Part 5. Experience Based on Common Use in Food before 1958

The conclusion of GRAS status for the use of shea stearin as an ingredient in select foods is based upon scientific procedures.

Part 6. Narrative

Historical Consumption and Regulated Use of Shea Butter and Shea Butter Derivatives

Historical Consumption

Refined and unrefined shea butter have been consumed extensively in Africa as cooking oil since at least the 19th century as summarized by Fuji Oil Co. Ltd. and also reported in the more recent published literature (FR, 1998; Honfo *et al.*, 2014). Exportation of shea butter from Africa to Europe has occurred since the latter part of the 19th century and it became more significant in the 1930s. In Europe, the oil was refined or fractionated and used as a cooking oil, as a cocoa butter substitute, and for making margarines. In the 1950s, the solvent fractionation process for shea butter was developed. This process allowed the manufacture of fractions suitable for the chocolate industry (FR, 1998). The fractioned product shea stearin derived from shea butter is used as a component in cocoa butter equivalents (CBEs) and cocoa butter improvers (CBIs) in the chocolate and confectionery industry.

Regulated Uses

United States

Sheanut oil was affirmed as GRAS by the FDA (21 CFR §184.1702) in 1998 for use in confections and frostings, coatings of soft candy, and sweet sauces and toppings at levels not to exceed cGMP; this GRAS affirmation was completed in response to a petition submitted by Fuji Oil Company Ltd. (GRASP 8G0343). As detailed in the Final Rule on the GRAS Affirmation of sheanut oil (FR, 1998), the product produced from crude oil derived from sheanut is described as "refined sheanut oil" and meets specifications consistent with other food-grade oils as described in the FCC at that time (i.e., FCC4).

Shea olein, another fractioned product from shea butter, was determined to be GRAS for use in select conventional foods as defined by BUNGE Loders Croklaan BV in 2019 (Bunge, 2019); FDA was notified of the GRAS conclusion (GRN 850) and responded with a "no questions" letter (FDA, 2020).

CODEX

Shea butter is the parent material from which shea stearin is derived. It is often consumed in its unrefined form in areas where it is harvested locally, but it is more typically subjected to refining, which reduces impurities to produce a clean tasting white paste. A regional standard

for shea butter was adopted by Codex in 2017 and amended in 2020; the standard applies to unrefined shea butter intended for direct consumption, or as an ingredient in the manufacture of food products (CXS 325R-2017 – CODEX, 2020).

European Union

There are no restrictions on the use of shea butter and its fractions in the EU, with the exception of foods with a standard of identity, such as chocolate. In 2000, the European Union provided regulatory clearance for the use of refined shea stearin as a partial substitute for cocoa butter fats used in chocolate production (up to 5% content by weight is allowed) (Directive 2000/36/EC) (EC, 2000).

Regulatory History of Other Plant-Derived Oils

Many plant oils consumed in the diet are GRAS by their history of safe use and not specified in 21 CFR, though several oils have food grade specifications defined in FCC. Examples of commonly consumed plant-derived oils include soy oil, corn oil, and also cocoa butter. Plant-derived oils with triglycerides that are compositionally and structurally similar to the shea stearin that is the subject of this GRAS dossier have been affirmed as GRAS for use in foods by FDA (21 CFR §184.1259; 21 CFR §184,1702) or FDA has been notified of a GRAS conclusion and responded with a letter of no concern (GRN 654). Additionally, numerous plant-based, edible oils have been concluded to be GRAS for use in foods and FDA responded to notice of the GRAS status of the intended use of these oils with letters of "no question".

Safety Evaluation of Shea Stearin

Introduction

The subject matter of this GRAS notice is shea stearin obtained from the fruit of *Vitellaria paradoxa.* Shea stearin is a fractionated vegetable fat composed of triglycerides and a small fraction of unsaponifiable matter. Analytical data demonstrate that triglycerides account for approximately 99% of the fat by weight, while the concentration of unsaponifiable matter is approximately 1% of the substance by weight. The safety of shea stearin was evaluated from evidence on the safety of dietary fats in general, and the specific components in shea stearin, namely triglycerides and unsaponifiable matter. As a means of establishing the safety of shea stearin, AAK examined information on the compositionally similar product shea olein, which is derived from the same source material as shea stearin. Data on studies of shea butter and shea butter extracts containing unsaponifiables were likewise used to bridge to the safety of the similar, albeit much lower, levels of the same unsaponifiables in shea stearin.

The evidence supporting the safety of the intended use of AAK's shea stearin is reviewed below. A series of literature searches was conducted in PubMed to identify information pertinent to the safety review; a summary of the PubMed search strings is provided in Appendix D. The searches were most recently updated in September 2022.

Absorption, Distribution, Metabolism and Excretion of Fat

The principal fatty acids in shea stearin, namely stearic acid and oleic acid, as well as the fatty acids present in substantially lower concentrations (e.g., palmitic, linoleic, and arachidic), are long-chain fatty acids with a well-known fate. Specifically, most of the consumed stearic acid is rapidly converted to oleic acid (C18:1n9) by stearoyl-CoA desaturase in the liver (Grundy 1994).

Shea stearin is a fat and it follows the normal metabolic pathway of other fats. The metabolic fate of fat is reported in the general nutrition literature (IOM, 2005; Lichtenstein and Jones, 2012; Nelson and Cox, 2008). Briefly, digestion of fat begins in the oral cavity. Lingual lipase is released, which hydrolyzes fatty acids from the sn-3 position of the triglyceride leaving sn-1,2 diacylglycerols. Activity of lingual lipase on food continues through the esophagus and into the stomach, where gastric lipase is secreted from gastric mucosal cells and cleaves triglyceride from the sn-3 position. Prior to entry of a food bolus into the small intestine, an estimated 10-30% of fat hydrolysis has occurred. The majority of triglyceride digestion and absorption therefore occurs in the small intestine portion of the gastrointestinal tract. Pancreatic lipase hydrolyzes the sn-1,3 ester linkages, resulting in a sn-2 monoacylglycerol and unesterified fatty acid products. Pancreatic co-lipase facilitates adhesion of pancreatic lipase to lipid droplets, while bile secreted from the gall bladder or liver serves to emulsify the intestinal contents.

The triglyceride digestion products as well as bile salts, phospholipids, cholesterol, and other fat-soluble substances then form micelles in the small intestine which are absorbed into the enterocyte. Within the enterocyte, triglycerides are reassembled and packaged with cholesterol and phospholipids into intestinally derived lipoproteins called chylomicrons. Chylomicrons, however, are secreted into lymphatic circulation and may be acted upon by the enzyme lipoprotein lipase on the surface of capillaries to hydrolyze triglycerides. Fatty acids freed from chylomicrons in circulation are primarily released into adipose for storage or taken up by muscle and oxidized for energy. Free fatty acids are then oxidized within mitochondria where they undergo sequential 2-carbon unit removal by β-oxidation.

Absorption of fatty acids is both chain length and saturation dependent, where short-chain fatty acids have a greater absorption efficiency relative to longer chains, and unsaturated fatty acids are better absorbed relative to saturated fatty acids. Thus, long-chain, saturated fatty acids are less readily solubilized into mixed micelles and absorbed across the intestinal epithelium. Once absorbed, the metabolism and distribution of longer chain fatty acids (>12 carbon units in

length), independent of saturation, is similar. Short- and medium-chain fatty acids can freely diffuse through mitochondrial membranes, while long-chain fatty acids require a specific transport system called the carnitine shuttle to access the inner mitochondrial membrane.

