Review of the Safety Data Available on \( p \)-Synephrine, Caffeine, and \( p \)-Synephrine-Caffeine Containing Combination Products

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April 12, 2013

Overview: Intertek Cantox has reexamined the safety and dosing guidelines of dietary supplements containing \( p \)-syynephrine, a direct-acting sympathomimetic amine derived from the plant *Citrus aurantium* (bitter orange), alone and in combination with caffeine. It was concluded that as a single one-time dose, up to 70 mg \( p \)-synephrine alone or 40 mg in combination with 320 mg of caffeine is not likely to cause adverse effects. If taken as divided doses spaced out over the course of a day, 100 mg of \( p \)-synephrine alone (e.g., 50 mg twice a day or 33 mg three times daily) or 70 mg in combination with 400 mg of caffeine is unlikely to be associated with adverse effects.
Review of the Safety Data Available on \( p \)-Synephrine, Caffeine, and \( p \)-Synephrine-Caffeine Containing Combination Products

At the request of Nutratech, Inc., Intertek Cantox has reviewed the safety of \( p \)-synephrine, a direct-acting sympathomimetic amine derived from the plant \textit{Citrus aurantium} (bitter orange) and caffeine. Specifically, Intertek Cantox evaluated the safety of consumption of a dietary supplement which would provide either \( p \)-synephrine alone or in combination with caffeine.

We have evaluated scientific information and data obtained from literature searches, pertaining to the safety of \( p \)-synephrine and caffeine, and of the use of these ingredients in combination products. A review of the safety data available on \( p \)-synephrine, caffeine, and \( p \)-synephrine-caffeine containing combination products is presented below, following which are provided our conclusions and/or recommendations.

**Synephrine**

\( p \)-Synephrine is the main active ingredient in \textit{Citrus aurantium} (bitter orange), a commonly marketed dietary herbal supplement for weight loss/management and sports performance. It is available alone or in combination with other ingredients, including caffeine. An industry survey of dietary supplement products on the market containing \textit{C. aurantium} extract indicates that of the products surveyed, the average single dose of \textit{C. aurantium} extract is approximately 290 mg (single doses ranged from 25 to 1,300 mg) and that the average total daily dose is approximately 675 mg/day (ranging from 60 to 3,600 mg/day). Extracts typically contain from 6 to 50\% \( p \)-synephrine with most daily ingestion of \( p \)-synephrine in the range of 15 to 50 mg.

There are 3 positional isoforms of synephrine, namely \emph{para}-synephrine (\( p \)-synephrine), \emph{meta}-synephrine (\( m \)-synephrine; also known as phenylephrine), and \emph{ortho}-synephrine (\( o \)-synephrine), each of which have two optical isomers or chiral forms (Allison et al., 2005). \textit{C. aurantium} primarily contains \( p \)-synephrine and \( m \)-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 \( \alpha1 \)-adrenoceptor agonist; however, the binding of \( p \)-synephrine to the \( \alpha1 \) and \( \alpha2 \) 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).

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). 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. These metabolic effects could reduce fat mass in obese humans (Jordan et al., 1987).

p-Synephrine is present in citrus fruits, such as Seville oranges, grapefruit, mandarin, and other species of oranges, and is consumed in the diet (reviewed in Stohs and Preuss, 2011). Typical levels of p-synephrine in orange juices are 5 mg per 8 ounce glass, although 10 samples of mandarin orange juice were reported to contain between 73 and 158 mg p-synephrine per litre.

Toxicological studies that examined C. aurantium extract or p-synephrine have reported effects primarily associated with the pharmacological action of the test compounds (e.g., reduced body weights and alterations in cardiovascular parameters); however, one study investigating effects on fetal growth and development was identified.

In rats, acute oral doses of up to 10,000 mg/kg body weight of a C. aurantium product (Douds, 1997) showed no effect on mortality. Various clinical signs were noted during the first few days of the study. No significant effects were observed at necropsy.

In a study that has not been reproduced, Calapai et al. (1999) evaluated the anti-obesity and potential cardiovascular toxic effects in male Sprague-Dawley rats that were administered 2.5, 5, 10, or 20 mg/kg body weight/day of C. aurantium extracts containing 4 or 6% p-synephrine via gavage for 7 or 15 consecutive days. Control animals were treated with saline (vehicle). Arterial blood pressure, heart rate, and electrocardiogram (ECG) were evaluated on days 5, 10, and 15, and clinical signs were observed daily. Body weight gain and food intake, which were examined in animals treated for 7 days, were significantly and dose-dependently reduced in treated animals compared to control animals. No other clinical signs were noted. Mortality was observed in all groups administered the C. aurantium extracts, whereas no mortality was observed in the control group. Although a significant trend between treatment and mortality was observed in animals treated only with the 6% p-synephrine C. aurantium extract, the authors reported that the data suggested that a causal relationship likely exists between treatment with C. aurantium extracts and mortality. No significant effects on arterial blood pressure were observed in rats administered C. aurantium extracts compared to the control group; however, analysis of ECGs revealed the presence of ventricular arrhythmias and enlargement of QRS complex in animals treated with C. aurantium extracts after 5 days of treatment, with this effect
achieving significance after 10 days of treatment and persisting after 15 days of treatment. The number of animals with ECG alterations was greater in those animals that received _C. aurantium_ extract containing 6% p-synephrine compared to those that received the 4% extract, thus suggesting that ECG changes were possibly correlated to p-synephrine dose. The results reported by Calapai et al. (1999) are difficult to extrapolate to p-synephrine directly since the other constituents of the _C. aurantium_ extract used were not identified.

