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GM Food Animals Coming

Foods derived from genetically modified animals are likely to be contaminated by potent vaccines, immune regulators, and growth hormones, as well as nucleic acids, viruses, and bacteria that have the potential to create pathogens and to trigger cancer

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Heritable vs non-heritable modifications

The Codex Alimentarius Commission of the United Nations is preparing guidelines for safety assessment of foods derived from recombinant-DNA animals [1], which is a sure sign that GM animal food is coming to our table.

Codex distinguishes between heritable and non-heritable genetic modification of food animals. Heritable genetic modification involves genetic changes that persist in sperm and egg while non-heritable modification involves the introduction of modified genes such as vaccines into the somatic tissue of animals. Codex asks: "Are there specific food safety questions (e.g. with regard to types of vectors) that should be considered relative to the assessment of safety of food from animals containing heritable versus non-heritable traits?"

We present an overview of heritable and non-heritable modifications, which are not as distinct as Codex thinks, and point to risks that have not been seriously considered. This article is base on a report we submitted to Codex [2], Genetically Modified Food Animals Coming, which contains all the detailed references.

Heritable modifications

Heritable alteration or genetic modification (GM) of food animals has been achieved since the early 1980s, mostly by injecting naked DNA. Between 1 and 20 million copies of the transgene (gene to be integrated into the animal genome) are injected into the embryo pronucleus (the nucleus before fertilization) or into the egg cytoplasm, with at most about one percent of injected embryos becoming transgenic animals. The transgenes integrate randomly, though rare instances of homologous recombination with host genes may occur.

A number of different vectors have been used to deliver transgenes in animals. Transposons (mobile genetic units capable of transferring genes) are not widely used in vertebrates. Lentivirus (lenti-, Latin for "slow"), a genus of slow viruses of the Retroviridae family characterized by a long incubation period, can deliver a significant amount of genetic information into the DNA of the host cell, and are among the most efficient gene delivery vectors. HIV (human immunodeficiency virus), SIV (simian immunodeficiency virus), and FIV (feline immunodeficiency virus) are all examples of lentiviruses that have been used successfully with farm animals such as chicken, pig and cow. They are about 50 times more efficient than DNA injection at producing transgenic animals. One problem encountered is that the long terminal repeats of the integration vector interfere with the inserted gene's promoter. Homologous recombination has been used to produce specific gene "knock outs" by replacing an active gene with an inactive one. "Knock in" refers to the integration of a foreign gene at a specific target, disrupting the target gene by inserting the transgene.

Transgenes are designed according to rules that result in gene expression in the host animal, such as the presence of at least one intron, exclusion of GC rich regions, particularly CpG rich motifs. Gene sequences called insulators are often included; these contain transcription enhancers and enhancer blockers to avoid cross talk with adjacent genes, and chromosome openers that modify histones to allow the transcription

machinery to be expressed. Finally, RNAi may be used to inactivate specific genes either as heritable transgenes or as non-heritable gene treatments. A vector based on HIV dramatically increased the efficiency of producing transgenic animals, thereby greatly reducing cost. Foetal fibroblast cells can be modified and then cloned to produce transgenic animals.

A novel approach was to transfect germ cell tissue in neonatal testis by electroporation, which was then grafted onto the backs of nude mice (nude mice are immune deficient and tolerate grafts from mammalian tissues). The nude mice, previously castrated, produced mature transgenic sperm that functioned well in *in vitro* fertilization to produce transgenic farm animals. The technique has been used successfully in cattle, pigs and even humans (though without producing an actual human as yet). The technique is promoted for humans as a means of allowing men requiring irradiation cancer treatment to set aside viable sperm for *in vitro* fertilization.

