



# Advisory Committee on Novel Foods and Processes

Annual Report 2002

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## Foreword

This is the final Foreword to an ACNFP report that I will be writing since my 6-year term of office ends on 31st August 2003. Looking back over six years, most of the Committee's work has focussed on evaluation of new products under the EC Novel Food Regulation (EC 258/97), which came into force in May 1997.

In July 2001 two closely linked proposals for new legislation concerning GMO's were issued. These are progressing toward a second reading in the European Parliament and will establish new procedures for the authorisation of foods derived from GMO's. In addition, the Novel Food Regulation is being reviewed and is likely to be revised during 2003-2004, not least to take account of the newly established European Food Safety Authority. Clearly, there will continue to be changes in the regulatory process and many challenges for my successor.

The primary role of the ACNFP continues to be the safety assessment of novel foods and processes. The rigorous and robust approach with close scrutiny of scientific data is fundamental to our activity. The wisdom and knowledge of our members is world class and essential to allow us to produce sound evidence-based advice; independent of commercial and political influence. We are also fortunate to have two consumer representatives and an ethicist on the Committee to help us in our deliberations.

Scientific rigour is one feature of our work, the other is transparency. Over the past 6 years we have moved forward considerably. In 1998 we published our minutes, in 1999 we encouraged debate and discussion by allowing disclosure of all non-confidential data relating to submissions. Public commentary on the dossier's prior to meetings is considered and our draft conclusions are available for comment. We also produce a corporate brochure with regular updates and 3 new fact sheets this year, and hold an annual open meeting, which this year was held in Cambridge. In March 2002 the FSA published a review of its Scientific Committees and I am delighted that the ACNFP is leading the way in terms of openness and dialogue with the consumer. I hope that we have helped to promote public knowledge and understanding of the issues and thus helped members of the public make their own informed decisions.

As you will see from the contents page of this annual report the Committee has dealt with a varied range of applications and with a range of issues. The sheer volume of the appendices is testament to the amount of high-quality support provided by our Secretariat; without them the Committee would be unable to function and deliver its opinion. I have enjoyed working with them all and thank them for their support.

In conclusion, I continue to uphold the view that transparency of the regulatory process, clear unambiguous advice, constant dialogue with consumers, industry, government, the broadcast media and our European neighbours will continue to be essential to ensure that we are able to reap the benefits of novel food technologies. The wide ranging and detailed programme of activities could never have been completed without the thoughtful and expert knowledge of my fellow Committee members who devote a large amount of their own time to their work. I would like to thank the Committee and Secretariat and send them every good wish for the future. I am confident that their robust approach will ensure that novel foods and processes continue to be thoroughly scrutinised for safety before they are considered for approval.

**Professor J.M Bainbridge O.B.E.**  
January 2003

## Introduction

This is the fourteenth Annual Report of the work of the Advisory Committee on Novel Foods and Processes (ACNFP).

The ACNFP considered a number of applications in 2002, details of which are in sections 1, 2 and 3 of this report. The summary reports of applications discussed by the ACNFP in 2002 have been split into 3 sections; applications for a full safety assessment initially received by the UK Competent Authority; those received where another Member State has provided the initial opinion on a full application; and requests for an opinion on Substantial Equivalence received by the UK Competent Authority. Those topics discussed during 2002 that were continuations of previous work are indicated as such.

During 2002 the Committee issued three new fact sheets for enclosure in its brochure, along with guidelines on the conduct of taste trials and use of human studies in the pre-market safety assessment of novel foods. Further details on these publications along with information on the ACNFP's second open meeting are given in section 5.

A cumulative index of topics considered in previous Annual Reports can be found in section 11. Hard copies of previous reports can be obtained from the Secretary to the Committee (see section 7), and all ACNFP reports, as well as other information on the committee can be found on its webpages on the FSA website at:

*[www.food.gov.uk/science/ouradvisors/novelfood](http://www.food.gov.uk/science/ouradvisors/novelfood)*



# 1. Full applications submitted to the UK Competent Authority

## 1.1 Unilever – an application under the Novel Foods Regulation to extend the range of uses of phytosterol esters in food products

The applicant previously submitted, via the Netherlands, a successful application under (EC) 258/97 for the use of a phytosterol ester component in a single product type (yellow fat spreads). Clearance was granted for use of the phytosterol ester ingredient in yellow fat spreads up to a maximum of 8%. The Committee commented on this application in 1999.

The ACNFP was asked to consider an application to extend the range of uses of the same phytosterol esters to include milk and yoghurt products. The Secretariat consulted the European Commission who were of the opinion that, as the initial approval was for incorporation into a single product, a full application under the terms and conditions of (EC) 258/97 was required before an extension could be granted. The application was considered by the ACNFP at its September meeting.

As the novel ingredient had already been subject to a safety assessment and granted approval up to a maximum level of use in spreads, Members' opinion was based on the existing conditions of approval and data from post-market monitoring. These data showed that intake from spreads had not reached the level anticipated in the previous application, and the applicant claimed that an extension of the product range would not lead to an intake of phytosterols above the level previously approved.

The ACNFP accepted the applicant's contention that an increased product range would not exceed the level of phytosterol ester consumption stipulated in the original approval. However, the Committee noted that a number of applications for products fortified with plant sterols had been made in the last two years and advised that the broader issue of the consumption of phytosterols, phytostanols and their esters from multiple dietary sources needed to be resolved before this, and any other plant sterol applications could be given approval under (EC) 258/97.

Members also commented on the extent of the company's intended post-launch monitoring scheme (PLM), although the company was able to clarify the points raised. The proposed PLM scheme will take account of all the applicant's products.

The Committee's positive initial opinion was forwarded to the Commission for consideration by other Member States in November 2002. A copy is attached at Appendix II.

## 1.2 DHA Gold™ – Update

In 2001, the ACNFP received an application from OmegaTech\*, seeking approval to market DHA Gold™, an oil rich in the unsaturated fatty acid DHA (docosahexaenoic acid).

The Committee was content that there were no nutritional or safety concerns with this product, since it was demonstrated that all components of the extracted oil are themselves present to some degree in the human food chain.

However, due to the lack of confirmatory data in humans, the Committee requested that the Company carry out a human clinical trial to demonstrate that there are no adverse effects from humans consuming the oil. The Company supplied these data in January 2002, and the Committee considered them at its meeting in February.

The Committee was content that this study demonstrated that a consumption of 1.5g DHA daily as DHA Gold™ resulted in the expected changes in serum lipids within the normal ranges. DHA Gold™ was well tolerated among the test group, with no adverse effects on liver function, cardiac enzymes, glucose metabolism, and haematology markers of inflammation or haemostatic function being recorded, apart from the statistically significant increase in Factor VIIc. However, an increase is also seen with fish oils and is a result of the compensatory increase in clotting in response to the known effects of DHA on platelet vessel wall interactions. Therefore, the Committee considered that this finding was of no toxicological concern.

The Committee was satisfied by the evidence provided by OmegaTech that DHA Gold™ is safe for use as a nutritional food ingredient. The Initial Opinion Report for DHA Gold™ can be found at Appendix III.

In the ACNFP's opinion, the level of 1.5g of DHA per day was considered to be an adequate upper level. However, safety data were provided to indicate that the oil was safe at higher intake levels.

The ACNFP considered there to be a large enough safety margin with consumers needing to consume approximately 7 average servings of DHA-enriched foods to reach the suggested upper limit of 1.5g DHA/day.

\*OmegaTech was taken over by Martek Biosciences in Spring 2002.

The positive scientific opinion was forwarded to the Commission in June, when it was circulated to other Member States for comment. The Novel Foods Competent Authorities Working Group discussed this application in October, when one Member State raised concerns with the use of this product in liquid milk products, suggesting that this may lead to over-consumption. A draft EU decision was considered in December.

*At the time of going to press, a final decision on authorisation had yet to be reached.*

### 1.3 Echium oil – update

This application was described in the 2000 Annual Report. In January 2002, Members considered the further data sent in response to the points raised at its meeting in November 2000. In particular they considered information concerning the protein content (an evaluation of which was necessary to investigate the absence/low content of Cytochrome C allergens), characterisation of unsaponifiable fraction, intended uses and possible effects in humans.

The Committee considered that the risk assessment regarding Cytochrome C allergens was not adequately justified and therefore, the protein content of the oil still needs to be established. Members also concluded that the available human studies were not robust or extensive enough to support the company's conclusions regarding the toxicological safety of the oil. Further information on non-sterol components of the unsaponifiable fraction of the oil was also requested. In conclusion Members felt that their earlier concerns had not been sufficiently addressed.

The Secretariat wrote to the company outlining the Committee's continued concerns and requesting further data to address these points, in particular the results from a human feeding study. The Company subsequently withdrew their application for novel food clearance.

## 2. Applications submitted to other Member States

### 2.1 Rapeseed oil (France)

The ACNFP was asked for its views on an application made to the French Competent Authority (CA) for approval of rapeseed oil high in unsaponifiable matter. This oil is derived from a conventional rapeseed line through a novel process. The novel food was considered to fall under Article 1(f) of Regulation 258/97 and the French CA produced a favourable Initial Opinion for use as a food ingredient at a dose of 1.5g per day.

While the ACNFP generally agreed with the Initial Opinion of the French CA it raised several additional points. The Committee was of the opinion that the applicant needed to provide a more accurate product specification, which provides at least a maximum and minimum range for each component. It was of the opinion that this should include the specific erucic acid content of the product, which should comply with the limit of 2% set in the relevant Codex Standard. The Committee was concerned that the applicant had not been specific enough in regards to the anticipated use of the ingredients quoting a very broad unspecified range of intended food products. The Committee did not consider that the applicant had satisfactorily demonstrated that consumption would be limited to a daily intake of 1.5g. The Committee agreed that the applicant should not be allowed to make cholesterol-lowering health claims. However, Members pointed out that susceptible, high risk members of the population may still be affected by this product, therefore the label should incorporate wording to indicate that the product contains phytosterols.

The UK Competent Authority objected to the marketing of this product until the concerns listed above have been addressed. In particular the UK was concerned that both the intake and likely use of the product need to be further clarified. The UK's opinion on this application was forwarded to the Commission in March 2002. A copy of this letter is attached at Appendix IV.

*At the time of going to press no further progress had been made.*

### 2.2 Maize germ oil (France)

This application was submitted at the same time and from the same applicant as the previous application for rapeseed oil high in unsaponifiable matter, and the concerns raised by the ACNFP were broadly similar.

The ACNFP was asked for its views on an application made to the French Competent Authority (CA) for approval of maize germ oil high in unsaponifiable matter. The application was considered to be a novel process and to fall under Article 1(f) of the Novel Food Regulation (EC) 258/97. The French CA carried out an initial assessment and produced a favourable initial opinion for use as a food ingredient at a dose of 1.5g per day.

While the Committee generally agreed with the Initial Opinion of the French CA it raised several additional points. Members were of the opinion that the applicant needed to provide a more accurate product specification, which provides at least a maximum and minimum range for each component. The Committee was also concerned that the applicant had not been specific enough in regards to the anticipated use of the ingredients quoting a very broad unspecified range of intended food products. Members did not consider that the applicant satisfactorily demonstrated consumption would be limited to a daily intake of 1.5g. The Committee agreed that the applicant should not be allowed to make cholesterol-lowering health claims. However, the UK points out that susceptible, high risk members of the population may still be affected by this product, therefore the label should incorporate wording to indicate that the product contains phytosterols.

The UK Competent Authority objected to the marketing of this product until the concerns listed above have been addressed. In particular the UK was concerned that both the intake and likely use of the product needed to be further clarified. The Committee's opinion on this application was forwarded to the Commission in March 2002. A copy of this letter is attached at Appendix V.

*At the time of going to press no further progress had been made.*

### **2.3 AR02 Multibene® (Finland)**

The ACNFP was asked to comment on the Finnish Competent Authority's initial opinion for an application by Multibene Health Oy Ltd for Multibene®, a range of crystalline plant sterol ingredients, which would be used to enrich dairy products, bakery products, processed meat products, edible fats, condiments (spices) sauces and soft drinks. In addition the Company intended to further enrich the product's mineral content (Ca, Mg, K). The applicant intends to use a mixture of phytosterols extracted from tall oil and vegetable oils, including soybean, corn, canola, sunflower seed, cottonseed and peanut oil. The plant sterols undergo standard extraction and purification procedures that are used traditionally in the food industry. The plant sterols are micronized to 20 microns or less before they are blended with food.

Although the Committee broadly agreed with the initial opinion of the Finnish Competent Authority, there were several concerns raised by Members. Many of these are broadly similar to the concerns previously

raised for other phytosterol products. Committee Members noted that some of the products (for instance soft drinks and ice cream) could be desirable to children although products fortified with sterols are not aimed at this section of the population. A general concern is that over consumption of phytosterols (above the dose of 2-3g per day that gives maximal reduction in blood cholesterol) could have a deleterious affect on the absorption of  $\beta$ -carotene. The Committee also found that the suggested labelling of the products containing the novel ingredient was unsatisfactory, as there was a potential for allergenicity since peanuts were one of the phytosterol sources used in the manufacture of Multibene®. They also felt that it was not clear whether the patterns of consumption described in the application would be predictive for the UK population and that the levels of phytosterols added to average intakes of particular food products should be limited to provide 1 serving of phytosterol.

The Committee does not support the marketing of the Multibene® product range until these concerns are addressed. The UK Competent Authority's views on the Finnish initial opinion were forwarded on to the Commission in April 2002, and a copy can be found at Appendix VI.

## **2.4 ADM plant sterols and sterol esters as novel food ingredients**

The ACNFP was asked to consider the Dutch Competent Authority's Initial Opinion on the application from the Archer Daniels Midland Company (ADM) for plant sterols and sterol esters as novel food ingredients.

The primary source of ADM plant sterols is crude edible soya oil, although some may be isolated from other edible oils, such as corn, rapeseed and palm. The plant sterols undergo standard extraction and purification procedures that are used traditionally in the food industry. It is intended that these compounds are used to enrich foods such as fat spreads, salad dressings, health bars, health drinks, yoghurt type products and processed meats, at a level designed to provide 1-3g of phytosterols per day.

The ACNFP broadly agreed with the Initial Opinion of the Dutch CA and therefore supported the marketing of the phytosterol-enriched yellow fat spread, subject to appropriate labelling of such products, as agreed for other phytosterol-containing products. However, the UK did not support the extension of the range to include salad dressings, health bars, health drinks, yoghurt-type products and processed meats, since the Committee felt that these products increased the opportunity for over consumption of phytosterols in the diet, and some of them were potentially desirable to children.

A letter was sent to the Commission in April 2002 describing the Committee's conclusions. This can be found at Appendix VII.

## 2.5 Iodine in eggs (Belgium)

In November 2002 the Committee was asked to consider an initial opinion from the Belgian Competent Authority for approval of iodine enriched eggs.

The applicant sought approval to market eggs enriched with iodine at a level of enrichment of 650µg (+/-50µg) per egg. The source of the enrichment was an iodine salt included in the chicken feed.

The Belgian Competent Authority concluded that the level of iodine fortification was sufficiently high that consumption of small quantities of the eggs may cause a health risk amongst the target population, namely iodine-deficient individuals.

The Committee agreed with the Belgian initial opinion and also noted that the chicken feed contained 100ppm “feed grade iodine salt ‘iodine’ equivalent” which exceeds the maximum authorised levels of iodine in feed (10ppm) as stipulated in Directive 70/524/EEC.

The Committee’s opinion was forwarded to the Commission in November 2002 and is attached at Appendix VIII.

*In light of similar responses from the Competent Authorities in other Member States, the European Commission advised the Belgian Competent Authority that it should inform the applicant that this product should not be placed on the market.*

## 2.6 Tahitian noni juice (*Morinda citrifolia*) – update

This application was described in the 2001 Annual Report. The European Commission’s Scientific Committee on Food (SCF) published a favourable opinion for this product in December 2002.

A copy of the SCF’s opinion can be found on their website at: [www.europa.eu.int/comm/food/fs/sc/scf/out151\\_en.pdf](http://www.europa.eu.int/comm/food/fs/sc/scf/out151_en.pdf)

## 2.7 Fresh and processed food products derived from Novartis Bt11 maize – update

This application was described in the 2000 and 2001 Annual Reports.

On the 17th April 2002 the Scientific Committee on Food (SCF) issued a favourable opinion on GM sweet maize line BT11.

*At the time of going to press the SCF opinion had not yet been considered by the Standing Committee on Food Chain and Animal Health.*

A copy of the SCF opinion can be found on their website at: [www.europa.eu.int/comm/food/fs/sc/scf/out129\\_en.pdf](http://www.europa.eu.int/comm/food/fs/sc/scf/out129_en.pdf)

## 2.8 Food and food ingredients derived from Monsanto GA21 GM maize – update

This application was described in the 2000 and 2001 annual reports.

On the 27th February 2002 the Scientific Committee on Food (SCF) issued a favourable opinion on GM maize line GA21.

*At the time of going to press the SCF opinion had not yet been considered by the Standing Committee on Food Chain and Animal Health.*

A copy of the SCF opinion can be found on their website at:  
[www.europa.eu.int/comm/food/fs/sc/scf/out121\\_en.pdf](http://www.europa.eu.int/comm/food/fs/sc/scf/out121_en.pdf)

## 3. Notifications

### 3.1 Phytosterol/Stanol – Forbes Medi-Tech

A submission was received from Forbes Medi-Tech in January 2002 seeking a scientific opinion on the substantial equivalence of FCP-3P7 to the active ingredients present in both the Benecol (phytostanol esters) and Flora pro.activ (phytosterol esters) yellow fat spreads. FCP-3P7 is a cholesterol-lowering ingredient containing a mixture of plant sterol esters and plant stanol esters proposed for incorporation into yellow fat spreads only.

The applicant claims that the phytosterol and phytostanol ester composition of FCP-3P7 is equivalent to a mixture of the active ingredients of the two yellow fat spreads fortified with phytostanol and phytosterol esters available in the European Union (Benecol and Flora pro.activ respectively).

Members concluded one product could not be claimed to be substantially equivalent to two different products. Therefore they were of the opinion that this application should not be dealt with under Article's 3(4) and 5 of the Novel Food Regulation (EC) 258/97 and that a full application should be submitted to gain pre-market approval for FCP-3P7.

The Committee also raised a number of issues relating to the dossier that would need to be addressed on submitting a full application. Evidence would be required to show that vitamin D absorption is not impaired.

Scientific studies supporting this application were carried out using a related product, FCP-3P1, rather than FCP-3P7. Therefore a full application would need to demonstrate that studies carried out using FCP-3P1 could be used in support of the application for FCP-3P7. This is due to the uncertainty surrounding the exact mechanism of action of phytosterols/stanols and whether esterification has any effect on this mechanism of action.

A full application would also need to include analyses for levels of any proteins derived from the source plant that might be present in FCP-3P7 so as to assess any allergic risk that this might pose.

Finally, when considering the intended Post-Market Monitoring study, some Members noted that if the purchase data related to households it might be difficult to determine which members of the household were actually consuming the product. Given the concerns that these products were not nutritionally appropriate for some groups of the population, such as children under five, this aspect would need to be clarified in any full application.

The Secretariat wrote to Forbes Medi-Tech with the Committee's views.

### 3.2 Monsanto GM Cottonseed

**RRC 1445 – modified to express herbicide tolerance (Roundup®- active ingredient glyphosate)**

**IPC 513 – modified to express insect resistance (Bt)**

As described in the 2001 Annual Report, the ACNFP first considered a request from Monsanto for scientific opinions on the substantial equivalence of oils derived from the seeds of two genetically modified cotton lines, in 1997. The lines had been modified to be tolerant of the effects of certain herbicides (line RRC 1445) and resistant to various lepidopteran insects (line IPC 513).

Since the original application, the Committee has requested further nutritional and compositional data on the oils, which the Company supplied. Data demonstrating the lack of DNA and protein in the derived oils was submitted by Monsanto and considered by the ACNFP at the end of 2001, when the Committee concluded that the oils derived from these lines are substantially equivalent to oil from conventionally bred cotton lines, in terms of composition, nutritional value, metabolism, intended use and level of undesirable substances. A formal Opinion was adopted by the Committee in July 2002. The ACNFP had a number of comments that it wished to make to the Company regarding the application, and these were addressed in a letter that accompanied the Scientific Opinions.

The Opinions of the ACNFP were forwarded to Monsanto on 5th July 2002. A copy of the opinions and the covering letter can be found at Appendices IX, X and XI

*A notification was published by the European Commission on 19 December 2002.*

## 4. Other issues considered by the ACNFP

### 4.1 Public Hearing on Chardon LL maize.

In 1995, the ACNFP considered the food safety of products derived from the genetically modified maize line T25, which had been developed by AgrEvo USA to be tolerant to glufosinate–ammonium based herbicides. The request related to products derived from the seeds of this line, together with products derived from inbred and hybrid lines developed using conventional cross breeding. The ACNFP gave a positive scientific opinion on the safety of foods derived from this maize line, in 1996. The Company subsequently made a notification on the 8th January 1998, under Articles 4(3) and 5 of the Novel Foods Regulation, citing the ACNFP’s scientific opinion in support.

Since then, the applicant has obtained regulatory clearance to grow this maize variety in the EU and in 2000, applied to DEFRA to have the seeds of the Chardon LL maize variety – which is derived from the T25 line – placed on the National List. This is the final step in the process for the Company to be able to grow this variety of maize in the UK.

Under the National List regulations, parties affected by the proposed decision to “list” a variety may make written representations to Ministers and request a Public Hearing. Friends of the Earth challenged the application for Chardon LL maize, and it was suspended. A Public Hearing began in October 2000 to enable interested parties to present evidence for and against Chardon LL maize being placed on the National List. The Hearing finished in June 2002.

The ACNFP was asked to consider the documents and oral presentations made to the Hearing. The Committee is content that no new evidence has been presented that would question the safety of foods derived from Chardon LL maize. In December, the ACNFP published a response to points raised at the Hearing. This can be found at Appendix XII.

### 4.2 T25 maize – update

In the ACNFP’s original safety assessment of T25 maize (see section 4.1 above), it was requested that the seed composition of this line of genetically modified maize should be monitored over time, to demonstrate the stable inheritance of the introduced trait and to determine any possible effect of genetic drift on the plant’s metabolism.

In September 2002, the company supplied these data, which the Committee considered by post.

The Committee was satisfied that the majority of components measured in the T25 seed fell within standard published reference ranges for maize, and that any statistically significant differences found between the transgenic and non-transgenic groups are not of biological significance when viewed in the context of these normal ranges.

The ACNFP's full assessment of these data can be found at Appendix XIII.

### **4.3 Scientific Steering Committee guidance document on the information needed for the risk assessment of GM plant-derived food and feed.**

The joint SCF/SCP/SCAN GM/Novel Food Working Group has been asked by the European Commission's Scientific Steering Committee to draw up a guidance document on the information needed for the risk assessment of genetically modified plants and derived food and feed. The document provides guidance to notifiers and risk assessors during applications for deliberate release of GM plants and derived cultivars under EC Directive 2001/18/EC and/or for commercial authorisation of novel (GM) food or feed. This preliminary guidance document and the UK Competent Authority on Novel Foods' draft response to the document were considered by the ACNFP at its meeting in September 2002. The Committee welcomed the document and agreed with the main comments made in the Competent Authority response, suggesting a number of minor revisions to the text. The response considered that the document did not fully reflect the concept of substantial equivalence within risk assessment and that guidelines adopted by the European Commission should be consistent with the draft Codex guidelines for the safety assessment of foods derived from GM plants. This response was forwarded to the Commission's Scientific Steering Committee.

### **4.4 Research on horizontal gene transfer**

When the first genetically modified maize was considered for approval in 1996 concern was expressed by the ACNFP regarding the retention of an antibiotic resistance marker and the potential for horizontal gene transfer from GM plants to bacteria that inhabit the gastrointestinal tract. The ACNFP considered that the risk of the antibiotic resistance gene being transferred from GM maize to bacteria was small but finite.

The Committee considered four completed R&D reports, which had been funded as part of the Food Standards Agency's research programme G01: Safety of Novel Foods. These research projects were commissioned in the UK to address the issue of DNA survival in the gastrointestinal tract and gene transfer into gut bacteria.

Overall, the research demonstrated that there may be some DNA survival when DNA (plasmid or genomic DNA released from transgenic material) is exposed to different environments identical to those in the animal or human gastrointestinal tract. However, the potential for uptake of functional transgenic DNA either by gut bacteria or by epithelial cells lining the gastrointestinal tract was found to be extremely low. Uptake is dependent on intact, functional DNA surviving the unfavourable conditions in the gastrointestinal tract in addition to gut bacteria or epithelial cells being capable of taking up the DNA. The implications of these findings, in relation to the safety assessment of GM foods were also discussed at the ACNFP open meeting in November 2002 and the Committee will consider whether the research has addressed their original concerns in 2003.

**(a) Project G01007:** Survival of ingested DNA in the gut and the potential for genetic transformation of resident bacteria (Rowett Research Institute)

This project looked at the survival potential of ingested DNA *in vitro* under simulated gut conditions and *in vivo* in the rat gut, and the potential for its transformation into gut bacteria using both plasmid and chromosomal DNA by *in vitro* methods. Results indicated that DNA has the potential to survive in the gut and undergo transformation. However, the probability of transformation of gut bacteria from fragments of GM DNA released from transgenic material will be influenced by the design of GM constructs since transformation depends largely on the presence of matching sequences in the host bacterium and GM DNA.

**(b) Project G01008:** Evaluating the risks associated with using GMOs in human foods (University of Newcastle)

This project examined the risk of gene transfer from GM plants and bacteria to other microorganisms and epithelial cells in the gastrointestinal tract. The project looked at the survival of transgenes in Soya DNA through the human gastrointestinal tract using ileostomy patients and healthy volunteers. Studies on 7 ileostomists showed that GM DNA survived passage through the small intestine and was detected in bacteria in the stoma. When microbes from the ileal digesta samples were grown up, trace amounts of the GM Soya transgene could be detected by PCR. However, bacteria containing the transgene could not be isolated indicating that only a minute proportion of the indigenous intestinal microflora had taken up the DNA. Furthermore, GM DNA was not found to survive passage through the complete gastrointestinal tract of healthy volunteers, nor was it detected in stool samples. Results from *in vitro* studies in a model intestinal cell line (Caco-2) indicated that gene transfer from GM material to the intestinal epithelium, either directly or via the intestinal microflora, is unlikely to occur.

