Application for the Placing on the Market of Cycloastragenol-TA65 as a Novel Food and Novel Food Ingredient pursuant to Article 4 of Regulation 258/97

Applicant:
TELOMERASE ACTIVATION SCIENCES, INC.
420 Lexington Ave., Suite 2900
New York, NY 10170, USA
Tel: +1-212-588-8805
www.tasciences.com

Application submitted by

K&L GATES

K&L GATES GP

Brussels, January 31, 2014

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A. EXECUTIVE SUMMARY

1. The novel food ingredient Cycloastragenol-TA65 (also hereinafter, the "**NF Ingredient", "NF"** or "**CAG-TA65**") is a white to off-white loose powder isolated from *Astragalus trojanus* Stev. (1853). By weight, the NF Ingredient is ≥98% Cycloastragenol, a cycloartane-type triterpenoid produced by many *Astragalus* spp. It will be marketed in the form of the bulk loose powder for intended use in food supplements in the sense of Directive 2002/46[1] in the European Union (hereinafter, the "**EU**").

2. Different species of the plant *Astragalus* have been traditionally used in a wide variety of herbal blends and "*natural*" remedies in China and other regions in Asia for thousands of years. They contain *Astragalosides* (including *Astragaloside IV*), which are the ultimate source of the purified Cycloastragenol-TA65, the proposed NF Ingredient.

3. The production process involves different steps, which are not novel in the food supplement sector and the final product of this process is carefully checked for its compliance with the proposed specifications. Appropriate quality control procedures are applied to every batch. Stability has been checked after different periods of storage.

4. According to the applicant's recommendations, daily intake of Cycloastragenol-TA65 in the EU is expected to be 8 mg/person for healthy adults (maximum 0.133 mg/kg bw/day). The maximum daily intake will be 8 mg of the NF Ingredient.

5. No toxically relevant adverse effects were observed in a 90-day sub-chronic toxicity study with Cycloastragenol-TA65. The only statistically significant effects noted were for various endpoints that were incidental or sporadic in nature, lacked a dose-response relationship and were not clinically relevant, or were also present in the control group, and/or were within the range of historical control data for rats of this age and strain. No effects attributable to administration of TA-65®[2] were identified in the in-life observations, ophthalmology, hematology, coagulation, clinical chemistry, urinalysis, gross pathology and histopathology for main toxicity or recovery group animals. In addition, no cardiac-related effects were identified. Under the conditions of this study, the no-observed-adverse-effect level (NOAEL) of orally administered Cycloastragenol was 150 mg/kg bw/day in male and female rats. Based on the NOAEL and on dietary exposure calculated from use levels a wide margin of safety (1100) can be calculated for extrapolation to a person of 60 Kg.

6. The genotoxicity studies provide overall support that Cycloastragenol-TA65 lacks mutagenic and clastogenic potential. The proposed NF Ingredient did not exhibit clastogenicity in the absence of metabolic activation and when clastogenic potential was evaluated in the in vivo erythrocyte micronucleus assay, it was not clastogenic and/or aneugenic and did not induce structural and/or numerical chromosomal damage in the immature erythrocytes of the mouse.

7. The human studies with Cycloastragenol-TA65, using daily doses much higher than the recommended dose, did not reveale adverse effects.

8. In an unpublished study examining carcinogenicity and in a published study on survival and tumor incidence in in vivo mice, both with Cycloastragenol from another species of Astragalus (purity ~95%, isolated from *A. membranaceus*) no effect on tumor incidence or growth for four different human cancer cell types, no effect on survival and no increase in malignant incidence were observed. In a study of the effects of a topically applied 5% preparation on wound healing, using experimentally created lesions in rats, no adverse effects were reported.

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1 Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements (OJ L 183, 12.7.2002, p. 51–57).

2 A dietary supplement containing Cycloastragenol has been commercialized in the United States since 2007 under the name of "TA-65®". This is the reason, why in several scientific studies the name "*TA-65®*" is present.

9. No allergenic reactions can be expected from Cycloastragenol-TA65 intake based on its chemical characteristics.

10. In summary, the assessment of all the information, included in proper structured schemes, suggests that the consumption of the NF Ingredient is neither nutritionally disadvantageous nor represents a danger for the consumer under the proposed conditions of use as a food supplement.

B. ADMINISTRATIVE INFORMATION

I. APPLICANT

TELOMERASE ACTIVATION SCIENCES, INC.
420 Lexington Ave., Suite 2900
New York, NY 10170
P: +1-212-588-8805
www.tasciences.com

II. CONTACT PERSON

Sebastián Romero Melchor
K&L GATES GP
The View Building | Nijverheidsstraat 26/38 | 1040 Brussels | Belgium
P +32 (0)2 336 1900
F +32 (0)2 336 1901
sebastian.romeromelchor@klgates.com
www.klgates.com

III. NAME OF THE NOVEL FOOD INGREDIENT:

CYCLOASTRAGENOL-TA65

IV. DATE OF APPLICATION:

January 31, 2014

C. IDENTIFICATION OF ESSENTIAL INFORMATION FOR ASSESSMENT OF THE NOVEL FOOD

11. The European Commission's (hereinafter, the "**EC**") and the scientific panels of the European Food Safety Authority's (hereinafter, the "**EFSA**"), recommendations concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients and the preparation of initial assessment reports under Regulation 258/97 concerning novel foods and novel food ingredients[3] (hereinafter, "**Regulation 258/97**") have been followed. Cycloastragenol-TA65 corresponds to Class 1 (pure chemicals or simple mixtures from non-GM sources).

12. *Species* of *Astragalus* are authorized for their use in food supplements in a number of Member States of the EU. According to the Belgian Royal Decree on Plants[4], *Astragalus membranaceus* Moench is authorized in food supplements. The Italian Decree of July 9, 2012[5] authorizes several *species* of the genius *Astragalus*: *Astragalus adscendens* B. et H., *Astragalus creticus* Lam., *Astragalus gummifer* Labill, *Astragalus membranaceus* Bung., *Astragalus microcephalus* Willd. and *Astragalus verus* Oliv. The draft French Order laying down the list of plants[6] authorizes *Astragalus membranaceus* Moench.

13. Pursuant to Commission Recommendation 97/618/EC concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods[7] (hereinafter, the "**Commission Recommendation**"), the following questions pertaining to the specifications of the NF Ingredient must be addressed:

    I. Specification of the NF Ingredient;
    II. Effect of the production process applied to the NF Ingredient;
    III. History of the organism used as the source of the NF Ingredient;
    IX. Anticipated intake/extent of use of the NF Ingredient;
    X. Information on previous human exposure to the novel food or its source
    XI. Nutritional information on the NF Ingredient;
    XII. Microbiological information on the NF Ingredient; and
    XIII. Toxicological information on the NF Ingredient.

I. SPECIFICATION OF THE NF INGREDIENT

I.1 General Description

14. The proposed NF is a white to off-white loose powder (main traits in Table I.1, complete specifications in Table I.2) isolated from *Astragalus trojanus* Stev. (1853). [redacted] *Astragalus trojanus* is a perennial flowering shrub of the Fabaceae (or legume) family with sprawling stems 5-30 cm in length that are sparsely covered by villous stipule bases[8]. Leaf rachis (the main stem) is spiny, 2-6 cm, and may be straight or curved with 5-7 paired 4-9 mm leaflets that are oblong-elliptical and hairy. When flowering, the 10-50 influorescences are 2-3 cm in diameter, and usually oblong with pink bracts and bracteoles resembling calyx lobes. *Astragalus*

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[3] Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients (OJ L 43, 14.2.1997, p. 1–6).

[4] *Arrêté Royal du 29 août 1997 relatif à la fabrication et au commerce de denrées alimentaires composes ou contenant des plantes ou préparations de plantes (Mon. 21.XI.1997).*

[5] *MINISTERO DELLA SALUTE - Decreto 9 luglio 2012 Disciplina dell'impiego negli integratori alimentary di sostanze e preparati vegetali (G.U. 21-7-2012 serie generale n. 169).*

[6] *Arrêté du [...] établissant la liste des plantes, autres que les champignons, autorisées dans les compléments alimentaires et les conditions de leur emploi.* TRIS notification No 2012/0728.

[7] Commission Recommendation 97/618/EC of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients and the preparation of initial assessment reports under Regulation (EC) No 258/97 of the European Parliament and of the Council (OJ L 253, 16.9.1997, p. 1–36).

[8] Available at http://www.treknature.com/gallery/photo252284.htlm.

trojanus is wild-harvested in the months of March through September [redacted]. The scientific faculty of the Department of Biology, [redacted], provides via visual and microscopic comparison to an authentic standard, authentication of the harvested materials.

Table I.1. General description of Cycloastragenol-TA65	
Appearance	White to off-white loose powder
Functionality	Dietary supplement
Molecular formula/molecular weight	C30H50O5/ 490,7
Packaging	Polyethylene bottle
Stability	Twelve months [a]
Storage	15-30°C

[a] see Annex IV.8 (Stability tables, lots 1,2,4,5,7,8,9,13)

15. By weight, the NF Ingredient is >98% Cycloastragenol (CAS #: 78574-94-4; CAS #: 84605-18-5). Cycloastragenol is a triterpene aglycone of the 9,19-cyclolanostane-type and one of the secondary metabolites found in all *Astragalus* spp.

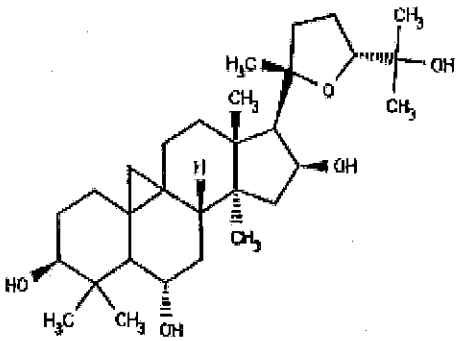

Figure 1. Structure of Cycloastragenol

16. Triterpene glycosides belong to the class of secondary plant metabolites known as saponins (glycosides that have a distinctive foaming characteristic) and are composed of a polycyclic aglycone (a choline steroid or triterpenoid) attached via C3 and an ether bond to a sugar side chain[9].

17. Cycloastragenol is the most common genuine aglycone[10] for the triterpenoid saponins[11] called astragalosides[12], which are found in *Astragalus* spp.[13]. As an example, Cycloastragenol is the

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9 Available at http://www.ansci.cornell.edu/plants/toxicagents/saponin.html.

10 Astragenol, an artifactual aglycone, can be produced during acidic hydrolysis of astragalosides (Kitagawa, I., H. K. Wang, et al. (1983). "Saponin and sapogenol. XXXIV. Chemical constituents of Astragali Radix, The Root of Astragalus membranaceus Bunge. (1). Cycloastragenol, the 9,19-Cyclolanostane-type Aglycone of Astragalosides, and the Atifact Aglycone Astragenol." Chemical & pharmaceutical bulletin **31**(2): 689-697.)

11 Saponins are a chemically heterogenous group consisting of sterol glycosides and triterpene glycosides; triterpene saponins include astragalosides (the triterpene glycosides) and soyasaponins.

12 Astragalosides are glycoside derivatives of the 20R,24S form of Cycloastragenol, also called astramembragenin. (Astramembrainnins are derivatives of the 20S,24R diasteroisomer of Cycloastragenol called cyclosieversigenin (Gan, L. X., X. B. Han, et al. (1986). "Astrasieversianins IX, XI and XV, cycloartane derived saponins from Astragalus sieversianus*." Phytochemistry **25**(6): 1437-1441.; Rios, J. L. and P. G. Waterman (1997). "A review of the pharmacology and toxicology of Astragalus." Phytotherapy Research **11**(6): 411-418.)

13 *Ibid*, Rios and Waterman (1997).

aglycone for ten out of the eleven astragalosides found in *Astragalus* membranaceus[14]. The systematic name for Cycloastragenol is 9,19-cycloanostane-3,6,16,25-tetrol, 20,24-epoxy-(3beta,6alpha, 16beta,20R,24S) (Figure 1). Cycloastragenol is also known as astramembrangenin and occasionally as cyclosieversigenin or cyclosiversigenin, although these last two designations should be used only to indicate the 20S,24R diastereoisomer which is not chemically identical to Cycloastragenol.

