

Expert Report by Dr. Christopher Preston, University of Adelaide
Re: Stephen William Marsh vs Michael Owen Baxter
Supreme Court of Western Australia – CIV 1561 of 2012

Prior to the preparation of this report I have been provided with a copy of the Code of Conduct for Expert Witnesses and certify this report complies with said code. In preparation of the report, I have responded to a set of questions put to me. I have relied on my own research both published and unpublished, my experience in the Australian agricultural sector and published research and reports from others.

1. Please attach your curriculum vitae and provide a summary of your qualifications in agricultural science, plant science and research.

I am an Associate Professor of Weed Management employed at the University of Adelaide. I have a Ph.D. in plant biochemistry in the course of which I investigated aspects of photosynthesis in salt-tolerant species. I spent 2.5 years working for the US Department of Energy on fundamental aspects of photosynthesis and the past 23 years working on herbicide resistant weeds at the University of Adelaide. I have published 96 peer reviewed research papers, as well as several book chapters.

In addition to my research and teaching activities, I sit on several industry committees including Chair of the Australian Glyphosate Sustainability Working Group, the National Integrated Weed Management Initiative and the Transgenic and Insect Management Strategies Committee Herbicide Technical Panel for the cotton industry. I also advise various agricultural companies, industry bodies and government agencies about herbicide resistance issues. I participate in the Herbicide Resistance Consultative Groups for Monsanto and Pioneer/Dupont and provide weed ecology and resistance management expertise to Monsanto's Institutional Biosafety Committee.

I have attached a brief CV.

- 2. Please outline the details of any studies and research in which you have been involved in relation to the survival rates of volunteer canola plants in fields, on the roadside and in bush land in the agricultural areas of Australia:**
- i. Where was such study or research conducted?**
 - ii. What methodology was employed in the conduct of the study or research?**
 - iii. What were the results of the study or research?**
 - iv. Were the results of the study or research published, and if so in what publication?**
 - v. Please provide your reasons for reaching the conclusions resulting from the study and research.**

I have designed and participated in research to address risk factors around canola volunteers in fields and on roadsides. This research has been conducted in South Australia, Victoria and New South Wales. Several pieces of research have been conducted. A study investigating persistence of canola in farmer's fields will be

addressed in the next section. In this section I will only address three studies examining canola on roadsides.

The first study was conducted in Victoria and South Australia in 2000-2002. In this study we collected seed pods from surviving canola plants growing along road sides. Pods were collected from up to 30 individual plants at individual locations separated by 400 km. For a total of 7 populations, DNA was separately extracted from one or more seed from each plant and a DNA fingerprinting technique called ISSR used to determine how many canola cultivars were present in each population. A set of canola cultivars grown in the region was provided by Mr Trent Potter, the SA canola breeder, to confirm that the technique was able to identify individual genotypes. The research also compared the genotype of the parent plant (by extracting DNA from the pod) with the progeny (DNA from seed inside the pod) to determine whether outcrossing was occurring in these roadside populations.

The results of this study were that small roadside canola populations (<5 individuals) shared a single genotype. Larger populations were composed of multiple genotypes (ranging from 2 to 5). The studies of parental and progeny DNA found no evidence of hybridization in the roadside populations.

Aspects of this research have been published in:

Baker, J., Hidayat, I. and Preston, C. 2007. Molecular tools for understanding distribution and spread of weed genotypes. *Crop Protection* 26, 198-206.

Baker, J. and Preston, C. 2004. Roadside canola in South Australia and Victoria: persistent or transient populations? In B.M. Sindel and S.B. Johnson eds. *Proceedings of the 14th Australian Weeds Conference*. Weed Society of New South Wales, Sydney, pp. 403-405.

I concluded from this research that canola populations on roadsides were the result of one or more spills from grain trucks rather than from persistent populations built up over time. The more complex populations were located near grain receival points where large numbers of grain trucks move, providing the opportunity for multiple spills. However, it was not possible to state whether the spills had occurred in a single year or over multiple years. I did conclude it was unlikely that the populations had persisted on the sites for many years, because if that were the case hybridisation between cultivars on roadsides would be expected. There was no evidence of this in the data collected.