'Improving' the nutritional value and health benefits of livestock

Transgenic clones of cattle producing milk with higher levels of beta casein and kappa casein proteins were created to improve emulsion, processing and heat stability. Rare natural forms of the caseins were used to transform embryonic fibroblasts, with as many as 84 copies of the genes integrated randomly in the genome, no doubt causing huge disruption. The fibroblasts were then used to produce clones of the cattle. Nine cows expressing the transgenes produced milk with up to 20 percent increase in beta-casein and double the level of kappa-casein. The overall health of the transgenic cattle was not discussed, let alone the health impacts of the milk used as food.

This is just one example in a whole range of genetically modified 'nutraceuticals', animals and animal products that are supposed to provide enhanced nutritional value.

Cloned transgenic pigs have been produced rich in the beneficial omega-3 fatty acids normally obtained by eating fish. The transgene consisted of a synthetic n-3 fatty acid desaturase from the roundworm *C. elegans* driven by an aggressive cytomegalovirus enhancer and chicken beta-actin promoter, accompanied by a selection marker gene for neomycin resistance. Such constructs are typical in attempts to make the transgenic animals over-express the gene product. Pig foetal fibroblasts were transformed and then used to clone transgenic pigs. The transgenic pigs produced high levels of omega-3 fatty acids and a significantly reduced ratio of n-6/n-3 fatty acids. As before, the overall health of the cloned transgenic pigs was not extensively discussed, nor the health impacts of the transgenic pig used as food.

Recombinant human protein C was expressed in the milk of cloned transgenic pigs, also created by transforming foetal pig fibroblasts. Human protein C is an anti-coagulant found in the blood, and serves as a therapy for many disease states. The transgenic pigs produced the therapeutic protein, which protected the pigs against blood clot, but with a risk of pulmonary embolism.

Pigs expressing an *E. coli* salivary phytase produced low phosphorus manure. Phytase increases the availability of feed phosphorous and decreases its release in manure, thereby eliminating environmental pollution by phosphorus.

Transgenic chickens expressing bacterial beta-galactosidase hydrolyze lactose in the intestine, using it as an energy source, which would have caused diarrhoea to normal chickens. Early chicken embryos were transformed using the spleen necrosis retrovirus vector (SNTZ). SNTZ is an avian immunosuppressive retrovirus that infects non-replicating cells, not only of birds but of some mammals as well. It has an extraordinarily high mutation rate, and that is not a defect in the replication-deficient vector.

Transgenic fish

Transgenic fish are poised for commercial release. These will either be produced in confined land-locked ponds, fish pens in confined fjords or sounds, or released to open seas or lakes. Landlocked ponds provide protection from environmental release while fish pens are notoriously unreliable and tend to harbour sea lice or other parasites and pathogens. It would seem most prudent to limit production of transgenic fish, if at all, to landlocked ponds, to avoid or reduce the potentially deleterious impact of transgenic fish on the general environment.

Fish genes are most frequently used in producing transgenic fish, but it would be a mistake to regard the transgenic fish "substantially equivalent" to the native fish, as even the Codex consultation document acknowledges that, "transgenic expression of non-native proteins in plants may lead to structural variants possessing altered immunogenicity."

AquaBounty Inc. first applied to the US FDA (Food and Drug Administration) in 1999 to release a transgenic Atlantic salmon. The transgenic Atlantic salmon contains a Chinook salmon growth hormone gene driven by the ocean pout antifreeze promoter, resulting in a dramatic increase in growth rate. AquaBounty announces that it is also developing fast growing strains of fin fish known as AquAdvantage™ fish, capable of reducing growth to maturity time by as much as 50 percent. It is expecting FDA approval in 2006 and commercial launch in 2009. Scientists have expressed concerns over the release of sexually reproducing transgenic fish; realistic models show that it can lead to the extinction of both the natural and the transgenic population. AquaBounty has produced triploid transgenic Atlantic salmon supposed to be 100 percent sterile; however, the sterility may be "leaky", and indeed some fertile animals have been produced [3] (Floating Transgenic Fish in a Leaky Triploid Craft).

Transgenic Coho salmon, carp, tilapia and mud loach are all in the pipelines. The transgenic mud loach grew 35 times faster than the wild type fish, resulting in giant mud loach that were ready for market after only 30 days.