**(c) Project G01010:** Assessment of the risks of transferring antibiotic resistance determinants from transgenic plants to microorganisms (University of Leeds)

The main aim of this project was to determine the likelihood that DNA from transgenic maize may be transferred to gut bacteria of food producing animals, and to establish the likelihood of transfer of an antibiotic resistance gene ( $bla_{TEM}$ ). Results from this work have shown that intact plasmid DNA can survive for measurable periods of time in ovine saliva, rumen fluids and silage effluent. DNA released from digested plant material could provide a potential source of DNA capable of transforming resident bacteria although such DNA has been demonstrated to be biologically active for less than one minute. Also, it was shown that the antibiotic resistance gene  $bla_{TEM}$  is stably incorporated into the GM maize, thus, the chances that it will escape easily and transfer to microbes associated with GM maize during silage production, or animals feeding on GM material, are slight.

**(d) Project G01011:** Dissemination of GM DNA and antibiotic resistance genes via rumen microorganisms (Rowett Research Institute)

The aim of this project was to assess the survival of DNA under rumen gut conditions by investigating *in vitro* the survival and uptake of DNA by rumen bacteria and investigating the potential for gene transfer between these bacteria. *In vitro* results from this work indicate that plant DNA can survive briefly in rumen fluid and some rumen bacteria are capable of incorporating and expressing foreign DNA. However, a component present in rumen fluid inhibits genetic transformation indicating that transformation in the rumen would be a rare event. Acquisition of foreign DNA by the bacterial chromosome is most likely when the sequences in the foreign DNA and the bacterium are similar. This shows that the possibility of rare acquisition of transgenes from ingested GM plant material by rumen bacteria cannot be completely ruled out. However, the observation that rumen fluid inhibits the transformation process in the rumen bacteria studied means that they are unlikely to acquire DNA under *in vivo* rumen conditions.

#### 4.5 ACRE guidance on best practices for molecular data presentation

The ACNFP discussed the draft guidance issued by the Advisory Committee on Releases to the Environment (ACRE) entitled 'Guidance on Best Practice for the Presentation of Molecular Data in Submission to Advisory Committee on Releases to the Environment'. This offers guidance on the scientific rigor sought by ACRE on the level of detail needed when presenting the molecular data in application dossiers. Dr. John Heritage was invited, as a molecular expert of the ACNFP with similar experience reviewing past consent applications, to contribute to the drafting of the guidance (the needs, experiences and expectations of the two committees for molecular data being very similar). The ACNFP welcomed the guidance, which will also be beneficial to its own work if companies follow its recommendations.

#### **4.6 OECD consensus documents on sunflower and rice.**

The OECD Task Force for the safety of novel foods and feeds has produced a series of consensus documents on various crops. The purpose of these documents is to provide a technical tool for regulatory officials as a general guide and reference source to the range and variability in conventional crops to aid in the safety assessment of modified crops.

In 2002 the Committee was asked to consider two new consensus documents on sunflower (drafted by France) and rice (drafted by Japan). Members felt that the documents would provide a useful source of information, although they queried whether a wider range of references was available for the sunflower document. The Committee's comments were forwarded to the OECD Task Force.

#### **4.7 Monsanto GM maize hybrid lines NK603 x MON810**

In May 2002 the Committee was asked to consider whether there were any human health issues arising from an application to import a GM maize line that had been submitted by Monsanto to DEFRA for authorisation under a Part C consent, under the Release of Genetically Modified Organisms (GMOs) Directive 90/220/EEC. The application is for maize hybrid lines NK603xMON810 derived from traditional crosses between GM maize lines NK603 (herbicide tolerant) and MON810 (insect resistant).

This application does not cover consent for food use. A separate application under the Novel Food Regulation (258/97) will be required for this purpose.

The Committee was concerned about the assumptions made in the dossier regarding a lack of any allergenic potential of the protein products of the introduced traits, when immunogenic properties clearly exist in order for antibodies to be raised for the ELISA test. They considered that further supporting data are required to justify statements made in the dossier regarding the potential allergenicity/toxicity in the hybrids.

Members noted that the dossier mentions feed use and other uses, such as in the production of food additives. They felt that following an incident in the US, where a large quantity of food had been recalled due to the accidental presence of a maize variety authorised only for use in animal feed, food and feed applications should be submitted simultaneously, as there is always the possibility of adventitious contamination. The current Commission proposals on GM Food and Feed as currently drafted would require, where a crop can be used for food and feed, that clearance is sought for both simultaneously.

The Committee highlighted a number of other aspects of the dossier that the Advisory Committee on Releases to the Environment (ACRE) may wish to consider in its evaluation of the application. In particular they felt that it was not sufficient just to cross reference to data contained in application dossiers relating to the parent lines. A complete dossier, containing all the relevant experimental data for the hybrid lines under consideration, is required.

The Committee's views were passed to ACRE, who considered the dossier at its meeting in May 2002.

#### 4.8 Argan oil (almond kernel oil)

The Commission was notified on the 01 August 2002 of the applicant's intention to market argan oil, extracted from kernels of the argan tree *Argania spinosa* L. which grows wild in regions of Morocco. The French Competent Authority had provided a scientific opinion concluding that argan oil had been demonstrated to be substantially equivalent to existing oils as regards its composition, nutritional value, metabolism, intended use, and contaminants.

The ACNFP considered this notification and expressed concerns regarding the allergenic potential of this product. Given the known allergenic potential of nuts, per se, there seems a particular risk of cross-reactivity with this product, which was not addressed either by the French opinion or in the company's dossier. Furthermore, the taxonomy of *Argania spinosa* L. is not sufficiently detailed for a clear indication of the allergic potential of this product to be drawn by comparison with closely related food sources. Without this information, Members were of the view that actual studies are needed in order to make an informed judgement about the likelihood of allergic reactions to the proteins present in the oil.

An additional concern identified by the Committee was that the trees are uncultivated and it is likely that there will be extensive genetic diversity, which may result in significant variability in the composition of the oil. However, the available data on composition appeared to result from the analysis of only a single batch. The Committee's view was that further data should be provided to demonstrate the range of the various constituents present in the oil.

It is also clear that the unsaponifiable fraction differs from the existing oils, both in terms of phytosterols and the flavour components. The present application relates to oil extracted from untoasted kernels and Members indicated that a separate evaluation would be required for oil extracted from toasted kernels.

The ACNFP's comments were transmitted to the European Commission and the French Competent Authority in December 2002. A copy of the letter can be found at Appendix XIV.

## 5. Other activities

### 5.1 ACNFP Open Meeting

The ACNFP held its second open meeting on the 13th November in Cambridge.

The aim of the meeting was to give the general public the opportunity to meet with the Committee and to discuss some of the issues that fall within the remit of the ACNFP.

The meeting was chaired by Professor Janet Bainbridge and was divided into four sections;

- the remit and role of the Committee;
- an open session with tabled questions;
- implications of the recently completed FSA research on horizontal gene transfer for the way in which the safety of GM foods is assessed; and
- the testing of novel foods (both GM and non-GM) for potential allergenicity.

Various stakeholders, representing those holding a broad range of views, attended the meeting.

The audience raised a number of interesting points and suggestions particularly on the divergence of views between regulators and consumers as to the definition of 'risk'.

The minutes of this meeting are available on the ACNFP pages of the FSA website: [www.food.gov.uk/science/ouradvisors/novelfood/acnfpmeets/meetings2002/open\\_meeting/111156](http://www.food.gov.uk/science/ouradvisors/novelfood/acnfpmeets/meetings2002/open_meeting/111156)

The Committee welcomed this opportunity to meet the various stakeholders, and found the meeting to be very useful.

### 5.2 ACNFP factsheets

The ACNFP Secretariat issues a corporate brochure to interested parties. This outlines the work of the Committee, its membership and functions and is in the form of a folder containing fact sheets.

During 2002, Members were asked to approve three new fact sheets. The first was an updated Membership fact sheet and the other two were on High Pressure Processing and the 2001 Annual Report.

*Copies of these fact sheets are available from the Secretariat, details are given on page 21.*

### **5.3 ACNFP guidelines on human studies and taste trials**

As described in the previous Annual Reports, revised ACNFP guidelines on the conduct of taste trials of novel foods (including Genetically Modified – GM-food) using human volunteers and new guidance on the role of human studies in the pre-market safety assessment of novel foods were issued for consultation in September 2001.

The summary of the responses to the consultation was published on the FSA website. The Committee considered the responses received and revised the guidelines at its meetings in January and March 2002 and the final guidelines were published in April 2002.

Copies of the final guidelines can be found at Appendices XV and XVI.

## 6. Developments elsewhere

### 6.1 FSA Review of Scientific Committees

The FSA reviewed the role, methods of operation and effectiveness of the independent scientific committees from which it seeks advice, this included the ACNFP.

As reported in the 2001 Annual Report, Professor Janet Bainbridge attended a meeting on the 27th September where she expressed the views of ACNFP Members regarding their work, openness and risk assessment.

These views along with those of the other Committees went forward to the Review Group, whose report – FSA Report on the Review of Scientific Committees was published in March 2002 and can be found on the FSA website at:

[www.food.gov.uk/multimedia/pdfs/CommitteesReview.pdf](http://www.food.gov.uk/multimedia/pdfs/CommitteesReview.pdf)

### 6.2 Review of the Novel Foods Regulation 258/97

The Novel Food Regulation (EC) No 258/97 came into force on the 15th May 1997, introducing a statutory pre-market approval system for GM and non-GM novel foods that did not have a significant history of consumption in the European Union. Article 14 of this Regulation stipulates that five years from the date of its entry into force, the Commission should forward a report on its implementation to the European Parliament and the Council. In accordance with this, a number of discussions took place between Member States and in early August the Commission published and invited comments on the Discussion Paper on the Evaluation of the Novel Food Regulation. At this time the FSA consulted a wide range of interested parties and informed them of the Commissions consultation. The ACNFP advised the FSA on this issue and took into consideration the comments they had been copied in on by UK interested parties. The UK comments were then forwarded to the Commission and are attached at Appendix XVII. The Commission has now published all the comments on the novel foods page of its website and held a stakeholder meeting in early 2003. ([http://www.europa.eu.int/comm/food/fs/novel\\_food/nf\\_index\\_en.html](http://www.europa.eu.int/comm/food/fs/novel_food/nf_index_en.html)).

### 6.3 Commission Proposals on the Traceability, Authorisation and Labelling of GM foods

In July 2001 the European Commission issued two closely linked proposals for new legislation concerning genetically modified organisms (GMOs), one covering Traceability and the other, the Food and Feed proposal, dealing with authorisation procedures and labelling issues.

On 3 July 2002 the European Parliament adopted 111 amendments to the Commission's original Food and Feed proposal. The Commission accepted 16 of the proposed amendments outright, 38 in part or subject to re-wording and rejected 57.

The EU Agriculture Council reached political agreement on the GM food and feed proposal on 28 November 2002. The proposal was agreed by a qualified majority with the UK, Austria and Luxembourg voting against.

Political agreement on the proposal on traceability and labelling was reached at the December Environment Council.

Following formal adoption of the Common Positions on these two proposals, they will be referred back to the European Parliament for their second reading.

Further details on these proposals can be found on the FSA website at: [www.food.gov.uk/science/sciencetopics/gmfoods/gmfoodfeedproposals](http://www.food.gov.uk/science/sciencetopics/gmfoods/gmfoodfeedproposals)

## 7. Contact points

For further information about the general work of the Committee or about specific scientific points concerning individual submissions (which have been made or are being made) contact in the first instance:

Dr Sandy Lawrie  
ACNFP Secretary  
Room 526B  
Aviation House  
125 Kingsway  
London  
WC2B 6NH

Tel (switchboard): 020 7276 8000  
Tel (Direct line ) : 020 7276 8565  
Fax: 020 7276 8564

The FSA Website can be found at <http://www.food.gov.uk>.  
Information on the ACNFP is at:  
<http://www.food.gov.uk/science/ouradvisors/novelfood>

Information can also be requested via e-mail at:  
[acnfp@foodstandards.gsi.gov.uk](mailto:acnfp@foodstandards.gsi.gov.uk).

## 8. References

1. Advisory Committee on Novel Foods and Processes. *Annual Report 1989*. Department of Health and Ministry of Agriculture, Fisheries and Food, 1990. (Available from the ACNFP Secretariat).
2. Advisory Committee on Novel Foods and Processes. *Annual Report 1990*. Department of Health and Ministry of Agriculture, Fisheries and Food, 1991. (Available from the ACNFP Secretariat).
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12. Advisory Committee on Novel Foods and Processes. *Annual Report 2000*. Food Standards Agency 2001 – FSA/0013/0301 (Available from the ACNFP Secretariat).
13. Advisory Committee on Novel Foods and Processes. *Annual Report 2001*. Food Standards Agency 2002 – FSA/0553/0302 (Available from the ACNFP Secretariat).

## 9. Glossary

**allergenicity:** the potential or ability to elicit an allergic response.

**erucic acid:** an unusual unsaturated fatty acid found in large quantities in rapeseed and other related plants.

**haemostatic:** relating to blood clotting.

**ileostomist:** a person who has had their large intestine removed, and their small intestine attached to a stoma.

**stoma:** *an opening in the body that is made surgically to replace a normal opening.*

**immunogenic:** causing an immune response.

**lepidopteran:** from the order of insects lepidoptera – butterflies and moths.

**micronized:** particles that have been reduced to a very small size, typically around 1 micron in size.

**micron:** a unit of length equal to one millionth of a meter.

**phytosterol esters:** compounds found in vegetable oil, seeds, nuts and coniferous trees that interfere with the absorption of cholesterol in the intestine due to their similar structure.

**plasmid:** small self-replicating circular DNA independent of the chromosome in bacteria. They are widely used in genetic engineering as vectors into which foreign genes are inserted for subsequent cloning or expression in bacterial cells.

**rumen:** the first stomach in ruminants (cud-chewing mammals) in which food is digested by bacteria.

**SCAN:** Scientific Committee on Animal Nutrition.

**SCF:** EC Scientific Committee on Food.

**SCP:** EC Scientific Committee on Plants.

**transgenic:** animal or plants that have had genes artificially introduced by genetic modification.

**unsaponifiable:** a fat which cannot be hydrolysed by an alkali to form a soap and an alcohol.

# APPENDIX I

## ACNFP – remit, membership and list of members’ interests, code of conduct and interactions with other committees

### Remit

The Advisory Committee on Novel Foods and Processes is an independent body of experts whose remit is:

*“to advise the central authorities responsible, in England, Scotland, Wales and Northern Ireland respectively on any matters relating to novel foods and novel food processes, including food irradiation, having regard where appropriate to the views of relevant expert bodies.”*

Officials of the Food Standards Agency provide the Secretariat. As well as formal meetings, the Committee organises workshops on specific topics related to its remit.

The interactions between the ACNFP and other independent advisory committees are outlined in Figure 1.

### Membership and Members’ Interests

The membership of the Committee provides a wide range of expertise in fields of relevance in the assessment of novel foods and processes. A list of the membership during 2002, together with the names of the FSA assessors can be found overleaf.

In common with other independent advisory committees the ACNFP is publishing a list of its members’ commercial interests. These have been divided into different categories relating to the type of interest:-

Personal:           a) direct employment or consultancy;  
                          b) occasional commissions;  
                          c) share holdings.

Non-personal: a) fellowships;  
                          b) support which does not benefit the member directly e.g. studentships.

Details of the interests held by members during 2002 can be found on page 27.

A copy of the code of conduct for ACNFP members can be found on page 29.

## MEMBERSHIP OF THE COMMITTEE DURING 2002

### Chairman

**Professor J Bainbridge** OBE, BSc, PhD, Grad.Cert.Ed (Tech), FRSA, SOFHT.  
Chief Executive of EPICC subsidiary of the University of Teesside,  
Middlesbrough.

### Deputy Chairman

**Professor P Dale** BSc, PhD, CBiol, FIBiol. (Molecular biologist/plant geneticist)  
Research Group Leader, Genetic Modification and Biosafety Assessment,  
John Innes Centre, Norwich.

### Members

**Professor P Aggett** OBE, MSc, MB, ChB, FRCPCH, FRCP(L)(E)(G) DCH  
(*Ex-officio* link to COT)  
Head of the Lancashire Postgraduate School of Medicine and Health.

**Miss J Brand** MPhil, FICSc (Consumer Representative)

**Professor R Chadwick** BA, BPhil, DPhil (Ethicist)  
Director of the Institute for Environment, Philosophy and Public Policy,  
Lancaster University

**Dr H Close** BSc, PhD, PG Dip (Consumer Representative)

**Mr N Craddock** MA, FIFST (Food Processing and Quality Assurance Expert)  
until 30 September: Group Regulatory and Environmental Affairs  
Manager, Nestle UK Ltd  
from 1 October: Non-Executive Director of Law Laboratories Ltd

**Professor J Dunwell** BA, MA, PhD (Plant Biotechnologist)

**Professor of Plant** Biotechnology, School of Plant Sciences, University of  
Reading.

**Dr J Fowler** BVM&S, PhD, FATS, CBiol, FIBiol, FRCPath, FRCVS (Toxicologist)  
Registered Toxicologist and Specialist of the Royal College of Veterinary  
Surgeons.

**Dr J Heritage** BA, DPhil, CBiol, FIBiol (Microbiologist)  
Senior Lecturer in Microbiology at the University of Leeds.

**Dr P Lund** BA, MA, DPhil (Plant Molecular Biologist)  
Senior Lecturer at School of Biosciences, University of Birmingham.

**Professor A Malcolm** MA, DPhil, FIFST, FIBiol, CBiol (Nutritionist)  
Chief Executive of the Institute of Biology

**Dr C Meredith** BA, MA, MSc, PhD (Toxicologist/Immunologist)  
Head of Immunology at TNO BIBRA International Ltd, Surrey.

**Professor M Johnston** BVM&S, DVet Med, Hon FRCVS, Dip ECVPH  
(ex-officio link to ACMSE)  
Professor of Veterinary Public Health, Royal Veterinary College

**Professor I Rowland** BSc, PhD. (Nutritionist/toxicologist)  
Director, Northern Ireland Centre of Diet and Health at the University of  
Ulster; Coleraine.

**Professor J Warner** MB, ChB, DCH, MRCP, MD, FRCP, MRCPCH, FRCPC.  
(Allergenicity Expert)  
Professor of Child Health at University of Southampton.

### **FSA Assessors**

Mr N Tomlinson      Food Standards Agency

Mrs J Whinney      Food Standards Agency (Wales)

Ms E MacDonald      Food Standards Agency (Scotland)

Mr G McCurdy      Food Standards Agency (Northern Ireland)

**Membership of the committee during 2002**

<b>Member</b>	<b>Company</b>	<b>Interest</b>	<b>Company</b>	<b>Interest</b>	<b>Company</b>	<b>Interest</b>
Professor J Bainbridge (CHAIRMAN)	None	None	Various	Departmental commissioned research and student placements	None	None
Dr P Dale (Deputy Chair)	John Innes Centre EU/United Nations Environmental Program/ United Nations Industrial Development Organisation/Defra/ OECD Agriculture and Environment Biotechnology Commission	Salary Advisor  Consultant Member	University of East Anglia Various Societies, Institutes and Steering Groups  Institute of Biology BBSRC/Defra/EU Rockefeller Maize biotechnology Programme	Honorary Professor Member  Fellow Research Funding Member of Advisory Committee	None	None
Professor P Aggett	None	None	Astra-Zeneca Smith & Nephew Nestec ILSI Abbott Wellcome Trust Yakult Wyeth SMA Int Copper Association	Departmental commissioned and education in medicine and health including safety and metabolism	None	None
Miss J Brand	None	None	None	None	None	None
Professor J Chadwick	Glaxo SmithKline	Occasional Consultant	Food Ethics Council	Member	None	None
Dr H Close	None	None	None	None	None	None
Mr N Craddock	Nestle UK Ltd (until 30/9/2002) Law Laboratories Ltd (from 1/10/2002)	Salary  Non-Executive Director	None	None	None	None
Professor J Dunwell	Syngenta	Minimal Shareholder	None	None	None	None
Dr J Fowler	Syngenta	Minimal Shareholder	None	None	None	None
Dr J Heritage	Ray A. Kroc Foundation	Visiting professor at East Virginia Medical School	None	None	None	None
Dr P Lund	Abbey National Celltech	Minimal Shareholder Minimal Shareholder	BBSRC	Departmental Research	None	None

**Membership of the committee during 2002 (continued)**

<b>Member</b>	<b>Company</b>	<b>Interest</b>	<b>Company</b>	<b>Interest</b>	<b>Company</b>	<b>Interest</b>
Professor M Johnston	Tesco	Consultant	Various	Departmental research on animal and public health issues	None	
Professor A Malcolm	Associated British Foods	Shareholder	None	None	None	
Dr C Meredith	None	None	Various	Departmental Commissioned Research	None	
Professor I Rowland	Colloids Naturels International Cerestar Abbey National Woolwich	Consultant Consultant Minimal Shareholder Minimal Shareholder	Robert Craig and Sons Valio (Finland) Alpro (Belgium) VK Muehlen (Germany) Biohit (Finland) Danone (France) Orafti (Belgium) Kelloggs UK Unilever UK Alpro Cultech UK	Funded Research Partners in EC funded Projects  PhD Studentships  Teaching Company Scheme	None  None	
Mrs E Russell	The Boots Company PLC	Shareholder	None	None	Husband Chief Executive of The Boots Company plc.	
Professor JO Warner	None	None	UBC Pharma Merck ILSI Europe	Research Research Editorial	None	

## A CODE OF CONDUCT FOR MEMBERS OF THE ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES (ACNFP)

### Public service values

The Members of the ACNFP must at all times:

- observe the highest standards of impartiality, integrity and objectivity in relation to the advice they provide and the management of this Committee;
- be accountable, through the Board of the Food Standards Agency and Health Ministers, to Parliament and the public for its activities and for the standard of advice it provides.

The Board of the FSA and Health Ministers are answerable to Parliament for the policies and performance of this Committee, including the policy framework within which it operates.

### Standards in Public Life

All Committee Members must:

- follow the Seven Principles of Public Life set out by the Committee on Standards in Public Life (Annex 1);
- comply with this Code, and ensure they understand their duties, rights and responsibilities, and that they are familiar with the function and role of this Committee and any relevant statements of Government policy. If necessary members should consider undertaking relevant training to assist them in carrying out their role;
- not misuse information gained in the course of their public service for personal gain or for political purpose, nor seek to use the opportunity of public service to promote their private interests or those of connected persons, firms, businesses or other organisations; and
- not hold any paid or high profile unpaid posts in a political party, and not engage in specific political activities on matters directly affecting the work of this Committee. When engaging in other political activities, Committee members should be conscious of their public role and exercise proper discretion. These restrictions do not apply to MPs (in those cases where MPs are eligible to be appointed), to local councillors, or to Peers in relation to their conduct in the House of Lords.

## Role of Committee members

Members have collective responsibility for the operation of this Committee. They must:

- engage fully in collective consideration of the issues, taking account of the full range of relevant factors, including any guidance issued by the Food Standards Agency or Health Ministers;
- in accordance with Government policy on openness, ensure that they adhere to the Code of Practice on Access to Government Information (including prompt responses to public requests for information); agree an Annual Report; and, where practicable and appropriate, provide suitable opportunities to open up the work of the Committee to public scrutiny;
- not divulge any information which is provided to the Committee in confidence;
- ensure that an appropriate response is provided to complaints and other correspondence, if necessary with reference to the sponsor department; and
- ensure that the Committee does not exceed its powers or functions.

Individual members should inform the Chairman (or the Secretariat on his or her behalf) if they are invited to speak in public in their capacity as a Committee member.

Communications between the Committee and the Board of the Food Standards Agency will generally be through the Chairman except where the Committee has agreed that an individual member should act on its behalf. Nevertheless, any member has the right of access to the Board of the FSA on any matter that he or she believes raises important issues relating to his or her duties as a Committee member. In such cases the agreement of the rest of the Committee should normally be sought.

Individual members can be removed from office by the Board of the FSA, if they fail to perform the duties required of them in line with the standards expected in public office.

## The role of the Chairman

The Chairman has particular responsibility for providing effective leadership on the issues above. In addition, the Chairman is responsible for:

- ensuring that the Committee meets at appropriate intervals, and that the minutes of meetings and any reports to the Board of the FSA accurately record the decisions taken and, where appropriate, the views of individual members;

- representing the views of the Committee to the general public; and
- ensuring that new members are briefed on appointment (and their training needs considered), and providing an assessment of their performance, on request, when members are considered for re-appointment to the Committee or for appointment to the board of some other public body.

## Handling conflicts of interests

The purpose of these provisions is to avoid any danger of Committee members being influenced, or appearing to be influenced, by their private interests in the exercise of their public duties. All Members should declare any personal or business interest that may, or may be *perceived* (by a reasonable member of the public) to, influence their judgement. A guide to the types of interest that should be declared is at Annex 2.

### (i) Declaration of Interests to the Secretariat

Members of the Committee should inform the Secretariat in writing of their current personal and non-personal interests, when they are appointed, including the principal position(s) held. Only the name of the organisation and the nature of the interest are required; the amount of any salary etc. need not be disclosed. Members are asked to inform the Secretariat at any time of any change of their personal interests and will be invited to complete a declaration form once a year. It is sufficient if changes in non-personal interests are reported in the annual declaration form following the change. (Non-personal interests involving less than £1,000 from a particular company in the previous year need not be declared to the Secretariat).

The register of interests should be kept up-to-date and be open to the public.

### (ii) Declaration of Interest and Participation at Meetings

Members of the Committee are required to declare any direct interests relating to salaried employment or consultancies, or those of close family members<sup>1</sup>, in matters under discussion at each meeting. Having fully explained the nature of their interest the Chairman will, having consulted the other members present, decide whether and to what extent the member should participate in the discussion and determination of the issue. If it is decided that the member should leave the meeting, the Chairman may first allow them to make a statement on the item under discussion.

<sup>1</sup> Close family members include personal partners, parents, children, brothers, sisters and the personal partners of any of these.

### Personal liability of Committee members

A Committee Member may be personally liable if he or she makes a fraudulent or negligent statement which results in a loss to a third party; or may commit a breach of confidence under common law or a criminal offence under insider dealing legislation, if he or she misuses information gained through their position. However, the Government has indicated that individual members who have acted honestly, reasonably, in good faith and without negligence will not have to meet out of their own personal resources any personal civil liability which is incurred in execution or purported execution of their Committee functions save where the person has acted recklessly. To this effect a formal statement of indemnity has been drawn up.

