COMMITTEE PAPER FOR DISCUSSION **ACNFP/113/10**

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

POSSIBLE TRANSFER OF DNA FROM FOOD TO HUMAN BLOOD

Issue

The Committee is invited to consider a recent article that reports on the potential presence of plant derived DNA sequences in human blood samples. It is suggested that these sequences may be derived from DNA fragments large enough to encode entire genes.

Background

1. Evidence from a large number of studies suggests that macromolecules such as DNA are degraded in the intestine before being taken up into the bloodstream and that only short fragments of DNA (100 to 200 nucleotides long) can be detected in the bloodstream; fragments that are generally too short to encode intact genes.

2. A paper published in July this year in the open access journal PLoS One (**Annex 1**) claims to have found evidence that, in fact, much longer fragments of foreign (non-human) DNA can be found in samples of human blood, a significant proportion of which are of plant origin.

3. The original aim of this study was to compare the sequence profiles of DNA present in the plasma of different cancer patients with that of normal plasma to determine if such profiles might be of use in cancer diagnostics.

4. Such DNA is referred to as “cell free DNA” as it is comprised of DNA present in blood once the cells have been removed. In an attempt to avoid external contamination of the DNA samples the authors used a contained blood collection and plasma separation system, which they claimed removed all potential sources of contamination.

5. The pooled DNA from four different groups (three from different categories of cancer patients and the fourth a normal, or control group) was size separated on agarose gels, isolated and sequenced. Three size fractions were recovered: (1) greater than 10 kilobases (kb), (2) between 10kb and 200 base pairs (bps) and (3) 200 bps in length.

6. These DNA fractions were then subjected to ‘next generation’ sequencing (NGS), a high throughput method of DNA sequencing that generates large numbers of DNA sequences long enough (50 bps) to allow identification by comparison with

existing DNA databases. This process generated 86.6 Gigabases of sequence information (1,732 Million sequence reads), but only 71.1% of these sequence reads could be mapped to the human reference genome.

7. Comparison of the foreign DNA sequences with the NCBI (National Centre for Biotechnology Information) chloroplast database revealed that over 25,000 sequence reads matched those of plant chloroplasts, the most frequent being potato and tomato.

8. Surprisingly most of the sequences that matched plant chloroplast DNA were derived from the DNA fraction larger than 10 kb, which the authors claim, suggests that food derived plant DNA can resist degradation in the gastrointestinal tract and be absorbed into the bloodstream by some unknown mechanism.

9. In an attempt to validate their findings, the authors carried out an analysis of archived NGS data from over 900 plasma samples and found similar matches to the chloroplast database that were log normally distributed. In these samples the most frequent matches were for soybean chloroplast DNA. Differences in the frequency of matches for different plant species between different samples (Fig. 4) is regarded as due to differing dietary intakes by individuals and evidence that the results are not due to external contamination. The absence of plant DNA sequences from foetal cord blood samples is also cited as evidence that the results of this study are not due to external contamination or a statistical artefact.

10. A detailed comment posted on the PLoS website by Dr R Lusk of the University of Michigan disputes the findings of the study (**Annex 2**), claiming that contamination of dilute DNA samples is a common problem with such projects and is the most likely explanation for the results presented in the article by Spisak et al.

11. Dr Lusk also contends that cord blood is not a suitable control to use in a comparison with maternal plasma, as the DNA concentration in cord blood will be much higher than in plasma, thus masking the problems encountered with dilute DNA samples.

12. Dr Lusk has performed a similar experiment to that carried out by Spisak *et al*., but using samples with no association with food. Matches to plant DNA sequences were found at a similar, or higher, frequency and with a similar distribution between samples, to those found by Spisak *et al*. The results of this study are currently being prepared for publication.

Committee action required

13. The Committee is asked to comment on the article and to indicate whether there are any safety concerns regarding the reported presence of plant DNA in human blood in relation to the risk assessment of GMOs.

Secretariat
November 2013

Annexes attached:

Annex 1: Spisak S, Solymosi N, Ittze´s P, Bodor A, Kondor D, et al. (2013) Complete Genes May Pass from Food to Human Blood. PLoS ONE 8(7): e69805. Doi:10.1371/journal.pone.0069805

http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0069805

Annex 2: Comment on the paper by R. Lusk of the University of Michigan
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