**COMMITTEE PAPER FOR DISCUSSION** **ACNFP/114/5**

**ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES**

# CYCLOASTRAGENOL

## Issue

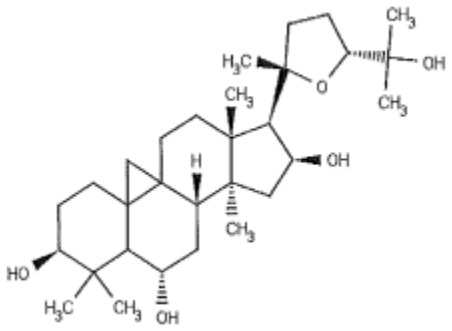
An application has been submitted to the UK Competent Authority for the use of cycloastragenol, which is classed as a novel food ingredient under the novel food regulation (EC) 258/97. The Committee is asked to advise whether the available data provides an adequate basis for a safety assessment, and if it recommends the authorisation of this ingredient for use in food supplements.

## Background

1. An application has been submitted by K&L Gates, on behalf of Telomerase Activation Science Inc., for the substance cycloastragenol, referred to in the dossier as cycloastragenol-TA65 or TA65, which is a novel food in the EU. The application was accepted by the UK Competent Authority on 10 February 2014 and, in accordance with Article 6(3) of Regulation (EC) No 258/97, the UK has 3 months to prepare an initial assessment report on this application. The European Commission will then circulate the initial assessment to the Competent Authorities in the other Member States for comment. The application dossier is attached at **Appendix 1**.

2. Cyloastragenol-TA65 is obtained from the *Astragalus trojanus* a perennial flowering shrub of the Fabaceae (or legume) family. Other species of *Astragalus,* most notably *A. membranaceus,* which also contain cycloastragenol are available in food supplements in the EU

3. Cycloastragenol is the most common a triterpede aglycone (often referred to as astragalosides) found in *Astragalus* sp. Triterpede aglycones belong to a class of secondary plant metabolites known as saponins The chemical structure of cycloastragenol is:



4. This application for cycloastragenol was prepared pursuant to the scheme set out in Commission Recommendation 97/618/EC. Cyloastragenol-TA65 is classified as a chemically defined substance which has a history of use in the Community (Class 1.1). The requirements for a submission of this class are detailed below.

| | | |
|---|---|---|
| **I** | **Specification of the NF** | **X** |
| **II** | **Effect of the production process applied to the NF** | **X** |
| **III** | **History of the organism used as the source of the NF** | **X** |
| IV | Effect of the genetic modification on the properties of the host organism | **-** |
| V | Genetic stability of the GMO | **-** |
| VI | Specificity of expression of novel genetic material | **-** |
| VII | Transfer of genetic material from GM microorganisms | **-** |

| | | |
|---|---|---|
| VIII | Ability to survive in and colonise the human gut | **-** |
| **IX** | **Anticipated intake/extent of use of the NF** | **X** |
| **X[1]** | **Information from previous human exposure to the NF or its source** | **X** |
| **XI** | **Nutritional information on the NF** | **X** |
| **XII** | **Microbiological information on the NF** | **X** |
| **XIII** | **Toxicological information on the NF** | **X** |

A non-confidential version of the application is being placed on the FSA website to allow the public to contribute to the assessment. The deadline for replies is 21 days from the date of publication and any comments received will be tabled at the next ACNFP meeting.

