**Special podcast feature: The status and future of CRISPR in agriculture, Part 2**

**PNAS:** Welcome back to a special feature episode of *Science Sessions*, the podcast of the *Proceedings of the National Academy of Sciences*. I’m Paul Gabrielsen. In part one of this episode, we talked to scientists using CRISPR gene editing to modify plants and animals and heard about how advancing technologies are opening up new possibilities for genetically engineering agricultural products.

The safety of gene-edited products is forefront on the minds of regulatory agencies, who are tasked with certifying products for commercial use or human consumption. Also forefront on their minds when evaluating genetically engineered organisms is the potential for off-target or unintended effects. Off-target genetic effects can occur when the guide RNA used in CRISPR mistargets a region of the genome. Other potential unintended effects include even on-target modifications that don’t go according to plan. CRISPR scientists, aware of these limitations, are improving the specificity of the method all the time.

Regulators also need to thoroughly investigate gene-edited organisms for the safety of the organism and for the consumer, if it’s a consumer product. Is there a chance the alteration could make the organism more susceptible to viruses? In animals, might it increase the risk of cancer? Could a change in gene or protein expression affect the composition of food in a way that could impact human health?

Laura Epstein is a senior policy advisor at the US Food and Drug Administration’s Center for Veterinary Medicine. The center evaluates the safety and efficacy of animal drugs and ingredients in animal foods, as well as the safety of food products made from treated animals. Epstein explains the role of regulators.

**Epstein:** Their role is defined by whatever law it is that Congress passed telling them what to do. In our case at FDA it’s the Food, Drug, and Cosmetic Act; that the alteration in animals is safe for the animal, that if it’s a food animal, it’s safe for anyone consuming food from the animal, and that it’s effective, meaning it does whatever the developer claims that it’s going to do. And we think that our role, in addition to protecting consumer and animal health, also gives consumers confidence that the products that they’re using are safe.

There’s been a lot of attention to the fact that CRISPR is pretty easy to do and that certain people can do it in their basement. So I think that’s one of the things that concerns us. Most of the developers that we work with, since they’re coming to us, are obviously wanting to be very responsible. They want to do the right thing. They want to make sure that their products are safe. But we don’t know what we don’t know.

**PNAS:** Epstein explains how regulators balance the entrepreneurial advances of agricultural technology with the potential risks to consumers.
**Epstein:** We know that people are really excited about this new technology and everything that it can do and that it's going to bring all these great innovative products to market. And we're excited about that also, but at the same time, we know that there are potential risks that we need to identify, figure out whether those risks actually exist, whether they pose a safety hazard, and it's FDA's role to balance those things. We want these products to come to market, but we want them to be safe when they get there.

**PNAS:** The US Department of Agriculture, which regulates crops, found the need to revise their rules for regulation as the gene-editing landscape changed with the advent of CRISPR. Neil Hoffman is a science advisor with the USDA's Animal Plant Health Inspection Services, or APHIS. He describes the drawbacks of the previous regulatory rule.

**Hoffman:** The trigger for regulation by USDA was whether the organism was genetically engineered and whether a plant pest or a plant pest sequence had been used to genetically engineer the organism. Conversely, genetically engineered organisms were not regulated if they were not themselves plant pests and were engineered without using a plant pest. USDA did not consider the plant pest risk of the organism until late in the regulatory process, namely after the organism had undergone several years of field testing and under confined conditions specified in a permit or notification.

All told, permitted field trials and the petition process added substantial costs and uncertainty for developers, and these costs and uncertainty occurred throughout this entire process. These costs and burdens have limited the ability of technology developers, particularly from small- and mid-sized companies and in academic research institutions, to navigate the regulatory process. Another feature in the legacy regulation was that there was an opportunity for riskier organisms to avoid the regulatory system. Because the regulatory trigger applied only to plants genetically engineered using a plant pest, the trigger created a situation where many lower-risk plants developed using a plant pest were subject to regulation while potentially higher-risk plants created without using a plant pest were not.

**PNAS:** The revised rule adopts a risk-based approach to regulation. The rule focuses on high-risk crops and exempts certain low-risk crops, even some produced using gene editing, from regulation.

**Hoffman:** So the revised biotech regulation establishes exemptions for plants modified by genetic engineering techniques where the modification could otherwise be achieved through conventional breeding techniques, ensuring that such plants are treated similarly to conventionally bred plants from a regulatory perspective. The revised biotech regulation uses a risk-based approach called regulatory status review to determine whether an organism is regulated rather than relying on whether the organism was developed using a plant pest or plant pest sequence. And it provides a mechanism for a rapid initial review to efficiently distinguish plants developed using
genetic engineering that do not pose plausible pathways to increase plant pest risk from those that do and thus require further evaluation.

Plants developed using conventional breeding have a history of safe use related to plant pest risk. Exempt plants could have been developed through conventional breeding. In the agency's experience, unintended effects are uncommon. And in the course of a normal breeding program, plants with unintended effects are discarded during the process of screening for desirable phenotypes and selecting lines for advancement.

**PNAS:** Similarly, he says, the USDA doesn’t consider off-target mutations in evaluating a gene-edited crop, reasoning that plant lines with adverse off-target mutations won’t be selected for development.

**Hoffman:** Off-target mutations are unintended mutations that arise during genome editing, when the targeting component of the editing nuclease is not as specific as intended. With current editing technology, the number of off-target edits is generally below the baseline mutation rate.

**PNAS:** The risk-based approach, Hoffman says, is designed to facilitate innovation.

**Hoffman:** Regulatory certainty at the outset of a project will make it easier to raise investment capital and minimize lost opportunity costs from regulatory delays. Lower regulatory costs will increase the return on investment, thereby increasing the types of crops and types of traits used for engineering. Use of genome editing will allow much more genetic combination per breeding cycle, and that will shorten the time it takes to release a finished crop.

