Expert Scientific Opinion
on
CSIRO GM Wheat Varieties

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Introduction

This document is provided to the Safe Food Institute. It contains my expert scientific opinion about whether CSIRO's GM wheat, which has been genetically modified to produce an RNA interference effect, may constitute a risk to animal or human health.

Expertise relevant to this report

I hold several degrees that are relevant to this expert scientific opinion. I graduated with a Bachelor of Science from the University of Adelaide, followed by an Honours Degree in Organic Chemistry from the same University. In 1989, I graduated with a PhD degree in Medicine (University of Adelaide) in the field of Metabolic Regulation/Nutritional Biochemistry, particularly in relation to cancer. This involved work on both isolated cells *in vitro* and animals *in vivo*. I performed many animal feeding studies during and after my PhD. In 1998, I obtained a Master of Public Health from the University of Sydney specialising in Epidemiology and Biostatistics.

I have worked in a number of positions with those relevant to this expert scientific opinion being: I have taught at Roseworthy Agricultural College (now part of the University of Adelaide); I was a researcher at the CSIRO (Commonwealth Scientific and Industrial Research Organisation) Division of Human Nutrition; I was a researcher at the Albion St (AIDS) Centre in Sydney and I was the Senior Epidemiologist in the Communicable Disease Control Branch of the South Australian Department of Human Services (now the South Australian Department of Health) investigating outbreaks of disease. While in the latter position, I investigated a considerable number of outbreaks of disease. Most outbreaks were food-borne. I was also the chief investigator of the multi-institutional investigation into whether Rabbit Calicivirus (Viral Haemorrhagic Disease of Rabbits) was able to infect people. I therefore have considerable expertise in animal and human biochemistry, nutrition and infectious diseases; and with food and how food consumption can affect the health of animals and humans.

For the last 12 years or so, I have worked in the safety of genetically modified (GM) crops, and in particular, whether these crops may affect the health of animals and humans that eat them. As a consequence of this work, I currently hold two positions that are relevant to this expert scientific opinion. First, I am Director of the Institute of Health and Environmental Research Inc. (IHER). This Institute is a private institution for health and environmental research, whose principal current focus is on GM foods. Second, I am an Associate Professor in Health and the Environment, School of the Environment, Flinders University, Adelaide.

I am the senior investigator of a research group comprising researchers within and external to IHER and Flinders University, undertaking research into whether GM foods are safe to eat. The research includes long-term feeding studies that measure endpoints that are relevant to human health.

I receive no funding from GM companies, either directly or indirectly, and I am completely independent of them.

The CSIRO GM wheat varieties

The aim of these GM wheat varieties

Unfortunately, the finer details of these wheat varieties are not available to me because (1) these GM wheat varieties are currently still under development and hence some information is not available for that reason, and (2) the CSIRO has retained crucial information from public view on the argument that it is Commercial Confidential Information. This information includes the names of genes expected to alter grain starch composition, the specific phenotypic changes occurring, when they are down-regulated and its application. This information is not known, and may never be known, to independent scientists such as myself or the general public. However, because they are GM varieties of wheat, they are subject to some regulation, so there is some information available publicly about them on the Office of Gene Technology Regulator (OGTR) website. Further information has been obtained from the CSIRO website and media releases. From these sources, it is clear that the CSIRO has permission to plant 1 ha of 3 different GM wheat varieties and 1 GM barley variety. As GM wheat is the topic of this report, I will refer only to the GM wheat varieties being developed by the CSIRO and not to the barley variety. It is clear from the OGTR website that these GM wheat varieties use a technology variously called RNAi, interference RNA, double-stranded (ds)RNA-mediated silencing, post-transcriptional gene silencing (PTGS) and transcriptional gene silencing (TGS) or various other names, involving short/small interfering RNAs (siRNAs), repeat-associated short interfering RNAs (rasiRNAs), microRNAs (miRNAs), or short-hairpin (sh)RNA. For the sake of simplicity, I will generally call this technology dsRNA in this document.

