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# Submission on **APPLICATION A524 FOOD DERIVED FROM HERBICIDE-TOLERANT WHEAT MON 71800**

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1. Introduction.

    1.1. This submission is the opinion of the submitter on Application A524 - Glyphosate tolerant wheat.

    1.2. The submitter is the New Zealand Institute of Gene Ecology (NZIGE), a research organisation (www.nzige.canterbury.ac.nz). The NZIGE has no commercial interest in the product at the focus of this application, no direct or indirect connections with the applicant, and has no connections to parties that seek to compete with the applicant by developing a similar novel food. Our submission is, however, informed by our extensive experience in the research areas discussed below. If there were to be a public hearing on the application, we would be pleased to present our view.

    1.3. Our submission specifically relates to "Scientific aspects of this application, in particular, information relevant to the safety assessment of food from wheat line MON 71800" as called for in the 18 February Initial Assessment Report.

    1.4. We have done our best to evaluate the scientific documents supplied by the applicant in support of the application. Some areas of uncertainty may have arisen from the poor reproduction of some material made available by FSANZ for our use.

    1.5. Disclaimer. As a research organisation it is partly in our own interest to identify issues worthy of public investment in research. We submit that, before the risks of the genetically engineered organism described in Application A524 can properly be assessed, better research is needed. Our interest in the research, however, is not a bias on how we view the applicant's product.

2. Overview. The Authority has made plain "the need for standards to be based on risk analysis using the best available scientific evidence". In the spirit of its equally important objective of protecting the "public health and safety and the provision of adequate information to consumers", the Authority must also determine if the best scientific evidence available is good enough. Our contribution has therefore been to help the Authority identify areas of scientific uncertainty in the application so that these uncertainties can be addressed during the Authority's development of a complete assessment.

Our submission has two primary themes. First, we will make the case that molecular characterization of MON 71800 wheat and/or its components is incomplete in certain important aspects. Second, the feeding trials are either too poorly described to meet our minimum standards for evaluation or are inadequate to meet our minimum standards for confidence in safety. Our view is that any conclusions drawn on the potential chronic toxicity or allergenicity are too speculative to justify with the science available to the applicant, and would therefore be below the certainty to which the public of Australia and New Zealand are entitled.

We encourage FSANZ to not be complacent in pursuing its case-by-case assessment policy[1] simply because, as Dr. Paul Brent is reported to have said on 10 March 2004 on New Zealand radio, FSANZ believes *a priori* that genetically engineered foods are safe and that wheat modified by CP4 EPSPS is unlikely to change that view because the protein has been approved in other contexts before.[2]

We strongly encourage the Authority to avail themselves of the recent report by Pryme and Lembcke (PRYME and LEMBCKE 2003) published in a prestigious, peer-reviewed journal. The degree of testing of CP4 EPSPS is cast in doubt by these researchers and thus, in the case of MON 71800, consideration based on precedent may be inappropriate.

According to Pryme and Lembcke, only two studies using glyphosate-resistant plants have ever been published. If we extrapolate from our experience of A524, then it is unlikely that past applications to amend the food code have added much in the way of numbers of feeding trials involving animals eating plants genetically engineered with CP4 EPSPS.

Of the two published studies, neither involves wheat. Moreover, one of the two was fundamentally flawed. The Hammond study used protein levels so high that the "diet would almost certainly mask, or at least effectively reduce, any possible effect of the transgene, particularly when the inclusion level of the GM soya in any case was low. It is therefore highly likely that all GM effects would have been diluted out" (p. 2 PRYME and

[1] http://www.biotechnology.gov.au/biotechnologyOnline/Food/Responsible/who_is_responsible.htm. Also Dr. Paul Brent in "Report of the 2nd Asean ILSI" published in 2002 by the Malaysian Department of Agriculture.

[2] http://www.bioscinews.com/files/news-detail.asp?NewsID=6678

LEMBCKE 2003). The second, or Teshima study, also had high concentrations of protein and was questioned because the rats did not gain weight during the trial despite the protein rich diet. Although neither study found evidence of harm, two studies can hardly be considered extensive experience with the material or exhaustive testing, *particularly when evidence of negative effects was found in nearly half of all published studies of animals eating genetically engineered plants* (PRYME and LEMBCKE 2003).