18. The NF Ingredient is defined as Cycloastragenol-TA65, presented in the form of the loose powder, for intended use as a food supplement.

I.2 Product Specifications

19. Cycloastragenol-TA65 (> 98% Cycloastragenol) is a white to off-white loose powder extracted and isolated from *Astragalus trojanus* Stev. (1853). Within the plant, Cycloastragenol is found as the aglycone and within the triterpene glycosides, including Astragaloside IV. The product specifications are shown in Table I.2.

20. All methods applied have been referenced (if they correspond to specific references), standardized and validated to ensure quality and consistency of the data) (see Annexes from I.1 to I.13).

Table I. 2. Product Specifications

Analysis	Method	Specification
Physical appearance	Visual inspection	White/off-white solid powder
Odor	Olfactory inspection	Odorless
Cycloastragenol (%)	HPLC/ELSD	NLT 98%
Water content (%)	Loss on drying	NMT 2%
Ash (%)	Residue on ignition	NMT 2%
Content (%)	Purity less *residual solvent*, water, and ash	NLT 98%
Structure	Proton NMR	Conforms
Mass	Mass Spec	490 ± 1.0 amu
Solvent residue (ppm)	Headspace GC	
n-Butanol		NMT 5000
Acetonitrile		NMT 400
Hexane		NMT 290
Methanol		NMT 3000
Ethanol		NMT 5000
Ethyl acetate		NMT 5000
Heavy metals (ppm)	ICP-MS[a]	
Arsenic		NMT 90
Cadmium		NMT 30
Lead		NMT 60
Mercury		NMT 90
Chromium		NMT 30
Selenium		NMT 30
Pesticides (304 total)[b]	GC/MS, LC-MS/MS[c]	USP
Microbiological (cfu/g)	USP Microbiological Examination of Nonsterile Products: "*Microbial Enumeration Test*", (1 g tested)	

[14] See # 10 *supra*; McKenna, D., K. Hughes, et al. (2002). "Astragalus." Alternative therapies in health and medicine 8(6): 34-40; quiz 41-32, 124.).

	(USP61); and specific organisms (USP62).	
Standard plate count (cfu/g)		NMT 1000
Yeast & mold (cfu/g)		NMT 100
Escherichia coli		Absent in 1 g
Salmonella		Absent in 1 g
Pseudomonus aeruginosus		Absent in 1 g
Staphylococcus aureus		Absent in 1 g

[a] US EPA (2007);(Şahan, Basoglu et al. 2007);
[b] Each sample was analyzed for 304 pesticides. Please see Annexes IV.1 to IV.7 for complete list of individual specifications, amu = atomic mass unit; AOAC = AOAC International (formerly known as the American Organization of Analytical Chemists); cm = colony forming units; g = grams; ELSD = Evaporative Light Scattering Detector; GC/MS = Gas Chromatography-Mass Spectrometry; HPLC = High Performance Liquid Chromatography; ICP-MS = Inductively Coupled Plasma-Mass Spectrometry; NLT = not less than; NMT = not more than; ppm = parts per million; USP = United States Pharmacopeia
[c] A&G PurLab (2013)

I.3 Specification Compliance

21. A total of six lots were analyzed and met the stated specifications, as shown in Tables I.3.a (averages and ranges) and I.3.b (lots individual results). Detailed individual specifications reports are in Annexes IV.1 to IV.7).

Table I.3.a. Specifications compliance: specification, average and range

Analysis	Specification	Batch Analysis Results (n = 6) Average [a]	Range
Physical appearance	White/off-white solid powder	White solid powder	
Odor	Odorless	Odorless	Odorless
Cycloastragenol (%)	NLT 98%	100.66	99.07- 102.37
Water content (%)	NMT 2%	0.64	0.32 - 0.90
Ash (%)	NMT 2%	0.26	0.08 - 0.40
Content (%)	NLT 98%	99.76	98.34- 101.24
Structure	Conforms	Conforms	Conforms
Mass	490 ± 1.0 amu	490.21	490.13 -490.38
Astragenol (%)	ND	ND	ND
Dehydrocycloastragenol (%)	ND	ND	ND
Solvent residue (ppm)			
n-Butanol	NMT 5000	30.75 (n=1)	ND - 30.75
Acetonitrile	NMT 400	73.44 (n=2)	ND - 137.84
Hexane	NMT 290	39.20 (n=1)	ND - 39.20
Methanol	NMT 3000	ND	ND
Ethanol	NMT 5000	ND	ND
Ethyl acetate	NMT 5000	5.77 (n=5)	ND - 13.34
Heavy metals (ppm)			
Arsenic	NMT 90	0.009	0.002 - 0.021
Cadmium	NMT 30	0.003 (n=4)	<0.001 -0.008
Lead	NMT 60	0.239	0.038- 1.017
Mercury	NMT 90	0.006 *(n=2)*	<0.003 - 0.009
Chromium	NMT 30	0.232	0.107-0.336
Selenium	NMT 30	0.054 *(n=3)*	<0.003 - 0.133

Pesticides (304 total)[b]	USP	ND for all	ND for all
Microbiological (cfu/g)			
Standard plate count (cfu/g)	NMT 1000	520 (n=1)	ND - 520
Yeast & mold (cfu/g)	NMT 100	ND	ND
Escherichia coli	Absent in 1 g	Absent in 1 g	Absent in 1 g
Salmonella	Absent in 1 g	Absent in 1 g	Absent in 1 g
Pseudomonus aeruginosus	Absent in 1 g	Absent in 1 g	Absent in 1 g
Staphylococcus aureus	Absent in 1 g	Absent in 1 g	Absent in 1 g

[a] n = 6, unless otherwise specified (Values below detectable limits were not included in the average.);
[b] Each sample was analyzed for 304 pesticides. See Annexes IV.1 to IV.7 for complete list of individual specifications, amu = atomic mass unit; AOAC = AOAC International (formerly known as the American Organization of Analytical Chemists); cfu = colony forming units; g = grams;
n = number of batches analyzed; ND = Not detected; NLT = not less than; NMT = not more than; ppm = parts per million; USP = United States Pharmacopeia.

Table I.3.b. Specification compliance: lots individual results

Assay	Specification	10/09/ 2011	14/11/ 2011	12/12/ 2011	20/01/ 2012	27/02/ 2012	05/03 /2012
Batch		13	1	2	3	4	5
Physical appearance	White, off-white solid	White solid	White solid	White solid	White solid	White solid	White solid
Odor	Odorless	Odorless	Odorless	Odorless	Odorless	Odorless	Odorless
Purity (% cycloastrogenol)	NLT 98%	99.07	99.50	102.37	101.17	100.29	101.55
Water content (%)	NMT 2%	0.66	0.77	0.90	0.32	0.63	0.55
Ash (%)	NMT 2%	0.08	0.35	0.24	0.20	0.32	0.40
Content (%)	NLT 98%	98.34	98.39	101.24	100.65	99.34	100.61
Structure	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms
Mass	490 +/- 1.0 amu	490.13	490.13	490.13	490.13	490.38	490.35
Astragenol (%)	ND	ND	ND	ND	ND	ND	ND
Dehydrocyclo-astrogenol (%)	ND	ND	ND	ND	ND	ND	ND
Solvent residue (ppm)							
n-Butanol,	NMT 5000	ND	ND	ND	ND	30.75	ND
Acetonitrile	NMT 400	137.84	ND	ND	ND	9.04	ND
Hexane	NMT 290	39.20	ND	ND	ND	ND	ND
Methanol	NMT 3000	ND	ND	ND	ND	ND	ND
Ethanol	NMT 5000	ND	ND	ND	ND	ND	ND
Ethyl acetate	NMT 5000	4.30	3.50	2.93	4.80	13.34	ND
Heavy metals (ppm)							
Arsenic	NMT 90	0.002	0.021	0.004	0.006	0.010	0.008
Cadmium	NMT 30	0.008	<0.001	0.002	0.002	<0.001	0.001
Lead	NMT 60	1.017	0.038	0.117	0.122	0.063	0.078
Mercury	NMT 90	0.009	0.003	<0.003	<0.003	<0.003	<0.003
Chromium	NMT 30	0.241	0.265	0.107	0.116	0.328	0.336

Table I.3.b. Specification compliance: lots individual results							
Assay	**Specification**	**10/09/ 2011**	**14/11/ 2011**	**12/12/ 2011**	**20/01/ 2012**	**27/02/ 2012**	**05/03 /2012**
Batch		**13**	**1**	**2**	**3**	**4**	**5**
Selenium	NMT 30	0.0081	0.133	<0.003	<0.003	<0.003	<0.003
Pesticides (304 total)[b]	USP	ND	ND	ND	ND	ND	ND
Microbiological (cfu/g)							
Total aerobic microbial count	NMT 1000 CFU/g	ND	ND	ND	ND	520	ND
Total yeast-mold	NMT 100 CFU/g	ND	ND	ND	ND	ND	ND
E. coli	Absent/1g	Absent	Absent	Absent	Absent	Absent	Absent
Salmonella	Absent/1g	Absent	Absent	Absent	Absent	Absent	Absent
Pseudomonas aeruginosa	Absent/1g	Absent	Absent	Absent	Absent	Absent	Absent
Staphylococcus aureus	Absent/1g	Absent	Absent	Absent	Absent	Absent	Absent

[b] Each sample was analyzed for 304 pesticides. Please see Annexes IV.1 to IV.7 for complete list of individual specifications, amu = atomic mass unit; AOAC = AOAC International (formerly known as the American Organization of Analytical Chemists); cfu = colony forming units; g = grams;
n = number of batches analyzed; ND = Not detected; NLT = not less than; NMT = not more than; ppm = parts per million; USP = United States Pharmacopeia.

22. Stability was also checked. The results of eight lots maintained during different periods under standard conditions of storage are in Annex IV.8. As an example, results of one of them are shown in Table I.4. The results show compliance with the Product's specifications.

Analysis	**Specification**	**Baseline 9/10/ 2011**	**6 Months 10/3/ 2012**	**12 Months[b] 13/09/ 2012**
Physical appearance	White/off-white solid powder	White solid	White solid	White solid
Odor	Odorless	Odorless	Odorless	Odorless
Cycloastragenol (%)	NLT 98%	99.07	99.15	99.60
Water content (%)	NMT 2%	0.66	1.12	1.12
Content (%)	NLT 98%	98.3	97.96	98.41
Structure	Conforms	Conforms	Conforms	Conforms
Astragenol (%)	ND	ND	ND	ND
Dehydrocycloastragenol (%)	ND	ND	ND	ND
Microbiological (cfu/g)				
Standard plate count (cfu/g)	NMT 1000	ND	ND	ND
Yeast & mold (cfu/g)	NMT 100	ND	ND	ND

Escherichia coli	Absent in 1 g	Absent in 1 g	Absent in 1 g	Absent in 1 g
Salmonella	Absent in 1 g	Absent in 1 g	Absent in 1 g	Absent in 1 g
Pseudomonus aeruginosus	Absent in 1 g	Absent in 1 g	Absent in 1 g	Absent in 1 g
Staphylococcus aureus	Absent in 1 g	Absent in 1 g	Absent in 1 g	Absent in 1 g

II. EFFECTS OF THE PRODUCTION PROCESS APPLIED TO THE NF INGREDIENT

23. The manufacturer is [redacted].

24. A graphical depiction of the manufacturing process is shown in Figure 2. The process involves different steps, which are not novel, e.g., not involving new types of heat processing or non-thermal preservation methods, new processes to chill or freeze products or to dehydrate products, the application of new processes catalyzed by enzymes, etc. All steps are well experienced in the food supplement industry and the final products of the described process are carefully checked to comply with the specifications given in Table I.2.

25. [redacted]

Figure 2. Manufacturing process of the NF

26. For storage and distribution, 300-400 g of the finished ingredient, a white to off-white loose powder, is packaged in a 2-liter polyethylene bottle and sealed with a screw cap.