The second study was conducted in 2009-2010. This study was conducted in Victoria. This study took advantage of the fact that only two grain receival sites were available for GM canola growers in western Victoria in the 2008 season. During early spring in 2009 a survey was conducted around Tatyoon and Lubeck in Victoria and leaf material collected from any canola plants growing on roadsides within 5 km of the grain receival points. The locations of the plants sampled were recorded using GPS. In total 50 samples were collected from 14 sites near Tatyoon and 51 samples collected from 17 sites near Lubeck. DNA was extracted from the leaf sample and tested for presence of the CP4 EPSPS using specific PCR primers. In 2010, the locations re-visited and leaf material collected from any canola plants occurring

within 50 m of the original locations. A total of 31 samples were collected at Tatyoon and 62 samples were collected at Lubeck. DNA was extracted from the leaf samples and tested for the presence of the C4 EPSPS using specific PCR primers.

In 2009, we identified GM canola at one location of the 14 sites near Tatyoon and at 5 locations of the 17 sites at Lubeck. In 2010, no sites at Tatyoon had GM canola present. At Lubeck, only one site that had GM canola present in 2009 had GM canola present in 2010. The remaining sites with GM canola present in 2009 had only non-GM canola present in 2010. At Lubeck, 4 of the sites that had no GM canola in 2009 had GM canola present in 2010. Across both sites, 14 of the 31 locations visited in 2009 had no canola present within 50 m in 2010.

This research is being prepared for publication and should be submitted by the end of 2013.

I concluded from this research that GM canola will be found on roadsides, particularly close to the grain receival points. Many of the locations where canola was present were on road bends, close to bridges and at intersections, areas where spills are more likely. I further concluded GM canola on road sides was not persisting in high numbers from year to year as most locations containing GM canola in one year had no GM canola the next year. Likewise, one location having only non-GM canola in 2009 had only GM canola in 2010. Therefore, a considerable portion of the canola on roadsides must be the result of spills from the previous year's harvest.

The third piece of research conducted was a survey of roadside canola populations across the canola-growing regions of Victoria and NSW in 2010. In this research, leaf samples were collected from canola volunteers growing on roadsides in spring 2010. A leaf was collected from a single plant at 292 locations. DNA was extracted from the leaf material and tested for the presence of the CP4 EPSPS using specific PCR primers.

The research was able to identify samples as GM canola in 49 samples out of the 292 tested. Therefore, across this area about 17% of the canola growing on roadsides was GM.

This research is being prepared for publication and should be submitted by the end of 2013.

I concluded from this research that GM canola present on roadsides was at a similar proportion to the area of the crop planted in the previous year. A market survey of 501 canola growers in NSW and Victoria in 2009 conducted by Hudson and Richards (2013) found 19% of the canola area planted by these growers was sown with Roundup Ready canola. Therefore, most canola growing on roadsides was likely to have arisen from spills from the previous year and a large population of canola was not persisting from year to year.

Hudson, D. and Richards, R. (2013) Evaluation of the agronomic, environmental, economic and co-existence impacts following the introduction of GM canola to Australia (2010-2012). Paper presented at GMCC-13, Lisbon 12-15 November 2013.

If large canola populations were to persist in roadside environments from year to year, then the amount of seed contributed by new seed spills would be considerably lower than the total amount of seed present in this environment. GM canola was first planted in NSW and Victoria over a very small area in 2008. Therefore, there would have been little opportunity for GM canola seed banks on road sides to build up to any size and nearly all of the seed present in seed banks would be from non-GM canola. Under such circumstances, the proportion of GM canola on roadsides in 2010 should have been much lower than the proportion of area planted to GM canola in 2009.

- 3. Please describe the details of any studies and research in which you have been involved as to the survival of the canola seed bank in farmer managed paddocks in the agricultural areas of Australia:**
- i. Where was such study or research conducted?**
 - ii. What methodology was employed in the conduct of the study or research?**
 - iii. What were the results of the study or research?**
 - iv. Were the results of the study or research published, and if so in what publication?**
 - v. Please provide your reasons for reaching the conclusions resulting from the study and research.**

From 2002 to 2005 research was conducted on commercial farms to examine the persistence of canola volunteers in commercial practice. Soil samples were collected from fields that had previously grown canola. The canola seed were removed from the soil by sieving and washing. Canola seed were identified visually. In 2005, seed was also tested for viability through a germination test.

This research determined that canola seed banks in the commercial fields decayed quickly after canola harvest and no viable seed remained after 2.5 years. There was an initial difference between minimum tillage (one pass prior to seeding) and no-till (direct drill with narrow points) systems in that higher seed banks were present in the minimum tillage system 6 months after harvest. However, this difference between tillage systems declined with time.

This research was published in:

Baker, J. and Preston, C. 2008. Canola (*Brassica napus* L.) seedbank declines rapidly in farmer-managed fields in South Australia. *Australian Journal of Agricultural Research* 59, 780-784.