From the OGTR website, it appears that these GM wheat varieties contain sections of DNA or genes from wheat, rice and some micro-organisms such as cauliflower mosaic virus, *Escherichia coli* and *Agrobacterium tumefaciens*. Moreover, it appears that the introduced DNA contains a promoter sequence from wheat and a reversed sequence from wheat, making it a chimera of sequences based on wheat. All these sequences combine to make a section of DNA in each GM wheat variety that has not been seen in nature before.

Essentially, it appears that the wheat is genetically modified to make a small piece of RNA which silences a gene in the wheat plant. The aim appears to be to prevent the wheat making a branched-chain version of starch. Instead, the wheat makes a straight-chain version of starch. This straight-chain version takes longer to be digested in the gastrointestinal tract of animals and people that eat it, when compared to the branched-chain version. From the documents that I have seen, I have concluded that the CSIRO and their commercial partners hope that (1) this will result in a slower release of glucose into the bloodstream, known as an improvement in the glycaemic index, and (2) that it will take so long for the wheat to digest, that some will survive digestion in the small intestine to enter the large intestine, where digestion will result in a change in the large intestine that could reduce the risk of colon cancer.

From the documents I have seen and my knowledge of the CSIRO, gained from my previous employment with that organisation, I believe that the CSIRO hopes to have these GM wheat varieties marketed with what is known as “health claims”. Health claims on food are marketing strategies that promote the health benefits of that food with the aim of increasing sales of that food. In this case, any positive effects of the wheat on the glycaemic index and bowel heath are likely to be promoted.

While these efforts to reduce the glycaemic index and improve bowel health are laudable, there are well-established ways of obtaining the same effect by eating plants other than wheat. In fact, a number of organisations including the CSIRO have done a considerable amount of research over many years and produced a large volume of literature on the benefits of eating other, non-GM grains that variously contain resistant starches, have a lower glycaemic index, improve large bowel health and improve cholesterol, such as oats, rye, lentils and peas. Consequently, there is no apparent benefit to people from eating this GM wheat variety that could not be obtained by eating other foods.

General risks from the technology

There are risks from using dsRNA technology to make food. When assessing these risks, an important factor to consider is that the greatest exposure to people will occur if the OGTR grants permission for these wheat varieties to be grown commercially, as that will give permission for farmers to plant it widely. That would likely result in widespread consumption by the community, particularly if health claims are present on any food packaging and a promotion campaign is conducted, as I expect would happen. I will therefore concentrate on the risks to the community from widespread exposure through eating these GM wheat varieties.

The first important factor in assessing the risk of this wheat to human health is to consider whether the dsRNA produced by the wheat plant can survive digestion in the organism that eats it. Having survived digestion, the next factor to consider is whether the dsRNA can then enter the tissues of the organism and affect the expression of its genes. There is scientific evidence that this chain of events can indeed occur with dsRNA and the evidence for this is given in Prof Jack Heinemann's report and also in his book (J Heinemann, 2009). In fact, employees from the world's largest GM company, Monsanto, have written at least one paper about how to commercially exploit the fact that dsRNA survives digestion in insects, in their attempts to try to control insect pests of plants. That is, the plant is genetically engineered to produce a dsRNA, which insects ingest when they eat the plant; the dsRNA survives digestion in the insect and then silences genes in the insect to stunt its growth and kill it (Baum et al, 2007). There are three main reasons why the CSIRO should know about these findings. First, the employees of the CSIRO who developed these GM wheat varieties would be reading the scientific literature in this area. Second, there is a close association between the CSIRO and Monsanto over many years (eg they collaborate to develop and commercialise GM cotton in Australia). Third, the paper described above was reviewed by Karl Gordon and Peter Waterhouse, both of whom have either worked for, or collaborated with, the CSIRO.

Furthermore, as summarized in Prof Heinemann's affidavit and book, novel dsRNAs cannot only be created in one organism and transferred through food to another organism, but they may be amplified in the recipient organism. Moreover, gene silencing caused by dsRNAs is sometimes heritable and self-replicating. There are suggestions that there is an amplification pathway where ingested dsRNAs are processed to siRNAs that may then prime the synthesis of more siRNAs. There is also evidence of a systemic spread of gene silencing beyond the cells in the gut to distant sites throughout the organism, suggesting that there is an as-yet unidentified mobile silencing signal that is an integral part of the RNA silencing pathway. The effect appears to be remarkably long-lived, being observed not only in the insect affected, but also in the offspring of the insect (Bernstein et al, 2001). For some genes studied, interference lasted for many generations of the organism. The effect therefore seems to be inherited, that is, dsRNA can cause heritable changes in gene expression.

