ILSI 2021 Annual Symposium Session 6: Alternative Proteins

Transcript of the presentation, Food Safety Considerations of Alternative Proteins, Clare Mills, PhD, University of Manchester, United Kingdom

My declaration of interests, funding that I get from various research councils and industrial collaborations and includes shares in a bio pharmaceutical startup reactor biotech. So, thinking about the title of the talk, I was also considering about where all these different types of alternative proteins could be sourced. And the two talks that proceed this have really introduced the topic very well. I've got a slightly different take on it, coming at it from a safety point of view, but we've got things like plant-derived proteins from existing or new crops. We've got microbes. So, this is the Fusarium venenatum, which is the filamentous algae that is in corn, but we also have other fungi bacteria. We've got micro-algae things like cultured meat and insects. So, what do we need to think about in terms of their safety?

Now, what I'm going to talk to is very much European based, but I know for example, in the USA, these kinds of novel foods, as we would call them in Europe, and I'm going to say we, because UK legislation is the same as European legislation at the moment, but in the U S you have the concept of generally recognized as safe. However, that still requires the same sorts of information. And you often find data that goes into grass applications in the U S is also seen in novel foods, dossiers within the EU. So, in the EU and the UK, we have two different groups whereby these alternative protein sources would be regulated. So, the first of these is traditional foods from what are called third countries, that includes the UK, but the UK would treat the rest of the world like that. This is applicable to foods with a 25-year history of use. And you have to demonstrate that they've been cooked in the customary diet of a significant number of people in at least one third country.

If not, if you don't satisfy that, then you have to go through the main novel foods regulation, where actually it covers food and food ingredients with a new or intentionally modified structure. And food and food ingredients consisting of, or isolated from microorganisms, or fungi, or micro algae, plants, or animal foods, except those obtained by traditional propagation or breeding.

And what I will say, because it was mentioned in the first talk about GM. GM has a completely separate regulatory framework. And I won't be talking about that today. Natural fact, all of these, all of the foods, you have classical hazards from microbiological to chemical contaminants, and they all need to be considered. So, when we look at all on you, alternative protein sources, I'm afraid actually, they are all going to count as novel foods. And there will need to be some kind of application and process, whether you're going in the USA where you need to grass status, or you're going through, biting the bullet, and going through novel foods.

So, what are the safety issues? Well, that depends on their origin and some of the new technology that we're getting to come through, very exciting and new processing technology, like the 3D printing also

gets regulated under the same framework. In actual fact, it depends on how you're making it. It depends on that origin. So, let's think about plant proteins from traditional plant sources. Now these are two food ingredients that have gone through this novel food process. The first is a protein isolate from all seed. It isn't the first one, it's the first one on my slide. And in actual fact that was approved. It was actually developed as an animal feed first, and then it gradually got upgraded. And it's now had a novel foods approval in Europe for human food use. And the sorts of things that had to be considered in that safety issue was things like orotic acid and glucosinolates and the way in which they address that it wasn't a barrier to the approval, it had to be addressed. And it had to be addressed using cultivars naturally low in those compounds and developing a production process that further reduce those compounds.

So, this is where you can address the food safety hazards often by managing them and by managing them right across your processes. So, you have to design your safety ed, right? From the very beginning. One of the first applications that got approved actually was a protein isolate from alfalfa. And here there were hazards such as phytoestrogens, but they were present in foods, similar levels to soybean and our canavanine, which is associated with systemic lupus erythematosus, but the levels are lower than other foods. And there was no evidence of DNA antibody generation. So, these both got approved. So yes, you do need to collect the information together, but I don't know many people in the food industry who actually want to hurt anybody with their products, but it's about having that safety engineered at the beginning. Now I've actually been working in food research and long enough to remember the development of corn.

