Possible attacks on the paper and rebuttals

Potential attack: The insecticidal Bt/Cry proteins made by GM crops have been safely used for decades – they are used in organic agriculture and there is no evidence that they are unsafe.

Reply
A bacterium called *Bacillus thuringiensis* may be used in organic agriculture when other methods of pest control have failed. It is sprayed as whole bacteria. The bacteria make naturally-occurring crystalline proteins, abbreviated to Cry proteins, or Bt proteins. These are degraded in sunlight and can be washed-off by the consumer, so that the consumer gets essentially no exposure to these proteins in their food. In contrast, GM crops are genetically modified to contain genes so that these proteins are made in every cell of the plant, including the edible part, and cannot be washed off by the consumer. There is therefore a huge difference in the amount of these proteins that the consumer is exposed to when eating these GM plants compared to eating non-GM plants.

In addition, there is a difference in the type of Cry protein that the consumer is exposed to. The Cry proteins made by GM crops are not the same as those produced by the bacteria. The naturally-occurring ones tend to be different, including usually being bigger and needing to be activated by being cut in the gut of an insect before they can function. In general, the proteins in GM crops are smaller and may already be in the active form (The International Service for the Acquisition of Agri-biotech Applications (ISAAA) GM Approval Database http://www.isaaa.org/gmapprovaldatabase). The reason for this is that the genes coding for the larger molecule do not give the plant a strong insecticidal property. The smaller proteins in the GM plant may therefore have lost species specificity to now have an effect on the stomachs of animals.

As we state in the paper, studies by other people have found that, in mammals, these proteins can bind to the surface of intestinal cells and can cause hyperpolarisation, consistent with the pore-forming action of Cry proteins in insects.

In our previous review paper (Zdziarski I M, Edwards JW, Carman JA, Haynes JI (2014). GM crops and the rat digestive tract: A critical review. Environment International, 73, 423-433. (https://doi.org/10.1016/j.envint.2014.08.018), we determined that we could not find a single study that was properly conducted or reported. We concluded that there was a lack of evidence that these crops were safe to eat.

Potential attack: The differences were due to the rats being starved

Reply
The rats were NOT starved. Starved animals lose weight. Neither group of rats lost weight. It was simply the case that the weight gain of both groups of rats was not as high as expected in the early stages of the experiment. The commercial manufacturer of the diets suggested that they may have made the diets too dry for the young rats to readily eat and suggested that the pellets be repelleted to a higher moisture content, starting with the GM diet, because they were the group with the lowest dietary intake of the two. The rats then had a typical weight gain for the rest of the study. This occurred for both groups of rats well before the end of the experiment. It is clear that there was no residual effect on the stomachs at the end of the experiment as evidenced by the fact that signs such as a thickening of the keratinized layer of the stomach were NOT present, confirming dietary and body weight observations that the rats were not starved.

There is no evidence that reduced dietary intake can cause any of the effects seen in this study, and those criticising the study have not produced any evidence that there is. Their view is therefore unsubstantiated conjecture.

A modest reduction in dietary intake, like the one that occurred in these rats, does not cause harm in animals or people. In fact, there are now hundreds of experiments that show that animals with modest dietary restriction have better health and live longer. Consequently, some people now voluntarily reduce their dietary intake in order to improve their health and increase their lifespan.

In a randomised controlled trial like this one, rats are randomised into two groups and then everything else is kept the same between the two groups except for the GM v non-GM aspect of the diet. It is the strongest study design there is, because any differences you see between those two groups at the end of the experiment must be due to the GM v. non-GM aspect of the diet and nothing else. The only way that a difference can be due to something else is if that "something else" happens to one group and not the other. Note that BOTH groups of rats had initial modest dietary restriction and BOTH groups of rats subsequently increased their dietary intake and had a typical weight gain thereafter. There was a difference as to WHEN this increased dietary intake happened between the two groups. It happened in the GM-fed group a little earlier than in the non-GM-fed group. This means that the rats on the GM diet had MORE time eating normally than the rats on the non-GM diet and hence weighed more at the end of the study. This means that if dietary restriction has an adverse effect on the health of the stomach, the rats on the GM diet should be healthier than the rats on the non-GM diet. They weren't.

Because rats on the GM diet had initial dietary restriction, they ate less GM corn than they would otherwise have eaten over the course of the experiment. Therefore, any adverse effects produced by the GM corn on the stomach would be lower compared to what could have occurred if they had eaten the predicted amount of corn. Therefore the effects of the GM corn on the stomach of the rats in this study has been underestimated. A repeat of the study using moister diets from the beginning may find an increase in adverse effects.

