two groups had the two lowest average daily food consumption totals. The decrease in food consumption appears to primarily be due to the presence of caffeine, because the 50 mg/kg BO group consumed significantly more food than did the 50 mg/kg BO + caffeine group.

The results obtained in this study suggest that 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. The addition of a bolus dose of 25 mg of caffeine/kg body weight with a dose of 50 mg of synephrine/kg body weight (using the BO extract) does not increase maternal or developmental toxicity.

3.2.6 Other animal safety studies

In a safety study, mice were treated daily 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 approximately 78 times a typical 50 mg human dose of *p*-synephrine. 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 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 with and without 25 mg caffeine/kg were determined (Hansen et al., 2012). Minimal, clinically insignificant effects were produced by these high doses of *p*-synephrine with respect to heart rate and blood pressure. As expected, caffeine alone and in combination with *p*-synephrine produced more pronounced but small increases in heart rate and blood pressure. *p*-Synephrine had no effect on the uncorrected QT interval. The adrenergic receptor binding of *p*-synephrine in rodents is as much as 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; Stohs & Shara 2013; Kaats et al., 2013, 2017; Shara et al., 2018; Stohs 2017; Ratamess et al., 2018), and therefore the 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 6% *p*-synephrine-containing bitter orange extract in the presence and absence of 25 mg/kg caffeine 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. Small increases in heart rate, blood pressures, and body temperature were reported due to caffeine, while *p*-synephrine exhibited small, 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. Furthermore, it should again be noted the adrenergic receptor binding of *p*-synephrine in rodents is as much as 10-fold greater than in humans, and adrenergic receptor binding is required to produce cardiovascular effects (Carpéné et al., 1999; 2014; Mercader et al., 2011) Therefore, cardiovascular effects seen in rodents cannot be directly extrapolated to humans.

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). 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 available upon request in a report from Intox Pvt Ltd. dated April 4, 2017. The statistically analyzed and summarized data were published by Deshmukh et al., 2017b.

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). 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.

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

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 4. 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 below.

Table 4. Summary of Published Human Clinical Studies Conducted with Advantra Z®

Product	Number of subjects	Duration	*p*-Synephrine Dose (mg)	Caffeine Dose (mg)	End Point	Adverse events	References
BOE	18	8 hrs	27	---	CV	No	Min et al., 2005
BOE	10	6 hrs	46.9	---	CV	HR	Haller et al., 2005
BOE	15	6 hrs	54	---	CV	NS	Bui et al., 2006
BOE	22	8 hrs	30	---	RQ	No	Gougeon et al., 2005
BOE	40	2 hrs	50	---	RMR, CV	No	Stohs et al., 2011c
BOE	30	15-30 days	100	---	Multiple	No	Kaats et al., 2017
BOE	40	60 days	98	---	Multiple	No	Kaats et al., 2013
BOE	18	1 day	49	---	Multiple	No	Shara et al., 2016
BOE	16	15 days	49	---	Multiple	No	Shara et al., 2017
BOE/Caf	12	3 days	100	100	Multiple	No	Ratamess et al., 2015
BOE/Caf	12	3 days	100	100	Multiple	No	Ratamess et al., 2016
BOE/Caf	24	1 day	46-104	232-337	CV	CR↑BP	Ratamess et al., 2017
Complex	9	42 days	58.5	528	Multiple	No	Colker et al., 1999
Complex	19	56 days	36	132	Multiple	No	Zenk et al., 2005
Complex	10	6 hrs	5.5	239	CV		Haller et al., 2005

Product	Number of subjects	Duration	*p*-Synephrine Dose (mg)	Caffeine Dose (mg)	End Point	Adverse events	References
Complex	10	7 hrs	12	150	RMR, Kcal Use	No	Sale et al., 2006
Complex	23	25 hrs	52	704	Kcal Use, RER	No	Seifert et al., 2011
Complex	25	1 day	20	284	Multiple	No	Jung et al., 2017a
Complex	27	56 days	20	284	Multiple	No	Jung et al., 2017b
BOE/Caf	24	1 day	46-104	232-337	Multiple	No	Bush et al., 2018

BOE=bitter orange extract; RMR=resting metabolic rate; CV=cardiovascular; RQ=respiratory quotient; NS = not studied; BP = blood pressure; HR = heart rate; RER=respiratory exchange ratio; CR=caffeine related

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 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. No adverse events were reported by any of the subjects. 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.