Studies demonstrating that shea butter absorption is comparable to absorption of other oils and fats are described by Thomasson (1956). The test product in this study was identified as shea butter with unsaponifiable matter of 5-7%. The study examined the rate of absorption of shea butter and 17 other natural oils and fats of vegetable and animal origin administered via oral gavage to male Wistar rats. At 3 to 11 hours following administration, the tested fats were examined at points along the digestive tract. The investigators categorized the fats and oils by rate of absorption, with shea butter exhibiting a slower rate of absorption but within the range of tested substances. Growth rates among rats administered one of the oils or fats were previously reported in feeding studies conducted by Thomasson (1955), with shea butter exhibiting similar growth indices albeit a reduced caloric value relative to other test materials.

As part of the GRAS petition review for sheanut oil, FDA reviewed the available studies on shea butter absorption (Thomasson, 1955; Thomasson, 1956) in addition to a third study (Sawadogo and Bezard, 1982) and concluded that the studies demonstrated that the absorbability of sheanut oil was comparable to that of other oils and fats concluded to be GRAS, and that animals fed sheanut oil demonstrated growth rates comparable to animals fed GRAS oils and fats (FR, 1998).

The digestive properties of shea butter including rate of hydrolysis, bioaccessibility, and micellar phases formed in an in vitro digestion model were recently examined (Chabni *et al.*, 2022). Relative to olive oil and microalgae oil, shea butter was observed to have a reduced rate of hydrolysis. Shea butter also was found to have larger micellar and precipitate phases relative to olive oil. These observations are not unexpected since the digestibility of a fatty acid is known to be related to its melting point. A lower melting point corresponds to greater digestibility since pancreatic lipase is more active against unsaturated fatty acids compared to saturated fatty acids (Gurr *et al.,* 2002).

Safety Evidence on Shea Stearin

Two *in vitro* genotoxicity studies were conducted on the shea stearin. A GLP, guideline compliant[1] Ames assay was performed by an independent laboratory with AAK's shea stearin conforming to specifications in Table 9 at levels ranging from 0 to 5000 µg/plate using the plate incorporation method ± S9 metabolic activation (Dubey, 2022). The test strains that were used

[1] OECD Guidelines for Testing of Chemicals, Section 4 (Test No. 471): "Bacterial Reverse Mutation Test (2020); U.S. FDA Toxicological Principles for the Safety Assessment of Food Ingredients, Redbook 2000, IV.C.1.a. "Bacterial Reverse Mutation Test" (2007); ICH S2 (R1) Guidance on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use (2012).

in the assay were *S. typhimurium* strains TA98, TA100, TA1535, and TA1537, and *E. coli* strain WP2 uvrA. The positive control substances induced the expected increases in revertant colony counts ± S9 metabolic activation, confirming the validity of the test and activity of the metabolic activation system. Overall, no concentration-related or test substance-related increases in the number of revertant colonies was observed in any of the tested strains ± S9 metabolic activation. Thus, shea stearin was determined to be not mutagenic under the stated experimental conditions.

A GLP, guideline compliant[2] *in vitro* mammalian micronucleus assay in human lymphocytes also was performed with AAK's shea stearin conforming to specifications in Table 9 in two experiments as summarized in Table 14 below (Graf, 2022).

Table 14. Study design for the in vitro mammalian micronucleus assay

	Without S9 metabolic activation		**With S9 metabolic activation**
	Experiment I	**Experiment II**	**Experiment I**
Exposure period	4 h	44 h	4 h
Cytochalasin B exposure	40 h	43 h	40 h
Preparation interval	44 h	44 h	44 h
Total culture period[a]	92 h	92 h	92 h

[a] Exposure started 48 h following culture initiation.

The concentrations of test material evaluated for micronuclei frequencies in experiment I (short-term exposure, 4 h), without and with metabolic activation, were 15.6, 31.3, and 62.5 µg/mL. The concentrations of test material evaluated for micronuclei frequencies in experiment II (long-term exposure, 44 h), without metabolic activation, were 3.13, 12.5, 37.5, and 75 µg/mL.

The test material was dissolved in tetrahydrofuran and diluted in cell culture medium to reach a final concentration of 0.5% v/v tetrahydrofuran in the samples. In experiment I without and with metabolic activation, precipitation was noted at the concentrations ≥ 62.5 µg/mL following 4 h of treatment. No precipitate of the test material was noted in experiment II without metabolic activation. It was concluded that the test substance, shea stearin, is not clastogenic and/or aneugenic under the stated experimental conditions.

In conclusion, evidence from unpublished studies conducted by AAK through an outside laboratory demonstrates that shea stearin, as defined in Table 9, is not mutagenic, and is not clastogenic and/or aneugenic in OECD-compliant tests conducted to examine these parameters.

[2] OECD Guidelines for Testing of Chemicals, Section 4, No. 487, "*In Vitro* Mammalian Cell Micronucleus Test", adopted 20 July. 2016; Commission regulation (EU) 2017/735 B.49 "*In Vitro* Mammalian Cell Micronucleus Test", dated February 14, 2017.

As unpublished data, these studies provide corroborative evidence of the safety of shea stearin. No pre-clinical studies on shea stearin were identified in the published literature.

Safety Evidence on Fat and Fatty Acids in Shea Stearin

Fat

Fat is a macronutrient with demonstrated essentiality in the diet. The typical human diet provides in the range of 25% to 35% of energy as fat, with triglycerides accounting for approximately 98% of total fat (Lichtenstein and Jones, 2012). In a daily diet of 2,000 kcal and assuming 30% energy from fat, an individual consumes 600 kcal as fat, or 67 grams fat, of which approximately 66 grams are consumed as triglycerides. The metabolic pathways of these substances are well understood as documented in the general nutrition literature and reviewed above (IOM, 2005; Lichtenstein and Jones, 2012; Nelson and Cox, 2008). As a macronutrient, fat, and fat as a triglyceride, has demonstrated safety in the diet.

Fatty Acids in Shea Stearin

The body of research on specific fatty acids, food matrix, and sources of fats is extensive and growing. The effects of saturated fatty acids on health are of particular importance, as evidence shows that replacing saturated fats with unsaturated fats can reduce serum total and low-density lipoprotein cholesterol (DGAC, 2020).

Stearic acid, the predominant fatty acid in shea stearin, is a saturated, long-chain fatty acid found in both animal and vegetable fat sources. This fatty acid constitutes approximately 8% of total fatty acids consumed by an average individual in the United States (USDA, 2020). Regarding stearic acid, evidence indicates that increased intake of stearic acid is not associated with alterations of serum lipid levels (Mensink, 2016; Sellem *et al*., 2021).

Oleic acid, a constituent of many vegetable oils, is the major monounsaturated fatty acid in the diet and a product of stearic acid desaturation. This fatty acid constitutes approximately 32% of total fatty acids consumed by an average individual in the United States (USDA, 2020). Oleic acid is also the primary component of triacylglycerol molecules stored in human adipose tissue (Tan *et al*., 2015).

