

COMPETING INTERESTS STATEMENT

The author declares competing financial interests: details accompany the full-text HTML version of the paper at <http://www.nature.com/naturebiotechnology/>

Rob C Aalberse

University of Amsterdam, Department of Immunopathology, Sanquin Research at CLB, Plesmanlaan 125, 1066 CX Amsterdam, The Netherlands.
email: r.aalberse@sanquin.nl

1. Goodman, R.E. *et al.* *Nat. Biotechnol.* **26**, 73–81 (2008).
2. FAO/WHO. *Evaluation of Allergenicity of Genetically Modified Foods. Report of a Joint FAO/WHO. Expert Consultation on Allergenicity of Foods Derived from Biotechnology* (Food and Agriculture Organization of the United Nations (FAO), Rome, 2001).

Richard E Goodman, Steve L Taylor, Ronald van Ree, Stefan Vieths, Motohiro Ebisawa & David Hill reply:

We thank Rob Aalberse for the interest shown in our Perspective. We acknowledge his impressive track record in studying the relation between allergen structure, cross-reactivity and criteria for establishing allergenicity of proteins. We do not, however, agree with most of his criticisms and would like to take the opportunity to answer to the objections he raised.

His arguments evoke a spirit of ‘scientific pureness in support of consumer safety’. Yet his reasoning and recommendations are quite hypothetical. We argue strongly against using mere theory to set regulatory guidance citing examples in the paper where limits or tests that were introduced based on expert opinion alone without proof of predictive value have led to unnecessary testing (e.g., six amino acid matches) or would allow a potentially risky product to pass (e.g., based only on animal model data). If a test is performed that misidentifies nine nonallergenic or weakly allergenic proteins as clear allergens, but ‘correctly’ identifies peanut Ara h 2 on the 10th test, the 10th test result might be viewed as valid, but clearly a 10% predictive value has no place in risk assessment. Using unproven tests or setting rigid, unnecessarily low levels of abundance, or new higher pH conditions (pH > 2.0) for pepsin digestion based on theoretical discussion will not improve the safety assessment. Although we agree strongly that discussing potential shortcomings helps to stimulate research, experimental data (from the literature or from specifically designed new studies) should be used to set regulatory guidelines. Providing recommendations with respect to an upper “safe limit of abundance” or of “allowable increased endogenous

allergenicity” without scientifically tested data could easily crystallize into regulations based on educated guesses. Aalberse’s aversion to validation is not rare among researchers, but is inappropriate in setting regulatory guidelines. Validation in this context is experimental proof of predictive value relative to human clinical allergic responses, which should be markedly above 60% overall (or over 90% positive or negative) to have regulatory value.

Aalberse focuses on three aspects of our paper: first, targeted serum screens, second, allergen abundance as determined by expression level and digestibility, and third, changes in endogenous allergenicity. Aalberse states that the purpose of the FAO/WHO recommendation for targeted serum testing is to identify previously unrecognized “pan-allergens” (proteins that are highly conserved across broadly diverse taxonomic groups). He gives some enlightening examples of pan-allergens present in “latex and banana, in birch and apple and in snail and mite.” Yet the examples given provide the best argument against the necessity of targeted serum screens. Pan-allergens have an evolutionary highly conserved structure. The relatively few identified pan-allergens include lipid transfer proteins, profilins, PR-10 proteins (Bet v 1 homologs), tropomyosins, hevein and hevein-like antifungal proteins, thaumatin and chitinases, which have been extensively studied between the early 1990s and now. *In vitro* IgE cross-reactivity within the pan-allergen groups is extensive and crosses broad taxonomic groups. Clinical cross-reactivity is much more restricted for individual subjects. Another confounding factor for IgE cross-reactivity is the common occurrence of similar modified asparagine-linked glycans that occur in many plants, insects and parasites. The broad cross-reactive binding to glycan has not proven to be clinically significant. Proteins sharing sequence and structure with the pan-allergens should be identified by the sequence identity (homology) search and would require specific serum testing, which requires testing of sera from those allergic to the source of the sequence-matched allergen. Such testing should be far more predictive than targeted testing. Our main reason for being cautious towards promoting targeted serum screens is that false-positive IgE test results are not rare. Low levels of clinically irrelevant IgE binding to a wide variety of proteins are commonly found in research and diagnostic studies. The clinical relevance of positive hits from a targeted serum screen will be very

difficult to verify or disprove, likely requiring human *in vivo* challenges for definitive proof.