I concluded from the research that volunteer canola would occur in cropped fields in the years after canola production, but that the seed bank would decay rapidly. The normal management practices adopted by the farmers in the area surveyed were effectively driving volunteer canola populations to extinction on their farms. We drew this conclusion from the rate of decline in populations between years and the fact that at 3.5 years no germinable canola seed remained.

- 4. Please describe the studies and research in which you have been involved relating to the capacity of canola plants to cross pollinate with other plant varieties in Australia:**
- i. Where was such study or research conducted?**
 - ii. What methodology was employed in the conduct of the study or research?**
 - iii. What were the results of the study or research?**
 - iv. Were the results of the study or research published, and if so in what publication?**
 - v. Please provide your reasons for reaching the conclusions resulting from the study and research.**

I have been involved in three trials investigating the capacity of canola to cross pollinate. One of the trials investigated the ability of canola to cross pollinate with wild radish under field conditions and was conducted in 1997-1999 in South Australia. The other two trials examined the ability of canola crops to cross pollinate and were conducted in 2000 in NSW, Victoria and South Australia and in 2008-2009 in NSW, Victoria and Western Australia.

For the wild radish trial, we grew test plots of canola (either susceptible or resistant to imidazolinone herbicides) with wild radish planted at 1 and 4 plants/m². Two wild radish populations were used: 1 susceptible to herbicides and 1 resistant to sulfonylurea herbicides. The susceptible wild radish was planted in the resistant canola plots and the resistant wild radish in the susceptible canola plots. At the end of the season, the wild radish was removed from the plots. Seed from the susceptible wild radish was kept and tested for resistance to herbicides. Seed from the susceptible canola plots was harvested and tested for resistance to herbicides. Any plants surviving the herbicide screen were further analysed through chromosome counts, RFLP analysis and morphologically to determine they were hybrids.

A total of 30,000 wild radish seedlings were screened and no hybrids identified. A total of 52 million canola seeds were screened, with 2 hybrids identified. These hybrids had 56 chromosomes, consistent with the hybrids being allohexaploids containing all canola and wild radish chromosomes. The hybrids were also intermediate in morphological characteristics between canola and wild radish and contained genetic material from both species. This work determined that hybrids between canola and wild radish would occur in the field, but at low frequencies. Where the hybrids occurred by crossing from wild radish to canola, it is most likely the seed would be harvested and removed.

This research was published in:

Rieger, M.A., Potter, T., Preston, C. and Powles, S.B. 2001. Hybridisation between *Brassica napus* L. and *Raphanus raphanistrum* L. under agronomic field conditions. *Theoretical and Applied Genetics* 103, 555-560.

Rieger, M.A., Preston, C., Potter, T. and Powles, S.B. 1999. Gene flow from transgenic canola to wild radish - a model system to determine the risks. In Lutman, P.J.W. ed. *Gene Flow and Agriculture: Relevance for Transgenic Crops*. British Crop Protection Council, Farnham UK, pp. 131-136.

I concluded from the research that viable hybrids between wild radish and canola were likely to occur in the field, but would occur at low frequencies. Where the canola carries a herbicide tolerance trait, the wild radish may become resistant to the herbicide through outcrossing. However, this is unlikely to become a major practical problem in the field, because it is likely resistance in wild radish would be selected by herbicide use faster in most cases than it would occur through gene flow.

A trial was conducted in 2000 in NSW, Victoria and South Australia to examine the potential of canola to cross-pollinate between crops. The research took advantage of the fact that imidazolinone-resistant canola (Clearfield canola) was sown commercially for the first time in 2000. Growers of Clearfield canola crops were identified and after crops had been windrowed, seed was collected from nearby non-Clearfield crops. The seed was collected from three different positions in each crop. Seed was sown, allowed to germinate and treated twice with a discriminating dose of a sulfonylurea herbicide.

A total of 63 non-Clearfield fields were visited. Herbicide resistant individuals were found in canola crops up to 3 km from the source crop. However, frequencies of resistance were low; below 0.2% in any collection. On a field basis, the level of resistant individuals averaged 0.009% of seed with a highest value of 0.07%.

This research was published in:

Rieger, M.A., Lamond, M., Preston, C., Powles, S.B. and Roush, R.T. 2002. Pollen-mediated movement of herbicide resistance between commercial canola fields. *Science* 296, 2386-2388.

