PI - 37Submission to FSANZ, Application A1274, GM banana QCAV-4
Due 6 November 2023, 6pm Canberra time.
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[redacted]

I request that Application A1274, which would allow QCAV-4 GM bananas to be used as a food, be *declined* for the reasons below.

1. The application states that QCAV-4 is not intended to replace current Cavendish banana cultivars grown in Australia, but rather to provide a "safety net" to the Australian banana industry, should it be heavily impacted by *Fusarium* wilt tropical race 4 (TR4), also known as Panama disease. This GM banana has not undergone any feeding studies that show it to be safe for eating by humans or animals. Assumptions of safety are no guarantee and should never be used to make safety assessments. The assumption of safety alone, as made by the applicant, does not answer the vital question of food safety with any of the essential observations or data. Therefore, the Application 1274 is not scientifically valid.

2. **Insertional mutagenesis**, i.e. disruption to DNA sequences, whether these are coding, regulatory or other sequences, always interferes with the genome of a plant during the process of any kind of genetic engineering. The result of this interference may or may not have been detected after *Agrobacterium*-mediated transformation, as has been used to produce QCAV-4. Unintended DNA modulation/s would result in altered metabolic processes within the cell, e.g. altered enzyme activity, which would have downstream consequences.

3. The application states that seven unintended open reading frames (ORFs) resulted from the insertion, as far as can be detected. According to this application, none contained the required regulatory elements necessary for expression of mRNA and protein biosynthesis.

While seven unintended open reading frames (ORFs) resulted from the insertion, none contained the required regulatory elements necessary for expression of mRNA and protein biosynthesis and this was confirmed by RNA-Seq. Analysis of the predicted amino acid sequences from these new ORFs showed that none had the potential to encode a protein with ***any significant amino acid sequence*** *similarity to known toxins or allergens. Using Southern blot analysis, the introduced genetic material was shown to be* ***stably inherited over five generations*** *of plants (p.1)*

Analysis of the predicted amino acid sequences from the new ORFs showed that none had the potential to encode a protein with any significant amino acid sequence to **known** toxins or allergens. However, any insertion (see 2. above), deletion or rearrangement of DNA in the GM banana cells, even as small as the change of a

single base pair, could result in the production of unknown and therefore undetectable toxins, allergens and/or carcinogens. Why is there no data provided that would enable one to assess whether the seven new proteins and their metabolites would be completely safe to eat?

4. Summarising the information provided in A1274, QCAV-4 cells have been selected for after *Agrobacterium*-mediated transformation, with antibiotic resistance markers (ARM). This methodology is essentially the same as has been used in laboratories since the 1980s. The T-DNA of the *Agrobacterium* plasmid, transferred directly to the genome of QCAV-4 during the genetic engineering process, contains two key genetic elements.

(a) **MamRGA2 coding sequence from the wild banana -** *Musa acuminata* ssp *malaccensis*. This gene is switched on by the NOS promoter from the enzyme nopaline synthase (NOS) of *Agrobacterium* and switched off by the 3' terminator, also derived from NOS.

(b) **The antibiotic resistance construct -** i.e. the nptII expression cassette. When expressed in the GM bananas, this genetic construct confers resistance to the antibiotic kanamycin, functioning as an antibiotic resistance marker gene. The promoter used to switch on the kanamycin resistance gene (CaMV35S) is derived from the RNA of cauliflower mosaic virus. This promoter is constitutive, i.e., it has the gene turned on at all times and drives the antibiotic resistance expression strongly. This promoter will be driving the expression of kanamycin resistance in all tissues of the banana plant, including the fruit.

Aside from the constitutive CaMV35S promoter, the gene conferring the antibiotic resistance could also cause problems. The nptII coding sequence has been isolated from the transposon Tn5 of *E. coli* strain K12. It is well documented that transposons are mobile genetic elements that can readily translocate in DNA. Why was this particular transposon gene sequence used? Are there experimental data documenting the safety of using such a DNA sequence in GM bananas?

When the fruit is eaten, the antibiotic resistance gene could be biologically active in the human gut. Research has shown that DNA from GM maize persists for a significant time in the rumen of sheep and may therefore provide a source of transforming DNA in the rumen. [1] *Agrobacterium* plasmid DNA extracted from the saliva of sheep was capable of transforming competent *Escherichia coli* to kanamycin resistance, implying that DNA released from the diet in the mouth may retain sufficient biological activity for the transformation of competent oral bacteria.[1]

DNA from GM banana tissue could potentially exchange genetic information with *E. coli* in the gut via the process of horizontal gene transfer (HGT). Antibiotic resistance passed on to gut bacteria could mean that people who have eaten the GM bananas would not respond to antibiotics of the kanamycin type, because of the

antibiotic resistance present in their gut flora. The lack of research on the horizontal transfer of DNA from GMOs, has been discussed by Ho (2014). [2]

Concern has also been addressed about whether HGT of genes from GM plant material to environmental microorganisms can take place, thereby – in the next step – compromising the therapeutic value of antibiotics in human and veterinary medicine.[3]

Antibiotic resistance could also be transported through *E.coli* into sewerage systems and waterways that deal with the treatment of wastewater. This water usually ends up in the sea or some other body of water, where aquatic ecosystems exist. Antibiotic resistance could potentially be transferred to any aquatic organisms and to any food gathered from these aquatic environments. Ho (2014) noted that scientific studies done in China demonstrated that a specific plasmid, used to make GM plants and coding for very potent beta-lactamases, was found in six Chinese rivers.[2]

Yet another route for antibiotic resistance to be transferred to organisms in the surrounding environment would be any waste from the GM banana itself (flesh and skins) or trimmings from banana plants. The leaves and other parts of the banana plants are palatable to animals. Composted waste could transfer antibiotic resistance to soil microorganisms via HGT, and into water runoff.