Potential attack: Graphs of dietary intake or body weights were not given

Reply
Such graphs are usually given to provide evidence that the GM diet influenced dietary intake or body weight. Because diets were changed to moister diets during the study, which influenced body weight, no conclusions can be drawn about the effects of the GM component of the diet on body weight. Any dietary intake or body weight graph would reflect the change to moister diets rather than the GM aspect of the diet, and hence would measure artefact rather than the issue under investigation. We concluded that no conclusions could be drawn about the effect of the GM diet on body weight.

Potential attack: 60% corn in the diet is too high. It will cause nutritional deficiencies in rats. People don't eat this much corn.

Reply
The FAO/WHO recommend that animal feeding studies should investigate the effects of a GM crop at a range of dose levels, including the highest dose level. The highest does is the maximum achievable dose that would not cause nutritional imbalances in the rat. According to the feed manufacturer, who makes laboratory feed for laboratories across Australia, 60% was the maximum achievable dose for corn in a semi-purified diet that would not cause nutritional disturbances and could form a food pellet for a rat.

The diet was approved for use by the Animal Welfare Committee of Flinders University, South Australia.

Some people do indeed eat this much corn, particularly in Africa. A third of the world's population subsists on maize as a staple food. It is the dietary staple for over 200 million people (Nuss ET and Tanumihardjo SA (2010). Maize: A paramount staple crop in the context of global nutrition. *Comprehensive Reviews in Food Science and Food Safety*, 9:417-436; https://onlinelibrary.wiley.com/doi/epdf/10.1111/j.1541-4337.2010.00117.x). In Malawi, maize consumption is 293 g/person/day (Ranum P, Peña- Rosas JP, Garcia- Casal MN (2014). Global maize production, utilization, and consumption, *Annals of the New York Academy of Sciences*, https://doi.org/10.1111/nyas.12396, https://nyaspubs.onlinelibrary.wiley.com/doi/full/10.1111/nyas.12396), it accounts for over 60% of total food production in Malawi (Mazunda J, Droppelmann K (2012), International Food Policy Research Institute, Washington) and 50% of the daily calorie intake (FAOSTAT, 2009; http://www.fao.org/3/a-aq168e.pdf). In Lesotho, consumption is even higher, at 328 g/person/day https://nyaspubs.onlinelibrary.wiley.com/doi/full/10.1111/nyas.12396 , being 54.8% of the average daily calorie intake (FAOSTAT, 2009; www.fao.org/3/a-aq169e.pdf).

Food regulators, such as Australia's FSANZ, have stated that corn containing one or more of these GM genes is as safe and wholesome as other corn. According to regulators, it should therefore be as safe to eat as non-GM corn at any level in the diet.

The 60% corn in the diet caused no problems if the corn was non-GM. It only caused problems if the corn was GM.

Potential attack: The diets were not analysed for compositional equivalence

Reply
The composition of the GM and non-GM diets fills two pages of the paper and covers 68 nutrients.

The GM and non-GM diets were made in exactly the same way by the same manufacturer. The only difference was the presence of non-GM corn in one diet and GM corn in the other.

Numerous food regulators have assessed GM corn with one or more of these three GM genes in them and have concluded that they are substantially equivalent and hence compositionally equivalent to non-GM varieties. (FSANZ, 2016. Current GM applications and approvals, Genetically modified (GM) foods. http://www.foodstandards.gov.au/consumer/gmfood/applications/Pages/default.aspx [accessed: 9th October 2017]. ANZFA, 2002. Final assessment report (Inquiry - s.17). Application A416. Glyphosate-tolerant corn line NK603. Australia and New Zealand Food Authority (ANZFA), Canberra, Australia. ANZFA, ND. Final analysis report. Application A346. Food produced from insect-protected corn line MON810. Australia and New Zealand Food Authority (ANZFA), Canberra, Australia. EFSA, 2008. Application for authorization of MON 863 × MON 810 × NK603 maize in the European Union, according to Regulation (EC) No 1829/2003 on genetically modified food and feed. Part II Summary. EFSA. FSANZ, 2003. Final assessment report: Application A484. Food from insect-protected MON863 corn. Food Standards Australia New Zealand (FSANZ), Canberra, Australia. FSANZ, 2006. Final assessment report. Application A548. Food from corn rootworm-protected & glyphosate-tolerant corn MON88017. Food Standards Australia New Zealand (FSANZ), Canberra, Australia.)