#### THE SEVEN PRINCIPLES OF PUBLIC LIFE

##### **Selflessness**

Holders of public office should take decisions solely in terms of the public interest. They should not do so in order to gain financial or other material benefits for themselves, their family, or their friends.

##### **Integrity**

Holders of public office should not place themselves under any financial or other obligation to outside individuals or organisations that might influence them in the performance of their official duties.

##### **Objectivity**

In carrying out public business, including making public appointments, awarding contracts, or recommending individuals for rewards and benefits, holders of public office should make choices on merit.

##### **Accountability**

Holders of public office are accountable for their decisions and actions to the public and must submit themselves to whatever scrutiny is appropriate to their office.

##### **Openness**

Holders of public office should be as open as possible about all the decisions and actions that they take. They should give reasons for their decisions and restrict information only when the wider public interest clearly demands.

##### **Honesty**

Holders of public office have a duty to declare any private interests relating to their public duties and to take steps to resolve any conflicts arising in a way that protects the public interests.

##### **Leadership**

Holders of public office should promote and support these principles by leadership and example.

## DIFFERENT TYPES OF INTEREST

The following is intended as a guide to the kinds of interests that should be declared. Where Members are uncertain as to whether an interest should be declared they should seek guidance from the Secretariat or, where it may concern a particular product which is to be considered at a meeting, from the Chairman at that meeting. **If Members have interests not specified in these notes but which they believe could be regarded as influencing their advice they should declare them.** However, neither the Members nor the Secretariat are under any obligation to search out links of which they might reasonably not be aware. For example, either through not being aware of all the interests of family members, or of not being aware of links between one company and another.

### Personal Interests

A personal interest involves the Member personally. The main examples are:

- **Consultancies and/or direct employment** any consultancy, directorship, position in or work for the industry or other relevant bodies which attracts regular or occasional payments in cash or kind;
- **Fee-Paid Work:** any commissioned work for which the member is paid in cash or kind;
- **Shareholdings:** any shareholding or other beneficial interest in shares of industry. This does not include shareholdings through unit trusts or similar arrangements where the member has no influence on financial management;
- **Membership or Affiliation** to clubs or organisations with interests relevant to the work of the Committee.

### Non-Personal Interests

A non-personal interest involves payment which benefits a department for which a member is responsible, but is not received by the member personally. The main examples are:

- **Fellowships:** the holding of a fellowship endowed by industry or other relevant body;
- **Support by Industry or other relevant bodies:** any payment, other support or sponsorship which does not convey any pecuniary or material benefit to a member personally, but which does benefit their position or department e.g.:

- (i) a grant for the running of a unit or department for which a member is responsible;
- (ii) a grant or fellowship or other payment to sponsor a post or a member of staff or a post graduate research programme in the unit for which a member is responsible (this does not include financial assistance for undergraduate students);
- (iii) the commissioning of research or other work by, or advice from, staff who work in a unit for which a member is responsible.

Members are under no obligation to seek out knowledge of work done for, or on behalf of, industry or other relevant bodies by departments for which they are responsible, if they would not normally expect to be informed. Where members are responsible for organisations which receive funds from a very large number of companies involved in that industry, the Secretariat can agree with them a summary of non-personal interests rather than draw up a long list of companies.

- Trusteeships: any investment in industry held by a charity for which a member is a trustee. Where a member is a trustee of a charity with investments in industry, the Secretariat can agree with the member a general declaration to cover this interest rather than draw up a detailed portfolio.

## DEFINITIONS

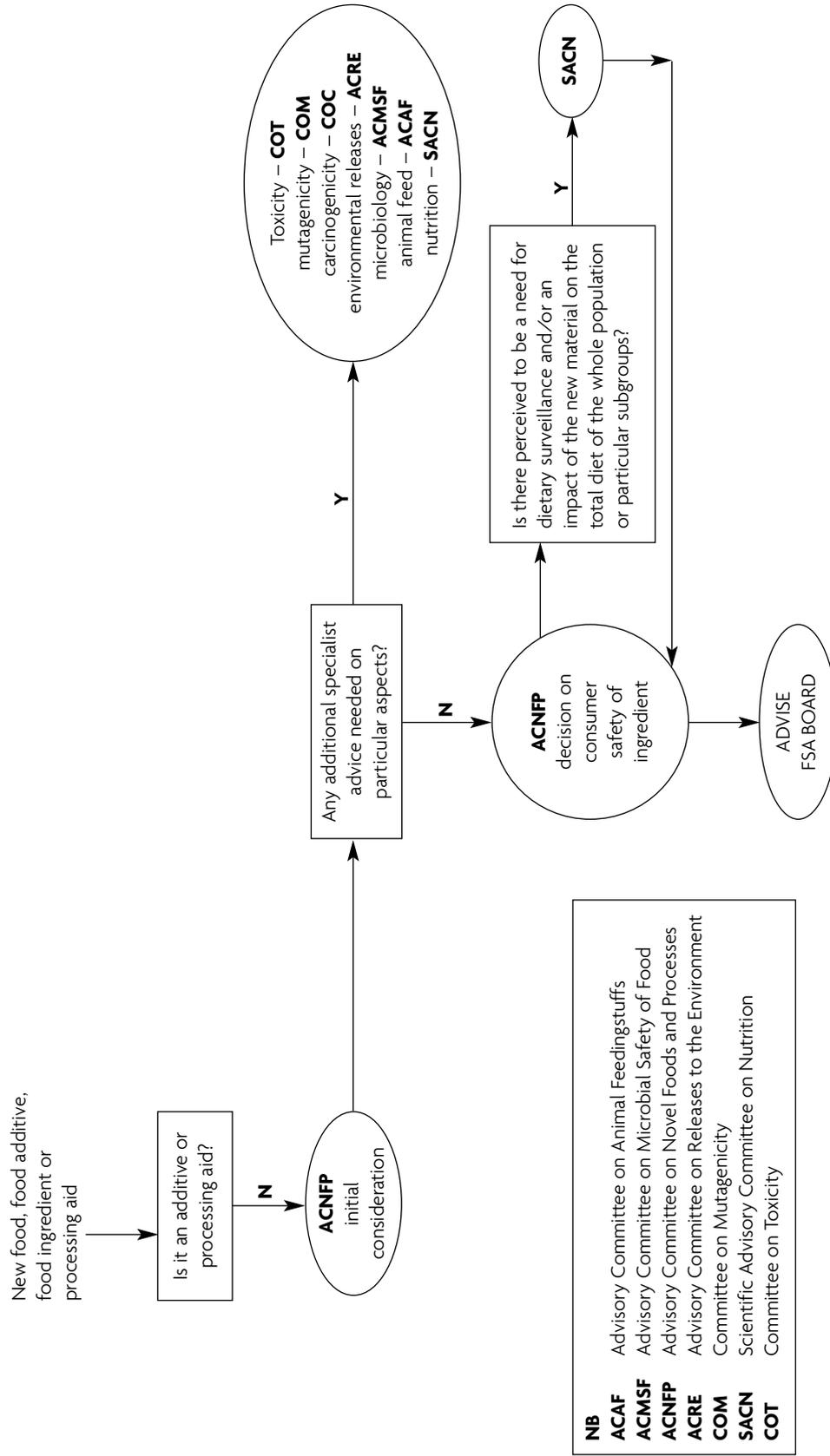
For the purposes of the ACNFP 'industry' means:

- Companies, partnerships or individuals who are involved with the production, manufacture, packaging, sale, advertising, or supply of food or food processes, subject to the Food Safety Act 1990;
- Trade associations representing companies involved with such products;
- Companies, partnerships or individuals who are directly concerned with research, development or marketing of a food product which is being considered by the Committee.

'Other relevant bodies' refers to organisations with a specific interest in food issues, such as charitable organisations or lobby groups.

In this Code 'the Secretariat' means the Secretariat of the ACNFP.

**Figure 1: Relationship of ACNFP with other expert committees involved in the assessment of food safety**



- NB** Advisory Committee on Animal Feedingstuffs
- ACAF** Advisory Committee on Microbial Safety of Food
- ACMSF** Advisory Committee on Novel Foods and Processes
- ACNFP** Advisory Committee on Releases to the Environment
- ACRE** Committee on Mutagenicity
- COM** Scientific Advisory Committee on Nutrition
- SACN** Committee on Toxicity
- COT**

## APPENDIX II

### ADVISORY COMMITTEE FOR NOVEL FOODS AND PROCESSES.

UK 2002/002

Opinion On An Application Under The Novel Foods Regulation To Extend The Range Of Uses Of Phytosterol Esters In Food Products

Applicant: Unilever

Responsible Person: Dr George Gordon

Novel Food: Extension In The Range Of Uses Of Phytosterol Esters

EC Classification: 1.1

### INTRODUCTION

1. An application was submitted by Unilever to the UK Competent Authority on 6th of August 2002 for approval of phytosterol esters for use in a range of food products. A copy of the application dossier was placed on the FSA website at the same time.
2. The applicant previously submitted, via the Netherlands, a successful application under (EC) 258/97 for the same phytosterol ester component in a single product type (yellow fat spreads). Approval was granted for use of the phytosterol ester ingredient in yellow fat spreads up to a maximum of 8% (Commission Decision 2000/500/EC). The UK discussed the issue of approval extension with the Commission who were of the opinion that, as the initial approval specified incorporation into a single product, a full application made under the terms and conditions of (EC) 258/97 was required before an extension could be granted.
3. The applicant is seeking permission to extend the current product range to include milk and yoghurt products. Although these products differ from their conventional counterparts only by the addition of phytosterol esters, the applicant is aware that this fortification will contravene EU and domestic regulations and mean that the products cannot be named milk or yoghurt. The applicant will comply with the regulatory position in Member States as necessary, and for the purposes of this opinion only, the products are referred to as milk 'type' and yoghurt 'type' products.

4. The novel ingredient has already been subject to a safety assessment and granted approval up to a maximum level of use in spreads only. Therefore, the application for an extension of the product range takes into account the existing conditions of approval. Data from post launch monitoring (PLM) that shows that intake from spreads has not reached the level anticipated in the previous application. The applicant uses data from the PLM, supplemented with dietary survey data and consumer purchase data to show that an extension of the product range will not lead to an intake of phytosterols above the level previously approved.

#### I. Specification of the Novel Ingredient

*Information on this aspect is provided on page 4 of the application dossier.*

5. The novel phytosterol esters ingredient is identical to that used in the yellow fat spreads that have been previously approved under (EC) 258/97. A full description of the ingredient and specification was given in the original application which was approved in 2000.

*Discussion. The Committee was satisfied that this ingredient is identical to that previously approved under (EC) 258/97.*

#### II. Effect of the production process applied to the novel food

*Information on this aspect is provided on page 5 of the application dossier.*

6. The production methods used to produce the phytosterol esters ingredient are identical to those used to produce the ingredient used in the yellow fat spreads that have been previously approved under (EC) 258/97.
7. The milk and yoghurt to be used are standard products. Storage and distribution temperatures are as for conventional counterparts and no additional controls are considered necessary. HACCP schemes are used to control product safety and quality. The only additional process required is to control the amount and quality of the phytosterol ester added.
8. Intended levels of fortification are the same as, or less than current yellow fat spread fortification levels of 8% and are as follows:

Yoghurt 1g free phytosterols per pot (125 – 150g)

Milk 1g free phytosterols in 250ml milk

*Discussion. The Committee was satisfied that the production process is controlled and that the in-process monitoring steps are appropriate to ensure safe and consistent products.*

### Production methods

*Information on this aspect is provided on page 5 of the application dossier.*

9. The source organisms used to produce the phytosterol esters are identical to those used in the yellow fat spreads that have been previously approved under (EC) 258/97. The phytosterols are extracted from edible oils (soya, maize, rapeseed, sunflower).

*Discussion. The Committee was satisfied that the sources of the ingredient are identical to those used in the phytosterol esters used in the yellow fat spreads previously approved under (EC) 258/97.*

### III. Anticipated intake/extent of use

*Information on this aspect is provided on page 7-19 of the application dossier.*

10. A condition of approval for yellow fat spreads fortified with phytosterol esters was that the applicant should carry out a post launch monitoring scheme (PLM). The purpose of this scheme was to ensure that the target population group was being reached and that exposure levels were within the maximum stipulated in the approval.
11. The results from the monitoring scheme, which have been presented to the Scientific Committee on Food indicate that the pre-market assumptions for daily intake of the yellow fat spread products, and also 95th percentile levels were significantly higher than the actual levels consumed. At the time of approval the predicted daily intake of the spread was 20-30g, whereas the PLM indicated that the daily spread intake was 15-18g.
12. The monitoring also confirms that the yellow fat spread product is reaching the target consumers (those over 45) and that intake per household remained constant irrespective of the number of people in the household, indicating that usage was predominantly by a single individual. The yellow fat spread product has a 0.1-2.5% market share and the applicant is of the opinion that as the new products are intended for the same target consumers, then there will not be any increase in the market share for each food category.
13. As pre-market assumptions regarding the target population are confirmed by the PLM data, and the daily consumption figures showed that spread intake is lower than anticipated, the applicant seeks approval to increase the product range, and can demonstrate that if the products are used as recommended on the labelling, then the intake would remain within the 2-3g free phytosterols per day range. This level of intake reflects the anticipated levels for use of yellow fat spreads containing 8% phytosterol approved in 2000.

14. In order to support this claim further, the applicant has used two types of data to predict the daily consumption of spreads, yoghurt and milk type products. These data are based on figures available for the daily consumption of similar (unfortified) products, and the consumption of individual product types, and also consumption in combination with the others can be estimated. In addition, data showing current intake of all similar (fortified) products are also presented.

**(a) Dietary Survey Data**

15. These data were obtained by consumers logging their food intake over a fixed time period. The applicant accessed data for the UK and the Netherlands. Survey data for other MS were not deemed sufficiently detailed, out of date or not available to third parties.

16. As the consumption information is obtained using un-fortified products the implicit assumption that all spreads, yoghurts and milk consumed are fortified, is made. Such an assumption is likely to lead to an overestimation of phytosterol fortified products as no allowance can be made for the purchase of both phytosterol fortified and unfortified products. In addition any restrictions on consumption of the fortified product such as effective labelling would not be seen.

17. As food consumption is not normally distributed, the applicant has used the median (50th percentile) and 95th percentile figures as a basis for comparison. In all cases data are presented as g phytosterols/person/day.

18. *UK NDNS data (Application dossier pp 11-15).*  
The highest potential intake is in the age range 65+. Median values were below 3g per day irrespective of whether 1 product or all three products were consumed. All consumers ate at least one of the product and 11% consumers all three. Consumption patterns are similar irrespective of age group and, as listed on p13 Annex 1, 95th percentile data are in the range 2.16–3.67 for consumption of any one product, and 3.22–5.01 for consumption of all three.

19. *Netherlands data (Application dossier pp 11-15)*  
The Dutch data are grouped according to sex, with higher potential intake by males. Median values were highest in the 46-65 age group at 4.58 (95th percentile 6.63). Although the highest 95th percentile value was for males aged between 6 and 16, because this group consumes the highest amounts of the unfortified products, the group is unlikely to be seeking cholesterol lowering products, and the applicant would not target products at such an age group.

**(b) Consumer Purchase Data**

20. The applicant has purchased consumer purchase data for all EU countries where such data are made available. No data were available for MS in Southern Europe. UK Consumer Purchase Data were obtained from AC Nielsen (Consumer Panel 2002) who collate information obtained from households scanning barcodes after purchase.
21. As for the dietary survey, data are presented as median (50th percentile) and 95th percentile values and potential intakes of phytosterols are calculated with the same assumption that all products purchased contain phytosterol. A number of other assumptions likely to lead to an over estimation of phytosterol intake have been made. These are: All the 'milk' and 'yoghurt' products that are purchased are consumed during the 12 or 26 week period of data collection; there is no spoilage or wastage of the food that is purchased; use is also by individuals that live in the house. A further assumption, that the products are not both purchased and consumed outside the home, is an unlikely scenario, given the limited availability of the products.
22. As the data are collected at household level it is not possible to determine who is actually consuming the product, if there is more than one person in the house. However as reasonable estimates of intake can be obtained from one member households these data are used.
23. Data showing consumption for one or all three products, were consistent with the dietary survey data indicating that the highest consumption would be in the target population group. The highest estimated intake of phytosterols in the UK was for all three products and were 2.44 (median) and 5.75 (95th percentile). Similar highest consumer age ranges and intake values were calculated for France and Germany.

**Consumption of existing cholesterol lowering foods**

24. Although data presented to date indicate consumption levels of phytosterols based on the consumption of all milk, yoghurt and yellow fat spreads, the consumer purchase data also enabled consumption of phytosterol (Flora pro.activ) and phytostanol (Benecol range) fortified products currently on the market in the EU to be assessed. These data are particularly useful in that they deal with a small, highly relevant product range, reflecting current purchase patterns for equivalent products.
25. The information was collected over a 26 week period. As there were insufficient numbers of one member households, throughout the age range, such data have only been included for individuals within the 45-64 (50-65 Finland) and 65+ age group, although 'per household'

data have also been included. In summary, median levels are not higher than 0.66g in the one member households, whilst 95th percentile values do not exceed 2g (2.75 in Finland).

26. It should be noted that these figures are significantly less than for “total” yoghurt, milk and yellow fat spread consumption and this is likely to be because non-fortified equivalent products were also purchased during this period. Up to 90% of single member households in the target group also purchased non-fortified spreads and yoghurt.

#### **Determination of the estimated daily intake from new product range**

27. This is discussed in para 39.

#### **Labelling**

28. The current approval for phytosterols in yellow fat spreads notes that they are not nutritionally appropriate for certain individuals such as infants and lactating or pregnant mothers.
29. In view of the need for clear and unambiguous labelling which must be maintained if the product range is increased, the applicant has sought to clarify the labelling of products in terms of recommended daily intake, and the amount of individual products that can be consumed. The applicant will also include a statement to the effect that extra servings will not provide any additional cholesterol lowering benefit.
30. In response to a large number of applications under (EC) 258/97 for approval of phytosterol ingredients in a range of food products, the SCF are currently considering the issue of elevated levels of phytosterols from multiple dietary sources. The use of appropriate labelling may be considered as a means of avoiding excess daily consumption and the applicant has indicated that the new product range will be covered by a comprehensive labelling regime. Furthermore the applicant has indicated a willingness to amend their application in response to the SCF discussions.

*Discussion. The Committee was content that the PLM data showed that consumption levels for fortified yellow fat spreads was lower than anticipated. The Committee reviewed the data obtained from the dietary surveys and was content that the increase in the product range would not lead to consumption of phytosterols at levels greater than those approved previously.*

*The Committee expressed concerns that although present levels of phytosterol consumption are currently limited by a small product range, as more fortified product become available the potential for over consumption increases and any clearance should reflect any decisions made by the European Commission.*

*The Committee also sought reassurance that the any labelling for additional product types should not only inform the consumer of the recommended number of daily servings, but should also make the consumer aware that there are a range of similar products on the market, including those from other manufacturers.*

#### **IV. Information on Previous Exposure**

*Information on this aspect is provided on page 20 of the application dossier*

31. Although there has been no exposure to phytosterol esters in 'milk' and 'yoghurt' products, the ingredient has been consumed in yellow fat spreads and there is low level consumption of 'free' phytosterols in a normal diet. Individuals have had significant exposure to phytostanol esters (hydrogenated phytosterols) in a range of products including 'milk' and 'yoghurt' products.

*Discussion. The Committee accepted that the novel ingredient has been consumed previously and that there were low levels of phytosterols in a normal healthy diet.*

#### **V. Nutritional Aspects**

*Information on this aspect is provided on page 21 of the application dossier*

32. The ability of phytosterols to reduce cholesterol absorption is well documented.
33. Data submitted by Unilever for the original approval have been considered previously, and the applicant commissioned research that addresses specific issues raised by the SCF. These include the PLM and the effect of consumption of phytosterols in conjunction with cholesterol lowering drugs. Studies have also looked at the effectiveness of phytosterols in children with familial hypercholesterolaemia. Research in both areas indicated the beneficial effect of phytosterols although the applicant does not intend to market the product to either group.
34. The cholesterol lowering effect of phytosterols is not influenced by the food matrix. The applicant has summarised research that shows that yoghurts and milk fortified with phytosterols, were as efficacious in the reduction of LDL-cholesterol as other foods containing this ingredient.

#### **Effect on carotenoids**

35. Studies indicate that the consumption of phytosterols can lead to modest reduction in carotenoids, particularly the most lipophilic carotenoids such as  $\beta$ -carotene. The applicant has updated studies in this field. Given that this application does not seek to increase the

amount of phytosterols in the diet beyond that originally approved, the previous risk assessment conclusions from the SCF in response to the original application are still appropriate.

*Discussion.* The Committee was content that the fortification of foods with phytosterols to help reduce cholesterol absorption is well recognised, provided appropriate labelling is included on the products indicating that they are not considered appropriate for certain subgroups of the population and that no new safety issues will be raised by the extension of this product range.

The Committee agreed that there was no effect of the food matrices on the effectiveness of the ingredient.

#### VI. Microbiological Information

Information on this aspect is provided on page 24 of the application dossier

36. The microbiological stability of the 'milk' and 'yoghurt' products containing phytosterol-esters is governed by the same principles as conventional products.
37. The accepted principles of Good Manufacturing Practice used for conventional milk and yoghurt will be used to control quality and safety during manufacture.

*Discussion.* The Committee was content with the information supplied by the applicant and considered the production process, the quality control measures and the nature of the final product to be sufficient to ensure no unintentional microbiological contamination of the products

#### VIII. Toxicological Aspects

38. There is a history of safe consumption for phytosterols within the normal dietary intake of 200-400mg/day. Given that the use of phytosterol esters in yellow fat spreads would lead to a 5-10 fold increase, a thorough toxicological examination of the product was carried out. Based on this assessment the SCF set the limit of fortification at 8% in yellow fat spreads. The toxicological examination of the novel ingredient did not identify any adverse health effects up to the maximum dose levels it was possible to test, and human trials involving large daily intakes of phytosterols have not reported any adverse health effects.

#### Determination of the estimated daily intake

39. The Joint FAO/WHO Expert Committee on Food Additives, (JECFA) NOAEL Safety Factor for free phytosterols is 137mg/kg/day, equivalent to 9.6g/person/day for a 70kg adult, although this is a conservative estimate as an effect level could not be established. The applicant notes that if the products are used as recommended on the

labelling then the intake will not exceed 2-3g free phytosterols per day. The applicant also estimated the highest intake levels if the labelling is ignored and all three fortified products are consumed. These data (irrespective of source) list the highest potential daily consumption for each country. The highest UK consumption is 2.7g/5.8g (median/95th percentile), (age group 45-64).

40. The applicant will extend their Post Launch Monitoring to take account of consumption of all fortified products by the target groups, to assess levels of phytosterol consumption and monitor any potential adverse health events.

*Discussion.* The Committee was content that providing the levels of fortification did not exceed those stipulated in the original approval, there were no specific toxicological issues that had not been covered in the previous approval.

*The Committee agreed that if the products were labelled as described in the application dossier no new toxicological issues would arise.*

*The Committee agreed that it would discuss consumption of phytosterols from multiple sources in the light of the views expressed by the Scientific Committee on Foods.*

## OVERALL DISCUSSION

41. This applicant company is seeking to extend their product range of phytosterol fortified products. Currently they have approval for a single product, yellow fat spreads. This product was approved under (EC) 258/97 in 2000 and they wish to extend the range to include two further product types. Phytosterol esters in yellow fat spreads have already been approved and the product specification data, and the production process are unchanged. Although the intention is to add the phytosterol esters to different products, the current production processes are well monitored, with quality control and safety measures in place. The products are manufactured using a standard method, which has been shown to be reliable and reproducible, the only additional process is to control the amount and quality of phytosterol esters added.
42. No nutritional, toxicological or microbial safety concerns have been raised. Phytosterols are already present in human diets, occurring naturally in foods at low levels and in fortified yellow fat spreads.
43. No issues were raised regarding the anticipated intake and extent of use as sufficient data were provided to demonstrate that an increase in the product range would not lead to an increase in the consumption of phytosterols beyond those previously approved.

44. If the product extension is approved, the Applicant Company will impose strict labelling criteria on the entire product range. These will clearly define the daily servings required to affect a reduction in cholesterol and will also state that consumption in excess of the recommended number of daily servings daily servings will not provide additional cholesterol lowering benefit. The Committee previously expressed their concerns regarding the over consumption of phytosterol fortified products when they have been asked to comment on previous applications made under (EC) 258/97. The Committee agreed that the issue of over consumption should take into account all products currently on the market, and those awaiting clearance under (EC) 258/97 and should be dealt with by the Commission. Members also acknowledged that the Scientific Committee for Foods had drafted a report entitled “View on the long term effects of the intake of elevated levels of phytosterols from multiple dietary sources”. The company has stated that they will act in accordance with any decisions made by the Commission in the light of this SCF report.
45. In view of the concerns of over consumption, and the stipulated requirement for PLM in the previous approval for the fortified yellow fat spreads the company are willing to undertake a similar exercise if approval for the extension of product range is given. The Committee recommends that any approval for this extension in the product range is subject to a requirement for further PLM.

## CONCLUSION

The Advisory Committee on Novel Foods and Processes is satisfied by the evidence provided by Unilever that the extension of the range of uses of phytosterol esters as described in the application dossier is acceptable, subject to the labelling and PLM requirements described above.

November 2002

## APPENDIX III

### ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

UK/2002/001

Opinion on an application under the Novel Food Regulation from OmegaTech for clearance of DHA Gold™, a DHA rich oil.

Applicant: OmegaTech

Responsible person: Mr Nigel Baldwin

Novel Food: DHA Gold™

EC Classification: 2

#### Introduction

1. An application was submitted by OmegaTech to the UK Competent Authority on 13th February 2001 for approval of DHA Gold', a DHA – rich oil. The full version of this dossier was placed on the UK competent authority website on 14th February 2001. During the course of the evaluation the UK Competent Authority sought further information to clarify certain aspects of the dossier.
2. DHA (docosahexaenoic acid) – rich oil is produced via an algal fermentation process using microalgae from the genus *Schizochytrium*, a member of the kingdom Chromista, which includes the golden algae.
3. *Schizochytrium* sp. has previously been assessed in the United States and was given GRAS (Generally Recognised As Safe) clearance as a nutritional food ingredient. In the United States, a daily intake of up to 1.5g of DHA was recommended (DHA-rich oil contains 35-45% DHA). Prior to this, the microalgae achieved GRAS status to be used in chicken feed at levels of incorporation of up to 2.8% for broilers and 4.7% in layers). There is evidence to suggest that each of the components of the oil is already present to a significant degree in the human food chain.
4. The production strain of microalgae used for DHA Gold™ has been developed using conventional improvement techniques of the wild type strain and no recombinant DNA technology was used.