And I started out working on trichothecene mycotoxins and actually it's very interesting because corn, which actually they can't make enough of, and it comes from the fact it was predicted back in the 1960s, that there would be a shortage of protein rich foods. And in response to that, something called mycoprotein was developed, which is based on fusarium venenatum. Now in actual fact, it's grown in very large scale. There was permission granted for its use in 1985, after a 10-year evaluation program, what the issues were with corn for the safety assessment were two. So first of all was mycotoxins; So, an actual fact, venenatum, like all fusarium species carries the genes for making mycotoxins such as deoxynivalinol and T2 toxin. Consequently, what they had to do is test every single batch, which is where I was involved because I was in a group that actually did that.

And actually, they've never had a problem with mycotoxin contamination in corn. It's been managed out of the entire system. The other risk was allergenicity, which was beginning to come onto the radar at that time. But in actual fact, what we can see based on what's been published in the literature is there is only one case report of an individual with IgE mediated, allergic reactions to the fusarium itself. Now obviously, there hasn't been the consumption of corn that has been of other types of foods like peanut, et cetera, that we commonly think of as food allergens, but it's actually safe, generally safe and, and have very few problems. So, in some really good precedents of how to manage it, when we come to think about microalgae, and I have the pleasure of working with Alison Smith from the University of Cambridge, who's an expert in algae and she's been teaching me a lot.

And I have to say, I did not realize how diverse they were. And that it, both embraces prokaryotic blue-green algae and diverse eukaryotic algae comprising 20 different phyla. They are very complicated and very diverse. So, we can't really put them all in one pot. And actually, that means the safety assessments that are done for one particular type of microalgae may not work for another, but there are some commonalities and things that we're going to have to watch out for. So again, toxins... And we've all heard about issues of algal blooms on water causing problems with toxins being produced. These

molecules are incredibly complex to analyze. These are just three of them. And it includes things like microcystin, which are hepatotoxins. You've got alkaloids such as saxitoxin, which is a neurotoxin and polyketides, such as ciguatoxin. And some of them are involved in shellfish poisoning, which we classically have heard of where they get concentrated up inside the shellfish.

All the algal species currently used for food do not make toxins, but it might be if you're going to look at algae, that it will be important to identify whether they have the capacity to produce toxins. And one of the things with microalgae is because they're small organisms, they have small genomes and the advances in genome sequencing make it much easier to do this sort of looking for toxin production and predicting it from the genome sequence. And you can manage it exactly the same as you potentially can for corn to ensure that your algae do not necessarily make nasty toxins. There are also other issues about accumulation of chemical contaminants. So, pCBs, dioxins, heavy metals, pesticides, all the classical chemical safety issues that we have for all the foods that we eat, they need to be considered. And that relates to understanding how your methods of cultivation and the control of water quality use for culture and the algal species used all work together to prevent or manage that accumulation.

And I think one of the things that I can see and going through this talk, actually, was very useful for me is you can see that actually to make food for humans to eat, you need high quality feed stocks that are going into your production. If they're not high quality, you will get problems in the final product. And it may well be that some of the things that have been published about using waste streams and using marginal land may actually be more difficult if you want to use these organisms for food.

Now, it's interesting about insects and the fact that consumers really don't like them. I find it fascinating because the insects of the sea that we eat all the time, which are called shrimp, which if you actually look at them, they really are quite hideous invertebrates of the seas and rivers.

And they are very the kind of insects that you might eat and have been eating in many parts of the world, are actually very closely related to them. We've also always eaten them because there are small levels of insects that are allowed in agricultural products because they're very difficult to get out. So, the sorts of safety considerations you need to think about with insects is, well, are you consuming them whole, do they have parts that actually could represent a choking hazard, all sorts of things you want to remove stingers. So, there's issues of removing body parts. They also can accumulate chemical contaminants, which is PCBs and dioxins. They can produce toxins and anti-nutritional factors. And there're also concerned about the potential for processing contaminants. Now, interestingly, the European food safety authority published recently their safety assessment of dried yellow mealworm larvae as a novel food and what they found in practice.