Transgenic zebra fish have been sold in United States pet shops since 2003 [4] (Transgenic Fish Coming). The transgenic zebra fish were projected to be capable of over-wintering in US southern and south-western waters. FDA allowed the release of the zebra fish because the animals did not fall into their jurisdiction. As the animals have been released, their presence in the natural environment should be monitored as a model for the release of transgenic food fish.

Non-heritable modifications

Non-heritable modifications of food animals include a number of applications such as DNA vaccination, transgenic probiotic bacteria as vector for vaccines and growth hormones, using RNAi (RNA interference) for epigenetic modifications, and stem cell chimeric animals whose somatic tissue but not the germ cells are transgenic. Non-heritable alterations are taking place or being implemented without full review of the impact on food and the environment, mainly because they do not fall under the rubric of genetic modification.

Naked DNA vaccines

It has been shown since the 1990s that ingested foreign DNA survives transiently in the gastrointestinal tract and enters the bloodstream of mice. Since then, naked DNA has found many applications, especially as DNA vaccines. DNA vaccines can be applied by a variety of routes including intradermal, intravenous, intramuscular, intraperitoneal, subcutaneous, sublinqual, intravaginal, intrarectal, via internasal inhalation, intranasal instillation, ocular and biolistic delivery. Gene vaccines are becoming commonplace and

have the advantage of raising antibodies to a target antigen specifically. However, DNA immunization can stimulate florid local inflammation. DNA vaccines are commonly delivered in polyethyenimine complexes, where the plasmid DNA remains active in cells at least 12 days after injection.

DNA vaccines are used in both farm animals and fish, and there has been no study on whether there is any carry over of the vaccine DNA into food prepared from vaccinated animals.

DNA vaccines have been created against pork tapeworms in pigs, bovine herpes virus 1 in cattle, and mastitis caused by Staphylococcus aureus in cows.

A recombinant plasmid DNA vaccine was made to control infectious bursal disease of young chickens characterized by immunosuppression and mortality generally at 3 to 6 weeks of age.

A recombinant plasmid DNA vaccine was prepared to control viral hemorrhagic septicemia, a systemic infection of various salmonid and a few non-salmonid fishes caused by a rhabdovirus (a single stranded RNA virus). A DNA vaccine was made to protect against *Mycobacterium marinum* that causes tuberculosis in fish and shellfish and cutaneous lesions in humans.

Recombinant vaccine vectors

Recombinant vectors have been developed from viruses or bacteria to deliver vaccine antigens. One fundamental concern over the use of such vectors is genetic recombination involving the vectors, resulting in novel pathogens. Not only are the vectors themselves already derived from pathogens, but they also carry transgenes from other pathogens.

A Newcastle disease virus was modified to express the H5 hemagglutinin of avian influenza. Newcastle disease is a highly contagious bird disease affecting many domestic and wild avian species, and is caused by a single stranded RNA virus. Its effects are most notable in domestic poultry, which are highly susceptible to the disease with the potential for severe epidemics that impact on the poultry industry. Avian influenza is endemic to many countries, and is a threat to both commercial and wild fowl as well as to humans. The virus can change to a form that causes serious disease in humans through reassortment, mutation and recombination [5-7] (Fowl Play in Bird Flu; Where's the Bird Flu Pandemic?; What Can You Believe About Bird Flu?). The chimeric vector vaccine is expected to protect against both influenza and Newcastle disease. It is clear that more extensive safety studies are needed.