5. The application was prepared according to the European Commission's guidelines. DHA-Gold™ was identified as belonging to class 2.2 ('complex novel food from a non-GM source', 'the source of the novel food has no history of use in the community'). The Committee's consideration of the data provided is presented according to these requirements.

#### I. Specification of the Novel Food

*Information on this aspect is provided in section 1 of the application dossier. Supplementary information was supplied in March 2001.*

6. DHA is a long chain polyunsaturated fatty acid, derived from heterotrophically grown microalgae. DHA Gold™, DHA-rich oil is described as a yellow to light orange-coloured oil derived from the heterotrophically grown marine microalga, *Schizochytrium* sp., intended for use as a nutritional food ingredient.
7. Quality control tests indicate that the production process is both reliable and reproducible and there is evidence to demonstrate that controls are in place to ensure individual batches meet manufacturing specifications.
8. Further information was sought regarding the compositional analysis of the oil. The Committee was concerned about the presence of components, particularly protein and carbohydrate, that may elicit an allergenic response.
9. Information was provided to demonstrate that the extraction process would not disproportionately concentrate any potentially toxic components.

**Discussion.** *The Committee was satisfied that the oil consists only of lipid components already present in other existing dietary forms. The data provided (Appendix A of original application) on a number of batches show that a consistent and reliable end product is produced.*

*The producers were able to demonstrate that there is a very low level of residual protein (less than 0.1%) and carbohydrate in the final refined oil. This indicates that the oil is likely to elicit only a low risk of allergenicity.*

#### II. Effect of the production process applied to the novel food

*Information on this aspect is provided in section 2 of the application dossier.*

10. The improvement of the *Schizochytrium* sp. was carried out using a classical mutagenesis/screening programme, which employs standard techniques commonly used in industrial microbial strain improvement (see section 2 of the application dossier). The production method is well defined, and a number of in-process

monitoring steps are included in the manufacturing procedure to ensure the safety and quality of the oil is maintained. DHA-rich oil is manufactured under the general guidelines of the food chemical Good Manufacturing Practices (Food Chemical Codex pp xxvii, 4th edition).

*Discussion.* The Committee was satisfied that the production process is well controlled and that the in-process monitoring steps are appropriate to ensure a safe and consistent product.

### III. History of source organism

*Information on this aspect is provided in section 3 of the application dossier.*

11. This class of microalga is primarily saprotrophic and is found throughout the world in estuarine and marine habitats. *Schizochytrium* sp. has a widespread distribution, and is consumed by a wide range of filter feeders. Although there are no reports of human consumption of *Schizochytrium*, the filter feeders (clams and mussels) that feed on this organism are part of the normal human diet.
12. *Schizochytrium* sp. belongs to the kingdom Chromista. This is not the same as the kingdom to which the bluegreen or dinoflagellate microalgae belong. This is significant since these two constitute the major known toxin producing microalgae, and most allergic responses to algal micro-organisms have been limited to exposure to these. Only two genera in the Kingdom Chromista are known to produce toxins, neither of which is in the same class as *Schizochytrium* sp. There have been no reports of toxins being found in this class.
13. The improved strain of *Schizochytrium* was developed from a patented wild-type parent strain, by using the standard chemical mutagen, NTG (1-methyl-3-nitro-1-nitrosoguanidine). Modified strains derived using this procedure sometimes acquire undesirable traits. Therefore, tests were conducted to characterise phenotypically the modified strain and its parent. The results indicate that the new strain performed equivalently to its parent and no adverse traits were observed due to the mutagenesis.
14. In addition, comparative compositional data of the oil from the parent (wildtype) and modified daughter strains demonstrated expected alterations in the balance in fatty acids, with the oil from the daughter strain having an increase in DHA content and a reduced level of palmitate. No unexpected fatty acids were found in the oil from the modified strain.

15. The sterol components of the parent and daughter strain oils were confirmed to be qualitatively constant when analysed. No unexpected sterols were identified in the daughter strain oil.

*Discussion. The Committee was satisfied that the parent organism has no history of toxin production. The Committee was also content that no unexpected phenotypical changes had been introduced, and that the composition of the oil obtained from the daughter strain was similar to that from the parent apart from the desired increase in DHA content, and a compensatory decrease in palmitate levels.*

#### **IX. Anticipated intake/extent of use.**

*Information on this aspect is in section 9 of the application dossier.*

16. In 1994, the UK Committee on Medical Aspects on Food Nutrition Policy (COMA) recommended that individuals should increase their intake of omega-3 fatty acids, including DHA, since raised intakes are associated with a reduced risk of coronary heart disease. It must be shown, however, that by increasing levels, no detrimental affects are introduced, either from the DHA or from the other components of the oil.
17. OmegaTech are intending to market DHA Gold™ only as an ingredient to food manufacturers, it will not be sold directly to consumers. The DHA-rich oil is, however, suitable for use in a wide range of food products. These include dairy products, fine bakery wares, confectionery, sauces, breakfast cereals and cereal bars, spreads, potato crisps and pasta. However, the company has agreed to include labelling in relation to the recommended intake of DHA, so that consumers can select products as appropriate. See paragraph below.
18. The level of incorporation of DHA Gold™ into these products is dependent on the existing background DHA levels. It is estimated that UK adults currently consume 107mg/day of DHA, with the 97.5th percentile consuming 401mg/day. However, the aim would be that the combined background and incorporated level of DHA would equal a daily mean intake of 550mg.
19. Under article 2 of Directive 90/496/EEC, it will be compulsory for food manufacturers to define the quantity of DHA per serving or per 100g in the final food. OmegaTech will recommend to their customers in Europe, the food manufacturers, that daily consumption should not exceed 1.5g of DHA, as was stipulated in the US GRAS approval. Many EU Member states have their own recommendations. For example, AFFSA (France), The Health Council of the Netherlands, and COMA (UK), and so usage levels would need to be determined for particular food products and may require adjustments for different markets in various Member States. The Company has agreed to ensure that labelling meets different national requirements.

*Discussion.* The Committee was content with the information provided by the applicant. However, it was considered that the producers of the oil should provide information concerning “recommended intake” to manufacturers intending to use DHA-Gold’ as a food ingredient. This information should then be included on the labelling, which would accompany the final food product, and so passed on to consumers.

#### **X. Information on previous human exposure.**

*Information on this aspect can be found in section 10 of the application dossier. Supplementary information was supplied in March 2001.*

20. DHA-rich oil contains a range of fatty acids, including eicosapentaenoic acid (EPA) and docosapentaenoic acid (DPA) as well as DHA, and traces of phytosterols. EPA and DPA both occur naturally in plant and animal products, and so estimates can be made to indicate current background levels of intake.
21. The principal sources of EPA and DHA in the diet are from oily fish. Only 35% of adults in the UK regularly consume oily fish, however. The absence of fatty fish in the diet greatly reduces the levels of DHA and EPA in the diet. The levels of fatty acid intake vary across Europe. This is probably due to the differences in trends of oily fish consumption. Most dietary DPA comes from offal.
22. Further information on previous human consumption was provided. The producers were able to give details of DHA being supplied to a customer who then sold the oil as a dietary supplement. The product has been on sale in the United States for over two years, and the information provided is in the form of a list of calls received from the after sales care helpline. Over 400 calls have been received by this helpline, none of which have recorded any adverse effects relating to this product.

*Discussion.* The Committee accepted the data provided on the background levels of consumption of the fatty acid and phytosterol components of DHA – rich oil already in the human diet. Although conscious that the information provided from the helpline call centre in the USA was anecdotal and not a formal structured study, the Committee noted that there have been no reported adverse effects in the two years that the product had been on sale.

#### **XI. Nutritional information**

*Information provided on this aspect is in section XI of the application dossier.*

23. DHA is considered to play an important role in maintaining a healthy heart. Several markers of the cardiovascular system are directly influenced by dietary DHA. These include triglyceride levels, platelet aggregation (may lower the risk of heart attack or stroke), cardiac rhythmicity and haemodynamics.

24. DHA is also considered to be vital for the development and function of brain and eyes. DHA oil is supplemented with Vitamin E for nutritional purposes.

*Discussion. The Committee accepted that nutritional advice is to increase intake of omega-3 fatty acids and was aware that this oil could improve the nutritional properties of foods to which it is added. The Committee agreed that foods containing DHA – Gold™ oil would have to be labelled to inform consumers of recommended intake levels. There is an increased nutritional need for vitamin E when increasing the intake of polyunsaturated fatty acids, and we note that the oil is supplemented with vitamin E to address this point.*

## **XII. Microbiological information**

*Information provided on this aspect is in section 12 of the application dossier.*

25. DHA Gold™ is manufactured under the general guidelines of food chemical Good Manufacturing practices. A combination of heat treatment, environmental conditions of oil extraction and processing and the extremely low water activity of the finished oil, contributes to the inhibition of typical food-borne microbes.

*Discussion. The Committee was content with the information provided by the applicant and considered the production process, the quality control measures and the nature of the final product to be sufficient to ensure no unintentional microbiological contamination of the oil.*

## **XIII. Toxicological information**

*Information on this aspect is provided in the application dossier in section 13. Additional data requested by the Committee was supplied in February 2002.*

26. A range of safety studies has been conducted with dried microalgae of the genus *Schizochytrium*. These studies were conducted in accordance to the 1982 FDA Redbook Guidelines and in compliance with the FDA Good Laboratory Practice (GLP) regulations to support the GRAS petition in the US.
27. The studies included a) a subchronic feeding study where dried DHA-rich microalga was fed to rats for at least 13 weeks, b) developmental toxicity evaluation in rats and rabbits, c) a single generation rat reproduction study and d) a mutagenicity study. Also an acute gavage study was conducted with extracted and refined DHA-rich oil, and a laying hen and a chicken broiler study were also conducted. The actual material tested in each of the studies is shown in the table attached below. The Company also presented the findings from a swine toxicity study carried out on the algal biomass, but the Committee did not feel this contributed any further information for the safety assessment of the oil for human consumption.

**Summary of the test material used in the toxicology studies.**

<b>Trial</b>	<b>Test Article</b>	<b>Strain</b>
<i>Acute Feeding</i> Mouse	oil	N230D (Daughter, production strain)
<i>Sub-chronic Feeding</i> Rat - 90 day <sup>a</sup>	algae	ATCC 20888 (Parental, wildtype strain)
Laying Hen -112 day <sup>d</sup>	algae	N230D
Broiler Chicken – whole life	algae	N230D
<b>Developmental &amp; Reproductive Toxicity</b>		
Rat – developmental toxicity <sup>b</sup> maternal offspring	algae algae	ATCC 20888 ATCC 20888
Rat – single generation reproduction <sup>c</sup> males females	algae algae	ATCC 20888 ATCC 20888
Rabbit – developmental toxicity <sup>b</sup> maternal offspring	algae algae	ATCC 20888 ATCC 20888

<sup>a</sup> Hammond et al. (2001b); <sup>b</sup> Hammond et al. (2001a); <sup>c</sup> Hammond et al. (2001c); <sup>d</sup> Abril et al. (2000);

**Summary of the genetic toxicity studies performed using dried algae of the genus *Schizochytrium* and oil containing DPA(n-6) and DHA.**

<b>Trial</b>	<b>Test Article</b>	<b>Strain</b>
Ames	oil (DHALIP-NS) <sup>NB</sup>	N230D
Ames <sup>a</sup>	intact algae	ATCC 20888
In Vitro Human Lymphocytes <sup>a</sup>	intact algae	ATCC 20888
Mouse Lymphoma <sup>a</sup>	intact algae	ATCC 20888
Ames <sup>a</sup>	lysed algae	ATCC 20888
AS52/XPRT Gene Locus <sup>a</sup>	lysed algae	ATCC 20888
Mouse Micronucleus <sup>a</sup>	lysed algae	ATCC 20888

<sup>a</sup> Hammond et al. (2001d)

<sup>NB</sup> DHALIP-NS refers to the Company's internal product code for commercial article.

28. A human clinical study on the DHA-Gold%<sup>o</sup> oil was supplied in 2002.

29. It was noted that the toxicology studies were carried out on either the parental (ATCC 20888) or the production daughter (N230D) strains. Evidence was provided to demonstrate that the oil from the two strains were comparable, with the exception of the expected increases in DHA content. For further information, see section III, The History of the Source Organism.

30. Each of the studies is summarised below.

**Safety Assessment of DHA-Rich Microalgae of the genus *Schizochytrium*: Part I: Sub-chronic Rat Feeding Study.**

31. The purpose of this study was to determine the effects of DHA-rich (docosahexaenoic acid) -rich microalgae (DRM) of the genus *Schizochytrium*, administered in the diet of rats for 13 weeks.

32. A dried preparation of DRM was administered in the diet to groups of 20 male and 20 female Sprague-Dawley rats to provide an intake of 0, 400, 1500 and 4000 mg/kg/day for at least 13 weeks. Untreated controls received basal diet only. An additional group of 20 males and 20 females received rodent diet mixed with fish oil to provide a target dose of 1628 mg/kg bw/day. In view of DRM's high fat content (41%, mainly unsaturated fatty acids), vitamin E acetate had been added (during manufacture of DRM) to all test substance and fish oil-treated groups to provide supplementary dietary antioxidant.
33. The stability and homogeneity of the diets were checked regularly during treatment periods. Animals were observed twice daily for mortality and clinical signs. Ophthalmoscopic examinations were carried out prior to start of study and prior to killing the controls, fish oil treated rats and the high-dose DRM treated animals. Blood collected under halothane anaesthesia was collected from 10 rats/sex/group during week 6-7 and prior to killing; urine was also collected during the latter periods from the same rats. Haematological measurements were carried out on 13 relevant parameters (including thromboplastin-clotting time). Serum clinical chemistry measurements were carried out on 24 parameters. Urinalysis was carried out on at least 13 end points. At the end of the study, the animals were anaesthetized by sodium pentobarbital and killed by exsanguination. Organ weight data were collected for the liver, spleen, heart, thymus, ovaries, testes, kidneys, adrenals, pituitary and brain. Complete sets of tissues were also collected for histopathology. All tissue slides from control, fish oil, and high dose DRM groups were examined microscopically. In addition, heart, kidneys and pituitary for males in all groups and liver from females in all groups were also examined microscopically.
34. Based on the results from a previous 13-week rat feeding study (CTBR study) with DRM, a Pathology Working Group was formed to review the heart slides from this study and CTBR study to resolve differences in terminology and severity scores between the two studies. The review also assessed the accuracy and consistency of the initial histopathological examinations of the hearts of male and female rats.
35. All animals survived during the study. There were no treatment-related clinical and ophthalmologic signs of toxicity. There were no treatment-related effects on body weight or food consumption compared with controls.
36. While there were a few significant intergroup differences in haematological parameters, these were not considered treatment-related, as there was no dose-response. The main clinical chemistry finding involved a drop in cholesterol and HDL levels in both sexes of the fish oil and the high-dose groups. A lowering in the latter two parameters was also noted in males receiving DRM at 1500 mg/kg bw/day.

37. At necropsy, no treatment-related effects on gross lesions and terminal body weights and absolute and relative organ weights were noted. Microscopic changes were mainly confined to the liver, kidneys and heart. In the liver, the incidence of periportal hepatocellular vacuolation was significantly increased in the female fish oil group (18/20) and all female treatment DRM groups (low dose, 16/20, mid-dose, 18/20 and high dose 19/20) when compared with the female untreated control group (8/20); there were no treatment-related differences in the severity of this observation. There was a significant increase in pelvic dilation of the kidneys in high dose DRM males (5/20) compared to control (0/20) and fish oil groups (2/20).
38. The nature of the histological changes affecting the heart (slight increases in the incidence and/or severity of inflammation and degenerative changes in the male rats) of animals from this study was similar to that reported in a previous study, although in this study, diets were not supplemented with vitamin E. A slight increase in the incidence, but not severity, of cardiomyopathy was observed in the 4000 mg/kg bw/day DRM dosed males in this study. The changes were characterised by small foci of mononuclear inflammatory cells and degeneration of myofibres, sometimes accompanied by fibrosis of the myocardium. The changes were reported to be identical to spontaneous 'cardiomyopathy' associated with ageing. The incidence and severity of cardiomyopathy in this study were greater in male rats than females.

**Safety Assessment of DHA-Rich Microalgae of the genus *Schizochytrium*: Part II: Developmental Toxicity Evaluation in Rats and Rabbits**

39. The developmental toxicity of DRM (supplemented with vitamin E during manufacture) was assessed in Sprague-Dawley rats (25/group, provided with dried DRM in the diet at 0, 0.6, 6, and 30% on gestation days (GD) 6-15) and in New Zealand White Rabbits, 22/group, dosed with DRM at levels of 180, 600, and 1800 mg/kg bw/day by gavage (in 2 equal daily doses, 6 hrs apart for 13 consecutive days on GD 6-19). An additional group of 22 rabbits dosed with fish oil (also supplemented with vitamin E) was used as a negative control to provide an equivalent amount of fat to that received by the high-dose DRM rabbits. Control animals (22 per group) received the vehicle (0.5% carboxymethyl cellulose and 0.1% polysorbate 80).
40. Sperm-positive female animals were weighed and food consumption determined on GD 0, 6, 9, 12, 16, 18, and 20 (rats) and GD 0 through to GD 29 (rabbits). Animals were observed twice daily for clinical signs and mortality. All rats were killed on GD 20 by asphyxiation with carbon dioxide. Rabbits were killed on GD 29 by a lethal injection of sodium pentobarbital. A complete gross necropsy was conducted on all rats and rabbits. At necropsy, the uterus and ovaries were excised from each animal and the number of corpora lutea recorded. The

uteri were examined for the location of foetus, resorptions and implantation sites. Live foetuses were dissected from their uterus, weighed and examined for morphologic and visceral abnormalities. All foetal carcasses were eviscerated and stained and examined for skeletal malformations.

#### Rats

41. No rats died during the course of the study. There were no treatment-related clinical signs. Animals in the 30% DRM group exhibited a reduction in weight gain from GD 16 to GD 18. Food consumption was also reduced in the latter group during GD 6 to GD 9 and between GD 16 and GD 18. Examination of the uteri confirmed that 88%, 88%, 92% and 80% of the mated animals in the control through to the high-dose DRM groups were pregnant and produced foetuses by GD 20. There were no treatment-related effects on corpora lutea, implantations, live foetuses, or in percent resorptions or late deaths. Statistical increases in the number of male foetuses and in the male sex ratio were noted in low- and mid-dose DRM groups (mainly due to a low percentage (39.1%) of male foetuses/litter in the control group). The incidence of foetuses with ossification centres in the first lumbar vertebrae (2%) was significantly lower in the high-dose-DRM group but was within the historical control range (1.5-15%). A statistically higher incidence of foetuses (but not litters) with reduced ossification of the ribs was seen in the mid- and high-dose DRM groups. This resulted from a single litter with a number of affected pups (mid-dose, 8 foetuses, high-dose, 5 foetuses). Treatment with DRM did not result in other skeletal and visceral anomalies in rats.
42. Since at the highest dietary concentration of DRM (30% – which equates to 22 g/kg/day) no treatment related clinical signs were observed, the authors proposed a NOEL to be the highest dose, 22 g/kg bw/day.

#### Rabbits

43. One animal in the 600 mg/kg bw/day DRM group died during GD 10 and a second was killed by an intubation error on GD 10 in the 1800 mg/kg/day DRM group. One female in the fish oil control group aborted on GD 23, and two females in the high-dose group aborted on GD days 25 and 26. No treatment-related clinical signs were reported in the DRM dosed groups. Reductions in body weight gain and food consumption were noted in the animals in the high-dose DRM group during GD 12-19 and when the entire treatment period was evaluated (there was reversal of this effect during the first half of the post-treatment period, GD 24-29). A similar loss in weight gain was noted in the fish oil-treated group.

44. Uteri examinations confirmed that 77%, 81%, 77%, 81%, and 89% of the artificially inseminated females in the control, fish oil and low through to high-dose DRM groups were pregnant when killed.
45. There were no significant differences between the DRM or fish oil treated groups and the control in mean number of corpora lutea, implantation sites, litter size, post implantation loss, and foetal body weight. Treatment with DRM did not result in skeletal and visceral anomalies in rabbits. The authors proposed a NOEL in rabbits of 600 mg/kg/day of DRM for maternal toxicity and 1800 mg/kg bw/day of DRM for developmental toxicity.

**Safety Assessment of DHA-Rich Microalgae of the genus *Schizochytrium*: Part III: Single generation rat reproduction study**

46. The reproductive toxicity of dried DRM was examined in Sprague-Dawley rats (30/sex/group) in the diet at concentrations of 0, 0.6, 6 and 30% (equivalent to a dose of 400, 3900, and 17800 mg/kg bw/day for F0 males and 480, 4600, and 20700 mg/kg bw/day for F0 females respectively). Treatment in males continued throughout mating and until termination. Females were treated throughout gestation and through lactation day 21. Females were killed after raising their offspring to weaning at 21 days of age.
47. Animals were observed twice daily for clinical signs and mortality. Body weight and food consumption data were recorded weekly. For each F1 litter, the number of live and dead pups, gross abnormalities and individual weight of live pups were recorded at birth and on days 4, 7, 14 and 21 of lactation. Culled F1 animals (4/sex/litter) were subjected to gross necropsy. F0 animals were terminated (males, 3 weeks after the end of mating period and females on days 21, 22 or 23 post partum) by carbon dioxide asphyxiation and gross necropsies performed. The left testes from F0 males were used to assess sperm count, motility and morphology. Uteri and ovaries from F0 females were used for assessment of implantation sites and implantation loss. In addition, histopathological examinations were conducted on epididymis, liver, ovaries, prostate, seminal vesicles, testis (right), uterus, vagina and tissues showing gross pathology.

**F0 generation**

48. Three male rats died during the study (a high-dose male died during week 5 and a control and two mid-dose DRM animals died, one during week 13 and one during week 14). These deaths were not related to treatment with DRM. Statistically non-significant increases in body weight in high dose males were noted from week 10 through to week 16. The body weight in female high-dose DRM group was increased significantly during pre-mating weeks 1 and 2 and throughout both gestation and lactation. Food consumption in the male DRM groups was reduced (only statistically significant in the high dose group during weeks 2-16). Food consumption was also reduced in high-dose DRM females during gestation. There were no

treatment-related adverse effects in reproductive performance, or in the duration of gestation, mean litter size, mean pup weight, number of litters with dead pups, or post implantation loss. Apart from an increased hepatocellular vacuolation in the mid- and high-dose DRM female groups, there were no treatment related histopathological changes noted. Dietary DRM treatment had no effect on epididymal weights, sperm counts, percent motility, sperm morphology or spermatogenic cycle.

#### **F1 generation**

49. There were no treatment-related clinical signs apparent in the F1 pups. Pup viability and survival was similar in control and treated animals. DRM treatment had no effect on F1 body weights recorded on lactation days 0, 4, 7, 14, or 21. No treatment-related internal or external gross abnormalities were noted in pups that were born dead or in those that were subjected to necropsy at the termination of the trial.

#### **An Evaluation Of The Mutagenic Potential Of DHALIP-NS In the Ames Salmonella/Microsome Assay (EX 4709).**

50. A precipitate was observed at doses of 500 and 1000 µg/plate. The plates dosed at 5000 µg/plate were not countable due to this precipitate. There was no toxicity observed at any test article concentrations. There appeared to be a two-fold increase in mean revertant colony numbers over that of the vehicle control (dimethyl sulfoxide) with strain TA98 at 500 and 1000 µg/plate without metabolic activation. This increase was neither dose-related nor reproducible in a repeat assay. There were no compound-related increases in the number of revertant colonies over the controls for the other 4 tester strains. The increases in the number of revertants as a result of treatment with the positive control compounds demonstrated the capability of the system to detect mutagens in this assay. It was concluded that DHALIP-NS is negative in the Ames test.

#### **Exploratory Acute Oral Limit Study of DHALIP-NS in Mice**

51. This (non-GLP) study was performed to assess the acute oral toxicity of DHALIP-NS (yellowish oil) in mice when administered as a single oral gavage dose. The compound (suspended in 0.5% carboxymethyl cellulose and 0.1% Tween 80 in distilled water) was dosed to 5 male and 5 female CD mice at 2000 mg/kg bw/day. The animals were observed for clinical signs at approximately 1, 2.5 and 4 hours after dosing and daily thereafter. Body weights were recorded before and after fasting on Day 0 and on post-dosing day 7. The animals were killed on day 7, post dosing.
52. There were no treatment-related deaths or clinical signs, or significant effects on body weight and gross necropsy related to administration of the test substance at a dose of 2000 mg/kg bw/day.

### Laying Hen Study

53. A target animal safety trial with laying hens was conducted using dried DHA-rich microalgae of the genus *Schizochytrium* at three different dose levels: 82, 240, and 408 mg DHA/bird per day. Each treatment consisted of 64 laying hens divided into eight replicates per group with a total of 320 animals. Body weights, food conversion, egg production, egg weight, shell thickness and interior quality were measures at the end of the month for each of the four dosing periods. Eggs were also collected and analysed at the end of months 2 and 4 for their weight, shell thickness, interior egg quality and fatty acid profile.
54. At the end of the four-month period, two randomly selected hens from each dose level and replicate were killed and evaluated for haematological and histopathological changes. Blood clotting time was also determined, since it is known that fatty acids lead to a decrease in platelet reactivity. Gross necropsy was completed on all layers that died during the study or killed for scheduled evaluation. Weights were determined for most of the major organs and a range of tissues were studied for fatty acid content. The results of the experimental diets were determined via statistical analysis.
55. The results showed that there were no significant differences in any of the organ weights measured. No alterations were noted in the histopathological study, and there were no significant differences between treatments for any of the haematological analyses.
56. It was concluded, based on this study, that dried DHA-rich microalgae of the genus *Schizochytrium* is safe as a feed ingredient for laying hens at 3,040 mg/kg body weight/day dried microalgae delivering 532mg DHA/kg body weight/day.