A safety assessment of fatty acids including stearic and oleic acids, the major fatty acids in shea stearin, was published in 1987 (CIR, 1987). The results of oral acute, sub-chronic and chronic studies in animals administered stearic acid or oleic acid by gavage or via the diet as summarized by the CIR Expert Panel are presented in Appendix E. No findings in these studies suggest that intake of the major fatty acids present in shea stearin, namely stearic acid and oleic acid, would be unsafe.

Fat and Fatty Acids from Shea Stearin in the Context of the Total Diet

Shea stearin has an overall composition that conforms to that of other edible fats with regard to triglyceride content, di- and mono-glyceride content, and unsaponifiable matter (FR, 1998). The metabolism of triglycerides in shea stearin produces mainly stearic acid, oleic acid, palmitic acid, linoleic acid, and 2-monoglycerides. These components are naturally part of glycerides, lipids, lipoproteins, and membranes of both plants and animals. Moreover, these specific fatty acids, monoglycerides, and glycerol components are found in a wide range of edible fats, oils, and emulsifiers that are GRAS, and therefore are part of the human diet and considered safe. Shea stearin therefore is similar compositionally and in metabolic fate to common fats in the diet.

The Institute of Medicine (IOM) identified acceptable macronutrient distribution ranges for fat as a percent of energy at 30-40% for children ages 1 to 3 years, 25-35% for children ages 4 to 18 years, and 20-35% for adults ages 19 and older (IOM, 2005) In a reference diet providing 2,000 kcal, adults may consume 400 to 700 kcal as fat per day, which is equivalent to 44 to 78 g of fat per day. The per user estimated daily intake of 48 g shea stearin for the U.S. population at the 90th percentile, which is approximately 99% fat as triglyceride, is well within the acceptable intake of fat established by the IOM. Thus, it is reasonable to conclude, based on the well-established safety of fat, that intake of triglyceride from the proposed use of shea stearin resulting in a per user 90th percentile intake of 48 g/person/day is safe.

Dietary guidance also recommends that saturated fat provide no more than 10% of energy (USDA/HHS, 2020), though usual nutrient intake data from USDA show that among the U.S. population age 1 year and older in 2015-2018, saturated fat accounted for 11.8% of energy on average and 14.5% of energy at the 90th percentile of intake (USDA, 2021), and, as already noted, will unlikely reach that level due to supply constraints and the functionality of shea stearin. Saturated fatty acids account for approximately 63.7% of the fatty acids in shea stearin based on the composition of representative batches (i.e., the average of values presented in Table 5 for 16:0, 18:0, and 20:0). With per user mean and 90th percentile intakes of shea stearin at 25 and 48 g/person/day, respectively, saturated fat from shea stearin is estimated to account for 7.2% and 13.8% of total energy, respectively, in a 2000 kcal diet, and therefore is in the range of intake in the current diet.

Safety Evidence on the Unsaponifiable Matter in Shea Stearin

Introduction

Shea stearin is a fractionated vegetable fat composed of triglycerides and a small fraction of unsaponifiable matter. An assessment of the safety of shea stearin vegetable fat therefore includes consideration of the safety of the unsaponifiable matter.

In the fractionation step of shea butter, the unsaponifiable fraction largely partitions into the shea olein fraction (Table 6), resulting in a significantly lower concentration of unsaponifiable matter in shea stearin compared with the concentration in shea olein. Analytical data demonstrate that the mean concentration of total unsaponifiable matter in shea stearin is approximately 1% (maximum of 3%) versus approximately 6% in shea olein (maximum of 9% as specified in GRN 850). Analytical data also indicate that total sterols/triterpenes account for approximately 50% of total unsaponifiable matter in shea stearin (i.e., 0.5 g of 1.0 g in 100 g shea stearin) and approximately 90% of total unsaponifiable matter in shea olein (i.e., 5.5 g of 6.0 g in 100 g shea olein). Furthermore, the normalized percent distribution of sterols/triterpenes in shea stearin and shea olein are similar (Table 6, also Appendix F Table 1). The concentration of the remaining components of the total unsaponifiable matter is similar in both stearin and olein fractions at approximately 0.5 g per 100 g

Likewise, the same sterols/triterpenes present in shea stearin are found in other shea-derived materials and in similar normalized percentage distributions, and relative to shea stearin, the other materials contain higher concentrations of sterols/triterpenes. Examples of other shea-derived materials include shea butter and shea butter extracts. The composition of a variety of shea-derived materials used in metabolic, pre-clinical, and clinicals studies is summarized in Appendix F. Given the compositional similarities of the unsaponifiable matter in all shea-derived materials (e.g., shea butter, shea stearin, shea olein, and shea sterol concentrate), metabolic, pre-clinical, and clinical studies using these test materials can be used to assess the fate of sterols/triterpenes in shea stearin.

ADME of Unsaponifiable Matter

ADME of the unsaponifiable matter in shea stearin can be derived through examination of a published study on the similar product, shea olein. The oral absorption and excretion of the unsaponifiable matter in shea olein was examined in both rats and humans (Earl *et al.*, 2002a). The investigators noted that the greatest proportion of unsaponifiable matter in shea olein is represented by sterols/triterpenes. Shea olein used in the studies was analyzed and reported to contain 8.2% sterols/triterpenes, with 97.4% of the sterols/triterpenes as 4,4-dimethyltriterpenols and 4,4-dimethylsterols, 2.1% as 4-desmethylsterols, and 0.5% as 4-α-methylsterols.

Two experiments were conducted using Wistar rats that received shea olein in a semisynthetic diet (Earl *et al.*, 2002a). In the first experiment (exposure to a dietary fat level of 5%), groups of 24 male rats received either control feed, feed containing 0.5% shea olein, or feed containing 5% shea olein for one week, with control feed administered to all rats the week prior and the week following the exposure week. Feces were collected and pooled weekly for each treatment group throughout weeks 2 and 3. In the second experiment (exposure to a high-fat diet), 2 groups of 15

rats per sex received either 10% or 20% shea olein in the feed for 3 weeks. Feces were collected and pooled for each treatment group in week 3.

The dried fecal matter of the rats was analyzed with thin-layer and gas-liquid chromatography for fecal lipid, total sterol, differential sterol levels, and sterols/triterpenes (reported in the study as 4,4-dimethylsterols), the main components of the unsaponifiable matter of shea olein. Excretion of sterols/triterpenes increased with the consumption of shea olein. Apparent absorption was estimated from the disappearance of sterols/triterpenes. The majority of the sterols/triterpenes was excreted unchanged and no preferential absorption was noted.

Earl *et al.* (2002a) also examined the oral absorption and excretion of sterols/triterpenes in shea olein, from which can be derived information on the absorption and excretion of sterols/triterpenes in shea stearin, in an 8-day study conducted in 4 male volunteers. On Day 3, the subjects consumed a single 25-g portion (~ 0.4 g/kg) of shea olein in mayonnaise. Shea olein contained approximately 8% sterols/triterpenes, therefore providing approximately 2 g sterols/triterpenes. Subjects did not consume any other vegetable fats during the course of the study. Feces were collected (days 3 to 8 inclusively), freeze-dried, weighed, and analyzed as described above. Excretion of triterpene alcohols increased with the consumption of shea olein, with a marked increase from baseline on Days 4 and 5 and a return baseline (approximate) on Day 8. Absorption of triterpene alcohols was estimated to be 13 to 49%. The sterols/triterpenes were largely excreted unchanged, and there was no preferential absorption of the sterols/triterpenes.

