Dossier Complément Alimentaire

TRICHOPIL ADVANCED HAIR ACTIVE - 25050700

I. NUTRITIONAL EVALUATION

I-1 L-CYSTINE

a) Nutritional information

The following table gives the L-cystine requirement provided by the recommended use of the finished product with the amino acid requirement (AAR) of L-cystine in Europe.

Table 1 : Comparison of the daily quanities of L-cystine provided by the finish product and the AAR.

Amino acid	Amino-acid requirements (AAR)*	Daily dose with the product (mg)
L-cystine	4 mg/kg bw/d (280 kg/d)**	400 mg

*According to FAO/WHO Proteins and amino acids requirements in human nutrition, 2007
**Assuming a body weight of 70 kg in accordance with guidance defined by EFSA.

b) Clinical and bibliographical data

- **Evidence accepted by independent expert bodies, national and international committees, recognized text books, monographs and reviews.**

Table 2 : Established health claims for L-cystine

References	Endorsed claim
EFSA	ID 597: Hair and nails health

- **Evidence from clinical trials**

Cystine is a sulfur amino acid which contributes to the formation of hair as well as other integuments such as the nails. It plays a complementary role with methionine. Deficiencies in sulfur amino acids provoke hair loss.

L-cystine plays an essential role in the keratinogenesis and maturation of the capillary structure. It is one of the essential components in the hair and nail keratins where it is an agent

of reticulation and solidification. The sulphur atoms unite to form a solid and lasting bond (formation of disulphide bridges). Its metabolism is closely linked to that of zinc and depends on vitamin B6 (Schmutz and Le Maitre, 2003). In addition, it has an anti-oxidant action fighting oxidation phenomena affecting the integuments (Schmutz and Le Maitre, 2003).
Cystine helps increase the diameter of the hair, protects the hair sheath and stimulates growth in case of loss for which there is no pathological cause.

The following table summarizes the clinical trials performed with L-cystine.

Table 3 : Clinical trial performed with L-cystine

Reference	Title	Method	Tested product, dose, duration, number of subjects	Results
Budde *et al.* 1993	Systemic therapy of diffuse effluvium and hair structure damage.	Controlled clinical study.	72 female patients with diffuse hair loss and hair structure damage were administered 3 capsules per day containing: - vitamin B5 (60 mg calcium pantothenate), - L-cystine (220 mg).	Cysteine and vitamin B5 are superior to placebo in terms of hair quality and hair growth.
Hertel *et al.* 1989	Low dosage retinol and L-cystine combination improve alopecia of the diffuse type following long-term oral administration.	Pilot and double-blind studies.	36 and 47 patients suffering from hair loss in the pilot and the double-blind studies respectively were administered L-cystine (70 mg) + gelatine (700 mg) + retinol (18 000 IE).	The pilot study demonstrated a significant improvement, with reduction of the telogen rate by 8.3%, an increase of the anagen rate by 11%, and an increase of the hair density by 6.9%. In the double blind study the trichogram showed a significant decrease of the telogen rate by 13.5% compared with pathological baseline values. There was no change in the placebo group. The lowered anagen rate of 47.2% was improved by 8%, whereas the mean value in the placebo group decreased from 47.7% to 39.9%. In addition, the percentage of dysplastic anagen hairs improved by 7.4%, as against further impairment with an increase of 26% in the placebo group. During oral therapy no systemic side-effects were detected.
Gehring and Gloor, 2000	Use of the phototrichogram to assess the stimulation of hair groth : an in vitro study of women	Randomized, double-blind, placebo-controlled study	40 women with androgenic alopecia (nverum=21, nplacebo=19) were administered L-cystine (12 mg) + millet (rich in sulfur	The active group, but not the placebo group, reached the study endpoint, thus establishing study product efficacy in female androgenetic alopecia.

Reference	Title	Method	Tested product, dose, duration, number of subjects	Results
	with androgenetic alopecia		amino acids, 840 mg) + Vitamin B5 (60 mg) for 6 months.	
Petri, 1990	The efficacy of drug therapy in structural lesions of the hair and in diffuse effluvium--comparative double blind study.		60 patients with diffuse effluvium capillorum and agnogenic structural aterations of hair wer administered Pantogar® (calcium pantothenate L-cystine, Vitamin B1, yeast, keratin and PABA) for 4 months.	Pantogar® was effective and improved quality of hair and retarded hair loss.
Rostin, 1989	Lobamine-cystéine* alopécies, séborrhée, bilan thérapeutique (étude multicentrique sur 403 patients).	Multicentric open clinical trial study.	355 subjects (142 men and 213 women) suffering from alopecia wereadministered Lobamine® (methionine +cysteine) 700 mg and 300 mg per capsule x6/day) for 3 to 6 months.	At 3 months, the hair-loss stopped for 62% of the subjects associating with hair re-growth.

Clinical trials have shown that daily supplements of cystine (or cysteine) and methionine have a beneficial effect on hair loss, with daily doses of sulphated amino acids of up to 2 to 3 g (Gehring and Gloor, 2000 ; Hertel *et al.* 1989).

I-2 L-METHIONINE

a) Nutritional information

The following table gives the L-methionine requirement provided by the recommended use of the finished product with the Amino Acid Requirement (AAR) of L-methionine in Europe.