3. Summary of recommendations.

3.1. The applicant should prepare or provide a complete proteomic analysis of the cultivars with the MON 71800 event accounting for all changes. Each change that is a variant of CP4 EPSPS should be identified. All variant forms of CP4 EPSPS should be characterized with respect to glycosylation.

3.2. The applicant should submit a complete DNA microarray analysis using MON 71800 RNA isolated from different tissues and plants grown under different but relevant conditions. Chips should represent the genomes of MON 71900, humans, and ecologically and culturally important animal species.

3.3. The applicant should apply the now established technique of NMR combined with chemometrics and univariate statistics to the characterization of differences in metabolites (LE GALL *et al.* 2003).

3.4. The Authority should require the applicant, at a minimum, to supply data on the digestibility of the MON 71800-derived protein using a protocol compliant with the FAO/WHO standard (FAO/WHO 2001) and the recommendations of Pusztai *et al.* (PUSZTAI *et al.* 2003). The FAO/WHO standard includes the requirement to measure the digestibility of the MON 71800-derived protein under the same conditions used to measure the *E. coli*-derived material.

3.5. The Authority should disregard the Bonnette study (Volume 5, Acute Oral Toxicity) until it is either supplemented with additional information, or is replaced by a study of satisfactory rigor. “The biological, immunological, hormonal properties and allergenicity of” MON 71800 wheat must be determined using the wheat product and not surrogate sources such as *E. coli* (PUSZTAI *et al.* 2003).

3.6. The Authority should insist that subsequent studies be conducted independently of the applicant. In a recent review of the literature on food safety studies measuring effects on animals that consume the genetically engineered plant (and not surrogate preparations), the authors found a significant discordance in the reports conducted or sponsored by the industry (directly or indirectly) and independent researchers (PRYME and LEMBCKE 2003). These authors also found that industry-conducted studies were more often operationally flawed (PRYME and LEMBCKE 2003).

3.7. The Authority should require the applicant to meet a higher standard of scholarship in presenting justifications for choice of analysis and protocol design, addressing the current literature and obvious criticisms.

3.8. We recommend that when and if the applicant satisfies this higher, but warranted, standard for conducting *in vitro* and animal *in vivo* safety tests, then human tests should be completed before another application is lodged with the FSANZ.

3.9. A plan for *effective* post-launch monitoring should be provided by the applicant and the plan should be subject to a transparent review through the independent scientific community.

4. <u>Molecular characterization of CP4 EPSPS and implications for other studies relevant to A524.</u>

The study by Lee *et al.* (Volume 2) detected by SDS-PAGE two forms of CP4 EPSPS protein isolated from event MON 71800 plants. These two forms differed in molecular weight from one another by 1kD, from theoretical prediction by ~2-3kD, but not at all from the protein produced by *E. coli*.

This characterization has failed to convince us that all glycoforms of CP4 EPSPS have been detected. Glycoforms of a protein are sugar-modified variants of the same primary amino acid polymer. We argue that all glycoforms must be characterized because different forms can have different biochemical characteristics.
The three main posttranslational protein modifications are N- and O-linked glycosylation and glycosyl phosphatidyl inositol (GPI) anchors (VAN DEN STEEN *et al.* 1998). Over half

of all proteins are glycosylated (VAN DEN STEEN *et al.* 1998). A single protein can emerge with a large variety of different glycoforms despite being synthesized in the same cell at the same time (RUDD and DWEK 1997).

There may be many more forms of glycosylation than discussed above (MANZI *et al.* 2000). Little is known about these other forms of modification, but procedures have been developed to isolate proteins with such modifications (MANZI *et al.* 2000).

*Glycosylation is a significant complexity in protein analysis.* It is critical that the applicant fully characterize glycosylation for two reasons. First, failing to do so can invalidate subsequent analyses that use procedures or reagents dependent on knowing the full glycosylation status of the wheat-derived protein. Second, the biochemical characteristics of proteins with different types or amounts of glycosylation are factors of safety.