Results from these studies demonstrate that the sterols/triterpenes in shea olein are largely excreted unchanged with no preferential absorption, from which it is reasonable to conclude that the sterols/triterpenes in shea stearin are largely excreted unchanged with no preferential absorption. AAK notes that these studies were previously discussed in GRN 850 (pp 39-40) as evidence of the metabolic fate of sterols/triterpenes in a shea-derived product.

Pre-Clinical Data on Unsaponifiable Matter

Sub-Chronic Toxicity of Unsaponifiable Matter

The sub-chronic toxicity of the unsaponifiable matter in shea stearin can be determined through examination of a published study on the similar product, shea olein. AAK notes that this study was previously discussed in the GRAS notice for the use of shea olein in foods (GRN 850, pp 42-43) for evidence of the safety of unsaponifiable matter from shea. In this 13-week rat feeding study reported by Earl *et al.* (2002b), Wistar rats received a diet containing 20 wt % (10 to 15 g/kg bw/day) shea olein or hydrogenated shea olein. The concentration of unsaponifiable material in shea olein was reported to be 7.44% as sterols/triterpenes, with 97.3% of the

sterols/triterpenes as 4,4-dimethyltriterpenols and 4,4-dimethylsterols, 2.1% as 4-desmethylsterols, and 0.6% as 4-α-methylsterols. As summarized therein, Earl *et al.*(2002b) concluded that shea olein given at 20% of the diet (10 to 15 g/kg bw/day, equivalent to levels of 740 to 1120 mg/kg bw/day of unsaponifiable matter as sterols/triterpenes) was well tolerated and appeared to have no adverse effect on growth. AAK agrees with this conclusion. Given the compositional similarities with shea stearin, results from this study provide pivotal evidence of the safety of up to 1120 mg/kg bw/day unsaponifiable matter as sterols/triterpenes in shea stearin. The study also supports the safety of shea stearin at 20% of the diet (10 to 15 g/kg bw/day).

Chronic and Reproductive Toxicity of Unsaponifiable Matter

The carcinogenicity and reproductive toxicity of the unsaponifiable matter in shea stearin can be determined through examination of two published studies on the similar product, shea olein. The carcinogenicity and reproductive toxicity of shea olein and unrefined shea butter (referred to as "crude sheanut oil" within the study) from which shea olein is derived were evaluated in dietary studies using Colworth-Wistar rats (Baldrick *et al.* 2001 and Carthew *et al.*, 2001, respectively). AAK notes that these studies were previously discussed in the GRAS notice for the use of shea olein in foods (GRN 850, pp 43-46) for evidence of the safety of unsaponifiable matter from shea.

In the carcinogenicity study, Colworth-Wistar rats in groups of 50 rats/sex received diets containing 15% (approximately 7.5 g/kg bw/day) shea olein, 15% unrefined shea butter, or 15% palm oil (control) for a duration of 104 weeks beginning at weaning (21 days) (Carthew *et al.*, 2001). The rats used in the study were the F_1 progeny of animals used in the reproduction study described below (Baldrick *et al.* 2001). The free fatty acid content of the materials administered was 0.2%, 11.3%, and 0.2%, respectively, and the unsaponifiable matter was measured at 6.4%, 5.0%, and 0.1%, with the unsaponifiable matter in shea olein and shea butter corresponding to sterols/triterpenes. The sterols/triterpenes in shea olein were reported to be 95.6% as 4,4-dimethyltriterpenols and 4,4-dimethylsterols, 3.7% as 4-desmethylsterols, and 0.7% as 4-α-methylsterols. There were no adverse toxicological findings or evidence of tumorigenic potential in Colworth-Wistar rats following long-term (104 weeks) consumption at levels up to 15% shea olein in the diet (7.5 g/kg bw/day) as reported by the authors, which is equivalent to 480 mg/kg bw/day unsaponifiable matter from shea olein.

In the two stage reproductive study, Colworth-Wistar rats were comprised of four groups of 20 rats/sex (Study 1) and five groups of 50 rats/sex (Study 2). The animals received diets containing 7% or 15% hardened shea olein in comparison with 7% or 15% unhardened shea olein and commercially available materials including sheanut and palm oils, cocoa butter, and toffee powder during pre-mating, mating, pregnancy and offspring weaning. Reproduction was

assessed by using the number of litters and pups born plus survival and body weights at birth and at weaning on day 21. Skeletal evaluation using X-ray, clinical pathology, and a macroscopic examination were also performed for F_1 rats. The authors concluded that there was no evidence of reproductive toxicity for unhardened or hardened shea olein in the rat at levels corresponding to 7.5 g/kg bw/day, which is equivalent to 480 mg/kg bw/day of unsaponifiable matter as sterols/triterpenes from shea olein.

The results of these studies demonstrate no adverse effects of 15% shea olein in the diet and its associated unsaponifiable fraction on reproductive and developmental toxicity parameters. AAK agrees with this conclusion. Given the compositional similarities with shea stearin, results from these studies provide pivotal evidence of the safety of up to 480 mg/kg bw/day unsaponifiable matter as sterols/triterpenes in shea stearin. The study also supports the safety of shea stearin at 15% of the diet (7.5 g/kg bw/day).

Acute Toxicity of Unsaponifiable Matter (Unpublished Data)

In 2003, a New Dietary Ingredient Notification (NDIN) was filed for use of a purified extract from the nut of *Vitellaria paradoxa* (formerly *Butyrospermum parkii*) (sheanut tree) marketed as BSP-201 to be encapsulated and used as a dietary supplement. Each capsule was intended to contain 750 mg of BSP-201, including 50% unsaponifiable matter. With a recommended intake of four capsules twice daily, the maximum daily intake of BSP-201 is 6.0 g or approximately 3.0 g unsaponifiable matter. The notification was accepted for filing by the FDA (FDA, 2004).

Results of an acute toxicity study on an extract from the shea butter preparation containing approximately 50% unsaponifiable matter, consisting of esterified triterpene alcohols and sterols (Appendix F Table 2), were reported by BSP Pharma A/S as part of the Premarket Notification for BSP-201 and SheaNature (BSP Pharma A/S, 2003). The study was carried out in Wistar rats following the OECD 420 testing guideline. Single gavage doses of 2,000 mg test substance/kg bw were administered to rats (5 rats/sex); the rats were observed for at least 1, 3, and 6 hours after dosing and daily thereafter for 14 days. There were no deaths or other signs of toxicity and body weight gains were normal during the study period. Piloerection was observed in 3 animals 1 hour after treatment and in 5 animals 3 hours after treatment, though the authors suggested this may have been related to treatment and handling procedures. Erythema was observed in the intestine of 1 male and discoloration of the liver, spleen, and lungs was seen in 1 female during gross necropsy examination; the significance of these findings was not discussed in the report. The authors concluded that shea olein containing 50% unsaponifiable matter was not acutely toxic and the minimal lethal dose was greater than 2,000 mg of the test substance/kg bw (BSP Pharma A/S, 2003). AAK agrees with this conclusion. Given the compositional similarities with shea stearin (Appendix F Table 2), results from this unpublished study provides

corroborative evidence of the absence of acute toxicity from the unsaponifiable matter under the conditions of the study.

