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The Food Safety of Transgenic Animals'

In the last 40 years a number of modern techniques for improving animal lines have been developed. Artificial insemination has already had an enormous impact on the dairy industry. Techniques such as *in vitro* fertilization, embryo cloning, nuclear transplantation and transgenesis are reaching maturity. These techniques and their potential effects on the environment, genetic diversity, animal production and society have been discussed by George Seidel (1989; 1991).

Introducing food products into the market place requires that the safety aspect be fully analyzed and documented so that healthy transgenic animals will be at least as safe as the traditional animals from which they were derived.

The classical breeding of familiar food animals has been practiced since antiquity and has never resulted in a hereditary trait that made animals unsafe as food. Traditional breeding is accomplished by focusing on a desirable trait, such as milk production or fat content, and breeding only those animals which best exemplify the trait. If the trait is quantitative, this practice moves the population mean in the desired direction. The cause of the improvement is unknown. Theprogeny results from thousands of selections between paternal and maternal genes and the genes responsible for the improvement in the phenotype are rarely, if ever, identified. There is little knowledge of the physiological mechanism underlying the phenotypic change. Yet, this approach has been safe and successful and is exemplified by the dairy industry where selecting semen from bulls with high-producing daughters more than doubled the milk output per cow in the twenty years following 1955. The genetic events associated with traditional breeding are safe; consequently, only the unique features of transgenesis are examined here.

One can organize the unique features of transgenesis into three categories: the genetic construct (i.e., the DNA introduced), changes resulting from the integration of the construct, and the nature of the gene product.

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* The views expressed are not necessarily those of the Food and Drug Administration.

SAFETY CONSIDERATIONS ASSOCIATED WITH THE GENETIC CONSTRUCT

There is little concern about the safety of orally consumed genes. The human diet, consisting of bacterial, animal and plant products, include all of the genetic material of those organisms. Digestive enzymes in the human gastrointestinal tract degrade DNA in the food and, since a single nick in a gene is enough to inactive the production of the gene product, the probability of a functional gene sequence surviving intestinal digestion may be considered near zero. On the off chance that some DNA does survive, it would only be excreted.

The increased purine and pyrimidine content of tissues resulting from the extra gene in transgenic animals will be negligible relative to the total tissue purine content. In mammals, the purine from a single gene is on the order of one millionth of the total genomic content of purines. Some plant breeds produced by traditional methods have resulted in large percentage increases in the nucleic acid content, i.e., increases in the somatic cell chromosome number. These considerations may be more important if the food product were a sole source of protein or energy.

The DNA of the construct is of concern only if it is infectious, i.e., if it can be propagated in the environment or transmitted by the food to susceptible cells in the gastrointestinal tract. Retroviruses are used to introduce genes into some species, particularly poultry. The viruses that come in contact with prospective transgene recipients are defective, likely carrying at least one deletion in a transacting gene. Rarely, through recombination with endogenous viruses or from functional retroviruses present in nature, could fully functional viruses emerge from the helper cell line. The probability of functional recombinants arising is small, but they have been observed. New helper cell lines with less homology between the defective viruses and the provirus will reduce the possibility of recombinant virus production (Miller, 1990; Temin, 1989). From the food safety perspective, even competent animal retroviruses pose no threat to human health because of the species specificity of viral infection.

SAFETY CONSIDERATIONS ASSOCIATED WITH INTEGRATION OF THE CONSTRUCT

The insertion of a transgene into a recipient genome is a safety consideration because the location and manner of insertion may increase or decrease the expression of host genes. The hypothesis is that the insertion process might activate latent toxin genes or increase levels of hormones or other substances detrimental to human health when the food is eaten. In healthy animals this is not a realistic concern. If the transgenic animal is not healthy, the cause must be investigated to be certain that the pathology has not resulted from something transmissible in the food. However, the possibility of activating a toxin gene is insignificant, as discussed below.

The genetic events causing the modulation of gene expression as a result of transgene insertion are not different from genetic events that occur naturally. Modifications of gene expression are caused by the generation of new connections between sequences that are not normally juxtaposed or by the separation of normally connected sequences. Chromosomal translocations, deletions and inversions occur continually in animals in nature as well as in food animals. Animals also contain interspersed sequences that transpose to new chromosomal locations, though the frequencies of transposition in food animals are not known. Written records of animal breeding go back as far as Aristotle (Sturtevant, 1965), and animal breeding has never been associated with the production of toxic lines of animals. This historical record is strong evidence for the food safety of translocations, inversions, deletions and insertions in animal chromosomes.