Table 4 : Comparison of the daily quanities of L-methionine provided by the finish product and the AAR.

Amino acid	Amino-acid requirements (AAR)*	Daily dose with the product (mg)
L-methionine	10 mg/kg bw/day (700 mg/kg)	100 mg

*According to FAO/WHO Proteins and amino acids requirements in human nutrition, 2007
**Assuming a body weight of 70 kg in accordance with guidance defined by EFSA.

b) Clinical and bibliographical data

- **Evidence accepted by independent expert bodies, national and international committees, recognized text books, monographs and reviews.**

Table 5 : Established health claims for L-Methionine.

References	Endorsed claim
EFSA	ID 597: Hair and nails health

- **Evidence from clinical trials**

Methionine is a sulfur amino acids which contribute to the formation of hair as well as other integuments such as the nails. It plays a complementary role with cysteine in eth metabolic process in particular in the biosynthesis of epidermal and integument keratins.
Methionine participates in the production of a great many basic substances (adrenaline, creatine). It is also a beneficial anti-oxidant playing a non-negligible anti-radicular role for the keratin structure. In fact, the hair and the integuments in general also undergo oxidation stress : some of the toxic effects induced by smoking (for example) are due to oxidation damage to the endothelial cells, induced by a deficiency in nitrogen oxide.

The following table summarizes the clinical trials performed with L-methionine.

Table 6 : Clinical trial performed with L-methionine

Reference	Title	Method	Tested product, dose, duration, number of subjects	Results
Goerz *et al.* 1996	Brittle and sparse hair with normal cystine content caused by methionine deficiency?	Report case	8-year-old girl with hair disorder.	The patient's hair has normal cystine content but is completely devoid methionine and reveals distinct changes of its viscoelastic parameters.
Rostin, 1989	Lobamine-cystéine* alopécies, séborrhée, bilan thérapeutique (étude multicentrique sur 403 patients).	Multicentric open clinical trial study.	355 subjects (142 men and 213 women) suffering from alopecia wereadministered Lobamine® (methionine +cysteine) 700 mg and 300 mg per capsule x6/day) for 3 to 6 months.	At 3 months, the hair-loss stopped for 62% of the subjects associating with hair re-growth.

I-3 L-ARGININE

a) Nutritional information

L-arginine is a protein amino acid present in the proteins of all life forms. It is classified as a semi-essential or conditionally essential amino acids. This means that under normal circonstances the body can synthesize sufficient L-arginine to meet physiological demands. L-arginine is essential for young children and for those with certain raregenetic disorders in which synthesis of the amino acid is impaired. Some stress conditions that put an increase demand on the body for the synthesis of L-arginine include trauma (including surgical trauma), sepsis and burns. Under these conditions, L-arginine becomes essential, and it is then very important to ensure adequate dietary intake of the amino acid to meet the increase physiological demands created by these situations.
L-arginine, even when it is not essential amino acid as defined above, is a vital one. In addition to participating in protein synthesis, it plays a number of other roles in the body. These include the detoxification of ammonia formed during the nitrogen catabolism of amino acids via the formation of urea. L-arginine is a precursor in the formation of nitric oxide, creatinine, polyamines, L-glutamate, L-proline, agmatin (a possible neurotransmitter in the brain) and the arginine-containing tetrapeptide tuftsin, believed to be immunomodulator. L-arginine is a glycogenic amino acid; it can be converted to D-glucose and glycogen if needed by the body or it can be catabolized to produce biological energy (Hendler, 2008).

b) Bibliographical data

Arginine is a precursor of nitric oxide (Saini *et al.* 2013). Nitric oxide is a vasodilator involved in the regulation of hair growth and acting on the proliferation and differentiation of keratinocytes, improving the vascularization of hair follicles helps to increase the number and induce hair growth (Wolf *et al.* 2003).

NO is synthesized by the intracellular enzyme, NO synthase (NOS), in a two-step oxidation of L-arginine, that produces equal parts of citrulline and NO (Palmer *et al.* 1988, Moncada and Higgs, 1993, Saini *et al.* 2013). NO is involved in regulating the activity of the hair follicle (Wolf *et al.* 2003). Androgen-dependent NO production by human dermal papilla cells is an important signaling pathway in the human hair follicle.

I-4 VITAMIN D3

- **Recommended Daily Allowances (RDAs)**

The following table compares the daily intake of Vitamin D3 provided by the recommended use of the finished product with the Recommended Dietary Allowances (RDA) of Vitamin D3 in Europe.

As a rule, 15% of the recommended allowance should be taken into consideration in deciding what constitutes a significant amount (Commission Directive 2008/100/EC of 28 October 2008).

Table 7: Comparison of the daily quantities of Vitamin D3 provided by the product and the RDAs and the Dietary Reference Intake (DRI).

	Daily dose with the Product	RDA (%)[1]	DRI[2]
Vitamin D3	5 µg	100	5 µg

- **Evidence accepted by independent expert bodies, national and international committees, recognized text books, monographs and reviews**

Table 8: Established health claims for Vitamin D3

References	Endorsed claim
Kragballe, 1997	Fight against hair drying
EFSA	Vitamin D contributes to normal cell division

- **Evidence from bibliographical data**

It participates in the process of cell division, including keratinization: it helps fight against the hair from drying (Kragballe, 1997; Ellison *et al.* 1997; Harmon and Nevins, 1994).