**PNAS:** The FDA also employs a risk-based approach to regulating gene-edited animals. Heather Lombardi, director of the division of animal bioengineering and cellular therapies at the FDA’s Center for Veterinary Medicine, explains how the regulatory process works.

**Lombardi:** Developers, they come and talk to us very early on in development, and they’ll ask us questions about their product. We meet with them very frequently early on to discuss their development work and to guide them through what sort of data we would be expecting to see for that particular product, whether it’s for enforcement discretion or for an approval. Some of that depends on what exactly the alteration is. Is it, for example, a transgenic sequence that's introduced using rDNA technology, or something like the gene edit, where you're maybe using a sequence that is already known to exist in the animal; it generally starts with molecular characterization, and that is the developer would give us some information about the experimental design. So for the gene edit, they would tell us things like what's the nuclease they were using, what sort of template they used, and what the exact
change is. And then they would have to do some sort of characterization to look at that alteration to say, yes, we have the alteration that we intended to make.

And then also a characterization of any potential unintended alterations that could impact safety. We're not as concerned about an unintended alteration being there per se, as much as we are concerned about having control over the process. So the other thing we would ask is, what do you plan to do during commercialization? Do you have controls in place that if you were to generate an unintended alteration that could impact safety, you would be able to detect it and remove it from production if necessary? And then they typically would characterize the phenotype that they're trying to generate, so some sort of indication that they have the alteration in place, but also that it's doing what they intended to do.

There would be a food safety component. So are you introducing any new proteins that could impact either the toxicity of the meat coming from the animal or the allergenicity? Is there anything about that alteration itself that could cause a potential concern? And then there's also the environmental piece. We look at a variety of different products. Some of them are highly contained and some of them are intended to be on farms all across America. And so it really depends on what the exact use of the product is, but we would be evaluating the impact on the environment of that particular alteration in those animals.

PNAS: The ultimate goal, Lombardi says, is to ensure product safety.

Lombardi: We want the public to feel very confident that the products that are out there are safe for them to eat.

PNAS: George Church of Harvard University and the Massachusetts Institute of Technology is a veteran of biotechnology entrepreneurship.

Church: I think it's extremely important that you go through the regulatory process to make sure that we don't have the ultimate barrier[, which] is when something really bad happens and then there's a backlash. Insisting on safety and efficacy in a step-wise manner where you start with a very small number of people is not a bad idea.

In terms of regulation, it may be more attractive that CRISPR and other advanced editing tools are more subtle. Rather than moving a big chunk of DNA from a completely different organism with uncertain baggage that it brought with it, now we're talking about a very subtle change that happens spontaneously like an A to a G or a C to a T or, you know, something simple, and that may be more attractive. It seems to already being considered more seriously for approval in nations that have historically been rightly concerned, as in Europe, for example.

PNAS: Luhan Yang, co-founder and CEO of Qihan Biotech, says that apart from the technical challenges of CRISPR gene editing, public and regulatory acceptance of the technology pose an entirely different challenge.
**Yang:** So, we are at the forefront of the technology breakthrough and trying to exercise that in pig[s] and also agriculture applications. We understand that technological breakthrough is always ahead of the policy, public awareness, and regulation and commercialization. So it’s actually a team work, among different communities, and actually, there shouldn’t be any national boundaries either to work together to really translate cutting-edge science into something really impactful to society.

**PNAS:** Here’s Rodolphe Barrangou again, of North Carolina State University.

**Barrangou:** However fast and far we’ve made progress in the technical side, I think on the regulatory side, we still have a ways to go. We need technological acceptance to be able to deploy commercially. And I think we should all be inspired by the US leadership from our own USDA that provides a great framework for others to use and inspire their regulatory regimes and policies from.

**PNAS:** Here’s Dana Carroll of the University of Utah.

**Carroll:** In agriculture, I think people don’t recognize that the current cultivars of the plants that we eat are all ones that have been developed through selective breeding and often after mutagenesis of a seed stock. And that mutagenesis caused not only the traits that were selected for, but other mutations in the genome, which we have little control over. And the key thing about genome editing is the precision with which it makes changes in the genome that are inherited. And so I think people don’t recognize that when it comes to agricultural applications, they seem to be concerned about making a modification in something they’re familiar with, not recognizing that what they’re familiar with is unnatural in all sorts of ways.

**PNAS:** Researchers and regulators alike envision a world where the agricultural problems of disease, drought, and yield can be precisely and rapidly addressed. Where crops can use less fertilizer and water. Where the quality of livestock can be enhanced safely and humanely. Where CRISPR and agriculture can together help address climate change.

Here’s Heather Lombardi.

**Lombardi:** There are so many new advancements that are just rapidly ongoing to improve the science. Nothing is a hundred percent fail safe or a hundred percent known. And so there are ways to overcome it. And that is through some of the things that we discussed: having proper controls in place, using high fidelity nucleases, having proper detection methods, and so forth.

**PNAS:** Daniel Voytas of the University of Minnesota echoes the sentiment.

**Voytas:** Well, I really see a very bright future for gene editing in agriculture. Breeding processes, they rely on genetic variation that has occurred spontaneously in nature. And
we have to find the genetic variation we want, we can't go out and create it. Gene editing allows us to go out and create it in a very precise and accurate fashion.

**PNAS:** And, finally, here’s Barrangou.

**Barrangou:** I think most of the CRISPR community is very confident that the biggest impact is yet to come. There's a lot of excitement about deploying that technology, repurposing the technology, and harnessing the disruptive power of CRISPR to work on sustainability, to work on farming, and to address the grand challenges that we have with regards to food and feed and with regards to forestry and beyond.

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