It is important to understand that these results come from a new area of research. Therefore, there is still much we don't know about how dsRNA affects a variety of organisms that consume it. Most

research to date has been done on insects and nematodes. Further research is required to determine how many other species in the animal kingdom may be affected, how they may be affected, and whether the list of affected organisms includes humans. It is also important to understand that insects and nematodes share many similar biochemical and regulatory mechanisms with other members of the animal kingdom, including humans, so there are grounds for concern that humans may be similarly affected.

As a result, there is a chain of evidence to show that there is a risk that the dsRNA from this GM wheat may survive digestion, enter the tissues of people that eat it and silence a gene or genes in those people. There is also evidence that any genetic changes so produced may be stable and become established in many cells of an organ. Furthermore, there a possibility that these changes may be passed-on to future generations.

It therefore concerns me that the CSIRO has decided to introduce this new technology into a crop that is widely eaten by people when we currently know so little about how the technology may affect the health of people; and without assessing the risks we do know about. Consequently, at a minimum, the possibility of the outcomes described above should be investigated in animals and humans using animal experiments before these GM wheat varieties enter the animal feed and human food supply.

The OGTR should also be aware of these factors, given that it should also be reading the peer-reviewed scientific literature. However, it appears that the OGTR is regulating these dsRNA molecules as if they were "ordinary" non-regulatory DNA or RNA, rather than fully understanding that these are regulatory pieces of RNA that are designed to silence genes. The OGTR acknowledges that the dsRNA could transfer into bacteria, plants, animals or other eukaryotes. (Note that this is a list that includes humans.) However, the OGTR regards the probability of this occurring as no greater than the transfer of any native gene. The OGTR appears to believe that the regulatory sequences are already widespread in the environment and hence are already available for transfer via natural mechanisms, without seemingly appreciating that the dsRNA comes from a genetically engineered, chimeric gene containing a reversed DNA sequence from wheat. Furthermore, the regulatory RNAs being produced have never been shown to exist in the human food supply or nature, and thus their safety when transmitted, at whatever frequency, has never been established. The OGTR then says that if gene transfer did occur, it could only result in the production of short protein fragments. Again, the OGTR does not seem to appreciate that these genes are designed to make a particular dsRNA molecule that could silence a gene that is crucial to the health and welfare of animals and humans, and has nothing to do with making proteins. Importantly, these regulatory RNAs appear to be much more capable of surviving digestion than DNA.

Throughout its risk assessment process, the OGTR discussed risks as theoretical probabilities that were not based on experimental proof or evidence. While experimental evidence is routinely required in medical research to measure risk, establish a lack of harm or establish cause and effect, such an "evidence-based" risk assessment does not seem to have been done by the OGTR. Instead, the OGTR has undertaken an "assumption-based" risk assessment. That is, the OGTR has made assumptions about matters that could have been measured instead. In sort, the OGTR has asserted the safety of these GM wheat varieties based on an absence of evidence instead of requiring evidence to establish the absence of adverse effects. This approach is inadequate in my view.

In his report, Prof Heinemann has described how the novel dsRNA in the GM wheat variety may silence a gene in the animal or human that eats it if there is a certain amount of matching between the dsRNA and a gene in the animal or human. The OGTR website acknowledges this, stating that homology of as little as 20 nucleotides can cause non-target genes to be silenced. The question is:

in the length and complexity of the human genome, how many matches would there be? In theory, the answer could range from nil to many. Hence the number of matches needs to be determined using experimental methods, rather than guessing. However, there is no mention of the CSIRO even considering this possibility as there is no mention of the CSIRO looking for any such matches in any documents I have seen about these wheat varieties. This concerns me.

A specific risk from the technology

Of the possible matches that may occur, my greatest concern lies with one that is the most obvious. The most likely match is for a gene that produces a very similar protein product in animals and humans. In my opinion, this likely match should have been checked. Yet it appears that neither the CSIRO nor the OGTR have even considered this likely match and the problems that may result from it.