When tested in mice for 28 days at daily doses of 30 or 300 mg/kg body weight/day, p-synephrine was reported to be without toxic effect on biochemical, hematological, or organ weight parameters. Treatment was, however, associated with a reduction in body weight gain (to be expected) and with an increase in reduced glutathione concentration and inhibition of glutathione peroxidise activity. The clinical significance of these changes was not clear (Arbo et al., 2009).

The administration of up to 100 mg/kg body weight/day p-synephrine from _C. aurantium_ or from a pure extract by gavage to pregnant Sprague-Dawley rats from GD 3 to 20 did not result in adverse effects on fetal growth or development (Hansen et al., 2011). It should be noted that a dose of 100 mg/kg of p-synephrine in rats is equivalent, based on a body surface to body weight ratio basis, to over 30-times a 40 mg daily dose in an 80 kg (176 lb.) human. The number of live implantations per litter was significantly reduced in the group receiving 100 mg/kg body weight/day p-synephrine from a pure extract compared to vehicle control; however, this effect was not observed in the group receiving 100 mg/kg body weight/day p-synephrine from _C. aurantium_, leading the authors to suggest that the reported effect was a spurious observation. The authors also examined the potential maternal and fetal effects of 50 mg/kg body weight/day p-synephrine from _C. aurantium_ or from a pure extract plus 25 mg/kg body weight/day caffeine or 25 mg/kg body weight/day caffeine alone. No evidence of fetal toxicity was observed, and the only effect on the dams was a significant decrease in body weight in the groups receiving 50 mg/kg body weight/day p-synephrine from _C. aurantium_ plus caffeine and the caffeine only group compared to vehicle control. The decreased body weight in these groups was attributed to decreased food consumption.

_C. aurantium_ (Zani et al., 1991) and p-synephrine (McGregor et al., 1988) have been reported to be non-mutagenic.

Numerous clinical studies have investigated the efficacy of _C. aurantium_ and p-synephrine, alone or in combination with other ingredients, for weight loss. While the focus of the majority of these studies was on efficacy, some parameters related to safety, including biochemical and hematological parameters, were evaluated in some studies. A review had been published that summarizes published as well as unpublished human studies involving bitter orange extract and p-synephrine (Stohs et al., 2012). In general, bitter orange extract (p-synephrine) alone or in combination with other herbal ingredients did not produce significant adverse events or alter serum chemistry, blood cell counts or urinalysis.

Conflicting evidence exists for cardiovascular effects of synephrine after acute infusion (4 mg/minute for 30 minutes) (Hofstetter et al., 1985) which cannot be equated with oral administration and repeated dosing (5 mg p-synephrine twice/day for 8 weeks) (Kalman et al.,
A wide range of acute doses of p-synephrine have been reported to elicit cardiovascular changes (e.g., increased peripheral vascular resistance, decreased heart rate, increased blood pressure) in humans (Zhao et al., 1978; Thomas et al., 1991; Grassi et al., 1997; Haller et al., 2005; Bui et al., 2006).

In other single dose human studies, no significant differences in SBP, DBP, cardiac index, systemic vascular resistance index, or PR, QRS, QT, RR, or QTc intervals over 8 hours were reported between placebo and an active product containing 27 mg p-synephrine from C. aurantium (Min et al., 2005), and no significant differences in heart rate, SBP, or DBP over 5 hours compared to baseline were reported following consumption of C. aurantium capsules providing 26 mg p-synephrine (Gougeon et al., 2005). In addition, SBP, DBP, and heart rate 75 minutes following the consumption of 50 mg synephrine in V-8 juice did not differ from baseline or compared to controls receiving placebo V-8 juice (Stohs et al., 2011c). Although Min et al. (2005) reported that the active constituent in C. aurantium was m-synephrine, it has since been demonstrated that C. aurantium contains only p-synephrine (Roman et al., 2007; reviewed in Stohs et al., 2011a,b). Thus, the discussion of m-synephrine’s effects on cardiac and hemodynamic parameters is not relevant.