Evidence of beneficial effect of vitamin D3 in hair health has been widely studied *in vitro* and in animals (Kragballe, 1997; Ellison *et al.* 1997; Harmon *et al.* 1994; Vegesna *et al.* 2002; Jimenez and Yunis, 1992).

Table 9: Studies performed with vitamin D3

Reference	Title	Method	Tested product, dose, duration, number of subjects	Results
Ellison *et al.* 2007	Evidence for 1,25-Dihydroxyvitamin	*In vivo* study (cellular and	Experiments were run on transgenic mice.	A functional vitamin D endocrine system is critical for maintaining

1Commission directive 2008|100|EC of 28 october 2008 amending council directive 90/496/EEC on nutrition labeling for foodstuffs as regards recommended daily allowances, energy conversion factors and definitions.

2Martin A. Apports nutritionnels conseillés pour la population française, 3eme édition Paris: edition tec&doc.

Reference	Title	Method	Tested product, dose, duration, number of subjects	Results
	D3-independent Transactivation by the Vitamin D Receptor.	molecular data)		appropriate skin homeostasis. The results show a transcriptional activation of the vitamin D-responsive 24-hydroxylase promoter by VDR in primary keratinocytes that is independent of the 1,25$(OH)_2$D3 ligand.
Harmon and Nevins, 1994	Biphasic effect of 1,25-dihydroxyvitamin D3 on human hair follicle growth and hair fiber production in whole-organ cultures.	*In vitro* study	A whole organ culture system was used to test the effect of 1,25(OH)2D3 on human hair follicle growth and hair fiber production.	Relatively low concentrations (1-10 nM) of 1,25$(OH)_2$D3 stimulated the cumulative growth of hair follicle and hair fibers, by 52% and 36% respectively (concentration producing 50% of the maximum response [EC50] values of 0.3 nM). This study suggests that vitamin D3 may play a physiological role in maintaining optimal hair follicle activity.
Jimenez and Yunis, 1992	Protection from Chemotherapy-induced Alopecia by 1,25-Dihydroxy vitamin D3	*In vivo* animal study	Sprague-Dawley rats with chemotherapy-induced alopecia were subjected to topical application of 1,25$(OH)_2$D3: 0.1 and 0.2 µg.	In three separate experiments, 0.2 µg of topical 1,25$(OH)_2$vitamin D3 protected rats from alopecia induced by etoposide, Cytoxan and an Adriamucin-cytoxan combination. In another experiment 0.1 microgram protected rats from etoposide-induced alopecia at the site of application. It is concluded that 1,25$(OH)_2$vitamin D3 may offer a new and exciting approach to the prevention of chemotherapy-induced alopecia.
Kragballe, 1997	The future of vitamin D in dermatology.	A review of the literature focused on the cellular targets of vitamin D3 in the skin and within the immune system.	Not available	The vitamin D receptor has been detected in most skin cells, which means that keratinization, hair growth, melanogenesis, fibrogenesis, angiogenesis, and immune-mediated processes are potential targets for vitamin D3.Vitamin D3 analogs have been synthesized with a higher therapeutic index or a higher degree of selectivity than the natural form of vitamin D3.
Vegesna *et al.* 2002	Vitamin D3 analogs stimulate hair growth in nude mice.	*In vivo* animal study	1.25dihydroxy-vitamin D3 was administered to beige/ /nude/xid (BNX) nu/nu	Vitamin D3 dramatically stimulated the hair growth of nude mice. Hair growth occurred in a

Reference	Title	Method	Tested product, dose, duration, number of subjects	Results
			(nude) mice exhibiting congenital alopecia at dose previously found to inhibit the clonal proliferation of cancer cells but not associated with hypercalcemia (which is the major toxicity of vitamin D3).	cyclinal pattern, accompanied by formation of normal hair follicles and increase expression of certain keratins (Ha7, Ha8 and Hb3). It is concluded that vitamin D3 analogs seem to act on keratinocytes to initiate hair follicle cycling and stimulate hair growth in mice that otherwise do not grow hair.k

I-5 VITAMIN B5

- **Recommended Daily Allowances (RDAs)**

The following table compares the daily intake of Vitamin B5 provided by the recommended use of the finished product with the Recommended Dietary Allowances (RDA) of Vitamin B5 in Europe.

As a rule, 15% of the recommended allowance should be taken into consideration in deciding what constitutes a significant amount (Commission Directive 2008/100/EC of 28 October 2008).

Table 10 : Comparison of the daily quantities of Vitamin B5 provided by the product and the RDAs and the Dietary Reference Intake (DRI).

	Daily dose with the Product	RDA (%)[3]	DRI[4]
Vitamin B5	6 mg	300	5 mg

- **Evidence accepted by independent expert bodies, national and international committees, recognized text books, monographs and reviews**

Table 11: Established health claims for Vitamin B5

References	Endorsed claim

3Commission directive 2008|100|EC of 28 october 2008 amending council directive 90/496/EEC on nutrition labeling for foodstuffs as regards recommended daily allowances, energy conversion factors and definitions.

4Martin A. Apports nutritionnels conseillés pour la population française, 3eme édition Paris: edition tec&doc.

