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**FOOD ADVISORY COMMITTEE**

**FdAC/Enzymes/43**

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**25 May 2000**

**Agenda Item 3**

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**IMMOBILISED LIPASE FROM *RHIZOPUS NIVEUS* ("Newlase")**

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**Purpose**

1. This paper advises the Committee of the latest recommendations of the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) with respect to a submission on the enzyme preparation "Newlase". The Committee is invited to consider whether, in the light of the COT's latest advice, the existing temporary clearance for the use of this enzyme preparation should be extended.

**Background**

2. There are currently no requirements for the use of enzyme products used as processing aids in food to be formally approved under UK regulations. However, as an enzyme preparation used in a novel process (interesterification) evaluated by the Advisory Committee on Novel Foods & Processes (ACNFP), this product falls within the scope of the Committee's limited review of enzyme preparations, which commenced in 1993.

3. In 1993, under the voluntary arrangements then in place, the ACNFP cleared an enzymically modified oil produced by the Fuji Oil company Ltd. (also known as Vamo Fuji). The modified oil was intended as a substitute for cocoa butter in chocolate and for confectionery products. It was prepared by enzyme-catalysed interesterification, a novel process similar to that approved for Unilever in 1992, in which vegetable oils such as safflower oil or sunflower oil are reacted with ethyl stearate in the presence of an immobilised lipase. The lipase enzyme, triacylglycerol acylhydrolase (EC 3.1.1.3), has a reaction specificity for the 1,3-positions of triglycerides. It incorporates stearic acid into the 1- and 3- positions of

triglycerides while leaving the 2- position unchanged. The ACNFP cleared the oil subject to the approval of the enzyme by the FAC and the COT.

#### **Previous COT and FAC consideration**

4. The enzyme preparation was first considered by the COT in January 1994. The Committee recommended temporary clearance for 12 months for use in the production of modified vegetable oil as a cocoa butter substitute in chocolate and confectionery, subject to the provision of additional information on a number of points, including a revised specification incorporating a limit for rhizoxin, a mycotoxin associated with some strains of *Rhizopus* species.

5. The FAC considered the COT's advice in April and July 1994 (FdAC/Enzymes/15 and FdAC/Enzymes/17), along with information on the manufacturing process, the company's process controls and a draft specification. The Committee agreed with the COT's recommendation and advised that the product should be given temporary clearance for one year for use in the production of modified vegetable oil as a cocoa butter substitute in chocolate and confectionery, pending review of the information requested by the COT. A product specification was temporarily cleared by the FAC in August 1994, with the method for rhizoxin testing still to be agreed.

6. The COT considered further information from the company at its meeting in September 1998. The Committee was content that all of its concerns had been addressed, apart from that relating to the method used to detect rhizoxin and routine testing for the mycotoxin. The opinion of the Committee was that the company had not provided sufficient evidence to demonstrate the presence or absence of rhizoxin. Furthermore, the COT did not accept the company's proposal not to test for rhizoxin on a routine basis. Accordingly it was unable to recommend full approval but recommended temporary approval for a further 12 months with the proviso that a satisfactory analytical method for the detection of rhizoxin would be developed and rhizoxin tested for on a routine basis. The COT Secretariat wrote to the company to advise it of this and the FAC was informed of the COT's decision (FdAC/Enzymes/41).

#### **Current status of submission and COT advice**

7. The company has now submitted data to answer this one outstanding area of concern and information on an analytical method to detect rhizoxin was considered by the COT at its meeting on 25 April 2000. A letter from the COT Secretariat,

accompanying the COT's detailed statement is attached at Annex 1. The COT has now recommended that this Committee extend, by two years, the temporary clearance this product. During this period the company should be asked to collate analytical data on the routine analysis of at least one in every four batches of Newlase for rhizoxin. At the end of two years these data should be submitted to the COT for review, so that the COT can be assured that the methodology is adequate for routine assay of production batches.

### **Conclusion**

8. In the light of the COT's advice (Annex 1), the Committee is asked whether the temporary clearance of this enzyme preparation can be extended by two years, during which the company should collate analytical data on the routine analysis of batches for rhizoxin in order to reassure the COT that the methodology is adequate for routine assay of production batches.

**Food Standards Agency  
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May 2000**

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## NEWLASE: ANALYTICAL METHOD TO DETECT RHIZOXIN

The Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) initially reviewed the enzyme preparation Newlase in 1994. The COT recommended that temporary clearance be granted while awaiting further data. These data were considered in September 1998 and the COT agreed to a further one year temporary clearance while awaiting a satisfactory analytical method to confirm the absence of the mycotoxin rhizoxin in Newlase. An analytical method for the detection of rhizoxin was submitted by the company in September 1999 but was considered inadequate by the COT.

At its 25<sup>th</sup> April 2000 meeting, the COT considered a revised analytical method to detect rhizoxin. The COT had two concerns about the revised methodology, namely:

1. The change in the retention time for rhizoxin between analytical batches was greater than the normal expected variation; and
2. The elution of rhizoxin from the clean-up column was not sufficiently characterised over a range of concentrations.

However, despite these reservations the COT considered that the overall methodology was acceptable provided that the protocol includes two Newlase samples spiked with rhizoxin at 0.1 ppm and 0.5 ppm to be analysed during each analytical run. The method recoveries of the spiked samples should be within 80-130% of an equivalent concentration analysed by direct injection as part of the same analytical run. This would ensure that the samples are assessed at the correct retention time and that the clean-up column is eluted properly, therefore addressing the two concerns regarding this methodology.

The COT recommended that at least one in every four batches be analysed using this revised methodology to confirm the absence of rhizoxin. This should form part of the specification for Newlase such that if rhizoxin is detected by this methodology that particular batch is discarded and subsequent batches are only accepted provided they conform to the specification. The COT considered that in the absence of adequate toxicological data they were unable to determine a concentration of rhizoxin which would not be considered a risk to human health.

**The COT recommended that the FAC extend, by two years, the temporary clearance of Newlase. During this period the company should be asked to collate analytical data on the routine analysis of at least one in every four batches of Newlase for rhizoxin. At the end of this two-year period these data should be submitted to the COT for review, so that the Committee can be assured that the methodology is adequate for routine assay of production batches of Newlase.**