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Update on "Evaluation of risks from creation of novel RNA molecules in genetically engineered wheat plants and recommendations for risk assessment"

An expert opinion of Professor Jack A. Heinemann, PhD

first issued 28 August 2012

Update 21 March 2013

On the 12th July 2012 I was contacted by the Safe Food Institute with a request to provide an expert scientific opinion on "GM wheat research being conducted by the CSIRO." Included in this correspondence was the additional request that: "If you do believe that there are risks, can you please provide recommendations for any studies that would provide information to confirm or exclude any risks to human health."

On 28 August 2012 I issued an opinion. That opinion:

1. Described the existing scientific evidence that the intended siRNAs (double-stranded RNA designed to alter gene expression in the GM wheat) produced in plants could transfer to other organisms (e.g., insects, wildlife or people);
2. Reviewed the existing scientific literature indicating that GM wheat could plausibly contain additional unintended dsRNA molecules unique to the GM wheat;
3. Described the existing scientific evidence that unintended siRNAs could transfer to other organisms (e.g., insects, wildlife or people) from GM wheat;
4. Reviewed the existing scientific literature indicating that GM wheat could plausibly contain additional unintended dsRNA molecules (2° dsRNA) unique to the GM wheat that might cause unintended changes in the plant and/or transfer to other organisms;
5. Described the existing scientific evidence that 2° dsRNA molecules could transfer to other organisms (e.g., insects, wildlife or people) from GM wheat;
6. Described the existing scientific evidence for the possibility that either primary intended or 2° unintended dsRNA molecules produced in either the GM wheat or an exposed organism may alter gene expression in organisms exposed to the GM wheat (e.g., through ingestion or inhalation).

I concluded that it was likely that the intended dsRNA molecule and unique 2° dsRNA molecules were present in the GM wheat; it was plausible that they would transfer to exposed organisms including people (Jiang et al., 2012); and it was possible that exposure could result in changes in exposed organisms. As a result, and because these conclusions were possible to reach well before the Office of the Gene Technology Regulator approved the release of the GM wheat into a field trial and for feeding to human volunteers, I suggested that these exposure pathways and potential for adverse effects should have been formally considered in the risk assessment (DIR093). However, I could not find any evidence that they were.

Finally, I suggested a series of tests that if followed could provide a high degree of confidence that all potential novel dsRNAs and adverse effects had been identified in the risk assessment. Since then, a modified version of this suggested procedure has been published in an international blind-reviewed journal (Heinemann et al., 2013).

The sequences

An obvious rejoinder to the need for a risk assessment — for example with regard to human health — is that wheat and humans (and other animals) have very different genomes and therefore very different DNA/RNA sequences. As the risks considered arise from the interactions between molecules of a high sequence similarity, there is no need to evaluate sequence-determined risks.

To establish that this assumption-based safety argument was not reliable, I conducted simple comparisons between the DNA sequence of the human genome and a DNA

sequence from the wheat *SBEI* gene provided to the database Genbank by CSIRO.[1] The original opinion made explicit, however, that the actual sequences used by CSIRO were unknown to me. Furthermore, it was and remains the obligation of the developer and regulator to ensure that a risk assessment was informed by the actual sequences. The use of the *SBEI* and surrounding DNA sequences by me merely established that a wheat target could create both intended and unintended dsRNA molecules with the potential to cause off-target effects on other genes in the plant or in other organisms.

New information

Madeleine Love of Australia contacted me subsequent to the issuing of my opinion with information she had obtained from CSIRO. According to emails released to me by her, all or some of the 'SE' sequences described in DIR093 as "confidential" relate to those disclosed in a publication (supplementary information published under

sense

ggcgggttgagtgagatctgggccactgaccgactcactcgctcgctgcgcggggatggcgacgttcgcggtgtccggcgcg
accctcggtgtggcgcggcccgccggcgccggcggcggactgctgccgcgatccggctcggagcggaggggcggggtgg
acctgccgtcgctgctcctcaggaagaaggactcctctcgcgccgtcctgagccgcgcggcctctccagggaaggtcctggtg
cctgacggtgagagcgacgacttggcaagtccggcgcaacctgaagaattacagatacctgaagacatcgaggagcaaacgg
ctgaagtaaacatgacagggggactgcagaaaaacttgaatcttcagaaccgactcaaggcattgtggaaacaatcactgatg
gtgtaaccaaaggagttaaggaactagtcgtgggggagaaaccgcgagttgtcccaaaaccaggagatgggcagaaaatatac
gagattgacccaacgctgaaagattttcggagccatcttgactaccg

reverse complement

cggtagtcaagatggctccgaaaatctttcagcgttgggtcaatctcgtatattttctgcccatctcctggttttgggacaactcgcgg
tttctcccccacgactagttccttaactcctttggttacaccatcagtgattgtttccacaatgccttgagtcggttctgaagattcaagtt
tttctgcagtcccccctgtcatgtttacttcagccgtttgctcctcgatgtcttcaggtatctgtaattcttcaggttgcgccggacttgc
caagtcgtcgctctcaccgtcaggcaccaggaccttccctggagaggccgcgcggctcaggacggcgcgagaggagtccttct
tcctgaggagcagcgacggcaggtccaccccgcccctccgctccgagccggatcgcggcagcagtccgccgccggcgccg
gcgggccgcgccacaccgagggtcgcgccggacaccgcgaacgtcgccatccccgcgcagcgagcgagtgagtcggtcag
tggcccagatctcactcaacccgcc

Figure U1: 'sense' and 'antisense' sequence assembled from GenBank accession no. Y11282 (not including intron 3).
Sense is nucleotides 96-635 of Y11282. The reverse compliment was assembled using http://www.geneinfinity.org.

