CropLife INTERNATIONAL

POSITION PAPER

May 9, 2024

Stacked Trait Product Food/Feed Risk Assessments: A Higher-Order Stack Is Sufficient to Assess Risk of All Lower-Order Stack Combinations

I. Overview and Background

With a growing world population, agricultural products containing genetically modified (GM) traits have provided a sustainable and safe way for farmers to meet the ever-increasing global food and feed demand. Over the last two decades, GM traits have been a critical tool for farmers to improve sustainability, implement best management practices, and to make choices that will directly increase both the output (food/feed) and efficiency of their operations (Brookes 2022a, Brookes 2022b). Seed containing multiple GM traits combined through conventional breeding (further referenced as stacked trait products) is an important means of enabling farmers to address several challenges encountered throughout a growing season.

With advances in GM trait availability, farmers' preferences have been toward access to multiple trait classes in a single seed product, including those that enable protection through different modes of action against damage from different insect pests. In addition, stacks of GM traits in a single seed product enable farmers' ability to manage weeds more effectively.

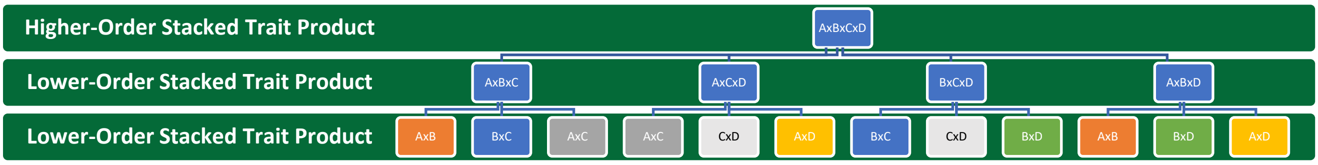
II. Scientific Justification

International safety assessments and independent publications conclude that stacking GM traits through conventional breeding poses no greater risk to food, feed compared to stacking non-GM traits using conventional breeding (summarized in Goodwin et al., 2021). Therefore, many regulatory agencies globally do not conduct an additional safety assessment on a stacked trait product produced by conventional breeding. To date, hundreds of stacked trait products have been approved for cultivation and food and feed use throughout the world (AgbioInvestor, 2022).

In many countries, stacked trait products receive regulatory approval based on the thorough scientific reviews of the parental single traits and do not require further safety assessments (see Table 1). This is a scientifically justified and sufficient approach to ensure the safety of traits stacked through conventional breeding (Goodwin et al., 2021, Kok et al., 2014, Pilacinski et al., 2011, Steiner et al., 2013). In jurisdictions that require a regulatory assessment of stacked trait products, these assessments provide only a redundant confirmation of the original safety assessments of the single traits, and dramatically increases the volume of risk assessments done by those agencies.

Stacked trait products can be described as higher-order or lower-order, depending on the number of included events. A higher-order stacked trait product contains three or more GM events and is derived from lower-order stacks that contain a subset of the events found in the higher-order stack. For example, as illustrated in Figure 1, a higher-order stack containing Event A, Event B, Event C and Event D (A x B x C x D) contains multiple lower-order stack combinations (e.g. A x B x C, A x C x D, A x D, etc.).

Figure 1. Higher-Order Stacks are Comprised of Lower-Order Stacks



Completing a risk assessment on the higher-order stack is sufficient for any lower-order stack as demonstrated by over two decades of history of safe use of stacked trait products.

Independent of the number of events stacked through conventional breeding, the safety assessments of the single parental events are sufficient to inform on the safety of the stacked trait product. In jurisdictions where a safety assessment of the stack is requested, the safety assessment of the higher-order stacked trait product is adequate to inform the assessment for all subcombinations of these traits in the potential lower-order stacks.

Hundreds of stacked trait products – including higher-order stacks and associated lower-order stacks – have been assessed for safety and approved globally. It is because of this demonstration of safety of the single and stacked trait products over the last two decades and thorough consideration of scientific and historical data generated on stacked trait products that several countries have adopted the approach of “high covers low”. While administrative processes may vary country to country, many regulatory authorities consider higher-order stacked trait product assessments sufficient to also cover assessments for the lower-order stacked trait products (Table 1).