### Broiler Chicken Study

57. A similar target animal safety trial with broiler chickens was carried out using 2240 birds. They were sexed and randomly assigned to one of four dietary treatments, including three different levels of DHA-rich microalgae and one control group. 560 broilers were included in each group, and then divided into eight replicates, with 70 birds in each, 35 each of both sex.
58. The same studies that were carried out with the laying hens were also used to analyse the diet affect on the broiler chickens.
59. The results indicated that there was no effect of treatment level on any of the evaluated broiler growth performance measures. No significant difference regarding weight gain, feed intake or feed conversion between treatment levels were noted. No histopathological or haematological differences were observed between the treatment groups.

60. Based on these results, it was concluded that dried DHA-rich microalga is safe as a feed ingredient for broiler chickens at 2331 mg/bird/day delivering approximately 408 mg DHA/bird/day.

#### Human Clinical Study

*Following consideration of the animal data, the Committee requested further confirmatory information in humans, and this was subsequently provided in January 2002.*

61. The aim of the study was to evaluate the effects of consuming 1.5g/day of DHA oil from DHA-Gold, considered to be in excess of estimated usage, on plasma lipids, haematology, biochemical markers of liver and cardiac functions, and certain haemostatic risk factors, which include plasma fibrinogen concentration, factor VII coagulant activity, C-reactive protein concentration, plasminogen activator inhibitor type 1 (PAL-1) activity and von Willebrand factor antigen concentrations (vWF). Platelet counts were also included in the study, since studies with fish oils have reported a fall in platelet count.

62. Seventy-nine individuals were divided randomly between two groups, the test group and the control, each containing approximately equal numbers of male and female subjects. Individuals in the test group consumed 4 capsules each containing 1000mg of oil per day, providing a daily total of 1.5g DHA from DHA Gold. The control group received an olive oil placebo, which has been shown to be inert.

63. Responses were assessed by measurements made on entry to the trial, day 1 and on days 28 and 29 of each treatment. Measurements included seated blood pressure and body weight. Fasting blood samples were collected on test days 1 and 28/29, and were used to determine blood counts, erythrocyte fatty acid composition, serum lipids, glucose, creatine kinase activity and liver function tests. Citrated samples were collected to determine PAL-1 activity, fibrinogen and factor VII coagulant activity. Further blood samples were also obtained for lipid analysis on these days.

64. Subjects recorded signs of illness, medication used, menstrual phase and deviations from the protocol in a diary. At the end of the study, subjects completed a questionnaire about their appreciation of the treatment, and any side effects experienced.

65. This study demonstrated that a consumption of 1.5g DHA daily as DHA Gold resulted in the expected changes in serum lipids within the normal ranges. A rise in plasma concentration of LDL cholesterol was noted, but this was accompanied by an increase in HDL cholesterol, and no overall net change in the LDL/HDL ratio was observed. DHA Gold was well tolerated among the test group, with no adverse effects on liver function, cardiac enzymes, glucose metabolism, and haematology markers of inflammation or haemostatic function being

recorded, apart from the statistically significant increase in Factor VIIc. However, an increase is also seen with fish oils and is a result of the compensatory increase in clotting in response to the known effects of DHA on platelet vessel wall interactions. Therefore, it is of no toxicological concern.

#### 66. Discussion and Conclusion

*The safety of fatty acids present in DHA-rich oil is based on four factors:*

- i) Extensive knowledge of fatty acid metabolism.*
- ii) Extensive previous exposure due to their high level in background human diet and the small quantities expected to be present in foods using DHA-rich oils.*
- iii) Published literature on the safety of fatty acid components and comparable oils.*
- iv) Confirmatory safety studies.*

*The safety of phytosterols found in DHA-rich oil is based on five factors. These factors allow a conclusion that an intake of phytosterols present in DHA-rich oil is safe:*

- i) Experience of use due to their natural abundance in food and low levels expected to be used.*
- ii) Extensive knowledge of the absorption, distribution, metabolism and excretion of phytosterols in mammalian species.*
- iii) Extensive safety information as the result of testing these and similar phytosterols.*
- iv) Easy identification of at risk populations.*
- v) Confirmatory safety studies.*

*A number of confirmatory toxicological studies have been conducted with the DHA-rich microalgae, including 90-day rat feeding studies, teratology studies in the rat and rabbit and a single generation reproduction study in the rat. The NOEL for DHA in these animal studies ranged from 153 – 1,868 mg/kg/day, although in some cases these levels were the top tested and the actual NOEL could be higher.*

*The periportal vacuolation seen in the 90-day rat study is likely to be the result of the high fat level in the diet and therefore does not represent an adverse effect as such. There is, however, a slight increase in the incidence, but not in the severity, of cardiomyopathy in the high dose male rats in this study, which may be a result of treatment. However, it was not within*

*the test protocol to histologically examine the heart in the single-generation rat reproduction study. The lesion seen was similar to that seen spontaneously in ageing rats of this strain. An expert panel in the United States reviewed the cardiomyopathy data. They concluded that the treatment related findings of the 13-week study had little relevance to the safety assessment of the use of DHA as a nutritional ingredient for humans. The ACNFP sought expert advice from an animal pathologist from the Committee on Toxicity. The same conclusion was drawn that the presence of heart lesions in the rat was of no significance in the safety evaluation of DHA for use in humans.*

*In the USA, DHA Gold™ has GRAS status at an intake level of 1.5g a day. This figure was based on the presence of DHA in human breast milk, although the safety data indicated the oil was safe at higher intake levels. The level of 1.5g a day is considered to be an adequate upper level of intake when considering individual Member States recommendations to achieve the intended effects, which include key cardiovascular and immune system health benefits.*

*The results of the 90 day rat feeding study indicates that the NOEL for DRM in the rat 90 day feeding study is at least 4000mg/kg/day (the highest dose tested), which is equivalent to an intake of 340mg/kg/day of DHA. No significant adverse effects were seen in either of the teratology studies or the single-generation reproduction study in the rat, other than slight reductions in food intake and body weight gain at high levels of incorporation of DRM in the diet. If the recommended dose for DHA were accepted to be 1500mg/person/day, this would equate to 25 mg/kg/day DHA for a 60-kg person, which would give a safety factor of 13.5 in relation to the rat study.*

*Published and unpublished scientific data relating to Schizochytrium sp., the species from which DHA-rich oil is derived, have not shown any adverse effects that are relevant to the safety of this oil for humans. There have been no reports of toxic compounds being produced by Schizochytrium sp., it occurs widely in the marine environment and is an indirect component of the human food chain.*

*The Committee agreed that a formal study in humans to confirm the safety of the oil was needed. The study provided by the Company was able to demonstrate that the inclusion of DHA Gold in the diet of human volunteers, at a level considered to be in excess of expected usage, had no effect on target parameters.*

*The study tested the Null Hypothesis that the mean values did not differ between treatments, since the aim of the safety testing is to demonstrate toxicity rather than to demonstrate equivalence. The study was designed to detect 1SD unit change in all the variables of interest, since a value of less than 1 unit is considered unlikely to be of clinical significance. A sample size of 32 subjects has the power to detect a 0.7 SD unit change, and so the study recruited 40 individuals to allow for drop outs. This Committee was satisfied with the statistical power of the study.*

## OVERALL DISCUSSION

67. The application dossier contains good product specification data and a detailed description of the production process. The process is well monitored, with quality control and safety measures in place. The product is manufactured using a standard method, which has been shown to be both reliable and reproducible.
68. No nutritional concerns have been raised, since the entire product components are already present to some degree, as background, in human diets, and there is a recommendation generally for an increase in the level of DHA in the diet.
69. The product has undergone toxicological testing, and there are data from animal and other studies to support the safety of the derived oil. The inclusion of a human clinical trial provided further reassurance as to the safety of the DHA-Gold™ ingredient.
70. If this product is approved, the Applicant Company will make recommendations to food manufacturers regarding appropriate intake levels for DHA and appropriate labelling for the final food product. The Applicant Company has agreed to make recommendations to food manufacturers as described in the application dossier. Final products will need to be labelled with the ingredient name and the prescribed nutritional labelling. Since the oil is to be used as a nutritional ingredient, any claims made on the food due to the inclusion of the oil must comply with the general criteria for making nutrient content claims. Also, any health claims made will have to comply with the appropriate legislation in this area, regardless of any nutritional claim made.

## CONCLUSION

The Advisory Committee on Novel Foods and Processes is satisfied by the evidence provided by Omegatech that DHA Gold™ is safe for use as a nutritional food ingredient, for the types of uses as described in the application dossier, subject to the labelling requirements described above.

## APPENDIX IV

Mr Andreas Klepsch  
European Commission  
DG-Sanco  
Rue de la loi 200  
B-1049 Brussels  
Belgium

18 March 2002

Reference: NFU 368

Dear Mr Klepsch

### **Laboratories Pharmascience: Rapeseed oil high in unsaponifiable matter**

The UK Competent Authority (CA) sought comments from the Advisory Committee on Novel Foods and Processes (ACNFP) on the Initial Opinion of the French CA on the application by Laboratories Pharmascience under the Novel Food Regulation (EC) 258/97. While the UK CA generally agreed with the Initial Opinion of the French CA they raised several additional points. These are listed below:

1. The French CA's recommendation that the applicant should reduce the daily intake of the novel food from 5g to 1.5g was based on their safe upper limit for vitamin E (a multiple of its recommended daily intake (RDI)). The Committee expressed doubts about this practice of setting safe upper limits for vitamins based on a multiple of the RDI since Member States are currently working to establish safe upper limits based on toxicity.
2. The French CA commented on the slight ambiguity in the specification of the product. However the UK CA was of the opinion that the applicant needed to provide a more accurate specification which provides at least a maximum and minimum range for each component. This should include the specific erucic acid content of the product since the UK CA believe the Codex Standard for the erucic acid is less than 2% rather than less than 5%.
3. The UK CA was concerned that the applicant had not been specific enough in regards to the anticipated use of the product quoting a very broad range of intended food products. The incorporation of this novel ingredient into an unspecified number and variety of foods is of particular concern when the company has been requested to limit consumption of this ingredient. The UK CA does not consider that the applicant has satisfactorily demonstrated that it can limit consumption to a daily intake of 1.5g.

4. The UK agree with the French CA that the applicant should not be allowed to make cholesterol lowering health claims. However the UK point out that susceptible, high risk members of the population may still be affected by this product, therefore the label should incorporate wording to indicate that the product contains phytosterols.

The UK CA did not have any specific safety concerns on this product. However the UK CA is concerned that the intake and likely use of the product needs to be clarified. Therefore the UK CA is objecting to the marketing of this product until this issue and the other concerns listed above have been addressed.

Yours sincerely

Sue Hattersley  
ACNFP Secretariat

## APPENDIX V

Mr A Klepsch  
European Commission  
DG-Sanco  
Rue de la Loi 200  
B-1049  
Brussels  
Belgium

18 March 2002

Reference: NFU 373

Dear Mr Klepsch

**Application by Laboratoires Pharmascience to place the ingredient maize-germ oil high in unsaponifiable matter on the market.**

The UK Competent Authority sought comments from the Advisory Committee on Novel Foods and Processes (ACNFP) on the initial opinion produced by the French Competent Authority (French CA) on the application to place the ingredient maize-germ oil high in unsaponifiable matter on the market. Although the Committee broadly agreed with the positive opinion given by the French CA, there were a number of concerns raised.

- The French CA's recommendation that the applicant should reduce the daily intake of the novel food from 8g to 2g was based on their safe upper limit for vitamin E (a multiple of its recommended daily intake (RDI)). The Committee expressed doubts about this practice of setting safe upper limits for vitamins based on a multiple of the RDI since Member States are currently working to establish safe upper limits based on toxicity.
- The Committee was concerned about how the company would limit the intake of the novel food to 2g/day given the number of foods that could potentially contain the novel food.
- The Committee agreed with the French CA on the ambiguity of the product specification supplied by the applicant. They stated that it was unclear how many batches of the novel food had been used to establish the specification, and that the ranges of each component should be given instead of using 'greater than' and 'less than' symbols.

- The Committee agreed that no nutritional claims should be made regarding the phytosterol content of the novel food, but stated that any foods or food supplements containing the novel food should state on their labelling that they contain phytosterols. This will ensure that the at-risk groups in the population are aware of the phytosterol content of these foods and therefore give them the opportunity to avoid them.

The UK Competent Authority would be content for consent for the marketing of maize germ oil high in unsaponifiable matter to be granted on the condition that the applicant addressed the above concerns.

Yours sincerely

Sue Hattersley  
ACNFP Secretariat

## APPENDIX VI

Mr Klepsch  
European Commission  
DG-Sanco  
Rue De La Loi 200  
B1049  
Brussels

30 April 2002

Reference: NFU 364A

Dear Mr Klepsch

### **Application under EC Regulation 258/97 Multibene Phytosterol products**

The UK Competent Authority, has sought the advice of the Advisory Committee on Novel Foods and Processes (ACNFP), on the initial assessment report produced by the Finnish Competent Authority on Multibene® phytosterol products into food groups (bakery, dairy and meat products, spice sauces, dietary fats and non-carbonated soft drinks). The Committee broadly agreed with the initial opinion of the Finnish Competent Authority, however there were several concerns raised by members. These are listed below:

1. Some of the products (for instance soft drinks and ice cream) to which it is proposed to add the phytosterol ingredient are perceived to be potentially desirable to children. There is concern that even if they are marketed at a premium price with an indication of the target population (middle aged people), there might still be consumption by children. Therefore, as agreed for other similar applications, products should be clearly labelled that they are not nutritionally suitable for certain sections of the population (pregnant and breast feeding women and children under the age of 5 years). In addition, there should be a consideration of whether there should be a restriction in the range of products to which phytosterols can be added.
2. There is a general concern that over-consumption of phytosterols above the dose of 2-3g per day that gives maximal reduction in blood cholesterol, could have an adverse affect on the absorption of  $\beta$ -carotene. The data on estimates of average consumption suggests that eating a combination of Multibene® products in a day could easily provide more than this maximally effective dose. For instance if the anticipated intake of 100g of bakery products and 75g of meat products were combined, this would provide 3.5g of phytosterol,

Similarly combining 100g bakery product, 25g dietary fats and 150g of yoghurt would provide 5g of phytosterol. Since there are no additional benefits, in terms of reducing serum cholesterol, from intakes of phytosterol above 2-3g/day, the levels of phytosterols added to an average serving of particular food product should be limited to provide 1 serving of phytosterol. In addition there should be labelling on the products to indicate that the recommended intake of such foods should be limited to 2-3 servings per day.

3. It was not clear whether patterns of consumption described in the application would be predictive for the UK population for all products (or in total). The data are based predominately on the patterns of consumption in Sweden, Finland & Denmark and as such might not be predictive for other EU MS due to the differences in intake patterns of these proposed food groups.
4. Concerns were raised about the possible allergenic potential of MultiBene® products because one of the sources of phytosterols could be peanuts. The Company should provide evidence to confirm that the processing of the vegetable oil derived plant sterols (phytosterols) consistently removes all traces of protein, at the limit of detection, using an acceptable method.
5. The fat levels in the proposed products appear to be low. Therefore the applicants should ensure that the products comply with international standards (e.g. CODEX) for the various product types.

The UK CA does not support the marketing of this ingredient in the range of products described until these factors have been addressed, and therefore formally objects to this application.

In addition we would wish to discuss in the Novel Food Competent Authority working group the outcome of the SCF consideration of the possible health implications of over-consumption of phytosterols/ stanols so that a strategy for managing any risks identified can be developed.

Yours sincerely

Dr Jane A Cockram  
Novel Foods Division

## APPENDIX VII

Mr Klepsch  
European Commission  
DG-Sanco  
Rue De La Loi 200  
B1049  
Brussels

25th April 2002

Reference: NFU 365

Dear Mr Klepsch

**Initial opinion from the Netherlands CA on the application by Archer Daniels Midland Company to place on the market Plant Sterols and Sterol Esters as Novel Food Ingredients.**

The UK Competent Authority (UK CA) sought advice from the Advisory Committee on Novel Foods and Processes (ACNFP) on the Initial Opinion from the Netherlands under the 60-day rule of the Novel Food Regulation (EC) 258/97. The following concerns were raised.

1. Several of the products, for which the applicant is seeking clearance, are perceived to be potentially desirable to children (for example yoghurts). Although it is acknowledged that the applicant has considered this matter, the issue of labelling the phytosterol-enriched products as being unsuitable for certain sections of the population is not fully discussed. As with previous consideration of other products that contain plant sterols, products should be appropriately labelled, for example, that they may be nutritionally unsuitable for certain sections of the population, including pregnant and breast-feeding women and children under the age of 5 years.
2. The applicant acknowledges that much of the intake data is based on calculations using the total phytosterol production and availability of foods and not on actual consumption. The Committee does not feel this to be acceptable, particularly when more accurate dietary survey information is available, especially for the UK. The basket consumption survey carried out in the Netherlands is also not applicable to the UK. The lack of intake data for salad dressings, health bars and health drinks has led to considerable variability and likely inaccuracy in projected phytosterol intakes. The Committee also considered that the intake of products such as sausages and salad dressings will differ widely across the EU, and, in the UK, are unlikely to contribute greatly to the total phytosterol intake.

3. The applicant is recommending a daily intake of between 1-3 grams of phytosterols, with up to 2.2g coming from an average serving phytosterol-enriched spread. The other products in the range will be fortified at a level providing 1g of phytosterol per serving. The applicant maintains that the target group for this product is nutritionally aware and is unlikely to consume multiple servings and exceed the recommended daily intake. The Committee did not agree with this statement, and considered the large diversity of products in the range will make it difficult for even nutritionally aware consumers to restrict their intake. Additionally, the information the applicant is suggesting to place on the label refers to advice given by Lipton's Generally Regarded As Safe (GRAS) panel in the US, which states a maximum intake of phytosterols of 7.8g/day. This is at variance with the recommended effective daily dose of 2-3g and is, therefore, not appropriate.
4. The applicant refers to Unilever's post market monitoring data to demonstrate that after one year of consuming phytosterols in yellow fat spread, a drop in plasma (- carotene levels is observed, although this lower level remains within normal ranges. However, the results from this survey are based on an average consumption of 2 grams of phytosterol per day, and the applicant is suggesting a possible daily intake of 7.4g, over 3 times greater than the amount consumed in Unilever's survey.
5. It is noted that toxicological and nutritional data are based on Unilever's application and post market monitoring data, and no studies were carried out on the actual product for which clearance is sought. However, it is acknowledged that the applicant claims "the extraction and purification steps used to manufacture phytosterols are similar to steps used traditionally by the food industry, including Unilever, in the EU".

The UK Competent Authority broadly agreed with the Initial Opinion of the Dutch CA and therefore supports the marketing of the phytosterol-enriched yellow fat spread, subject to appropriate labelling of such products, as agreed for other phytosterol-containing products. However, the UK does not support the extension of the range to include salad dressings, health bars, health drinks, yoghurt type products and processed meats.

Yours sincerely

Ruth Dadswell  
Novel Foods Division  
Food Standards Agency

## APPENDIX VIII

Andreas Klepsch  
European Commission  
DG-SANCO  
Brussels

22nd November 2002

Reference: NFU 417

### **Iodine Enriched Wild Type Eggs**

Dear Mr Klepsch

As the UK Competent Authority, the Food Standards Agency has sought comments from the Advisory Committee on Novel Foods and Processes (ACNFP) on the Belgian Initial Opinion for the application under the Novel Foods Regulation (EC) 258/97 by Belovo SO for the above product at a meeting on the 14th November 2002.

The Committee agreed with the Initial Opinion of the Belgian Competent Authority that the levels of iodine found in the product are sufficiently high for there to be safety concerns. We also note that the levels of iodine fortification in the feed appear to exceed those permitted in Council Directive 70/524/EEC.

We therefore agree with the Belgian CA that this product should be given a negative opinion, and does not support the marketing of this product.

Yours sincerely

Dr Chris Jones  
ACNFP Secretariat

## APPENDIX IX

Dr. Bruno Tinland  
Monsanto Services International S.A  
Avenue De Tervuren 270-272  
1150 BRUSSELS  
BELGIUM

5th July 2002

Reference: NFU 194

Dear Dr. Tinland

**Request for Scientific Opinions on the Substantial Equivalence of Cottonseed Oil Derived from Genetically Modified lines.**

The UK Competent Authority took advice from the Advisory Committee on Novel Foods and Processes (ACNFP), and considered your applications under Articles 3(4) and 5 of the Novel Food Regulation, (EC) 258/97, on the substantial equivalence of oil derived from two distinct genetically modified cotton lines, Insect Protected line 531 and Roundup Ready line 1445. The Committee agreed that processed oils derived from these lines were equivalent, in composition, to oils from conventional cottonseed varieties. The scientific opinions of the ACNFP on these two oils are attached. The two opinions, together with this letter, will appear on the ACNFP pages of the Food Standards Agency website.

Although the ACNFP is content that the oils are substantially equivalent to their conventional counterparts, the Committee did have a number of comments on the presentational quality of the molecular biology characterisation data.

1. Members regarded the inclusion of Roundup Ready line 1698 as a control in many of the Southern blots to be misleading and unnecessary. The Committee was of the view that some of the information was confusing, and although the molecular biology experts on the ACNFP could understand the significant points, it was felt that the lay person would have some difficulties. Please be mindful of this point when making applications of this nature in the future.
2. As previously discussed, the explanation in the text describing the genetic components of plasmid PV-GHGT06, which is present in RRC line 1698, is not clear. The Committee recommends the following text be substituted for the penultimate sentence under section IV, part D. Characterisation of the insertion event:

“Plasmid PV-GHGT06 is a derivative of Plasmid PVGHGT07. PV-GHGT06 lacks a region of 2.492 kbp containing the entire gox gene cassette. This cassette contains a CmoVb promoter, a ctp chloroplast targeting region, a gox open reading frame and a NOS 3' termination signal.”

3. It was noted that several blots showed unequal intensity of signal and no explanation for this was given. Members felt that it would have been beneficial to see images of the original gels in order to demonstrate equal sample loading.
4. Members commented that the images in the original application were poorly labelled and unclear. The Committee accepts that many of the blots were carried out nearly 10 years ago, however, they felt that such standards would be unacceptable in an application submitted today. You may be aware that the Advisory Committee on Releases to the Environment is developing molecular data requirement guidelines, and in the future, you would be advised to submit data of the standard indicated in this document.
5. Members also wished it to be noted that they had concerns about the use of the aad marker gene in the transformation construct. However, since it has been demonstrated that there is no detectable DNA or protein in the oils, this is not a food safety issue. As you are aware, this gene confers resistance to the antibiotics spectinomycin and streptomycin. This is of particular concern since spectinomycin is sometimes used in the treatment of *Neisseria gonorrhoeae*. For further information on the ACNFP's opinion on the use of this marker gene, please look on the ACNFP pages of the FSA website, at the following address:

<http://www.food.gov.uk/science/ouradvisors/novelfood/acnfpapers/cottonseed>

However, despite the lack of clarity in some of the molecular data, the Committee agreed that, because it had been demonstrated that there was no detectable DNA present in the processed oils, this did not detract from the food safety assessment. Members are therefore content that the oils derived from the two GM cotton lines are equivalent in terms of composition to their counterparts derived from non-GM sources.

Yours sincerely

Sue Hattersley  
Novel Foods Division  
Food Standards Agency.

## APPENDIX X

### ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

#### REQUEST FOR AN ARTICLE 5 OPINION ON THE SUBSTANTIAL EQUIVALENCE OF COTTONSEED OIL AND FOOD INGREDIENTS DERIVED FROM ROUNDUP® READY COTTON

Company	Monsanto Europe S.A.
Product	Cottonseed oil
Application	Substantial equivalence
Modification	RRC 1445 – Herbicide tolerant (herbicide Roundup® (- active ingredient glyphosate)
EC guidelines category	3.1 (the host plant used for the genetic modification has a history of use as a source of food ingredients)

#### BACKGROUND

1. In June 1997, the UK Competent Authority received a request from Monsanto Europe SA for a scientific opinion on the substantial equivalence as regard their composition, nutritional value, metabolism, intended use and the level of undesirable substances contained therein of food and food products derived from Roundup Ready Cottonseed, under article 5 of the Novel Foods Regulation (EC 258/97).
2. Monsanto originally requested an opinion on two products derived from cottonseed: oil and linters. In November 1997, the Company provided information that linters are processed to the food additives carboxy methylcellulose (E 466) and methylcellulose (E461). Since additives are exempt from the Novel Food Regulation, the Committee confirmed that consideration need only be given to the oil derived from this line. None of the aspects of the dossier that apply directly to cottonseed linters have been considered in this opinion.
3. The approach taken by the Company in their supporting dossier, was to fully describe the genetic modification event, to demonstrate that the composition of the cottonseed from the modified line was comparable to seed from conventional line and to provide further evidence on the composition of the oil derived from the modified seed, including evidence to demonstrate the absence of DNA and

proteins in the refined oil. The premise of this approach was that if the seed was shown to be comparable, then derived oil would also be comparable.

#### DESCRIPTION OF THE GM LINE

##### The Host Plant

4. Genetic material was stably inserted into the genome of the host Coker line 312 cultivar of cotton (*G. hirsutum* L.). The host is a commercial breeding line, that has been grown in the United States for over 10 years and has a history of safe use in foods for human consumption, including oil derived from cottonseed.
5. The modified line is comparable to the parental variety in morphology, disease- and pest-resistance and agronomic performance, except for the genes and proteins that were introduced to the plant and the tolerance to Round-up® herbicide, conferred by the introduced CP4 EPSPS protein.

##### The Introduced trait

6. RRC line 1445 was modified by the addition of the cp4 epsps gene from the common soil bacterium *Agrobacterium* subsp. CP4. The modified plants produce the 5-enolpyruvylshikimate-3-phosphate synthase protein (CP4 EPSPS), the presence of which confers resistance to Roundup® herbicide. The genetically modified line also contains two antibiotic resistance marker genes: nptII and aad.

##### The Transformation System

7. The T-DNA, which includes the cp4 epsps, nptII and aad genes, was stably transferred into the genome of cotton using *Agrobacterium tumefaciens* mediated transformation. The use of *Agrobacterium* in transformation ensures that only T-DNA is integrated in the plant genome and the border sequence, (which contains the necessary genetic elements for transfer) is not. Therefore, once integrated, the insert is no longer functional as a T-DNA, and cannot be remobilised into the genome of another plant.
8. After the plant transformation, residual *Agrobacterium* cells were killed using specific antibiotics.