Toxin genes are rare in animals. Animals are generally safe as food. The dangers in eating animal products usually stem from parasites or microbiological contaminants; these are inactivated by cooking. The overwhelming majority of animal species can be eaten without harm. There have been reports of dogs being poisoned by eating polar bear liver, but the poisonings are caused by high levels of vitamin A in the livers (Russel, 1966). Although this is an example of toxicity from the ingestion of animal tissues, the accumulations of high levels of vitamin A in the liver is a complex trait and is not induced by a single genetic event. The genomes of the common food animals do not carry toxin genes that can be activated.

A classical case of acute "animal" poisoning is the biblical case of quail poisoning (brought to my attention by John Kirschman) described in *Numbers, Chapter 11.* During the Exodus the Israelites became tired of eating manna and wanted "flesh."

> And there went forth a wind from the Lord, and brought across quails from the sea...and the people gathered quail; he that gathered the least gathered ten heaps...While the flesh was yet between their teeth, ere it was chewed, the anger of the Lord was kindled against the people, and the Lord smote the people with a very great plague. And...they buried the people that lusted.

The investigation of modern cases of quail poisoning have been attributed to coniine, the hemlock neurotoxin that killed Socrates. The quail feed on hemlock during their migration from Africa to Europe, are resistant to the toxin, and are able to consume enough hemlock to poison predators. The toxin itself is a plant product, not an animal product.

An important consideration in animals is that toxic genetic effects with adverse human health effects, unexpected or otherwise, are likely to produce visible signs in the development or growth of the transgenic animal. Transgenic animals are themselves an important demonstration of their food safety. The fact that an animal has gone through normal intrauterine development,

birth and growth in the presence of the transgene and its product is a strong indication of the safety of the derived food. For the food to be toxic, the animal would have to produce a species-specific toxin that is inactive in the species of origin, but orally active in the species consuming the food. No such toxins from land food-animals have been described.

THE SAFETY OF THE GENE PRODUCT

The essence of the safety review of transgenic animals must be an examination of the gene product. The safety of gene products may be reviewed in the same way the safety of drugs or pesticides are classically reviewed, i.e., the important food safety matter is the presence of a pharmacologically or toxicologically active residue. Because the product of the transgene is completely characterized, one can use traditional methods to evaluate its safety. This is an advantage over traditional breeding because the knowledge of the exact genetic change directs the safety inquiry to the correct gene product and its effects. Traditional breeding is accomplished empirically by focusing on a desirable trait with little knowledge of the physiological mechanism underlying the phenotypic change.

Gene products may have both direct effects resulting from the action of the gene products themselves and indirect, secondary or compensatory effects brought about in response to the direct effects of the gene product. For example, somatotropin stimulates the secretion of IGF-1 from the liver and other tissues. IGF-1 is responsible for many of the effects formerly attributed directly to growth hormone and this was taken into account in evaluating the safety of milk from bovine somatotropin-treated cows (Juskevich and Guyer, 1990). Such reasoning is normally part of the review of the food safety of feed additives and new animal drugs. Routine toxicology testing is designed to detect all effects of a compound, direct and indirect.

Once the safety of the transgene product is established, transgenic animals may be considered as safe as traditional animals. Some of the food safety considerations may change as the technology advances. Richa and Lo (1989) produced "transomic" mice by introducing chromosome fragments dissected from metaphase spreads into fertilized ova. Chromosome fragments known to be associated with desired traits can be used selectively. Large numbers of genes (10 megabases) are introduced rather than selected genes. For intraspecies transfers, the results are likely to be similar to naturally-occurring cases of trisomy. Transomic animals are likely to be safe also, but too few have been studied to make conclusions about the food safety considerations.

If we imagine that we are many years in the future when livestock are routinely improved by recombinant DNA techniques, traditional breeding, in retrospect, will seem far too hazardous. To allow all the genetic changes to occur by chance and then never know what genes or genetic changes were re-

sponsible for the new phenotypes is likely to seem far more risky than transgenesis. Cattle have 30 pairs of chromosomes. Thus, *in the absence of recombination,* a single mating has a potential of producing 2^® or 1.07 billion genetically different eggs or sperm. Surely the introduction of a single well-characterized known gene is less risky!

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