Collected scientific data supports the position of the higher-order stacked trait product assessment being sufficient to also ensure the safety of lower-order stacked products (Bell et al., 2018, Wu et al., 2018). In those countries where additional assessments are required for stacks, the higher-order stacked trait product represents the sum effect of all the stacked products. Therefore, assessments for compositional or protein expression changes in the higher-order stacked trait product are sufficient for lower-order stack assessments. Furthermore, as discussed in the below paragraphs, stacked trait products do not show significant changes in protein expression or composition relative to the single events. These effects/changes have not been observed as it would not be expected based on the mechanism of action (MoA) of the traits.

More than 15 years of field and laboratory data on higher-order stacked trait products and their sub-stacks demonstrates that no substantial changes occurred in the composition of harvested material when stacking the single traits through conventional breeding (Kramer et al., 2016). Grain from the GM single event and from lower-order sub-stack products were proven to be compositionally equivalent to the conventional controls demonstrating that what was observed in the highest-order stacked trait product applies to the lower-order sub-stacks as well (Bell et al., 2018, Ridley et al., 2011, Venkatesh et al., 2014, Xu et al., 2014). Furthermore, in the case of hybrid crops such as corn, the grain that is tested for composition is the F2 grain from F1 plants and therefore contains the lower-order stacks subcombinations as a result of the segregation of traits present in the F1 plants.

Together these data demonstrate that the stacking of the traits has no significant impact on crop composition, as any differences observed are only due to natural variation seen in the crop and not the presence of the traits (Harrigan et al., 2010; Herman and Price, 2013). This applies unless the trait is intended to modify the composition, e.g. modified oil profiles in soybeans.

Another consideration of agencies that regulate stacked trait products is whether the level of protein expression in the product is changed due to trait stacking. Any variation of protein expression will be due to environmental factors and is not an indication of trait interactions (Chinnadurai et al., 2018; Wu et al., 2019). When the natural variability in protein expression is accounted for, the expression values in single events predicts those in the stacked trait products that contain those single events (Gampala et al., 2017). The wide range of expression variability is also observed in endogenous proteins (Geng et al., 2017; Hill et al., 2017). Therefore, protein expression levels are not expected to change from those observed for the single trait independent of the number of additional traits present in the higher-order stacked trait product and, if required, the values in the higher-order stack would cover those in the lower-order ones as well.

Finally, authorities should take into account the proposed uses of the lower-order stacked trait products. For example, one use of lower-order stacked trait products is as breeding intermediates which may be required to produce the final, commercial, higher-order stacked trait product; in these cases, a safety assessment of the breeding intermediates should not be required given their limited use compared to the commercial product.

Table 1. Examples of Agencies with Streamlined Stacked Trait Product Regulatory Policy for Food/Feed

Country	Agency (Use)	Approach to Stacked Trait Products[1]	High-Covers-Low[2]
Australia	FSANZ (Food/Feed)	Voluntary notification	N/A[3]
Argentina	SENASA (Food/Feed)	Mandatory submission – Interaction based on Mechanism of Action (MoA)[4]	Yes
Brazil	CTNBio (Food/Feed)	No submission required[5]	N/A
Canada	Health Canada (Food)	No submission required	N/A
	CFIA (Feed)	Mandatory notification	Yes
Colombia	Instituto Colombiano Agropecuario (ICA) (Feed)	Mandatory submission	Yes
	Instituto Nacional de Vigilancia de Medicamentos y Alimentos (INVIMA) (Food)	Mandatory submission	Yes
Japan	MHLW (Food) MAFF (Feed)	Mandatory notification for Cat1xCat1 stacks Mandatory submission – Interaction based on MoA for Cat2xCat1 stacks[6]	Yes

		For all other stacks mandatory submission	
Nigeria	National Biosafety Management Authority (NBMA) (Food / Feed)	Mandatory notification	Yes
Paraguay	The Biosecurity Commission (CONBIO) (Food/Feed)	Mandatory submission – Interaction based on MoA	Yes
Philippines	BPI (Food/Feed)	Mandatory notification	Yes
Singapore	SFA (Food/Feed/Processing)	Mandatory submission – Interaction based on MoA	Yes
United States	FDA (Food/Feed)	No submission required	N/A
Thailand	THFDA (Food/Feed)	Mandatory submission	Yes