Additional Genotoxicity and Pre-Clinical Studies of Unsaponifiable Matter

Genotoxicity of Unsaponifiable Matter (Unpublished Data)

As previously reviewed, AAK's shea stearin is not mutagenic, and is not clastogenic and/or aneugenic. Genotoxicity testing of other materials derived from the parent material shea, namely shea olein and shea butter extract, also demonstrate the materials are not mutagenic and are not clastogenic and/or aneugenic.

A bacterial reverse mutation test and an i*n vitro* micronucleus test were conducted on the BUNGE shea olein material (Usta, 2018; van der Wijngaard, 2018). AAK notes that these unpublished studies were previously discussed in the GRAS notification for the use of shea olein in foods (GRN 850, pp. 41-42) for corroborative evidence of the safety of unsaponifiable matter from shea. Shea olein was determined to be not mutagenic and not clastogenic and/or aneugenic under the stated experimental conditions. Results of an Ames bacterial mutagenicity assay and an *in vivo* mouse micronucleus test on an extract from shea butter containing 50% unsaponifiable matter were reported by BSP Pharma A/S as part of the Premarket Notification for BSP-201 and SheaNature (BSP Pharma A/S, 2003). AAK notes that these unpublished genotoxicity studies on the BSP-201 material were previously discussed in the GRAS notification for the use of shea olein in foods (GRN 850, pp. 46-47) for corroborative evidence of the absence of genotoxicity of unsaponifiable matter from shea and it was concluded that the test substance was not genotoxic under the conditions of this study. AAK agrees with the conclusions from these unpublished studies. Further, the conclusions of these studies agree with findings from AAK's own genotoxicity studies.

Additional Pre-Clinical Data on Unsaponifiable Matter

In a study reported by Kao *et al.* (2016), an anterior cruciate ligament transection with medial meniscectomy was used to induce osteoarthritis in 40 male Wistar rats. Different doses of a shea butter extract referred to as SheaFlex75™ (112 mg/kg, 223 mg/kg, and 446 mg/kg), with 50-70% unsaponifiable material as sterols/triterpenes (Appendix F Table 2) were then intragastrically administered daily for 12 weeks after surgery. Body weight and the width of the knee joint were measured weekly. Additionally, incapacitance tests were performed at weeks 2, 4, 6, 8, 10, and 12 to measure the weight bearing of the hind limbs, and the morphology and histopathology of the medial femoral condyles were examined and evaluated using the Osteoarthritis Research Society International (OARSI) scoring system. This study showed that SheaFlex75™ reduced the swelling of the knee joint with osteoarthritis and restored its weight bearing after anterior cruciate ligament transaction with medial meniscectomy surgery in rats.

No abnormal behavior or alteration of body weight of the rats was seen. All rats survived for 12 weeks until sacrifice.

An additional study of shea-derived material (SheaFlex75™) was summarized in GRN 850. As detailed in GRN 850, Weidner (2003) compared the gastric effects of ibuprofen to the effects of unsaponifiable matter of shea butter. In a four-day study, 12 Sprague-Dawley rats were administered 2000 mg/kg of SheaFlex75™ dissolved in peanut oil via gavage. Additional groups of rats were administered either the control vehicle or 200 mg/kg of the positive control ibuprofen. Overnight fasted rats received intravenous injections of 1 mL of Evans blue (percentage unspecified) saline 30 minutes prior to sacrifice. The stomach and small intestines were scored for lesions. No adverse clinical signs were observed, and no adverse effects on body weight were reported except for a single rat treated with the ibuprofen positive control. In addition, no gastrointestinal lesions were observed in the negative control or in SheaFlex75™ treated rats. Conversely, a significant number of lesions were observed in ibuprofen treated rats compared to controls. The majority of these small intestinal lesions were observed in the aboral part of the jejunum and in the ileum. Four-day exposure to SheaFlex75™, at a dose of 2000 mg/kg bw/day did not results in any ulcerogenic effects in the rat. Taken together, the absence of gastrointestinal effects and treatment-related adverse clinical signs support the safety of an acute intake of up to 1500 mg/kg bw of unsaponifiable matter derived from shea.

Chen *et al.* (2019) investigated the effect of SheaFlex75™ on attenuating the progression of early and chronic osteoarthritis in a rat model of chronic osteoarthritis progression. Specifically, an anterior cruciate ligament transection with medial meniscectomy-induced osteoarthritis was employed using adult male Wistar rats. The surgery (32 rats) or sham surgery (8 rats) was performed at week 0 of the study. Fourteen days following surgery, the animals were randomized to receive a non-feed control or SheaFlex75™ oil extract at a dose of 223 mg/kg bw/day for 10 weeks. The SheaFlex75™ oil extract was administered via oral gavage. As reported by Kao *et al*., (2016), SheaFlex75™ contains 50-70% unsaponifiable material as sterols/triterpenes (Appendix F Table 2). Ten weeks following treatment, knee joint samples were obtained from the sham-operated (2 rats), non-feed controls (4 rats), and SheaFlex75™-treated rats (4 rats). Non-feed controls were then reassigned to receive SheaFlex75™ or continued as non-feed controls for another 12 weeks. SheaFlex75™-treated rats either discontinued treatment or continued to receive SheaFlex75™ for 12 weeks. Following study completion (i.e., week 24 post-surgery), rats were euthanized, and samples were obtained from the right knees for histologic assessments. The study authors showed that SheaFlex75™ attenuated osteoarthritis deterioration in rats that presented with osteoarthritis symptomatology and active histological damage. A protective effect of SheaFlex75™ was observed in both early and chronic stages of osteoarthritis in rats. In addition, protective effects on cartilage were observed for up to 12 weeks after treatment was discontinued. Long-term dietary ingestion of SheaFlex75™ in the osteoarthritis-induced rats was determined to be safe.

Summary of Pre-Clinical Data on Unsaponifiable Matter

In order to bridge to the safety of shea stearin, AAK examined pre-clinical studies on the compositionally similar product shea olein, which is derived from the same source material as shea stearin. Pre-clinical studies on shea butter, and shea butter extracts containing 50% unsaponifiables, also were used to bridge to the safety of the similar, albeit much lower, levels of the same unsaponifiables in shea stearin.

Pre-clinical studies assessing the sub-chronic, reproductive, and carcinogenic effects of shea olein in rats are reported in the peer-reviewed literature (Baldrick *et al.*, 2001, Carthew *et al.*, 2001, Earl *et al.*, 2002b). These studies provided shea olein at levels up to 20% in the diet (from 7.5 to 15 g/kg bw/day across the studies). The authors of each study concluded that diets containing shea olein were well tolerated at all tested doses, and no adverse effects were attributed to shea olein. The unsaponifiable material in shea olein, present as sterols/triterpenes, was tolerated and did not produce adverse effects in rats consuming approximately 480 mg/kg bw/day in the carcinogenicity study (Carthew *et al.*, 2001) to 1120 mg/kg bw/day in the 13-week rat-feeding study (Earl *et al*., 2002b). Given the compositional similarities with shea stearin, results from these studies provide pivotal evidence of the safety of up to 480 mg/kg bw/day unsaponifiable matter as sterols/triterpenes in shea stearin in the carcinogenicity study to 1120 mg/kg bw/day as sterols/triterpenes in the 13-week study.