## I Specification of the Novel Ingredient (NI)

Appendix 1, p 4-7

5. The applicant provides both a general description and a detailed specification for Cyloastragenol-TA65, together with methods of analysis, (refer to Appendix 1, Table I.1 and I.2). The specification is summarised below:

| **Analysis** | **Specification** |
|---|---|
| Physical appearance | White/off-white solid powder |
| Odour | Odourless |
| Cycloastragenol (%) | 98% min |
| Water content (%) | 2% max |
| Ash (%) | 2% max |
| Structure | Conforms |
| Mass | 490 ± 1.0 amu |
| **Solvent residue (ppm)** | |
| n-Butanol | NMT* 5000 |
| Acetonitrile | NMT 400 |
| Hexane | NMT 290 |
| Methanol | NMT 3000 |
| Ethanol | NMT 5000 |
| Ethyl acetate | NMT 5000 |

| **Heavy metals (ppm)** | |
|---|---|
| Arsenic | NMT 90 |
| Cadmium | NMT 30 |
| Lead | NMT 60 |
| Mercury | NMT 90 |
| Chromium | NMT 30 |
| Selenium | NMT 30 |
| **Pesticides (304 total)** | USP |

*NMT: not more than

6. The product is crystalline and analysis of six independent batches confirms that the purity is at least 98%, with the remainder made up of ash and water.

## II Effect of the production process applied to the NI

Appendix 1 pp10-12 (PROTECT COMMERCIAL)

7. Certain aspects of the extraction process for Cyloastragenol-TA65 are commercially sensitive and Members are advised to refer to the Pages 10-12 of Appendix 1 for additional details about the process employed.
8. The applicant has provided details of a real time stability test which shows good stability of Cyloastragenol-TA65 over a 12 month period when kept under the applicant's standard conditions of storage (Table I.4).

## III History of the source of the NI

Appendix 1 1 p12-14 (PROTECT COMMERCIAL)

9. The applicant advises that *Astragalus* (*A. membranaceus*) is a herb that has traditionally been used in a wide variety of herbal blends and "natural" remedies in China or other regions of Asia. The dried root of *A. membranaceus* is used in traditional Chinese medicine primarily as a tonic, especially for the spleen and lungs. However, the MHRA[1] has confirmed that Cyloastragenol-TA65 has no major pharmacological effect that would result in products being classed as medicinal. Assessment as a novel food is therefore required before it can be marketed in the EU as a food supplement
10. Cyloastragenol-TA65 is extracted from the roots of the related species, *A.trojanus.*

## IX Anticipated intake and extent of use of the NI

Appendix 1 p14-15

11. The applicant intends to restrict the use Cyloastragenol-TA65 to food supplements (single dose of 8mg/day) which will be targeted at adults >25 years old. In regard to the purported claims for this product (see para 34 below) the applicant intends the product to be used for the maintenance or enhancement of physiological functions in the body that are associated with wellbeing and health.

[1] The UK's Medicines and Healthcare products Regulatory Authority

The applicant also notes that the company is particularly interested in the use of Cycloastragenol-TA65 in regard to physiological processes and functions that may decline with age and that may be mediated by telomerase activity. The company is carrying out a number of studies to substantiate a health claim in this respect.

## X. Information from previous human exposure to the NF or its source

Appendix 1 p15-17

12. As noted in Section III, above, Astragalus is a widely available herbal supplement. Cycloastragenol-rich extracts are also widely consumed in China and in the USA and the applicant has provided information, such as the recommended dose and purity, of a number of commercially available products. (Appendix 1 Table X.1) The applicant has also marketed the novel ingredient in the US and China and 46 other counties, including Australia, Canada and Switzerland.

13. The applicant also highlights three human studies on Cyloastragenol-TA65, at higher doses than is proposed here. These are briefly summarised in the following table:

| **Authors** | **Study** | **Parameters** | **Headline finding** |
|---|---|---|---|
| Montgomery et al 2013 | 16mg/day, 30day, 125 subjects | Range of blood chemistry parameters | No reports of adverse side effects or interactions. |
| Harley et al 2011 | 10-50mg /day,12 months, 114 subjects | Immunological effects, | No adverse effects seen, but 'positive' immunological effects (lengthens telomeres and changes proportion of leukocytes observed |
| Harley et al 2013 | 10-50mg /day, 5 yr | Range of blood chemistry parameters | No adverse effects seen. Positive effect seen on markers for metabolic bone and cardiovascular health observed. |

## XI Nutritional information on the novel food

Appendix 1 p16

14. The applicant notes that Cyloastragenol-TA65 does not supply any significant dietary macro- or micro-nutrients, nor does it affect any healthy dietary pattern. No significant anti-nutritional factors (e.g. inhibitors of mineral absorption or bioavailability) are present and as a consequence no nutritional concerns are foreseen by the applicant.