In order to describe this likely problem, it is important to describe the structure of various forms of starch. Starch is comprised of glucose molecules that are arranged like beads on a string. They can be arranged either as a linear form of beads or as a branched form of beads. In plants, the least branched form of starch is called amylose. It is essentially a linear form of glucose molecules with very few branches. Because of its tightly packed structure, amylose is more resistant to digestion than other starch molecules. Plants can also produce a branched form of starch, called amylopectin, where branching takes place every 24 to 30 glucose units, resulting in a soluble molecule that can be quickly broken down into individual glucose units to provide energy. Starch from plants is about 70-80% amylopectin by weight, though it varies depending on the plant source. The aim of these GM wheat varieties appears to be to use a dsRNA process to silence a gene so that less branching enzyme is produced in the wheat, so that the plant produces much less branched starch and much more linear starch. The CSIRO is not publicly saying much about which of the wheat's branching enzymes it is trying to silence, possibly due to patent implications, but it is clear from DIR054/2004 on the OGTR website, that the CSIRO is trying to silence one or both of the branching enzymes it calls SEI and SEII, and that the CSIRO's aim is to produce wheat that has less amylopectin and more amylose in it.

It is important to understand that humans and animals also produce a form of highly branched starch to store glucose for future use. In humans, the starch is called glycogen. Glycogen is stored by the body after eating, mostly in liver and muscle, and then released in the fasted state and during exercise to maintain the blood glucose level. For example, we use glycogen as a source of energy in the morning after an overnight fast, or for a burst of energy to run across a road. Glycogen has many branch points resulting in a soluble molecule that can be quickly broken down into glucose units for energy. Glycogen has much the same composition and structure as amylopectin in plants, but with more extensive branching that occurs every 8 to 12 glucose units. Branching is also essential to increase the solubility of the glycogen and in reducing the osmotic pressure within the cell. A fed, 70kg man should have enough glycogen for about 8,500 kilojoules of energy. This information is widely available from a number of biochemistry and medical text books.

The question therefore arises: if a person eats one of these GM wheat varieties, could the dsRNA engineered to be produced in the GM wheat to silence wheat branching enzyme, also silence human branching enzyme? It is likely that it could. The branching enzyme seems to be be fairly highly conserved along the evolutionary pathway from plants to animals and humans. That is, the branching enzyme has many homologues in many species and performs much the same function in much the same way across these species. The OGTR website shows a theoretical understanding that this might be a problem, stating that more than one gene in a gene family can be silenced by dsRNA and that the risk of this occurring increases if the dsRNA targets a section of those genes

that is highly conserved between the members of the gene family. But the OGTR website then goes on to talk only about wheat and fails to link this knowledge to the fact that animals and humans also have a branching enzyme that could be affected. However, as a result of the information on the OGTR website and in the peer-reviewed literature, it is likely that there could be sufficient similarity between the human and plant genes for the wheat dsRNA to also silence the human gene.

The next question therefore arises: what health effects would result if eating one of these GM wheat varieties silences the human branching enzyme? Clearly, humans would then make little of the branched form of glycogen. Instead, they would make a linear, unbranched form of it. Some people are born with a genetic error that causes them to do just this. Of the different types of glycogen storage diseases in people, one of them involves not having a functioning branching enzyme so these people do not make a branched form of glycogen. Instead, they make very long, linear, unbranched glucose chains, like amylose. This form of glycogen has a low solubility in human cells, so it precipitates out of solution to form deposits. These deposits build-up in the tissues of the body, especially in the heart and liver. The disease that results is variously called Glycogen Storage Disease IV, glycogenosis type IV, glycogen branching enzyme deficiency (GBED), polyglucosan body disease, amylopectinosis or Anderson Disease (named after Dorothy Hansine Anderson, an American physician). It results in an enlarged liver, cirrhosis of the liver, and failure to thrive. Children born with the disease usually die at about 5 years of age. In adult polyglucosan body disease, the activity of the branching enzyme is a little higher and so symptoms do not appear until later in life. A similar disease can also affect horses, where it is called glycogen branching enzyme deficiency. This information is readily available to the public and scientists – it even appears on Wikipedia.