These studies provided the pivotal data from which a conclusion that the intended use of shea olein is safe, with a per user 90th percentile intake of 30 g/person/day shea olein (0.56 g/kg bw/day) as detailed in GRN 850. This level of shea olein intake (0.56 g/kg bw/day) corresponds to approximately 31 mg/kg bw/day sterols/triterpenes assuming a typical concentration of 5.5 g per 100 g in shea olein, or approximately 48 mg/kg bw/day sterols/triterpenes assuming a maximum unsaponifiable concentration of 9 g per 100 g in shea olein, and 90% of unsaponifiable matter present as sterols/triterpenes.

Information from the unpublished but publicly available studies submitted to FDA for a new dietary ingredient derived from shea shows a lack of genotoxicity and absence of acute toxicity at the tested doses. Findings from additional published and unpublished studies provide corroborative evidence of safe intake of shea-derived fractions and also safe intake of sterols/triterpenes at the levels tested.

Clinical Data on Unsaponifiable Matter

Clinical studies have been conducted on shea butter extracts. These extracts provide a highly concentrated source of the sterols/triterpenes in the unsaponifiable matter that is compositionally similar to the unsaponifiable matter in shea stearin (Appendix F).

Six clinical trials with test products containing shea sterols/triterpenes are summarized in Table 15. Five of these studies (Chen *et al.*, 2013; Cheras *et al.*, 2010; Sierksma *et al.*, 1999; Weststrate and Meijer, 1998; Vissers *et al.*, 2000) were summarized in GRN 850 (pp. 48-51) as evidence supporting the safe intake of shea-derived sterols from the unsaponifiable material in shea; AAK agrees with those conclusions.

In three clinical trials, sheanut sterols/triterpene concentrates were added to margarine and consumed in quantities delivering 2.6 to 3.2 g sterols/triterpenes daily for 3 weeks (Sierksma *et al.*, 1999; Weststrate and Meijer, 1998; Vissers *et al.*, 2000); the interventions had no effects on measures including clinical chemistries, blood count, or lipids. The normalized percent distributions of sterols/triterpenes in these concentrates are similar to the distribution in shea stearin (Appendix F Table 3).

Daily intake of approximately 1.6 g sterols/triterpenes from shea butter (i.e., shea butter extracts) identified as SheaFlex70 or SheaFlex75 for 15-16 weeks in patients with knee osteoarthritis had no adverse effects on symptoms or markers of function, though adverse effects were not discussed (Chen *et al.*, 2013; Cheras *et al.*, 2010). In one study, healthy men consuming 3 g sheanut oil extract (BSP-201) daily for 22 days following intensive eccentric exercise reported no adverse effects (Arendt-Nielsen *et al.*, 2009). As previously noted, the SheaFlex and BSP-201 materials also are concentrated sources of unsaponifiable material (as sterols/triterpenes), with normalized percent distributions similar to those present in shea stearin (Appendix F Table 2).

Overall, no adverse effects were observed at doses of shea sterols/triterpenes ranging from 1.6 to 3.2 g/day, which is equivalent to consumption of approximately 300 to 600 grams shea stearin assuming a typical concentration of 0.5% sterols/triterpenes. The estimated daily intake of sterols/triterpene from the proposed use of shea stearin was estimated at 0.71 g/person/day at the 90th percentile of intake for the population ages 2 years and older, which is below the levels of intake reported in clinical studies.

Table 15. Clinical trials with test products that contain shea sterols/triterpenes: Pivotal evidence

Reference	Study Design	Population Characteristics	Shea Sterols/Triterpenes Test Article	Key Findings
Weststrate and Meijer, 1998	DB, cross-over RCT; 3.5 weeks/ arm	95 Generally healthy, normo- or mildly hyper-cholesterolemic, 45 ± 12.8 y	3.1 g total sterols/triterpenes per day in margarine fortified with sheanut oil sterol concentrate (30.4 g margarine x 10.15% sterols) consisting of a mixture of sterols esterified to cinnamic acid (69%), acetic acid (25%), and fatty acids (4%); sterol concentration	No treatment-related effects on blood chemistries (GGT, ALT, AST, ALP, albumin, glucose, urea, creatinine), serum total bile acids, and complete blood counts; no effect on

Reference	Study Design	Population Characteristics	Shea Sterols/Triterpenes Test Article	Key Findings
			10.15% in final product, including predominantly sterols/triterpenes (α-amyrin, butyrospermol, lupeol), other shea sterols, and other sterols.[a] Sheanut concentrate source: Loders Croklaan, Wormerveer, The Netherlands.	blood lipids compared to control margarine.
Sierksma *et al.*, 1999	DB, cross-over RCT; 3 weeks/ arm	76 Generally healthy, 44 ± 11 y	3.2 g total sterols/ triterpenes[b] per day in margarine fortified with sheanut oil sterol concentrate (24.7 g margarine x 133 mg sterols/kg) consisting of a mixture of sterols esterified to cinnamic acid (69%) and acetic acid (25%); sterol concentration 13.3% in final product, including 95.6% 4,4-dimethylsterols, 3.5% 4-desmethylsterols, and 0.9% 4-monomethylsterols.[c] Sheanut concentrate source: Loders Croklaan, Wormerveer, The Netherlands).	No treatment-related effects on blood chemistries (GGT, ALT, AST, ALP, albumin, glucose, bilirubin, creatinine) or hematology; no effect on blood lipids compared to control margarine.
Vissers *et al.*, 2000	DB, cross-over RCT; 3 weeks/ arm	60 Generally healthy, 18-59 y	2.6 g total sterols/ triterpenes per day in margarine fortified with sheanut oil sterol concentrate; total known plant sterols concentration 0.4% and triterpene alcohols (free sterol or triterpene alcohol equivalents) concentration 8.9%.[c] Sheanut concentrate source: Not specified.	No effect on blood lipids among the total population. No adverse effects were mentioned.
Arendt-Nielsen *et al.*, 2009	DB, parallel RCT; 22 days	20 Healthy men, 26 ± 0.61 y	3 g/day oral sheanut oil extract (BSP-201) (4 × 750 mg soft gel capsules). Each BSP-201 capsule contained 50% unsaponifiables.	None of the participants reported any adverse effects. Compared to placebo, reduced subjective muscle soreness on some though not all days post exercise.
Cheras *et al.*, 2010	DB, parallel RCT; 15 weeks	89 Patients with osteoarthritis in the hips or knees, mean 64.0 y (39 in the shea sterol	2.25 g 100% shea butter extract with 75% triterpene esters (1.575 g; SheaFlex70).	No adverse effects on biomarkers of inflammation, cartilage degradation, cartilage aggrecan synthesis, or bone

Reference	Study Design	Population Characteristics	Shea Sterols/Triterpenes Test Article	Key Findings
		group; 50 in the control group)		formation; adverse effects not mentioned.
Chen *et al.*, 2013	unblinded, intervention, no control; 16 weeks	33 Patients with knee osteoarthritis, 63.6 ± 5.8 y	6 SheaFlex75 pills, reported to provide 2.16 g sheanut oil extract (1.62 g; SheaFlex75).[c]	No adverse effects on symptoms or muscle function; adverse effects not mentioned.

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DB, double blind; GGT, L-γ-glutamyltransferase; RCT, randomized controlled trial; y, years

[a] See Appendix F for further details on the composition of the sheanut oil sterol concentrate; assume similar test article used in studies by Weststrate and Meijer, 1998; Sierksma *et al*., 1999; and Vissers *et al*., 2000.