My co-authors and I concluded from this research that co-existence of GM and non-GM canola should be possible in the Australian agricultural environment with delivery below market thresholds for adventitious presence, so long as a small buffer was in place between crops to limit mixing of seed at harvest. This conclusion was based on the low levels of herbicide tolerant canola present in the sink crops. My co-authors and I also concluded that sporadic low frequency gene movement was likely to occur and growers should manage the impact of this through effective management of canola volunteers.

In 2008 and 2009 we repeated the above trial using Roundup Ready canola crops as source crops. The methodology used was similar to the 2000 trial; except we used glyphosate herbicide to detect resistance and further confirmed resistance by PCR amplification of the CP4 EPSPS gene. This trial was conducted in NSW and Victoria in 2008 and Western Australia in 2009.

Samples were collected from a total of 121 non-GM canola crops located near 45 Roundup Ready crops. The frequency of herbicide resistant individuals ranged from 0 to 5.5% in any collection. High numbers were from samples collected close to the source GM crop (within 20 m). Resistance was detected up to 4.3 km from the source crop. On a field basis, resistance varied between 0 and 0.8% of canola seed. The amount of gene flow was higher in this trial than in the 2000 trial; averaging 0.05% in 2008 and 0.07% in 2009 compared with 0.009% in 2000.

This research has been submitted to Nature Biotechnology for review.

I concluded from this trial that even though the amount of gene flow was higher in 2008 and 2009, compared with 2000, that co-existence should still be practical in the Australian cropping system provided an appropriate buffer zone is in place. I came to this conclusion based on the evidence that the highest amount of resistance detected was still under the allowed adventitious presence limits (0.9%) for non-GM canola as stipulated by the European Union (EU). In addition, the research conducted in 2008 and 2009 showed there was a significantly higher frequency of gene flow to the windrow closest to the Roundup Ready crop than to elsewhere in the field. I concluded from this that the methodology used was likely to over-estimate the amount of GM canola present in non-GM fields.

5. To what extent are the findings resulting from your studies and research as to the survival rates of volunteer canola plants and seeds on roadsides, in the field, and in bush land relevant to the survival rates of GM canola volunteers and seeds?

The data from research I have been involved in concerning decay of canola seed banks in farmer fields is entirely relevant to the survival rates of canola seed in cropped fields in the Australian environment. The research on roadside canola is less relevant, but supportive of the fact that seed banks of canola decline rapidly with time. Adequate management of canola volunteers so that seed set is prevented should lead to extinction of the canola in less than 3.5 years. In our study above, the growers were implementing no additional practices to their normal weed control in order to limit the canola seed bank. Therefore, some canola plants were likely flowering each year and setting seed. Had these flowering canola plants been more rigorously controlled the growers may have been able to reduce their canola seed bank more quickly.

6. Does GM canola have the capacity to cross pollinate with other varieties of canola? If so, what practices are followed in the agricultural areas of Australia to enable GM canola and non-GM canola to co-exist?

The research I have been involved in discussed above has demonstrated that GM canola can cross pollinate with other varieties of canola. On a field basis, the rates of cross pollination are typically low, but can be as high as 0.8%. Cross pollination is more likely when crops are planted within 10 m and declines rapidly with distance.

The Roundup Ready Crop Management Plan recommends a minimum distance of 5 m between GM canola and other canola types to minimise adventitious presence.

Further under the Roundup Ready Crop Management Plan, if GM and non-GM canola are grown within 5 m of each other, growers are required to take action to reduce the impact of adventitious presence. These actions are to either slash and/or cultivate a 5 m band of the Roundup Ready crop prior to the onset of flowering or to deliver the first 5 m of the non-GM crop as GM canola.

7. In Australia what tolerance is there for the presence of GM canola seeds in non-GM canola seed before delivery to the market?

The Australian Oilseed Federation (AOF) produces oilseed delivery standards for Australia under the auspices of Grain Trade Australia, previously NACMA. The AOF canola delivery standard for non-GM canola CSO 1-a, states that under the standard:

“The adventitious presence of up to 0.9% of GM events approved by the Australian Government Office of the Gene Technology Regulator is permitted”.

...

8. In Australia what tolerance is there for GM canola seed in non-GM canola seed in seed for sowing?

The industry through the Australian Seeds Federation has established a standard for adventitious presence of approved GM canola events be below 0.5% in non-GM canola seed for sowing.