27. The product is evaluated in accordance with the product specifications provided in Table I.2.

28. Appropriate quality control procedures are applied to every batch.

29. Quality controls that are applied to the different steps of the process production, from the very beginning (harvesting), to the intermediary steps, and to the very end (packaging process) and including the reference to the tests to the final product such as those detailed in the product specifications. A scheme of the quality controls used from harvest through production is shown in Figure 3. In the Annex IV.14 a detailed description of the specific procedures and certificates are included.

Quality Control Steps	Quality Control Techniques

Raw material inspection: It is carried out a) To authenticate the plant material b) To confirm that it conforms to pesticide residue requirements.	Organoleptic/Macroscopic/Microscopic analyses Pesticide Residue Analysis by LC-MS (GC-MS)
In-process control for extraction: It is carried out to confirm completion of the extraction process.	Thin Layer Chromatography
In-process control of reaction: It is carried out to confirm completion of hydrolysis reaction.	Thin Layer Chromatography
In-process control of liquid-liquid extraction: It is carried out to confirm completion of the liquid-liquid extraction (partitioning).	Thin Layer Chromatography
In-process control of column chromatography: It is carried out to select the pure fractions and pool them.	Thin Layer Chromatography
In-process control of precipitation and washing: It is carried out to confirm purity of the precipitated and washed compound.	Thin Layer Chromatography
Quality control of the end product: In order to ensure that the end product meets the specifications.	Organoleptic analysis HPLC/ELSD Thin Layer Chromatography LC-MS/MS Proton NMR Karl Fischer Residue on Ignition Heavy Metal Analysis by ICP-MS Solvent Residue Analysis by Headspace GC Microbiological Analyses

Figure 3. - Scheme of the quality controls used from harvest through production

III. HISTORY OF THE ORGANISM USED AS THE SOURCE OF THE NF INGREDIENT

30. [redacted] *Astragalus trojanus* Stev (1853) is a perennial flowering shrub of the Fabaceae (or legume) family[15]. *A. trojanus* is wild-harvested in the months of March through September [redacted]. The scientific faculty of the Department of Biology, [redacted] via visual and microscopic comparison to an authentic standard, provides authentication of the harvested materials.

31. Cycloastragenol-TA65 (≥98% Cycloastragenol) is isolated from the plant material and Cycloastragenol is found as the aglycone and within the triterpene glycosides, including Astragaloside IV.

32. *Astragalus* (namely *Astragalus membranaceus*) is an herb that has traditionally been used in a wide variety of herbal blends and "natural" remedies in China or other regions of Asia for thousands of years. According to (McKenna, Hughes et al. 2002) and references therein, the first mention of *Astragalus* spp. is in the Divine Husbandman's Classic of the Materia Medica, an ancient Chinese medical text. In traditional Chinese medicine the dried root of *A. membranaceus* is used primarily as a tonic, especially for the spleen and lungs[16]. It is said to benefit the deficiency of *qi* (vital energy) of the spleen that symptomatically presents with fatigue, diarrhea, and lack of appetite.

33. It has been also considered important for the treatment of infections of the mucous membranes, especially the urinary and respiratory tracts. In China, it is used as a prophylactic against colds.

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[15] See #8 *supra*.

[16] Bensky, D., S. Clavey, et al. (2004). Chinese Herbal Medicine: Materia Medica, Eastland Press Incorporated. Hsu, H. Y. (1986). Oriental Materia Medica, Keats Pub.

Astragalus is also believed to function as a cardiotonic and to increase contraction in normal hearts. In hearts affected by fatigue or poison, the improvement is reportedly more dramatic[17].

34. The roots of *Astragalus membranaceus* (Common Names on CZ Kozinec blanitý, EN: milk vetch, NL: *Astragalus*, DE: Tragant, FR: Astragale) have been used in food supplements before May 15, 1997.

35. Different aspects of the plant have been investigated more recently[18], due to its potential cardio-protective effects, its anti-inflammatory effects, the potential for *Astragalus* extract to enhance longevity and lifespan (through its action on telomerase), to support immune system[19], for preventing colds and upper respiratory infections[20], and other suggested effects[21] as reducing fatigue in athletes[22]. In some studies, *Astragalus* based food supplements seem to help faster recovery[23] and allow cells to live longer[24]. The telomerase activator Cycloastragenol elongates short telomeres and increases health span of adult /old mice without increasing cancer incidence[25].

36. As reported by (Sevimli-Gur, Onbasilar et al. 2011)[26], [redacted] an aqueous extract of *Astragalus* is traditionally used for its wound-healing properties. Known biologically active constituents of *Astragalus* represent two major classes of chemical compounds, polysaccharides and cycloartane type saponins[27].

37. *A. trojanus* is preferred over *A. membranaceous* or other *Astragalus sp* as a source of Cycloastragenol because (i) larger amounts of Cycloastragenol can be extracted from *A. trojanus* than from *A. membranaceous* and (ii) [redacted].

38. About four hundred cycloartane-type saponins were determined and one hundred and sixty of them were isolated from *Astragalus* genus[28]. Investigations of [redacted] *Astragalus* species resulted in the isolation of over sixty cycloartane glycosides including five different aglycones[29], and four

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17 Hsu, H. Y. (1986). Oriental Materia Medica, Keats Pub. references in McKenna, Hughes et al. 2002.

18 Available at http://www.umm.edu/altmed/articles/*Astragalus*-000223.htm#ixzz2NujWBnMO-- .

19 Shao, B. M., W. Xu, et al. (2004). "A study on the immune receptors for polysaccharides from the roots of Astragalus membranaceus, a Chinese medicinal herb." Biochemical and biophysical research communications **320**(4): 1103-1111.

20 Matkovic, Z., V. Zivkovic, et al. (2010). "Efficacy and safety of Astragalus membranaceus in the treatment of patients with seasonal allergic rhinitis." Phytotherapy research : PTR **24**(2): 175-181.

21 Duan, P. and Z. M. Wang (2002). "[Clinical study on effect of Astragalus in efficacy enhancing and toxicity reducing of chemotherapy in patients of malignant tumor]." Zhongguo Zhong xi yi jie he za zhi Zhongguo Zhongxiyi jiehe zazhi = Chinese journal of integrated traditional and Western medicine / Zhongguo Zhong xi yi jie he xue hui, Zhongguo Zhong yi yan jiu yuan zhu ban **22**(7): 515-517.

22 Chen, K. T., C. H. Su, et al. (2002). "Reducing fatigue of athletes following oral administration of huangqi jianzhong tang." Acta pharmacologica Sinica **23**(8): 757-761.

23 *Ibid.*

24 Bernardes de Jesus, B., K. Schneeberger, et al. (2011). "The telomerase activator TA-65 elongates short telomeres and increases health span of adult/old mice without increasing cancer incidence." Aging cell **10**(4): 604-621..

25 *Ibid.*

26 Sevimli-Gur, C., I. Onbasilar, et al. (2011). "In vitro growth stimulatory and in vivo wound healing studies on cycloartane-type saponins of Astragalus genus." Journal of ethnopharmacology **134**(3): 844-850.

27 Tang, W. and G. Eisenbrand (1992). Chinese drugs of plant origin: chemistry, pharmacology, and use in traditional and modern medicine, Springer-Verlag.

28 Mamedova, R. P. and M. I. Isaev (2004). "Triterpenoids from Astragalus Plants." Chemistry of Natural Compounds **40**(4): 303-357.

29 Bedir *et al.* (1998a). "Cyclocephaloside I: A Novel Cycloartane-Type Glycoside from *Astragalus microcephalus*." J. Nat Prod. **1998**, *61*, 503-505, Bedir *et al.* (1998b). "Cycloartane Triterpene Glycosides from the Roots of *Astragalus brachypterus* and *Astragalus microcephalus*" J. Nat Prod. **1998**, *61*, 1469- 1472, Bedir *et al.* (1999a) "Secondary Metabolites from the Roots of *Astragalus trojanus* " J. Nat Prod. **1999**, *62*, 563-568, Bedir *et al.* (1999b). " Trojanoside H: a cycloartane-type glycoside from the aerial parts of *Astragalus trojanus*", Phytochemistry 51 (1999) 1017-1020, Bedir *et al.*

cycloartane type saponins that are present in [redacted] *Astragalus* species as major chemical entities (astragaloside IV, cycloastragenol, cyclocephaloside I and cyclocanthoside E)[30].

IX. ANTICIPATED INTAKE/EXTENT OF USE OF THE NF INGREDIENT

39. The product will be marketed in the form of the bulk loose powder for intended use in food supplements in the sense of Directive 2002/46 in the European Union. The target population is the healthy adult population and the product is not intended to diagnose, treat, cure or prevent any disease but will be directed for the maintenance or enhancement of physiological functions in the body that are associated with wellbeing and health. Of particular interest are some physiological processes and functions, whose performance may decline with age and may be mediated by telomerase activity, which is participating in the mechanism of action of low doses of Cycloastragenol. Various on-going studies have been designed to substantiate a health claim in this respect.

40. Projections of anticipated intakes are to evaluate the dietary and nutritional significance of the NF Ingredient. As previously described (section I.i)), the recommended daily intake (8 mg/day) will be very clearly labeled together with all key information related to Cycloastragenol-TA65. Historical and current consumption patterns of Cycloastragenol in non-EU countries (available in US since 2007) were considered first in order to derive appropriate daily intakes as food supplements in the EU.

41. In the EFSA database there is information from five EU countries on chronic consumption patterns of supplements (Table IX.1).

Table IX.1. Chronic consumption of Supplements (Units)[31]							Percentile	
Country	Age Group	Consumers	% Cons	Mean	Std	Median	95	99
Finland	Adults	663	42,1	3,2	9,4	1,9	8,5	17,0
Finland	Elderly	215	46,4	3,5	4,4	2,4	12,1	25,7
Ireland	Adults	246	25,7	3,0	6,5	1,3	10,0	40,9
Italy	Adolescents	4	1,6	3,1	2,2	3,4	5,2	5,2
Italy	Adults	98	4,2	5,4	9,9	2,1	22,4	78,0
Italy	Elderly	12	4,1	2,6	3,2	1,2	10,0	10,0
Italy	Very elderly	17	7,5	6,6	7,5	4,0	26,7	26,7
Sweden	Adolescents	178	17,5	1,1	2,0	0,8	2,8	15,0
United Kingdom	Adults	420	24,4	5,1	36,7	1,1	9,0	42,9
Mean				**3,7**	**9,1**	**2,0**	**11,9**	**29,0**

42. Therefore, the recommended pattern of consumption (8 mg per day in one daily dose) is within the normal range of food supplements intake patterns in the EU. There is no reason to expect overconsumption of the NF Cycloastragenol-TA65.

43. The NF is intended for healthy adults, over the age of 25 years. The daily intake is taken from one capsule containing 8 mg of the NF.

44. The Product is not intended to replace other food supplements, food components or foods in the diet, and it does not supply significant dietary macro- or micro-nutrients.

(2001a). "Two novel cycloartane-type triterpene glycosides from the roots of *Astragalus prusianus*", Tetrahedron 57 (2001) 5961-5966, Bedir *et al.* (2001b) "Trojanosides I—K: New Cycloartane-Type Glycosides from the Aerial Parts of *Astragalus trojanus*", *Chem. Pharm. Bull.* 49(11) 1482—1486 (2001), Polat *et al.* (2009) "Cycloartane-type glycosides from *Astragalus amblolepis* ", Phytochemistry 70 (2009) 628-632, Polat *et al.* (2010) "Triterpenoid saponins from *Astragalus wiedemannianus* Fischer ", Phytochemistry 71 (2010) 658-662, Horo *et al.* (2010) "Triterpene glycosides from *Astragalus icmadophilus*", Phytochemistry 71 (2010) 956-963.

[30] See # 26 *supra*.

[31] EFSA comprehensive consumption database. Last revised 04-10-2013.

X. INFORMATION ON PREVIOUS HUMAN EXPOSURE TO THE NOVEL FOOD

45. The NF Ingredient is pure (>98%) Cycloastragenol and it has been widely commercialized and consumed in a number of countries outside the EU. In particular, it has been widely consumed in China and in the USA. In addition, Cycloastragenol has been consumed as part of a number of extract formulations of *Astragalus*, as for example in Astragaloside IV supplements.