In a randomized, placebo-controlled cross-over study, 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, 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 effect 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. In this study, caffeine and *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 to ensure that all subjects had ingested the bitter orange extract and to estimate consumption of coffee and caffeinated beverages. Serum levels indicated

that all subjects complied regarding ingestion of the bitter orange extract. Two of the subjects were found to be non-caffeine users while caffeine consumption varied markedly among the other 14 participants. No significant differences were observed in caffeine and *p*-synephrine levels between days 7 and 14 which were approximately 860 ng/mL and 2.6 ng/mL, respectively.

In a 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. 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. Statistically significant increases in energy and appetite/eating control were reported 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 Xenadrine EFX® (12 mg *p*-synephrine) for 56 days. The product also contained 400 mg caffeine/capsule. 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 pre-workout dietary supplement for 8 weeks containing 284 mg caffeine with or without 20 mg *p*-synephrine (Advantra Z®). The pre-workout dietary supplement (PWS) contained beta-alanine (3 g), creatine nitrate as a salt (2 g), arginine alpha-ketoglutarate (2 g), N-acetyl-L-tyrosine (300 mg), caffeine (284 mg), *Mucuna pruiriens* extract standardized for 15% L-Dopa (15 mg), vitamin C as ascorbic acid (500 mg), niacin (60 mg), folate as folic acid (50 mg), and Vitamin B12 as methylcobalamin (70 mg); or, the PWS supplement with *Citrus aurantium* extract containing 20 mg of synephrine (PWS + S) once per day for 8-weeks during training. The group receiving the product with *p*-synephrine contained 27 male subjects while the other two groups contained similar numbers of subjects. 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.

In addition to the above studies, a number of other studies have addressed the safety and mechanisms associated with bitter orange and *p*-synephrine (Advantra Z®). The potential for interactive effects of *p*-synephrine with caffeine regarding effects on heart rate and blood pressure are frequently cited in the published literature, with no definitive human studies to address or substantiate this supposition. In order to address this question, Ratamess et al. (2017) conducted a study in groups of eight subjects who received single doses of placebo, *p*-synephrine (103 mg), caffeine 240 mg or 325 mg) or a combination of *p*-synephrine and caffeine (104 mg + 233 mg; and 46 mg + 337 mg, respectively). A washout period of at least one week was employed between various arms of the study. The subjects received the various products in a double-blinded, randomized cross-over fashion such that the eight subjects received all six of the products. Cardiovascular effects including diastolic and systolic blood pressures, heart rates and ECGs were determined with time.

In the study by Ratamess et al. (2018) *p*-synephrine consumption alone (103 mg) did not significantly affect systolic blood pressure or heart rate at any time point. Compared to other controls, *p*-synephrine alone resulted in small 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). Only the two trials consisting of higher caffeine doses (325 mg and 337 mg) resulted in significantly elevated systolic blood pressures. No augmented blood pressure or heart rate responses were observed when *p*-synephrine was added to caffeine beyond the effects of caffeine alone. In fact, no changes in heart rates were observed during consumption of any of these supplements. Responses were similar in both habitual caffeine consumers and those who did not routinely consume caffeine. These data support previous studies and indicate that *p*-synephrine on its own does not induce cardiovascular stress and does not augment the cardiovascular effects when combined with caffeine (Ratamess et al., 2018).

In addition to assessing the cardiovascular effects with respect to *p*-synephrine in combination with caffeine, 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 indicated that consumption of *p*-synephrine did not negatively impact acute blood parameters, did not augment the effects of caffeine, and did not produce stimulant-like perceptual mood effects.

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.

A placebo-controlled, randomized cross-over study examined the effects of a standardized bitter orange extract (Advantra Z®) at a dose of 49 mg *p*-synephrine in capsule form in 18 male and female subjects. 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 at 2 and 8 hours (Shara et al., 2016). No significant changes occurred in ECGs, heart rates, blood pressures, 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 dose used in the study.