Regina et al., 2006) that appeared some 5 years before CSIRO removed its claim of confidentiality, and 3 years before it submitted that information as confidential to OGTR.[2]

[1] I assumed that the SBEI activity was the or one of the targets chosen by the CSIRO based on, for example, the sentences: "The GM wheat lines contain partial sequences of the *SEI* gene (details of which are CCI) and the GM barley line contains partial sequences of the *SEI* and *SEII* genes (details of which are CCI), which are both involved in starch biosynthesis"; "The *SEI* and *SEII* genes were isolated from the wheat *Aegilops tauschii* (donor of the D genome in wheat) and *Triticum aestivum*, respectively" (from DIR093).

[2] Email from Rachel Fitzgerald to Madeleine Love dated 28 September 2012: "In the DIR093 [issued in 2009] application to the OGTR, the names of the genes referred to as SEI, SEII, SEIII, SEIV was commercial in confidence information. CSIRO removed this restriction in December 2011. In both the Regina publication [of 2006] and the DIR111 risk assessment and risk management plan and licence, these genes were named…". Setting aside the ambiguity of what was intended to be kept secret (DNA sequence, gene names, or both), it remains unclear what is the scope of the four DNA sequences SEI-SEIV. They may refer to the sense and 'antisense'

Using Regina et al. (2006) as a guide, I reconstructed at least some of the intended novel DNA sequences used to create the GM wheat described in DIR093 (Figure U1). I interrogated both the human genome and selected parts of the human genome using this sequence. The methods used were similar to those reported in my original report of 28 August 2012. The sequence in Figure U1 was compared using blastn (default settings). As I found previously, there are matches to the human genome that are in the size range which may affect gene regulation (Table U1). Therefore again this update reinforces my previous argument that the risk assessment conducted on the GM wheat should be informed by both bioinformatic and experimental evidence that off-target effects do not arise or if they do, they will not cause adverse effects on animals or humans, or any adverse effects can be mitigated.

sequences for both *SBE2a* and *SBE2b* on the same strand, respectively, or they may refer to additional targets beyond that discussed in Regina et al. (2006). At a minimum, I understand the DIR093 covers the described exon sequences from both *SBE2a* and *SBE2b* (and their reverse complements) as well as intron 3 of *SBE2a*. It should also be noted that Regina et al. (2006) reported an unintended off-target effect of the SBE2a silencing construct in many of the derived GM wheat plants (but the authors did not comprehensively search for others) and thus SEI-SEIV may have intentionally or inadvertently included other targets.

Table U1: Selected potential strong matches between sequences derived from Genbank Y11282 and the human genome.

Sense (96-635 from Y11282) to human genome	Description
Query 479 GGGCAGAAAATATACGAGATT 499 Sbjct 52921480 GGGCAGAAAATATACGAGATT 52921500	Homo sapiens chromosome 1 genomic scaffold, alternate assembly CHM1_1.0 Sequence ID: ref|NW_004077990.1|
Query 468 AACCAGGAGATGGGCAGAAAA 488 Sbjct 43885503 AACCAGGAGATGGGCAGAAAA 43885523	Homo sapiens chromosome 3 genomic scaffold, alternate assembly CHM1_1.0 Sequence ID: ref|NW_004078011.1|
Antisense (of 96-635 from Y11282) to human genome	
Query 53 TTTTCTGCCCATCTCCTGGTT 73 Sbjct 22278981 TTTTCTGCCCATCTCCTGGTT 22279001	Homo sapiens chromosome 3 genomic scaffold, alternate assembly HuRef SCAF_1103279188143 Sequence ID: ref|NW_001838877.2|
Query 42 AATCTCGTATATTTTCTGCCC 62 Sbjct 36052534 AATCTCGTATATTTTCTGCCC 36052514	Homo sapiens chromosome 1 genomic scaffold, alternate assembly HuRef SCAF_1103279188432 Sequence ID: ref|NW_001838579.2|
Sense and antisense (of 96-635 from Y11282) to LDLRAP1	
Query 227 CCAGGGAAGGTCC 239 Sbjct 9204 CCAGGGAAGGTCC 9216	Homo sapiens low density lipoprotein receptor adaptor protein 1 (LDLRAP1), RefSeqGene (LRG_276) on chromosome 1 Sequence ID: ref|NG_008932.1|

References[3]:

Heinemann, J. A., Agapito-Tenfen, S. Z. and Carman, J. A. (2013). A comparative evaluation of the regulation of GM crops or products containing dsRNA and suggested improvements to risk assessments. Environ Int *in press*.

Jiang, M., Sang, X. and Hong, Z. (2012). Beyond nutrients: food-derived microRNAs provide cross-kingdom regulation. BioEssays *34*, 280-284.

Regina, A., Bird, A., Topping, D., Bowden, S., Freeman, J., Barsby, T., Kosar-Hashemi, B., Li, Z., Rahman, S. and Morell, M. (2006). High-amylose wheat generated by RNA interference improves indices of large-bowel health in rats. Proc. Natl. Acad. Sci. USA *103*, 3546-3551.

[3] The author thanks Brigitta Kurenbach, Sara Agapito-Tenfen, Stinus Lindgreen and Belinda Martineau for comments and review.