EFSA	ID 2874: hair and nails care

- **Evidence from clinical trials and bibliographical data**

Pantothenic acid (Vitamin B5) is involved in the Krebs cycle with the formation of glucose, an essential element in the functioning of the hair follicle (Fabre 1987). This vitamin is essential, among other factors, for the good health of the scalp (Gehring, 2000). In animals, signs of pantothenate (vitamin B5) deficiency have been examined. All species studied (humans, calves, pigs, dogs, rodents, cats, poultry, fish) exhibited deficiency signs affecting hair color, feather development, skin or gills. Development of normal feathering in birds (quails, pheasants, turkeys, chick, etc) required a higher dietary intake of pantothenate (Smith, 1996).

Table 12: Clinical trials performed with Vitamin B5 in humans

Reference	Title	Method	Tested product, dose, duration, number of subjects	Results
Brzezinska-wcislo, 2001	The clinical and trichological evaluation of vitamin B6 and calcium pantothenate influence on hair growth in feminine diffuse alopecia.	Open clinical study	46 women with symptoms of diffuse alopecia were administered vitamin B5 (100 mg calcium pantothenate twice daily for 4-5 months) + vitamin B6 (i.m. daily injections for 20-30 days).	On the basis of clinical and trichological studies it was revealed that vitamin B6 administered parenterally for a period of several weeks induces improvement in the hair condition in a number of women and it reduces the hair loss especially in alopecia of telogenic pathomechanism.
Budde *et al.* 1993	Systemic therapy of diffuse effluvium and hair structure damage.	Controlled clinical study.	72 female patients with diffuse hair loss and hair structure damage were administered 3 capsules per day containing: - vitamin B5 (60 mg calcium pantothenate), - L-cystine (220 mg).	Cystine and vitamin B5 are superior to placebo in terms of hair quality and hair growth.
Gehring *et al.* 2000	The phototrichogram as a method for assessing hair growth promoting preparations the example of a combination of millet fruit extract, L-cystine and calcium	Randomized double-blind study.	40 women with androgenic alopecia (nverum=21; nplacebo=19) were administered L-cystine (12 mg) + millet (rich in sulfur amino acids, 840 mg) + Vitamin B5 (60 mg) for 6 months.	The study endpoint was defined as change of anagen hair rate, as determined by phototrichogram, from abnormal (<80%) at baseline to normal (>85%) at the follow-up (after three months) and final visits (after 6 months). The active group, but not the placebo group, reached the study endpoint.

Reference	Title	Method	Tested product, dose, duration, number of subjects	Results
	pantothenate: results of an in vivo study in women with androgenetic alopecia.			
Petri, 1990	The efficacy of drug therapy in structural lesions of the hair and in diffuse effluvium--comparative double blind study.		60 patients with diffuse effluvium capillorum and agnogenic structural aterations of hair wer administered Pantogar® (calcium pantothenate L-cystine, Vitamin B1, yeast, keratin and PABA) for 4 months.	Pantogar® was effective and improved quality of hair and retarded hair loss.

I-6 VITAMIN B6

- **Recommended Daily Allowances (RDAs)**

The following table compares the daily intake of Vitamin B6 provided by the recommended use of the finished product with the Recommended Dietary Allowances (RDA) of Vitamin B6 in Europe.

As a rule, 15% of the recommended allowance should be taken into consideration in deciding what constitutes a significant amount (Commission Directive 2008/100/EC of 28 October 2008).

Table 13: Comparison of the daily quantities of Vitamin B6 provided by the product and the RDAs and the Dietary Reference Intake (DRI).

	Daily dose with the Product	RDA (%)[5]	DRI[6]
Vitamin B6	5.4 mg	386	1.5-1.8 mg

5Commission directive 2008|100|EC of 28 october 2008 amending council directive 90/496/EEC on nutrition labeling for foodstuffs as regards recommended daily allowances, energy conversion factors and definitions.

6Martin A. Apports nutritionnels conseillés pour la population française, 3eme édition Paris: edition tec&doc.

- **Evidence accepted by independent expert bodies, national and international committees, recognized text books, monographs and reviews**

Table 14: Established health claims for vitamin B6

References	Endorsed claim
EFSA	ID 74: Bone/teeth/hair/skin and nail health

- **Evidence from clinical trials and bibliographical data**

Vitamin B6, in the form of pyridoxal phosphate, is an active coenzyme intervening in many enzyme reactions involved in the metabolism of sulfur amino acids (methionine, taurine and cysteine). It is required for the transformation of homocystine into cysteine. As a result, the metabolism of methionine and cysteine closely depends on vitamin B6 (Sturman, 1981). Therefore a vitamin B6 deficiency induces a reduction in the incorporation of cysteine in the hair (Sturman, 1981; D'agostini, 2007). Since cysteine and methionine participate in keratogenesis, in the maturation of the capillary structure and thereby in the formation of good hair quality, vitamin B6 is necessary to maintain the natural integrity of the hair. A deficiency of this coenzyme can lead to many manifestations including alopecia (SCF, 2000).