A crossover, open-label, cardiovascular toxicity study (that also monitored caffeine intake) in 12 normotensive, healthy young adults examined heart rate, systolic and diastolic blood pressures, and mean arterial pressure after 2 oral doses (8 hours apart) of 8 ounces of C. aurantium juice (containing 13 to 14 mg synephrine) (Penzak et al., 2001). Approximately one week later, the test was repeated with the crossover treatment, which consisted of water. The synephrine content in this study was reported as m-synephrine, rather than p-synephrine, even though the analytical methods employed were unable to separately quantify the respective isomers. Cardiovascular parameters were measured immediately prior to the second dose and once per hour over 5 hours following the second dose; adverse effect reporting was not included as part of the study design. Systolic and diastolic blood pressures, mean arterial pressure, and heart rate were not affected by p-synephrine treatment.

Blood pressure, heart rate, and ECGs assessed in the first 8 hours following the first dose and at Weeks 1 and 2 were not significantly different in healthy subjects consuming 50 mg p-synephrine/day in capsules for 14 days compared to placebo (Shara and Stohs, 2011). These results were further extended in a double-blinded, placebo-controlled study (Kaats et al., 2013). The study was of a double-blind, placebo-controlled design involving 75 healthy subjects. Three groups of 25 subjects (15 males and 60 females; average age 51.3 years, weight 185.6 lbs, and BMI 30.8) consumed, in capsule form, either: a) a placebo, b) bitter orange extract twice daily to provide 98 mg of p-synephrine, or c) bitter orange extract twice daily (98 mg of p-synephrine/day) in combination with naringin (~100 mg/day) and hesperidin (~576 mg/day). Subjects consumed their daily allotments for 60 consecutive days. At baseline and the completion of the study, subjects were evaluated with respect to resting perception of quality of life, heart rate, blood pressure, and a comprehensive blood chemistry and hematology panel for evaluation of hepatic, renal, and cardiac function. A total of 67 subjects completed the study, with similar numbers of losses in each of the 3 groups. After 60 days of treatment, there were no adverse effects on any of the parameters evaluated. Small, statistically significant increases in resting heart rate (+2.9 bpm) were noted between the p-synephrine and
naringin/hesperidin group vs. p-synephrine alone and placebo groups. This finding was considered of no clinical significance. In addition, no such differences were noted between the p-synephrine alone group and the placebo controls. There were no reports of adverse events in any of the 3 groups. Efficacy of treatment, in the form of weight loss, was not evaluated. The study was designed and conducted as a safety study *per se*.

No adverse events were reported following consumption of 30.6 mg p-synephrine/day from *C. aurantium* for 28 days by 12 young healthy adults (Gurley *et al*., 2004). Furthermore, the lack of an effect on phenotypic ratios of cytochrome P450 (CYP) enzymes CYP1A2, CYP2D6, CYP2E1, or CYP3A4 suggests minimal risk of herb-drug interactions via these drug metabolizing enzymes.

It should be noted that case reports exist in the scientific literature that allege adverse cardiovascular events in persons consuming p-synephrine or *C. aurantium* (Keogh and Baron, 1985; Health Canada, 2004; Firenzuoli *et al*., 2005; Gange *et al*., 2006; Stephensen and Sarlay, 2010). Many of these have been recently reviewed (Rossato *et al*., 2011). 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). Concerns about the cardiovascular effects of p-synephrine, particularly at high doses (e.g., 100 mg/day or more) that could be consumed based on the suggested serving size of some supplements, were re-iterated in a recent review article (Inchiosa, 2011). The present knowledge of the pharmacology of p-synephrine is inconsistent with it having a causal role in the case reports of cardiovascular incidents especially at serving sizes/doses recommended on most product labels.

**Caffeine**

The toxicology of caffeine has been extensively studied in both animals and in humans.

Caffeine binds and inhibits the action at adenosine A₁ and A₂a receptors (Benowitz, 1990; Fredholm, 1995; Donovan and DeVane, 2001; Nawrot *et al*., 2003). When normally occupied by adenosine, activation of the A₁ receptor results in the inhibition of adenylate cyclase activity (*i.e.*, the G protein blocks adenylate cyclase rather than activating it), and decreased subsequent activity of protein kinase A, with resultant downstream pharmacological effects such as decreased energy metabolism, decreased smooth muscle contraction, vasodilatation, and decreased neurotransmitter release (Benowitz, 1990; Fredholm, 1995; Donovan and DeVane, 2001; Orton, 2005). Occupation of the A₁ receptors by caffeine in the tissues in which this receptor is located results in increased adenylate cyclase activity and is responsible for caffeine’s inhibition of depressant effects in the CNS, positive inotropic effects on the heart (possibly weak chronotropic as well), vasoconstriction, increased blood pressure and, in the kidney, diuresis, vasodilatation and sodium reabsorption in response to increased glomerular filtration rate due to increased blood pressure (Benowitz, 1990; Donovan and DeVane, 2001; Nawrot *et al*., 2003; Orton, 2005). At normal levels of consumption, approximately 100 to 400 mg/day, caffeine concentrations in plasma attain levels known to be associated with the antagonism of the A₁ receptor.
Acute and chronic symptoms of caffeine exposure include anxiety, tension, headache, insomnia, diarrhea, irritability, loss of appetite, dizziness, and a decrease in hand steadiness (Abbott, 1986). Cardiovascular effects include palpitations, extra-systoles, tachycardia, arrhythmias, flushing, and with extremely large doses, marked hypotension and circulatory failure (Abbott, 1986). Additionally, gastrointestinal effects include nausea, vomiting, diarrhea, epigastric pain, and occasionally peptic ulcer and hematemesis. Prolonged consumption of high levels of caffeine may result in tolerance to some of the pharmacological actions (Leonard et al., 1987; Myers, 1988; Sweetman, 2002; Nawrot et al., 2003), and abrupt discontinuation may lead to physical symptoms of withdrawal such as irritability, lethargy, and headache (Sweetman, 2002).