Aalberse suggested that “there is an unfortunate misprint in the paper.” This is, however, not the case as can be checked in the reference listed with reported allergen levels indeed being in the mg/g range for important food allergens.

We have tried to give a balanced view of pepsin digestion assays and the role of variations of pH between 1 and 3, including an example of cod fish parvalbumin for which stability is clearly increased under influence of a minor shift in pH from 2.5 to 2.75. Pepsin digestibility assays have been validated at pH 2 and pH 1.2, with little difference in results. According to the supplier, Sigma Chemical (St. Louis, MO, USA), pepsin activity is optimal at pH 2.2 and only 90% efficient at pH 1.5 and 35% effective at pH 4.5. As discussed in articles referenced in our paper, the pepsin resistance assay (at pH 1.2 and 2.0) provides a scientifically justifiable correlation between stability and the propensity of dietary proteins to act as allergens.

The comment by Aalberse that he would be comfortable to restrict GM proteins to a limit of 1 mg/day dose, even if highly digestible, unless or until an animal model could prove safety, is in our view an extremely precautionary position that is not scientifically defensible. First, a predictive animal model may never be validated. Second, such hypothetical numbers are not protective for consumers and are dangerous for producers of new products as the theoretical value is likely to turn into a regulatory threshold.

The same type of issue is raised with respect to changes in endogenous allergenicity. We are certainly not against measuring changes in IgE-binding potency of wild-type and GM food if there is scientific rationale and a basis for making a sound judgment (regulatory decision). What we pointed out is that a very wide degree of variability is observed for allergen levels in some non-GM crop varieties. We need to know more about endogenous allergen levels and natural variation and have so far not seen data that demonstrates an enhanced risk to the consumer based on the observed variation. Tests to quantitatively evaluate differences in endogenous allergenicity are fraught with potential bias by selection of a limited number of subjects and assay design. Furthermore, a misconception about genetic modification is that the GM plant that will be produced contains only one single

genetic difference from the comparator (parent line, sibling line or null segregant). In fact, the plants that are transformed are not homozygous at every locus. Many generations of back-crossing and/or selective crossing are needed to produce a viable commercial line from the original transgenic plant. After approval, the event is usually crossed into many diverse varieties of the same crop. The genetic diversity will include expression of various allergens. We do agree that measuring endogenous allergenicity might provide useful information if the introduced gene is a transcription factor or is intended to alter the expression of

an allergen. We simply pointed out the magnitude of natural variability and urge that additional data are needed to establish criteria that would be useful. Statistically significant differences are much less important than biologically meaningful differences. Overall, we think that we have given a balanced view that can improve the assessment compared with some current regulatory demands.

COMPETING INTERESTS STATEMENT

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at <http://www.nature.com/naturebiotechnology/>

Field-evolved resistance to *Bt* toxins

To the Editor:

An article by Tabashnik *et al.*¹ in your February issue states that, for the first time, the frequency of resistant alleles has increased substantially and that there is field-evolved *Bacillus thuringiensis* toxin (*Bt*) resistance in bollworm, *Helicoverpa zea* (Boddie), in the United States because of the extensive use of *Bt* cotton. Tabashnik *et al.* base their conclusions on two publications by Randy Luttrell's laboratory in which Cry1Ac toxicity to numerous *H. zea* populations was evaluated: Luttrell *et al.*² (before introduction of *Bt* cotton) and Ali *et al.*³ (after introduction of *Bt* cotton).