In a randomized, placebo-controlled crossover designed study involving 12 male athletes, each subject was randomly assigned (in double-blind manner) to a treatment sequence consisting of the use of three supplements in the form of two chews: (1) 100 mg of *p*-synephrine (Advantra Z®); (2) 100 mg of *p*-synephrine with 100 mg of caffeine); and (3) placebo (Ratamess et al., 2015; Ratamess et al., 2016). The supplements were consumed for three days. The subjects performed a controlled resistance exercise protocol. Each supplement treatment was separated by a one-week washout period. No adverse effects were observed or reported with respect to *p*-synephrine consumption in the presence or absence of caffeine.

In summary, the effects of bitter orange extract, containing *p*-synephrine, alone or in combination with other ingredients, particularly caffeine, have been investigated in over 30 human clinical studies (Stohs, 2017; Stohs & Ratamess, 2017; 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, and approximately 43% of all subjects consumed a product containing *p*-synephrine (12-104 mg/day) in conjunction with caffeine (132-528 mg/day). 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 patented product Advantra Z®.

Overall, based on the information from the clinical studies, there appears to be lack of occurrence of cardiovascular effects, as evaluated by increase in heart rate or blood pressure, with various studies lasting for up to 12 weeks with doses of *p*-synephrine up to 104 mg/day. In one short-term study, the investigators reported that *p*-synephrine increased heart rate and blood pressure (Bui et al., 2006). The results from this study are clinically insignificant and have not been duplicated. Furthermore, a statistical manipulation was required to demonstrate the small but clinically insignificant increase in cardiovascular effects. Of the studies that involved subjects consuming *p*-synephrine plus caffeine, only one study by Haller et al. (2005) reported an increase in heart rate, a response 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 that contained 21 mg of *p*-synephrine and a high level (304 mg) of caffeine, and the increase in diastolic blood pressure cannot be attributed to the *p*-synephrine. In this study, only 10 subjects were involved. In contrast to these studies, all other studies with and without caffeine reported no effect on heart rate or blood pressure involving over 700 subjects (Stohs & Ratamess, 2017). Taken together, these results indicate that *p*-synephrine and bitter orange extract do not result in cardiovascular effects at commonly used doses of *p*-synephrine.

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. This cardiovascular receptor response may explain why an increase in heart rate or blood pressure is not seen when *p*-synephrine is used alone or in combination with caffeine in dietary supplements, in spite of the fact that caffeine alone may produce modest increases in these parameters under some conditions. 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; Shara et al., 2018; Ratamess et al., 2018).

3.2.10 Summary of Review Articles

Various peer reviewed and published articles have summarized the published and unpublished clinical studies involving bitter orange extract and *p*-synephrine alone or in combination with other ingredients (Marles, 2011; Stohs et al., 2012; Stohs & Shara, 2013; Lynch, 2018; Stohs, 2017; Suntar et al., 2018; Stohs et al., 2019).

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). The Health Canada report was prepared prior to the publication of several human and animal studies, which have demonstrated safety at higher dosage levels and have shown no interactions with caffeine. 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." The report also concluded that

use of products containing 40 mg per day or less of *p*-synephrine in combination with 320 mg per day or less of caffeine was also not likely to cause adverse effects. It should be noted that the guidelines for consumption of *p*-octopamine were identical to *p*-synephrine in the Health Canada report.

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 in combination with caffeine, and provided recommended guidelines for their use (Lynch, 2018). The report concluded that "the available data indicate that *p*-synephrine does not act as a cardiovascular stimulant and does not augment the cardiovascular effects of caffeine at commonly used doses. As a result, specific warnings regarding the combination of *p*-synephrine with caffeine are not warranted" (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 report also noted that the no-observed-adverse-effect-level [NOAEL] in a 90-day study in rats was 1000 mg/kg body weight for a *C. aurantium* extract containing 50% *p*-synephrine (Advantra Z®) which is 500 mg/kg of *p*-synephrine/day. For a 60 kg human, 500 mg/kg/day equals 30,000 mg *p*-synephrine/day. For a dose of 125 mg p-synephrine/day the NOAEL provides a safety factor of 240-fold, keeping in mind that the average weight of an adult female is closer to 70 kg and an adult male closer to 80 kg."

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).