A recombinant pseudorabies virus expressing a fusion protein of pig circovirus type 2 was made. Pseudorabies viral disease in swine is endemic in most parts of the world, and is caused by porcine herpesvirus 1. The name pseudorabies comes from the similarity of symptoms to rabies in dogs. Secondary hosts are infected through direct contact with swine, or via infected pork. Porcine circovirus (PCV) is a member of the virus family Circoviridae; and there are two serotypes, PCV1 and PCV2. These relatively small, non-enveloped, circular DNA viruses are quite stable in the environment and resistant to many common disinfectants. PCV2 is associated with postweaning multisystemic wasting syndrome in piglets, characterized by progressive loss of body condition, visibly enlarged lymph nodes, difficulty in breathing, and sometimes diarrhoea, pale skin, and jaundice. The vaccine appears to protect against both circovirus and psuedorabies virus infection, but its safety remains to be ascertained.

The use of lactic acid bacteria as vehicles to delivery antigens to immunize animals appears promising. When genetically modified, these bacteria can induce a specific local and systemic immune response against selected pathogens. Gastric acid and bile salts tolerance, production of antagonistic substances against pathogenic microorganisms, and adhesive ability to gut epithelium are other important characteristics

that make these bacteria useful for oral immunization. By the same token, genetically modifying these bacteria has the potential to turn them into serious pathogens.

Lactobacillus isolated from the gastrointestinal tract of broiler chickens is being used to live oral vaccines to immunize broilers against infectious diseases. A number of such oral vaccines have been successfully tested in mice.

Using GM probiotic bacteria as vaccine vectors requires special caution. These bacteria are natural beneficial symbionts of the gastrointestinal tract, and have adapted to their human and animal hosts over millions if not billions of years of evolution. Genetically modifying them as vectors could easily turn them into pathogens pre-adapted to invade the human and animal gut. Furthermore, the gastroinstestinal tract is an ideal environment for horizontal gene transfer and recombination, the major route to creating pathogens. For these reasons, we have proposed that any genetic modification of probiotic bacteria should be banned [8-10] (Ban GM Probiotics; GM Probiotic Bacteria in Gene Therapy).

There is increasing evidence that infectious disease epidemics, such as bird flu, are created by intensive industrial farming of livestock and the globalised trade in livestock, meat and animal products [5]. Vaccines are risky on the whole, and cost a lot to develop; and may well not be necessary if much more effort were devoted to establishing farming practices that reduce stocking rates while improving animal welfare, nutrition and health to build up the animals' natural immunity to disease.

RNAi in epigenetic gene modification in food animals

Among the major discoveries of molecular genetics in the 1990s is RNA interference (RNAi), how very small RNA molecules - around 21 to 25 nucleotides or shorter - can inhibit expression of specific genes in all organisms [11] (Subverting the Genetic Text). RNAi regulates basic biological processes, including transition from one stage of development to another. Furthermore, RNAi is used as a form of immunity to protect the cell from invasion by foreign nucleic acids introduced by mobile genetic elements and viruses. RNAi soon found applications in human gene therapy, as it appeared to offer the ability to shut down any chosen gene specifically without affecting any other.

But the technique hailed as "breakthrough of the year" in 2002 was found not to be so specific after all. There were substantial "off target" effects on other genes and proteins [12] (Controversy over Gene Therapy 'Breakthrough'). In May 2006, RNAi gene therapy was found to kill mice by the dozens [13] (Gene Therapy Nightmare for Mice). The mice died of liver failure from RNAi overload. There are reasons to believe that RNAi therapy is unsafe, because the effects are not, and cannot be specific. Numerous RNA species interfere at every level of gene function, and it is impossible to target the effects precisely because the RNA interference underworld is huge, comprising some 97 to 98 percent of the transcription activity in the cell, and specificity depends on low levels of the correct sequences being produced at the right time in the appropriate places. Extreme caution is needed as these RNAi species have the potential to affect the animals adversely, and can also be passed onto humans through food.

RNAi has been used as a tool to study gene function in bovine oocytes, to target the sheep parasite *Trichostrongylus*, developmental control genes in chicken embryos and to prevent avian influenza. RNAi specifically silenced genes in fish embryos, and specific gene knockout appeared effective in medaka, zebra fish and rainbow trout. Silencing the myostatin gene led to giant zebra fish. The tiger frog iridovirus also attacks fish; and RNAi was effective in inhibiting replication of the virus in fish cells.