##### Plasmid Vector

9. RRC line 1445 was transformed using the single border binary transformation vector, PV-GHGT07. The vector contains well-characterised DNA segments required for selection and replication of the plasmid, as well as a right border for initiating the region of T-DNA integrated in to the plant genome. The plasmid also contains a gox gene, which encodes for glyphosate oxidoreductase. This was not transferred into RRC line 1445.

10. The Company has provided a list of all genetic elements contained in this vector, together with their sizes and functions. These are described below.

#### Nature, Function and Expression of Inserted Genes

The *cp4 epsps* gene

11. This gene confers resistance of the herbicide Roundup® to the modified cotton plants and is under the control of the constitutive promoter CmoVb (isolated from the cauliflower family of viruses, specifically the figwort mosaic virus). The gene is derived from *Agrobacterium* subsp. *CP4*. A synthetic version of this gene, with plant-preferred sequences, was used in the production of RRC line 1445. The activity of the synthetic CP4 EPSPS protein was compared with that from the native *cp4 epsps* gene and was found to be identical.
12. The region that codes for the chloroplast transit peptide from the native CP4 EPSPS is fused to the region of DNA that codes for the synthetic protein. This enables the protein to be targeted to the chloroplast where aromatic amino acid biosynthesis takes place.

Antibiotic selection

13. RRC line 1445 contains *nptII* gene under the control of the constitutive CaMV 35s promoter and the *aad* gene, which is driven by its own bacterial promoter and, as such, is not expressed in the modified cotton line.

#### Characterisation of the Insertion Event

14. Southern blot analyses were conducted to characterise the inserted T-DNA in terms of insert number (number of integration events), copy number (number of T-DNA copies at a particular genetic locus) and insert integrity (gene size, composition and linkage). The characterisations were carried out on genomic DNA isolated from leaf tissue from both the modified and parental (control) lines.
15. Analyses of RRC line 1445 were carried out in parallel with another, non-commercial RRC line, 1698, which was transformed with the plasmid PV-GHGT06, a derivative of PV-GHGT07. PV-GHGT06 lacks the *gox* gene cassette, which is contained in PV-GHGT07. In some of the Southern blot experiments, PV-GHGT06 was used as a probe or a control, rather than plasmid PV-GHGT07. This is not likely to have any bearing on the outcome of these experiments.

Insert Number

16. One T-DNA insert was transferred into the genome of RRC line 1445, which is not present in the genome of the parental line. Data on this

section indicates that RRC line 1445 has a single locus containing DNA from PV-GHGT07. Segregation data provided also supports this conclusion.

#### Insert Integrity and Copy Number

17. Southern blot analysis confirms the presence of the *cp4 epsps* gene and the CMo Vb promoter in the PV-GHGT07 plasmid and digested genomic DNA from RRC line 1445.
18. The presence of the *nptII* and *aad* genes in the genomic DNA of RRC line 1445 was also demonstrated, with no hybridising bands being seen in the genomic DNA from the parental, demonstrating the absence of these two genes in the control line.
19. Southern analysis indicates that the *ori-V* sequence was partially truncated before or during the insertion event.
20. A defined band of the expected size of the *gox* gene was seen in the digested plasmid PV-GHGT07. The absence of this band in the genomic RRC line 1445 DNA demonstrates that this gene was not integrated into the genome of the cotton plant when it was transformed to produce the RRC line 1445. It was also absent from the DNA of Coker 312, control line.

#### Genetic Stability of RRC line 1445

##### Segregation

21. Segregation ratios observed in R1 plants (selfed progeny from the initial transformant) supports the conclusion that the inserted T-DNA segregates as a single Mendelian locus. The Southern blot analysis demonstrates that the T-DNA was stably maintained for three generations.

##### Stability of the insert

22. For RRC line 1445, Southern blot analysis shows that the *cp4 epsps* gene is stably maintained from the R3 through to the R5 generations. The *nptII* gene is also shown to be stably maintained for three generations.

#### Stability of expression

##### Stability of gene expression

23. Data on levels of introduced CP4 EPSPS and NPTII proteins demonstrate that that production in leaves and seed are comparable in 1993 and 1994. The results from these analyses indicate that levels of expression of the introduced *cp4 epsps* and *nptII* genes are consistent from one generation to the next.

#### Stability of phenotypic expression

24. RRC line 1445 has exhibited consistent levels of Roundup-tolerance in field conditions since it was first tested in trials in 1991. The trait has been stably maintained through subsequent generations of plant propagation and breeding in different genetic backgrounds and under different environmental conditions. This was confirmed on a commercial scale in 1997 in the US. This demonstrates stable maintenance of the phenotype under different field conditions over 5 years.

#### EFFECT OF PRODUCTION PROCESS ON NOVEL FOOD

25. A description of the typical production process for cottonseed oil was provided and it is intended that oil from the RRC cotton line 1445 will be processed in the same way. In order to eliminate naturally occurring toxicants in cotton, the oil undergoes extensive processing during production.
26. The Committee sought further information regarding the processing conditions to ensure that they will eliminate protein and DNA from the refined oil. The Company provided a reference with a more detailed description of the production methodology of the oil. This information satisfied the Committee's concerns.

#### COMPOSITION OF THE COTTONSEED OIL

27. The Committee sought further assurance regarding the sensitivity of the original protocol to detect DNA and protein. At this request, the Company revised the study protocol for DNA extraction from refined cottonseed oil. The new method demonstrated a limit of detection of approximately 100pg of DNA (equivalent to 1ng/100ml control oil spiked with genomic cotton DNA). Refined oil from RRC line 1445 was analysed using this method, and no DNA was detected.
28. The refined cottonseed oil from RRC line 1445 and Coker 312 was found to be comparable in quality to commercially processed cottonseed oil from non-GM sources. These data indicate that the levels of the important fatty acids, as well as the toxic cyclopropenoid fatty acids, are comparable in refined cottonseed oil fractions produced from RRC line 1445 and the control line. There was no detectable gossypol in refined oil, and levels of alpha tocopherols were also comparable in both lines and were within the range published for other commercial varieties.
29. The insertion of the genes to provide herbicide tolerance did not alter the processing characteristics of the cottonseed or the quality of refined oil.

30. At the further request by the Committee, a report was submitted in August 2001 regarding the detectability of amino acids in refined oil from RRC line 1445. The results demonstrated that there is no detectable protein in refined oil at the limit of detection, which is 0.082µg/ml oil, and that the processing of oil removes protein to non-detectable levels. The Committee was content with these data.

#### COMPOSITION OF THE COTTONSEED

31. The Company argues that the composition of the modified seed, in terms of protein, oil, carbohydrate, moisture, ash and calories, is comparable to the parental, non-GM variety, and, as such, the resulting oil will also be comparable.
32. Extensive compositional analyses were performed on cottonseed from Roundup® Ready cotton line 1445 and the parental line, from field trials carried out in 1993 and 1994 at 6 field sites across the US cotton belt.
33. Although there were clear fluctuations between the control and test lines over the two years, observed differences still fell within the limits of previously reported ranges found in the literature for refined cottonseed oil. It was also demonstrated that the introduction of the EPSPS protein, which catalyses a step in the aromatic amino acid biosynthetic pathway, and the application of Roundup® herbicide on the modified line had no effect on the amino acid profile of the seeds.
34. The values obtained for the major toxicants were shown to be statistically equivalent between the two lines throughout the field trials.

#### CONCLUSION

The Committee was satisfied that:

- line RRC 1445 has been well characterised regarding the genetic modification event;
  - the T-DNA has been stably inserted and;
  - the composition of cottonseed oil derived from line RRC 1445 is comparable in terms of protein, oil, carbohydrate, moisture, ash and calories in composition to the parental line and to commercial varieties, and that there is no novel genetic material present in the refined oil.
35. The Committee is therefore of the opinion that oil derived from herbicide tolerant cottonseed line RRC 1445 is substantially equivalent to oil from conventional cottonseed lines, in terms of composition, nutritional value, metabolism, intended use and level of undesirable substances.

## APPENDIX XI

### ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

#### REQUEST FOR AN ARTICLE 5 OPINION ON THE SUBSTANTIAL EQUIVALENCE OF COTTONSEED OIL AND FOOD INGREDIENTS DERIVED FROM INSECT PROTECTED COTTON

Company	Monsanto Europe S.A.
Product	Cottonseed oil
Application	Substantial equivalence
Modification	IPC line 531 – Insect protected (Bt)
EC guidelines category	3.1 (the host plant used for the genetic modification has a history of use as a source of food ingredients)

#### BACKGROUND

1. In June 1997, the UK Competent Authority received a request from Monsanto Europe SA for a scientific opinion on the substantial equivalence as regard their composition, nutritional value, metabolism, intended use and the level of undesirable substances contained therein of food and food products derived from Insect Protected Cottonseed, under article 5 of the Novel Foods Regulation (EC 258/97).
2. Monsanto originally requested an opinion on two products derived from insect protected cottonseed: oil and linters. In November 1997, the Company provided information that linters are processed to the food additives carboxymethyl cellulose (E466) and methylcellulose (E461). Since additives are exempt from the Novel Foods Regulation, the Committee confirmed that consideration need only be given to the oil derived from this line. None of the aspects of the dossier that apply directly to cottonseed linters have been considered in this opinion.
3. The approach taken by the Company in their supporting dossier, was to fully describe the genetic modification event, to demonstrate that the composition of the cottonseed from the modified line was comparable to seed from conventional line and to provide further evidence on the composition of the oil derived from the modified seed, including evidence to demonstrate the absence of DNA and proteins in the refined oil. The premise of this approach was that if the seed was shown to be comparable, then derived oil would also be comparable.

## DESCRIPTION OF THE GM LINE

### The Host Plant

4. Genetic material was stably inserted into the genome of the host Coker line 312 cultivar of cotton (*G. hirsutum* L.). The host is a commercial breeding line that has been grown in the United States for over 10 years and has a history of safe use in foods for human consumption, including oil derived from cottonseed.
5. The genetically modified line is comparable to the parental variety in morphology and agronomic performance, except for the genes and proteins introduced to the plant for protection against damage from lepidopteran insects, conferred by the introduced CryIA(c) protein.

### The Introduced trait

6. Insect-Protected line 531 was modified by the addition of the *cryIA(c)* gene from the common soil bacterium *Bacillus thuringiensis* subsp. *Kurstaki* (*B.t.k.*). The modified plants produce the CryIA(c) protein, which is insecticidal to specific lepidopteran target pests. The genetically modified line also contains two antibiotic resistance marker genes: *nptII* and *aad*.

### The Transformation System

7. The T-DNA, which includes the *cryIA(c)*, *nptII* and *aad* genes, was stably transferred into the genome of cotton using *Agrobacterium tumefaciens* mediated transformation. The use of *Agrobacteria* in transformation ensures that only T-DNA is integrated in the plant genome and the border sequence, (which contains the necessary genetic elements for transfer) is not. Therefore, once integrated, the insert is no longer functional as a T-DNA, and cannot be remobilised into the genome of another plant.
8. After the plant transformation, residual *Agrobacterium* cells were killed using specific antibiotics.

### Plasmid Vector

9. IPC line 531 was transformed using the single border binary transformation vector, PV-GHBK04. The vector contains well-characterised DNA segments required for selection and replication of the plasmid, as well as a right border for initiating the region of T-DNA, integrated into the plant genome.
10. The Company has provided a list of all genetic elements contained in this vector, together with their sizes and functions. These are described below.

### Nature, Function and Expression of Inserted Genes

The modified *cryIA(c)* gene

11. The *cryIA(c)* gene used in this transformation was constructed by combining the first 1398 nucleotides of the *cryIA(b)* gene with nucleotides number 1399 – 3534 of the naturally occurring *cryIA(c)* gene. With the exception of 6 amino acids, the region derived from the CryIA(b) protein is identical to the analogous region of the CryIA(c) protein. The modified *cryIA(c)* gene encodes for a protein that is identical to the CryIA(c) protein found in nature, with the exception of one amino acid at position 766. This discrepancy was unintentional. However, the amino acid at this position is degraded upon exposure to trypsin, or the proteases found within the insect gut, and so will not affect the host range or the active portion of the protein.

Antibiotic selection

12. IPC line 531 contains *nptII* gene under the control of the constitutive CaMV 35s promoter and the *aad* gene, which is driven by its own bacterial promoter and, as such, is not expressed in the modified cotton line.

### Characterisation of the Insertion Event

13. Southern blot analyses were conducted to characterise the inserted T-DNA in terms of insert number (number of integration events), copy number (number of T-DNA copies at a particular genetic locus) and insert integrity (gene size, composition and linkage). The characterisations were carried out on genomic DNA isolated from leaf tissue from both the modified and parental (control) lines.

Insert Number

14. Molecular characterisation of Bollgard cotton event 531 demonstrated there are two T-DNA inserts. The primary functional insert contains single copies of the full-length *cryIAc* gene, the *nptII* gene and the *aad* antibiotic resistance gene. This T-DNA insert also contains an 892 bp portion of the 3' end of the *cryIAc* gene fused to the 7S 3' transcriptional termination sequence. This segment of DNA is at the 5' end of the insert, is contiguous and in the reverse orientation with the full-length *cryIAc* gene cassette and does not contain a promoter. The second T-DNA insert contains 242 bp of a portion of the 7S 3' polyadenylation sequence from the terminus of the *cryIAc* gene and is not functionally active in the plant genome.

#### Insert Integrity and Copy Number

15. Restriction digest analysis demonstrated that the larger insert containing an intact fragment containing the *cryIA(c)* and *nptII* genes has been integrated into the cotton genome. This T-DNA copy is no larger than 8.2 Kb, and so maximally contains the *cryIA(c)*, *nptII* and *aad* genes, as well as part of the *oriV* site.
16. Further restriction analysis indicates that this insert also contains a second, but smaller T-DNA derived copy (1.7kb, which hybridised to the *cryIA(c)* gene probe, but not to the *nptII* gene probe. Since the origin of transfer typically initiates from the right border, this fragment contains 0.44kb of 7S 3' termination sequence, and 0.89kb of the 3' end of the *cryIA(c)* gene. This portion of the *cryIA(c)* gene is not insecticidally active and so will have no impact on the action of the introduced trait.
17. The results from these analyses established that the two T-DNA copies are contiguous to each other, and in a tail-to-tail arrangement.
18. The second insert has been fully characterised and has been shown to be 242bp in length. It contains only a portion of the transcriptional termination sequence from the *cryIAc* gene and is functionally inactive in the plant genome.

#### Genetic Stability of IPC line 531

##### Segregation

19. The *cryIA(c)* gene segregated in a manner consistent with a single intact gene insertion that is stably transferred with crossing. The selfed data from crosses further demonstrates the stability of the insert during transfer over subsequent generations, with the structural and local maintenance of the inserted genes being conserved over four back crossed generations of derivatives of IPC line 531 in several elite cultivar lines.
20. Segregation data for the R1 plants (selfed progeny of the initial transformant – referred to as RO) and the progeny of the R1 plants is presented. These data demonstrate that a single active copy of the *cryIA(c)* gene has been inserted in IPC line 531.

##### Stability of the insert

21. Stability of the T-DNA insertions in Bollgard cotton event 531 was determined by analysing the R5 and R6 generations, as well as two commercial cotton lines containing Bollgard cotton event 531 by Southern blot analysis. The results from these experiments indicated that the functional insert was present in all the generations of Bollgard cotton event 531 that were analysed. However, the 242 bp T-DNA segment containing a portion of the 7S 3' genetic element was

detected in the R5 and R6 inbred generations, but was not detected by Southern blot analysis in the two commercial lines containing Bollgard cotton event 531 that were tested. A likely explanation for the absence of the 242 bp insertion in the commercial lines is that it segregates independently of the functional insertion since the commercial lines were derived from the original Bollgard cotton event 531 through traditional breeding methods.

#### Stability of expression

##### Stability of gene expression

22. Data presented on the levels of introduced CryIA(c) and NPTII proteins demonstrate that production in leaves and seed are comparable in 1992 and 1993 (and 1994 for CryIA(c) only). When IPC line 531 was backcrossed, no significant differences, within the limits of the assay, were observed between protein levels. This demonstrates that the levels of expression of the introduced *cryIA(c)* and *nptII* genes are consistent from one generation to the next.

##### Stability of phenotypic expression

23. IPC line 531 has expressed the introduced trait of insect-resistance in field conditions since it was first planted in trials in 1990. Subsequent generations further demonstrated the stable maintenance of this trait in plant propagation, breeding in field trials under differing environmental conditions and on a commercial scale in 1996, in the US and Australia. This demonstrates stable maintenance of the insect-protected phenotype over 6 years.

#### EFFECT OF PRODUCTION PROCESS ON NOVEL FOOD

24. A description of the typical production process for cottonseed oil was provided and it is intended that oil from the IPC line 531 will be processed in the same way. In order to eliminate naturally occurring toxicants present in conventional cotton, the oil undergoes extensive processing during production.
25. The Committee sought further information regarding the processing conditions to ensure that they will eliminate protein and DNA from the refined oil. The Company provided a reference with a more detailed description of the production methodology of the oil. This information satisfied the Committee's concerns.

#### COMPOSITION OF THE COTTONSEED OIL

26. The Committee sought further assurance regarding the sensitivity of the original protocol to detect DNA and protein. At this request, the Company revised the study protocol for DNA extraction from refined cottonseed oil. The new method demonstrated a limit of DNA detection of approximately 100pg (equivalent to 1ng/100ml control oil spiked with genomic cotton DNA). Refined oil from IPC

line 531 was analysed using this method, and no DNA was detected. The limit of detection for the protein assay was determined as being 0.082(g/ml of oil).

27. The refined cottonseed oil from IPC line 531 and Coker 312 was found to be comparable in quality to commercially processed cottonseed oil from non-GM sources. These results indicate that the levels of the important fatty acids, as well as the toxic cyclopropenoid fatty acids, are comparable in refined cottonseed oil fractions produced from IPC line 531 and the control line. There was no detectable gossypol in refined oil, and levels of alpha tocopherols were also comparable in both lines and were within the range published for other commercial varieties.
28. The insertion of the genes to provide insect resistance did not alter the processing characteristics of the cottonseed or the quality of refined oil.
29. A further field study carried out in 1998, also demonstrated that oil derived from Bollgard™ (commercial line of insect-protected cotton) was compositionally equivalent to that derived from the non-genetically modified control line, and three other commercial lines.

#### COMPOSITION OF THE COTTONSEED

30. The Company argues that the composition of the modified seed, in terms of protein, oil, carbohydrate, moisture, ash and calories, is comparable to the parental, non-GM variety, and, as such, the resulting oil will also be comparable.
31. Extensive compositional analyses were performed on cottonseed from Insect-Protected cotton line 531 grown in both 1992 and 1993. Field sites were selected in major cotton growing regions of the US, under a variety of environmental conditions and insect pressures from agronomically important insect pests. Both test and control lines were grown under identical conditions at each location, with the agronomic practices and conditions being monitored and recorded.
32. The level of the major components was shown to be compositionally equivalent between the two lines over the two field trials. All values obtained for these components were within published ranges.

#### CONCLUSION

33. The Committee was satisfied that
  - line IPC 531 has been well characterised regarding the genetic modification event;

- the T-DNA has been stably inserted and;
- the composition of oil derived from line IPC 531 is comparable in terms of protein, oil, carbohydrate, moisture, ash and calories to the parental line and to commercial varieties, and that there was no novel genetic material present in the refined oil.

34. The Committee is therefore of the opinion that oil derived from insect protected cottonseed line IPC 531 is substantially equivalent to oil from conventional cottonseed lines, in terms of composition, nutritional value, metabolism, intended use and level of undesirable substances.

## APPENDIX XII

### Advisory Committee on Novel Foods and Processes

#### Response to the food safety issues raised in the evidence submitted to the Chardon LL Hearing.

##### SUMMARY

1. The Advisory Committee on Novel Foods and Processes (ACNFP) has agreed to provide advice on the food safety related issues raised from the recent Chardon LL Public Hearing regarding processed products derived from this genetically modified maize line.
2. The Committee is content that no new evidence was submitted to the Hearing that would question the safety of foods derived from Chardon LL maize.

##### INTRODUCTION

3. The ACNFP is an independent body of experts, whose remit is to advise the central authorities responsible, in England, Scotland, Wales and Northern Ireland respectively on any matters relating to novel foods, having regard, where appropriate, to the views of relevant expert bodies.
4. The Committee has considered the documents submitted to the T25 Public Hearing. The Committee has also addressed criticisms levied by some of those giving evidence to the Hearing, regarding the manner in which the safety assessment was conducted. These fall into the broad areas of the regulatory procedure, substantial equivalence and composition, the scientific assessment, the use of GM technology and the possible presence of GM pollen in honey.
5. The Advisory Committee on Animal Feedingstuffs (ACAF) has recently considered the safety of T25 maize as an animal feed. Their conclusions were addressed in a letter to the ACRE Secretariat, dated 5th September 2001. The implications of this GM variety entering the human food chain via the human consumption of products derived from animals fed on T25 maize was also addressed by ACAF. Points arising from the Hearing relating to animal feed issues have not been addressed by the ACNFP in this advice.
6. The Advisory Committee on Releases to the Environment (who consulted with the Advisory Committee on Animal Feedingstuffs) is conducting a similar exercise on the evidence presented at the Hearing on environmental issues, and other matters relating to the assessment carried out as part of the Part C clearance under 90/220/EEC. DEFRA's Plant Varieties and Seeds Division are also commenting on issues relating to the listing of T25 on the national seeds list.

## BACKGROUND

7. In 1995, the ACNFP considered the food safety of products derived from the genetically modified maize line Chardon LL (T25), which had been developed by AgrEvo USA to be tolerant to glufosinate–ammonium based herbicides. The request related to products derived from the seeds of this line, together with products derived from inbred and hybrid lines developed using conventional cross breeding. The ACNFP approved the application in 1996 and provided AgrEvo with a positive scientific opinion. The Company subsequently made a notification on the 8th January 1998, under Articles 4(3) and 5 of the Novel Foods Regulation, citing the ACNFP's scientific opinion. In accordance with Article 5, this notification was copied to all other Member States within 60 days of the Company notifying the Commission.
8. Following comments raised by one Member State regarding the notification route used for oils and other processed products derived from this and other GM crops, the Scientific Committee on Food reviewed the ACNFP opinion on T25 maize. The SCF concluded in September 2000, that the use of products derived from T25 maize did not endanger human health.
9. In 1996, AgrEvo applied to the French Competent Authority for marketing consent for T25 maize seed under Directive 90/220/EEC. Scientific advisory committees in other Member States, including ACRE in the UK, conducted their own independent safety assessments. Following this assessment, it was agreed that marketing consent should be granted, and in 1998, this was issued by France.
10. In 2000, the Company submitted an application to have Chardon LL placed on the UK National List. Under the National List regulations, parties affected by the proposed decision to “list” a variety may make written representations to Ministers and request a public Hearing. Friends of the Earth challenged this application, and it was suspended. A public Hearing began in October 2000 to enable interested parties to present evidence for and against T25 being placed on the National List. The Hearing was chaired by Alun Aylesbury, a senior barrister, who was appointed by Ministers. The Hearing finished in June 2002.

## RESPONSE

### Regulatory procedure

11. AgrEvo applied to the ACNFP for clearance of food and feed products derived from this line in 1995, two years before the implementation of the Novel Foods Regulation (EC 258/97). Clearance was sought for starch, oil, and all heat processed or fermented food products obtained from maize meal and flour derived from the T25 maize line.

12. Until Regulation EC 258/97 came into effect, the UK operated a voluntary scheme under which companies could submit applications for food safety assessment. This system had been in place since the ACNFP was set up in 1988, to provide a forum in which the safety of novel foods could be assessed. In 1994 the ACNFP developed a structured decision tree for use in the safety assessment of novel foods. This was adopted following extensive public consultation.
13. The ACNFP carried out a full safety assessment and provided a scientific opinion that concluded that the processed products obtained from the GM maize line T25 were safe to use in food and any differences in composition from those of conventionally-bred maize were not considered to be of biological significance.
14. On the 15th May 1997, the EC Novel Foods and Novel Food Ingredients Regulation (EC 258/97) came into effect, introducing a statutory pre-market approval system for novel foods across the whole of the European Union. To ensure that all Member States follow a consistent approach to the safety assessment for novel foods, the Commission published a series of guidelines to accompany the regulation. These guidelines were largely based on the earlier approach taken by the ACNFP, in its voluntary assessments.
15. Articles 3(4) and 5 of the Novel Foods Regulation detail a simplified procedure whereby it is possible for companies to notify the Commission that they are placing a novel food on the market. To make use of this simplified procedure, a company requires a scientific opinion delivered by a Competent Authority in one of the Member States concluding that their novel food is substantially equivalent to existing foods or food ingredients, as regards its composition, nutritional value, metabolism, intended use and level of undesirable substances contained therein. The responsibility lies with the company to notify the Commission when the food is placed on the market.
16. Article 5 states that when a company submits such a notification, the Commission shall forward a copy to other Member States within 60 days. Member States have the option to request further details, such as the supporting scientific opinion and any data supplied by the company.
17. On the 15th December 1997, the ACNFP had written to the European Commission suggesting that in the future, opinions in accordance with Article 3(4) of the Novel Food Regulation, should only be provided for products where it could be demonstrated that novel DNA or protein was not present. The letter went on to say that for certain products, such as flour, a full safety assessment would be required since the processing that they undergo may not completely remove or destroy DNA and protein. In the case of the food products derived from T25 maize, these had already undergone a full safety assessment by the ACNFP.

