



Letter to the Editor

Comment on “Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Townships of Quebec, Canada” by A. Aris and S. Leblanc [Reprod. Toxicol. 31 (2011) 528–533]

The title of this article suggests the authors had accumulated compelling evidence to demonstrate that in Canada dietary exposure to pesticides came from the consumption of food derived from GM crops. A logical investigative approach to reach such a conclusion would have been to confirm the amount of GM food actually present in the diet of the volunteers and then match the corresponding levels of pesticides in blood. An alternative approach may have been to measure novel GM crop-specific pesticide metabolites, such as N-methyl glyphosate or N-acetyl-L-glufosinate, in sera. However, disappointingly the authors of this paper do not present any convincing data to link the consumption of GM food with detectable levels of agricultural pesticides/metabolites in the blood. Instead their approach is to quantify three pesticides in the sera of pregnant and non-pregnant women and newborn infants without defining the food intake of the subjects beyond saying that it was a representative food market-basket. To confuse matters further these three pesticides are commonly used in conventional agriculture. Cry1Ab and Cry1Ac proteins are produced by the soil bacterium *Bacillus thuringiensis* subsp. *kurstaki* (*Btk*) which is permitted for use in organic farming in many countries including Canada [1] and the U.S.A. [2] because it is a naturally occurring, non-pathogenic bacterium. There are published studies which show that microscopically visible *Bacillus thuringiensis* and residues of *Bacillus thuringiensis*-based insecticides are present on fresh fruit and vegetables such as grapes, lettuce and tomatoes, which are all non-GM crops [3]. Consequently the assertion implied in the title of this paper that the detection of glyphosate, ammonium glufosinate or Cry1Ab/Cry1Ac in sera is somehow linked to ingested GM food could, we believe, at best be considered speculative.

The detection of glyphosate and glufosinate was done via GC–MS analysis while Cry1Ab (the only Cry protein specified by the authors) was detected using an ELISA assay. A point of concern relates to a number of methodological anomalies with the use of the ELISA assay to detect and quantify Cry1Ab and Cry1Ac. These anomalies are of such a magnitude that the reliability of the results is, in our view, in doubt. The ELISA plates were sourced from Agdia (Elkhart, IN, USA). Website literature published by Agdia indicates that these ELISA plates can detect and measure not only Cry1Ab protein but also Cry1Ac. Additionally, it is stated that the plates are designed for testing of plant tissue. As highlighted by Paul et al. [4] and reiterated by Paul et al. [5], commercial kits designed for transgenic protein detection in plant material require suitable assay validation before use for protein analysis in animal systems. Agdia make no claim that these kits are suitable to detect Cry1Ab/Cry1Ac in serum but Aris and Leblanc claim to have followed the manufacturer's instructions. Aris and Leblanc did undertake a validation procedure for the GC–MS analyses of the two herbicides which

included determining the limit of detection (LOD) for each pesticide and metabolite but surprisingly this was not done for the ELISA assay kit. This is an important consideration because, while the Agdia recommended standard curve ranges from 0.1 ng/ml to 10 ng/ml, the mean concentration reported for umbilical cord sera is 0.04 ng/ml with a range of 'not detected' to 0.14 ng/ml ($n=30$). This means that nearly all of the values reported for cord blood are derived from an extrapolated standard curve between 0 and 0.1 ng/ml. This extrapolation would only be valid if the LOD was zero. A LOD equal to zero is not achievable and a LOD at the lowest level on the standard curve seems unlikely. Similarly, based on the observation that the reported mean levels of Cry1Ab/Cry1Ac in the sera of pregnant and non-pregnant women were 0.19 ng/ml and 0.13 ng/ml respectively and the ranges included 'not detected', it follows that many of these positive detections reported by Aris and Leblanc must have been calculated using the same flawed extrapolation procedure.

We note that other investigators such as Petit et al. [6] used an Agdia test kit to measure Cry1Ab in bovine serum spiked with purified Cry1Ab protein and report a LOD of 1 ng/ml. Assuming that the Agdia ELISA assay used by Aris and Leblanc had an equivalent LOD, there would have been very few detections of Cry1Ab/Cry1Ac in non-spiked human sera. Lutz et al. [7] used the ELISA kit to measure Cry1Ab/Cry1Ac levels in the rumen, jejunum and colon of cattle. These authors could not reconcile the results of the ELISA assay with those of an immunoblotting assay and concluded that ELISA is not a suitable method for drawing definitive conclusions because of its inability to provide information on the molecular weight of the protein/peptide being detected. They report that “results on Cry1Ab protein content based solely on ELISA measurements have to be interpreted carefully”. If one accepts that the Agdia ELISA assay used by Aris and Leblanc, is capable of detecting full length Cry1Ab/Cry1Ac proteins at 1 ng/ml then it does not necessarily follow that it will be capable of detecting any absorbed Cry1Ab/Cry1Ac peptides from the GI tract, or at least not at the same LOD. This comes from the knowledge that the sensitivity of any polyclonal antibody-based assay for a protein will be less for a cross-reacting peptide because there will be fewer epitopes available for antibody binding. The effect of fewer epitopes will, in turn, impact on the colour yield in the ELISA assay and hence its ability to detect peptides absorbed from Cry1Ab/Cry1Ac degradation in the GI tract.

References

- [1] Government of Canada. Organic production systems permitted substances lists. Gatineau: Canadian General Standards Board; 2009, report no. CAN/CGSB-32.311-2006.
- [2] USDA. National organic program. United States Department of Agriculture, Agricultural Marketing Service; 2011.
- [3] Frederiksen K, Rosenquist H, Jorgensen K, Wilcks A. Occurrence of natural *Bacillus thuringiensis* contaminants and residues of *Bacillus thuringiensis*-based insecticides on fresh fruits and vegetables. *Applied and Environmental Microbiology* 2006;72(5):3435–40.

- [4] Paul V, Steinke K, Meyer HHD. Development and validation of a sensitive enzyme immunoassay for surveillance of Cry1Ab toxin in bovine blood plasma of cows fed Bt-maize (MON810). *Analytica Chimica Acta* 2008;607: 106–13.
- [5] Paul V, Guertler P, Wiedemann S, Meyer HHD. Degradation of Cry1Ab protein from genetically modified maize (MON810) in relation to total dietary feed proteins in dairy cow digestion. *Transgenic Research* 2010;19: 683–9.
- [6] Petit L, Baraige F, Bertheau Y, Brunshwig P, Diolez A, Duhem K, et al. Detection of genetically modified corn (Bt176) in spiked cow blood samples by polymerase chain reaction and immunoassay methods. *Journal of AOAC International* 2005;88(2):654–64.
- [7] Lutz B, Wiedemann S, Einspanier R, Mayer J, Albrecht C. Degradation of Cry1Ab protein from genetically modified maize in the bovine gastrointestinal tract. *Journal of Agricultural and Food Chemistry* 2005;53:1453–6.

Utz Mueller

Janet Gorst*

*Food Standards Australia New Zealand, 55 Blackall
Street, Barton, ACT 2600, Australia*

* Corresponding author. Tel.: +61 2 6271 2222;

fax: +61 2 6271 2278.

E-mail address: janet.gorst@foodstandards.gov.au
(J. Gorst)

18 August 2011

Available online 21 February 2012