1. Refers to the approach of the indicated regulatory agency to stacked trait products when all component single events have been approved.
2. "High Covers Low" – whether the higher-order stacked trait product assessment sufficiently covers the assessments for all lower-order stacked trait products.
3. "N/A" indicates not applicable given approach to regulation of stacked trait products.
4. "Interaction based on MoA" - the regulatory agency assesses if there is a likelihood of GM traits interacting based on the MoA of each trait, without requiring data from the stacked trait product. If there is not a likelihood of interaction based on MoA, then no data is required from the stacked trait product.
5. "No Submission required" - there is no additional information submitted to the regulatory agency for the stacked trait product if all the single events are approved.
6. Category 1 (Cat1) traits do not have a metabolic impact. Category 2 (Cat2) traits include those that alter the metabolic pathways of the host plants. For full description of Japanese policy please reference Iizuka, 2020.

III. Conclusion

More than 20 years' of global experience aimed at the risk assessment of stacked trait products, combined with extensive scientific literature related to the topic, establishes the appropriateness of streamlining stacked trait product requirements. This approach is grounded in the notion that the combinatorial effects of stacking events are predictable, and that the safety profile of a higher-order stack can be informative for any lower-order stacked trait product. It recognizes that the individual risks associated with each trait have already been assessed and that no new hazards are expected in the stacked-trait product. This includes the opportunity to apply the conclusions of the risk assessment of higher-order stacked trait products to any possible lower-order stacked trait products. While doing so it is important to consider the practical considerations of the higher-order stack evaluation and any processes or regulations put into practice as it relates to the number of lower-order stack trait products that may be shared across higher-order stacked trait products.

By adopting “high covers low” policies, regulators at agencies that assess safety of stacks are able to dramatically reduce the number of assessments that need to be conducted without compromising the scientific rigor of their evaluations. In the example in Figure 1, safety assessments of the four single event products and the highest-order stack trait product are sufficient to conclude on the safety of all 16 lower-order stacks. As the number of traits that can be stacked is expected to increase, the number of traits in a single stacked trait product will also increase, and therefore the number of lower-order stacked trait products will grow exponentially.

The principle of high covers low reflects the scientific consensus on the predictability of stacked trait product outcomes and is administered by many regulatory agencies globally. With careful consideration, implementation of using the regulatory safety review of the higher-order stacked trait product to cover the safety assessment of lower-order stacked trait products (“high covers low”), has been successful and continues to ensure the food and feed safety of GM products.

Glossary

Event - The insertion of DNA into the plant genome as a result of a single transformation process.

Higher-Order Stacks – A stacked trait product containing 3 or more events.

Lower-Order Stacks – A subset of two or more GM events within a higher-order stack; may also be referred to as a subcombination.

Mechanism of Action (MoA) – The biochemical process(es) through which genetic material determines a trait.

Stacked Trait Products – GM product containing multiple genetically modified events which have been brought together through conventional breeding.

Trait – An observable characteristic of an organism. A GM trait is a trait produced through the genetic modification of the organism.

References

AgBioInvestor "AgbioInvestor GM Monitor" https://gm.agbioinvestor.com/approvals-database. Accessed 1 September 2022.

Bell, E., Nakai, S., & Burzio, L. A. (2018). Stacked genetically engineered trait products produced by conventional breeding reflect the compositional profiles of their component single trait products. *J Agric Food Chem*, *66*(29), 7794-7804.

Brookes, G. (2022a). Farm income and production impacts from the use of genetically modified (GM) crop technology 1996-2020. *GM Crops Food*, *13*(1), 171-195. https://doi.org/10.1080/21645698.2022.2105626

Brookes, G. (2022b). Genetically Modified (GM) Crop Use 1996–2020: Impacts on Carbon Emissions. *GM Crops Food*, *13*(1), 242-261. https://doi.org/10.1080/21645698.2022.2118495

Chinnadurai, P., Stojšin, D., Liu, K., Frierdich, G. E., Glenn, K. C., Geng, T., Schapaugh, A., Huang, K., Deffenbaugh, A. E., Liu, Z. L., & Burzio, L. A. (2018). Variability of CP4 EPSPS expression in genetically engineered soybean (Glycine max L. Merrill). *Transgenic Research*, *27*(6), 511-524. https://doi.org/10.1007/s11248-018-0092-z