Somatic gene therapy in farm animals using vectors or naked DNA

Gene therapy has been used in farm animals to transform somatic cells without affecting the germ cells, at least in theory. Most of the applications are to increase milk yield or

growth rate, or to protect the animals from disease. At least some of the farm animals may be serving as models for human gene therapy, so experimental animals too, may be passed off as food for humans.

Retrovirus mediated gene transfer in lungs of living feta sheep has been demonstrated. A Moloney murine leukemia retrovirus vector incorporated a marker gene and either beta-galactosidase, or human interleukin receptor antagonist gene. Gene integration was observed in cells of the airway epithelia.

A plasmid vector highly efficient at releasing growth hormone was introduced into the skeletal muscle of pigs using electroporation. The transgenic pigs showed enhanced weight gain and improved body composition at low DNA plasmid dose. An adenovirus vector was used to deliver a human gene angeopoein-1 into the pig heart in animals affected by chronic myocardial ischemia. The implanted gene helped the pigs recover from the condition. A DNA plasmid encoding somatostatin fused with an antigenic protein of a pig reproductive and respiratory syndrome virus induced antibodies to the viral protein and promoted growth in immunized pigs,after a single injection of the plasmid.

Continuous infusion of bovine growth hormone releasing factor increased milk production by as much as 46 percent; so a vector was created from the bovine leukemia virus carried the gene for growth hormone release factor driven by a mouse whey acidic protein promoter, or alternatively, a mouse mammary tumour virus promoter.

A fowl adenovirus vector was used to insert chicken interferon gene controlled by the fowl adenovirus late promoter and SV40 polyA site. Chickens treated with the recombinant vector showed increased weight gain, and less weight loss when challenged with the parasite causing coccidiosis.

A live fowlpox virus vector was constructed carrying a chicken mylomonocytic growth factor gene. Chickens treated with the vector had elevated monocyte levels and a high proportion of active monocytes. Another vector containing chicken interferon, when combined with an antigen (sheep red blood cells), resulted in enhanced antibody response. Using the interferon vector alone increased weight gain and improved resistance to disease.

Recombinant microbes in the rumen

Genetic modification of the microbes in the rumen is a seductive topic. In theory the microbes can be modified to make fodder much more digestible, thus making more efficient use of grazing land. However, it has not proven effective as yet, because rumen ecology is complex. On the other hand, if the recombinant microbes succeed, they may unbalance the ecology of the rumen and cause disease to the animals and to the human beings that use the animal and animal products as food. Genetic engineers should learn much more about the ecology of the rumen before proceeding..

A recombinant rumen bacterium, *Butyrivibrio fibrisolvens*, expressing a fungal xylanase gene and erythromycin resistance marker gene was inoculated into a sheep's rumen. The recombinant bacterium disappeared from the rumen of hay-fed sheep within 12 hours of being introduced, but flourished when inoculated into autoclaved rumen fluid; showing that the recombinant bacteria were eliminated by living organisms. The main fibre-digesting bacteria in the rumen, *Ruminococcus* and *Fibrobacter*, have proved refractory to genetically modification, leaving only *Butyrivibrio* that can be modified. The recombinant bacteria were less effective at digesting fibre than the native fibre digesters. Protozoan predation was the main cause of the introduced bacteria disappearing.

The toxin flouroacetate accumulates to high levels in some Australian plants, becoming lethal to grazing sheep. A gene for flouracetate dehalogenase was isolated from the bacterium *Moraxella* and used to modify *Butyrivibrio fibrisolvens*. Sheep exposed to

flouracetate showed markedly reduced poisoning symptoms after being inoculated with the recombinant bacteria.

Interestingly, over 75 percent of the genes for carbohydrate in rumen ciliates originated by horizontal gene from rumen bacteria. Many of the permanent bacterial residents of the rumen have not yet been cultured. Wild animals may have acquired microbes not seen in domestic animals because they are exposed to more severe dietary conditions. Such microbes and their enzymes may be useful for applications in future.