46. Table X.1 shows some examples of Cycloastragenol marketed as food supplement in China and USA, including the recommended doses, purity and format. Expanded information on these products consisting of Cycloastragenol is available in the referred web pages, also reporting information on Astragalosides containing Cycloastragenol.

Table X.1. Previous human exposure

Company	Brand name	Country	mg of Cycloastragenol* per Capsule	Recommended daily dose	Purity	Product Format	Sold On
GUANGZHOU ASTRAGLAXO BIOPHARMACEUTICAL CO	Astraglaxo Cycloastragenol	China	5/10/20/25 & 50 mg	2-4 capsules/day	> 98%	Capsules	http://www.astraglaxo.com/en/products.php
BEIJING MEDICASS TECHNOLOGIES CO. LTD.	Cycloastragenol	China	5 mg	1-2 capsules/day	> 98%	Capsules	http://www.medicass.com/default_en.asp
KING TIGER	Cycloastragenol	China	5-50 mg	ND	50% - 98%	Capsules	http://www.astragalosideiv.cn/
CRACK-AGING	Cycloastragenol	USA	5/10/25 & 50 mg	10-50 mg/day	98%	Capsules	http://www.crackaging.com/
TERRATERNAL	Cycloastragenol	USA	10/25 mg	10-50 mg/day	67%	Capsules	http://www.terraternal.com/

ND: Not described

47. In the Annex IV.9, graphic information on these products is reported.

In addition to USA and China, the applicant has sold various amounts of the NF in 46 additional countries: Afghanistan, Argentina, Australia, Bangladesh, Belize, Brazil, Cambodia, Canada, Chile, Colombia, Dominican Republic, Egypt, Gibraltar, Grand Cayman, Hong Kong, India, Indonesia, Israel, Japan, Kazakhstan, Lebanon, Malaysia, Mexico, Moldova, Mongolia, New Zealand, Nigeria, Northern Mariana Islands (Saipan), Pakistan, Philippines, Puerto Rico, Russia, Saudi Arabia, Singapore, South Africa, South Korea, Sri Lanka, Switzerland, Taiwan, Thailand, Trinidad, Turkey, United Arab Emirates, US Virgin Islands, Venezuela and Yemen.

X.I Human studies with purified Cycloastragenol-ta65.

48. Three following human studies with Cycloastragenol-TA65 have been conducted in humans using daily doses much higher than the recommended dose by the applicant. No adverse events have been reported.

49. i) Montgomery, M.; Pine, D.; Roberts, C. Preliminary Safety and Effectiveness Study for TA-65MD for Human Use. (T.A. Sciences 2013, unpublished). In this study the safety and effectiveness of Cycloastragenol-TA-65 have been evaluated for short-term use in a DBPC study involving 125 healthy adult humans. TA-65 was administered in the form of capsules containing 8mg Cycloastragenol-TA65 b.i.d (two capsules per day) resulting in a total of 16 mg Cycloastragenol). Subjects were randomized into either the active product group of 100 subjects or the placebo group of 25 subjects for the entire 30-day study. The placebo group was provided a green bottle with generic labeling to match the active product group. The methodology included baseline data collection (adrenal blood draws, vital signs, history and intake); 1 week washout; 1 capsule b.i.d.

for 30 days by each subject; and final day adrenal blood draws for biochemistry data. The safety and efficacy measurements assessed were: vital signs; *safety parameters*, including blood pressure, body temperature, blood glucose, liver □functionality (asparate aminotransferase (AST), alanine aminotransferase (ALT), albumin), kidney function (blood urea nitrogen (BUN)), thyroid-stimulating hormone, pH blood, pH saliva, and pH urine; *efficacy parameters*, including homocysteine, C-reactive protein, T-cell (CD4 and CD8), nitrates (urine), nitrites (urine), and oxidation reduction potential (blood, urine, and saliva). Each of these tests was run on arterial blood drawn following standardized protocol for the procedures and was completed by healthcare professionals. Over the course of the 30-day study, all subjects complied with the protocol. There were no dropouts and no reports of adverse side effects or interactions. There was also no observed organ or system damage in any study participant. This preliminary clinical analysis of safety and effectiveness parameters of TA-65MD shows that the product is safe and effective for short-term human use.

50. ii) Harley CB, Liu W, Blasco M, Vera E, Andrews WH, Briggs LA, Raffaele JM. A natural product telomerase activator as part of a health maintenance program. Rejuvenation Res, 2011, 14(1):45-56. This study was based on the hypothesis that most human cells lack sufficient telomerase to maintain telomeres and hence these genetic elements shorten with time and stress, contributing to aging and disease. Thus, in January 2007, a commercial health maintenance program, PattonProtocol-1, was launched that included a natural product-derived telomerase activator (TA-65®, 10–50 mg daily), a comprehensive dietary supplement pack, and physician counselling/laboratory tests at baseline and every 3–6 months thereafter. The present study reports analysis of the first year of data focusing on the immune system. Low nanomolar levels of TA-65® moderately activated telomerase in human keratinocytes, fibroblasts, and immune cells in culture; similar plasma levels of TA-65® were achieved in pilot human pharmacokinetic studies with single 10- to 50-mg doses. The most striking in vivo effects were declines in the percent senescent cytotoxic (CD8+/CD28-) T cells (1.5, 4.4, 8.6, and 7.5% at 3, 6, 9, and 12 months, respectively; p=not significant [N.S.], 0.018, 0.0024, 0.0062) and natural killer cells at 6 and 12 months (p = 0.028 and 0.00013, respectively). Most of these decreases were seen in cytomegalovirus (CMV) seropositive subjects. In a subset of subjects, the distribution of telomere lengths in leukocytes at baseline and 12 months was measured. Although mean telomere length did not increase, there was a significant reduction in the percent short (<4 kbp) telomeres (p = 0.037). No adverse events were attributed to PattonProtocol-1. The study concluded that the protocol lengthens critically short telomeres and remodels the relative proportions of circulating leukocytes of CMV+ subjects toward the more "youthful" profile of CMV- subjects.

51. iii) Harley, CB, Liu W, Raffaele JM, Flom PL. A natural product telomerase activator as part of a health maintenance program: Metabolic and cardiovascular response. Rejuvenation Research (06/2013; DOI:10.1089/rej.2013.1430). In this study the authors indicated that in over a 5-year period and an estimated 7000 person-years of use, no adverse events or effects have been attributed to TA-65® by physicians licensed to sell the product. The study reports on changes in metabolic markers measured at baseline (n=97-107 subjects) and every 3-6 months (n=27-59 subjects) during the first 12 months of study. Rates of change per year from baseline determined by mixed effects ANOVA were -3.72 mg/dL for fasting glucose (p=0.02), -1.32 mIU/mL for insulin (p=0.01), -13.2 and -11.8 mg/dL for total- and LDL-cholesterol (p=0.002, p=0.002, respectively), -17.3 and -4.2 mm Hg for systolic and diastolic blood pressure (p=0.007 and 0.001, respectively), and -3.6 umole/L homocysteine (p=0.001). In a subset of individuals with bone mineral density (BMD) measured at baseline and 12 months, density increased 2.0% in the spine (p=0.003). The author concluded that in addition to apparent positive immune remodeling, PattonProtocol-1 (Harley et al., 2011) may improve markers of metabolic, bone and cardiovascular health.

XI. NUTRITIONAL INFORMATION ON THE NF INGREDIENT

52. The composition and the production process of the Product are described in Table I.3.b and Figure 2, respectively.

53. Cycloastragenol-TA65 is not intended and will not replace other food supplements, food components or foods in the diet, and it does not supply leading significant dietary macro- or micro-nutrients, nor does it affect any healthy dietary pattern. No significant anti-nutritional factors

(e.g. inhibiting mineral absorption or bioavailability) are included in the composition of the NF. Consequently, no nutritional concerns are foreseen and it cannot be expected the food supplement containing the NF to be nutritionally disadvantageous to the consumer.

(i) Absorption, distribution, metabolism and elimination (ADME)

54. The intestinal absorption of Cycloastragenol was studied using TAT2 (Cycloastragenol of approximately 90-95% purity generated by acid hydrolysis from commercially available astragaloside IV) in the Caco-2 cell monolayer model with additional examination of metabolism in rat and human liver microsomes[32]. Because passage through the Caco-2 monolayer proceeded by passive diffusion[33] with minimal metabolism (only two oxidized metabolites and four glucuronide conjugates identified in the apical and basolateral sides of the monolayer), the possibility of rapid first-pass metabolism of TAT2 through the intestinal epithelium was suggested. The possibility of extensive metabolism was, however, also suggested when 30-minute incubations in rat and human liver microsomes revealed only 17.4% and 8.2%, respectively, of the parent remaining. In the liver samples, TAT2 metabolites were primarily monohydroxylated with additional hydroxylation occurring post-oxidation. Based on these findings that indicate efficient first-pass metabolism and extensive hepatic metabolism, oral bioavailability of TAT2 is expected to be low. Supportive of these expectations, the typical plasma levels reported in humans 4-8 hours after oral ingestion of 5-100 mg Cycloastragenol (equivalent to 0.08-1.67 mg/kg bw for a 60-kg individual) are in the 1-20 nM range[34] (see also Annex II.20).

XII. MICROBIOLOGICAL INFORMATION ON THE NF INGREDIENT

55. Taking into account the nature and purity of the NF and its manufacturing process, the risk of bacterial proliferation is considered very low. In any case, as included in the specifications (Table 2.B.1.) a careful microbiological control is in force.

XIII. TOXICOLOGICAL INFORMATION ON THE NF INGREDIENT

56. This scheme covers the set of toxicological information to assess the NF.

XIII.1. Sub-chronic studies

XIII.1.a) Evaluation of Cycloastragenol-TA65 in a 13-week subchronic toxicity study with a 4-week recovery period (Annex IV.14)

57. This study was performed in compliance with Good Laboratory Practices (GLP) in accordance with OECD Principles of Good Laboratory Practice (as revised in 1997)[35, 36] and US FDA GLP[37]

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[32] Zhu, J., S. Lee, et al. (2010). "In vitro intestinal absorption and first-pass intestinal and hepatic metabolism of cycloastragenol, a potent small molecule telomerase activator." Drug metabolism and pharmacokinetics **25**(5): 477-486.

[33] Whether applied to the apical (absorptive) or basolateral (secretory) side, ¬ about 9.1% of applied TAT2 (50 uM) reached the receiver side within 10 min. Further, the transported amount quickly increased to about 38% at 60 minutes of exposure and from 60 to 180 minutes, transport slowed and approached a plateau.

[34] Harley, C. B., W. Liu, et al. (2011). "A natural product telomerase activator as part of a health maintenance program." Rejuvenation research 14(1): 45-56.

[35] OECD Environmental Health and Safety Publication, Series on Principles of Good Laboratory Practice and Compliance Monitoring – Number 1. Environment Directorate, Paris 1998.

[36] Due to a refrigerator failure, the quality control (QC) materials, calibrators and/or reagents used to validate hematology and urinalysis parameters (excluding microscopic sediment examination), as well as lactate dehydrogenase and total creatine kinase from recovery animals only (Groups 5 and 6) on Day 118 only were exposed to temperatures > 10 °C for six days. All sample results were reported acceptable based on QC results and/or performing laboratory Standard Operating Procedures for daily control evaluation. This information is documented in the Good Laboratory Practice Compliance Statement (PSL (2012). TA-65®: A 90-day Repeated Dose Oral Gavage Study in Rats with Cardiac Assessment. Report Number: Study No. 32721, Product Safety Labs (PSL): p. 1 - 616.).

[37] US FDA, 21 CFR 58, 1987.

and in general compliance with the OECD Guidelines for Testing of Chemicals and Food Ingredients[38].

58. Cycloastragenol-TA65 was evaluated in a 13-week subchronic toxicity study with a 4-week recovery period in Hsd: Sprague-Dawley SD® rats (8 weeks old at study initiation) In the main toxicity study, a total of 80 rats were randomly assigned to one of four groups (10 males and 10 females/group): 0 (Group 1; vehicle control[39]), 40 (Group 2), 80 (Group 3) and 150 (Group 4) mg/kg bw/day Cycloastragenol-TA65. In the recovery study, 20 additional rats were randomly assigned to one of two additional groups (5 males and 5 females/group): 0 (Group 5; vehicle control) and 150 (Group 6) mg/kg bw/day Cycloastragenol-TA65. In the main toxicity and recovery studies, vehicle control and treatment doses[40] were administered to rats via oral gavage once per day for 91 or 92 days. At the end of the treatment period, rats in the recovery study entered the 4-week recovery period. Food was provided ad libitum to all animals, except during scheduled periods of fasting prior to blood/urine collection. Water was provided ad libitum to all animals at all times.

