

**ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES****PHYTOSTEROLS PRODUCED BY DDO PROCESSING****Issue**

The Committee is asked to consider additional information from the applicant in response to the public comments made on their substantial equivalence dossier.

**Background**

1. The public comments received on the DDO Processing dossier are listed in paper ACNFP/75/2. The Secretariat invited DDO Processing to consider points (i) and (iii). The applicant's response was received by the Agency on 17 January 2006 (Annex C) and is summarised in the paragraphs below.

**Point (i) Inadequate analytical method - Result in excess of 100%, minor sterols not detected and not accounting for moisture content.**

2. The applicant used Gas Chromatography-Flame Ionisation Detection (GC-FID) for determining the composition of its phytosterol ingredient, as recommended by the European Commission for the analysis of existing phytosterols derived from sources other than vegetable oil. The six tested batches of this ingredient have a purity of more than 99%, which complies with the specification laid down in Commission Decision 2004/845/EC for Forbes Medi-Tech's phytosterols. Four out of the six batches tested have a total phytosterols content between 100% and 104%. The applicant explains that individual phytosterol levels are estimated by comparison with a corresponding internal standard of known quantity. This methodology assumes that all the compounds have precisely the same response factor, but the applicant suggests that differences in the response factor could give rise to measurement inaccuracies of +/-5 %. The applicant cites a reference as a justification that the variability seen is not atypical<sup>1</sup>.

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<sup>1</sup> The applicant's response includes a reference to a paper on analysis of phytosterols by GC-FID that reports response factors for 4 underivatised phytosterols (beta-sitosterol, campesterol, stigmasterol and brassicasterol) in the range 0.75-0.81, compared with a non-hydroxylated internal standard (cholestane). The GC-FID method used by DDO is described on pages 22-25 of their dossier (see Annex A of paper ACNFP/74/4) and uses derivatisation with BSTFA. The results are calculated using a stigmasterol reference standard, assuming the same response factor for the other sterols.

3. The applicant will provide some additional analytical data on minor sterols present in their ingredient. This data will be tabled at the ACNFP meeting on 25 January.

**Point (iii) Heptane solvent – legality of its use under Directive 88/344<sup>2</sup> (as amended) and solvent residues**

4. As stated in the ACNFP paper 75/2 (paragraph 3(iii)), the Secretariat has sought advice on the use of heptane from colleagues with expertise in legislation on extraction solvents and will be advising the ACNFP accordingly, in due course.
5. Regarding the level of solvent residues, the applicant indicated in their dossier that the residues of heptane would be below 10 mg/kg. The applicant has now stated that residues are very low or undetectable (although no data have been provided) and has specified that material will only be shipped to Europe with a heptane residue level below 0.1 mg/kg.

**Committee Action Required**

6. The Committee is asked to consider the answers provided by DDO Processing as part of its assessment of this dossier (ACNFP/75/2).

**ACNFP Secretariat  
January 2006**

**Annexes attached**

**Annex C:** DDO Processing additional information.

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<sup>2</sup> Council Directive 88/344/EEC of 13 June 1988 on the approximation of the laws of the Member States on extraction solvents used in the production of foodstuffs and food ingredients.

**ACNFP/75/2 Annex C**

**ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES**

DDO Processing additional information.

**Secretariat  
January 2006**

## **Response to Questions on Sterol Analytical Results**

### **Issue: Results in excess of 100%.**

Response: In general, a result in excess of 100% should be viewed with skepticism. However, when the material being tested is expected to exceed 99%, a result slightly in excess of 100% should not be cause for alarm based on the reasons below. (We would point out that USP typically allows for assays up to 102%). As a matter of scientific principle, a careful analyst should check results, and if no gross problems are found, should report the findings as they are, which we have done throughout our application.

An assay totalling more than 100% can be caused by two factors:

1. In the method, the individual peaks are integrated by the computer. The integrations are very good approximations. As such, especially when the material is very pure, these approximations, when totalled, can add up to more than 100%.
2. The different sterol isomers are measured by comparing their peak size to the internal standard peak size. While in general this is valid, close attention should be paid to the fact that the various sterols have a slightly different response factor about +/- 5%. Again, at very high purity, this difference in response factor may result in a total in excess of 100%. (Ref: Journal of Food and Drug Analysis 1999.7(4)279-290; *A Rapid Gas Chromatographic Method for Direct determination of Free Sterols in Animal and Vegetable Fats and Oils—Page 284 is of particular interest*)

### **Issue: Minor sterols not detected**

We note that the EU Generic specification does not require the minor (<3%) sterols that are permitted to be present to be enumerated in detail. We are in the process of conducting additional analyses and will forward additional information on this issue by e-mail prior to the next Committee meeting on January 25th.