Table 15: Clinical trials performed with vitamin B6 in humans

Reference	Title	Method	Tested product, dose, duration, number of subjects	Results
Brzezinska-wcislo, 2001	The clinical and trichological evaluation of vitamin B6 and calcium pantothenate influence on hair growth in feminine diffuse alopecia.	Open clinical study.	46 women with symptoms of diffuse alopecia were administered vitamin B5 (100 mg calcium pantothenate twice daily for 4-5 months) + vitamin B6 (i.m. daily injections for 20-30 days).	On the basis of clinical and trichological studies it was revealed that vitamin B6 administered parenterally for a period of several weeks induces improvement in the hair condition in a number of women and it reduces the hair loss especially in alopecia of telogenic pathomechanism.

I-7 VITAMIN B8

- **Recommended Daily Allowances (RDAs)**

The following table compares the daily intake of Vitamin B8 provided by the recommended use of the finished product with the Recommended Dietary Allowances (RDA) of Vitamine B8 in Europe.

As a rule, 15% of the recommended allowance should be taken into consideration in deciding what constitutes a significant amount (Commission Directive 2008/100/EC of 28 October 2008).

Table 16: Comparison of the daily quantities of Vitamin B8 provided by the product and the RDAs and the Dietary Reference Intake (DRI).

	Daily dose with the Product	RDA (%)[7]	DRI[8]
Vitamin B8	450 µg	900	50 µg

- **Evidence accepted by independent expert bodies, national and international committees, recognized text books, monographs and reviews**

Table 17: Established health claims for Vitamin B8

References	Endorsed claim
EFSA	ID 2874: hair and nails care

- **Evidence from clinical trials and bibliographical data**

Biotine is the coenzyme of the carboxylases involved in the metabolism of carbohydrates, proteins and fatty acids (EHPM, 2000). This vitamin has been shown to have an antiseborrhoeic action and is recommended in case of seborrhea of the face and scalp (Fabre, 1987). It is necessary for healthy skin as well as or good quality of hair and nails (EHPM, 2000). Major clinical manifestations in biotin deficiency as well as biotinidase deficiency (the enzyme that recycles biocytine into biotin) are seborrheic dermatitis, dry skin, fine and brittle hair, and alopecia (Mock, 2001; Arslan, 2009; Fritsche, 1991).

7Commission directive 2008|100|EC of 28 october 2008 amending council directive 90/496/EEC on nutrition labeling for foodstuffs as regards recommended daily allowances, energy conversion factors and definitions.

8Martin A. Apports nutritionnels conseillés pour la population française, 3eme édition Paris: edition tec&doc.

Table 18: Clinical trials performed with Vitamin B8 in humans

Reference	Title	Method	Tested product, dose, duration, number of subjects	Results
Clinical studies				
Floersheim 1992	An examination of the effect of biotin on alopecia and hair quality	Open clinical study	93 patients with the symptoms hair loss (mostly androgenetic alopecia) and reduced hair quality were administered 2.5 mg of biotin per day for 7.9±2.8 months.	An obvious improvement of hair-loss (mostly androgenetic alopecia) and reduced hair quality.
Hornfeldt, 2015	The Safety and Efficacy of a Sustainable Marine Extract for the Treatment of Thinning Hair: A Summary of New Clinical Research and Results from a Panel Discussion on the Problem of Thinning Hair and Current Treatments	Review	Marine protein (600 mg), Vitamin C (77 mg), Niacin (16 mg), Biotin (156 µg), Iron (13,6 mg), Zinc (14,9 mg), Horsetail extract (49 mg), Millet extract (10 mg).	Clinical studies have demonstrated the effectiveness of a nutraceutical supplement to provide essential nutrients that aid in stimulating existing hair growth and reducing hair shedding.
Case reports				
Ananth, 2003	Biotinidase deficiency-Diagnosis by enzyme assay and a follow-up study	Case report	3 month-old male child with complaints of skin rashes, developmental delay, seizures, seborrheic dermatitis, alopecia and mild acidosis was administered 10 mg per day of biotin for 6 months.	A biotinidase deficiency was highlighted and the efficacy of biotin supplementation in biotinidase deficiency was demonstrated. Cutaneous manifestation resolved quickly followed by a regression of seizures.
Khalidi *et al.* 1984	Biotin deficiency in a patient with short bowel syndrome. during home parenteral nutrition.	Case report	A 54-year-old woman with short bowel syndrome which developed biotin deficiency with complete hair loss following a parenteral nutrition without biotin.	After 3 weeks, serum and urine biotin levels were 650 pg/ml and 35.6 ng/mg creatinine, respectively. New hair growth was evident and all of her other symptoms resolved.
Shelley, 1985	Uncombable hair syndrome:	Case report	3 children with uncombable hair	Significant improvement in one patient with increased growth

Reference	Title	Method	Tested product, dose, duration, number of subjects	Results
	observations on response to biotin and occurrence in siblings with ectodermal dysplasia.		syndrome consisting of slow-growing, straw-colored scalp hair.	rate and with strength and combability of the hair.

I-8 VITAMIN B9

- **Recommended Daily Allowances (RDAs)**

The following table compares the daily intake of Vitamin B9 provided by the recommended use of the finished product with the Recommended Dietary Allowances (RDA) of Vitamin B9 in Europe.

As a rule, 15% of the recommended allowance should be taken into consideration in deciding what constitutes a significant amount (Commission Directive 2008/100/EC of 28 October 2008).

Table 19: Comparison of the daily quantities of Vitamin B9 provided by the product and the RDAs and the Dietary Reference Intake (DRI).