59. All rats were observed twice daily for viability and once daily for behavioral changes and signs of gross toxicity. Functional observational battery and motor activity tests were performed on all surviving animals in the main toxicity study during Week 12. Blood pressure was assessed during Week 12 near the end of the test period for all surviving main toxicity animals as well as during Week 16 for all recovery animals. Body weights of all animals were measured during the acclimation period, on Day 1 prior to study initiation, once per week during the study periods, and prior to termination. Individual food consumption for all animals was recorded on the same schedule with mean daily food consumption calculated weekly. All study rats were subjected to ophthalmologic examination prior to study initiation; on Day 90 the eyes of main toxicity rats were re-examined. Urine[41] and blood (hematology[42] and clinical chemistry[43]) were collected on Day 86 from main study animals that had been fasted overnight and on Day 118 from fasted recovery animals. In both studies, blood for coagulation parameters (i.e., prothrombin time and activated partial thromboplastin time) was collected from fasted animals prior to termination. Gross necropsy and histopathological evaluation occurred for all decedent and surviving rats at death or termination (Day 92 for males, Day 93 for females) in the main toxicity study. Although animals in the recovery study were examined via gross necropsy (including collection of organ weights) at termination (Day 120), tissues were not subjected to histopathologic evaluation[44].

60. Due to the widely reported cardiotonic effects of extracts from *Astragalus* species[45] (in addition to other species not identified in the genus) in traditional medicine[46], a cardio-component consisting

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38 Concentrations of the test substance at up to the Maximum Tolerated Dose (MTD) were not tested. OECD Guidelines for Testing of Chemicals and Food Ingredients, Section 4 - Health Effects, *Repeated Dose 90-Day Oral Toxicity Study in Rodents* (1998). Dose concentrations for the study were selected based on previously conducted pilot studies in rodents and tolerance already demonstrated in humans (see # 34 *supra*).

39 Aqueous 0.5% weight/volume (w/v) methylcellulose and 2% volume/volume (v/v) Tween 80 vehicle control mixture in deionized reverse osmosis water.

40 Dose preparations of 0.4%, 0.8% and 1.5% Cycloastragenol-TA65 were prepared as w/v suspensions in the vehicle to provide, respectively, 40, 80 and 150 mg/kg bw Cycloastragenol-TA65.

41 Urinalysis parameters included quality, color, clarity, volume, pH, glucose, protein, specific gravity, ketone, bilirubin, blood, urobilinogen and microscopic urine sediment.

42 Hematology parameters included erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration, red cell distribution width, absolute reticulocyte count, platelet count, total white blood cell and differential leukocyte count.

43 Clinical biochemistry parameters included aspartate aminotransferase, alanine aminotransferase, sorbitol dehydrogenase, alkaline phosphatase, total bilirubin, blood urea nitrogen, blood creatinine, total cholesterol, triglycerides, total creatine kinase, fasting glucose, total serum protein, albumin, globulin, calcium, inorganic phosphorus, sodium, potassium, chloride, and lactate dehydrogenase.

44 The recovery phase is not a required component of the 90-day subchronic toxicity study.

45 Cardiotonic effects are primarily associated with *A. membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao and *A. membranaceus* (Fisch.) Bge., although other unspecified members in the genus are reported to also produce the same or similar effects (WHO, 1999; Ma *et al.*, 2002; McKenna, *et al.*,2002; Yu *et al.*, 2007; Sevimli-Gür *et al.*, 2011).

of blood pressure measurement via tail-cuff and the serum biochemistry parameters aspartate aminotransferase (AST), total creatine kinase (CK) and lactate dehydrogenase (LDH) was included in the 13-week rodent study.

61. Daily administration of the NF Ingredient at levels up to 150 mg/kg bw/day was well tolerated by the rats in the main toxicity and recovery studies. No treatment-related mortalities occurred in either study. One control male in the main toxicity study was euthanized on Day 65 for humane reasons. Earlier that same day, the animal had sustained internal injury from the gavage needle while escaping from the technician performing dosing. Macroscopic and microscopic findings at necropsy were consistent with injury incurred from a gavage needle. Neither injury nor death of this animal was related to the test substance. Ingestion of Cycloastragenol-TA65 was not found to affect health or growth as measured by viability, condition, behavior, body weight, body weight gain, food consumption, or food efficiency in main toxicity or recovery study groups.

62. No treatment-related effects were identified in the ophthalmology, hematology, clinical chemistry (including the cardiac biomarkers AST, CK and LDH), or urinalysis of animals in any group of either study. Although statistical significance was shown for several parameters, none was attributable to ingestion of the NF Ingredient because the changes were incidental/sporadic in nature, were not clinically relevant, were not correlated with other clinical or histopathologic changes, also occurred in the controls, and/or were within the ranges historically observed in the age and strain of rats used in this study.

63. These observations are summarized below:

    (i) Changes noted only sporadically with no dose-response relationship: (a) a statistically significant decrease in mean daily body weight gain for Days 22-29 in Group 2 males compared to Group 1 control males ($P < 0.01$); (b) a corresponding statistically significant decrease in mean food efficiency for Days 22-29 in Group 2 males compared to Group 1 control males ($P < 0.05$); and (c) a statistically significant increase in mean daily food consumption on Days 36-43 in Group 2 females compared to Group 1 control females ($P < 0.01$) and on Days 36-43 ($P < 0.01$), Days 50-57 ($P < 0.05$) and overall (Days 1-91) ($P < 0.05$) in Group 4 females compared to Group 1 control females.

    (ii) Changes that were considered incidental and not specifically related to the test substance because the magnitude of the changes were not clinically relevant or correlated with any other clinical or histopathological change or were within the range of historically observed changes in the age and strain of rat used[47]: (a) a statistically significant increase in MCH (Day 86) in Group 4 males compared to the corresponding Group 1 control males ($P < 0.05$); (b) a statistically significant increase in absolute reticulocyte count (Day 86) in Group 3 females compared to corresponding Group 1 females ($P < 0.05$); (c) statistically significant increases in total white blood cell count and absolute lymphocyte concentration (Day 118) in Group 6 females, compared to corresponding Group 5 control females ($P < 0.05$ for both); (d) statistically significant decreases in alkaline phosphatase and cholesterol on Day 86 in Group 3 males, triglycerides in Group 2 and Group 3 males, and bilirubin in Group 2, 3 and 4 males, when compared to the corresponding Group 1 control males ($P < 0.05$ for all). (e) statistically significant decreases in creatinine and cholesterol, along with statistically significant increases in calcium, potassium, and inorganic phosphorus concentrations on Day 118 in Group 6 females compared to Group 5 control females ($P < 0.05$ for all).

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[46] WHO (1999). "WHO monographs on selected medicinal plants."; Ma, X. Q., Q. Shi, et al. (2002). "Chemical analysis of Radix Astragali (Huangqi) in China: a comparison with its adulterants and seasonal variations." Journal of agricultural and food chemistry **50**(17): 4861-4866.; McKenna, Hughes *et al.* # 14 *supra*; Yu, Q. T., L. W. Qi, et al. (2007). "Determination of seventeen main flavonoids and saponins in the medicinal plant Huang-qi (Radix astragali) by HPLC-DAD-ELSD." Journal of separation science **30**(9): 1292-1299; see # 14 *supra*.

[47] Derelanko, M. J. (2000). Toxicologist's pocket handbook, CRC Press.; Pettersen, J. C., R. L. Morrissey, et al. (1996). "A 2-year comparison study of Crl:CD BR and Hsd:Sprague-Dawley SD rats." Fundamental and applied toxicology : official journal of the Society of Toxicology **33**(2): 196-211..

(iii) In the cardiac assessment, no effect on blood pressure[48] (i.e., systolic, diastolic, or mean arterial pressure) was found between the dose groups and their corresponding control groups for either sex in the main toxicity study or in the recovery study. In addition, the Week 16 values for Groups 5 and 6 and Week 12 values for Groups 1 and 4 are comparable, which indicates that the test substance was without influence and the test criteria are reliable. Regarding the serum biochemistry parameters, no statistically significant changes in AST, CK or LDH were identified in male or female rats in the main toxicity (Day 86) or recovery (Day 118) studies.

(iv) Compared to their respective control groups, statistically significant changes for absolute or relative organ weights in the dose groups included increased absolute liver weight in Group 4 males ($P < 0.05$); increased heart-to-brain weight ratio in Group 6 males ($P < 0.05$); and in Group 3 and 4 females, increased absolute heart weight ($P < 0.05$ for both groups), heart-to-body weight ($P < 0.01$, Group 3; $P < 0.05$, Group 4) and heart-to-brain weight ratios ($P < 0.01$, Group 3; $P < 0.05$, Group 4). These findings had no clinical or histopathologic correlate; therefore, they were considered incidental and toxicologically insignificant.

(v) Macroscopic findings in main study males (Day 92) included small, bilateral testes due to slight germ cell atrophy and a small, bilateral epididymis corresponding to slight hypospermia in a Group 1 control male; a soft mass in the left epididymis due to the presence of a sperm granuloma in a Group 2 male; and a discolored liver with a supernumerary lobe in a Group 4 male that corresponded to chronic liver infarction consistent with liver lobe torsion. One additional Group 4 male had a damaged tail. No macroscopic observations were reported in recovery males (Day 120). Macroscopic findings in females, included fluid-filled uteri/oviducts consistent with variations in the estrous cycle in a total of ten main study females on Day 93 (five in Group 1, two in Group 2, and three in Group 3) and five recovery females on Day 120 (three in Group 5 and two in Group 6); an endometrial cyst on the lower left horn of the uterus in one Group 1 female; and multiple mass-like lesions embedded in fat and connective tissue dorsal to the thymus, anterior to the lungs in one Group 1 control female. Microscopic evaluation of the latter finding is not confirmed, but is considered toxicologically insignificant in a control animal.

(vi) Microscopic findings in control and high dose animals of the main study included minimal chronic progressive neuropathy of minimal intensity in nine Group 1 males, three Group 1 females, ten Group 4 males, and four Group 4 females (incidental finding commonly observed in SD rats); a small, poorly demarcated neoplasm in the cortex of one kidney in a Group 4 female that had morphologic features consistent with renal mesenchymal tumor (spontaneous lesion, incidental); myofiber degeneration of minimal to slight intensity in the esophageal wall in males and females of Groups 1 and 4 (attributed to tissue trauma associated with daily gavage procedures). No macroscopic or microscopic observations related to the test substance were noted for the heart.

64. In summary, although statistically significant effects were noted for several endpoints, these were incidental or sporadic in nature, had no dose-response relationship, were not clinically relevant, were also present in the control group, and/or were within the range of historical control data for rats of this age and strain. No effects attributable to administration of the NF Ingredient were identified. Under the conditions of this study, the no-observed-adverse-effect level (NOAEL) of orally administered Cycloastragenol-TA65 was > 150 mg/kg bw/day in male and female rats.