Has the applicant carried out any experimental work that would provide a data set on any or all of the above probabilities? If not, there is no way that any assumptions about consumer and post-consumer safety can be made, both for human and animal consumption, and in the environment. The life cycle of food should always include agricultural production and food waste in all forms.

5. Has the GM banana been tested by human tasters for palatability? This seems like an obvious question, but given the amount of assumptions made in the application, it may have been completely overlooked, as I could not find a mention of it. Even if a banana is disease resistant, it will not be a commercial success if the taste or texture is unpleasant.

6. Has the applicant examined the history of other GM fruits that have been marketed as a success, but have failed on counts of food safety, unpleasant taste and undesirable texture? This was well documented with the GM Flavr Savr tomato, the world's first GM crop, engineered by Calgene in the 1990s to have a longer shelf life. Calgene carried out three feeding studies with rats and sent the studies to the FDA. FDA scientists were concerned about the presence of haemorrhagic stomach lesions in these rats. Seven out of forty female rats that ate Flavr Savr tomatoes had lesions. None were found in the control rat group that ate natural tomatoes. FDA repeatedly asked Calgene to provide additional data in order to resolve what they regarded as outstanding safety questions. A senior FDA director wrote "… the data fall short of 'a demonstration of safety' or of a 'demonstration of reasonable certainty of no harm',

which is the standard we typically apply to food additives. To do that we would need, in my opinion, a study that resolves the safety question raised by current data."[4]

Flavr Savr also contained the kanamycin resistance marker gene construct (as has been used for the GM banana QCAV-4). FDA scientists were asked to evaluate Calgene's use of this gene. On December 3, 1992, the Division of Anti-Infective Drug Products submitted to the FDA Biotechnology Coordinator their response to Calgene's proposal with the key sentence of their conclusion emphasized in capital letters: " IT WOULD BE A SERIOUS HEALTH HAZARD TO INTRODUCE A GENE THAT CODES FOR ANTIBIOTIC RESISTANCE INTO THE NORMAL FLORA OF THE GENERAL POPULATION." [5]

To further emphasize his concern, the division's director sent the document to another FDA official adding, "The Division comes down fairly squarely against the [kanamycin] gene marker in the genetically engineered tomatoes. I know this could have serious ramifications". [5]

ARM genes are not the only method to confirm that a foreign gene has successfully been transferred to the host DNA. At this point in time FDA microbiologist Albert Sheldon wrote, "Other markers… are available and should be used." He also wrote in a further communication in 1993, "In my opinion, the benefit to be gained by the use of the kanamycin resistance marker in transgenic plants is outweighed by the risk… If we allow this proposal, we…will probably assure dissemination of kanamycin resistance."[6]

Given that this was already regarded as a serious issue in the 1990s, there should have been much discussion and research around it. The presence of the antibiotic resistant marker gene will lead to unpredictable environmental hazards. [7] On the basis of economic incentives and safety concerns, there are already several methods, such as site-specific recombination, homologous recombination and co-transformation that have been developed to eliminate these genes.[7]

The use of the kanamycin resistance gene as a selective marker for these QCAV-4 GM bananas, poses another biosafety concern that has been overlooked by the applicant.

7. **Eco-friendly solutions to *Fusarium* wilt**.

Biological control agents (BCAs) have been applied to bananas with diverse procedures (e.g. spore suspension, organic amendments, bio-formulations etc.) and at different stages of plant development. Many of the BCAs in use greatly contribute to limit the damage caused by *Fusarium* banana wilt.[8]

In a recent comprehensive review of sustainable, eco-friendly management of *Fusarium* wilt in bananas, control of the disease using classical and essential oil

approaches is described in detail. Such practices target the bio-film formed by the fungus and suppress the pathogen.[9]

References

1. Duggan, P.S. et al. (2003) Fate of genetically modified maize DNA in the oral cavity and rumen of sheep. Br. J. Nutr. Feb; 89(2):159-166

2. Ho, M.W. Horizontal transfer of GM DNA widespread. Why is almost no one looking? Microb. Ecol. Health Dis. 2014; 25:25918

3. Midtvedt, T. (2014) Antibiotic resistance and genetically modified plants. Microb. Ecol. Health Dis. 25 doi:10.3402/mehd.v25.25918

4. Robert J. Scheuplein to the FDA Biotechnology Coordinator and others, "Response to Calgene Amended Petition," Oct 27, 1993 www.biointegrity.org

5. Murray Lumpkin to Bruce Burlington, "The tomatoes that will eat Akron," Dec 17, 1992 www.biointegrity.org

6. Albert Sheldon to James Maryanski, Biotechnology Coordinator, "Use of Kanamycin Resistance Markers in Tomatoes," Mar 30, 1993 www.biointegrity.org

7. Natarajan, S and Turna, J. Excision of selectable marker genes from transgenic crops as a concern for environmental biosafety. J. Sci Food Agric. 2007 Nov; 87 (14): 2547-54

8. Bubici, G. et al (2019) Biological Control Agents against *Fusarium* wilt of banana. Front Microbiol. 10:616

9. Ismaila, A.A et al (2023) *Fusarium* wilt of banana:Current update and sustainable disease control using classical and essential oil approaches. Horticultural Plant Journal 9 (1) Feb 2023:1-28