Do whole-food animal feeding studies have any value in the safety assessment of GM crops?

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1. Introduction

1.1. Objective of feeding studies

The use of whole-food (grain meal contained in feed) animal-feeding studies to inform the safety assessment for genetically modified (GM) crops is quite controversial. Part of this controversy stems from differences in perspectives concerning the objectives of such studies. These perspectives may include: (1) that whole-food animal-feeding studies are of no or little value in the safety assessment of GM crops no matter what their purported purpose; (2) that these studies are useful for detecting unexpected compositional changes that might cause adverse effects in animals; (3) that such studies are useful in evaluating expected compositional changes that may cause adverse effects; (4) and/or that these studies are useful in a subset of cases where large multiples of exposure for transgene products (e.g., proteins) are possible in animal feed compared with human food (EFSA, 2008; Knudsen and Poulsen, 2007; Kok et al., 2008).

1.2. Unexpected effects

The issue of unexpected compositional changes having adverse health effects has been discussed extensively in the literature, and from a scientific, evidence-based perspective, it appears that transgenesis is less likely to cause such effects compared with traditional breeding (Herman et al., 2009; Herman and Price, 2013; Ricroch, 2013). This calls into question, not only the general use of compositional equivalence studies to uniquely inform the safety assessment of GM crops, but by extension, the use of whole-food animal-feeding studies for the same purpose.

1.3. Expected effects

The issue of whether whole-food animal feeding studies might be useful in certain circumstances to evaluate potential adverse effects of intended or expected compositional changes, including expression of novel food proteins, has been less discussed. Here we briefly outline the potential merits of such studies in evaluating the safety of GM crops and discuss how such studies might address reasonable hypotheses concerning adverse effects.
2. Transgene products

2.1. Background

GM crops are engineered to contain genes inserted using recombinant DNA technology. Most often these genes produce novel proteins in terms of their previous history as constituents in food crops. Common examples include expression of enzymes that degrade certain herbicides or are homologues of endogenous plant enzymes that are insensitive to certain herbicides (rendering plants tolerant to these herbicides), and insecticidal toxins (providing insect pest protection). Transgenes may also produce transcription factors (also proteins) that modulate other plant genes, or RNAs that can also modulate plant genes or insect-pest genes, with the latter enabling traits that can be designed to intoxicate pest insects (Parrott et al., 2010). If novel gene products are expressed in food crops, then many believe that the safety of these products should be evaluated for human safety.

2.2. Exposure

Human health risk is a function of hazard (intrinsic toxicity) and exposure (e.g. how much of the transgene product ends up in the food we eat) (Kok et al., 2008). For commercial GM crops, the transgene products have thus far been found to be inactivated during food processing and/or cooking, so exposure to an active transgene product is negligible (Hammond and Jez, 2011). Proteins are most commonly able to exert their function based on their specific folding pattern or three-dimensional conformation. Elevated temperatures typically cause an irreversible denaturation (unfolding) of the protein accompanied by loss of function (Privalle et al., 2011). In such cases, exposure to an active protein is absent in food rendering the denatured protein a nutrient source that is digested into the amino acid subunits required by animals to survive (Delaney et al., 2008).

2.3. Hazard

If exposure to an active transgene product is reasonably expected in food, the hazard should be evaluated (likewise if no hazard is identified, exposure is irrelevant). The potential for adverse effects can be informed by a number of factors. These include: (1) history of safe consumption; (2) similarity to other dietary constituents with a history of safe consumption; (3) mode or mechanism of action; and (4) relationship to proteins with known adverse effects (e.g. toxins or allergens) (Delaney et al., 2008). If these factors are insufficient to establish low risk, then empirical toxicity studies may be warranted to investigate the human-health hazard. In cases where the active transgene product can be isolated or significantly enriched, controlled-dose animal feeding studies with the purified or enriched transgene product provide the most scientifically robust approach (Kok et al., 2008). In such cases, the transgene product in the purified preparation must be shown to be biochemically and functionally equivalent to that produced by the GM crop (Raybould et al., 2012). Use of high-dose animal toxicity studies with purified compounds has an established history of value in the safety evaluation (Delaney et al., 2008). The ability to dose at many multiples of the expected exposure levels present in real-life scenarios provides a robust evaluation of hazard.