XIII.1.b) Subchronic (ip/iv) toxicity study in rats and dogs of a product (Radix Astragali extract) containing Cycloastragenol

65. This study was on Radix Astragali extract (RAE) obtained from Astragalus membranaceus (Yu, Ouyang et al. 2007). The extract consisted of Astragalus polysaccharide (88.96% glucose) and Astragalus membranaceus saponins (including Astragaloside IV, Isoastragaloside IV, Astragaloside I, Isoastragaloside I, Astragaloside II, Isoastragaloside II, and Acetylastragaloside I

[48] All surviving study rats were acclimated to the blood pressure tail cuff procedure over a two-week period before testing. Each rat was evaluated five times with an average of five data points (30 seconds each) collected *per* trial.

which all have Cycloastragenol as the aglycone). The subchronic toxicity of RAE was observed in Sprague–Dawley rats and beagle dogs to evaluate the safety dosage range in clinical application. Subjects were daily administered of RAE by intra-peritoneal injection (rats) or intravenous injection (dogs) for three consecutive months. General index were observed such as food-intake, behavior, body weight, hematological parameters, etc. Body weight, the absolute and relative weights of the principal organs, clinical signs, and hematology index were not significantly different between experimental groups and control groups. The hematological biochemistry examination and histopathology examination of experimental groups were similar to control groups and did not reveal any dose-dependent or test article-related associations. In conclusion, the authors determined that RAE administered daily for three months by intra-peritoneal injection (rats) or intravenous injection (dogs) was safe without any distinct toxicity or side effects; the safety dosage ranges were 5.7–39.9 g/kg for rats and 2.85–19.95 g/kg for beagle dogs (Yu, Ouyang et al. 2007). The authors reported a median effective dose (MED) (dose that produces the desired effect in 50 % of the consuming population) of 0.57 g/kg for RAE in humans (administration route not specified); the safety dosage ranges in the rats and beagle dogs are equivalent to 5–70 times the human MED, which provides indirect corroboration of safety.

XIII.2 Genotoxicity Studies

XIII.2.a) Bacterial reverse mutation assay

66. The mutagenic potential of Cycloastragenol-TA65 as facility-designated ingredient "DTI-001"[49] was evaluated by bacterial reverse mutation assay (i.e. Ames test) using the two-phase plate incorporation method in the presence and absence of Aroclor 1254-induced rat liver S9[50]. The initial toxicity-mutation assay (first phase) provided a preliminary mutagenicity evaluation and established the dose-range for the second phase. The second phase (the confirmatory mutagenicity assay) was then conducted to evaluate and confirm the mutagenic potential of the test article. Tester strains included *Salmonella typhimurium* TA98, TA100, TA1535 and TA1537 and Escherichia coli strain WP2 uvrA. In the absence of activation, sodium azide served as positive control for *S. typhimurium* test strains TA100 and TA1535, 2-nitro-fluorene for test strain TA98, 9-aminoacridine for test strain TA1537 and methylmethanesulfonate for E. coli WP2 uvrA. In the presence of S9 activation, the positive control for all bacterial strains was 2-aminoanthracene. The vehicle (negative) control for all tests was dimethyl sulfoxide (DMSO). The assays were conducted under GLP conditions[51] and in compliance with the international guidelines of the OECD (1998) and the International Conference on Harmonisation (ICH 1996; ICH 1997). Based on pre-experiments to evaluate cytotoxicity, 5000 µg/plate DTI-001 in DMSO was selected as the highest dose for all test strains and conditions. For the initial 5000 µg/plate series, seven lower dose levels (1500, 500, 150, 50, 15, 5.0 and 1.5 µg/plate) were prepared and tested. In the initial toxicity-mutation assay, no cytotoxicity[52] and no mutagenic response was observed. In the confirmatory mutagenicity assay, five dose levels (5000, 1500, 500, 150 and 50 µg/plate) were prepared and tested. Dose-dependent increases in the number of revertant colonies of greater than two times (TA98, TA100, WP2 uvrA) or three times (TA1535, TA1537) the negative control values were not observed in any strain treated with DTI-001 regardless of the presence or absence of metabolic activation. Precipitation was observed at the 1500 µg/plate dose levels and higher in all test strains with and without S9 activation in assays of both the initial and secondary phases, but no cytotoxicity and no mutagenic response was observed. Under the conditions of this study, test article DTI-001 (90% Cycloastragenol) was not a mutagen.

XIII.2.b) In vitro chromosomal aberration assay

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[49] DTI-001 is ~90% Cycloastragenol from acid-hydrolyzed astragaloside IV from *A. membranaceus*. (The NF Cycloastragenol ingredient is ≥ 98% Cycloastragenol.)

[50] Wagner, V. O. and M. L. Klug (2003). Bacterial Reverse Mutation Assay. Report Number: Study AA68SB.503.BTL BioReliance: p. 1-55.

[51] US EPA GLP: 40 CFR 792 and 40 CFR 160, 1989 and US FDA GLP: 21 CFR 58, 1987.

[52] The background lawn was unaffected.

67. The clastogenic potential of the NF Cycloastragenol-TA65 to induce structural changes in a cell's genetic material was evaluated in an *in vitro* mammalian chromosome aberration test in Chinese hamster V79 cells (Bioservice 2012. a). The assay was performed under GLP conditions[53] and in compliance with international guidelines.[54] Cycloastragenol-TA65, prepared one hour before treatment, was dissolved in DMSO (ultrasound, five minutes) then diluted in culture medium until the concentration of DMSO was 1%. At dilution in cell medium, precipitation occurred and necessitated a second ultrasound step (five minutes). Due to a lack of solubility, Cycloastragenol-TA65 was applied as suspensions of selected concentrations to V79 cells in the presence and absence of metabolic activation.[55] Negative controls consisted of 1% DMSO in cell culture medium. Positive controls in the absence of metabolic activation consisted of ethylmethanesulfonate (EMS) (400 and 600 µg/ml) prepared in nutrient medium. Positive controls in the presence of metabolic activation consisted of cyclophosphamide (CPA) (0.83 µg/ml) also prepared in nutrient medium. Cultures were prepared in duplicate for all controls and at each selected test concentration of Cycloastragenol-TA65 in all experiments. After the cells were fixed and stained, at least 200 metaphases per test concentration and validity control sample were scored for cytogenic damage to determine the incidence of structural chromosomal aberrations (i.e., breaks, fragments, deletion exchanges, chromosomal disintegrations and gaps). In addition, 1000 cells per test culture and validity control were evaluated for cytotoxicity to determine the mitotic index (percent of cells in mitosis). As an additional measure of cytotoxicity, relative cell density was calculated as the mean of 20 cell counts per test group (cells within the visual field at a 400-fold magnification).

68. Based on results of the solubility test and in accordance with the guidelines, the highest dose in the pre-experiment for toxicity was 10 mM. In the pre-experiment, the following concentrations were tested in the presence and absence of metabolic activation: 0.016, 0.031, 0.063, 0.125, 0.25, 0.50, 1.25, 2.5, 5, and 10 mM. In the absence of metabolic activation, precipitation was observed at concentrations of 0.125 mM and higher. In the presence of activation, precipitation was observed at concentrations of 0.25 mM and higher. Toxicity, as evaluated by a biologically relevant decrease of the relative mitotic index (below 70%) and cell density (below 50%) was observed at concentrations of 0.125 mM (63% rel. mitotic index) and 0.25 mM (12% rel. cell density), respectively, and higher without metabolic activation and at concentrations of 2.5 mM (24% rel. mitotic index; 11% rel. cell density) and higher with activation.

69. In Experiment I, V79 cells were exposed to selected concentrations of Cycloastragenol-TA65 for four hours without metabolic activation (0.025, 0.05, 0.10, 0.125, 0.150, 0.175, 0.200, 0.225, and 0.250 mM) and with metabolic activation (0.05, 0.10, 0.20, 0.50, 1.25, 1.50, 1.75, 2.00, 2.25, and 2.50 mM). In the absence of activation, the concentrations 0.05, 0.10, and 0.125 mM were selected for microscopic evaluation; in the presence of activation, 0.05, 1.25, 1.50, 1.75, and 2.00 mM concentrations were selected. The findings for Experiment I are summarized in Table XIII.1. Precipitation of the test substance was noted without metabolic activation at concentrations of 0.10 mM and higher and with metabolic activation at concentrations of 1.25 mM and higher. Toxicity was observed at concentrations of 0.125 mM (47% rel. mitotic index) and 0.10 mM (53% rel. cell density) and higher without metabolic activation and at concentrations of 1.50 mM (53% rel. mitotic index; 30% rel. cell density) and higher with activation. Clastogenicity as indicated by the aberration rates of the cells[56] was within the historical control data range (0.0% - 4.0%) of the

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[53] Chemicals Act ("*Chemikaliengesetz*") of the Federal Republic of Germany, Appendix 1 to §19a as amended and promulgated on June 20, 2002 (BGB1. I Nr. 40 S. 2090), revised October 31, 2006 (BGB1. I Nr. 50 S. 2407) and OECD Principles of Good Laboratory Practice (as revised in 1997); OECD Environmental Health and Safety Publications; Series on Principles of Good Laboratory Practice and Compliance Monitoring - Number 1. Environment Directorate, Organisation for Economic Co-operation and Development, Paris 1998.

[54] OECD Test No. 473: In vitro Mammalian Chromosome Aberration Test, OECD Publishing, adopted July 21, 1997 and Commission Regulation (EC) No. 440/2008 B.10, "*Mutagenicity* – In vitro *Mammalian Chromosome Aberration Test*", dated May 30, 2008.

[55] The S9 microsomal fraction was prepared from the livers of male Wistar rats induced with phenobarbital (80 mg/kg bw) and β-napthoflavone (100 mg/kg bw) provided orally on each of three consecutive days. Bioservice (2012. a). In vitro Mammalian Chromosome Aberration Test in Chinese Hamster V79 cells with TA-65®. Report Number: Study No. 114190 BSL Bioservice.

[56] One hundred metaphases *per* test concentration duplicate were evaluated (total of 200 metaphases) for all controls and most dose groups. A total of 400 metaphases (200 per duplicate) was scored for Experiment I test concentration 1.50 m*M*

testing facility for the negative and solvent controls and all dose groups in the absence of metabolic activation. With activation, the aberration rates for the negative and solvent controls and for all but one intermediate test group, the 1.50 mM group (5.3% from a total of 400 scored metaphases), were within the historical control data range. The inconsistency observed in the 1.50 mM test group may be an effect of the nature of the test substance and its solubility. No dose-response relationship was observed, as the aberration rates for the 1.75 and 2.00 mM dose groups both fell within the range of the historical control data.

with metabolic activation and Experiment II test concentrations 1.6 and 2.1 m*M*, also with metabolic activation. All aberration rates were normalized to 100 metaphases.

Table XIII.1. Summary of results for chromosome aberration assay conducted in Chinese hamster V79 cells			
Dose Group	**Mitotic Index (percent rel. index)**	**Cell Density (percent rel. density)**	**Aberration Rate [a] (mean percent)**
Experiment I - without S9			
Negative control	96	105	2.5
Solvent control	100	100	2.5
0.05 mM	91	99	0.5
0.10 mM [b]	80	53	2.0
0.125 mM [b]	47	47	2.5
EMS, 600 µg/ml	95	90	8.0
Experiment I – with S9			
Negative control	102	99	1.5
Solvent control	100	100	1.5
0.05 mM	105	97	2.5
1.25 mM [b]	75	78	1.5
1.50 mM [b]	53	30	5.3
1.75 mM [b]	54	24	2.0
2.00 mM [b]	39	20	1.0
CPA, 0.83 µg/ml	72	96	8.5
Experiment II – without S9			
Negative control	106	102	1.0
Solvent control	100	100	3.0
0.065 mM	86	84	1.5
0.070 mM	82	81	0.5
0.075 mM	45	51	1.5
EMS, 400 µg/ml	99	93	8.0
Experiment II – with S9			
Negative control	97	100	2.0
Solvent control	100	100	2.5
0.4 mM [b]	72	97	1.0
1.0 mM [b]	63	86	2.0
1.6 mM [b]	63	62	1.8
2.1 mM [b]	44	30	2.3
CPA, 0.83 µg/ml	78	100	11.5

[a] Range of historical laboratory control data: 0.0% - 4.0% (with and without metabolic activation);
[b] Precipitation observed.

