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CSIRO SEI/SEII SHRNA GM WHEAT FOR PRODUCING GRAINS WITH A LOWER CONTENT OF BRANCHED STARCH MOLECULES

Appraisal of statements by Prof Jack Heinemann and Prof Judy Carman

Background

A now well established level of regulation of gene expression is via the interfering RNA (RNAi) pathway, which includes small interfering RNAs (siRNAs) and micro RNAs (miRNAs). Recently it has been demonstrated that plant miRNA in food can survive digestion and enter the bodies of animals and humans who consume them. Furthermore, plant miRNAs that possess sequence identity to animal mRNAs can result in the knock-down (partial silencing) of the later (Zhang L *et al*. *Cell Res*. **22**: 107-126, 2012; see Hirschi KD, *Trends Plant Sci*. **17**: 123-125, 2012). These findings were totally unexpected findings but which have major nutritional, health and safety consequences. Therefore, based on these recent findings it is scientifically valid to raise safety concerns regarding the GM wheat produced by the Australian CSIRO, which is engineered to suppress expression of the SEI and SEII genes in the endosperm via an RNAi approach. The SE genes encode the SBE starch branching enzymes and are responsible for producing soluble branched starch that is rapidly digested instead of linear starch that is less soluble and less rapidly digested.

The health concerns for this GM wheat stem from two main areas; (i) the siRNA engineered into the GM wheat as short hairpin RNA (shRNA) entities in question can to be taken up by human and animal consumers and in principle silence the human (or animal) equivalent of the SEI and SEII genes and (ii) the siRNA approach used to generate this GM wheat is not a perfect technology and possesses an inherent risk of silencing not only the intended target gene or genes but also others with similar DNA sequence, so called "off-target effects". This can result in disturbed gene function in the wheat giving rise to, for example, novel allergens. More worryingly multiple gene systems could be interfered with in the human and animal consumer resulting in disturbances to multiple organ systems.

Comments on statements by Prof Jack Heinemann and Prof Judy Carmen on the CSIRO SEI/SEII shRNA GM Wheat

Generally, in my opinion the statements of both Prof Jack Heinemann and Prof Judy Carman are both technically and conceptually scientifically accurate. They comprehensively cover all

possible risks that could arise from the consumption of the CSIRO SEI/SEII shRNA GM wheat and put forward sound and essential generic evaluation of this product for off-target effects and possible allergenicity and toxicity.

The findings of Prof Jack Heinemann and Prof Judy Carman that I find of particular concern from a health risk perspective are as follows.

Prof Jack Heinemann

Prof Heinemann has comprehensively and accurately described the RNAi systems and their known mechanisms of action in the regulation of gene expression. The various mechanisms by which siRNAs and miRNAs lead to gene silencing at an mRNA and transcriptional (epigenetic) level are very clearly described and illustrated.

Unfortunately, the CSIRO took the position of not disclosing essential molecular biological details of the SEI/SEII shRNA GM wheat. Most importantly they did not make available the sequences of the shRNA(s) engineered into the GM wheat and whether the SEI or SEII genes or both are being targeted. As a result Prof Heinemann was forced to take a generic approach to evaluating effects that could arise from the consumption of this GM food crop.

Using the best available bioinformatics computing tools to search the DNA sequence databases, Prof Heinemann found that the SEI and SEII wheat genes in numerous regions share a high degree of DNA sequence identity with the human equivalent enzyme known as *GBE* (pages 10-11 of the report). Based on this finding the possibility of a cross-species shRNA effect resulting from the consumption of this GM wheat cannot be ruled out.

A similar analysis comparing the SE gene sequences to the entire human genome also identified many other gene regions that could be subject to an SE shRNA off-target effect (pages 11-12; Table 1; Appendix 1).

Taken together these bioinformatics predictions suggest that there is a very high probability of target and off-target effects arising from the consumption of the GM wheat depending on the amount consumed and circulating levels of the SEI/SEII shRNA(s).

In addition, Prof Heinemann rightly points out that initial shRNA effects can be amplified and give rise to the synthesis of secondary shRNAs, which will have their unknown gene targets.

However, again as rightly stated bioinformatics is a predictive and not definitive analytical tool. Predictions based on this approach must therefore be experimentally validated. Prof Heinemann provides a comprehensive series of experiments with which I agree must be conducted prior to any field release and human feeding studies (pages 12-13).