Overdose can occur at >300 mg caffeine and may result in restlessness, tremor, and elevated reflex excitability, and be accompanied by vomiting and abdominal spasm. The available data do not indicate that moderate caffeine intake of <400 mg/day adversely affects cardiovascular health or bone and calcium economy (Nawrot et al., 2003). Estimates of a fatal dose of caffeine in humans are greater than 10 g, equivalent to 140 mg/kg body weight (Abbott, 1986; Nawrot et al., 2003). This quantity of caffeine would be approximately equivalent to that found in 75 cups of coffee, 125 cups of tea, or 200 cola beverages (Curatolo and Robertson, 1983). The cardiovascular effects of caffeine have been extensively studied. The rapid development of tolerance to caffeine greatly complicates the interpretation of cardiovascular data. Caffeine has been reported to raise, lower, and not affect blood pressure and heart rate, as well as lower or not affect levels of epinephrine (Robertson and Curatolo, 1984; LeBlanc et al., 1985; Poehlman et al., 1985; Abbott, 1986; Leonard et al., 1987; Myers, 1988; Astrup et al., 1990; Arciero et al., 1995; Bracco et al., 1995; Green et al., 1996; Nawrot et al., 2003). Following review of more than 50 acute studies, results indicate that caffeine increases both systolic and diastolic blood pressure at doses >250 mg/person in adults of both sexes, irrespective of age, although the effect is more pronounced in the elderly (Nawrot et al., 2003). However, these effects were seen less consistently in repeat-dose studies (Nawrot et al., 2003). It is generally agreed that the pressor effects of caffeine subside within 1 to 3 days due to tolerance, although tolerance is partially lost with as little as 12 hours of abstinence, which may partially explain conflicting results (Leonard et al., 1987; Myers, 1988; Nawrot et al., 2003). The possibility that heavy coffee consumption may adversely affect the incidence of heart disease cannot be ruled out (Nawrot et al., 2003). Also, recent case reports of adverse cardiovascular effects have appeared in the scientific literature in regard to the use of caffeine as a weight loss supplement in combination with ephedra (Saper et al., 2004), and more recently with synephrine or synephrine containing botanicals (Health Canada, 2004; Nykamp et al., 2004; Bouchard et al., 2005).

Due to caffeine’s extensive use in foods and beverages, there exists an enormous amount of genotoxic and mutagenic data. The scientific literature reviewed utilized various animal, prokaryotic, eukaryotic and mammalian cell culture systems, and the mutagenic activity of caffeine varied between test systems. There is little evidence of mutagenic activity in mammalian cells in vivo, despite the fact that caffeine has been found to be mutagenic both alone and in combination with other DNA damaging agents in various in vitro tests systems (Haynes and Collins, 1984; Abbott, 1986; D’Ambrosio, 1994). A large number of studies in
experimental animals investigating the carcinogenicity of caffeine have concluded that caffeine is not carcinogenic (Zeitlin, 1972; Bauer et al., 1977; Mohr et al., 1984; Pozniak, 1985).

Overall, clinical studies have indicated that single doses of <450 mg of caffeine do not increase the severity or frequency of cardiac arrhythmia (Nawrot et al., 2003). The available data do not indicate that moderate caffeine intake of <400 mg/day adversely affects cardiovascular health (Nawrot et al., 2003). Caffeine may cause anxiety, tachycardia, palpitation, insomnia, restlessness, nervousness, tremor, and headache at doses >600 mg/day (Health Canada, 2008). Health Canada (2008) has indicated that hypersensitivity/allergy to caffeine is known to occur, in which case, the use of caffeine should be discontinued.

**Synephrine/Caffeine Combination Products**

Toxicological studies that examined *C. aurantium* extract or *p*-synephrine in combination with caffeine are lacking. In a review of human published and unpublished studies, approximately half of the studies used a product that contained *p*-synephrine plus caffeine (Stohs et al., 2012). A single animal study examining the cardiovascular effects of the combination was identified (Hansen et al., 2012). A study conducted with a combination product that included other alkaloids also was identified and is discussed below (Schmitt et al., 2012).