Potential attack: The results were not due to the GM component to the diet, but to mycotoxins in the corn.

Reply
There is no evidence that the results were due to mycotoxins. We will measure the level of mycotoxins in a follow-up study.

If mycotoxins were the cause, they must have been only in the GM corn.

Potential attack: The non-GM corn used was not isogenic to the GM corn used

Reply
An isogenic corn variety might be possible to find for the triple-stack GM corn if the developers of the GM corn had inserted three GM genes at once into a non-GM plant. This did not happen. Instead, the triple stack GM corn variety was made by conventionally crossing several GM varieties. Therefore, there is no non-GM isogenic corn available for this GM corn variety and there never has been.

Using even one of the parental lines as a control was not possible because we could not find any of them in commercial production. Hence they were not available to us to feed to rats.

Numerous food regulators have assessed GM corn with one or more of these three GM genes in them and have concluded that they are substantially equivalent to non-GM varieties.

Potential attack: The rats ate raw corn when people eat cooked corn.

Reply

The diets were made by pelleting using a high compression die at 60^{o}C. According to the diet manufacturer, pelleting needs to occur below 85^{o}C, or casein starts to cook, denature and undergo a Mallard reaction. In fact, it is best to keep pelleting below 65^{o}C, which we did.

The industry does not cook corn before feeding it to animals in its studies.

The industry should be, according to CODEX, showing that it is safe to eat in the home for each country and culture within it, stating: "The potential effects of food processing, including home preparation, on foods derived from recombinant-DNA plants should also be considered "

Potential attack: The severity score that was used was an unpublished method.

Reply
The score simply added up the number of things that were adverse in the tissue. This is a valid thing to do. Severity scores are commonly used in anatomy research e.g. Howarth, G.S., Francis, G.L., Cool, J.C., Xu, X., Byard, R.W., Read, L.C., 1996. Milk growth factors enriched from cheese whey ameliorate intestinal damage by methotrexate when administered orally to rats. Journal of Nutrition 126, 2519-2530.

Potential attack: There was inflammation in the pig stomachs but not in the rat stomachs. This means that the results are inconsistent and should therefore be ignored.

Reply
This study is a follow-up study to the study we did on pigs, where pigs were fed a mixed diet of GM soy and GM corn. In this follow-up study, we wanted to look at just the GM corn component of that diet, and feed it to rats because the rat is the standard laboratory animal.

The difference may be due to the different animal species used. Pigs may react to the corn in a way which includes inflammation while rats may not.

The difference may be due to the type of diet used. Pigs were fed a combination of GM soy and GM corn while the rats were fed GM corn. Soy can make the gastrointestinal tract more fragile.

The difference may be due to the fact that the pigs were fed a diet that was finely ground, as per normal US piggery practices, while rats were fed rat pellets, as per normal laboratory practice. A finely ground diet is known to promote inflammation, which the GM component of the diet added-to.

Potential attack: The authors are inconsistent about whether dysplasia is occurring.

Reply
There is no inconsistency. We noted that some cells in the glands of GM-fed rats showed signs of dysplasia. This is a condition where cells are abnormal. It may progress to cancer. Many tests that screen for cancer, actually screen for dysplasia (e.g. a Pap smear for cervical cancer). In these rats, dysplasia took the form where some glands in the GM-fed rats contained cells that would not normally occur in that location. This kind of change often happens after injury or a drastic change in the stomach environment. Often, once such a trigger is removed, these abnormal cells can disappear. However, sometimes the damage is too severe for the tissue to recover and it can further develop into something more serious, such as cancer. Future studies should investigate the risk of these kinds of serious conditions developing. Meanwhile other cells elsewhere in the stomach, lying mostly at the base of the pits (i.e. not in the glands) in the stomach, showed a statistically significant 21% reduction in the number of dividing cells in rats fed the GM diet compared to those fed the non-GM diet.