9. Has scientific research or studies been conducted in Australia to determine the level of success in the management of the co-existence of GM canola and non-GM canola in keeping the GM canola within the permissible thresholds in non-GM canola crops? If so:

- i. Please identify the studies and research;**
- ii. What conclusions did you reach as a result of the studies and research?**

In Australia, co-existence of GM and non-GM canola is an industry managed issue. It is managed through requirements in the Roundup Ready Crop Management Plan, Crop Declaration requirements for growers delivering canola, and testing of canola in the supply chain for adventitious presence.

The AOF has published three reports assessing co-existence practices covering the 2008/9 to 2010/11 seasons:

Market Choice in the Canola Industry: 2008/9 Final Stakeholders Report

Market Choice in the Canola Industry: 2009/10 Season Performance Report

Market Choice in the Canola Industry 2010/11 Season: Performance Report October 2011

These reports indicate no significant problems with co-existence of GM and non-GM canola in Australia in terms of delivery of specified products to markets.

Additionally, McCauley et al. (2012) published a paper that examined the introduction of GM canola into Western Australia:

McCauley, R., Davies, M., and Wyntje, A. (2012). The Step-wise Approach to Adoption of Genetically Modified (GM) Canola in Western Australia. *AgBioForum*, 15: 61-69.

This paper addressed the issues of adventitious presence and concluded:

“There was effective segregation of non-GM canola from GM canola in 2009 and 2010. There was a coexistence-related event where an organic grower lost certification of a portion of his property due to the presence of GM plant material. This case highlights the need for realistic thresholds in biological systems to enable coexistence of different production systems.”

From these surveys the conclusion I have drawn is that the arrangements for co-existence in the market place in Australia are currently working well for delivery of GM and non-GM canola to customers.

10. Were canola plant material and/or the volunteer canola plants which germinated on Eagle Rest scientifically capable of:

- i. Infecting, poisoning or doing other damage to soil, crops, plants and sheep on Eagle Rest?**
- ii. Transmitting genetic material to soil, crops, plants and sheep at Eagle Rest?**

Canola contains glucosinolates, which on enzymatic breakdown produce isothiocyanates. Isothiocyanates can be harmful to various organisms in the soil or to animals if large amounts of canola foliage are eaten. Glucosinolates occur in all members of the Brassicaceae plant family (contains canola, mustard, cabbage, radish, turnip and other crop and vegetable species as well as numerous agricultural weeds) studied and in 15 other plant families (Fahey et al. 2001). The type and content of glucosinolates present vary among plant species. Glucosinolate content is also affected by plant age.

Fahey, J. W., Zalcmann, A. T., and Talalay, P. (2001). The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry* 56, 5-51.

Among *Brassica napus* crops, glucosinolate content is generally lower in oilseed crops, such as canola, than it is in leafy crops, such as rutabaga (Velasco et al. 2008). Within the *Brassica* genus, *B. napus* has lower glucosinolate content in leaves than other members, such as *B. oxyrhina* and *B. carinata* (Potter et al. 1999)

Velasco, P., Soengas, P., Vilar, M., Cartea, M. E., and del Rio, M. (2008). Comparison of glucosinolate profiles in leaf and seed tissues of different *Brassica napus* crops. *Journal of the American Society for Horticultural Science*, 133, 551-558.

Potter, M. J., Davies, K., & Rathjen, A. J. (1998). Suppressive impact of glucosinolates in *Brassica* vegetative tissues on root lesion nematode *Pratylenchus neglectus*. *Journal of Chemical Ecology* 24, 67-80.

With respect to Roundup Ready canola, research has demonstrated there is no significant difference between Roundup Ready canola and conventional canola in glucosinolate content of grain (Daun 1999; Nickson and Hammond 2002)

Nickson, T.E., and Hammond, B.G. (2002). Case Study: canola tolerant to Roundup® herbicide. In Atherib, K., ed. Genetically Modified Crops. Assessing Safety. Taylor & Francis, pp 138-163.

Daun, J. K. (1999). Comparison of the Quality of Genetically modified Canola Varieties with Other Canola Varieties Grown in Western Canada in 1996/97. In New Horizons for an old crop Proceedings of the 10th International Rapeseed Congress, The Regional Institute Ltd, Canberra, Australia.
<http://www.regional.org.au/au/gcirc/4/223.htm>

The OGTR assessment of Roundup Ready canola (OGTR 2003) included data on glucosinolate content of Roundup Ready canola seed and a conventional canola variety Westar. The assessment concluded:

“The levels of the naturally occurring toxicants of canola, erucic acid and glucosinolates, do not vary between GM and conventional canola”

OGTR (2003) General Release of Roundup Ready® canola (*Brassica napus*) in Australia. Risk Assessment and Risk Management Plan. Final Version. DIR020/2002.