Are they safe?

Health risks from GM food animals

Food derived from genetically modified animals pose several kinds of health risks, whether heritable or not, and we do not recommend using them as food unless and until these risks have been assessed, and comprehensive studies show that they are safe beyond reasonable doubt.

The health risks of food derived from genetically modified animals come from the specific proteins encoded by the transgenes, from the transgenic nucleic acids and vectors used for genetic modification, and from unintended effects of transgenesis and the cloning procedures used to produce a herd of transgenic animals, as the transgenic animals are often sterile or else do not breed true [14].

Non-heritable traits, in particular, include potent synthetic antigens for vaccination and powerful immune regulators with well-described side effects, while both heritable and non-heritable traits include growth hormones. The ingestion of foods with growth factors, vaccine antigens or immune regulators is likely to have untoward impacts on the immune system and development of human beings, especially the young.

Many of the genes used to create transgenic food animals are synthetic approximations of the original gene, but deemed, mistakenly, to be “substantially equivalent” to the natural genes. The synthetic genes contain DNA sequences that have never existed in evolution, and by no stretch of the imagination can they be presumed safe.

Synthetic genes are used, first of all, because bacterial genes are not readily translated in animals and plants. Bacteria use different codons for the same amino acids (codon bias), and so the gene sequence has to be modified to allow for that. Transgenes are often composites of different genes. For example, a synthetic transgene was made up of an antibacterial gene from *Staphylococcus* (lyphostatin) joined to a gene from a *Streptococcus* bacteriophage (virus of bacteria) encoding endolysin, which dissolves bacteria. The synthetic composite gene was used to modify cows, so they would produce milk that kills bacteria [15].

One main problem discussed was allergenic potential of the protein in milk. Proponents assured us that the cows modified with the synthetic gene were unlikely to be allergic to the toxin because it is a part of their genome, and thus recognized as self. But they failed to mention that children drinking the milk would not recognize the protein as ‘self’, and might well mount immune reactions against the protein, including allergy.

Efforts were made to ‘humanize’ transgenic proteins by altering the genes specifying a protein’s glycosylation pattern to avoid immune reactions including allergy (allergy sites on proteins often have specific glycosylation), but that approach was only partly effective. In view of the recent finding that a normally harmless bean protein turned into a potent immunogen when transferred to pea [16, 17] (Transgenic Pea that Made Mice Ill), there is a case for banning all GM food products until and unless they can be proven safe by adequate tests. This applies all the more so to transgenic animal food products, especially milk, which are consumed predominantly by infants and children.

The profligate use of nucleic acids (RNAs and DNAs) in livestock is a source of deep concern, as it is already well known that they are to varying degrees capable of horizontal gene transfer and recombination with attendant risks of creating new viruses and bacteria that cause diseases, and of triggering cancer by integrating into genome sites that activate oncogenes as gene therapy clinical trials have made all too clear [18] (Gene Therapy Woes). Similarly, RNAi overload proved lethal to mice [13]; and it is not safe to presume that the RNAi used to modify animals will not affect those consuming the treated animals.

The dangers of genetic engineering, especially the use of recombinant viral vectors and bacteria have been recognized by genetic engineers themselves before the lure of commercial exploitation swept aside these concerns [19] (Gene Technology and Gene Ecology of Infectious Diseases). We have continued to warn of the dangers of environmental releases of genetically modified nucleic acids in subsequent years, and constructs with recombination hotspots such as viral promoters [20-23] (Slipping through the regulatory net; Cauliflower Mosaic Viral Promoter - A Recipe for Disaster?; Hazards of Transgenic Plants Containing the Cauliflower Mosaic ...; CaMV 35S promoter fragmentation hotspot confirmed, and it is ...)