The European Food Safety Authority (EFSA) published a scientific opinion on the safety of caffeine (EFSA 2015). The opinion states that "single doses of caffeine up to 200 mg (about 3 mg/kg body weight for a 70 kg adult) do not give rise to safety concerns." Furthermore, "habitual caffeine consumption up to 400 mg per day does not give rise to safety concerns for non-pregnant adults." The opinion further stated that "the short- and long-term effects of caffeine and synephrine on the cardiovascular system have not been adequately investigated in humans." The opinion did not review current literature regarding over 20 human studies that had been conducted on *p*-synephrine in the presence and absence of caffeine after 2015. Furthermore, numerous safety studies have been published since the publication of this scientific opinion demonstrating a lack of interaction between *p*-synephrine and caffeine (Shara et al., 2018; Ratamess et al., 2015; 2016; 2017) and have been summarized in a review (Stohs and Ratamess, 2017). Thus, the EFSA document is not a reflection of current knowledge regarding caffeine and *p*-synephrine and their lack of interaction.

A detailed review assessed the safety, efficacy, and mechanisms of action of *Citrus aurantium* (bitter orange) extracts and *p*-synephrine (Stohs, 2017). A subsequent review summarized more recent studies regarding the safety and efficacy of bitter orange extracts and *p*-synephrine (Stohs et al., 2019). 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.

3.2.11 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. 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. 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 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.

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 and caffeine (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 poorly written 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. 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 review the literature, and cited other poorly written case reports and speculative review articles. The authors appeared not to know how much *p*-synephrine was in the product, that no controlled studies have ever shown adverse cardiovascular effects associated with *p*-synephrine, and that this amount of *p*-synephrine is widely consumed on a daily basis by many individuals in citrus juices and other food products without adverse effects. It is impossible to show a cause and effect relationship between the product and the ascending aortic dissection, much less a relationship with any ingredient including *p*-synephrine. Caffeine is known to exhibit cardiovascular activity, while as discussed above, *p*-synephrine is not.

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, while *p*-synephrine does not contribute to or augment the cardiovascular effects of caffeine (Ratamess et al., 2018).

In this case study, as in other case reports, the authors failed to review the literature, citing another case study and no 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 noted that mixing caffeine with synephrine can "lead to an increase in the adverse effects profile of each," contrary to recent research (Unnikrishnan et al.,

2018), while citing 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 specifically demonstrating an adverse reaction to bitter orange 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).

3.2.12 Other relevant studies for safety assessment

There are three positional isoforms of synephrine, namely para-synephrine (*p*-synephrine), meta-synephrine (*m*-synephrine; also known as phenylephrine), and ortho-synephrine (o-synephrine), each of which have two optical isomers or chiral forms (Allison et al., 2005). *C. aurantium* primarily contains *p*-synephrine, but *m*-synephrine is not a constituent (Roman et al., 2007; reviewed in Stohs et al., 2011a, and Stohs and Preuss, 2011). Although structurally similar to ephedrine, norepinephrine, and epinephrine, *p*-synephrine also has structural differences, such as a para-hydroxy group and lack of a methyl side chain, which alter the stereochemistry and receptor binding properties of *p*-synephrine relative to the other adrenergic agonists (reviewed in Stohs and Preuss, 2011; Stohs et al., 2011a,b). *p*-Synephrine has been reported to be a direct-acting α1-adrenoceptor agonist; however, the binding of *p*-synephrine to the α-1 and α-2 adrenoreceptors was 1,000-fold less active than norepinephrine (Brown et al., 1988). In addition,

p-synephrine was reported to induce only 55% of the maximal response of *m*-synephrine at human α-1a-adrenoreceptors, and even lower responses at α-2a and α-2c adrenoreceptors (Ma et al., 2010). Little or no β-1 and β-2-adrenoreceptor activation was reported in guinea pig atria and trachea, as *p*-synephrine was 40,000-fold less potent than norepinephrine at binding to β-1 and β-2 adrenoreceptors (Jordan et al., 1987).