As glucosinolates do not differ between Roundup Ready and conventional canola and are lower in canola than in some other species of the same family, I consider it unlikely that a small number of canola plants will have a major effect on soil organisms.

With respect to other components of Roundup Ready canola, the OGTR assessment of Roundup Ready canola concluded:

“Compositional analyses of Roundup Ready® canola show no significant differences to conventional canola as a result of the genetic modifications”

The lack of differences in composition between Roundup Ready canola and conventional canola (with the exception of the proteins introduced by the genetic modification) make it highly unlikely that Roundup Ready canola volunteers would damage soil, or poison crop plants or livestock to any greater extent than conventional canola.

Canola is not pathogenic, so it would not be able to infect other plants or animals. Roundup Ready canola is not more pathogenic than conventional canola.

It is very unlikely that genetic material will be transferred from the Roundup Ready canola to sheep on Eagle Rest. Free DNA is readily degraded in the acidic stomach of mammals. Alexander et al. (2002; 2004) examined the fate of the canola plant DNA and the CPE EPSPS gene in processing and digestion.

Alexander, T. W., Sharma, R., Okine, E. K., Dixon, W. T., Forster, R. J., Stanford, K., and McAllister, T. A. (2002). Impact of feed processing and mixed ruminal culture on the fate of recombinant EPSP synthase and endogenous canola plant DNA. FEMS Microbiology Letters, 214, 263-269.

Alexander, T. W., Sharma, R., Deng, M. Y., Whetsell, A. J., Jennings, J. C., Wang, Y., Okine, E., Damgaard, D. and McAllister, T. A. (2004). Use of quantitative real-time and conventional PCR to assess the stability of the *cp4 epsps* transgene from Roundup Ready® canola in the intestinal, ruminal, and fecal contents of sheep. *Journal of biotechnology*, 112, 255-266.

Their research showed that canola plant DNA was rapidly degraded in ruminal batch cultures once released from cells. Plant DNA could not be detected in the supernatant of the culture. Likewise the CP4 EPSPS gene was present as an intact gene for 0.5 minutes in simulated digestive fluid at pH 7, but not at all at pH 5.

Where measurements were made on the solid component of the ruminal cultures, Plant DNA could be detected for extended periods of time (Alexander et al. 2002; Sharma et al. 2004) depending on the digestibility of the food source. However, this plant DNA is still inside plant cells and is degraded rapidly once the cells are broken.

Sharma, R., Alexander, T. W., John, S. J., Forster, R. J., and McAllister, T. A. (2004). Relative stability of transgene DNA fragments from GM rapeseed in mixed ruminal cultures. *British Journal of Nutrition*, 91, 673-682.

Some research has concluded that plant DNA sequences and sequences from CP4 EPSPS can be detected in pig organs, but not in sheep (Sharma et al. 2006). However, only one fragment of the gene could be detected in organs, indicating the gene was no longer intact. Also the authors point out that examination of genomic libraries from the organs that tested positive for the CP4 EPSPS gene fragments could find no evidence that the DNA had become incorporated into the cellular genome.

Sharma, R., Damgaard, D., Alexander, T. W., Dugan, M. E., Aalhus, J. L., Stanford, K., and McAllister, T. A. (2006). Detection of transgenic and endogenous plant DNA in digesta and tissues of sheep and pigs fed Roundup Ready canola meal. *Journal of Agricultural and Food Chemistry*, 54, 1699-1709.

A similar study examining pigs fed Bt maize feed (Mazza et al. 2005) identified fragments of maize genes in all tissues tested except muscle and fragments of the Cry1Ab gene in several tissues.

Mazza R, Soave M, Morlacchini M, Piva G, Marocco A (2005) Assessing the transfer of genetically modified DNA from feed to animal tissues. *Transgenic Res* 14: 775–784

However, other research (Walsh et al. 2011; 2012) has failed to identify the presence of DNA fragments of transgenic Cry genes in pig organs, although Walsh et al. (2012) were able to detect small fragments of rubisco, a maize gene, in various organs.