Hansen et al. (2012) examined cardiovascular effects (*i.e.*, SBP, DBP, heart rate, QT interval) of isolated *p*-synephrine (90% *p*-synephrine) and synephrine provided in a *C. aurantium* extract (6% *p*-synephrine) alone and in combination with caffeine in rats. Female Sprague-Dawley rats were administered by gavage 0, 10, or 50 mg/kg body weight/day of *p*-synephrine from each source with or without the addition of 25 mg/kg body weight/day of caffeine for a period of 28 days and SBP, DBP, heart rate, and ECG endpoints were continuously monitored. In comparison to baseline values, SBP and QT interval were not significantly changed in any of the treatment groups at the end of the 28 days, and DBP was not significantly different in the *p*-synephrine only groups; however, DBP was significantly decreased in all groups administered caffeine, except for the 50 mg/kg body weight/day *p*-synephrine extract plus caffeine. In addition, heart rate was significantly decreased in all groups receiving *p*-synephrine, except for the 50 mg/kg body weight/day group of *p*-synephrine from *C. aurantium* extract. When comparing the effects over the first 8 hours following dosing (day of assessment not reported) between groups, SBP was significantly elevated at 1, 2, and 4 hours in the groups receiving *p*-synephrine at a dose of 50 mg/kg body weight/day from either source and also at 8 hours in the group administered 50 mg/kg *p*-synephrine from *C. aurantium* extract compared to the vehicle control. SBP also was significantly increased in the group receiving 50 mg/kg body weight/day *p*-synephrine from *C. aurantium* at 1, 2, 4, and 8 hours compared to the group receiving 50 mg/kg body weight/day *p*-synephrine from the purified extract, leading the authors to suggest that other components of the *C. aurantium* extract had an adverse effect on blood pressure. The addition of caffeine significantly increased SBP at 1, 2, and 4 hours in all *p*-synephrine groups compared to *p*-synephrine alone. DBP was not significantly affected by *p*-synephrine, except for transient increases compared to controls at 4 and 8 hours in the group receiving 50 mg/kg body weight/day *p*-synephrine from *C. aurantium* extract. *p*-Sympetrine did not affect heart rate in a consistent manner. Heart rate was significantly increased at 1, 2, 4, and 8 hours in the group receiving 10 mg/kg body weight/day *p*-synephrine from the purified extract.
compared to control, but not in the 50 mg/kg body weight/day group. In the groups receiving p-synephrine from *C. aurantium* extract, heart rate was significantly increased at 2 hours in the 10 mg/kg body weight/day group and at 1, 2, and 4 hours in the 50 mg/kg body weight/day group compared to controls.

The addition of caffeine significantly increased heart rate 1, 2, 4, and 8 hours in all groups provided caffeine compared to vehicle control. The addition of caffeine also significantly increased heart rate compared to p-synephrine alone at 1, 2, 4, and 8 hours in both 50 mg/kg body weight/day dose groups and in the 10 mg/kg body weight/day p-synephrine from *C. aurantium* dose group. In the 10 mg/kg body weight/day p-synephrine from purified extract plus caffeine dose group, heart rate was significantly increased at 2 and 4 hours compared to p-synephrine alone. Uncorrected QT interval was not affected by p-synephrine alone in any of the dose groups compared to control, and no consistent effects attributable to caffeine or p-synephrine source were observed. Based on the reported effects, the authors concluded that “p-synephrine, either as the purified component or as part of a botanical extract with other components, can increase heart rate and blood pressure. These effects tend to be more pronounced when the source of p-synephrine is a multi-component product or when caffeine is added.” In addition, it was noted that in contrast to the results of Calapai *et al.* (1999), there was no weight loss, decrease in food consumption, or differences in mortalities in the present study in rats that received up to 50 mg/kg body weight/day p-synephrine for 28 days compared to control. It should also be noted that the doses of p-synephrine used in these studies were up to 20-times the typical daily doses consumed by humans, and although the cardiovascular changes were statistically significant, because they were small it is doubtful that they would be clinically significant.

The acute administration of a mixture of p-synephrine, caffeine, ephedrine, and salicin in a 10:80:4:6 ratio at doses totalling 300, 350, or 400 mg/kg body weight/day to male and female albino CF1 mice (6/group) resulted in 1 death in mid-dose males and 5 deaths in high-dose males (Schmitt *et al*., 2012). Following necropsy, cardiopulmonary hemorrhage was observed in the animals that died. Clinical signs of toxicity such as a reduction in locomotor activity and ptosis were significantly increased in all treated groups, whereas observations of other clinical signs of toxicity, such as piloerection, gasping, tearing, salivation, agitation, tremors, and muscle spasms reached statistical significance mainly in high-dose groups. Due to the presence of ephedrine and salicin, it is not possible to determine what contribution p-synephrine made to the observed effects; however, in a separate acute test of spontaneous locomotor activity, the administration of 30 mg/kg p-synephrine to male albino CF1 mice resulted in decreased locomotor activity compared to controls. The p-synephrine used in these studies was (±)-p-synephrine and was obtained from MP Biomedicals; thus, it was likely synthetic and not isolated from *C. aurantium*. The doses of p-synephrine and ephedrine ranged from approximately 4 to 8 times an equivalent average human daily dose.