Various glycosylation patterns can lead to different biochemical and antigenic properties. For example, different strains of prions (e.g. the causative agent of Mad Cows Disease and CJD) derive from different glycosylation patterns (discussed in RUDD *et al.* 1999).

4.1. It is not made adequately clear from the METHODS or Appendix 1 how glycosylation might affect the isolation procedure and whether all glycosylated forms of the protein would be fairly represented in the final stock solution.

4.2. That the protein was not glycosylated was confirmed by Western blot analysis of proteins isolated from grain. The authors used goat anti-CP4 EPSPS polyclonal antibodies for the Western blots (47/71). It is not made adequately clear how it was confirmed that antibodies raised to CP4 EPSPS in goats would detect all possible glycosylated forms of the protein produced in wheat. If they were isolated using antibodies raised to the reference protein expressed in *E. coli*, then subsequent analyses could be blinded to the full range of species being produced in the plant.

*4.3.* Glycosylation or its absence can create different antigenic epitopes from those present in otherwise identical polypeptides.

4.4. For example "O-linked glycosylation has a profound effect on the antigenic properties of peptides. O-linked glycosylation can generate a neo-epitope (e.g., CII),

or can have as an effect *the hiding of an epitope* (e.g., VF13N). O-linked glycosylation can mimic other epitopes (molecular mimicry of cytokeratins). *It can change the properties of an epitope even without really being part of the epitope* (CD43 and GPA)" [emphasis ours] (VAN DEN STEEN *et al.* 1998).

4.5. Alternative ways to study glycosylation are known, but all are not equally acceptable. So the applicant must take care in considering how to seek better data for the Authority. "Only comparisons of the same protein structure with or without carbohydrate can be conclusive about the role of sugars. Currently, there exist several means to generate such glycoprotein variants for comparative glycosylation studies. Enzymatic deglycosylation under native conditions (e.g., with PNGase F or with sialidase plus endo-β-*N*-acetylgalactosaminidase), expression of recombinant glycoproteins in cell lines with specific defects in the glycosylation machinery and expression in the presence and absence of glycosylation inhibitors (e.g., tunicamycins, benzyl- α-GalNAc or monensins) are all examples of relevant methods for this purpose. Chemical deglycosylation is much less ideal because the protein structure is most often damaged or destroyed" (VAN DEN STEEN *et al.* 1998).

4.6. The analysis of wheat-derived CP4 EPSPS has, in our view, fallen below the standard of reporting necessary to confirm the applicant's conclusions or the standard required to form the applicant's conclusions. This again is a criticism that has come from the scientific community previously (e.g. p. 358 of PUSZTAI *et al.* 2003) but has been inexplicably ignored by the applicant. We recommend that the applicant prepare or provide a complete proteomic analysis of the cultivars with the MON 71800 event accounting for all changes. Each change should be identified as either a variant of CP4 EPSPS or an unintended change in the modified plant. All variant forms of CP4 EPSPS should be characterized for glycosylation.

5. Substantial equivalence data.

The proteomic data recommended above should be supplemented with microarray data to complete the description of MON 71800, and update the compositional study by Obert *et al.* (Volume 3). In addition, recent work by Le Gall *et al.* has shown that "NMR combined with chemometrics and univariant statistics can successfully trace even small differences

in metabolite levels between plants" (LE GALL *et al.* 2003). This technique has been successfully tested using modified tomatoes. Their findings make obsolete comments in several recent review articles that metabolomics is still an uncertain science for assessing risk, and we recommend that this analysis be supplied to the Authority by the applicant.

5.1. There are significant effects of RNA and DNA that are not measured through a description of the average content of ribo- and deoxyribo-nucleotides, nor even through the average content of polymers. Small RNA molecules, on the order of <30 nucleotides, for example, are potent gene regulators (e.g. COGONI and MACINO 2000; HANNON 2002).

5.2. The creation of novel RNA regulatory molecules is too high by chance to ignore. They can be created by insertion of the transgene into a previously transcribed region (and not all transcripts emanate from ORFs), by aborted transcripts of the new transgene, or through fortuitous sequence similarity with an endogenous transcript.