Consequently, it is clear that there is an obvious risk to animals and people who eat these GM wheat varieties. That is, there is evidence that dsRNA from GM plants may survive digestion to enter the tissues of the body, where it may silence a gene in the host. Furthermore, there is evidence of a specific gene that may be silenced in the host – a similar gene to the one that is silenced on purpose in the GM wheat variety. There is also evidence that if this gene was silenced in animals and people, it could cause serious ill-health and even death. As this dsRNA does not appear in nature, there is no appropriate way to dismiss this scenario through reference to any non-GM food or by using assumption-based reasoning.

Safety assessments planned and the regulatory framework under which they will be completed

Given this possible dire outcome in people who eat these GM wheat varieties, it is important to determine how well this GM wheat will be safety tested, and whether it will be specifically tested to determine if this outcome could occur in people who eat the GM wheat.

The first thing to consider here is the regulatory and other requirements that the CSIRO has to fulfill in order to gain approval for these wheat varieties to be planted on a commercial scale and to enter the Australian food supply. If the CSIRO wants to do any animal or human studies, it first needs to gain approval from an animal ethics committee for animal studies and a human ethics committee for human studies. In a University setting, the researchers must apply to the relevant ethics committee for approval to do the study, and in many universities, everyone (not a majority) on the committee needs to give approval, before the study can start. These committees usually contain people from outside of the university, including people from the community, to provide additional oversight. This process is so rigorous that many researchers can find the process frustrating and slow. It is not uncommon to take several meetings of the ethics committee over several months to get approval for a particular study, while the committee requests and gets further information from the researchers.

It is also not uncommon for the committee to give approval with a range of conditions attached. In comparison, the CSIRO appears to have used its own in-house ethics committee to gain approval for its own feeding studies for a product from which it hopes to gain considerable financial benefit. There is a lack of available information about the composition of the committee and whether it contains members that are external to the CSIRO, including members of the community, and consequently if there is any outside or community oversight of these studies to ensure that they are carried-out correctly. Consequently, there may be a serious conflict of interest, and a lack of rigour, in a CSIRO committee approving its own study.

The second body to consider is Food Standards Australia New Zealand (FSANZ). Approval is not needed from this body to undertake animal or human feeding studies or to plant the wheat in fields. It therefore has no oversight over these matters. The OGTR gives those approvals. Approval is needed from FSANZ to allow any of these GM wheat varieties to come into the Australian food supply, usually after the OGTR has given approval for the crop to be planted on a commercial scale. However, even at that point, FSANZ does not require any human or animal studies to be undertaken to determine if this or any other GM crop is safe to eat. I have seen one GM crop where FSANZ did indeed assess the crop to be safe to eat without seeing any animal feeding studies. FSANZ only requires a compositional comparison to be done between a GM crop and a similar non-GM crop. Furthermore, the results of the comparison do not need to show that the GM crop has exactly the same composition as the non-GM crop. I have seen a GM corn variety that FSANZ assessed as being "substantially equivalent" and therefore safe to eat, when almost half of the amino acids (the building blocks of proteins) were significantly different in the GM crop compared to an equivalent non-GM crop. However, these GM wheat varieties are designed to have a different composition to non-GM wheat. They are therefore designed to fail a compositional comparison test. As a result, I doubt that FSANZ will require these GM wheat varieties to pass even this test. Consequently, it is my expert opinion that FSANZ's safety assessment process is inadequate to determine if these GM wheat varieties are safe to eat before they come into the Australian food supply.

The final body that has regulatory oversight over these GM wheat varieties is the OGTR. Indeed, it is the only regulatory body with oversight of these wheat varieties at the current stage of development. It is therefore the body that is currently assessing the safety of these crops. It is clear from the OGTR website that feeding studies are planned for these GM wheat varieties on rats, pigs and humans. The question is: what is the aim of these studies and how well designed are they? From the OGTR website, the aim of the studies is to: "assess the effect of the compositional changes in the wheat and barley grains on their nutritional value using both *in vitro* and *in vivo* experiments including controlled nutritional trials with GM wheat and barley products in rats and pigs. Products made from GM wheat may also be consumed by a small group of volunteers as part of a carefully controlled nutritional study." Furthermore, it appears that human studies would only take place if the animal studies showed good results. It is therefore clear that these studies are assessing whether the GM wheat produces the desired nutritional benefits to the animals and humans in the trials. That is, the studies are narrowly designed to see if the starch in a GM wheat variety is more slowly digested and hence has a lower glycaemic index, and whether it may improve bowel health.