And so, this is for mealworms, either given as snacks, I must admit, I'm not sure I'd want to eat them as snacks, but that's a level of 10% as a food ingredient as that and biscuits, pasta and legume dishes and sports nutrition supplements. So, what did they find? Well, bioaccumulation of dioxins and PCBs. So, that had to be managed by ensuring the feed had no detectable dioxin or PCB. So, you have to monitor the feed going into the insects. Arsenic was actually accumulated, and they did have reasonable levels of both arsenic and a mycotoxin called ochratoxin. But the way to assess this was by exposure assessments based on this 10% in foods, was that really the contribution to the diet with the intended use was negligible and other mycotoxins and pesticide levels were not an issue. Toxins and anti-nutrients were present at similar levels to other food.

So really good news. And they did conclude that it was generally safe for us to eat yellow mealworms. There was a concern about chitin and the chitin content. So, it's a new thing. If you start eating a lot of it, we have issues that we don't have methods for determination of chitin that are properly validated. And there are issues over trying to convert to protein content. These are nitty gritty things that affect the safety assessment but will also follow on into how you use this material as a food ingredient. And there are potentially immune modulating effects. So, one of the things that's not clear is whether the observation on the chemical contaminants and natural toxins for mealworm are generalizable to other insect species in the same way as algae are very diverse so were insects. And I think we just have to see what happens with some of the other dossiers that are being assessed at the moment.

So, we've already heard about cultured meat, and this is really quite fascinating from a regulatory point of view, because we haven't ever made cell cultures to eat before. And these issues of cell isolation, immortalization, materials use for cell culture hygiene and the nutritional quality. There are some legal issues that we don't know what we're going to call it. Is it meat or isn't it neat, but safety issues that relate to how you grow these and an issue such as viral contamination of cell cultures, we have to develop a safety framework for actually looking at cultured meat. It does fall within the novel foods' regulations, but it will present some interesting questions, which actually we probably should deal with in research projects rather than expecting applicants to sort them out. Now, the second part of my talk is really relating to one of the big things that really faced anybody going to do a safety assessment on an alternative protein and that's to do with its allergenicity.

So the idea really came about, the issues for novel foods, came from GM, where in order to improve the nutritional quality for soybean back in the 1990s and molecular biologist put the 2S albumin storage protein from Brazil into soybeans, there was this amazing paper published in the new England journal of medicine, where the team at the university of Nebraska showed that if you had a Brazil nut allergy and you had an IgE, this is an immuno blot showing that the IgE from the patient binds to the 2S albumin in Brazil nuts, so that's allergenic and actually lo and behold, your soybean, which is expressing that protein was also allergenic. So, it made it the ultimate hidden allergen, and we've moved on an awful lot since then, we have a very detailed framework for assessing genetically modified organisms. And actually, that has been very much used in built on within Europe to produce the weight of evidence approach, that is also being applied to the allergenicity risk assessment of novel proteins.

And this has a whole series of things that we work through is the new protein... To kind of conclude is the new protein likely to be allergenic going through various different tests. So since almost all food allergens are proteins, the allergenic potential for a novel food, such as a highly refined oil or hydrolysate is very low, no protein, no problem. However, if you have a novel protein, you have to think about where it's come from, the source, the production process, and experimental and human data. Through the cost action in price, this put together this kind of right, nice, clear framework. If you like the first step of your risk assessment is really to take your note new protein and say, well, how's it related to anything we know that's an allergen.

What are the proteins in it? How are you going to use it? Could it cause a reaction in somebody who's already got a food allergy? So, does it cause a reaction in somebody with a peanut allergy? And in actual fact, we can do step one and step two A with a reasonable level of assurance. What is very difficult to do is to understand whether a new protein has the potential to generate a new De novo sensitization. This schema does not involve using animal models, but undoubtedly in the risk assessment, one would use, potentially, animal models if you needed to. And that is a big problem for a lot of the food industry

where animal models are a no-no. And we do need to get more, better cell-based models that perhaps could be an alternative to animal models in this process.