5.3. Although these RNA molecules are seen as acting in a sequence-specific way, they can have unpredictable but still specific additional targets (JACKSON *et al.* 2003).

5.4. Importantly, the RNA effects are heritable even in multicellular animals, and transmit through food (COGONI and MACINO 2000; TIMMONS *et al.* 2001). Small RNA molecules developed in the food may have no effect on the plant itself, but could transmit to both somatic and germ cells in animal consumers. Therefore, microarray data is required to detect unintended and unanticipated effects on gene expression in both the modified wheat cultivar and on consuming organisms.

5.5. Microarray descriptions should be capable of detecting novel RNA species in the modified plant, with the RNA source being the plant grown under a variety of relevant field conditions. The microarray should comprehensively represent the genomes of:

5.5.1. the cultivar of wheat modified and unmodified;

5.5.2. the human genome; and

5.5.3. the genome of plants and animals, of cultural and ecological significance to Māori and other inhabitants of New Zealand, that might consume MON 71800 by design or by chance.

6. Digestion studies from Volume 4 and the George *et al.* study from Volume 5.

The digestion studies were submitted in part to reassure FSANZ and the public that the modified wheat has low potential as a novel source of allergens. It is our view that these digestion studies were fundamentally flawed with regard to conclusions of allergenicity. Our argument is that:

- the techniques used to assess the additional protein introduced with the MON 71800 event were below international minimum standards;
- these standards were set by FAO/WHO, the organization that establishes the principles FSANZ's assessments are supposed to be based upon;[1]
- *no studies were performed on the genetically engineered wheat*, making unintended effects creating potential allergens completely undetectable (PUSZTAI *et al.* 2003);
- the fact that similar studies have been reported before is not a good reason to pursue a protocol with known faults[3].

The digestion studies reported by the applicant fail to meet the minimum standards set by FAO/WHO (FAO/WHO 2001), despite conducting the studies after the FAO/WHO standards were published. These minimum standards are not, in our view, luxuries, but essential for making a legitimate risk assessment.

At a minimum, the applicant should have used CP4 EPSPS derived from MON 71800 wheat. Clearly this material was available because stocks of it were used in the Lee *et al.* study (Volume 2) and the Leach *et al.* study (Volume 5). We can see no justification for including the studies discussed below because it is not clear how the use of *E. coli*-derived

[3] Several studies link to previous studies of the same type, such as did the George *et al.* study (Volume 4) to the Ream *et al.* study in 1993. Not only has the 1993 study been the subject of significant criticism, the citation is to an unpublished document not included with the submitted materials making it impossible for scientific referees such as ourselves to verify claims and conditions.
Another example is provided by the Leach *et al.* study (Volume 5) and others of this type that justify (section 5) the test system as "In vitro digestion models are used widely…" and "A previous study has demonstrated that digestibility is a factor relevant…". Not only is this below standards of scholarship for justification, but the single published study upon which these statements are based was written by one of the authors of the Leach study 8 years ago.

material matches or increases confidence in the findings using material derived from MON 71800. We recommend that the Authority require the applicant, at a minimum, to supply data on the digestibility of the MON 71800-derived protein under the same conditions used to measure the *E. coli*-derived material, and that those conditions achieve the FAO/WHO standard.

6.1. The FAO/WHO state that "the expressed protein should be assessed in its principal edible form under identical pepsin degradation conditions to those used to examine the expressed protein" (p. 12). The applicant only tested the latter, i.e. expressed protein isolated from *E. coli*. The equivalence of the *E. coli* preparation has not been established to our satisfaction (see above).

Further arguments that the sources are equivalent remain just that, arguments. Since it is technically possible to isolate the protein in its edible form there is no reason to leave this point simply to argument.

6.2. The FAO/WHO state that "Both known non-allergenic (soybean lipoxygenase, potato acid phosphatase or equivalent) and allergenic (milk beta lactoglobulin, soybean trypsin inhibitor or equivalent) food proteins should be included as comparators to determine the relative degree of the expressed proteins pepsin resistance" (p. 12). We could not find these controls in the materials supplied to FSANZ.