There also were limited human data available specifically on combination products containing only p-synephrine and caffeine. The limitations of the available data have raised some concern with respect to the adequacy of demonstration of product safety (Haaz *et al*., 2006; Rossato *et al*., 2011).
In the one double-blind, placebo-controlled crossover study that assessed the effects of caffeine (304 mg) and \( p \)-sympatrine (21 mg) on vital signs, serum electrolytes, and oxygen consumption during rest and after intense exercise (30 minutes and 75 to 80% maximum heart rate) in 10 healthy adults, slight increases were reported in post-exercise diastolic blood pressure (71.7 mmHg vs. 63.0 mmHg) and postprandial glucose concentrations (121.0 mg/dl vs. 103.7 mg/dl with the placebo treatment) (Haller et al., 2008). There was no effect at rest or during exercise on heart rate, systolic blood pressure, or exercise-related changes in oxygen consumption, serum lactate, or insulin. With supplementation subjects did perceive the exercise routine to be less strenuous. No significant adverse events were reported to have occurred.

Haller et al. (2005) previously conducted a randomized, placebo-controlled double blind, cross-over trial in which 10 healthy non-smoking adults consumed single doses of \( C. \) aurantium (Advantra Z) to provide a \( p \)-sympatrine dose of 46.9 mg, a multi-ingredient product (Xenadrine EFX) to provide 5.5 mg of \( p \)-sympatrine along with 5.7 mg of octopamine and 239.2 mg of caffeine, or a placebo. A 1-week washout was incorporated between treatments. Effects on heart rate and blood pressure were recorded at regular intervals. Xenadrine EFX was found to increase both systolic and diastolic blood pressure by about 9 mmHg 2 hours following dosing. The \( p \)-sympatrine-only containing product, Advantra Z had no effect on blood pressure. Both products induced a slight increase in heart rate (Xenadrine EFX 16.7 bpm and Advantra Z 11.4 bpm). The lack of effect of Advantra Z on blood pressure, despite containing nearly 10-fold higher concentrations of \( p \)-sympatrine was attributed to caffeine and other stimulants present in Xenadrine EFX. Another human trial in healthy overweight males conducted by Sale et al. (2006) on Xenadrine EFX (to provide 6 mg \( p \)-sympatrine, 150 mg caffeine, and 150 mg catechin polyphenols) failed to show any effects of treatment on either blood pressure or heart rate.

Most of the human trial data that have been published pertain to combination products containing several or many ingredients in addition to \( p \)-sympatrine and caffeine. In relation to safety, these clinical trials have assessed potential effects on heart rate, blood pressure, mean arterial pressure, and glucose tolerance/energy metabolism. The results of the studies have been mixed, with some showing no direct effects of combination products on cardiovascular parameters with others demonstrating slight to moderate effects. The more notable studies are summarized below.

Seifert et al. (2011) conducted an acute, double-blind, placebo-controlled, cross-over study in 23 mildly overweight (BMI 26.6 ± 3.8) volunteers to investigate the cardiovascular effects of a product containing caffeine and bitter orange extract (\( p \)-sympatrine), as well as green tea extract, bee pollen, white willow bark powder, \( Panax \) ginseng root, \( Garcinia \) cambogia extract, and vanadium. On the study day 1, heart rate, blood pressure, and non-protein respiratory exchange ratio (RER) were determined following an overnight fast. Subjects were then provided with 3 study capsules and instructed to consume 1 at each meal of the day. The following day, subjects returned to the laboratory following an overnight fast, consumed the 4th capsule, and blood pressure, heart rate, and RER were determined 60 to 75 minutes following consumption of the capsule. Following a 1-week washout period, the subjects returned to the laboratory and followed the same procedure on the alternate capsule. Each active product capsule contained 176 mg caffeine (from guarana) and 13 mg \( p \)-sympatrine (from \( C. \) aurantium), providing a total dose of 704 mg caffeine and 52 mg \( p \)-sympatrine over the
24-hour period. No adverse effects were reported by any of the subjects, and there were no significant between-group or within-group differences in heart rate, systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), or RER for the entire population or when the data were separated by gender. When high caffeine users were separated from low caffeine users, a significant decrease in RER compared to placebo and baseline values was reported for the low caffeine users; however, there were no significant differences in heart rate, SBP, DBP, or MAP.

Significant increases in SBP at 2 hours and in the 3-hour average and in DBP at 2 hours following the consumption of 1.5 cups of an enriched coffee providing 450 mg caffeine and 21.6 mg p-synephrine compared to regular coffee were reported in 10 healthy & physically active young adults (Hoffman et al., 2006). Although the authors concluded that “the 150 mg higher caffeine dose found in JavaFit™ in combination with p-synephrine likely contributed to the modest 3 mmHg average increase in systolic blood pressure”, the enriched coffee also contained Garcinia cambogia and chromium polynicotinate. Thus, it is not possible to determine which ingredients were responsible for the observed effects.