15. The applicant has considered the absorption of cycloastragenol, using a preparation TAT2 (containing 90-95% cycloastragenol, produced from

commercially available astragaoloside IV, a triterpede aglycone). in the Caco-2 cell monolayer model. Passage through the Caco-2 monolayer proceeded with minimal metabolism (only two oxidised metabolites and four glucuronide conjugates were identified in the apical and basolateral sides of the monolayer) and rapid passage of cycloastragenol 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 test material remaining. In the liver samples, metabolites were primarily monohydroxylated with additional hydroxylation occurring post-oxidation. The applicant suggests that efficient first-pass metabolism and extensive hepatic metabolism, oral bioavailability of cycloastragenol is expected to be low. The applicant also notes that 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 (refer to Appendix 1 Annex II.20).

## XII Microbiological Information

Appendix 1 p17

16. The applicant provides a specification for microbial contamination (Appendix 1 Table I.2). Analysis of 6 batches of Cyloastragenol-TA65 (Appendix 1 Table I.3.A) shows no significant levels of microbial contamination. The applicant notes that the nature of the manufacturing process and the purity of Cyloastragenol-TA65 mean that the risk of bacterial proliferation is considered very low

## XIII Toxicological information

Appendix 1 pp17-28

17. The applicant has presented a number of studies in support of the safety of Cyloastragenol-TA65.

### Toxicity Studies (Animal)

<u>13-week subchronic toxicity study with a 4-week recovery period (Appendix 1 Annex IV.14).</u>

18. This study was carried out in accordance with GLP and OECD guidelines on the testing of chemicals and food ingredients. In the main study, a total of 80 Sprague-Dawley rats were given daily doses of up to 150 mg/kg bw by oral gavage Cycloastragenol-TA65 and in the recovery study, 20 additional rats were given similar doses. Due to reports of ‘cardiotonic’ effects of extracts of Astragalus sp., additional parameters consisting of blood pressure measurement and serum biochemistry (aspartate aminotransferase (AST), total creatine kinase (CK) and lactate dehydrogenase (LDH)) were also included in the study.

19. No treatment-related effects were identified in the ophthalmology, haematology, 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 Cycloastragenol-TA65 because the changes were incidental or sporadic in nature, were not clinically relevant, were not correlated with other clinical or histopathological 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. These observations are summarised in paragraph 63 of Appendix 1.

20. 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.

Subchronic toxicity study in rats and dogs of a product (Astragalus membranaceus extract) containing Cycloastragenol (Yu, Ouyang et al. 2007).

21. This study used an *A. membranaceus* extract which was administered by intra-peritoneal injection (rats) or intravenous injection (dogs) for three months. The test material was not highly purified and it contained a high proportion of polysaccharides as well as a mixture of saponins. The extract contained unquantified amounts of cycloastragenol in the form of glycosides.

22. A number of parameters were observed (food-intake, behaviour, body weight, haematology parameters). The results showed no significant differences between experimental groups and control groups in body weight, the absolute and relative weights of the principal organs, clinical signs, and haematology indices. Haematological, biochemical histopathology examination of experimental groups did not reveal any dose-dependent or test related associations.

23. The study authors concluded that the extract showed no distinct toxicity or side effects at the doses used in these experiments (5.7–39.9 g/kg for rats and 2.85–19.95 g/kg for beagle dogs). The applicant notes that these doses are 5–70 fold less than a perceived effective dose of the extract for humans, as cited by the authors of the study, and suggests that this provides additional, if indirect, reassurance of safety. However, the Secretariat notes that these studies are only of limited relevance to the evaluation of novel ingredient Cycloastragenol-TA65.