18. On the 8th of January 1998, AgrEvo notified the Commission of their intention to market products derived from T25 maize, and used the scientific opinion of the ACNFP to support their notification. Although regarded by some as a “fast track route” to approval, the Article 5 notification procedure involves a full safety assessment by the Competent Authority responsible for the initial opinion. In the case of T25, a full safety assessment was carried out by the ACNFP.
19. AgrEvo developed T25 maize as a processing line, and, as such, it would be unsuitable for human consumption in an unprocessed form. The ACNFP considered and gave food safety clearance only to products derived from this line and so, under regulation 5(1) of the Novel Foods and Novel Food Ingredients Regulations 1997 (UK Statutory Instrument), it would be an offence to sell unprocessed T25 maize for human consumption in the UK.
20. It is within ACAF’s remit to assess any risk to humans of eating meat and/or dairy products from animals fed GM feed. Therefore, this response does not address animal feed issues. ACAF recently addressed the safety of T25 maize as an animal feed in a letter to the ACRE Secretariat dated 5th September 2001. ACAF was content that there was no evidence to indicate that T25 maize grain or its products pose any more risk to animals or humans if used in animal feed than non-GM maize varieties.
21. It has been suggested by some of those giving evidence to the Hearing that the safety assessment of T25 maize should have been carried out using independent data, and should not have been based on data submitted by the Company. However, the role of the scientific experts on the ACNFP is to scrutinise data supplied in support of an application, and this was the case with data submitted by AgrEvo to determine the safety of products derived from T25 maize. It is common practice in many areas of safety evaluation (such as for medicines, pesticides, food additives and industrial chemicals) to use toxicological data generated by the company seeking approval. Laboratories conducting such studies are subject to regular inspections to ensure that operating procedures are satisfactory and individual studies may be audited to ensure compliance with such procedures. Thus data produced to these standards are considered to be robust. The T25 application dossier has been deposited in the British Library, and so is available for scrutiny by interested parties.
22. The ACNFP’s original assessment requested that the seed composition, including the amino acid profile and the fatty acid profile of the oil from the GM line and hybrid lines, should be monitored over time, to demonstrate the stable inheritance of the introduced trait and to determine any possible effect of genetic drift over successive generations on the plant’s metabolism.

23. Seed composition data, collected from field trials carried out over two years, were submitted to the Committee in September 2002. These data demonstrated that some statistically significant differences were seen for seeds from non-transgenic and herbicide treated and untreated transgenic lines. However, in all cases, the nutritional impact of these differences is considered to be negligible, since the levels of the analysed components generally fall within previously reported reference ranges for conventionally-bred maize. Conventionally-bred plant varieties have a spectrum of chemical and nutritional compositions, and no regulatory process is in place to analyse or monitor these. The Committee was content that these data demonstrated the stability of the introduced trait and assessed the impact of the genetic modification event on the composition of the seed. The Committee is satisfied that this information does not alter the original safety assessment of T25 maize.
24. In May 2001, the Company supplied additional characterisation data for the Committee to consider. These data consisted of detailed sequence information of the flanking regions of the inserted DNA, and Northern blot analysis. A letter from Aventis, describing the findings of this analysis can be found on the ACNFP pages of the FSA website. The Committee was content that this information did not alter the original safety assessment of T25 maize derived products, and therefore a re-assessment of the dossier was not required at that time.

**Substantial equivalence and composition.**

25. The concept of substantial equivalence is used to structure the safety assessment; it is not a safety assessment in itself. The safety assessment of GM foods requires an integrated and stepwise, case by case approach. An approach in which a GM food is compared with a conventional counterpart having a history of safe use is considered the most appropriate strategy for the safety assessment of GM foods.
26. The FAO/WHO expert consultation (May 2000) considered the concept of substantial equivalence as part of its review on the current safety assessment procedure for GM foods and recognised 'that there were presently no alternative strategies that would provide a better assurance of safety for genetically modified foods than the appropriate use of the concept of substantial equivalence' and that 'substantial equivalence should be seen as a key step in the safety assessment process'. The ACNFP fully supports that view.
27. As part of its initial application, AgrEvo supplied compositional and nutritional analysis data from material harvested from 2 US field sites (4 pairs of trials, with at least 3 samples taken from each pair) in 1994, which demonstrated that the silage and grain were not materially different from current commercial varieties in essential nutrients, including moisture, crude fat, crude protein, carbohydrate, acid

detergent fibre, neutral fibre and ash. The approach adopted by the Company in its application was to demonstrate that the GM grain was equivalent in compositional terms to conventional grain and then to conclude that any products derived from such grain would also be comparable.

28. The Committee recognised that there were some statistically significant differences between the fat, carbohydrate, amino acid and fatty acid contents of the GM and non-GM lines. However, Members considered that although the differences were occasionally statistically significant, the levels of the components still fell within previously published reference ranges for maize. As a result, the Committee regarded the differences not to be biologically significant. Further data was supplied in September 2002, which is described in paragraph 23.
29. The original study to determine the levels of the anti-nutritional substance phytic acid was conducted on silage produced from T25 maize. However, it is considered that the contribution of phytic acid from a processing maize variety to the human diet will be negligible. ACAF has recently reviewed the compositional analysis of maize grain, and was content that there were no important differences from other maize varieties in terms of essential nutrients and anti-nutrients.
30. Further compositional characterisation data (see paragraph 23) demonstrated that the level of phytic acid in the T25 maize line was at the bottom end of the range that is normally found for conventionally-bred maize.

### Scientific Assessment

#### Toxicology

31. Toxicological assessments were based on the evaluation of any differences between the experimental lines and their parental counterparts i.e. the toxicity of the genes themselves, the introduced protein, and the consequences of any secondary effects arising from the modification.
32. The ACNFP considers each application individually and requests animal test data in the context of the overall toxicological profile of the food in question, where it is considered necessary. Since the products derived from T25 maize were demonstrated to be comparable to their conventional counterparts, no animal studies were required for the safety assessment.
33. The FAO/WHO joint report (Safety aspects of genetically modified foods of plant origin, 2000) notes that the practical constraints of obtaining meaningful information from animal-based toxicology studies on the safety of whole foods have been well recognised for

many years. Foods are complex mixtures of compounds, which have a wide variation in their composition and nutritional value. They can only be fed to animals at low multiples of the amounts that might be present in the human diet. Identifying potential adverse effects and relating these to the food and not other factors can therefore be extremely difficult for a number of reasons.

34. The FAO/WHO joint report goes on to say that in certain cases, where insufficient data are presented for a safety assessment, animal testing may be deemed necessary.
35. Some additional animal studies (in broiler chickens and rats) were submitted to the French Competent Authority as part of the Part C evaluation under Directive 90/220. However, these studies were designed to support the wholesomeness and nutritional value of products derived from this maize line for use as animal feed ingredients and not as toxicity studies. As such, they would not have been relevant to a novel food application and would not have been considered as primary evidence in the food safety assessment. It is extremely important that studies of this nature should only be used for the purpose for which they were intended.
36. ACAF recently reviewed (September 2001) the broiler chicken and rat feeding studies. Although they were critical of some aspects of the information provided, Members saw nothing to indicate that T25 maize grain or its products pose any more risk to animals or humans if used in animal feed than non-GM maize varieties.
37. T25 maize was modified with the introduction of the pat gene that confers tolerance to glufosinate-ammonium based herbicides. The pat gene, which encodes for the enzyme phosphinothricin acetyl transferase (PAT), was originally isolated from a soil-borne bacteria, but the T25 maize line was produced using a synthetic version of this gene.
38. Evidence was provided to demonstrate that the PAT protein was degraded at temperatures above 35°C, whereas milling of the grain takes place at temperatures in excess of 100°C. In addition, the PAT enzyme was shown to be readily inactivated at pHs below 5. The food products obtained from T25 maize all undergo either wet milling, which involves steeping before milling and then processing by conventional means, using pressure, heat, and solvent extraction, or dry milling. The processes that are applied prior to consumption will either further degrade or remove protein. This, together with evidence of the rapid breakdown of the protein under the conditions experienced in the gut, satisfied the Committee that the PAT protein did not represent a food safety concern. The PAT protein was found to be absent from the maize oil and starch derived from this line, but was detected at 20 –300 ppm of the crude protein of wet and dry milled hulls, grits, meal and flour.

39. The PAT protein is present at very low levels in T25 maize, and so, to be able to detect any potential toxicity, isolated protein was used in the rat feeding study. The protein used for this experiment was extracted from a strain of *E.coli*, modified to express the pat gene. There is a small, theoretical risk that the PAT protein derived from *E.coli* is different from the PAT protein from maize and so it is possible that the protein used for toxicological studies could differ from that produced by T25 plants. This would be due to a post-translational modification, resulting in changes in the properties of the target molecule. Such modifications would, however, affect the electrophoretic mobility of the PAT proteins, with the modified protein being retarded in a gel compared with the unmodified protein. Since the protein from bacteria and maize show the same electrophoretic mobility; the PAT protein is unlikely to have undergone significant post-translational modification. However, the rat feeding assessment is only part of the overall safety evaluation and evidence was supplied that demonstrated that the PAT protein was not present in processed food products. Therefore, any possible differences between the PAT protein from different sources is not relevant to the safety evaluation of the processed food products derived from T25 maize.
40. Post-translational modification is a term used to describe the changes that occur to proteins after peptide bond formation has occurred. Examples include glycosylation, acylation, limited proteolysis, phosphorylation, and isoprenylation. The most reliable way in which the effects of post-translational-modification can be assessed is by evaluating morphological and agronomic data. The gross phenotype and general health of a mature plant gives the best indication of how proteins function. As was stated in the executive summary of the ACNFP report on processed products derived from T25 maize, the data supplied to the Committee satisfied Members that no unintentional changes had taken place at the molecular level.

#### Allergenicity

41. The first decision tree for use to detect allergenicity in novel foods was published in 1996 and was further refined by the FAO/WHO in 2001. Searching for amino acid sequence homology with known allergens is a significant part of allergenicity detection, but this is supported by other methodologies, including the assessment of protein stability and digestibility.
42. The safety of products derived from T25 maize were assessed prior to the implementation of these decision trees. However, in the initial application searches of the EMBL and SWISSPROT databases were conducted and it was concluded that there was no evidence to suggest any homology between the PAT protein and known allergens. In addition, as mentioned above, there was evidence to demonstrate rapid breakdown of the PAT protein during both processing and digestion.

### Genetic Modification technology

43. The application from AgrEvo sought clearance of food products derived from the T25 maize line. The Committee was content that the processing that these products undergo would destroy any functional DNA present.
44. When the Committee first considered this application, there was considerable discussion regarding the presence of the antibiotic resistance marker. However, after Members had sought and interrogated further data supplied by AgrEvo, the Committee was satisfied that the GM line contains a single, truncated copy of an ampicillin resistance gene, and the data provided demonstrated that it was inactivate. This was therefore not a safety concern in relation to food products intended for human consumption.
45. In 2001, Aventis supplied the Committee with further characterisation data on the T25 maize line. The Committee was asked to consider these data and to discuss whether there were any issues that had implications for the food safety assessment of this line. The Committee was content that they did not. A letter from Aventis explaining these data is on the ACNFP pages of the Food Standards Agency website, together with the minutes from the ACNFP meetings when they were discussed.
46. To address the possibility of unexpected effects such as gene silencing, pleiotropic effects or genetic instability, the assessment for the safety of GM foods addresses both intentional and unintentional effects that may occur as a result of the particular genetic modification.
47. The cauliflower mosaic virus is found world-wide in temperate regions and is common in commercial crops of cabbage, Brussels sprouts, and cauliflower. Therefore, plant material infected by this virus has been eaten for centuries and no ill effects have been reported.
48. There is limited published scientific evidence that suggests that the CaMV 35s promoter, which drives pat gene expression, is functional in *E.coli*. It was noted, however, that functional pat genes are found in bacteria in nature. The Committee was content that this is not a food safety concern.
49. The potential that honey may contain pollen collected from GM maize is not considered to be a cause for concern. AgrEvo provided analysis of the whole plant, leaves, roots, seeds and mature pollen from the GM maize line and its non-GM counterparts grown in Europe. The analysis was concerned with detection of the PAT protein. Although a small amount of the PAT protein was found in unprocessed seeds, leaves, stems and roots, none was found in mature pollen, and so there is no evidence that honey will contain this protein.

50. Research conducted by Laboratory of the Government Chemist into the pollen content of honey, estimated that consumers would ingest a maximum 1 part-per-billion of transgenic protein from this source. In December 1999 the ACNFP looked again at the advice it issued in 1991 to take account of the results from further research in this field. The Committee endorsed the advice given in 1991 and confirmed that it was still content that the presence of very small amounts of GM pollen in honey did not represent a risk to consumers.

#### **CONCLUSION**

51. The ACNFP has considered the information submitted to the Public Hearing on Chardon LL maize, and it is content that no new evidence has been presented that would question the safety of foods derived from Chardon LL maize.

**December 2002**

## Appendix XIII

### ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

#### ADDITIONAL DATA ON THE NUTRITIONAL IMPACT ASSESSMENT OF T25 MAIZE

##### BACKGROUND

1. In the original ACNFP safety assessment of T25 maize, it was requested that seed composition be monitored over time to demonstrate the stable inheritance of the introduced trait and to determine any possible effect of genetic drift on the plant's metabolism.
2. In September 2002, Bayer CropScience (previously Aventis) provided the ACNFP with these data. The Committee considered them by post.
3. The data represent compositional and nutritional analysis using raw agricultural commodity grain generated from 15 field sites over a two-year period. Three different treatment groups of maize were used in the analysis: 1) non-transgenic, unsprayed, 2) transgenic, unsprayed and 3) transgenic, sprayed with Liberty herbicide. A total of 135 samples were taken from each site, with 92 components being analysed in each sample.
4. Statistical analysis was performed by analysis of variance and a 95% confidence interval. In each case, the first statistical comparison carried out was between the three treatment groups grown within the same site (site-by-site analysis). Following this, comparisons were made with data collected from across all 15 sites (over-all-sites analysis).
5. To determine compositional equivalence between the transgenic line and the non-transgenic line, pooled over-all-sites data were compared to published reference ranges. The non-transgenic line was also used as a reference value since it provides the most accurate control, being the same variety of maize grown in the same field trial as its GM counterpart.

## DISCUSSION

### Proximate analysis (moisture, fat, protein, fibre, ash and carbohydrates)

6. Homogeneity within the non-transgenic, unsprayed reference line grown across all sites, could not be demonstrated for proximate components.
7. For most of the 15 sites, site-by-site analysis showed no significant differences between the three treatment groups for moisture, fat, protein, ash, total and available carbohydrates.
8. There were statistically significant differences at several sites in the level of total dietary fibre in the sprayed, transgenic line. However, these differences were not seen in the over-all-sites analysis using pooled data.
9. With the exception of moisture and total dietary fibre, the levels of the proximate components across all three treatment groups fell within published reference range. The three treatment groups had moisture levels averaging 27.4% (non-transgenic), 27.5% (transgenic, non-sprayed) and 27.1% (transgenic, sprayed), compared with the published range of 7-23%. For total dietary fibre, only one published reference figure was available.

### Minerals

10. The analysis of minerals in the non-transgenic, unsprayed reference line did not show homogeneity across all of the sites.
11. With the exception of chloride, consistently higher levels of minerals were found in the sprayed transgenic lines when compared to the reference group within the same site. This was also the case when the sprayed and unsprayed transgenic lines were compared, thus indicating that this was not a result of the genetic modification event.
12. Using pooled data from all 15 sites, no statistically significant differences were found for the minerals phosphorous, potassium, magnesium, manganese, zinc, chloride, calcium and iron between the three treatment groups. Higher levels of copper were present in the transgenic lines: 1.8 mg/kg and 2.0 mg/kg respectively in the unsprayed and sprayed transgenic groups, compared with 1.5 in the reference group. However, all three groups were within the published range of 0.8-10 mg/kg.
13. Most of the levels of minerals found in the three treatment groups fell within published reference ranges, except for magnesium and calcium. The levels of calcium were significantly lower than those reported in the published reference ranges, but were consistent within the three treatment groups, including the non-transgenic line.

### **Vitamins**

14. The levels of most of the 11 vitamins measured in the study revealed considerable variation within each site for all three treatment groups.
15. Although there were some statistical differences when individual vitamins were compared between the three treatment groups grown at individual sites, these were not consistent across all sites, and no uniform pattern was evident.
16. Across all sites, no statistically significant differences were seen between the transgenic and reference groups for 10 of the 11 vitamins, namely vitamin B1, vitamin B2, Niacin, pantothenic acid, folic acid, alpha tocopherol, beta tocopherol, gamma tocopherol, alpha tocotrienol vitamin E activity.
17. The mean level of delta-tocopherol was significantly lower in the non-transgenic group than in the unsprayed transgenic group (0.16 and 0.18mg/100g dry matter respectively). The value for the sprayed transgenic group was intermediate.
18. The levels of all the vitamins in the pooled over-all-sites data fell within published reference ranges, with the exception of folic acid. The levels of folic acid in all three treatment groups were higher than the upper figure for the published reference range for this vitamin, with the highest level being evident in the non-transgenic unsprayed reference group. However, there were no significant differences in folic acid between the non-transgenic and two transgenic groups, in either the site-by-site or the over-all-sites analyses.

### **Anti-nutrients (phytic acid)**

19. The phytic acid levels were very variable in all three treatment groups and the non-transgenic reference group did not show homogeneity across all of the sites. A slightly higher level of phytic acid was found in the transgenic samples at a number of the individual sites. However, no statistically significant differences were found between the three treatment groups in the over-all sites analysis.
20. The levels of phytic acid in the over-all-sites pooled data were found to fall within the published reference ranges.

### **Total Amino Acids (after hydrolysis)**

21. Homogeneity of the levels of total amino acids was demonstrated for the non-transgenic reference group across all sites. The site-by-site analysis showed no differences between sites for all the amino acids for the three treatment groups.

22. Although no significant differences were observed among the field data, small differences were observed in 7 of the 18 amino acids included in the analyses when compared with published reference ranges. In all cases, the differences were represented across all three treatment groups, and were not considered to be significant.

#### **Free Amino Acids**

23. The site-by-site analysis did not show homogeneity in the non-transgenic reference group for these components, with the exception of asparagine, tryptophan and the sum of all 18 free amino acids.
24. No consistent differences were seen in the statistical analysis carried out on the transgenic plants either in the site-by-site or over-all-sites analyses.
25. No published reference ranges are available for free amino acids, and so a comparison was not possible.

#### **Total Fatty Acids (after hydrolysis)**

26. Homogeneity in the non-transgenic reference group could not be established for total fatty acids across all of the sites.
27. Significant differences between the three treatment groups were only observed for 2 of the 10 fatty acids, namely palmitoleic acid and eicosenoic acid. The differences between treatment groups were mixtures of increases and decreases depending on the site and no consistent changes were observed. The three treatments can be considered equivalent with respect to the over-all-sites analysis.
28. Total fatty acids in the non-transgenic and transgenic lines were in good correspondence with published reference ranges, where these were available.

#### **Free Fatty Acids**

29. Levels of 12 free fatty acids were analysed in all of the samples but 5 of these were consistently below the limit of detection. The remaining 7 – myristic, palmitic, palmitoleic, oleic, linoleic, linolenic and behenic acids – were detectable in only a minority of the samples and at very low levels. Where statistical comparisons were possible, there was no uniform tendency in the differences between the three treatment groups. No published reference values were available for free fatty acids.

#### **CONCLUSION**

30. The results of this study have shown that there is considerable variability in the composition of crops grown on different sites or at different times. Most of the values obtained from the transgenic and non-transgenic lines in this study fall within the published reference

values. Where they do not, this may either reflect differences in the measurement method or the limited range of the analyses reported in the literature.

31. The Committee is content that the data presented demonstrates stable inheritance of the introduced trait and do not suggest that genetic changes are affecting the modified plant's metabolism.
32. The Committee is satisfied that the majority of components measured in the T25 seed fall within standard reference ranges for maize and that any statistically significant differences found between the three different test groups are not of biological significance when viewed in the context of the normal ranges.

December 2002

## APPENDIX XIV

Mr A Klepsch  
European Commission  
DG SANCO  
Rue de la Loi 200  
B-1049  
Brussels  
Belgium

21 November 2002

Reference: NFU 408

Dear Mr Klepsch

### **Argan Oil (*Argania spinosa* L.)**

As the UK Competent Authority, the Food Standards Agency has sought expert advice from the Advisory Committee on Novel Foods and Processes (ACNFP) on the notification submitted to the Commission by a French company on the 26 August 2002 regarding the marketing of argan oil under Article 5 of regulation 258/97. We wish to make the following comments:

#### (i) allergenicity

The ACNFP has expressed concerns regarding the allergenic potential of this product. No studies have been provided on the proteins present in the oil and the applicant has supplied no information on allergenicity. Given the known allergenic potential of nuts per se there seems a particular risk of cross-reactivity with this product which do not appear to have been addressed. Furthermore, the taxonomy of *Argania spinosa* L is not sufficiently detailed for a clear indication of the allergic potential of this product to be drawn by comparison with closely related food sources. Without this information, we are of the view that actual studies should be provided.

#### (ii) undesirable substances

No information has been provided on the levels of hydrocyanic acid which breaks down to form cyanide and has been a concern with similar products. The applicant has specified that argan oil contains relatively small amounts of heavy metals for example less than 0.5mg/kg of lead and arsenic. However oils such as sesame oil, to which the applicant claims argan oil substantial equivalence, comply with the Codex Standard for Named Vegetable Oils which specifies that the lead and arsenic content should not exceed 0.1mg/kg.

(iii) variability in composition

An additional concern identified by the ACNFP is that the trees are uncultivated and it is likely that there will be extensive genetic diversity, which may result in significant variability in the composition of the oil. However, the available data on composition appear to result from the analysis of only a single batch. We believe that further data should be provided to demonstrate the range of the various constituents present in the oil.

(iv) substantial equivalence

We are of the opinion that this notification does not meet all of the criteria set out in Article 3(4) of the novel food regulation (EC) No 258/97. It is difficult to see how the oil can be substantially equivalent to two or more different products. It is also clear that the unsaponifiable fraction differs from the existing oils, both in terms of phytosterols and the flavour components. We therefore believe that a full application should be used to gain pre-market approval for argan oil. In the interests of consistency it should be noted that similar concerns were raised in the UK over a recent Article 5 notification for phytosterols and phytostanols.

We also note that argan oils can be produced from either toasted or untoasted kernels. The present application relates to oil extracted from untoasted kernels and we consider that a separate evaluation would be required for oil extracted from toasted kernels.

In conclusion, the UK Competent Authority considers that argan oil cannot be regarded as substantially equivalent to the named vegetable oils and that a full authorisation is required. In order for an assessment to be carried out, the company should provide additional information to address the points mentioned above. We would therefore welcome the opportunity to discuss this product at the next Competent Authority meeting.

Yours sincerely

Dr Sandy Lawrie  
Novel Foods Division

## APPENDIX XV

### ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

#### GUIDELINES ON THE CONDUCT OF TASTE TRIALS INVOLVING NOVEL FOODS OR FOODS PRODUCED BY NOVEL PROCESSES

##### INTRODUCTION

1. The Advisory Committee on Novel Foods and Processes (ACNFP) is an independent Committee of experts that advises the central authorities responsible, in England, Scotland, Wales and Northern Ireland respectively on any matters relating to novel foods and novel food processes, having regard to the views of relevant expert bodies where appropriate.
2. The EC Novel Foods Regulation (EC 258/97) defines a 'novel' food as a food or food ingredient which has not previously been used for human consumption to a significant degree within the European Community. Amongst the foods covered by this definition are low calorie fat replacers, GM foods and an increasing range of dietary products such as some functional foods. All foods that are deemed novel are required to undergo a rigorous pre-market safety assessment. Pre-market acceptability trials as described by these guidelines are not generally regarded as 'placing on the market' within the context of the EC Novel Food Regulation 258/97.
3. In 1991, the ACNFP issued Guidelines (1) on the assessment of novel foods and processes, to assist those wishing to develop and/or market such foods in the UK. Included in those Guidelines is a brief section on human studies, such as taste trials and marketing and acceptability trials.
4. In 1992, the ACNFP published general guidance relating to ethical, as well as safety, criteria for the conduct of taste trials on novel foods or foods produced by novel processes (2).
5. The ACNFP guidelines on the assessment of novel foods and processes (1) were superseded by the EC Novel Foods Regulation (3) which came into force in May 1997 with accompanying guidelines on the provision of information, including that relating to previous human exposure to the novel food or its source (4). Additional guidelines on novel foods and novel food ingredients legislation were produced by the Ministry of Agriculture, Fisheries and Food/Department of Health (5). However, none of these address the issues of taste trials or human studies *per se* and, therefore, the need for such guidance remains.

6. The Committee is preparing guidelines on the use of human studies in the pre-market safety assessment of novel foods (6). These focus on the circumstances in which such studies might be appropriate and the issues that need to be considered when conducting human studies on novel foods. In view of these, and given that the existing guidelines on taste trials (2) were produced some time prior to the Novel Foods Regulation, it was considered prudent to reconsider and update them at this time.
7. The Committee wishes to stress that these guidelines relate solely to taste trials, and not to exercises related to preliminary marketing/monitored sales, nor to studies designed to assess safety (which are addressed elsewhere (6)). The guidelines are intended for use by those developing novel foods.

#### General Guidance

8. The ACNFP is of the opinion that, in general, there is no need for protocols for taste trials to be referred to it for consideration provided that certain conditions are met:
  - (i) Those carrying out the trial are satisfied, after taking suitable professional advice, that it poses no hazard to human health;
  - (ii) The protocol for the taste trial had been referred to, and cleared by, an independent Ethics Committee (see paragraph 10);
  - (iii) Appropriate records are kept (see paragraph 13);
  - (iv) If the trial could involve the release of genetically modified organisms into the environment, the appropriate notification and clearance procedures are followed (see paragraph 14).

#### *Assessment of Risk to Human Health*

9. In considering whether to proceed with a taste trial, it is necessary to carry out a risk assessment, taking into account the likely levels of intake from a taste trial and the extent of information on the safety of the product. Where there is limited information on the safety of the product, taste trials should not proceed. It is recommended that individuals with known food allergy or intolerances or individuals with gastrointestinal disorders should be excluded from taste trials.

#### Local Ethics Committees

10. Irrespective of the guidance obtained from this document, any relevant legal requirements relating to the performance of studies on human subjects should be adhered to. Detailed guidance on the legal and ethical considerations of studies in human subjects is available elsewhere (7, 8, 9, 10, 11, 12). One of the prime requirements relates to

the need for all research involving healthy volunteers to be approved by an independent Ethics Committee. Such Committees exist within major industrial companies or, alternatively, organisations can refer their research to the local or regional Ethics Committees that already exist at many Universities and Medical Schools for assessment. However, it is recognised that these Ethics Committees produce their own guidance on what products should and should not be referred to them for consideration and such guidance must be borne in mind in any such referral. The UK Government has produced guidelines advising on the structure and function of local research ethics committees (13, 14).