Cardiovascular function was assessed in a 2-week study in which 27 healthy overweight adults (17 females, 10 males) consumed a placebo (n=8) or a commercial weight loss supplement (n=19) containing 335 mg Ma Huang (20 mg ephedrine alkaloids), 910 mg guarana (200 mg caffeine), and 85 mg bitter orange (5 mg p-synephrine) per 2 capsules (1 serving) (Kalman et al., 2002). For the first 7 days of the study, each subject was instructed to ingest 1 capsule twice a day, while for the second 7 days of the study, each subject was instructed to ingest 2 capsules twice per day. Cardiovascular parameters that were evaluated on days 0, 7, and 14 of the study included systolic and diastolic blood pressures, heart rate, and serial ECG, while a Doppler echocardiogram was conducted on day 14 in all subjects. No serious adverse events were reported throughout the 14-day study; however, minor adverse events, such as dry mouth, feeling hyperactive, headache, increased thirst, and difficulty initiating sleep, were reported, with no significant differences between treatment and placebo groups. In addition, there were no significant differences between the treatment and placebo groups with respect to heart rate, blood pressure, serial ECG, Doppler echocardiogram, left ventricular ejection fraction, and heart valve function.

In a 6-week, double-blind, randomized, placebo-controlled study, 23 healthy obese adults received daily either a combination product (528 mg caffeine, 900 mg St. John’s Wort) containing 975 mg of C. aurantium extract (6% p-synephrine or 58.5 mg p-synephrine) (n=9), a placebo (maltodextrin) (n=7), or nothing (control) (n=4) (Colker et al., 1999). The study also included diet and exercise components. Study parameters included body composition and metabolic variables (taken at baseline and at 6 weeks), plasma lipid levels, and mood states. ECG was monitored at baseline and at 3 and 6 weeks. Twenty out of 23 subjects completed the study, with reasons for discontinuation reportedly unrelated to treatment. No significant adverse effects were reported. Significant reductions in body weight, percentage body fat, and fat mass were observed in the treated group compared to baseline values. The basal metabolic rate of the treated subjects was significantly increased, whereas the placebo group showed a significantly decreased metabolic rate compared to baseline, which, in a review by Preuss et al. (2002), was suggested to be related to the implementation of caloric restriction throughout the
study. No significant changes in ECG, serum chemistry, urinalysis, cholesterol, triglyceride levels, fatigue and vigor (as assessed by a Profile of Mood States questionnaire), heart rate, or blood pressure were observed in any treatment group.

To examine the effects of an ephedrine- and p-synephrine-based product on body mass, body composition, metabolic variables, and mood states in healthy overweight adults, 30 subjects were randomly assigned to the experimental or placebo group (Kalman et al., 2000). For 8 weeks, the experimental group received a capsule containing 20 mg ephedrine alkaloids, 5 mg p-synephrine, 200 mg caffeine, and 15 mg salicin twice daily. The study included diet and exercise components and examined body mass, body composition, metabolic parameters, mood states, ECG, blood pressure, and pulse monitoring at baseline and weeks 4 and 8. Twenty-five out of 30 subjects completed the study, with reasons for dropouts reportedly unrelated to treatment. The experimental group had a significantly greater weight loss compared to the placebo group, with a 16% decrease in body fat compared with a 1% increase for the placebo group. No significant changes in blood pressure, serial ECGs, heart rate, serum chemistry, or caloric intake were noted throughout the study.

No significant differences in heart rate, SBP, DBP, or overall incidence of adverse events were reported between healthy overweight subjects consuming a multi-ingredient product providing 36 mg p-synephrine and 308 mg caffeine per day for 8 weeks or those consuming a rice powder placebo in a study designed to assess the effects of the product on resting metabolic rate (Zenk et al., 2005). Of the 7 adverse events reported in the active product group, 2 (1 case each of nausea and urticaria) were considered to be possibly related to treatment. The multi-ingredient product also contained 3-acetyl-7-oxo-dehydroepiandrosterone, coleus forskohlii extract, yerba mate, piper nigrum, and dandelion leaf and root powder.

As with the use of p-synephrine containing products, there have been several case reports with combination products containing synephrine/caffeine and/or other ingredients of adverse cardiovascular effects (Health Canada, 2004; Nasir et al., 2004; Nykamp et al., 2004; Bouchard et al., 2005; Holmes and Tavee, 2008; Smedema and Müller, 2008; Thomas et al., 2009; Moaddeb et al., 2011). The relationship of these effects to the consumption of the dietary supplements involved is difficult to assess given the lack of details regarding concomitant exposures to other products, existing medical conditions, and lifestyle factors.