	Daily dose with the Product	RDA (%)[9]	DRI[10]
Vitamin B9	200 µg	100	300-330 µg

- **Evidence from bibliographical data**

Folic acid (vitamin B9) is required in the cellular process of hair follicle (Bouvet *et al.* 1988). A folate deficiency, which causes hair loss, is one of the most common vitamin deficiencies in industrialised countries. This vitamin is thus supported as an effective nutritional supplement for hair quality and vitality (Bouvet *et al.* 1988).

9Commission directive 2008|100|EC of 28 october 2008 amending council directive 90/496/EEC on nutrition labeling for foodstuffs as regards recommended daily allowances, energy conversion factors and definitions.

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I-9 ZINC

- **Recommended Daily Allowances (RDAs)**

The following table compares the daily intake of zinc provided by the recommended use of the finished product with the Recommended Dietary Allowances (RDA) of zinc in Europe.

As a rule, 15% of the recommended allowance should be taken into consideration in deciding what constitutes a significant amount (Commission Directive 2008/100/EC of 28 October 2008).

Table 21: Comparison of the daily quantities of zinc provided by the product and the RDAs and the Dietary Reference Intake (DRI).

	Daily dose with the Product	RDA (%)[11]	DRI[12]
Zinc	10 mg	100	10-12 mg

- **Evidence accepted by independent expert bodies, national and international committees, recognized text books, monographs and reviews**

Table 22: Established health claims for zinc

References	Endorsed claim
EFSA	ID 4293: Contributes to protein synthesis, namely keratine and collagen which belong to hair, skin and nail structure

- **Evidence from report cases and clinical data.**

Zinc is known to be essential for the growth of hair and other epidermal tissue. It is involved in the synthesis of keratin, the main component of hair. This accounts for its effect on the speed of hair growth, since the growth involves an optimum level of zinc in the follicles (EMEA, 1996). Zinc inhibits the 5α-reductase, present in the root and sebaceous gland, an enzyme responsible for the transformation of testosterone into its active form, dihydrotestosterone. These androgens are usually the cause of hair loss (EMEA, 1996; EVM, 2003). Clinical studies, notably randomised controlled trials, have demonstrated the effectiveness of inhibitors of 5α-reductase in the treatment of androgenic alopecia (Prager, 2002). A zinc deficiency (relatively common) induces hair loss or fragility as well as skin lesions (EMEA, 1996; EVM, 2003). In case of alopecia, dry and brittle hair or brittle nails,

11Commission directive 2008|100|EC of 28 october 2008 amending council directive 90/496/EEC on nutrition labeling for foodstuffs as regards recommended daily allowances, energy conversion factors and definitions.

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symptoms that have been attributed to a zinc deficiency, have been improved with an oral zinc supplementation (Slonim, 1992; Nurnberger, 1987).

Table 23: Clinical trials performed with zinc in humans

Reference	Title	Method	Tested product, dose, duration, number of subjects	Results
Clinical studies				
Park, 2009	The therapeutic effect and the changed serum zinc level after zinc supplementation in alopecia areata patient who had a low serum zinc level.	Open clinical study	15 alopecia areata patients were administered zinc gluconate tablet (50 mg/tablet/day) for 12 weeks.	The serum zinc levels increased significantly from 56.9 µg/dl to 84.5 µg/dl. Positive therapeutic effects were observed for 9 out of 15 paients (66.7%) although this was not statistically significant.
Hornfeldt, 2015	The Safety and Efficacy of a Sustainable Marine Extract for the Treatment of Thinning Hair: A Summary of New Clinical Research and Results from a Panel Discussion on the Problem of Thinning Hair and Current Treatments	Review	Marine protein (600 mg), Vitamin C (77 mg), Niacin (16 mg), Biotin (156 µg), Iron (13,6 mg), Zinc (14,9 mg), Horsetail extract (49 mg), Millet extract (10 mg).	Clinical studies have demonstrated the effectiveness of a nutraceutical supplement to provide essential nutrients that aid in stimulating existing hair growth and reducing hair shedding.
Report cases				
Nurnberger, 1987	Zinc deficiency in artificial nutrition.	Case report	A 35-year-old patient suffering from acute zinc deficiency syndrome.	Symptoms such as loss of hair were improved by zinc therapy.
Slonim, 1992	Clinical response of alopecia, trichorrhexis nodosa, and dry, scaly skin to zinc supplementation.	Case reports	Two children who had alopecia trichorhexis nodosa, dry, scaly skin, short stature, and neurosecretory growth hormone deficiency were administered zinc (18-20 mg of zinc per day) for 2 years.	Patient 1 was shown to have zinc deficiency, whereas no clear zinc deficiency could be demonstrated in patient 2. However, the skin and hair abnormalities improved with zinc therapy.

I-10 COPPER

- **Recommended Daily Allowances (RDAs)**

The following table compares the daily intake of copper provided by the recommended use of the finished product with the Recommended Dietary Allowances (RDA) of copper in Europe.

As a rule, 15% of the recommended allowance should be taken into consideration in deciding what constitutes a significant amount (Commission Directive 2008/100/EC of 28 October 2008).

Table 24 : Comparison of the daily quantities of copper provided by the product and the RDAs and the Dietary Reference Intake (DRI).

