OPINION

A new paradigm for regulating genetically engineered animals that are used as food

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Over the past 20 years, transgenic or genetically engineered (GE) plants have been routinely approved for human consumption, with close to 80 varieties successfully navigating the regulatory process. This is in stark contrast to GE animals, where only one has been approved for human consumption: the AquAdvantage salmon (1). The different regulatory trajectories for GE plants and GE animals for food raises the following questions: Why are the two regulatory tracts so different in outcome? Are the differences between GE plants and animals for use as food more significant than the similarities? We suggest that the two situations are more similar than different, that their regulatory paths should be harmonized, and that the regulations for genetically modified animals should be altered on multiple fronts.

Policy Approach

In the United States, GE animals for use for food production are regulated by the Food and Drug Administration (FDA), as outlined in Guidance #187 (2). The first GE animals were reported in 1985 (3) and the first application for approval for food, the AquAdvantage salmon (4), was submitted 10 years later. A limited number of other applications for approval have also been submitted (5–8). On November 19, 2015 the US FDA approved the AquAdvantage salmon for use as food (1), the first such approval anywhere in the world; a regulatory time frame that saw the patent protection held by AquaBounty Technologies Inc. expire before FDA approval was granted.

Over this same time frame, many GE plants became widely used for food in the United States and other countries. Regulation of GE plants falls to three agencies: the Department of Agriculture Animal and Plant Health Inspection Service (USDA/APHIS), the FDA, and the Environmental Protection Agency. Since 1995, GE plants have been used for food and feed with no documented adverse reactions in humans. Starting in 1993, USDA/APHIS began deregulating (allowing commercialization) some varieties of GE plants, with at least 78 varieties now deregulated (9).

Differences between plants and animals include life cycles, generation intervals, public perception of the health and well-being of the organism, and the potential of the organism to impact the environment. However, these factors do not impact food safety. The health and well-being of the organism and environmental impact are equally of concern whether or not the organism is GE. Any practice of agriculture impacts these issues and if a GE rapeseed plant, a GE salmon, a highly selected non-GE rapeseed plant, or a non-GE domesticated salmon fertilizes a wild relative there are potential environmental impacts. The similarities between GE plants and animals with respect to food safety issues include the presence of new DNA sequences, insertional mutagenesis, activation of quiescent viruses, and retained selectable markers. The expression product of the transgene depends on the construct used, but the potential effects of the expression of a new gene on the level of expression of endogenous genes is relevant in both plants and animals.

The concepts of "generally recognized as safe," or GRAS, and substantial equivalence should be applied

Transgenic animals, such as these goats, should face regulations much more akin to those faced by transgenic plants. Image courtesy of Karin Higgins (University of California, Davis, CA).

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to the regulation of GE animals. Both concepts are grounded in the recognition of two biological realities. First, food is an undefined, exceedingly complex mixture of organic compounds and inorganic minerals, which results in no two meals being identical in chemical composition; thus, no two steaks or bottles of milk are identical chemically or at the DNA level. Second, humans have been developing new food sources for millennia. GRAS and substantial equivalence are a direct reflection of these two realities.

GRAS states that, for example, as our ancestors ate aurochs, the ancestor of domestic cattle, a new breed of cattle developed through selective breeding would be safe for use as food and would not require any new safety testing. The same goes for a new cultivar of a plant commonly used for food. In both cases the DNA sequences would be different, even though all DNA contains the same four nucleotides. The consumption of DNA is considered GRAS, even though the base composition and sequence varies between individuals and between different cells within an individual. GRAS also recognizes that no two individual food items—let alone meals—are chemically the same. It is not possible to chemically define a food unless it is something like crystalline sugar; for example, the composition of cows' milk at the protein, lipid, carbohydrate, nucleic acid, and mineral levels varies between breeds of cows, between lactations of a cow, over the course of a lactation, and is affected by the health of the animal, the feed consumed, and environmental conditions. Cows' milk commonly contains around 150 different lipids, but over 500 different lipids have been found in milk (10). The reality is that one cannot chemically define food. However, it is possible to define normal ranges for the amount of nutrients found in a food and acceptable ranges for the presence of toxic or antinutrient compounds in that food.

The concept of substantial equivalents is incorporated into the Codex Alimentarius, food safety standards established by the World Health Organization and the United Nation's Food and Agriculture Organization (www.fao.org/fao-who-codexalimentarius/standards/en/). It states that if the specific transgene product is deemed safe and the gross levels of the various classes of nutrients in the GE food are within the normal ranges found in the same non-GE food, then the food item is deemed substantially equivalent and thus safe; that is, there are no significant changes in the nutritional value of the food item. The concept of substantial equivalence recognizes the biological reality that even a single food item cannot be chemically defined.

Animal Product Paradigms

So where does this leave the regulation of GE animal products destined for use as food? The current trigger for regulation of GE animals is the introduction of DNA, regardless of application. This paradigm lumps together all applications of GE animals without regard to whether the product of the transgene will actually be consumed or not. The following two examples underscore how the current paradigm fails in the case of GRAS and substantial equivalence.

The first example is a case where the food derived from the transgenic animal would contain only the transgene DNA and not the protein product of the transgene. Bovine α-lactalbumin GE pigs express increased levels of α-lactalbumin in the mammary gland epithelial cells during lactation. α-Lactalbumin is one of the six main proteins found in cows' milk and functions to produce lactose, which dictates the volume of milk produced. Expression of bovine α-lactalbumin in GE pigs increases the production of lactose in the milk and consequently the production of milk by the GE sow (7). Milk production in modern sows is not adequate to feed the average number of offspring born per litter. Consequently, the smaller pigs in the litter do not receive sufficient nutrients and often do not survive, decreasing productivity. Additionally, all of the pigs in the litter grow less efficiently than their potential throughout their life, also decreasing feed efficiency. This increases the environmental footprint of raising pigs.