There have been no studies addressing the unintended changes of genetic modification in transgenic animals, which may well create unexpected toxins or immunogens [14] (Fatal Flaws in Food Safety Assessment: Critique of the Joint FAO ...).

Similarly, the cloning process is already known to result in unintended gross morphological as well as genetic defects [24] (What's Wrong with Assisted Reproductive Technologies?) that may compromise the safety of transgenic meat.

Non-heritable may be more risky than heritable

It may appear that the food safety issues of heritable transgenic traits and non-heritable traits are different. Non-heritable traits are mainly based on DNA plasmids, bacterial vectors or viral vectors that do not theoretically integrate into the germline genome, though there is always a small probability that any DNA introduced into an organism may integrate into the germline genome, as the germ cells are not separated from somatic cells by any real physiological barrier. On account of the unjustified presumption that the foreign genetic material will not be incorporated into the germline, there is a tendency for relaxed regulation, which is equally unjustified.

Many of the recombinant DNA plasmids, bacterial vectors or viral vectors have been subject to clinical trials or even approved with little fanfare and public notification. It has been presumed that the recombinant genes and their protein products are not present in the milk or meat of treated animals but there is little published information to support that assumption, and that is perhaps the main danger.

Non-heritable genetic modifications are more threatening than heritable modifications because of its widespread use without the necessary risk assessments. It is also highly likely that meat or milk of recombinant animals will not even be labelled in the market, as they do not fall under the rubric of genetic modification.

"Substantial equivalence"

Valueless and highly misleading

In line with current risk assessment guidelines, Codex Draft Guideline states:" The concept of substantial equivalence is a key step in the safety assessment process."

We take issue with that statement. "Substantial equivalence" is often used as a starting point to structure the safety assessment of a new food in the most undiscerning and reductionist way. For example, comparisons are made in the gross composition of proteins, carbohydrates and fats, or in amino acid compositions, which generally show

little or no difference; and so it allows the proponent to focus on the transgene product(s) only [14]. Moreover, the comparators are completely arbitrary. Instead of comparing the transgenic variety with the variety from which it has been derived, companies have been allowed to compare the transgenic variety with the entire species, or indeed with whole category of foodstuffs from many different species, as in the case of edible oils for example.

Although there have been attempts to improve on establishing substantial equivalence by incorporating profiles of total protein, metabolites and transcripts, the technical hurdles involved in comparing and interpreting patterns are insurmountable, and no official requirements are enforced. In this way, unintended, untoward effects of the modifications will not be revealed unless specific tests other than those used for establishing substantial equivalence are carried out. Examples are tests for toxicity, allergenicity and immunogenicity. Substantial equivalence therefore has nothing to say about the safety of the transgenic food product, and it would be highly misleading to claim that it does.

Synthetic genes not substantially equivalent to the natural

One important fact ignored by the Codex guidelines, which also disposes of the concept of substantial equivalence is that the recombinant animals are constructed using synthetic versions of natural genes that often involve composites of different genes, with different nucleic acid sequences as well as changes in amino acid sequence. The changes in nucleic acid sequence will lead to differences in the recognition of the gene by nucleosomes and histones, proteins that regulate gene activity. Changes in amino acids will result in proteins with different conformations that would affect the proteins' interactions with other proteins, and are likely to be regarded as foreign by the host's immune system, as well as by humans eating the transgenic food. Furthermore, these proteins specify potent antigens, growth factors, cytokines or other signal proteins that have potent biological effects and can in no way be regarded as safe.

Transgenes exchanged between closely related species not substantially equivalent

Even when genes are transferred between closely related species, glycosylation patterns of the proteins change as mentioned earlier, and could have catastrophic consequences for the human consumer.

Codex should abolish the discredited concept of substantial equivalence once and for all, in recognition that it is highly misleading when used as a key concept in safety assessment.

We do not recommend using genetically modified animals and animal products as food, until and unless they can be proven to be safe by comprehensive safety evaluations, whether the genetic alterations are heritable or non-heritable.