70. In Experiment II, V79 cells were exposed to selected concentrations of Cycloastragenol-TA65 for four hours with metabolic activation (at Cycloastragenol-TA65 concentrations of 0.026, 0.064, 0.16, 0.4, 1.0, 1.2, 1.4, 1.6, 1.8, and 2.1 mM) and for 20 hours without metabolic activation (at Cycloastragenol-TA65 concentrations of 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.065, 0.070, 0.075, 0.080, 0.085, 0.090, and 0.100 mM). In the absence of activation, the concentrations 0.065, 0.070, and 0.075 mM were selected for microscopic evaluation; in the presence of activation, 0.4, 1.0, 1.6, and 2.1 mM concentrations were selected. Precipitation of the test substance was not observed in the test without metabolic activation; with metabolic activation precipitation was present at all concentrations. The findings for Experiment II are summarized in Table XIII.1. Toxicity was observed at concentrations of 0.075 mM (45% rel. mitotic index; 51% rel. cell density) without metabolic activation and at concentrations of 1.0 mM (63% rel. mitotic index) and

1.6 mM (62% rel. cell density) and higher with activation. Clastogenicity (as indicated by cell aberration rates) was within the historical control data range (0% - 4%) for the negative and solvent controls and all dose groups in the absence and in the presence of metabolic activation. Due to inconsistency in the results obtained for the 1.6 and 2.1 mM groups, 400 metaphases were scored for each of these concentrations. At both concentrations, one out of the four slides evaluated showed an aberration rate (5% for both) above the historical negative control data range (0.0% - 4.0%). Because the remaining three slides were in the negative control range, the reported means were within the historical control data range. As in Experiment I, no dose-response relationship was observed. No biologically relevant increase in the occurrence of polyploid cells was found after treatment with the test substance in any dose group in Experiment I or II. The positive controls in Experiments I and II with and without metabolic activation exhibited biologically relevant increases in aberrant cell values are presented in Table XIII.1.

71. In summary, the NF Ingredient did not exhibit clastogenicity under the conditions of the *in vitro* chromosome aberration assay in the V79 Chinese hamster cell line in both experiments without metabolic activation. With metabolic activation, inconsistent results, likely related to the nature of the test substance and its solubility properties were observed in both experiments that led to a final increased aberration rate in one intermediate test group (1.50 mM) in Experiment I. Because the clastogenic effect was relatively moderate and in a concentration range where precipitation occurred and a dose-response relationship was not observed, the results for the in vitro chromosome aberration assay were determined to be equivocal.

XIII.2.c) "*In vivo*" erythrocyte micronucleus assay

72. The clastogenic potential of Cycloastragenol-TA65 was also evaluated in an in vivo erythrocyte micronucleus assay in the mouse (Bioservice 2012. b). The assay was performed under GLP conditions[57] and in compliance with international guidelines[58]. Prior to the main experiment, a range-finding study[59] was used to determine the maximum tolerated dose (MTD). Prepared in cottonseed oil, Cycloastragenol-TA65 at a dose of 2000 mg/kg bw was administered via intraperitoneal (i.p.) injection to Crl:NMRI mice (n = 3/sex; 7-13 weeks old) in two injections over three hours, with each injection equivalent to 1000 mg/kg bw. Symptoms of systemic toxicity were observed in the mice.[60] In accordance with OECD guideline 474, 2000 mg/kg bw was selected as the MTD; this dose was then used in the main study.

73. In the micronucleus assay, Crl:NMRI mice (n = 5/sex/group; 7-13 weeks old) were administered Cycloastragenol-TA65 in cottonseed oil at levels of 400 (0.2 MTD), 1000 (0.5 MTD) and 2000 (1 MTD) mg/kg bw via i.p. injection (10 ml/kg bw). Administration of the low- and mid-level doses was provided in a single injection (10 ml/kg bw). Administration of the highest dose was provided in two injections over three hours, with each injection equivalent to 1000 mg/kg bw. Negative control animals (n = 5/sex) were given a single dose of the cottonseed oil vehicle. Positive control animals (n = 5/sex) received a single dose of 40 mg/kg bw cyclophosphamide via i.p. injection. Peripheral blood was collected from all animals at 44 hours and a second time from mice in the negative control and high dose groups at 68 hours. A minimum of 16 hours after fixing and staining, the blood cells were evaluated by flow cytometry. To determine the incidence (percentage) of polychromatic (micronucleated immature) erythrocytes (PCE) in peripheral blood, a minimum of 10,000 immature erythrocytes from each mouse were examined and scored. To assess cytotoxicity, relative (rel.) PCE [61] was determined for each mouse.

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57 OECD Environmental Health and Safety Publication, Series on Principles of Good Laboratory Practice and Compliance Monitoring – Number 1. Environment Directorate, Paris 1998.

58 *Ibid.*

59 OECD Test No. 474: Mammalian Erythrocyte Micronucleus Test, OECD Publishing.

60 Symptoms included reduced spontaneous activity, constricted abdomen, piloerection, bradykinesia, half-closed eyes, weight loss, kyphosis (hunching) and recumbency.

61 The proportion of PCEs among total erythrocytes.

74. Moderate and strong toxicities were observed in mice of the low- and mid-dose groups, respectively, with mice in the high-dose group demonstrating symptoms of systemic toxicity.[62] The mean rel. PCE values for the negative control and dose groups at 44 and 68 hours were within the ranges of the historical negative control values (see Table 4). The values for the low- and mid-level female groups at 44 hours were significantly decreased when compared to the study's female negative control ($P < 0.05$). At 68 hours, the values for the high-level dose groups were significantly decreased when compared to the corresponding negative controls ($P < 0.05$). As a supportive endpoint used to assess cytotoxicity, the decrease in rel. PCE with exposure to the test substance demonstrates target cell exposure to the test substance.

75. The mean micronucleus frequency values for all dose groups and times (44 hour and 68 hour) were within the ranges of the negative control and historical negative control values (see Table XII.2). Although no statistically significant increase of micronucleus incidence occurred in the dose groups, a significant increase in micronucleus frequency was induced in the positive controls ($P > 0.05$) which served to validate the assay. Under the conditions of this study, Cycloastragenol-TA65 was not clastogenic and/or aneugenic; the NF Ingredient did not induce structural and/or numerical chromosomal damage in the immature erythrocytes of the mouse.

Table XIII.2. Mean rel. PCE values and incidence of micronucleus induction in peripheral erythrocytes

	Mean rel. PCE [a]		Micronucleus Induction (%) [a]	
Study Groups	**Male**	**Female**	**Male**	**Female**
44 Hours				
Negative Control	3.18	3.77	0.18	0.22
Positive Control	2.30	2.12*	1.75*	1.34*
400 mg/kg bw	3.06	2.10*	0.20	0.17
1000 mg/kg bw	2.68	2.54*	0.19	0.12
2000 mg/kg bw	2.56	2.92	0.19	0.14
68 Hours				
Negative Control	3.28	3.89	0.24	0.20
2000 mg/kg bw	0.96*	1.03*	0.16*	0.13
Historical (2007-2010)				
Negative Control [b]	1.43-3.97	1.19-3.85	0.08-0.43	0.08-0.34
Positive Control [c]	0.30-2.21	0.30-2.83	0.93-3.76	0.68-2.84

* $P < 0.05$, compared to the corresponding negative control
[a] n = 5, unless otherwise indicated
[b] n = 78
[c] = n = 72; rel. PCE = relative polychromatic erythrocytes

XIII.3 Carcinogenicity

76. In an unpublished non-GLP study[63] monitoring the effect of TA-65® (~95% Cycloastragenol, isolated from *A. membranaceus*) on human tumor growth in mice, four different human cancer cell types were xenografted into nude female mice (n = 20 mice/cell type). Cell types included lung (H460), colon (HT29), breast (MDA-MB-435), and prostate (PC3). Ten mice inoculated with each cell type were randomly selected to the treatment group, the other ten served as controls. Starting on Day 1 (day of inoculation), TA-65® was administered to treatment group mice by oral gavage at a rate of 5 mg/kg bw/day for 40 days or until tumor size reached 1500 mm^2 in volume. Endpoints included mortality, clinical observations, body weight (weekly) and tumor incidence and size (twice per week). Over the course of the study, no mortalities occurred in the treatment or control groups inoculated with MDA-MB-435 or PC3; animals in these groups were terminated on

[62] Symptoms included reduced spontaneous activity, constricted abdomen, bradykinesia, recumbency, ataxia and half-closed/closed eyes.

[63] Perry Scientific Inc (2008). Efficacy of compound TA-65® on human tumor xenograft growth in nude mice. Report Number: 05-0060.06. San Diego, Perry Scientific Inc.

Day 42. Mice in the HT29 treatment and control groups were terminated at Day 38, presumably due to tumor burden. Five animals in the H460 treatment group and seven H460 control animals died or were terminated at Day 32 (cause of death not specified); the remaining five treatment and three control mice were terminated at Day 38, presumably due to tumor burden. The difference in mortality was not reported as statistically significant. No statistically significant changes in body weight, tumor incidence, or tumor size between the treatment and control groups for any of the four examined cell lines were identified. In summary, under the conditions of this study, 5 mg/kg bw/day TA-65® had no effect on tumor incidence or growth for four different human cancer cell types xenografted into nude mice.

77. In a blinded in vivo study using two adult murine cohorts[64], mature (1-year old) and aged (2-year old) female C57BL/6JOlaHsd mice (n = 4/group) were randomized to receive either a fruit mash vehicle-control (100 µL) or 25 mg/kg bw of the test substance, designated TA-65® (~95% Cycloastragenol, purified from *A. membranaceus*) in fruit mash once a day for four months. Any mouse presenting with illness or tumors was terminated prior to study end. TA-65® was determined by the researchers to be well-tolerated. No treatment-related deaths or overt pathologies were observed. Body weight was maintained in the treatment groups and did not vary from that of the controls. Three months after the study ended, leukocytes from both mature and aged treated cohorts were found to have significantly fewer "very short" telomeres than the corresponding age-matched control groups ($P < 0.001$ for both cohorts). Although several tissues were examined (i.e., liver, heart, kidney, muscle, lung, brain), it was only in the livers of treated aged and mature mice that mTERT[65] mRNA and protein levels were significantly increased in comparison to those of the corresponding control cohorts ($P < 0.05$ for all). The increase in hepatic mTERT mRNA was also accompanied by a significant increase in c-Myc mRNA levels compared to the control groups ($P < 0.05$) which suggest that regulation of telomerase by TA-65® occurs at the transcription level, possibly through the MAPK pathway. Six months and twelve months after the study, glucose sensitivity in the mature treated mice was significantly better than glucose tolerance in the control group at either time point ($P < 0.005$ and $P < 0.05$, respectively). At death (variable periods of time after the study) measured subcutaneous and epidermal adipose layers were significantly thicker in the skins of the mature treated cohort members compared to age-matched controls ($P < 0.05$ and $P < 0.01$, respectively). Although a similar difference in skin was not found among the aged cohort, bone mineral density in the femurs of the aged treated mice was significantly greater than in the controls ($P < 0.05$), a difference not found in the mature cohort. Although not conducted under a validated carcinogenicity study protocol, compared with the control groups, no significant treatment-related effect on survival (mean or maximum) was observed in the treatment cohorts. Moreover, there was no statistically significant difference in the incidence of malignant cancers among the treatment and control groups of either cohort. In summary, dietary TA-65® at a level of 25 mg/kg bw/day for four months was well-tolerated in mature and aged mice with no effect on survival and no increase in malignant incidence.

XIII.4 Topical study

78. The effect of topically applied Cycloastragenol on wound healing was studied in vivo using experimentally created lesions in 21 male SD rats (12 weeks of age)[66]. Under general anesthesia, circular full-thickness skin wounds (8 mm in diameter) were made using a biopsy punch. Test materials applied daily to the wounds on each animal included placebo gel to wound 1, gel containing 5% Cycloastragenol to wound 2, gel containing 1.25% Cycloastragenol to wound 3. On Days 3, 7 and 14 after initial surgery, the wounds were removed under anesthesiology from seven rats for blinded histological examination and scoring of reepithelization, neovascularization, and presence of inflammatory cells, granulation tissue amount and maturation. Based on the histological findings, a 5% Cycloastragenol preparation applied to wounds in vivo showed significantly greater cell density, significantly more regularly organized dermis and significantly more newly formed blood vessels than placebo-treated wounds. The authors reported no Cycloastragenol-related adverse effects in the injured cells or animals.

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64 See # 24 *supra*.

65 Telomerase reverse transcriptase.

66 See # 24 *supra*.

XIII.5 Potential allergenicity

79. No allergenic reactions can be expected from Cycloastragenol-TA65. Regulation 1169/2011 on food information to consumers[67] enumerates 14 classes of food products that are potential allergens: cereals containing gluten; crustaceans; eggs; fish; peanuts; soybeans; milk (including lactose); nuts; celery; mustard; sesame seeds; sulphur dioxide and sulphites, lupins and molluscs. None of them is included in the composition of the Product.