[b] Value of 3.2 g shea-nut oil sterols as reported in the text of Sierksma *et al.*, 1999 (corresponds to 24.4 g/day spread and a sterols concentration of 133 g/kg); a value of 3.3 g/day shea-nut oil sterols from shea was reported in the abstract.

[c] Amount of extract as reported in GRN 850.

Additional Studies on Shea Butter or Shea Oil

Shea butter and fractions derived from shea are concentrated sources of stearic acid. Given their natural fatty acid profile, test articles prepared with shea butter/shea oil have been used in studies examining the nutritional impact of stearic acid intake on various measures of health. Although not directly designed to assess the safety of shea products, AAK concludes that they provide corroborative information for the overall conclusion on shea stearin safety.

As summarized in Appendix G, six clinical studies report use of test products identified as sheanut butter, shea butter, sheanut oil, or shea oil a component of a test article; these products were consumed daily for periods of 21 to 40 days (Berry *et al.*, 2007; Dougherty *et al.*, 1995; Park *et al.*, 1996; Snook *et al.*, 1999; Tholstrup *et al.*, 1994; Storm *et al.*, 1997). Details on the test products are limited and naming of the test products does not appear consistent, though based on the descriptions and time period in which the studies were conducted, the products likely represent refined shea butter. Daily intake of the shea products was not specified in all of these repeat intake studies, but among the studies that did provide information to estimate intake, daily intake ranged from 24 g to 139 g of product per day (Berry *et al.*, 2007; Snook *et al.*, 1999; Tholstrup *et al.*, 1994). Three additional trials were identified in which the effects of a test article containing shea(nut) butter/ oil as a source of stearic acid were examined following a single intake or intake over the course of a single day (Maljaars *et al.*, 2009; Sanders & Berry, 2005; Tholstrup *et al.*, 2003 & Tholstrup *et al.*, 1996), and one repeat intake study also included an acute assessment subsequent to the repeat intake intervention (Berry *et al.*, 2007). Intake of shea(nut) butter/ oil was approximately 42 to 50 g per meal in assessments of effects on lipids (Maljaars *et al.*, 2009; Sanders & Berry, 2005; Tholstrup *et al.*, 2003 & Tholstrup *et al.*, 1996), and an emulsion providing 6 g of fat from shea oil was administered in an assessment of satiety (Maljaars *et al.*, 2009). As noted, these studies were not designed to study the safety of shea(nut) butter/oil; the absence of reported adverse effects and the observed efficacy outcomes suggest

that shea products as provided in these interventions do not adversely impact biochemical measures.

Given the limited details on composition of test articles, these substances cannot be directly compared to shea stearin. Nonetheless, the studies provide evidence that consumption of shea butter (or shea oil), which is the source of both shea stearin and shea olein, can be consumed as a substantial fraction of fat in the diet without adverse effect. These studies provide corroborative evidence for the safety of in the intended use of shea stearin.

Safety of Phytosterols of Other Oils

The safety of consumption of phytosterols and stanols common to other oils (e.g., desmethylsterols including but not limited to sitosterol, sitostanol, campesterol, campestanol, stigmasterol, brassicasterol, and esters of these) has been considered by authoritative bodies outside the U.S. (WHO, 2009). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) critically evaluated the safety of phytosterols. Given that phytosterol and phytostanol esters and mixtures of phytosterols and phytostanols generally show similar effect profiles, the Committee considered establishing a group acceptable daily intake (ADI). In their review, toxicological studies with a range of phytosterols, phytostanols and their esters (focusing on desmethylsterols), were evaluated in double-blinded, placebo-controlled human studies in which these substances were added to the diet were evaluated. The Committee determined the acceptable daily intake (ADI) to be 0-40 mg/kg bw/day for phytosterols, phytostanols and their esters, and concluded that dietary exposure to phytosterols and phytostanols would typically be within the ADI range of 0-40 mg/kg bw/day. Based on the estimated intake of AAK's shea stearin by the U.S. population ages 2 years and older, the EDI of sterols/triterpenes from the proposed use is 6 mg/kg bw/day at the mean and 12 mg/kg bw/day at the 90th percentile (Table 13). This is well within JECFA's ADI.

Potential Allergenicity

The FDA requires listing of products derived from tree nuts including shea as allergenic, with the exception of highly refined oils such as shea stearin as defined in the Food Allergen Labeling and Consumer Protection Act (FALCPA) of 2004. AAK's shea stearin is refined, bleached, degummed and deodorized.

Data in the published literature indicate that shea butter does not contain any detectable IgE-binding protein residues as measured by Western blot and ELISA tests (Chawla *et al.,* 2011). Shea stearin is produced from refined shea butter which further reduces the risk for an allergenic reaction. The PubMed search for literature on shea (Appendix D) provided no evidence to

indicate that any allergic reactions have been reported from shea butter or from shea kernels or fractionated derivatives of these products.

Using the DNA from plastids, Anderberg *et al.* (2002) demonstrated a relationship between shea nut and Brazil nut. The latter cross-reacts with almond, hazelnut and peanut (Sharma *et al*. 2009). AAK analyzed the shea stearin described in this dossier (Table 9) for Brazil nut, almond and peanut allergens. Brazil nut allergen was analyzed using a PCR method and all other allergens were analyzed using an ELISA method. No allergen was detected in any of the tests.

In an Expert Opinion Statement from the University of Nebraska's Food Allergy Research and Resource Program the authors concluded that "shea nut butter does not pose any known or likely allergenic risk to consumers including individuals with pre-existing peanut or tree nut allergies" (Taylor *et al*., 2018).

GRAS Criteria

The regulatory framework for determining whether a substance can be considered Generally Recognized As Safe (GRAS) for its intended use in accordance with section 201(s) (21 U.S.C. § 321(s)) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. § 301 et. Seq.) ("the Act"), is set forth at 21 CFR 170.30, which states:

> General recognition of safety may be based only on the view of experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food. The basis of such views may be either (1) scientific procedures or (2) in the case of a substance used in food prior to January 1, 1958, through experience based on common use in food. General recognition of safety requires common knowledge about the substance throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food.
>
> General recognition of safety based upon scientific procedures shall require the same quantity and quality of scientific evidence as is required to obtain approval of a food additive regulation for the ingredient. General recognition of safety through scientific procedures shall ordinarily be based upon published studies, which may be corroborated by unpublished studies and other data information.

In the preamble to the final rule for GRAS notifications, FDA stated that a GRAS conclusion, based on scientific procedures, may be supported by scientific data (such as human, animal, analytical or other scientific studies), information, methods and principles, published or unpublished, appropriate to establish the safety of a substance under the conditions of intended use. The safety standard requires that there be a reasonable certainty of no harm under the conditions of intended use of the substance. To be eligible for a GRAS conclusion based on scientific procedures, there must be evidence of a consensus among qualified experts that the

proposed use is safe and the pivotal data and information supporting the safety of the ingredient's intended use must be publicly available.

Safety Assessment

Shea stearin that is the subject of this notice is a fractionated solid derivative of shea butter. Shea stearin is composed of triglycerides comprising primarily stearic and oleic acids, with smaller concentrations of other fatty acids. Shea stearin is produced from shea butter under cGMP using processes commonly used in the production of fats and oils. Analytical data from non-consecutive, representative lots of the subject material demonstrate that AAK's shea stearin product can consistently be manufactured so as to meet the established specifications.