	Daily dose with the Product	RDA (%)[13]	DRI[14]
Copper	1 mg	100	1.5-2.0 mg

- **Evidence accepted by independent expert bodies, national and international committees, recognized text books, monographs and reviews**

Table 25: Established health claims for copper

References	Endorsed claim
EFSA	ID 1724: Maintenance of skin and hair pigmentation

- **Evidence from bibliographical data**

The role of copper in the pigmentation of skin, hair, and eyes is related to the requirement of the cuproenzyme tyrosinase (monophenol oxidase) for melanin synthesis. Mutational loss of this catalytic function leads to albinism. Achromotrichia is observed in domestic and laboratory animals consuming diets low in copper (Arredondo and Nunez, 2005; Linder, 1991).

I-11 ROCKET AERIAL PART (*Eruca sativa* Mill.)

a) Food use / Historical use

13Commission directive 2008|100|EC of 28 october 2008 amending council directive 90/496/EEC on nutrition labeling for foodstuffs as regards recommended daily allowances, energy conversion factors and definitions.

14Martin A. Apports nutritionnels conseillés pour la population française, 3eme édition Paris: edition tec&doc.

Eruca sativa (rocket) is an annual or biannual herb, and is one of the varieties of mustard. It is a native plant of Israel, documented in the old literature. A survey was conducted by Yaniv *et al.* of the old literature of ancient Israel, including Jewish, Classical and Islamic sources up to the Middle ages. It was found that rocket was used as a garden crop and spice. It was also known as a medicinal plant and was used as an aphrodisiac, for eye infections, and for digestive and kidney problems (Yaniv *et al.* 1998).
The medicinal properties of rocket were recognized by the ancient Greeks and Romans (Barazani and Ziffer-Berger, 2014).
The plant originated in the Mediterranean region, but is presently found around the world. It is extensively consumed in some European countries, e.g., Italy and is also used in Indian cooking. The leaves and sprouts of the plant are widely used in salads for their hot pungent taste and can add flavour to any boring salad. The seeds yield oil, which is a substitute for rapeseed oil. The plant also has a wide spread medicinal use. Traditionally, its use as astringent, diuretic, digestive, emollient, tonic, depurative, laxative, rubefacient, and stimulant is well documented (Sarwar Alam *et al.* 2007).
The data presented above are in accordance with the European commission opinion who considered that rocket does not take part of novel food since it was on the market as a food or food ingredient and consumed to a significant degree before 15 May 1997 (European Commission).

Eruca sativa is included some lists of plant authorized in food (i. e. Belfrit project[1]).

b) Clinical and bibliographical data

- **Evidence accepted by independent expert bodies, national and international committees, recognized text books, monographs and reviews.**

[1] Allegato 1.bis (attuale lista Belfrit) (http://www.unerbe.it/attivita/2014/decreto_27_marzo_2014_all_%201_bis.pdf).

Table 26: Established health claims for *Eruca sativa*

References	Endorsed claim
Sher *et al.* 2011	Hair loss and hair tonic

- **Evidence from ethnopharmacological data**

Extensive ethnopharmacological survey of medicinal herbs showed that *Eruca sativa* would be used for treating skin disease, hair loss (Said *et al*. 2002; Lev and Amar, 2002) or as a hair tonic (Sher *et al*. 2011).

Rocket contain sulfur amino acids (Nurzynska-Wierdak, 2015, Villatoro-Pulido *et al.* 2013) essential in keratin formation.

I-12 COMMON HORSETAIL AERIAL PART (*Equisetum arvense* L.)

a) Food use / Historical use

Horsetail is primarily used as a dietary supplement for mineral content, also in diuretic formulations, including teas, tinctures, capsules, tablets, and so on (Foster and Duke, 1990).

For a long time, *E. arvense* has been used as a folklore medicine for treatment of various conditions such as tuberculosis, as a catarrh in the kidney and bladder regions, as a hematostatic for profuse menstruation, nasal, pulmonary and gastric hemorrhages, for brittle fingernails and loss of hair, for rheumatic diseases, gout, poorly healing wounds and ulcers, swelling and fractures and for frostbite (Sandhu *et al.* 2010).

Equisetum arvense is included in several lists of plant authorized in food supplements (i.e. French order of June 24, 2014[15], Belgium royal order of August 29, 1997[16] and Belfrit project[17]).

b) Clinical and bibliographical data

- **Evidence accepted by independent expert bodies, national and international committees, recognized text books, monographs and reviews.**

Table 27: Established health claims for *Equisetum arvense* L.

References	Endorsed claim
EFSA	ID: 2438 improves skin, hair and nail condition, promotes hair growth and strengthening

- **Evidence from clinical and bibliographical data**

Table 28: Clinical trials performed with *Equisetum arvense* L. in humans

Reference	Title	Method	Tested product, dose, duration, number of subjects	Results

[15] Arrêté du 24 Juin 2014 établissant la liste des plantes, autre que les champignons, autorisées dans les compléments alimentaires et les conditions de leur emploi. JO République Française.
[16] Arrêté Royal du 29 août 1997 relatif à la fabrication et au commerce de denrées alimentaires composées ou contenant des plantes ou préparations de plantes.
[17] Allegato 1.bis (attuale lista Belfrit) (http://www.unerbe.it/attivita/2014/decreto_27_marzo_2014_all_%201_bis.pdf).