The bovine α-lactalbumin pigs perform as expected with increased milk production, resulting in larger, healthier offspring, which are more feed efficient (11), with the transgene only expressed in the mammary gland during lactation (12). If meat from a lactating transgenic pig were consumed, the α-lactalbumin protein would not be present, only the DNA sequence encoding the transgene. In males or nonlactating females the transgene is not expressed, so even the mammary gland tissue would be free of the bovine α-lactalbumin protein. Even though the transgene product would not be consumed, and the consumption of DNA is considered GRAS, the current regulatory process assumes there is risk based merely on the presence of transgene DNA, failing both the concept of GRAS and substantial equivalence. It is inconsistent to consider DNA GRAS yet regulate transgene DNA as potentially hazardous.

The second example concerns regulation when the transgene product is consumed, but is a protein already consumed in the diet. Human lysozyme (hLZ) transgenic goats express hLZ in milk for potential use as a food supplement to limit the incidence or severity of diarrheal infections (7). Lysozyme is a naturally occurring antimicrobial found at 1,500-times greater amounts in human milk than in goat milk. Substantial data demonstrate both that the GE animals producing hLZ at levels approaching human milk are healthy and that consumption of pasteurized hLZ-containing goats' milk by young pigs results in positive benefits and can aid in recovery from an *Escherichia coli*-caused diarrheal infection (13). There is no production of the transgene product in muscle; so, when consuming meat from these animals, only the DNA is being eaten. The transgene product would be consumed in the milk but because hLZ is naturally present in saliva it is thus eaten daily by everyone. Lysozyme from hen egg whites is extensively used in the food industry to prevent spoilage and has GRAS status. This application represents an example of a transgene product that is normally consumed and present in food, and as such, should not pose any

greater risk. Furthermore, the current regulatory pathway becomes murky with respect to animal food products containing an expressed transgene in that it is not clear if additional FDA agencies, such as the Center for Food Safety and Applied Nutrition, would be involved.

Note that for each transgenic line discussed, the original animal was produced more than 10 years ago, meaning that the animals available today are 6 to 20 generations removed from the transgenic founder that was injected with the transgene. The animals grow to a normal size, reproduce normally, transmit the transgene to offspring, and are healthy. A part of the USDA Food Safety Inspection Services (USDA-FSIS) meat inspector's function is to perform an antemortem inspection on livestock and poultry before slaughter to ensure the animals are normal and healthy, with inspection personnel directed "to observe the overall condition of the animal, including the head, with attention to the eyes; the legs; and the body of the animal" (14). A healthy animal is deemed to be a wholesome animal for human consumption.

Regulating based on the process, in the absence of any scientifically defined risks, is not useful and dilutes the scrutiny required for truly novel GE products designed for use as food.

The consumption of GE plant-derived food has demonstrated that the technology does not pose a food safety issue. Rather, it is the product of the transferred gene that needs to be assessed for potential problems, which is also true for GE animals. In cases where the transgene product is a protein already found in food, unless it is a known allergen it should not present a risk. The level of transgene product in a given tissue should not be the issue, but rather the amount consumed in the course of a meal. If food safety is regulated based on scientific grounds, then where are the data or evidence to suggest that the transgenes discussed above may pose a food safety risk? Short of such evidence, we are regulating the process based on the perception that there may be an unknown, and likely, unknowable food safety risk. It is time to review the approach for regulating GE animals in the United States to bring it in line with a traditional, scientifically founded, product-based, risk-benefit analysis.

First, although the fact that an animal is transgenic may be the trigger for regulatory review, the initial review should be based solely on whether or not the transgene product is expressed in the food parts of the animal. If not, then because DNA is considered GRAS, the product should be moved to the status of enforcement discretion or deregulated. Expression should be determined by the presence of detectable mRNA for the transgene product based on regular PCR. Trace amounts of transgene product, especially if the product is normally found in food, should be acceptable rather than a requirement for no or zero tolerance of the product.

Second, if the transgene product is found in food products derived from the transgenic animal, the regulatory review should ask if the product is normally found in nontransgenic food and whether the level in the GE-derived product significantly exceeds the normal level. If the level contributed by the GE-derived product to food is within the normal range of that product in that food or in food products in general, then the application should be moved to discretionary enforcement or be deregulated.

Third, in the case where the transgene product is either found at levels significantly greater than normally found in food or if the transgene product is an orally active compound, then the regulatory process should require further review.

Fourth, mandatory time limits should be established for each phase of the review. Developers need to know the time frame for a regulatory decision, as well as what kind of data will be required. Additionally, the regulatory guidance should also include guidelines on what classes of GE animals could expect enforcement discretion following an initial review.

At present, a segment of the population has a biased perception of what GE does and what potential risks it may cause within the context of food safety. The regulatory process in place should assure these individuals, and the general population, that their food is safe and wholesome. At the same time, the regulatory process should make scientifically defensible decisions in a timely manner. Right now, we have neither. Suggestions, such as those made herein should help lead to a robust regulatory process, based on a clear and defensible understanding of the potential risks and benefits. They would also help to ensure regulatory decisions move forward in a timely manner. Regulating based on the process, in the absence of any scientifically defined risks, is not useful and dilutes the scrutiny required for truly novel GE products designed for use as food.

Biotechnology has an important role to play in the future of agriculture for food security, animal health and welfare, and the improvement of the nutritional benefits of various foods. This can only be realized through appropriate investment and this will only occur when developers have the confidence of an appropriate regulatory process for those animals and food products. With many nations poised to take steps forward and many benefits clearly in sight, the eyes of the world are on the United States to lead the way.

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