References

1. Codex Alimentarius Commission Request for comments to the provisional draft guidelines for the conduct of food safety assessment of foods derived from recombinant DNA animals Food and Agriculture Organization-World Health Organization CL 2006/27-FBT July 2006 http://www.codexalimentarius.net
2. Cummins J and Ho MW. Genetically modified food animals coming. ISIS press release, 25 September 2006, http://www.i-sis.org.uk/Genetically_Modified_Food_Animals_Coming.php
3. Cummins J. Floating transgenic crops in a leaky triploid craft. Science in Society 24, 32, 2003.
4. Cummins J. Transgenic fish coming. Science in Society 21, 26-7, 2004.
5. Ho MW. Fowl play in bird flu. Science in Society 30, 17-19, 2006.

6. Ho MW. Where's the bird flu pandemic? Science in Society 30, 20-21, 2006.
7. Ho MW. What can you believe about bird flu? Science in Society 30, 22-25, 2006.
8. Cummins J and Ho MW. Genetically modified probiotics should be banned. *Microbial Ecology in Health and Disease* 2005, 17, 66-68.
9. Cummins J and Ho MW. Ban GM probiotics Science in Society 26, 29, 2005.
10. Cummins J and Ho MW. GM probiotic bacteria in gene therapy, Science in Society 31, 26-27, 2006.
11. Ho MW. Subverting the genetic text. Science in Society 24, 6-8, 2004.
12. Ho MW. Controversy over gene therapy 'breakthrough'. Science in Society 26, 38, microRNA/short hairpin RNA pathways. Nature. 2006 May 25;441(7092):537-41.
13. Ho MW. Gene therapy nightmare for mice, could humans be next? Science in Society 31, 25, 2006.
14. Ho MW and Steinbrecher R. Fatal flaws in food safety assessment: Critique of the joing FAO/WHO Biotechnology and Food Safety Report. *Journal of Nutritional and Environmental Interactions* 1998, 2, 51-84.
15. Wall RJ, Powell AM, Paape MJ, Kerr DE, Bannerman DD, Pursel VG, Wells KD, Talbot N and Hawk HW Genetically enhanced cows resist intramammary *Staphylococcus aureus* infection. *Nat Biotechnol.* 2005, 23(4), 445-51.
16. Prescott VE, Campbell PM, Moore A, Mattes J, Rothenberg ME, Foster PS, Higgins TJV and Hogan SP. Transgenic expression of bean a-amylase inhibitor in peas results in altered structure and immunogenicity. *J Agricultural and Food Chemistry* 2005, 53, 9023-30.
17. Ho MW. Transgenic pea that made mice ill. Science in Society 29, 28-29, 2006.
18. Ho MW. Gene therapy woes. Science in Society 26, 36-37, 2005.
19. Ho MW, Traavik T, Olsvik R, Tappeser B, Howard V, von Weizsacker C and McGavin G. Gene technology and gene ecology of infectious diseases. *Microbial Ecology in Health and Disease* 1998, 10, 33-59.
20. Ho MW, Ryan A, Cummins J and Traavik T. Slipping Through the Regulatory Net, 'Naked' and 'Free' Nucleic Acids, TWN Biotechnology and Biosafety Series 5, Third World Network, Penang, 2001.
21. Ho MW, Ryan A and Cummins J. Cauliflower mosaic viral promoter – a recipe for Disaster? *Microbial Ecology in Health and Disease* 1999 11, 194-7.
22. Ho MW, Ryan A and Cummins J. Hazards of transgenic plants with the cauliflower mosaic viral promoter. *Microbial Ecology in Health and Disease* 2000, 12, 6-11.
23. Ho MW, Ryan A and Cummins J. CaMV35S promoter fragmentation hotspot confirmed and it is active in animals. *Microbial Ecology in Health and Disease* 2000, 12, 189.
24. Ho MW. What's wrong with assisted reproductive technologies? Science in Society 20, 42-43, 2003.