Stohs (2010) conducted a critical review of 22 adverse event reports received by the U.S. FDA between April 2004 and October 2009 and 10 clinical case reports published during the same time period. In each of the reports, C. aurantium or p-synephrine were considered as the most probable cause of the adverse event; however, all cases involved the use of products that contained multiple herbal ingredients and multiple alkaloids, thus it is not possible to ascribe the reported effects to a single ingredient. Furthermore, in the majority of the cases, additional confounding factors were present and may have contributed to or caused the observed adverse event. Many of the discussions in the clinical case reports centered on the structural similarity between ephedrine and p-synephrine; however, Stohs (2010) highlighted the structural differences that are responsible for their different pharmacological activities. Stohs (2010) also noted that p-synephrine is consumed in the diet at levels ranging from 6 mg from a typical sweet orange to 35 mg from an 8 ounce glass of mandarin orange juice with no reports of adverse
events. Based on the totality of the afore-mentioned information, Stohs (2010) concluded that *C. aurantium* and *p*-synepherine had been unjustifiably singled out as the causative agent in the adverse event and case reports.

Based on a report available on the Internet, it appears that Health Canada has recently conducted an assessment of the risk to human health of natural health products containing *p*-synepherine and caffeine (Marles, 2011). Following review of information pertaining to the structure, receptor binding properties, preclinical pharmacology and toxicology, conditions of use in humans, dose-response, adverse reaction reports, and exposure to *p*-synepherine, Health Canada concluded that doses up to 50 mg/day of *p*-synepherine alone and up to 40 mg/day *p*-synepherine plus 320 mg/day caffeine would not likely cause any adverse health consequences. Health Canada noted that in single ingredient products, the risk of cardiovascular adverse effects “had been overstated in the literature and probably incorrectly linked causally to case reports”. Nonetheless, a limit of 50 mg/day *p*-synepherine was determined, as at doses above 50 mg/day, the dose-response curve was considered not adequately characterized to assess safety.

**Discussion and Conclusions**

Although *p*-synepherine and caffeine have been assumed to produce similar clinical effects, but through different mechanisms, recent data regarding the isoform of synephrine present in *C. aurantium* extracts and from receptor binding studies indicates that *p*-synepherine is unlikely to have significant effects on inotropy, vasoconstriction, or blood pressure. At physiologically attainable serum concentrations, therefore, additive or synergistic effects on inotropy, vasoconstriction, and blood pressure also are unlikely. Although additive or synergistic effects on cardiovascular functioning are not anticipated based on the receptor binding properties of *p*-synepherine, it is prudent use submaximal amounts of *p*-synepherine in combination products that also include caffeine. This remains the conservative approach in the absence of specific data to support safety of a wide range of *p*-synepherine/caffeine dosage combinations.

Overall, it is concluded that as a single one time dose, up to 70 mg *p*-synepherine alone or 40 mg in combination with 320 mg of caffeine is not likely to cause adverse effects. It should be noted that the half-life of *p*-synepherine is approximately 3 hours (Haller et al., 2005; 2008). As a result, if dosing is spaced by at least 6 hours, slightly higher amounts of *p*-synepherine could be consumed. If taken as divided doses (2 or more) spaced out over the course of the day, 100 mg of *p*-synepherine (e.g., 50 mg twice a day or 33 mg thrice daily) alone, or 70 mg *p*-synepherine in combination with 400 mg caffeine (e.g., 35 mg *p*-synepherine and 200 mg caffeine twice per day), is unlikely to be associated with adverse effects. Total exposure to *p*-synepherine and/or caffeine should not exceed these limits. The human clinical data that are available do indicate that there may be some slight increase in blood pressure, and to a lesser extent heart rate, in some consumers of *p*-synepherine/caffeine combination products. Also, as noted by Rossato *et al.* (2011), Health Canada (Marles 2011), and Inchiosa (2011), there are several case reports of adverse cardiovascular effects of *p*-synepherine or *p*-synepherine/caffeine combination products. While these reports do not provide causal association, they do highlight the need for appropriate labeling of the product.
It is recommended that the product be labeled to indicate that: clearance from a physician is strongly 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 various medical conditions, taking certain medications, other dietary supplements, or wish to consume other caffeine-containing products. In particular, contraindicated populations would include: pregnant and nursing women; children; individuals with high blood pressure, cardiac arrhythmias, or heart, kidney, or liver disease; people with psychiatric or epileptic disorders; and those currently taking blood pressure medications, sympathomimetics, or over-the-counter dietary supplements (containing ephedrine, pseudoephedrine, or phenylpropanolamine). Also, the product should provide a dosing formulation (e.g., break the dose down into 2 or 3 daily servings) so as to allow for a “tolerance test” at the initiation of dosing and to reduce the likelihood of a pharmacological effect of bolus dosing. The incorporation of a tolerance period (i.e., consumption of 1/2 or 1/3 the suggested serving size) should also appear on the label.

Based on the foregoing, under conditions prescribed by the label, the use of p-synephrine alone or in combination with caffeine within the specified limits, is not considered to pose significant concerns. Under conditions of widespread use, there remains some potential (not quantifiable) for cardiovascular effects, or adverse events in persons with unrecognized sensitivity to caffeine or who have undiagnosed cardiovascular conditions; hence, the importance of the inclusion on the label of a strong recommendation for clearance by a physician prior to the use of the product.

Sincerely,

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References