**Genotoxicity Studies**

Bacterial reverse mutation assay

24. The mutagenic potential of cycloastragenol (in the form of a ‘facility-designated ingredient’ with a cycloastragenol level of >90%) was evaluated by bacterial reverse mutation assay (i.e. Ames test). Although some precipitation was observed at high dose levels, both with and without S9 activation, no cytotoxicity

and no mutagenic response was observed and the test material was considered not to be mutagenic.

*In vitro* chromosomal aberration assay

25. The clastogenic potential of Cycloastragenol-TA65 was evaluated in an *in vitro* mammalian chromosome aberration test in Chinese hamster V79 cells.

26. The rates of chromosomal aberration were within the historical control data range (of the 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 (at 1.50 mM) were within the historical control data range. The inconsistency observed in this one 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 higher dose groups (1.75 and 2.00 mM) both fell within the range of the historical control data.

*In vivo* erythrocyte micronucleus assay

27. The clastogenic potential of Cycloastragenol-TA65 was also evaluated in an *in vivo* erythrocyte micronucleus assay in the mouse

28. Under the conditions of this study, Cycloastragenol-TA65 was not regarded to be clastogenic and/or aneugenic, as the test material did not induce structural and/or numerical chromosomal damage in the immature erythrocytes of the mouse.

**Carcinogenicity Study**

29. Cycloastragenol (~95% Cycloastragenol *A. membranaceus* extract*)* administered orally to mice at rate 5 mg/kg bw/day 40 days had no effect on tumour incidence or growth for four different human cancer cell types (lung, colon, breast and prostate) xenografted into nude mice.

30. In a separate, 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 the incidence of malignant tumours up to twelve months after the administration period ended.

**Allergenicity and Labelling**

31. The applicant states the 14 classes of foods which require identification as known allergens, in accordance with food labelling regulations, are not present in the composition of the product, and that no allergenic reactions can be expected.

32. However, as a member of the legume family, *Astragalus*, sp. and extracts thereof could potentially elicit an allergic response. While the applicant has not carried out any investigation into the level of residual protein present in the novel

ingredient the Secretariat notes that the nature of the novel ingredient and the, extraction and purification methods employed mean that proteins are unlikely to be present in detectable amounts.

**Access and Choice**

33. The Secretariat has considered the issues of access and choice in relation to Cyloastragenol-TA65. If authorised, the novel ingredient would be available for use in food supplements, which will be targeted at adults >25 years old. In practical terms, access to novel foods is limited by a high price or by limited geographic distribution, which are both driven by commercial considerations which cannot be predicted at this stage. It is envisaged that the introduction of supplements containing Cyloastragenol-TA65 will increase existing consumer choice.

**Health and Nutrition Claims**

34. The Committee's assessment focuses on safety and labelling and does not address any nutrition or health benefits that may be claimed for the novel ingredient or for foods that contain it. Nutrition or health claims may only be made if they are specifically authorised under Regulation (EC) No 1924/2006.

**Committee Action Sought**

35. Committee is asked:

- whether the available data are adequate to determine whether the request to authorise Cyloastragenol-TA65 complies with the criteria for acceptance under the novel food regulation, namely:
    - It does not present a danger to the consumer
    - It does not mislead the consumer
    - It is not nutritionally disadvantageous compared with foods which it might replace.
- If so, the Committee is asked whether it is content to recommend approval for the use of Cyloastragenol-TA65 in food supplements.
- If not, the Committee is invited to identify what further data should be provided.

36. Members are asked for their immediate comments at the meeting or afterwards in writing, by 24 February 2014, in order that the applicant is given sufficient time to respond to any questions or concerns before the next meeting in April.

**Secretariat**
**February 2014**

**Appendix attached:**

**Appendix 1:** Application dossier for the approval of Cyloastragenol-TA65 (Protect - Commercial)
A non-confidential version is publicly available via the Committee's website
http://acnfp.food.gov.uk