Reference	Title	Method	Tested product, dose, duration, number of subjects	Results
Hornfeldt *et al.* 2015	The Safety and Efficacy of a Sustainable Marine Extract for the Treatment of Thinning Hair: A Summary of New Clinical Research and Results from a Panel Discussion on the Problem of Thinning Hair and Current Treatments.	Review	Marine protein (600 mg), Vitamin C (77 mg), Niacin (16 mg), Biotin (156 µg), Iron (13,6 mg), Zinc (14,9 mg), Horsetail extract (49 mg), Millet extract (10 mg).	Clinical studies have demonstrated the effectiveness of a nutraceutical supplement to provide essential nutrients that aid in stimulating existing hair growth and reducing hair shedding.

According to Fukuda and Kidena (2001), hair preparations containing *Equisetum arvense* extracts and silicones or cationic polymers would improve the texture and tone of hair. The extracts also showed hair growth-stimulating effect in an aged man with alopecia (Ikemitsu *et al.* 2001).

I-10 CONCLUSION

The bibliographical data presented support the nutritional and beneficial effects of the active ingredients contained in the finished product:

- cystine and methionine are amino acids naturally present in human body.
- vitamin B8 and zinc contribute to hair growth.
- vitamin D3 participate in keratinization
- vitamin B6 and B9 are essential in amino acid metabolism
- vitamin B5 is essential for the good health of hair.
- copper plays a role in hair pigmentation.
- arginine contribute to NO formation involved in hair growth.
- rocket is rich in amino acid sulfur
- horsetail contain silice which contribute to tone of hair.

II. TOXICOLOGICAL EVALUATION AND SAFETY IN USE

II-1. AMINO ACIDS

The quantity of amino acids provided by the finished product does not exceed the safe intake as presented in the following table.

Table 29: Comparison of the daily quantities of amino acids provided by the finish product and their safe intakes

Amino acids	Safe intakes of indispensable amino acids*
L-cystine	5 mg/kg bw/day (350 mg/day
L-methionine	4 mg/kg bw/day (280 mg/day)
L-arginine	20 g/day (SHAO, 2008)

*the safe levels of intake for the indispensable amino acids are 24 % higher then the values for average requirement according to FAO/WHO proteins and amino acids requirements in human nutrition, 2007.

II-2. VITAMINS

Upper safety levels

The quantity of vitamins provided by the product does not exceed the daily maximal acceptable doses in France or upper safety levels (ULs) as presented in the following table.

Table 30: Comparison of the daily quantities of vitamins provided by the product and the maximal acceptable daily intakes.

	Daily amount with the product	Maximal acceptable daily intake*	UL
Vitamin D3	5 µg	10 µg	50 µg/d^{+}
Vitamin B5	6 mg	5.4 mg	200 mg/d^{++}
Vitamin B6	5.4 mg	2 mg	25 mg/d^{+}
Vitamin B8	450 µg	30 µg	0.9 mg/d^{+++}
Vitamin B9	200 µg	400 µg	1 mg/d^{+}

* FAO/WHO Vitamins and Minerals requirements in human nutrition, 2004.
$^{+}$ European Food Safety Authority, Tolerable upper intake levels for vitamins and minerals. February 2006.
$^{++}$Food Standard Agency, Expert Group on Vitamins and mmmMinerals (EVM) Safe Upper Levels for Vitamins and Minerals, 2003.
$^{+++}$No Safe upper Level has been established However, for guidance purposes only, a safe dose is proposed by the Expert Group on Vitamins and Minerals (EVM), 2003.

II-3. MINERALS

Upper safety levels

The quantity of minerals provided by the product does not exceed the daily maximal acceptable doses in France or upper safety levels (ULs) as presented in the following table.

Table 31: Comparison of the daily quantities of minerals set by the Scientific Committee on Food (SCF, 2006).

	Daily amount with the product	Maximal acceptable daily intake in France*	UL (SCF)
Zinc	10 mg	15 mg	25 mg/d
Copper	1 mg	2 mg	5 mg/d

* Arrêté du 9 mai 2006 relatif aux nutriments pouvant être employés dans la fabrication des compléments alimentaires. Journal Officiel de la République Française. 28 mai 2006.

II-4. ROCKET LEAF (*Eruca sativa* Mill.)

Rocket is a food largely consumed all around the world and no safety concern has been identified in scientific literature.

II-5. COMMON HORSETAIL LEAF (*Equisetum arvense* L.)

a) Safety data

Acute toxicity

A diet with increasing quantities of horsetail (0.1 to 0.8 mg/kg bw/day) was not found to be toxic to voles (rodents) in term of liver and kidney function.

Feeding experiments reported in 1904 investigating the toxicity of several Equisetum species found *E. arvense* to be harmless (Mills, 2005).

Repeated-dose toxicity

No adverse effects were observed in rats intraperitoneally administered 50 mg/kg bw of a hydroalcoholic extract of horsetail daily for 8 weeks (Gardner, 2013).

b) Warnings / Precautions of use

Safety in children under 12 years old has not been established. In the absence of sufficient data, **the use in children under 12 years old is not recommended.**

II-6. CONCLUSION ABOUT SAFETY

In the light of the data about the safety of each active ingredient of the product, it can be concluded that its use as recommended presents no foreseeable toxic risk for human Health.

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