However, it is also clear from the OGTR website that there is no mention whatsoever of checking to see if the wheat causes any adverse effects in animals and humans that eat it. More specifically, there is no mention of looking to see if any of the following may occur: (1) any uptake of the dsRNA into the animal or human that eats it; (2) any silencing of any genes in animals or people; (3) any silencing of the branching enzyme; (4) any toxic effects such as any damage to liver, kidneys, or any other organ; (5) any increased risk of any reproductive problems (including to see if any dsRNA-related changes are inherited); (6) any increased risk of cancer; or (7) any increased risk of an allergic reaction to wheat. In fact on its website, the OGTR has clearly stated that it will

not be conducting some of these studies, stating specifically: "***Conclusion:*** The potential for increased weediness, allergenicity or toxicity due to expression of the introduced RNAi constructs for altered grain starch composition improving the survival of the GM wheat and barley lines is **not an identified risk** and will not be assessed further." (Emphases come from the OGTR) The OGTR appears to have made this conclusion without any *in vivo* experimental proof. That is, there appears to be no *in vivo* evidence from anywhere to support this conclusion. This situation is particularly worrying, given the increasing evidence that GM crops may cause these sorts of adverse effects. In particular, a GM variety of pea made by the CSIRO that was close to commercial release, was found to cause serious allergy-type reactions in mice after only a few weeks of exposure. The results of this study were published for all to see (Prescott et al, 2005). It is important to note that those animal experiments appear to have been done voluntarily by the CSIRO as it is my understanding that they were not required by any regulatory agency in Australia. Consequently, the OGTR (1) is, or should be, aware of evidence that the CSIRO can produce GM crops that can provoke a strong allergy-like reaction in animals that eat it and (2) the OGTR has chosen to ignore this evidence to not require similar studies to be done for these GM wheat varieties.

The "gold standard" for human safety assessments is the clinical trial process. In this process, safety assessments are first carried out on animals. If the agent being assessed passes animal tests, it then enters the four phases of human trials. Phase I of human trials involves safety testing, to see if the agent produces any adverse effects on the health of people. If it passes this phase, the agent then undergoes Phase II trials to see if the agent has any health benefits to people. However, the CSIRO studies, as they are described on the OGTR website, do not appear to complete animal studies before being tested on people. That is, the CSIRO does not appear to be doing any assessment for harmful effects on animals before moving on to human trials. The CSIRO studies also appear to be skipping Phase I of the human clinical trial process before moving on to Phase II of the process. That is, they appear not to be looking for any adverse effects in people, but intend to go directly to look for any benefits.

Also of importance is the number of animals and people used in the experiments, particularly for trying to find any adverse effects. The number of animals used in these experiments is given on the OGTR website as:

Study	Study type	Treatment period duration (days)	Number of animals or volunteers/ treatment group	Genetically modified wholemeal flour required (kg)
Rat	Bowel health	28	10	8.4
Pig	Bowel health	28	8	336
Human	Trial 1	1	16	1.0
	Trial 2	1	8	0.4
	Trial 3	28	20	56.0

Note that, in the absence of actively looking to see if the GM wheat varieties harm the health of the animals and people that eat them, any adverse effects would need to be quite obvious and hence serious to be noted. The death of experimental animals would be one example that would still be noticed. However, the use of such small numbers of animals and humans for each experiment means that a high proportion of the animals would need to become seriously ill before the result would reach statistical significance and hence be regarded as a serious problem. For example, if death were an outcome of interest in an experiment on 10 rats in a group, as per the rat experiment shown in the table above, half of the animals in the GM-fed group would have to die and none in

the non-GM-fed group before this biologically highly serious outcome reached statistical significance. As scientists generally only consider results to be valid if they are statistically significant, even this sort of obvious adverse result could be ignored and result in the GM wheat variety continuing on to human trials and being released into the community to eat, where it could cause an epidemic of serious ill-health, simply because the sample size of these experiments is too low. Moreover, the amount of wheat eaten in these trials appears to be quite small when compared to the amount that some teenagers and physically active males can eat. Consequently, adverse effects that may only appear from eating large amounts of this wheat variety could be missed in these experiments. Furthermore, the GM wheat is only eaten for a few days in these experiments. Consequently, adverse effects that may only appear after accumulated, long-term exposure could be missed in these experiments.