6.3. It is difficult from the applicant's description (Leach *et al*., Volume 4) to say with certainty how much pepsin was used in the applicant's assay. However, we calculate that the applicant used approximately 3,000 times more pepsin than called for by FAO/WHO to make standard comparisons, effectively rendering the assay inadmissible. Our reasoning is as follows:

6.3.1. The applicant used 1µg CP4 EPSPS/10 pepsin AU. Every 1mg of pepsin had 3,460 AU. Thus, the applicant would have used 3µg of pepsin for every 1ug CP4 EPSPS.

6.3.2. FAO/WHO recommends 200µl of 0.32% (w/v) pepsin per 500µg CP4 EPSPS (0.32% is 0.32mg/100ml, or 0.64µg in 200µl). The FAO/WHO recommendation is 0.001µg of pepsin/1ug CP4 EPSPS.

6.3.3. The applicant therefore used 3,000 times the amount of pepsin recommended by the FAO/WHO, rendering the assay inadmissible. Extrapolations of the relation between increases in the amount of pepsin and relative digestion times is not provided, and probably cannot be determined in the absence of empirical testing.

7. Digestion study by Leach *et al.*, Volume 5, and comments pertinent to all digestion studies submitted by the applicant.

The Leach *et al.* digestion study differed from those described above in that the CP4 EPSPS protein was isolated from the plant and the concentration of pepsin was different.

7.1. The isolation of the protein from the wheat partially complies with the FAO/WHO standard that "the expressed protein should be assessed in its principal edible form under identical pepsin degradation conditions to those used to examine the expressed protein" (p. 12).

7.2. However, the study still failed to meet the standard that "Both known non-allergenic (soybean lipoxygenase, potato acid phosphatase or equivalent) and allergenic (milk beta lactoglobulin, soybean trypsin inhibitor or equivalent) food proteins should be included as comparators to determine the relative degree of the expressed proteins pepsin resistance" (p. 12), as far as we could tell from the materials supplied to FSANZ.

7.3. Although the METHODS section of this study matches the other Leach *et al.* study (Volume 4) using protein from *E. coli*, for this study 61% more pepsin was used. We are uncertain how this increase is justified. Presumably, this is to account for the additional protein supplied by the MON 71900 matrix. However, if high amounts of pepsin had to be added when the experiment followed a protocol marginally more relevant to the FAO/WHO standard, then we have added concerns about the uniform failure of the applicant to adhere to the FAO/WHO standard in any digestion study.

7.4. From the applicant's description, we extrapolate our calculations from above and conclude that the applicant used approximately 2.5 million times more pepsin than

called for by FAO/WHO to make standard comparisons. Our reasoning is as follows:

7.4.1. Per weight CP4 EPSPS, the applicant used 61% more pepsin (0.4197mg/ml vs. 0.2557mg/ml) and only 1ng CP4 EPSPS (vs. 500 ng).

$$(3{,}000 \times 500) \div 0.61 = 2{,}459{,}016$$

We recommend that the Authority require the applicant, at a minimum, to supply data on the digestibility of the MON 71800-derived protein using a protocol compliant with the FAO/WHO standard and the recommendations of Pusztai *et al.* (PUSZTAI *et al.* 2003).

The importance of *in vitro* digestion studies is not doubted by this submitter. However, their relevance to potential health hazards has never been accepted unequivocally. They have been viewed as the best that could be done at certain times. That is no longer the case (PUSZTAI *et al.* 2003).

The conclusion the applicant wishes to draw from these studies was most eloquently summarized by Goodman *et al.* (Volume 5) who said that "The conclusion of the bioinformatics and digestive fate assessments is that the CP4 EPSPS protein is not likely to cause allergic disease in consumers of wheat products" (p. 9/95). The central and most stringent case for the conclusion is therefore made based upon the digestibility studies, because bioinformatic tools have never been validated as comprehensively predictive. We believe that fundamental flaws in those studies should leave the Authority with no confidence in claims regarding allergenicity.