11. Ethics Committees should be able to draw on sufficient technical competence and informed judgment to be able to assess the consequences of participation in the trial, in the context of the welfare of the subject and the objectives of the investigation. However, the Committees also need to accommodate respected lay opinion so as to provide effective representation of community, as well as scientific interests.
12. Other important issues include the method of recruitment of volunteers and the need for full informed written consent. A copy of the explanatory information to be given to volunteers should be submitted to the ethics committee, which should be satisfied that the information is adequate and in a form that would be understood readily by the volunteers. The information supplied to volunteers *must* include details of any known adverse reactions to the novel food/ingredient. The company conducting the taste trials should also send a letter to the GP's of the volunteers providing the details of the study.

#### Records

- 13 Records should be kept on the conduct of, and results from, taste trials and should include the names and particulars of the individuals involved, including their health status, and also details of the novel food involved in the trial. These records should be retained for 30 years. Any adverse effects reported by the volunteers should be recorded and followed up for a suitable period, with medical investigation if necessary. Classification and reporting of adverse effects is dealt with in a report on Adverse Reactions to Food and Food Ingredients published by the Committee on Toxicity (COT) (15).

#### Release to the Environment

14. (i) If the production of the novel food has taken place in the UK and involves the contained use of a live genetically modified organism<sup>1</sup>, then the centre will have been notified to the Health and Safety Executive (HSE) under The Genetically Modified Organisms (Contained Use) Regulations 2000.
- (ii) If the novel food contains live genetically modified organisms (GMOs) then, under The Genetically Modified Organisms (Contained Use) Regulations 2000, any contained use, including taste trials, must be undertaken in premises that have been notified to HSE. Individual activities such as taste trials will only need to be notified to HSE if, for GM plants and animals, the GMO is more harmful to humans than the non-modified organism. For GM micro-organisms (GMM), the individual activity only requires notification if the GMM is likely to cause adverse effects on humans or the environment. Consequently, it is highly unlikely that individual taste trials would require notification, although the requirement for the premises to be notified must be complied with.
- (iii) The Genetically Modified Organisms (Deliberate Release) Regulations 1992 as amended in 1996 and 1997, together with Part VI of the Environmental Protection Act 1990, implement EC Directive on the Deliberate Release into the Environment of Genetically Modified Organisms (90/220/EEC)<sup>2</sup>. They require specific consent for release from the Secretary of State for the Environment in England (or the devolved authorities in Scotland and Wales). Taste trials involving the release of genetically modified organisms, including via waste, will require such consent.
- (iv) The contact point for contained use of genetically modified organisms is:

Health and Safety Executive (HSE)  
Health Directorate  
Room 6.19 Rose Court  
2 Southwark Bridge  
London SE1 9HS

<sup>1</sup> Defined in the EC Directive on the Deliberate Release into the Environment of Genetically Modified Organisms (90/220/EEC) (as amended by Directive 2001/18)

<sup>2</sup> Directive 2001/18 entered into force on the 17 April 2001, the date of its publication in the Official Journal of the European Communities. Implementation of, and compliance with, most provisions in the Directive will, however, require Member States to take measures at national level. Member States have until 17 October 2002 to bring such measures into force. Directive 90/220 will also be repealed on that date. Until then, the relevant provisions of Directive 90/220 will remain in force, although the UK intends as far as possible to apply the principles of the new Directive ahead of formal implementation.

The contact point for releases of genetically modified organisms to the environment is:

Joint Regulatory Authority  
Department of the Environment, Food and Rural  
Affairs (DEFRA)  
GM Policy and Regulatory Unit  
Room 3/G9 Ashdown House  
123 Victoria Street  
London SW1E 6DE

#### Other Points

15. The Committee has indicated that where the above conditions are met there is no need, on a routine basis, for protocols for taste trials to be referred to it consideration. However, the Committee is willing to give advice in individual instances, particularly those involving difficult or complex issues.
16. Those seeking further information, or wishing to obtain advice from the Committee should, in the first instance, contact Mrs Sue Hattersley at:

Food Standards Agency  
Room 526B  
Aviation House  
London  
WC2B 6NH

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  15. Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) Report on Adverse Reactions to Food and Food Ingredients (2000). Available from the COT Secretariat on request.

## APPENDIX XVI

### ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

#### CONSIDERATION OF THE USE OF HUMAN STUDIES IN THE PRE-MARKET SAFETY ASSESSMENT OF NOVEL FOODS

##### INTRODUCTION

1. This paper briefly reviews the role of human studies in the pre-market safety assessment of novel foods and focuses on the circumstances in which such studies might be appropriate and the issues that need to be considered when conducting human studies on novel foods. It should be noted that such studies are performed to support other safety studies and not to investigate potential toxicity.

##### Definition of Novel Foods

2. The EC Novel Foods Regulation (EC 258/97) defines a 'novel' food as a food or food ingredient which has not previously been used for human consumption to a significant degree within the European Community. Amongst the foods covered by this definition are low calorie fat replacers, GM foods and an increasing range of dietary products such as some functional foods. All foods that are deemed novel are required to undergo a rigorous pre-market safety assessment. Pre-market safety trials as described by these guidelines are not generally regarded as 'placing on the market' within the context of the EC Novel Food Regulation 258/97.

##### Safety Assessment of Novel Foods

3. Safety assessments of novel foods should be carried out on a case-by-case basis, including, where appropriate the results of conventional animal toxicological studies. In such studies the test compound is normally fed to animals at a range of doses, some several orders of magnitude greater than expected human exposure. However, foods are intended to be consumed by humans at levels that approach the maximum dose that could be used in animal studies and therefore for many novel foods such studies may not be feasible (1). In such circumstances risks should be characterised as completely as possible by comparison with closely related existing food products e.g. consideration of the key nutrients and toxicants. This approach is termed 'substantial equivalence' and identifies any similarities and differences between a novel food and its conventional counterpart. Where differences are found the safety assessment will focus on any potential safety and nutritional issues arising from these differences. Where a novel food can be

demonstrated to be substantially equivalent to a conventional counterpart, i.e. no differences are found in the comparison, it is considered to be as safe as this conventional counterpart and no further safety assessment is required. This conclusion is based on the assumption that since individual ingredients have an extensive history of consumption a new combination of such ingredients would not raise any new safety issues.

4. If a novel food is not substantially equivalent, either because the differences cannot be defined or because there is no existing conventional counterpart, this does not mean that the food is not safe. In such cases a detailed data package is required to facilitate a rigorous pre-market safety assessment.

#### ACNFP Decision tree

5. In 1990 the UK Advisory Committee on Novel Foods and Processes (ACNFP) developed a decision tree (2) to indicate the types of data that were likely to be required for assessing the safety of individual novel foods. This tree, which was reviewed and extended in 1994 (3), includes 15 possible information categories, one of which refers to “Human Studies.”
6. The ACNFP, in addressing the role of human studies in the safety assessment of novel foods, acknowledged that, ‘There is a wide diversity of studies that may need to be performed in humans on novel foods or products derived from novel foods, including the tasting of a new variety of an existing food organism, large scale acceptability and marketing trials and tests for intolerance or allergenicity. These studies in humans are to confirm acceptability and tolerability, not to investigate potential toxicity’ (2). Such studies can be considered under the following study types:
  - (i) Tasting/palatability;
  - (ii) Single dose/short term repeated dose studies for digestibility and tolerance;
  - (iii) Allergenicity, including observations of any allergic reactions in occupationally exposed personnel;
  - (iv) Acceptability/marketing trials; and
  - (v) Post-marketing surveillance.
7. Tasting/palatability and acceptability/marketing trials do not constitute safety assessment studies and are therefore outside the scope of this paper (the Committee has published guidelines on the conduct of taste trials involving novel foods elsewhere (4)). Clearly, post-marketing surveillance studies, with the intention of providing further public reassurance of the safety of novel foods, also fall

outside the scope of this paper. Human studies covered by these guidelines would be relatively short term, and therefore would be unlikely to detect low incidences of allergic reaction. Detection of such effects would require Post Market Surveillance. Research on the feasibility of the post-marketing surveillance of novel foods is currently being conducted by the FSA.

#### **Guidelines accompanying the EC Novel Foods Regulation**

8. Following the introduction of the Novel Foods Regulation, the European Commission (5) published a detailed set of guidelines setting out the type of information that would be expected to support an application for approval of a novel food to ensure that all member states follow a similar approach to the safety assessment of novel foods. These guidelines draw upon the structured approach developed by the ACNFP and require a detailed data package to facilitate a rigorous safety assessment. Not all the data requirements will be relevant to every novel food submitted and the appropriateness of human studies as part of this overall data package should be assessed on a case by case basis.

#### **When are Human Studies Justified?**

9. A comprehensive framework for the safety assessment of novel foods already exists but, given the wide variety of foods and food ingredients that are potentially covered by the Novel Foods Regulation, it is not possible to draw up a comprehensive list of foods/ingredients for which data from human studies would be required. However, there are certain circumstances when such testing is likely to be particularly appropriate and it is hoped that the following may serve as a guide for when human studies are applicable.

#### *Safety Considerations*

10. If substantial equivalence to a conventional counterpart can not be established the toxicological assessment, which will include a systematic review of the relevant existing information, may identify potential concerns. For example, in the safety assessment of novel fats it is important to address health outcomes known to be associated with dietary fats, such as possible thrombogenic potential. Given that such a novel food is likely to be consumed by individuals at risk for coronary heart disease (CHD) and thus susceptible to any potential thrombogenic activity, participants at moderately increased risk of CHD e.g. middle-aged, overweight, need to be investigated. The safety assessment of novel dietary fibres will also need to address issues such as digestibility in the human gut and effects on normal gut flora, which may be difficult to predict using data from in vitro and animal studies.

#### *Nutritional Considerations*

11. The overall safety assessment must consider the nutritional implications of the novel food both at expected and high intakes, taking into account the effects of storage, further processing and cooking. If substantial equivalence cannot be established (see para. 4) and the novel food is anticipated to have an important role in the diet, while appropriate preliminary assessments should be made in animal models to establish some aspects of nutritional quality, a full nutritional assessment needs to be carried out in humans. Nutritional, including metabolic, outcome measures should be relevant to the objective of the study (for example, the effect of fat replacers on the absorption of fat soluble vitamins) and to the anticipated consumer groups (for example, particular attention should be paid to the nutritional requirements of specific population groups, including infants and children, pregnant and lactating women, and the elderly).

#### *Allergenic Considerations*

12. It is not always possible to make a full assessment of allergenic potential of a novel food without challenge testing in humans. As a general principle if the novel food is similar to or derived from a conventional counterpart associated with food allergy, sera from individuals with confirmed allergies to that conventional food can be used for specific *in vitro* immunological tests. If such tests are negative, skin prick tests and oral challenges of such individuals may be carried out (5).
13. If there are no similarities with conventional foods with associated allergies, a number of other factors can serve as indicators of possible allergenicity e.g. sequence homology of the novel protein with known allergenic proteins. Additional evidence might include reports of workers' sensitisations. However, while it is realised that the current assessment of the allergenic activity of novel foods is problematic, human studies should only be carried out to confirm the lack of allergenicity in those novel foods that are considered potentially allergenic but have proved negative in subsequent *in vitro/in vivo* immunological tests as opposed to a general screen for allergenicity.

### **ISSUES TO BE CONSIDERED IN THE CONDUCT OF HUMAN STUDIES ON NOVEL FOODS**

#### **Ethics of Human Studies**

14. As well as the need for clearly defined, scientific justification for conducting human studies on novel foods (see para. 19), careful consideration must be given to the ethical aspects of such studies. This latter aspect is of particular importance when there is no direct benefit to the participating subject i.e. non-therapeutic research,

which encompasses research on novel foods, as opposed to therapeutic research<sup>3</sup>. The rights, safety and well being of participants taking part in human studies are protected by the principles laid down in the Declaration of Helsinki (6, 7). One of the important principles established by this code is the need to assess possible risk (see para. 16) compared to potential benefit. In such instances where there is no direct benefit to the participant, benefits to the population at large should be considered. The following discussion on the ethical aspects of human research is only a guide to those intending to carry out research on novel foods. The issues are addressed in greater detail elsewhere (8, 9, 10, 11) and anyone intending to conduct such studies should refer to these guidelines. For example, the Council of Europe has set out 16 principles on the ethics of research in humans (10), which address the need for inter alia respect for the individual, informed consent, an appreciation of the benefit relative to the risk involved, and ethic review procedures.

15. All human studies on novel foods must receive approval of an independent Ethics Committee. Such committees exist in hospitals, universities and industrial companies. The UK Government has produced guidelines advising on the structure and function of local research ethics committees (12, 13). The Royal College of Physicians has published Guidelines on the Practice of Ethics Committees in Medical Research involving Human Subjects (14). As a minimum such ethics committees will need to know:
  - (i) Has the scientific merit of the proposal been properly assessed?
  - (ii) How will the health of the research participants be affected?
  - (iii) Are there possible hazards and, if so, adequate facilities to deal with them?
  - (iv) What degree of discomfort or distress is foreseen?
  - (v) Is the investigation adequately supervised and is the supervisor responsible for the project adequately qualified and experienced?
  - (vi) What monetary or other inducements are being offered researchers, participants or anyone else involved?
  - (vii) Are there proper procedures for obtaining consent from the participants or where necessary their parents or guardians?
  - (viii) Has an appropriate information sheet for the participants been prepared?

<sup>3</sup> Therapeutic research encompasses both treatment and prevention of disease, offering direct and possibly immediate benefit to the participant; in non-therapeutic research such benefits are either long delayed or unlikely.

### Assessing the risks to human health

16. Before conducting human studies on novel foods a risk assessment must be performed to determine whether these studies would pose a risk to human health. **It is important to stress that these studies in humans are to support other safety studies, not to investigate potential toxicity.** In this context therefore, risk means the risk of causing physical disturbance, discomfort or pain, or psychological disturbance to the participant, as opposed to the risk of serious harm, which no ethics committee would approve in any case. Whilst judgements will have to be made as to what is an acceptable level of risk in each case, in general the risks to those participating in human studies should be minimal i.e. studies that cause more than minimal anxiety, distress and lowering of self-esteem should be avoided. It is also recommended that all individuals with known food allergies or intolerances or individuals with gastrointestinal disorders should be excluded from such studies. However, it may be appropriate to include them at a later stage but only after suitable screening for cross-reactivities etc.

### Informed consent

17. No research may be carried out without the informed, free, express and specific consent of all the participants in the study. Furthermore, such consent may be freely withdrawn at any phase of the research, and the subject undergoing the research should be informed, before being included in it, of their right to withdraw their consent. Therefore, those conducting the study have a duty to explain, in language that is understandable to the lay person, the nature and the purpose of the study and to inform volunteers of possible risks and inconveniences involved in participating in the study. Participants should be clear about what is expected of them during the study e.g. there may be restrictions on the type of food they can eat or they may be asked to refrain from consuming alcohol. Any procedures that may be performed during the study e.g. taking blood samples should also be clearly explained. The Royal College of Physicians guidelines (14) recommend that such information be given in written form, for example as a Subject Information Sheet (see para. 15). Participants in the study should be assured of confidentiality. Any blood or tissue samples taken must be used only for the study for which consent has been given, and must be randomised so that they cannot be traced back to a specific volunteer (for more information see reference 20). The Company carrying out the study should also send a letter describing the study to the GPs of each volunteer to ensure that they are aware of any potential health risks associated with the study, and therefore give them the opportunity to advise the volunteer about taking part in the trial.

18. Children should not be the subjects of research that might equally well be carried out in adults. However, if studies on novel foods are deemed necessary in children (para. 15), as well as the child's consent, parental consent must also be obtained in all cases, even when a child is competent to consent.

#### Study Design and Protocol

19. There must be a clearly defined question or hypothesis accompanied by scientific justification for conducting human studies on novel foods. Such studies should be designed, conducted, analysed and reported according to sound scientific principles to achieve their objectives. The objectives should be clearly and explicitly stated at the outset and each part should be defined in a written protocol before the study starts. The following principles are discussed in greater detail elsewhere (15).

- (i) The objective(s) of the study, on the basis of effects that may be expected to occur as predicted from the pre-clinical data, should be clearly and explicitly stated at the outset of the study.
- (ii) The appropriate study design should be chosen to achieve the study objective(s) effectively. Control or reference groups are used to allow for the effect of natural variability in the outcome measures, as proper randomisation (see paragraph 19iii) will ensure similar natural variability between control and treatment groups. In parallel trials groups of participants fed either the novel food (treatment group) or its conventional counterpart (control group) are compared to detect potential differences in the selected outcomes. Cross-over trials, where groups receive both the novel food and the conventional counterpart at random, are used to reduce natural variability of the outcome measures even further by eliminating inter-individual variability as participants act as their own controls for treatment comparisons. While this design reduces the number of participants required to achieve a specific statistical power, a carry-over effect of the treatment i.e. a residual influence of the novel food in the subsequent period when the participant receives the conventional counterpart can compromise the study although such a possibility can be minimised by the use of a 'wash-out' period between treatments. In such cases a parallel study may be more appropriate.
- (iii) The protocol should specify methods to minimise bias. Random allocation to comparative groups will ensure that factors, which are known to be associated with the outcomes, as well as those which are not known, are distributed without bias between the groups being compared. Blinding is an effective way of minimising bias. A trial where the subject is unaware of the treatment assignment is referred to as a single blind study. When the clinical investigator is also unaware of treatment assignment the study is double blind.

- (iv) Outcome measures should be defined based on the study objective(s). The outcome measures chosen should be assessed for their accuracy, precision, reproducibility, reliability, validity, feasibility and cost, and they should be relevant. Baseline measurements are essential and routine clinical observation and monitoring and regular clinical chemistry measurements should be maintained throughout the study even when it is not anticipated that these parameters will be altered by the novel food. Arrangements for dealing with abnormalities detected during the study should be in place from the outset
- (v) The sample size should be based on the consideration of differences between comparative groups in outcome measures regarded as clinically significant, the anticipated variability in these outcome measures within each group, and should be large enough to give sufficient statistical power to the study. Procedures to calculate sample sizes are presented elsewhere (16).
- (vi) At the outset of the trial there should be defined selection criteria taking into account pre-clinical knowledge. If the investigation can be performed in healthy adult volunteers it should be. However, there may be instances when studies on special sub-groups of the population are required e.g. participants with a particular disease that may benefit or be adversely affected by the consumption of the novel food, for example people with increased risk of coronary heart disease and foods intended to lower blood cholesterol levels. In such instances the ethical considerations of conducting studies in populations such as these will be different to that for healthy adults. With regards to research in children the British Paediatric Association have published ethical guidelines (17).
- (vii) All human studies on novel foods should be conducted in accordance with the principles of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human use Guideline for Good Clinical Practice (ICH/GCP) (18). ICH/GCP is an international ethical and scientific quality standard for designing, conducting, recording and reporting studies that involve human participants. The clinical investigator must be scientifically and professionally competent and aware of the principles of the study. There should be adequate resources of time, staff and data recording equipment, and safeguards for confidentiality. Further investigations should also be in accord with the principles of Good Laboratory Practice (GLP) to ensure that laboratory staff are appropriately qualified and that the equipment is reliable. A quality assurance scheme that monitors the laboratory analysis can provide further reassurance of the adequacy of the study.

- (viii) In the event of any adverse events encountered during human studies, these should be reported clearly, with the adverse reaction being appropriately classified, for example toxic or non-toxic, allergic reaction or intolerance reaction. Classification of adverse reactions is dealt with in a report on Adverse Reactions to Food and Food Ingredients published by the Committee on Toxicity (COT) (19).
- (ix) The study protocol should have a specified analysis plan that is appropriate for the objectives and design of the study taking into account the specific hypotheses to be tested, the analytical methods of the outcome measures, and approaches to common problems including protocol violations.
- (x) All results should be analysed and adequately documented and be publicly available. The study report should include results presented as absolute numbers. The statistical power of the study should be stated as well as the confidence limits of any differences observed.

## CONCLUSIONS

- 20. Human testing may form an important part of the safety assessment of certain novel foods but the need to conduct such studies should be considered on a case by case basis. There must be sound scientific justification for conducting such studies and the permission of a suitable ethics committee must be obtained. In general human studies on novel foods should be carried out on healthy adult volunteers and should be conducted in accordance with the principles of Good Clinical and Laboratory Practice.

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19. Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) Report on Adverse Reactions to Food and Food Ingredients (2000). Available from the COT Secretariat on request.

Other Useful Information may be obtained from:

20. <http://www.doh.gov.uk/research/rd3/nhsrandd/researchgovernance/ethics/ethics.htm>

## APPENDIX XVII

Mr Andreas Klepsch  
European Commission  
DGIII  
Rue de la Loi 200  
B-1049  
Brussels  
Belgium

26 September 2002

Reference: NFU 374

Dear Mr Klepsch

### **Evaluation of Regulation (EC) 258/97**

Following on from useful discussions on the draft discussion document at the Novel Foods Competent Authorities meetings and further discussions within the Food Standards Agency, the UK Competent Authority now wishes to provide written comments on the discussion document published by the Commission in July 2002. Detailed comments are attached at annex 1.

It would be helpful to have the facility to 'stop the clock' whilst information is being sought from applicants. This does happen in practice and leaves the regulation open to criticism, as deadlines may appear not to be adhered to. Conditions for 'stopping the clock' would need to be clearly set out.

We hope these comments, which outline the UK position, are useful and I am happy to discuss the contents further.

As you know I will be moving jobs within the Agency and will be taking up my new responsibilities on the 30th September 2002. Dr Sandy Lawrie will be replacing me, but will not be taking up the post until the beginning of November. Thank you for all your help in the past and best wishes.

Yours sincerely

Sue Hattersley  
For the UK Competent Authority

- 1.3.1 Further clarification is required regarding what specifically the term “generally available” refers to. Herbal products are retailed through many outlets (including mail order) and are subject to diverse regulatory classifications throughout the EU.
- 1.3.3 This section needs to be expanded to address the border between novel foods and supplements. i.e. medicinal ingredients, PARNUTS, and Food Supplements Directive. In addition it should be noted that where a product is agreed to be exempt from (EC) 258/97 because of a previous history of consumption it may still be caught by the Food Supplement Directive (EC) 46/2002.
- 1.4.3 It is necessary to insert the phrase “if that food is already on the market “ in the 2nd sentence after “In addition”.
- 1.4.5 The second paragraph is not correct. Some early decisions were made by the Standing Committee before the NF CA group was set up, eg on psyllium.
- 1.6 The last sentence should be redrafted to read “Certain products, however, may be considered under a simplified procedure.”
- 1.6.1 Under the section “*Initial Assessment – comments and objections on the report*” reference is made to “broader, non scientific issues”. An explanation as to what these issues might be is required. The second paragraph assumes that the initial Member State is recommending approval of the novel food. However no reference has been made to a scenario when it is recommended that the novel food should not be approved (this was the case with Stevia which was still referred to the Standing Committee which could be seen as unnecessary). Is it necessary for the Standing Committee On The Food Chain And Animal Health to place conditions on approval or is it possible this could be imposed in the terms of the approval from the Initial Member State, if all other Member States agree? In the final paragraph should something be added to cover what will happen when the EFSA becomes fully operational? If as discussed previously decisions will still be made by the Standing Committee perhaps this needs to be included here?
- 1.7 Do these requirements cover the provisions of information to the final consumer? For example, with trehalose there has been some debate regarding the labelling requirements as the applicant granted the approval, produces only an ingredient and is not the producer of the final food.
- 3.1.1 The UK agrees that whole animals, including insects, should be covered.

- 3.1.2 Should the definition of the ‘production process’ novel food category include a reference to microbial contamination or does the reference to undesirable substances include microbial as well as chemical contamination? The UK would suggest that one way to deal with the question of novel processes would be to include processes that either give rise to significant changes in the food being processed (with an expansion on the meaning of ‘significant changes’) or that have implications for public health. Guidelines such as those suggested in option 2 are likely to prove difficult to implement in practice.
- 3.1.3 Option 2 should be expanded to define more clearly the border between novel foods and dietary supplements. Examples of foods, which have fallen outside the current category based definitions, but that perhaps should have been treated as novel should be included for reasons of clarity.
- 3.2 There are a number of issues regarding whether decisions should be addressed to the applicant and who can be an applicant. However, the key point is that conditions (such as labelling) attached to the approval of a novel food or ingredient should also be binding on the food manufacturers marketing the final product. Similarly any defined operating conditions for a novel processing technique should be applicable to those actually using the equipment.
- 3.3.1 Option 1 suggests that a history of safe use in third countries or a satisfactory safety evaluation by an approved third country might be grounds for using a simplified procedure to save the Community time and resources. Evaluations elsewhere in the world may be useful background information, but it is important that the standards of those evaluations are comparable to those in the EU. Therefore in each case there would be a need to investigate the supporting evidence ourselves. In addition differences between populations may make reliance on such data unsound; for example with Nangai nuts the lack of allergic reactions in the native population does not preclude the possibility of such reactions occurring if the food was introduced into Europe. The scenario described in option 2 already exists (with the use of decision trees to determine the differing extents and types of data required). Therefore it would be reasonable to have common procedures for all novel foods but with supporting information requirements that relate to the particular food in question. In Option 3 the supporting information needed currently for notifications is similar to that needed to conduct a normal safety evaluation. Therefore the suggestion to remove the simplified procedure will not result in a more resource intensive approach but will be dependent on how in practice the task is undertaken by Member States and the EFSA.

- 3.3.2 The UK often has considerable discussion with a company before an application is formally lodged but it is not clear if the EFSA will have the resources for this. This problem could be overcome by the EFSA being the initial contact point and referring the applicant to a rapporteur Member State who would take forward the detailed discussions and the evaluation. Further expansion on the remit of the EFSA and its likely working relationship with the Commission should be included in this section.
- 3.3.3 In option 1 we would like to see the whole dossier (minus any commercially confidential information) being made available to the public, not just the summary. We would also seek clarification as to whether it is the role of the EFSA or the rapporteur Member State to make the information available?
- 3.4 The labelling requirements were not formulated on the basis that all novel foods were likely to contain GMOs. It would also be important to label conventionally derived foods if their composition is significantly different (e.g. oils with a different fatty acid composition or fat replacers with different calorific values). Does there need to be some text dealing with possible claims in this section?

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The Advisory Committee on Novel Foods and Processes (ACNFP)  
is an independent body of experts whose remit is:

*'to advise the central authorities responsible, in England, Scotland, Wales and Northern Ireland respectively on any matters relating to novel foods and novel food processes, including food irradiation, having regard where appropriate to the views of relevant expert bodies.'*

**Further copies may be obtained from:**

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