2.4. Risk

Where: (1) exposure to the functional gene product is reasonably expected; (2) there are reasonable uncertainties as to the hazard of the gene product based on other knowledge; and (3) the transgene product cannot be purified or enriched in an active form equivalent to what is produced in the GM crop (Madduri et al., 2012), then the use of whole-food animal-feeding studies might be considered to inform the human-health risk assessment, if certain criteria can be met; (1) reasonably high multiples of exposure can be obtained for the active transgene product compared with expected human exposure, and (2) matched isogenic non-transgenic controls can be produced (under equivalent environmental conditions) (Knudsen and Poulsen, 2007).

2.5. Multiples of exposure

As an example, for soybean in the European Union, expected dietary inclusion in high-exposure populations is <3% soy in the diet (WHO GEMS Cluster Diet F; http://www.who.int/foodsafety/chem/gems/en/index1.html). Since inclusion of >30% soy in animal feed is possible without adverse effects in certain species (e.g. rats and broiler chickens), a 10-fold multiple of exposure is possible (Herman et al., 2011a). If a transgene product can survive in an active form in processed soy (which must be heated to inactivate endogenous protein antinutrients), then whole-food animal feeding studies may provide some value in the human-health risk assessment due to increased inclusion of the commodity in animal feed compared with human diets. A second example of where whole-food animal-feeding studies might be useful is in situations where a transgene product is largely inactivated by processing (e.g. in processed or cooked corn-based food products), but in which some active protein might remain. Since raw corn meal can be fed to some animal species at high levels in the diet, and human consumption of raw corn grain is negligible, very large multiples (>100-fold) of the active transgene product may be fed in whole-food animal-feeding studies compared with human exposure, again potentially providing value in the human-health risk assessment (EFSA, 2008; Herman et al., 2011b).

2.6. Justification

Whole-food animal-feeding studies for evaluating the safety of transgene products (e.g. proteins) have been criticized for being too insensitive to detect adverse effects. However, some proteins are quite potent as oral toxicants (e.g. as little as 100 ng of orally ingested botulinum toxin can be fatal in humans) (Schantz and Johnson, 1992), and other food proteins are quite toxic at natural levels in food crops (e.g. raw kidney beans can kill rats due to the presence of kidney bean lectin, while humans get sick if they consume under-cooked kidney beans) (de Oliveira et al., 1988). From a practical standpoint, if reasonable multiples of exposure are achieved, then the concentration of the transgene product in the study is appropriate for evaluating safety since expected exposure is greatly exceeded (Knudsen and Poulsen, 2007).

It is noteworthy that, for experimental purposes, the kidney bean lectin was actually expressed in transgenic rice at 0.067% (30 mg lectin in 45 g of rice, which is 3 to 15-fold lower than the concentration reported in various kidney bean cultivars), and when this rice was fed to rats at an incorporation rate of 60% in the diet for 90 days, statistically significant increases in the weights of the small intestine, stomach, and pancreas were observed compared with non-transgenic rice (expected adverse effects of kidney bean lectin) (Knudsen and Poulsen, 2007; Muzquiz et al., 1999; Poulsen et al., 2007). This provides an example of where a whole-food animal-feeding study can detect adverse effects even when the concentration of the transgene-product is considerably below the level that occurs naturally in a food crop.

Another common assertion is that no feeding study has raised a safety issue for a GM crop intended for human consumption, so
they are not useful. However, by that standard (since no safety study of any kind has raised a substantiated health issue for a GM crop intended for consumption), it would follow that no safety studies are useful (which some have proposed, but many reject).

2.7. Context

Novel gene products can and have been introduced into food crops through non-GM breeding methods. Cross-breeding with rare alleles and wild relatives, as well as intentional mutagenesis, is qualitatively similar to transgenesis (with the exception that changes due to traditional breeding are often multiple, unknown, and uncharacterized) and our experience with assessing the safety of traditionally bred crops should be considered in our approach to assessing the safety of GM crops (Herman and Price, 2013).