XIII.6 Summary of toxicological information

80. Although statistically significant effects were noted for several endpoints in the 90-day subchronic toxicity study, these were incidental or sporadic in nature, lacked a dose-response relationship, were not clinically relevant, were also present in the control group, and/or were within the range of historical control data for rats of this age and strain. No effects attributable to administration of the NF Ingredient were identified in the in-life observations, ophthalmology, hematology, coagulation, clinical chemistry, urinalysis, gross pathology, and histopathology for main toxicity or recovery group animals.[68] In addition, no cardiac-related effects as indicated by changes in blood pressure, AST, CK, LDH, and gross and histopathologic examination were identified. Under the conditions of this study, the no-observed-adverse-effect level (NOAEL) of orally administered Cycloastragenol-TA65 was 150 mg/kg bw/day in male and female rats. Based on the NOAEL a safety margin of 1100 can be calculated for a 60 kg person.

81. The genotoxicity studies provide overall support that Cycloastragenol-TA65 lacks mutagenic and clastogenic potential. Under the conditions of the bacterial reverse mutation assay DTI-001 (Cycloastragenol of ~90% purity from acid-hydrolyzed astragaloside IV isolated from *A. membranaceus*) was not a mutagen. Although the NF Ingredient in the in vitro chromosome aberration assay in the V79 Chinese hamster cell line gave an inconsistent and equivocal response in the presence of metabolic activation, the equivocal finding occurred in only one intermediate test group in one of two experiments, was not consistent with a dose-response relationship, and was likely related to the nature of the test substance and its limited solubility properties. Further, in the same study Cycloastragenol-TA65 did not exhibit clastogenicity in the absence of metabolic activation. When clastogenic potential was evaluated in the in vivo erythrocyte micronucleus assay, Cycloastragenol-TA65 was not clastogenic and/or aneugenic and did not induce structural and/or numerical chromosomal damage in the immature erythrocytes of the mouse.

82. In a published topical application study in rats wounded by biopsy punch, Cycloastragenol was reported to not adversely affect the injured cells or animals. In an unpublished study examining carcinogenicity, the Cycloastragenol (~95% Cycloastragenol isolated from A. membranaceus) extract orally administered at a rate 5 mg/kg bw/day 40 days had no effect on tumor incidence or growth for four different human cancer cell types (lung, colon, breast and prostate) xenografted into nude mice. In a published study, the dietary supplement (~95% Cycloastragenol, purified from dried *A. membranaceus*) ingested at a rate of 25 mg/kg bw/day for four months was well-tolerated in mature and aged mice with no effect on survival and no increase in malignant incidence up to twelve months after the administration period ended.

83. Based on the nature and composition of the NF Cycloastragenol-TA65 allergenic reactions are extremely unlikely

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[67] Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004 (OJ L 304, 22.11.2011, p. 18–63).

[68] Although all other evaluations were made, the tissues of recovery animals were not subjected to microscopic assessment.

D. CONCLUSION

84. After having considered the information corresponding to all structured schemes as required by the EFSA Scientific Panel for the assessment of the matching novel food class, it can be concluded that the consumption of Cycloastragenol-TA65 is not nutritionally disadvantageous and does not present a danger for the consumer, under the proposed conditions of use as a food supplement.

85. This conclusion is supported mainly by the composition of the NF Ingredient, a pure chemical supplement, >98% Cycloastragenol and that the other components are authorized additives; the long history of safe use in non EU countries of different sources of the NF Ingredient; the assessment of the food production process, which do not involve novel processes in the food sector and whose final product has been accurately checked for its stability, quality characteristics and to meet the product specifications; the lack of any evidence on potential toxicity, potential allergenicity and of adverse effects in humans, and the toxicological information, from which a derived NOAEL provides a wide margin of safety use.

E. REFERENCES

- Bedir et al. (1998a). "Cyclocephaloside I: A Novel Cycloartane-Type Glycoside from Astragalus microcephalus." J. Nat Prod. 1998, 61, 503-505.
- Bedir et al. (1998a). "Cycloartane Triterpene Glycosides from the Roots of Astragalus brachypterus and Astragalus microcephalus" J. Nat Prod. 1998, 61, 1469- 1472.
- Bedir et al. (1999a) "Secondary Metabolites from the Roots of Astragalus trojanus " J. Nat Prod. 1999, 62, 563-568, Bedir et al. (1999b). " Trojanoside H: a cycloartane-type glycoside from the aerial parts of Astragalus trojanus", Phytochemistry 51 (1999) 1017-1020.
- Bedir et al. (2001a). "Two novel cycloartane-type triterpene glycosides from the roots of Astragalus prusianus", Tetrahedron 57 (2001) 5961-5966.
- Bedir et al. (2001b) "Trojanosides I—K: New Cycloartane-Type Glycosides from the Aerial Parts of Astragalus trojanus", Chem. Pharm. Bull. 49(11) 1482—1486 (2001).
- Bensky, D., S. Clavey, et al. (2004). Chinese Herbal Medicine: Materia Medica, Eastland Press Incorporated.
- Bernardes de Jesus, B., K. Schneeberger, et al. (2011). "The telomerase activator TA-65 elongates short telomeres and increases health span of adult/old mice without increasing cancer incidence." Aging cell **10**(4): 604-621.
- Bioservice (2012. a). In vitro Mammalian Chromosome Aberration Test in Chinese Hamster V79 cells with TA-65®. Report Number: Study No. 114190 BSL Bioservice.
- Bioservice (2012. b). Mammalian Micronucleus Test of Murine Peripheral Blood Cells with TA-65®. Report Number: Study no. 114191, BSL Bioservice.
- World Health Organisation WHO (1999). "WHO monographs on selected medicinal plants."
- Chen, K. T., C. H. Su, et al. (2002). "Reducing fatigue of athletes following oral administration of huangqi jianzhong tang." Acta pharmacologica Sinica **23**(8): 757-761.
- Derelanko, M. J. (2000). Toxicologist's pocket handbook, CRC Press.
- Duan, P. and Z. M. Wang (2002). "[Clinical study on effect of Astragalus in efficacy enhancing and toxicity reducing of chemotherapy in patients of malignant tumor]." Zhongguo Zhong xi yi jie he za zhi Zhongguo Zhongxiyi jiehe zazhi = Chinese journal of integrated traditional and Western medicine / Zhongguo Zhong xi yi jie he xue hui, Zhongguo Zhong yi yan jiu yuan zhu ban **22**(7): 515-517.
- Gan, L. X., X. B. Han, et al. (1986). "Astrasieversianins IX, XI and XV, cycloartane derived saponins from Astragalus sieversianus*." Phytochemistry **25**(6): 1437-1441.
- Harley, C. B., W. Liu, et al. (2011). "A natural product telomerase activator as part of a health maintenance program." Rejuvenation research **14**(1): 45-56.
- Harley, C. B., W. Liu, et al. (2013). "A natural product telomerase activator as part of a health maintenance program. B. Metabolic and cardiovascular response" Rejuvenation research (doi: 10.10189/rej.2013.1430): 1-52.
- Horo et al. (2010) "Triterpene glycosides from Astragalus icmadophilus", Phytochemistry 71 (2010) 956-963.
- Hsu, H. Y. (1986). Oriental Materia Medica, Keats Pub.

- ICH (1996). S2A: Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals, The International Conference on Harmonisation. Safety Guidelines, S2.
- ICH (1997). S2B: Guidance on Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals, The International Conference on Harmonisation. Safety Guidelines, S2.
- Kitagawa, I., H. K. Wang, et al. (1983). "Saponin and sapogenol. XXXIV. Chemical constituents of Astragali Radix, The Root of Astragalus membranaceus Bunge. (1). Cycloastragenol, the 9,19-Cyclolanostane-type Aglycone of Astragalosides, and the Atifact Aglycone Astragenol." Chemical & pharmaceutical bulletin **31**(2): 689-697.
- Ma, X. Q., Q. Shi, et al. (2002). "Chemical analysis of Radix Astragali (Huangqi) in China: a comparison with its adulterants and seasonal variations." Journal of agricultural and food chemistry **50**(17): 4861-4866.
- Mamedova, R. P. and M. I. Isaev (2004). "Triterpenoids from Astragalus Plants." Chemistry of Natural Compounds **40**(4): 303-357.
- Matkovic, Z., V. Zivkovic, et al. (2010). "Efficacy and safety of Astragalus membranaceus in the treatment of patients with seasonal allergic rhinitis." Phytotherapy research : PTR **24**(2): 175-181.
- McKenna, D., K. Hughes, et al. (2002). "Astragalus." Alternative therapies in health and medicine **8**(6): 34-40; quiz 41-32, 124.
- Montgomery, M; Pine, D. ; Roberts, C. (2013). "Human Clinical trial. A Randomized, Placebo Controlled, Double-Blind Trial Evaluating the Safety and Efficacy of Product: TA-65MD™.
- OECD Test No. 473: In vitro Mammalian Chromosome Aberration Test, OECD Publishing.
- OECD Test No. 474: Mammalian Erythrocyte Micronucleus Test, OECD Publishing.
- Perry Scientific Inc (2008). Efficacy of compound TA-65® on human tumor xenograft growth in nude mice. Report Number: 05-0060.06. San Diego, Perry Scientific Inc
- Pettersen, J. C., R. L. Morrissey, et al. (1996). "A 2-year comparison study of Crl:CD BR and Hsd:Sprague-Dawley SD rats." Fundamental and applied toxicology : official journal of the Society of Toxicology **33**(2): 196-211.
- Polat et al. (2009) "Cycloartane-type glycosides from Astragalus amblolepis ", Phytochemistry 70 (2009) 628-632,
- Polat et al. (2010) "Triterpenoid saponins from Astragalus wiedemannianus Fischer ", Phytochemistry 71 (2010) 658-662,
- PSL (2012). TA-65®: A 90-day Repeated Dose Oral Gavage Study in Rats with Cardiac Assessment. Report Number: Study No. 32721, Product Safety Labs (PSL): p. 1 - 616.
- Rios, J. L. and P. G. Waterman (1997). "A review of the pharmacology and toxicology of Astragalus." Phytotherapy Research **11**(6): 411-418.
- Şahan, Y., F. Basoglu, et al. (2007). "ICP-MS analysis of a series of metals (Namely: Mg, Cr, Co, Ni, Fe, Cu, Zn, Sn, Cd and Pb) in black and green olive samples from Bursa, Turkey." Food chemistry **105**(1): 395-399.
- Sevimli-Gur, C., I. Onbasilar, et al. (2011). "In vitro growth stimulatory and in vivo wound healing studies on cycloartane-type saponins of Astragalus genus." Journal of ethnopharmacology **134**(3): 844-850.

- Shao, B. M., W. Xu, et al. (2004). "A study on the immune receptors for polysaccharides from the roots of Astragalus membranaceus, a Chinese medicinal herb." Biochemical and biophysical research communications **320**(4): 1103-1111.

- Tang, W. and G. Eisenbrand (1992). Chinese drugs of plant origin: chemistry, pharmacology, and use in traditional and modern medicine, Springer-Verlag.

- Wagner, V. O. and M. L. Klug (2003). Bacterial Reverse Mutation Assay. Report Number: Study AA68SB.503.BTL BioReliance: p. 1-55.

- Yu, Q. T., L. W. Qi, et al. (2007). "Determination of seventeen main flavonoids and saponins in the medicinal plant Huang-qi (Radix astragali) by HPLC-DAD-ELSD." Journal of separation science **30**(9): 1292-1299.

- Yu, S. Y., H. T. Ouyang, et al. (2007). "Subchronic toxicity studies of Radix Astragali extract in rats and dogs." Journal of ethnopharmacology **110**(2): 352-355.

- Zhu, J., S. Lee, et al. (2010). "In vitro intestinal absorption and first-pass intestinal and hepatic metabolism of cycloastragenol, a potent small molecule telomerase activator." Drug metabolism and pharmacokinetics **25**(5): 477-486.

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