Products derived from shea are recognized as safe ingredients globally and in the United States. Shea butter is consumed in Africa where it has a long history of use. A regional standard for shea butter was adopted by Codex that applies to unrefined shea butter intended for direct consumption or as an ingredient in the manufacture of food products. The European Union allows for use of refined shea stearin as a partial substitute for cocoa butter fats used in chocolate production (Directive 2000/36/EC) (EC, 2000).

In the United States, sheanut oil was affirmed as GRAS by the FDA (21 CFR §184.1702) in 1998 for use in confections and frostings, coatings of soft candy, and sweet sauces and toppings at levels not to exceed cGMP. Additionally, shea olein, which is made from the fractionation of shea butter and meeting specifications as defined in GRN 850, was concluded to be safe for use as an ingredient in select foods and FDA had no questions regarding the GRAS conclusion (FDA, 2020).

Shea stearin is a fractionated vegetable fat from shea butter composed of triglycerides and a small fraction of unsaponifiable matter. Analytical data demonstrate that triglycerides account for approximately 99% of the fat in shea stearin by weight, while the concentration of unsaponifiable matter is approximately 1% of the substance by weight.

The safety of shea stearin was evaluated from evidence on the safety of dietary fats in general, and the specific components in shea stearin, namely triglycerides and unsaponifiable matter. As a means of establishing the safety of shea stearin, information on the compositionally similar shea olein, shea butter, and shea butter extracts was examined. Shea olein, shea butter, and shea butter extracts are derived from the same source material as shea stearin, and studies on these materials were used to bridge to the safety of the similar, albeit much lower, levels of the same unsaponifiables in shea stearin.

Shea stearin has an overall composition that conforms to that of other edible fats with regard to triglyceride content, di- and mono-glyceride content, and unsaponifiable matter. Unpublished data from OECD-compliant tests conducted by AAK also demonstrate that shea stearin is not mutagenic and is not clastogenic and/or aneugenic. The metabolism of triglycerides in shea stearin produces mainly stearic acid, oleic acid, palmitic acid, linoleic acid, and 2-monoglycerides. These components are naturally part of glycerides, lipids, lipoproteins, and membranes of both plants and animals. Moreover, these components are found in a wide range of edible fats, oils, and emulsifiers that are GRAS, and therefore are part of the human diet and considered safe. Shea stearin therefore is similar compositionally and in metabolic fate to common fats in the diet. Furthermore, intake of total fat from the proposed use of AAK's shea stearin is within acceptable levels of fat, and intake of energy from saturated fat is within current ranges.

Shea stearin contains a small fraction of unsaponifiable matter. Analytical data demonstrate that the mean concentration of total unsaponifiable matter in shea stearin is approximately 1% (maximum of 3%), and that total sterols/triterpenes account for approximately 50% of total unsaponifiable matter in shea stearin (i.e., 0.5 g of 1.0 g in 100 g shea stearin). Published studies support the safety of the intake of the sterols/triterpenes component in the unsaponifiable matter of shea stearin (provided as shea olein/hardened shea olein) in rats consuming approximately 480 mg/kg bw/day in a carcinogenicity study (Carthew *et al.*, 2001), and up to 1120 mg/kg bw/day in the 13-week rat-feeding study (Earl *et al.*, 2002b). Evidence from unpublished studies corroborates these findings.

The per user 90th percentile estimated intake of shea stearin is 48 g/day, or 0.8 g shea stearin/kg bw/day, for the U.S. population ages 2 years and older. At the maximum concentration of 1.5% sterols/triterpenes in shea stearin, the per user 90th percentile estimated intake of sterols/triterpenes by the U.S. population ages 2 years and older is 0.71 g/person/day, or 12 mg/kg bw/day (i.e., 48 g shea stearin/day x 1.5 g sterols/triterpenes/100 g shea stearin, or 0.8 g shea stearin/kg bw/day x 1.5 g sterols/triterpenes/100 g shea stearin). Based on the measured concentration of sterols/triterpenes in shea stearin (0.5%), the per user 90th percentile estimated intake of sterols/triterpenes by the U.S. population ages 2 years and older is 0.24 g/person/day or 4 mg/kg bw/day. The estimated intake of sterols/triterpenes from the proposed use is well below levels demonstrated to be safe in published pre-clinical studies, and therefore can be concluded to be safe. The intended use of shea stearin thus has been determined by AAK to be GRAS on the basis of scientific procedures, relying on data in the published literature and corroborated by unpublished data.

Safety Conclusion

Collectively, the publicly available data related to shea stearin provide evidence that the proposed use of shea stearin resulting in a per user 90th percentile intake of 48 g/person/day is safe. This level of shea stearin intake is below the upper bound of the acceptable macronutrient distribution range established by the IOM for fat. Additionally, this level of shea stearin intake provides exposure to sterols/triterpenes below levels examined in pre-clinical studies and demonstrated to be well tolerated with no adverse effects, and below levels observed to be tolerated with no adverse effects in clinical trials. It is therefore reasonable to conclude that the proposed use of shea stearin in plant-based meat & poultry analogues including burgers/ground meat and sausages; plant-based dairy alternatives and dairy analogues (including butter, cheese, cream cheese, creamers, frozen desserts, milks, sour cream, and yogurt); fillings for cookies & wafers and confectionery; nut/seed spreads and butters; margarines/spreads; and bakery products including bars, biscuits, cakes, cookies, laminated dough products (e.g., Danish pastry/croissants), muffins, and pie crust, are safe, and safe within the meaning of the Act, i.e., the proposed use meets the standard of reasonable certainty of no harm under the conditions of intended use.

General Recognition of Safety

General recognition of safety through scientific procedures requires common knowledge throughout the scientific community knowledgeable about the safety of food ingredients that there is a reasonable certainty that a substance is not harmful under the intended conditions of use in foods. The regulatory and scientific reviews related to the consumption and safety of shea stearin are published in the scientific literature, and therefore are generally available and generally known among the community of qualified food ingredient safety experts. There is broad-based and widely disseminated knowledge concerning shea stearin and related substances and the common dietary components in these substances with the same metabolic fate, e.g., fatty acids and sterols/triterpenes. The data and publicly available information supporting the safety of the proposed use of shea stearin in select foods, as described herein are widely known and disseminated and are also commonly accepted among qualified food safety experts.

The GRAS Panel convened by AAK independently and critically evaluated all data and information presented herein, and concluded that shea stearin is GRAS for the intended uses in select foods based on scientific procedures. It is also the unanimous consensus opinion of this GRAS Panel that other qualified experts would concur with these conclusions. The GRAS Panel Signed Consensus Statement is located in Appendix H.

The intended use of shea stearin has been determined to be safe following the scientific procedures set forth in 21 CFR §170.3(b), thus satisfying the so-called "technical" element of the GRAS determination. Because this safety evaluation was based on generally available and widely accepted data and information, it also satisfies the so-called "common knowledge" element of a GRAS determination.

Discussion of Information Inconsistent with GRAS Determination

No information has been identified that would be inconsistent with a finding that the proposed use of shea stearin in foods, meeting appropriate specifications specified herein and used according to cGMP, is safe and GRAS.

Part 7. List of Supporting Data and Information in GRAS Notice

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