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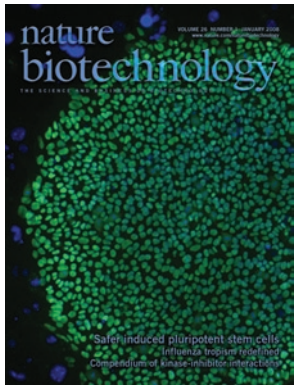
Allergenicity testing of GM crops

To the Editor:

I would like to respond to the Perspective on "Allergenicity assessment of genetically modified crops—what makes sense" by Goodman *et al.* in your January issue[1]. A recurring theme is "validation" of tests, or rather, the lack of validation. In fact, this is the most important argument in the case of targeted serum screens. I am not an expert on regulatory affairs and do not know the fine details on the regulatory aspects of test validation. It is undoubtedly extremely important. However, if a test has not been validated, its results are not necessarily invalid. There is a problem with 'targeted serum screens': the terminology is unclear and not well defined, and was introduced at the World Health Organization (WHO; Geneva)/Food and Agricultural Organization (FAO; Rome) meeting[2]. It is used particularly in relation to a specific situation to address a very specific problem. The situation is: the source of the genetically modified (GM) protein is not a known allergen source and has no significant homology to a known allergen in the database. The problem is: might the GM protein be a member of a pan-allergen family, that is, might it be cross-reactive with allergenic proteins that are not closely taxonomically related?



Several such unexpected cross-reactivities have been described due to pan-allergenic families; for example, rubber latex with banana, birch with apple and snail with mite. Cross-reactivity among mold allergens is often also not tightly restricted by taxonomic barriers. So, if the GM protein is taken from a nonallergenic mold, the FAO/WHO proposal is to take sera with IgE to various molds and test these for reactivity with the GM protein. Goodman *et al.* dismiss this type of test because of a "potentially high rate of false-positive and low probability of true-positive results." For the false positives, the problem is not any different for the other, well-accepted, cross-reactivity assays. The experience with profilins, tropomyosins and so forth has told us that the scope of immune recognition is not necessarily restricted by our taxonomic rules. So, our screening system also needs to have a broader scope. We cannot yet do without the targeted serum screen. However, a better name would certainly be welcome.

I agree that the predictive value of the current animal models is low. This may change. In the meantime, the risk of introducing a novel allergen can be minimized by ensuring a low level of expression of the GM protein, combined with high digestibility. There is an unfortunate misprint in the paper, which suggests that allergen levels are in the mg/g range. The authors presumably intended micrograms/gram. It would have been interesting to learn from these experts with what level of expression of the GM protein they would feel comfortable with: a ball-park number of 1 mg/daily dose would sound reasonable to me (but, alas, is not validated). In relation to digestibility, the authors indirectly suggest to make the pepsin digestion test more stringent (using a pH below 2). This does not seem wise. We already know that some food allergens are digested at pH > 2. Moreover, in infants and subjects using anti-acids the pH in the stomach will often not go below 2.

In the concluding statement ("there is no scientific justification for inclusion of the following tests in allergenicity assessment…"), the measurement of a GM-induced increase of endogenous allergenicity is one of the tests listed for dismissal. As reported in the Goodman article, substantial natural variability in endogenous allergenicity exists. In apple cultivars, differences in allergen content up to 100-fold were found. One of the arguments in favour of not measuring changes in endogenous activity is, that "patients allergic to the food will (should) avoid eating [the GM food] anyway, GM or not, to avoid allergic reactions." However, accidental exposure is all too common. So, if the same type of accident might result in a 100-fold higher exposure, it is time to tighten the rules. Personally, I would start worrying if the GM process increases the endogenous allergenicity more than threefold, and would start ringing bells if the increase is more than tenfold. These numbers are, obviously, not intended to be made into rules by some regulatory agency, but to evoke a response, hopefully with a sounder scientific basis than what I have to offer right now. The statistical evaluation of changes in allergen levels may not be completely straightforward, but this analysis can undoubtedly be done in a way that is acceptable to all parties, taking natural variability into account. Not to measure changes in endogenous activity because you don't know how to do the statistics does not make a convincing argument.

Goodman *et al.* note an additional problem: how to "evaluate changes in endogenous allergenicity of foods for which it is virtually impossible to find sufficient truly allergic patients for a well-powered study." With all the expertise present among the authors, it is disappointing that no alternatives to the use of human material for the measurement of endogenous activity are mentioned. To take corn as an example of a transgenic crop, a biotech company involved in generating GM corn may be expected to know the proteins involved in the endogenous allergenicity of corn. Proteomics-based assays and/or immunoassays based on animal antibodies to corn allergens can surely be devised that would be adequate for answering questions on expression levels.

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/

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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 anti-fungal proteins, thaumatins 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