3. Expected changes in crop composition

3.1. Background

In addition to expressing gene products, GM crops may be engineered to have a modified composition or otherwise be expected to have an altered crop composition (Kok et al., 2008). This may be through modulation of endogenous plant biochemical pathways via transcription factors, RNAi, or expression of enzymes that catalyze reactions within plants (Parrott et al., 2010). If the composition of the crop is expected to be altered, hypothesis-driven compositional analysis of the relevant crop tissues (e.g. grain) is an obvious approach to understanding the nature and magnitude of the changes (Herman and Price, 2013). After characterizing these compositional changes, their biological relevance should be evaluated. It is noteworthy that whole-food animal-feeding studies may be needed to evaluate the claimed beneficial effects of GM crops designed for enhanced nutrition, but here we focus on safety assessment. To assess safety, any changes to crop composition are first evaluated compared with the breadth of composition for varieties of the same crop to determine if they represent a novel change or simply a different method of achieving a similar composition already present in that crop (and having a history of safe use). A next step might be to compare the novel composition with other crops or foods used for similar purposes (e.g. does the oil from a GM crop resemble the oil from an alternative crop – canola oil vs. olive oil). Finally, the biological relevance of any compositional change should be evaluated within the context of human diets (Herman et al., 2010).

3.2. Reasonable uncertainty

If the above evaluation leaves reasonable doubt as to the safety of the GM crop with altered composition, whole-food animal-feeding studies may be appropriate to evaluate potential adverse health effects. For example, broilers are extremely sensitive to nutritional imbalances due to their rapid growth (approximately 60-fold weight gain in 6 weeks) (Herman et al., 2011a,b). However, if an individual composition component is in question regarding its safety, and if this component can be isolated in a form equivalent to what is present in the crop, then high-dose animal feeding studies with the component in question will likely be more informative compared with whole-food testing.

3.3. Justification

Whole-food animal-feeding studies for evaluating safety have been criticized for being too insensitive to detect adverse effects of altered composition, but it seems reasonable to conclude that, if whole-food animal-feeding studies can be designed to detect the benefits of nutritionally enhanced GM crops, then they could also be designed to detect potential adverse effects of altered crop composition.

3.4. Context

It should be noted that compositional changes have been effected in crop varieties through conventional breeding, and the safety of such crops should be considered when evaluating GM crops. Specifically, the novelty and potential risk of a trait should govern the appropriate steps in assessing risk, rather than the method of achieving the final product (Kok et al., 2008).

4. Design of feeding studies

By far the most common whole-food animal-feeding studies used for evaluating the safety of GM crops are the 90-day rat study and the 42-day broiler chicken study. The 90-day rat study has been adapted from chemical toxicology approaches without significant modification (EFSA, 2008). This has caused much resistance to the use of whole-food animal-feeding studies because the breadth of endpoints measured in the standard chemistry-based 90-day rat study and its lack of modification to address the limited number of endpoints known to reflect adverse effects with foods or proteins. As such, many endpoints are measured without any known example of a food or protein that can adversely affect these endpoints. The typical endpoints measured in a 42-day broiler study are more appropriate for evaluating food safety in that performance parameters (general health, weight gain, feed consumption, feed conversion, carcass measurements, etc.) are measured rather than toxicological endpoints (which is more consistent with evaluating foods and diets) (Herman et al., 2011a,b). However, a formal survey of relevant adverse endpoints that might be caused by foods or proteins is needed to better determine those endpoints that might be useful to measure in animal-feeding studies aimed at assessing the safety of whole foods. In addition, clear and reasonable hypotheses should be articulated so that meaningful studies can be designed rather than conducting exploratory studies devoid of specific hypotheses. Finally, animal welfare should be considered in conducting any animal study. It is not consistent with animal welfare ethics to conduct studies that are unlikely to yield meaningful results relevant to safety.

5. Summary

It is important to clearly articulate the hypotheses that are being tested in any animal study so that animals are not needlessly sacrificed. A general search for unexpected adverse compositional changes due to transgenesis is not warranted based on a survey of the considerable empirical data that are available, or our understanding of the mechanisms that cause such changes (Herman and Price, 2013). For expected compositional changes, exposure to the changed constituents should be estimated and documented before whole-food animal-feeding studies are considered. In addition, other factors and knowledge should be examined and reasonable doubt should be present regarding the safety of the compositional change or component (Delaney et al., 2008). Efforts should also first be made to isolate the active components in question for inclusion in controlled-dose animal diets before whole foods are considered for use in such studies. Finally, when whole-food animal-feeding studies are deemed beneficial for evaluating the safety of a specific GM-crop, reasonable hypotheses should drive their design and the types of data collected.
Conflict of interest statement

The authors are employed by Dow AgroSciences LLC which develops and markets transgenic seed.

References

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