In conclusion, based on Prof Heineman's analysis I would say it is not a question of *if* there will be gene function disturbances arising from the consumption of the SEI/SEII shRNA GM wheat but to what *degree* they will take place and with currently unknown health consequences. The generic experiments suggested by Prof Heinemann will go a long way to providing answers to these vital questions.

Prof Judy Carman

Given her expertise Prof Carman provides invaluable clinical insight as to what might be the outcomes from consuming the SEI/SEII shRNA GM wheat. Worryingly, if the SE shRNA(s) in the GM wheat were to silence the human equivalent *GBE* gene, this could give rise to

conditions similar to the naturally occurring group of genetically inherited ailments known collectively as glycogen storage diseases (page 7).
Prof Carman also points out that epigenetic mediated gene silencing by siRNA can be stable and in certain circumstances be inherited transgenerationally. This raises major concerns that any ill-health arising from targeted or off-target effects of the engineered shRNS(s) in the GM wheat may become a stable feature in the human (and animal) population and thus difficult to eradicate (page 4).

The comments provided by Prof Carman into the manner in which the CSIRO, FSANZ and OGTR have evaluated the safety of the SEI/SEII shRNA GM wheat I found particularly insightful. I found it incomprehensible that such expert bodies could make claims that contained fundamental scientific errors about shRNA technology and the GM wheat product itself.

For example, the claim by FSANZ that the SEI/SEII shRNA GM wheat is substantially equivalent to the non-GM wheat parent is clearly wrong since apart from the differences in starch composition as intended the GM wheat had significant differences in its amino acid composition (page 8), lower protein content, a higher free sugar content and changes in a number of enzymes (page 10). These major differences between the GM wheat and its non-GM parent are of great concern as they imply that aspects of the core biochemistry of the GM wheat has been markedly disturbed and that this may be arising from off-target effects of the engineered shRNA(s) in the GM wheat or the inherent mutagenic effect of the GM transformation process or a combination of the two. Such a disturbed biochemistry needs to be analysed in detail using modern molecular profiling methods as they may, for example, lead to the production of novel allergens in the GM wheat that need to tested for with human subjects.

That the OGTR can state that the shRNA(s) as normal RNAs rather than sequences with a gene regulatory function and which if translated would give rise to only short protein fragments (page 5) suggests a fundamental lack of understanding of the molecular biology of the RNAi system. The lack of appropriate pre-clinical and Phase 1-4 clinical toxicity testing, by the OGTR for this GM wheat (pages 8-9) also reflects a lack of contemporary understanding of the shRNA system in question. It is clear that an inadequate number of animals and human subjects are to be used in these studies to reach meaningful statistically significant values (Table, page 9). It would appear that the OGTR is merely interested in evaluating “nutritional” value rather than toxicity of this GM product. This implies that they have taken the position in advance that there are no health risks from this GM wheat, presumably because it has been passed (erroneously) as substantially equivalent to the non-GM parent wheat (pages 8 and 10).

From this and other evidence presented I am led to conclude that the members of FSANZ and OGTR are not familiar with the latest developments in the field of shRNA biology and technology. Most crucially, there appears to be no acknowledgement of the recent findings that miRNAs can survive digestion and enter the circulation of the consumer and elicit biological effects. This alone should raise concern that the SEI/SEII shRNA(s) in the GM wheat could also survive digestion and bring about a biological response via gene silencing in the animal or human consumer with as yet unknown health consequences.

As in case of Prof Heinemann’s statement, Prof Carman provides a comprehensive and appropriate series of experiments that need to be conducted in animal model systems and to be followed in human subjects. These are essential to generically evaluate the potential toxicity from the consumption of this GM wheat that could arise from gene disruption from the ingested shRNA(s) and other.

Summary

It is clear from the up-to-date, comprehensive, and scientifically sound evidence provided by Prof Carman and Prof Heinemann in their statements that there are genuine, significant safety issues connected with the consumption of the CSIRO SEI/SEII shRNA GM wheat that need to be evaluated comprehensively and generically as they suggest. Most worryingly from what Prof Carmen has discovered is that the regulatory bodies overseeing the risk assessment and approval of GM crops in Australia (FSANZ, OGTR) seem to be out of step with the latest developments in the field of RNAi biology and technology and therefore not taking the necessary steps to properly evaluate the safety of the SEI/SEII shRNA GM wheat.

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