The literature in the past several years has indicated that extrapolations from *in vitro* studies is no substitute of tests using animals (PUSZTAI *et al.* 2003). For example, Chowdhury *et al.* demonstrated that Cry1Ab protein from genetically engineered corn survived digestion in the stomach of pigs because it could be detected by ELISA, immunochromatography and immunoblot in the intestine, despite it being shown to be highly digestible through the type of *in vitro* studies reported for CP4 EPSPS (CHOWDHURY *et al.* 2003). Similary, large fragments of the *cry1Ab* gene found in corn survived digestion and were detected in fecal material (CHOWDHURY *et al.* 2003). Interestingly, DNA and protein from the natural source (*Bacillus thuringiensis* subsp. *kurstaki*) was not detected in control pigs, indicating that the concentration of this

material, or its structure, when present in feed corn differs from the material introduced into food through natural contamination by soil microorganisms. The differences are worthy of investigation. Many other studies also based on *in vivo* data of protein or DNA stability, and undoubtedly well known to the Authority, have made claims similar to the Chowdhury study. It is our view that *in vivo* data from reliable studies will always be more trustworthy than extrapolations from *in vitro* data.

Furthermore, the applicant and Authority need to address possible effects of novel foods on the intestinal flora. *In vitro* studies cannot do this. Few if any *in vivo* studies have been designed to do this (aside from looking at microbial recombinants arising from transgenic DNA surviving digestion (NETHERWOOD *et al.* 2004)). Data are accumulating that soil flora can be affected by some forms of transgenic crops, such as Bt rice (WU *et al.* 2004). If soil flora is affected, then intestinal flora could be affected. Although Bt rice and the type of modification made to it are different from MON 71800, the wheat variety will be introduced with other changes to traditional wheat cultivation techniques (e.g. the use of Roundup). The intestinal flora is critical to good health. Disease states arise from subtle shifts in population structure, not just the introduction of new pathogenic species (BERG 1995; BERG 1996).

8. Toxicity study.

The study by Bonnette (Volume 5) examined the acute oral toxicity of CP4 EPSPS isolated from *E. coli*. The study was conducted on 100 mice, 10 of each sex per group, with each group receiving CP4 EPSPS at 0, 100, 300 or 1000 mg/kg body weight. The control group received 1000 mg/kg BSA. Each received a single dose of the protein by gavage followed by *ad libitum* feeding on commercial feed for the 14 day trial. No significant weight changes were detected over the trial.

8.1. The source of the protein should have been MON 71800 wheat, not *E. coli*. This is a criticism that has been made repeatedly by the scientific community (PUSZTAI *et al.* 2003) but has gone unheeded even in these studies which should have by now benefited from the extensive contribution of the independent scientific community. The reasons for ignoring this criticism need to be placed before the community for evaluation. This study should be supplemented with additional information, or

replaced by a study that accounts for this and the remaining criticisms, before FSANZ amends the food code.

"With regard to the last point, toxicity testing of the whole crop or derived plant products might be required. For example, cases where the composition of the whole crop has been changed significantly compared with the traditional counterpart, or where there is a need to further investigate potential unintended side effects of the genetic modification, warrant additional toxicity testing" (p. 440 of KOK and KUPIER 2003). In our view, there is need to further investigate unintended side effects because such have been found in recent tests of other genetically engineered plants (PUSZTAI *et al.* 2003). Toxicity tests on the whole crop are warranted in this case.

8.2. We seek clarification on the question of the degree of significance in the differences between weights on Day 0 and Day 14. If the overall weight gain of the mice over the 14 day period is negligible, then the mice were not of suitable age for this study. The ~10% variance in starting body weights within groups would exceed what is likely to be detected from all but extremely potent toxins (PUSZTAI *et al.* 2003).

8.3. In addition to using MON 71800 and protein isolated from MON 71800 in a toxicity trial, it is essential that both MON 71900 and MON 71900 spiked with CP4 EPSPS be included as controls. Failing this, unintended effects of the modification will not be detected.

