

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES**LYCOPENE-RICH OLEORESIN FROM TOMATO****ISSUE**

At the January meeting, Members requested further information to be provided to be able to complete the assessment of LycoRed's application. Members are therefore invited to consider the responses provided to their comments and are asked whether they recommend authorisation of this application.

Background

1. An application has been submitted by LycoRed for the authorisation of an lycopene-rich oleoresin derived from tomato, as a novel food ingredient (NI). This application was accepted by the UK Competent Authority, on 7 September 2004. In accordance with Article 6(3) of the novel foods regulation (EC) 258/97, the UK had 3 months to prepare an initial assessment report. This was extended as the ACNFP asked for additional information from the applicant at the Committee's meeting in November 2004. Once the UK initial assessment on LycoRed's application is finalised, the European Commission will circulate this to the other European Competent Authorities for comment.
2. At the January meeting, Members considered the LycoRed's response to the comments they made in November 2004. They were not completely satisfied with all the additional information provided by the applicant and made the following comments:
 - (i) Members were still concerned that the nutritional benefits provided by the addition of the oleoresin to foodstuffs such as ice-cream, cakes or biscuits would be compromised by the presence of high level of sugar and fats in such products;
 - (ii) They also stated that the applicant did not address their concerns on the potential over- consumption of the aforementioned products by children;
 - (iii) Members asked the Secretariat to seek advice from a specialist in animal pathology on the significance of the increased lung weight of female rat observed in the semi-chronic toxicity study and
 - (iv) Members stated that the gel chromatograph used to investigate the level of protein in the oleoresin was of poor quality and further analysis should therefore be carried out.

3. The Secretariat wrote to the applicant on 7 February 2005, seeking clarification of each of these issues (see Appendix 1)
4. Regarding points (i) and (ii) above, the Secretariat has advised the applicant that these concerns would be reflected in the Committee's opinion.
5. Concerning point (iii), the detailed expert's advice stated that the observed increased absolute lung weights was not indicative of a target organ toxic effect and related to the body weight increases for rat females, caused by treatment (see Appendix 2).
6. Finally, the applicant has acknowledged that further protein analysis should be carried out on its oleoresin and is currently working on providing this data to the Committee.

Committee Action required

7. The Committee is asked to note the expert's advice provided in Appendix 2. If not, the Committee is asked to indicate what additional data would be required.
8. New data on the protein content of the oleoresin should be shortly provided by LycoRed and will be circulated to Members for comments.
9. In the meantime, the Committee is also asked to consider and comment on the text of the draft opinion attached as Appendix 3, which will be finalised at a future date once the applicant has provided the outstanding data and that Members are satisfied with this data.

**Secretariat
March 2005**

Appendices attached:

- **Appendix 1:** Letter detailing ACNFP comments
- **Appendix 2:** Advice on animal pathology
- **Appendix 3:** Draft Opinion (restricted)

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

**APPLICATION FOR THE APPROVAL OF LYCOPENE-RICH OLEORESIN
FROM TOMATO AS A NOVEL FOOD INGREDIENT**

LETTER DETAILING ACNFP COMMENTS
(07 February 2005)

**Mr Peter Berry Ottoway
Berry Ottoway and Associates Ltd
1A Fields Yard
Plough Lane
Hereford HR4 0EL**

e-mail: boa@berryottaway.co.uk

7 February 2005

Reference: NFU 482

Dear Peter,

ACNFP COMMENTS

On 26 January 2005, the Advisory Committee on Novel Foods and Processes (ACNFP) considered your response of 17 January 2005 to the concerns they raised at their December meeting on LycoRed's novel food application.

The ACNFP was content with the additional data you provided on previous human exposure assessment on the oleoresin and Members were satisfied that you had shown the absence of tomatine in the oleoresin.

However, Members remained concerned that the nutritional benefits provided by the addition of the oleoresin to foodstuffs such as ice-cream, cakes or biscuits, would be compromised by the presence of high level of sugar and fats in such products. The Committee also drew attention to the possible over-consumption of the oleoresin by children as a result of LycoRed's intention to use its oleoresin in products such as fruit juices, yoghurts, ice-cream, desserts, biscuits and cakes. These concerns will be reflected in the UK opinion.

The Committee did not reach any conclusion concerning the increased lung weights of female rats in the semi-chronic toxicity study and has sought additional expert advice on the significance of these findings. I have already contacted the COT Secretariat and will inform you, after 21 February 2005, of their comments.

Finally, Members indicated that the gel used to detect the level of proteins in the oleoresin was of very poor quality as explained below:

- Staining of the gel is not uniform and crucially the area of the gel containing the oleoresin samples does not appear to be stained at all,
- There only appears to be a single molecular marker and this was only run in one lane. It is customary to run molecular ladders at either end of such a gel to demonstrate the resolution of the gel. This should be down to about 10 kDa.
- The picture of the gel has been presented without the sample wells being visible. It is also not clear if the whole of the length of the gel is represented in the picture as its bottom edge is not visible and there are no molecular markers smaller than 67 kDa.