### **Issue: Not accounting for the moisture content results in an exaggerated sterol content.**

The moisture of prilled sterols is less than 1%. This will therefore affect the overall sterol content report by a factor of  $100 \div 99+$ : a trivial amount. In fact, the moisture of prilled sterols is typically so low that no published analytical method, including that reported in the Reducol Novel Food application, adjusts the sterol assay for moisture content.

### **Issue: Use of Heptane Solvent Residuals and Directive 88/344 -**

Directive 88/344 (as amended) applies to extraction solvents used in the production of foodstuffs or food ingredients, including those imported into the Community. However, the 2<sup>nd</sup> paragraph of Article 1.1 of this Directive states explicitly that *“this Directive shall **not** apply to extraction solvents used for the production of food additives, vitamins and **“other nutritional additives”** that are not listed in the annex to the Directive”*.

The category of “other nutritional additives” are not legally defined either in this Directive, or subsequent EU food legislation, and we would contend that phytosterols fall within this group. Since, in addition, “phytosterols” are not listed in the annex, we do not believe the Directive is applicable.

Furthermore, the 3<sup>d</sup> paragraph of Article 1.1 contains a specific requirement that *“Member States must, however, ensure that the use of food additives, vitamins and **other nutritional additives** does not result in foodstuffs containing extraction solvent residue levels that are dangerous to human health”*. There would have been no reason for the regulators to include this paragraph if it were not their intention to permit other extraction solvents to be used during the purification of these materials, subject only to the general safety provision related to their residue levels in the final foodstuffs.

In view of both these arguments, we conclude that the requirements of Directive 88/344 do not apply to the use of heptane in the production and purification of tall oil phytosterol mixtures.

### **Heptane Residues in Sterols**

We are aware of the phytosterol processing methods of several American manufacturers that have in the past used, and are very likely to continue to use, heptane as a solvent in their process for phytosterol production that is marketed in the EU. For example, the original application by ADM, submitted to the Dutch authorities, states that its materials are the same as those used by Unilever in their yellow fat spreads and clearly refers to the sterols being re-crystallised from heptane. The application from Unilever to extend the use of phytosterols, ([www.food.gov.uk/multimedia/pdfs/d02\\_018.pdf](http://www.food.gov.uk/multimedia/pdfs/d02_018.pdf)) contains the following extract: “The production methods used to produce the phytosterol-esters are identical to those used to produce the phytosterol-esters used in Unilever’s Yellow Fat Spreads.” Heptane has been used historically in phytosterol production because its higher boiling point makes the separation easier and a major pharmaceutical customer specified heptane in its drug master file as the process solvent.

Therefore, it is our contention that the use of heptane in phytosterol production is a commonly used practice that is not at all unique to our production; what appears to be unique is our full disclosure of production solvents.

Prior to dispatch, the finished sterols are checked for residual heptane using EPA method 8260b. In most cases, this analysis shows any residual heptane level to be below the limit of detection. No material is shipped with a heptane residue level over 100 ppb. A heptane residue level that is significantly below that permitted for residual hexane in vegetable oils under the EU legislation (1 ppm) and has a comparatively low anticipated intake in the sterols, is not considered to pose any health concerns.

The safety in use of heptane as a residual solvent is further supported by guidance regarding its use in drug manufacturing. The International Conference on Harmonization has issued guidance on residual solvents in pharmaceuticals (Q3C *Impurities: Residual Solvents*, 1997 at [www.fda.gov/cder/audiences/iact/ICH\\_Q3C.htm](http://www.fda.gov/cder/audiences/iact/ICH_Q3C.htm)). It has also presented lists of solvents and classification as to their acceptability as residues in drugs in Q3C *Guidance: Tables and Lists* at <http://www.fda.gov/cder/guidance/Q3CT&Lrev1.pdf>. Heptane is listed as a Class 3 solvent. Class 3 includes no solvent known as a human health hazard at levels normally accepted in pharmaceuticals (at least 50 mg/day intake minimum) and may be set higher based on manufacture and cGMP requirements.

Lastly, JECFA has evaluated heptane as an extraction solvent and has set the ADI as “limited to GMP.” ([www.inchem.org/documents/jecfa/jecval/jec\\_891.htm](http://www.inchem.org/documents/jecfa/jecval/jec_891.htm)). We believe the limit set for residual heptane of 100 ppb in our product is fully consistent with GMP.