In addition to the reasons given above, results on the OGTR website provide other reasons to thoroughly safety test these GM wheat varieties. That is, the process of genetically modifying the wheat appears to have caused unexpected changes in the composition of the wheat, specifically a lower protein content, a higher free sugar content and changes in a number of enzymes. Such changes are highly unlikely to be due to silencing a branching enzyme in the wheat and indicate that the process of genetically modifying the wheat may have led to unexpected and unexplained changes in the expression of other genes in the wheat plant. These changes should be investigated at the DNA/RNA level using the tools of molecular biology. The changes should also be assessed on the health of animals and people that eat the wheat variety, as the changes may have resulted in the production of toxic or allergic substances in the wheat.

It is therefore my conclusion that there has been a poor risk assessment process applied to these GM wheat varieties by both the CSIRO and its regulatory overseer, the OGTR. It appears that neither organisation has appreciated or properly safety assessed this wheat in the light of the fact that the dsRNA produced in these GM wheat varieties may survive digestion, enter the tissues of the body and silence a gene or genes in the recipient. It also appears that neither organisation has "joined the dots" to appreciate that, of all the genes that could be silenced, the most likely one is a similar branching enzyme in animals and people and that silencing it could seriously impair or even kill those that eat it.

Recommendations for further safety testing

I therefore recommend that all animal and human feeding studies be stopped until the pre-feeding studies recommended by Prof Jack Heinemann are completed.

If these pre-feeding studies show no adverse effects, I further recommend that animal feeding studies then be completed before any human studies are undertaken. The animal studies should include studies to investigate whether there is: (1) any uptake of the dsRNA into the animal or microbes in the gut, (2) any silencing of any genes in the animal or microbes in the gut, (3) any silencing of the branching enzyme, (4) any toxic effects such as any damage to liver, kidneys, or any other organ, (5) any increased risk of any reproductive problems (including to see if any dsRNA-related changes are inherited) over two generations, (6) any increased risk of cancer, or (7) any increased risk of an allergic reaction to wheat. When investigating (4), it is important that:

- A control group of rats, fed non-GM wheat, is included for comparison.
- There are sufficient animals in each group for a statistically significant result to be found for biologically significant outcomes, eg 25 male and 25 female rats per dietary group.
- The animals are fed from just-weaned for at least six months and preferably for the lifespan of the animals.
- Sub-groups of animals are fed with various doses of the wheat, including high doses.

- At a minimum, full biochemistry and haematology analyses on blood are done on every rat. Other analyses may also be required.
- A full autopsy is conducted on the rats at the end of the experiment, which includes histology on all major organs, with particular emphasis on the liver.
- Tests specific for Anderson disease are conducted.

If those animal studies fail to find any adverse effects and demonstrate the hoped-for benefits, I recommend that the wheat undergo the four phases of a clinical trial in humans. In Phase I, where adverse effects are investigated, I recommend that the study should investigate whether there is: (1) any uptake of the dsRNA into people or microbes in their gut, (2) any silencing of any genes in people or microbes in the gut, (3) any silencing of the branching enzyme, (4) any toxic effects such as any damage to liver, kidneys, or any other organ, or (5) any increased risk of an allergic reaction to wheat. When investigating (4), above, it is important that:

- A control group of people, fed non-GM wheat, is included for comparison.
- There are sufficient numbers of people in each group for a statistically significant result to be found for biologically significant outcomes, eg at least 25 males and 25 females per dietary group.
- People are fed for at least six months.
- Sub-groups of people are fed with various doses of the wheat, including high doses.
- At a minimum, full biochemistry and haematology analyses on blood are done on every participant. Other analyses may also be required.
- Tests specific for Anderson disease are conducted.

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