Potential attack: You measured so many things that something is bound to come up as statistically significant by chance alone

Reply
Such critiques usually argue that a statistical adjustment should be made for the number of statistical tests done, using techniques such as a Bonferroni correction. This argument fails for three reasons. First, it goes

against OECD guidelines for analysing data from experiments like this. Second, it is a misunderstanding of how adjustments like this should be applied. Third, even if such critiques are correct, it should be noted that we undertook a global test for significance, combining all the adverse findings into one score, and doing a statistical test on that score. No statistical adjustment is ever required if only one statistical test is done for the experiment. We found that the rats on the GM diet had a severity score that was 33% higher than rats on the non-GM diet and this was statistically significant at p=0.027.

If people are arguing that our results were obtained by chance alone, then they should test their hypothesis by repeating the experiment and measuring only one outcome for the experiment, such as tight junction apposition loss, and doing just one statistical test on that one outcome, to determine if the outcome is statistically significant.

Potential attack: Attacks on the credibility of the researchers

Reply
The overarching study design was reviewed by an international panel of experts and then reviewed by a steering committee of Australian experts. The paper also underwent peer-review in order to be published in a peer-reviewed journal.

Animal husbandry and observations were undertaken by people who were blinded as to whether the rats were fed the GM or non-GM diet.

The research was conducted by researchers from Adelaide University, Flinders University and the Institute of Health and Environmental Research, all based in South Australia. All authors have PhDs. Two have been, or continue to be, Associate Professors. Between them, the authors have training and experience in: anatomy, histology, histopathology, toxicology, biochemistry, epidemiology, biostatistics and animal feeding studies.

Most attacks have traditionally been on Judy Carman and not on any other co-authors. Here are specific rebuttals for attacks on her:

- Dr Judy Carman has a Batchelor of Science, an Honours Degree in Organic Chemistry, a Ph.D. in Medicine in the field of nutritional biochemistry and metabolic regulation, and a Master of Public Health specialising in epidemiology and biostatistics.
- She has worked in the fields of human nutrition and nutritional biochemistry (including at Australia's CSIRO) and disease investigation and control. She has held senior population health positions in Australia, including as the Senior Epidemiologist in the Communicable Disease Control Branch of the South Australian Department of Human Services, investigating outbreaks of disease in the State. In that role, she conducted a government-funded, multi-State investigation into whether Rabbit Calicivirus (Viral Haemorrhagic Disease of Rabbits) could infect people after it had escaped from quarantine to spread through Australia.
- She has taught chemistry, biochemistry, epidemiology, research methods and statistics over many years at various tertiary institutions, including at an agricultural college and Adelaide and Flinders Universities. She has held senior academic positions in the latter two including as an Affiliated Associate Professor. She is the Director of the Institute of Health and Environmental Research, based in South Australia.
- Dr Carman is a world expert on the risk assessments of genetically modified crops and has conducted research in the area for over a decade. She conducted and published one of the first long-term safety assessments of GM crops by independent scientists by feeding GM crops to animals for many months and then looking for outcomes that are relevant to human health. She has served on the Ad Hoc Technical Expert Group on Risk Assessment and Risk Management of Living Modified Organisms under the Cartagena Protocol on Biosafety of the United Nations.

Rebuttals for attacks on Dr Irena Zdziarski:
Irena has a Bachelor of Health Sciences (majoring in anatomical science and pathology), an Honours Degree in Anatomy and a PhD in Medicine from the Faculty of Health and Medical Sciences at the University of Adelaide. She has taught gross anatomy and histology at the Adelaide Medical School, University of Adelaide for over six years. She has been a research scientist since 2005, i.e. for 13 years at the time of the

publication of the study. She has over 10 years' experience in processing tissues for light, confocal and electron microscopy.

Rebuttals for attacks on Dr John Edwards:
Dr Edwards has these qualifications: BSc (University of Adelaide); BSc (Hons) (University of Adelaide); PhD (University of Adelaide); Grad. Cert. Tert. Ed. (Flinders University); COH; MAIOH. He is a professionally registered toxicologist (UK and European registers of toxicologists) and a Certified Occupational Hygienist (Member or the Australian Institute of Occupational Hygienists). He is an Associate Professor at Flinders University, South Australia. At that university, amongst other positions, he has been Associate Dean (Teaching and Learning), School of the Environment. He is a Member of the Commonwealth Government's Advisory Committee on Chemicals Scheduling (meeting jointly with the Advisory Committee on Medicines Scheduling) and a Member of the Commonwealth Government's Advisory Committee on Complementary Medicines.

Look at the people criticising the paper. See if they have vested interests in GM crops. See if they have any training or experience in medical research. If they have no training or experience in medical research, they do not have the expertise to be able to criticise the paper.