The ACNFP is therefore asking LycoRed to repeat this assessment showing the absence of potentially allergenic proteins in the oleoresin. As the next ACNFP meeting will be held on 30 March 2005, could you please let me know whether you will be able to provide this analytical data by 23 February.

Yours sincerely,

(By e-mail only)

Annie-Laure Robin
Novel Foods, Additives and Food Supplements

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

**APPLICATION FOR THE APPROVAL OF LYCOPENE-RICH OLEORESIN
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ADVICE ON ANIMAL PATHOLOGY
(Phil Carthew, FRCPath, 8/02/2005)

Review of the lung weight increases in female rats after 90 days treatment with lycopene-rich oleoresin derived from tomato.

A ninety day study was performed in 1994-1995 at Pharmacia LSR (Eye, UK) testing the toxicity of Oleoresin by gavage in corn oil to Charles River CD rats. The doses administered were 45, 450 and 4,500 mg/kg bw/day.

There was a significant increase in the absolute lung weights of female rats, only at doses of 450 and 4,500 mg/kg bw/day. When the lung weights were expressed as a percentage of body weight the increases were not significant.

The study director concluded that because the female rats, treated with Oleoresin had increased body weight gains compared to the controls, the increased absolute lung weights were not toxicologically relevant, as they were no longer significant, if corrected for the increased body weight gain.

The body weight and absolute lung weight increases for female rats were,

Group	% body weight increase	% lung weight increase
45 mg/kg bw/day	3.5	7
450 mg/kg bw/day	5.7	13
4500 mg/kg bw/day	3.5	10.4

Are the increased absolute lung weights indicative of a target organ toxic effect?

The distinction of adverse from adaptive effects, which are normally regarded as non-adverse, is the key to the question of whether the increased lung weights are toxicologically significant.

In distinguishing between these two possibilities the following criteria are normally applied.

Were there any microscopic lesions in the lung indicating a toxic effect?

Commonly these would include cell death (respiratory or alveolar epithelium), regeneration of these epithelia (squamous metaplasia, or Type II cell hyperplasia), recruitment, activation or degeneration of alveolar macrophages. Infiltration of polymorphonuclear cells to phagocytose debris from epithelial damage, granuloma formation, or interstitial infiltration and fibrosis.

The report makes clear that the only evidence of any of these types of pathologies occurred in only one of twenty animals in the 4500 mg/kg bw/day group (animal 154) with a focal alveolar epithelial hyperplasia graded as slight. This would be regarded as an incidental lesion, probably not related to treatment, because of the low incidence and the low severity grading of this lesion. It is not unknown for the occasional rat to show such lesions, which may have been related to accidental misdosing (or reflux) into the lungs of the test material, during the study. The other animal with any finding (animal 159), had occasional punctate dark foci on the lungs. This was not confirmed by any significant pathology at the microscopic level, which could have been because the occasional nature of the lesion was of too low an incidence to be picked out on the sections prepared. This could have been due to accidental misdosing (or reflux) into the lungs. Again, this would be regarded as an isolated, incidental finding, not regarded as treatment related.

Possible limitations of the study.

In the methods section the statement regarding examination of tissues reads,

Microscopy

Microscopic examination was performed as follows:

- i) The tissues specified in Section 3.4.4 were examined for all animals of Groups 1 and 4 sacrificed on completion of the scheduled treatment period.
- ii) Tissues reported at macroscopic examination as being abnormal were examined for all rats.

This means that the high dose and controls were reviewed, and since there were no significant, treatment related, findings in the lung, only animals with macroscopic lesions were examined microscopically, in groups 2 and 3. This means that not all lungs in all treatment groups were examined microscopically. Animal 130 in group 3 (450 mg/kg bw /day) was examined microscopically because it had multiple punctate dark foci on the lungs and pleural adhesions (fibrosis) replacing the right lung lobes. Microscopically there was interstitial pneumonitis (moderate grade) and this was probably detected more easily than with animal 159 due to the severity (lesions probably more frequent in tissue). Again this could have been due to misgavage, or because of the extent of the lesions, perforation of the oesophagus during dosing.

Study design.

A better study design would have included a recovery group of animals at the top (or all) dose(s), to see if any organ weight increases (more commonly seen in the liver and kidney) were reversible in a treatment free period of a month or so, at the end of the study. Unfortunately this was not included in this particular study.

Conclusion.

Given that the increased lung weights were not significant when corrected for body weight, and there was no significant increase in degenerative pathologies (other than the probable incidental alveolar hyperplasia in one animal) the normal convention would be to regard the increased absolute lung weights as a non-adverse finding, related to the body weight increases for females, caused by treatment.

Given the absence of any significant incidence of lung lesions at the top dose, I would agree with the 4500 mg/kg bw/day exposure level being consistent with the NOAEL for lung toxicity in this study.

Phil Carthew FRCPATH 8/02/2005