Walsh, M.C., Buzoianu, S.G., Gardiner, G.E., Rea, M.C., Gelencsér, E, Jánosi, A., Epstein, M.M., Ross, P.R. and Lawlor, P.G. (2011) Fate of Transgenic DNA from Orally Administered Bt MON810 Maize and Effects on Immune Response and Growth in Pigs. *PLoS ONE* 6(11): e27177. doi:10.1371/journal.pone.0027177

Walsh, M.C., Buzoianu, S.G., Rea, M.C., O'Donovan, O., Gelencsér, E., Ujhelyi, G., Ross, R.P, Gardiner, G. P. and Lawlor, P.G. (2012). Effects of feeding Bt MON810 maize to pigs for 110 days on peripheral immune response and digestive fate of the cry1Ab gene and truncated Bt toxin. *PloS ONE*, 7(5): e36141.
doi:10.1371/journal.pone.0036141

The various studies indicate that small pieces of plant DNA can occasionally be absorbed from the digestive system of monogastric species, such as pigs, and be detected by sensitive PCR tests. High copy number DNA sequences are more likely to be detected than single copy DNA sequences. There is currently no evidence that the pieces of DNA are integrated into the animal's genome.

Canola can cross pollinate and share genetic material with other canola crops, black, white and oriental mustard, radish vegetables and with *Brassica rapa* and *Brassica oleracea* vegetable crops, such as cauliflower, broccoli, Brussels sprouts, cabbage, swede and turnips (Salisbury 2002). If any of these crops are grown at Eagle Rest there is a probability that crossing between the volunteer canola and these crops would occur, provided they flowered at the same time as the volunteer canola.

Salisbury P.A. (2002). Gene flow between *Brassica napus* and other Brassicaceae species. Report PAS 0201, Institute of Land and Food Resources, University of Melbourne.

The greatest probability of out-crossing would occur to *Brassica napus* crops, followed by *Brassica rapa* and *Brassica juncea* crops. Out-crossing to the other species is possible, but has not been identified under field conditions.

Volunteer canola could also cross pollinate with several weed species if they were present at Eagle Rest. These weeds include: feral and volunteer versions of the species mentioned above, *Raphanus raphanistrum*, *Hirschfeldia incana*, *Sinapis arvensis*, *Brassica fruticulosa*, *Brassica tournefortii*, *Diplotaxis muralis*, *Diplotaxis tenuifolia* and *Rapistrum rugosum* (Rieger et al. 1999; Salisbury 2002). Crosses between canola and *Raphanus raphanistrum*, *Hirschfeldia incana* and *Sinapis arvensis* have been identified in field experiments at low frequencies.

Rieger MA, Preston C, Powles SB (1999) Risks of gene flow from transgenic herbicide-resistant canola (*Brassica napus*) to weedy relatives in southern Australian cropping systems. *Aust J Agric Res* 50: 115-128

Horizontal gene transfer is common among bacteria. Plasmids containing genes are frequently transferred from one bacterium to another, even when the bacteria are of different species. Several studies have identified the incorporation of transgenic material from plants into bacteria at low frequencies under artificial conditions (Nielsen et al. 2007; Simpson et al. 2007). However, these examples are restricted to specific circumstances where bacterial antibiotic resistance genes were being taken up by bacteria containing a non-function version of the same gene and then being selected by the antibiotic.

Nielsen, K. M., Johnsen, P. J., Bensasson, D., and Daffonchio, D. (2007). Release and persistence of extracellular DNA in the environment. *Environmental Biosafety Research*, 6, 37-54.

Simpson, D.J., Fry, J.C., Rogers, H.J., and Day, M.J. (2007) Transformation of *Acinetobacter baylyi* in non-sterile soil using recombinant plant nuclear DNA. *Environmental Biosafety Research*, 6, 101-112.

Free DNA is typically present in the environment for short periods of time before it is degraded, reducing the potential for it to be incorporated by soil bacteria. The current evidence suggests bacterial antibiotic genes used as selectable markers in transgenic plants are the most likely genes to be acquired by soil bacteria. Roundup Ready canola does not contain any antibiotic genes.

In the case of the CP4 EPSPS and *gox* genes present in Roundup Ready canola, these were originally bacterial genes. These genes are abundant in the environment and there is no obvious selection pressure to drive the acquisition by bacteria of the plant versions. Even if a bacterium were to acquire one of these genes, there would be no advantage to it and it is extremely unlikely that any harm would ensue.

11. What if any practical measures could have been taken by Mr Marsh to remove or reduce the presence of GM canola volunteers on Eagle Rest.