We emphatically disagree with the conclusions of Tabashnik *et al.* that the data published in these two articles demonstrate field-evolved resistance in *H. zea* for four reasons: first, the definition of *Bt* resistance used by Tabashnik *et al.* is purely laboratory based, whereas field efficacy and larval survival on plant tissues are the ultimate criteria for contextualizing laboratory-based estimates of resistance, and no change in *Bt* cotton efficacy has been documented during the past decade; second, larval samples should not be collected from *Bt* crops because they will not be representative of the population as a whole, especially for highly mobile insects such as *H. zea*; third, the data from Luttrell's laboratory on which Tabashnik *et al.* base their conclusions have been evaluated using LC₅₀ (median lethal dose; 50%) values to

measure resistance, which introduces artifacts into the analysis; and fourth, the baseline comparator used to assess variability in these laboratory assays is not representative of field susceptibility; when a more appropriate comparator colony is employed, results from Luttrell's laboratory bioassays indicate no change in susceptibility. We discuss each of these aspects in turn below.

Pest resistance can be defined in two very different ways, either based on laboratory conditions, or based on real-world, field conditions. Laboratory resistance is defined as a statistically significant, genetically mediated reduction in sensitivity of the target organism to the controlling agent, relative to a susceptible laboratory strain. In this setting, resistance is typically

observed as an increase in population LC₅₀, or as enhanced growth and/or survival at a discriminating concentration, compared with a susceptible colony. Although laboratory-based estimates of resistance are essential in proactive resistance management programs to achieve early warning of reduced larval susceptibility and thus potential resistance problems, proof of resistance ultimately must rest on field efficacy. Laboratory tests are unable to accurately predict or interpret the impact that any given frequency and/or intensity of resistance will have on pest (or population) survival and fitness in a field environment. Field resistance can be defined as a genetically mediated increase in the

ability of a target pest to feed and complete development on one or more commercial line(s) of *Bt* cotton under field conditions. This definition incorporates the potential for incomplete resistance (e.g., increased feeding but delayed or incomplete development to adult) and fitness costs. In the case of *H. zea*, not only have there been no reports of widespread control failures, as confirmed in Tabashnik *et al.*, but also there has been no documented change in efficacy of *Bt* cotton anywhere in the US Cotton Belt. Although control of *H. zea* by *Bt* cotton has never been complete (http://www.epa.gov/scipoly/sap/meetings/2000/october/brad4_irm.pdf), no further increase in survival has been observed since *Bt* cotton was introduced in 1996. The most recent monitoring data from 2007 parallel results from previous years⁴. In other words, no real-world field data support the laboratory-based conclusions made by Tabashnik *et al.* regarding a mounting resistance problem with *H. zea*. In contrast to *H. zea* in *Bt* cotton, fall armyworm, *Spodoptera frugiperda*, has developed resistance to Cry1F *Bt* corn in Puerto Rico (A. Reynolds, Entomological Society of America Annual Meeting, December 2007). In this case, there was a change in field performance resulting in field failures, and subsequently it was demonstrated that *S. frugiperda* showed no mortality at the highest concentration of Cry1F tested in laboratory bioassays. As a result, there was an immediate voluntary discontinuation of commercial cultivation of Cry1F *Bt* corn in Puerto Rico.

With regard to choosing the most appropriate method for measuring changes in susceptibility, the US Environmental Protection Agency (EPA) mandated annual surveys of *H. zea* susceptibility to Cry1Ac across all cotton production regions as part of the overall post-commercial monitoring process. The susceptibility monitoring protocol was developed and accepted by experts from EPA, academia, extension and industry. The methods involved collecting insects from non-*Bt* crops, rearing them to subsequent generations (F₁–F₂), conducting neonate bioassays using Cry1Ac (MVP II) at diagnostic concentrations, including the use of 'failure to molt into second instars' as the definition of mortality, and comparing results to a susceptible laboratory colony and previous years' results. (Note that Luttrell *et al.*² used Cry1Ac toxin for conducting bioassays, which has significantly different toxicity against *H. zea* compared to MVP II (ref. 5), which was used for official resistance monitoring and by Ali *et al.*³.) These procedures are similar to those used