So, step one; the first thing you do, so it could be a transgene sequence, or it could be the sequences of your proteins in your normal source. And you look for a database and you look to see whether there's an allergen that looks like your protein, your new protein. And that gives you a very good idea of potential allergen risk.

So, this is what they did for the shrimp, the tropomyosin. So, tropomyosin is a major allergen in shrimps. And if you look in insect, there is a similar tropomyosin and it has been known for some time that the IgE in people with shrimp allergy binds to tropomyosin, also binds to the tropomyosin from insects and it causes cross-reactive allergy syndromes. And this is nice work on the IgE reactivity from three different mealworm preparations, and this is the shrimp extract, and you can see the IgE from patient one patient, five binds very strongly to the shrimp, but it also what binds to the equivalent protein in the mealworm. And that's also shown here in another type of essay, demonstrating allergic potential. This went one step further. And in actual fact, the team and you tracked fed mealworm protein to people with shrimp allergy and showed that it causes an allergic reaction.

So, there is actually no doubt that mealworm protein is allergenic. However, I suspect, and we don't know yet, because DG Saunte has yet to deliberate, but the evidence is it's allergenic. So, that becomes a risk management problem. And of course, one of the ways of managing allergenic risks is just putting it on the label. So, what they did with the world's seed rate protein was that it was very similar to mustard protein, and DG Saunte just put it on the label. So, I think it's highly likely that we will end up with an approval on, bets in a sealed envelope, I have no inside knowledge on this, but I think it's highly likely that it will get approved for use and it will end up having to be labeled. And we will need tools for managing mealworm proteins as allergens. So, this is just a quick example about predicting sensitization and the uncertainty of it.

So, using chia seeds, so we've done many, many applications for novel foods where chia seeds had been approved and obviously their seeds, they're full of protein. And you've got the full complement of proteins there. Chia seeds are not from a food that's on annex two, which is the labeling list of priority allergenic foods in Europe. And taxonomically, it's not to any known allergen, or allergen family. So, it got its approval. However, kind of like five, six years down the line, actually, there is some evidence that chia seeds can act as allergens.

This is a patient who ate chia every day in yogurt and develop eczema. They've got multiple sensitizations to other foods, including tree nuts and legumes. It looks like this sensitized to the storage proteins in the chia seeds. So allergenicity risk assessment is an uncertain process. Now there are lots of management decisions that have to be made. I'm involved in providing scientific advice through the advisory committee on novel foods and processes to the UK food standards agency. Our role is to provide the science, they do the risk management. But I think there are a number of things we really need to square this off and avoid it becoming a barrier and feeding into uncertainty over consumer acceptance of foods. Because if they get worried that it's going to cause an allergy people might be reluctant to adopt new technology, which we urgently need. So, we need better models, cell models, that can predict sensitization potential. We need far better ways of monitoring allergens after they'd been introduced onto the market.

This is just to show you, this is a summary of the fatal reactions that was published earlier on this year in the UK, going back to 1992 through to 2013 and 2015, what's interesting is peanut, it kind of goes up and down, wiggles around. What's been very interesting was that between 1992 and 1996, there was no record of for fatality to milk. But actually in 2013, 2015 milk was the most significant cause of allergic reactions. So, whether there are things changing in our allergic population, and this is where food allergens challenge safety paradigms, dioxins are carcinogens, whatever time of year you might've had them. The problem with allergies is they change, and they will change over time as a consequence of our change in dietary habits, change in exposure, climate change, all sorts of things. The last thing I think we need to consider is labeling for consumers to enable that monitoring to be joined up and we need to have patient and clinical community involved in this. So, that's it a bit of a whistle-stop tour. Thank you very much for listening.