8.4. In any supplemental study, it is essential to weigh and observe the organs of the sacrificed animals.

We recommend that the Authority disregard the applicant's claims on acute oral toxicity until either the Bonnette study (Volume 5, Acute Oral Toxicity) is supplemented with the information we believe is essential, or is replaced by a study of satisfactory rigor. "The biological, immunological, hormonal properties and allergenicity of" MON 71800 wheat must be determined using the wheat product and not surrogate sources such as *E. coli* (PUSZTAI *et al.* 2003).

9. Allergenicity study and comments pertinent to both the toxicity and allergenicity studies.

The study by Goodman *et al.* (Volume 5) tested the human immune response using sera challenged with MON 71800, its parent MON 71900 and similar red spring wheat varieties grown in North Amercia. Ostensibly the study was "designed to evaluate potential changes that may have occurred in the endogenous allergenicity of" MON 71800, "due to direct or indirect effects of the transformation" (p. 9/95).

"Serum IgE-immunoblotting and serum IgE-inhibition ELISA were chosen to evaluate the potential qualitative and quantitative IgE binding differences" (p. 9/95). Serum was donated by 10 volunteers with histories of "IgE-mediated allergic responses to dietary exposure to wheat" (p. 10/95).

Although this report stood out as being one of the more thoughtful submitted by the applicant, we have a low confidence in its predictive value.

9.1. The study used sera from people exposed to conventional wheat, not MON 71800. These individuals would not have mounted an immune reaction to an unknown allergen unique to MON 71800.

9.2. Therefore the study only provides baseline data about the generic allergenicity of red spring wheat.

9.3. The study was limited to dietary exposure. People could also be exposed to MON 71800 products through inhalation of the flour. Therefore, the study should include an assessment of inhalation challenge.

9.4. Allergenicity is arguably the most complex and difficult assessment to make (PUSZTAI *et al.* 2003). That is why we believe that human trials are ultimately essential. To be conducted responsibly, they must be conducted in controlled settings, not on consumers.

Since neither the toxicity nor the allergenicity study have convinced us that they have strong predictive power, we recommend that a risk management plan should be submitted to the Authority by the applicant. That plan should include a description of how the applicant will conduct post-launch monitoring (SCHILTER and CONSTABLE 2002). Failure to do so would, in our minds, undermine the Authority's objective of ensuring the consumers receive "adequate information relating to food" so that they may make informed choices in the long-term.

Until now, a *de facto* passive surveillance of novel foods has substituted for an active monitoring of genetically engineered foods. For example, it is common to hear the claim that "Americans have been eating GM for years and they seem alright." However, passive surveillance of consumers falls short of what we would recommend for New Zealand, because passive surveillance:

9.5. detects only "serious acute adverse effects" and is not appropriate for the kinds of effects most likely to arise from novel foods (SCHILTER and CONSTABLE 2002);

9.6. lacks an intrinsic control population from which baseline data of chronic conditions can be extrapolated;

9.7. could incur delays if chronic conditions were mis-diagnosed;

9.8. is not designed as a failsafe for unanticipated errors, such as, for example, the unintended contamination of novel foods with novel feeds approved only for animals.

10. Summary. Our conclusion is that significant additional information should be provided by the applicant before the Australia New Zealand food standards are altered. Since the time that other genetically engineered food and genetically engineered plant-derived materials have been included in amendments to the Code, there have been significant advances in biosafety and risk assessment science. The studies submitted in support of A524 no longer uniformly meet what we see as the standard of the science.

    Moreover, while the standard of the science has advanced, the tools available to the applicant have also improved (KUIPER *et al.* 2003; LE GALL *et al.* 2003; PUSZTAI *et al.*

2003). Indeed, all data that we recommend be made available to the Authority can be obtained using commercially available materials or using protocols developed in the public domain.

Respectfully submitted,

Dr. Jack Heinemann
Director

**Bibliography**


BERG, R. D., 1995 Bacterial translocation from the gastrointestinal tract. Trends Microbiol. **3:** 149-154.

BERG, R. D., 1996 The indigenous gastrointestinal microflora. Trends Microbiol. **4:** 430-435.