p-Synephrine's poor binding affinity to α and β-1 and β-2 adrenoreceptors provides a mechanistic explanation for the lack of observed effects on blood pressure and heart rate in several animal and human studies (Stohs et al., 2011b). As a result, cardiovascular effects that have been reported to be associated with ephedrine and norepinephrine are not relevant to the safety profile of *p*-synephrine (Stohs, 2017). *p*-Synephrine is hypothesized to bind primarily to β-3 adrenergic receptors, as it was reported to induce lipolysis in vitro, and to decrease blood glucose levels, increase insulin secretion, and decrease concentrations of cholesterol and triglycerides in serum of rats and mice (reviewed in Stohs and Preuss, 2011; Stohs et al., 2011a,b; Stohs, 2017). β-3 adrenoreceptors are located in white and brown adipose tissues and muscles, and 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). These metabolic effects could reduce fat mass in obese humans (Jordan et al., 1987). Of significance is the finding that the ability of *p*-synephrine to bind to adrenoreceptors is upwards of 10-fold greater in rodents than in humans (Carpéné et al., 1999, 2014; Mercader et al., 2011; Stohs , 2017). As a consequence, the small effects of *p*-synephrine and bitter orange extract observed in rodents cannot be directly extrapolated to humans. This is important with respect to the evaluation of slight changes in cardiovascular parameters in rodents administered high doses of either *p*-synephrine or bitter orange extract.

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 Section 6 ("Comments")). Thus, the sum of these minor alkaloids represents less than 7% of the total protoalkaloidal content of 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 very small amounts, its contribution to the effects of standardized bitter orange extracts will be very small.

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) [emphasis added]. 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). 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 i*n 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.

- As a single one-time dose, up to 100 mg *p*-synephrine is unlikely be associated with adverse effects in healthy populations.
- 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 90-day study in rats was 1000 mg/kg for a *Citrus aurantium* extract containing 50% *p*-synephrine [Advantra Z®].
- *p*-Synephrine does not act as a cardiovascular stimulant at commonly used doses because it does not act directly or indirectly as a α-1, α-2, β-1 or β-2 adrenergic receptor agonist.
- *p*-Synephrine does not augment the cardiovascular effects of caffeine at commonly used doses. Therefore, when determining a safe dose of *p*-synephrine in combination with caffeine each should be considered independently in the context of the full product formula.
- *p*-Synephrine and *Citrus aurantium* (bitter orange) extract are non-mutagenic and non-teratogenic.
- 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. No adverse effects have been reported.
- Extensive in vitro studies have demonstrated that *p*-synephrine functions as a metabolic enhancer without cardiovascular stimulant effects.

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®). The NOEL was determined as 300 mg/kg bw/day in the same study.

4.2 Determination of the Estimated Daily Intake (EDI)

The dosage recommendation for Advantra Z® containing 50% *p*-synephrine is 100 mg per day for dietary supplements. FDA assumes an average of 60 kg body weight for an adult, the EDI for Advantra Z® containing 50 mg *p*-synephrine would be: 100 mg/day ÷ 60 kg = 1.67 mg/kg bw/day. If the average weight is assumed to be 75 kg (70 kg for women and 80 kg for men), the EDI for 100 mg Advantra Z® would be 1.33 mg/kg bw/day.

4.3 Determination of the margin of safety

The margin of safety between the NOAEL and the EDI for Advantra Z® containing 50% *p*-synephrine would be:
1000 mg/kg bw/day ÷ 1.67 mg/kg bw/day = 599 for a 60 kg individual, and 752 for a 75 kg adult.

The margin of safety between the NOEL and the EDI for Advantra Z® containing 50% *p*-synephrine would be:
300 mg/kg bw/day ÷ 1.67 mg/kg bw/day = 180 for a 60 kg individual, and 226 for a 75 kg adult.

4.4 Safety narrative and conclusion

The margin of safety between the NOAEL and the EDI is over 600, and over 180 between the NOEL and the EDI for Advantra Z® containing 50% *p*-synephrine. These margins of safety are adequate to conclude that bitter orange extract (Advantra Z®) is reasonably expected to be safe under its intended conditions of use in dietary supplements.

In conclusion, the margins of safety are adequate to conclude that Advantra Z® (bitter orange extract) is 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

6. Comments

6.1 Analytical data from three non-consecutive manufacturing lots of Advantra Z® (Bitter Orange Extract)

The following Certificates of Analysis for Advantra Z® (50% *p*-synephrine) are included in attached Reference 110 **(Confidential Commercial Information)**: