

writing, albeit in their description of our profession as ethereal medical communicators. It's almost flattering to read that we have the power to spin *P*-value straw into statistically significant gold. That, however, would be spooky.

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/>

Lanie M Adamson¹, Mary Whitman², Adam Jacobs³ & Tracy E Bunting-Early⁴

¹Independent consultant, 26532 Montecito Lane, Mission Viejo, California 92691, USA. ²Centocor Ortho Biotech Services LLC, 200 Great Valley Parkway, Malvern, Pennsylvania 19355, USA.

³Dianthus Medical Ltd., 4 Lyon Road, London, SW19 2RL, UK. ⁴Independent consultant, 412

Stafford Ave., Newark, Delaware 19711, USA.
e-mail: lanie@ewordpro.com

1. Anonymous. Nothing to see here. *Nat. Biotechnol.* **26**, 476 (2008).
2. Ross, J.S., Hill, K.P., Egilman, D.S. & Krumholz, H.M. *J. Am. Med. Assoc.* **299**, 1800–1812 (2008).
3. AAMC Task Force. *Industry Funding of Medical Education Report of the AAMC Task Force* (AAMC, Washington, DC, June 2008).
4. International Committee of Medical Journal Editors. Uniform requirements for manuscripts submitted to biomedical journals. *ICMJE* <<http://www.icmje.org/>> (2007).
5. Moher, D., Schulz, K.F. & Altman, D. *J. Am. Med. Assoc.* **285**, 1987–1991 (2001).
6. Altman, D.G. *et al. Ann. Intern. Med.* **134**, 663–694 (2001).
7. 110th Congress of the USA. Food and Drug Administration Amendments Act of 2007. <<http://www.fda.gov/oc/initiatives/HR3580.pdf>>
8. Hamilton, C.W. & Royer, M.G. *AMWA Journal* **18**, 13–15 (2003).
9. Jacobs, A. & Wager, E. *Curr. Med. Res. Opin.* **21**, 317–321 (2005).

1% of Brazilian agricultural land is currently used. Increasing Brazilian ethanol production by tenfold (replacing 10% of the world's petroleum) would displace just 3% world cropland. Foreign investment in Brazilian bioethanol under carbon trading has been used to offset CO₂ emissions under the Clean Development Mechanism. Indeed, Brazilian ethanol prices are now comparable to current oil prices⁵.

At present, the United States uses 14% of its current corn grain for bioethanol fermentation, but this displaces only a few percent of current transport fuel⁶. A very much smaller amount of biodiesel is derived from soy. Careful systems and life cycle analyses show that corn grain bioethanol and soy biodiesel generate more energy than consumed in production⁷, contradicting earlier contrary claims. Products made from starch-depleted grain residues (e.g., corn oil and protein-rich animal feed) help ensure overall energy gain. Second-generation fuels from grain starch, like dimethyl furan, require no fermentation at all⁸.

But if all the current US grain and soy crops were used, no more than 6–12% of US transport fuel would be produced. And the effect of nitrogen fertilizer on soil N₂O emissions suggests there may be no saving of GHG emissions either⁹. Despite these caveats, government subsidies are increasing the amount of corn grain directed to refineries; corn prices are rising as bioethanol prices decrease¹⁰. In parallel, world food prices, underpinned by US corn, are rising reversing a beneficial downward trend in the past 50 years that benefited the poorest most. Food aid may also come under pressure if this trend continues.

The imaginative alternative is to derive ethanol from cellulose feedstocks and plant waste¹¹. A detailed US survey uncovered a projected 1.3 billion tons waste, excluding all food grains¹² by 2030 sufficient to provide 30% of US transport fuel when fermented while continuing to meet food, animal feed and export demands. Forestry will supply sustainably nearly 400 million tons from timber treatment residues; the rest is from agriculture as annual and/or perennial crop residues, manures, process residues and from food consumption and energy crops. Second-generation refineries are planned which will use different microbes to generate other useful chemicals. Research targets include cellulase reconstruction to improve activity, sustainable solubilization of cellulose and isolation of yeasts able to tolerate much higher levels of ethanol than presently. The net energy gained/net

Fuelling the 9 billion

To the Editor:

The editorial in your February issue¹ refers to the International Assessment of Agricultural Science and Technology for Development (IAASTD; <http://www.agassessment.org/>) initiated by the World Bank (Geneva) and the United Nations Food and Agricultural Organisation (Rome) to report on the potential of agricultural knowledge, science and technology for reducing hunger and poverty. Although you focus on IAASTD's vision for biotech and genetically modified (GM) crops, the main thrust of the assessment was concerns over the sustainable future of humankind, which center on population increase, food production and global warming.

There are enormous uncertainties about food production for a projected nine billion people and whether a rightful desire to remove poverty, the scourge of disease and food insecurity will result in increased greenhouse gas (GHG) emissions. As such, IAASTD recognized that an agri-biofuel economy can offer many benefits when the level of future oil reserves remains uncertain and some countries wish to remove their dependence on imported fossil fuel. By recycling carbon through biofuel, GHG emissions could be reduced and dependence

minimized. Indeed, the UN has specified criteria for sustainable biofuel production (Box 1)².

Critics have argued that biofuels will further increase human exploitation of fully stretched natural resources and that they will increase, not reduce, emissions and require greater energy input than energy gained³. These critics regard low-yielding organic agriculture as mankind's future because intensive methods supposedly reduce biodiversity and are unsustainable, despite contrary evidence from long-term agricultural experiments⁴. The challenge

for creative agriculture is to grow more food, supply biofuel and conserve the environment on the same area of land. Low-yielding agriculture is not obviously a way forward.

Bioethanol is the major renewable energy product but ethanol produces less energy per unit volume than petroleum. The commonest use will probably be as ethanol-fossil fuel (gasohol) mixtures. Sugarcane is an efficient tropical/sub-tropical grass with nitrogen-fixing symbionts, thus needing little fertilizer. Sugarcane bioethanol accounts for 40% of Brazilian transport fuel and the residue from extraction is burnt to generate electricity⁵. Competition between food production and sugarcane has not yet been substantial; only



GHG emissions for cellulose bioethanol is estimated to be 100-fold better than that of grain bioethanol⁷. Intense research interest is focused on the termite gut. These insects eat wood and with an array of some 200 gut microbe species digest it into sugars in 24 h, producing both hydrogen and methane as side products¹³. Lignin interferes with cellulose digestion but levels have been advantageously reduced by genetic modification¹⁴. Termite microbe investigation may also solve the lignin digestion problem and produce a useable product.

Even so, the billion ton project requires, first, a continued annual yield increase, and projections based on past yield increases suggest this is feasible. Recent corn yield improvements owe much to insect-resistant GM crops. New GM corn plants with greater stability to disease and water stress are currently under trials to improve yield again. Asexual cereal reproduction is anticipated to be developed soon, enabling individual corn, wheat or rice clones to be tailored to individual farm microclimates, increasing yield further.

Second, it also requires a continued advance of no-till agriculture that currently occupies 10% of US crop area. No-till eschews the plough, leaves crop residues on the soil and seeds by direct drill. Although no-till and intensive yields are similar, GHG emissions, including N₂O, are one-third of organic agriculture and close to emission levels of undisturbed woodland. Farm traction fuel is reduced by half, run-off of nitrogen or pesticides into water courses is either greatly diminished or disappears altogether and increased biodiversity has been recorded (summarized in ref. 15). GM herbicide-resistant crops have enabled the huge advance of US no-till. Although all de-grained shoot residues are left on the soil eliminating erosion, the billion ton project asks for research to optimize the actual amount needed. Successful no-till may only require leaving crop roots and stubbles undisturbed.

In Europe, short rotation coppicing of willow has been exploited for energy generation. Using detailed life cycle analysis, Bertilsson¹⁶ showed that farms with >10% of area in willow produce more energy than the farm uses¹⁶. Willow provides fourfold the energy yield/hectare of crops like rape or sunflower and if used for cellulose-bioethanol could supply all UK transport fuel using just 25% of arable land¹⁷. The half-million hectares of UK set-aside farmland could be used for willow. Breeding programs

Box 1 The UN's nine goals for biofuels

UN-Energy is the principal interagency mechanism at the UN that helps ensure coherence in the UN's response to sustainable development and collective agreement with non-UN stakeholders. The organization report published last year cited nine key issues for a successful biofuels industry.

- Integrated bioenergy generation must alleviate poverty in the poorest nations.
- Agro-industrial development of biofuels must occur locally for job creation.
- Health benefits will become evident with national biofuel use because urban and domestic air quality will improve.
- The structure of the agricultural industry must change to accommodate the development of integrated biorefineries.
- Food security will be assured if new agronomic and biological technologies are employed to improve food yields, conserve land use and use non-food crop feedstocks for bioenergy production.
- Government budgets of the poorest nations will be more able to invest in public services and food production if their bioenergy economy is secure.
- Changes in trade, foreign exchange balances and energy security will be sustainable if second-generation biofuel technologies are employed.
- Biodiversity and natural resources can be conserved if integrated agricultural methods are used to produce bioenergy feedstocks.
- Climate change mitigation will be an outcome of a successful bioenergy economy.

and biotech can also be used to improve willow yield.

The European Commission (Brussels) has unveiled its blueprint for streamlining and further modernizing the European Union's (EU; Brussels) Common Agricultural Policy (CAP)¹⁸. The so-called 'Health Check' of the CAP is questioning the current use of set-aside land in Europe given new incentives for biofuel production, such as the compulsory EU member nation bioenergy targets. The EU target is to generate bioethanol (e.g., from wheat and sugar beet) and biodiesel (e.g., from rape and sunflower) sufficient to displace 5.75% fossil fuels by 2010 (ref. 19), but few think this can be achieved. The UK government has recently introduced the Renewable Transport Fuel Obligation (RTFO) as a means of achieving the EU target. The UK Renewable Fuels Agency (RFA; London) will implement the RTFO and is currently consulting on the impacts of different types of biofuel (<http://www.dft.gov.uk/rfa/>).

The EU estimates suggest 150 million tons of biomass waste might be available for biofuel¹⁹. Some European cellulose-ethanol refineries are already operational. The EU is half the land area of the United States and with a similar proportion of forest. What characterizes the difference between the US 1.3 billion tons and EU 150 million tons is the lack of aspiration in the EU for continued yield increases and encouragement to use no-till. Some integrated farms in the UK use no-till or minimum-till but development is

hampered from lack of suitable herbicide-resistant GM crops freely available to US farmers. On this basis, there is no scientific reason for delaying the introduction of GM crops for European no-till²⁰.

EU biofuel development is mired in complex regulations, a stifling of innovation from precautionary attitudes and lack of political will to deal with reactionary activists who damage agricultural trials. Humankind is part of nature, not separate from it. The only way that nine billion will live in harmony with nature is through scientific and technological development, not by removing themselves from it altogether. By targeting only biomass, not grain, for transport fuel and increasing no-till agriculture, food production will increase, GHG emission substantially decline and transport continue sustainably, conserving biodiversity as well. These solutions best fit the UN criteria (Box 1). We predict that, as with the Green Revolution, technology can defeat Malthusian attitudes to energy and food security.

Wayne Martindale¹ & Anthony Trewavas²

¹Food Innovation, Sheffield Hallam University, Surrey Building, Howard Street, Sheffield S1 1WB, UK. ²UK Institute of Molecular Plant Science, Mayfield Road, Edinburgh EH9 3JH, UK. e-mail: w.martindale@shu.ac.uk

1. Anonymous. *Nat. Biotechnol.* **26**, 247 (2008).
2. United Nations. *Sustainable Bioenergy: A Framework for Decision Makers*. (UN, Geneva, 2007). <<http://esa.un.org/un-energy/pdf/susdev.Biofuels.FAO.pdf>>
3. Pimentel, D. & Patzek, T.W. *Nat. Res. Research* **14**,

- 65–76 (2005).
- Rasmussen, P.E., Goulding, K.W.T., Brown, J.R., Grace, P.R. & Janzen, H.H. *Science* **282**, 893–896 (1998).
 - Goldemberg, J. *Science* **315**, 808–810 (2007).
 - Hill, J., Nelson, E., Tilman, D., Polasky, S. & Tiffany, D. *Proc. Natl. Acad. Sci. USA* **103**, 11206–11210 (2006).
 - Farrell, A.E. *et al. Science* **311**, 506–508 (2006).
 - Schmidt, L.D. & Dauenhauer, P.J. *Nature* **447**, 914–915 (2007).
 - Crutzen, P.J., Mosier, A.R., Smith, K.A. & Winiwarter, W. *Atmos. Chem. Phys. Discuss* **7**, 11191–11205 (2007).
 - Odling-Smee, L. *Nature* **446**, 483 (2007).
 - Lynd, L.R. *et al. Nat. Biotechnol.* **26**, 169–172 (2008).
 - Somerville, C. *Science* **316**, 1277 (2006).
 - <http://genomicsgl.energy.gov/pubs/Biofuels_Flyer_2007-2.pdf>
 - Chen, F. & Dixon, R.A. *Nat. Biotechnol.* **25**, 759–761 (2007).
 - Trewavas, A.J. *Crop Prot.* **23**, 757–781 (2004).
 - Bertilsson, G. Environmental consequences of different farming systems using good agricultural practices. *Proceedings of the Fertiliser Society, Proceeding No. 332.* (Fertiliser Society, Cambridge, UK, 16–17 December 1992).
 - Eyre, N., Fergusson, M. & Mills, R. *Fuelling Road Transport.* (Energy Saving Trust, IEEP and National Society for Clean Air, London, 2002).
 - European Commission. 'Health Check' of the Common Agricultural Policy. *Fit for New Opportunities* (EC, Brussels, 2007). <http://ec.europa.eu/agriculture/healthcheck/index_en.htm>
 - European Commission. *An EU Strategy for Biofuels* (EC, Brussels, 2006). <http://www.ec.europa.eu/energy/res/biomass_action_plan/green_electricity_en.htm>
 - Dewar, A.M. *et al. Proc. R. Soc. Lond. B* **270**, 335–340 (2003).

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 issue¹. 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) meeting². 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.