CHOWDHURY, E. H., H. KURIBARA, A. HINO, P. SULTANA, O. MIKAMI *et al.*, 2003 Detection of corn intrinsic and recombinant DNA fragments and Cry1Ab protein in the gastrointestinal contents of pigs fed genetically modified corn Bt11. J. Anim. Sci. **81:** 2546-2551.

COGONI, C., and G. MACINO, 2000 Post-transcriptional gene silencing across kingsoms. Curr. Opin. Genet. Develop. **10:** 638-643.

FAO/WHO, 2001 Allergenicity of Genetically Modified Foods. Report of a Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology., pp. Food and Agriculture Organisation of the United Nations, Rome.

HANNON, G. J., 2002 RNA interference. Nature **418:** 244-251.

JACKSON, A. L., S. R. BARTZ, J. SCHELTER, S. V. KOBAYASHI, J. BURCHARD *et al.*, 2003 Expression profiling reveals off-target gene regulation by RNAi. Nat. Biotechnol. **21:** 635-637.

KOK, E. J., and H. A. KUPIER, 2003 Comparative safety assessment for biotech crops. Trends Biotechnol. **21:** 439-444.

KUIPER, H. A., E. J. KOK and K.-H. ENGEL, 2003 Exploitation of molecular profiling techniques for GM food safety assessment. Curr. Opin. Biotech. **14:** 238-242.

LE GALL, G., I. J. COLQUHOUN, A. L. DAVIS, G. J. COLLINS and M. E. VERHOEYEN, 2003 Metabolite profiling of Tomato (Lycopersicon esculentum) using 1H NMR

spectroscopy as a tool to detect potential unintended effects following a genetic modification. J. Agric. Food Chem. **51:** 2447-2456.

MANZI, A. E., K. NORGARD-SUMNICHT, S. ARGADE, J. D. MARTH, H. VAN HALBEEK *et al.*, 2000 Exploring the glycan repertoire of genetically modified mice by isolation and profiling of the major glycan classes and nano-NMR analysis of glycan mixtures. Glycobiology **10:** 669-689.

NETHERWOOD, T., S. M. MARTÍN-ORÚE, A. G. O'DONNELL, S. GOCKLING, J. GRAHAM *et al.*, 2004 Assessing the survival of transgenic plant DNA in the human gastrointestinal tract. Nat. Biotechnol. **22:** 204-209.

PRYME, I. F., and R. LEMBCKE, 2003 In vivo studies on possible health consequences of genetically modified food and feed - with paticular regard to ingredients consisting of genetically modified plant materials. Nut. Health **17:** 1-8.

PUSZTAI, A., S. BARDOCZ and S. W. B. EWEN, 2003 Genetically modified foods: potential human health effects, pp. 347-371 in *Food Safety: Contaminants and Toxins*, edited by J. P. F. D´MELLO. CAB International.

RUDD, P. M., and R. A. DWEK, 1997 Glycosylation: heterogeneity and the 3D structure of proteins. Crit. Rev. Biochem. Mol. Biol. **32:** 1-100.

RUDD, P. M., T. ENDO, C. COLOMINAS, D. GROTH, S. F. WHEELER *et al.*, 1999 Glycosylation differences between the normal and pathogenic prion protein isoforms. PNAS **96:** 13044-13049.

SCHILTER, B., and A. CONSTABLE, 2002 Regulatory control of genetically modified (GM) foods: likely developments. Toxicol. Lett. **127:** 341-349.

TIMMONS, L., D. L. COURT and A. FIRE, 2001 Ingestion of bacterially expressed dsRNAs can produce specific and potent genetic interference in Caenorhabditis elegans. Gene **263:** 103-112.

VAN DEN STEEN, P., P. M. RUDD, R. A. DWEK and G. OPDENAKKER, 1998 Concepts and Principles of O-Linked Glycosylation. Crit. Rev. Biochem. Mol. Biol. **33:** 151-208.

WU, W., Q. YE and H. MIN, 2004 Effect of straws from Bt-transgenic rice on selected biological activities in water-flooded soil. Eur. J. Soil BIol. **40:** 15–22.