My understanding from news reports is that cut mature canola plants from windrows were moved by wind onto the farm of Mr Marsh. Based on my experience in weed management my recommendations for action to minimise the potential for GM canola volunteers on the farm would have been to take the following steps:

1. Remove as many of the cut canola plants as possible from the fields and burn them in a suitable place or otherwise dispose of them. This would greatly reduce the amount of spilt seed remaining in the fields.
2. After harvest, avoid cultivating the fields over summer. This will keep any spilt seed on the surface, where it may fatally germinate after summer rain, or if plants do establish they can be removed. In the Australian environment, most of the loss of canola from the seed bank occurs in the first 6 months after harvest.
3. Scout the fields during the next winter to detect any canola plants and control or remove them to stop seed set. Canola is easily identified from a distance as a volunteer in early spring because of their distinctive flowers. Failure to stop volunteer canola plants setting seed at this time will replenish the seed bank and lead to a continuing problem.
4. Further monitoring in subsequent years should be undertaken to ensure all the canola is removed.

12. Are there any scientific tests capable of detecting the presence of particles or residues of GM canola plant material in the soil, sheep, plants or the cereal grain seed grown on Eagle Rest (apart from any volunteer canola seed which may have been harvested with the cereal crops):

- i. If the answer is yes, please describe the relevant scientific tests;**
- ii. If the answer is no, are no such tests capable of detecting GM canola particles or residues?**

There are two types of tests that are commonly used to detect GM canola material in various circumstances. These are an ELISA (enzyme-linked immunosorbent assay) test that detects the CP4 EPSPS protein produced by the gene introduced into Roundup Ready canola or various PCR-based tests that detect the inserted genetic material.

The ELISA test uses an antibody to detect presence of the C4 EPSPS protein. It can be conducted in a laboratory setting or in the field using the so-called stick tests. Because it relies on detecting the C4 EPSPS protein, it is best conducted on grain samples or living leaf tissue. Once cells have been broken and the protein degraded, the ELISA test is unable to reliably detect the protein. The ELISA test typically has a quantification limit of 0.1%, although tests can be calibrated for lower sensitivity.

PCR-based methods employ the polymerase chain reaction to amplify DNA from the genome. The choice of primers to be used will depend on the material to be tested and the likelihood of similar sequences being present that might provide false positives. Typically primers are selected that will amplify part of the introduced gene or of regulatory sequences, such as the gene promoter or termination sequences. PCR requires a properly equipped laboratory, is time consuming and expensive. The theoretical quantification limit is 0.005%, but the practical detection limit is much higher than this value.

Variations on the PCR technique can be used to make the process quicker or more sensitive. The use of qPCR can increase the theoretical quantification limit to 0.001%. qPCR uses fluorescent probes to detect the amplified DNA after each cycle of amplification. Through this process the amount of sequence can be quantified. qPCR requires very specialised laboratory equipment and is more expensive than conventional PCR.

PCR-based tests can be used on any material from which DNA can be extracted. This includes plants, animals and soil. PCR does not require the tissue to be alive at the time of collection, as DNA can be extracted from dead and dried plant material. There are techniques in handling material that can enhance the probability of detection of the target DNA in a sample, for example through sieving large grain to concentrate smaller materials that would be more likely to contain canola seed or seed components.

However, there are limitations to PCR-based methods. PCR-based tests suffer from false positives, unless the amplified DNA is sequenced. Therefore, positive and negative controls need to be included. The choice of primers has to be done with care. If the primers amplify sequences from other organisms, soil bacteria for example, this will lead to false positives.

13. Is it practical to screen canola seed from cereal grain seed? If so:

- i. By what means can the canola seed be screened out?**
- ii. Is such screening method commonly practical in farming?**

During the harvesting operation, farmers typically harvest weed seed with their crop seed. There are price penalties for delivering grain with too much weed seed present. Therefore, farmers on occasions do remove weed seed from their harvested grain in order to meet delivery specifications.

Where the weed seed is a considerably different size to the grain, such as canola in cereals, a well set-up harvester will remove most of the small seeds from the harvested grain through the sieves. If further cleaning is required, the grain can be put through seed cleaning equipment that uses a combination of blowers and sieves to separate seeds by weight and size. If desired, further cleaning can be conducted with a gravity table. The gravity table cleans seed by density. Under normal circumstances, a gravity table would not be used to clean small seeds from cereal grain, because it would be unnecessary.

All of this equipment is used in commercial farming practice in Australia. However, the amount of seed cleaning that occurs varies depending on the market for the grain and the specification for delivery. Grain cleaning is a balance between the need to deliver grain to set specifications and the cost of the grain cleaning.

I Christopher Preston have made all inquiries which I believe are desirable and appropriate and that no matters of significance which I regard as relevant have, to my knowledge, been withheld from the Court.

Christopher Preston

4th December 2013