Gampala, S. S., Fast, B. J., Richey, K. A., Gao, Z., Hill, R., Wulfkuhle, B., Shan, G., Bradfisch, G. A., & Herman, R. A. (2017). Single-Event Transgene Product Levels Predict Levels in Genetically Modified Breeding Stacks. *J Agric Food Chem*, *65*(36), 7885-7892. https://doi.org/10.1021/acs.jafc.7b03098

Geng, T., Stojšin, D., Liu, K., Schaalje, B., Postin, C., Ward, J., Wang, Y., Liu, Z. L., Li, B., & Glenn, K. (2017). Natural Variability of Allergen Levels in Conventional Soybeans: Assessing Variation across North and South America from Five Production Years. *J Agric Food Chem*, *65*(2), 463-472. https://doi.org/10.1021/acs.jafc.6b04542

Goodwin, L., Hunst, P., Burzio, L. A., Rowe, L., Money, S., & Chakravarthy, S. (2021). Stacked Trait Products Are As Safe As Non-Genetically Modified (GM) Products Developed By Conventional Breeding Practices. *Journal of Regulatory Science*, *9*(1), 22-25. https://doi.org/https://doi.org/10.21423/jrs-v09i1goodwin

Hill, R. C., Fast, B. J., & Herman, R. A. (2017). Transgenesis affects endogenous soybean allergen levels less than traditional breeding. *Regulatory Toxicology and Pharmacology*, *89*, 70-73. https://doi.org/http://dx.doi.org/10.1016/j.yrtph.2017.07.013

Iizuka, W. (2020). Current Understanding of Food Safety Assessment Procedure for Stacked Trait Products Derived from Conventional Breeding among Approved GM Plants. *Food Saf (Tokyo)*, 8(1), 1-3. https://doi.org/10.14252/foodsafetyfscj.D-20-00003

Kok, E. J., Pedersen, J., Onori, R., Sowa, S., Schauzu, M., De Schrijver, A., & Teeri, T. H. (2014). Plants with stacked genetically modified events: to assess or not to assess? *Trends in biotechnology*, *32*(2), 70-73. https://doi.org/10.1016/j.tibtech.2013.12.001

References

Pilacinski, W., Crawford, A., Downey, R., Harvey, B., Huber, S., Hunst, P., Lahman, L. K., MacIntosh, S., Pohl, M., Rickard, C., Tagliani, L., & Weber, N. (2011). Plants with genetically modified events combined by conventional breeding: An assessment of the need for additional regulatory data. *Food and Chemical Toxicology*, *49*(1), 1-7. https://doi.org/http://dx.doi.org/10.1016/j.fct.2010.11.004

Ridley, W. P., Harrigan, G. G., Breeze, M. L., Nemeth, M. A., Sidhu, R. S., & Glenn, K. C. (2011). Evaluation of compositional equivalence for multitrait biotechnology crops. *J Agric Food Chem*, null-null. https://doi.org/10.1021/jf103874t

Steiner, H.-Y., Halpin, C., Jez, J. M., Kough, J., Parrott, W., Underhill, L., Weber, N., & Hannah, L. C. (2013). Editor's Choice: Evaluating the Potential for Adverse Interactions within Genetically Engineered Breeding Stacks. *Plant Physiology*, *161*(4), 1587-1594. https://doi.org/10.1104/pp.112.209817

Venkatesh, T. V., Breeze, M. L., Liu, K., Harrigan, G. G., & Culler, A. H. (2014). Compositional analysis of grain and forage from MON 87427, an inducible male sterile and tissue selective glyphosate-tolerant maize product for hybrid seed production. *J Agric Food Chem*, *62*(8), 1964-1973. https://doi.org/10.1021/jf4041589

Wu, A. J., Chapman, K., Sathischandra, S., Massengill, J., Araujo, R., Soria, M., Bugas, M., Bishop, Z., Haas, C., Holliday, B., Cisneros, K., Lor, J., Canez, C., New, S., Mackie, S., Ghoshal, D., Privalle, L., Hunst, P., & Pallett, K. (2019). GHB614 × T304-40 × GHB119 × COT102 Cotton: Protein Expression Analyses of Field-Grown Samples. *Journal of Agricultural and Food Chemistry*, *67*(1), 275-281. https://doi.org/10.1021/acs.jafc.8b05395

Xu, Y., Goodacre, R., & Harrigan, G. G. (2014). Compositional equivalence of grain from multi-trait drought-tolerant maize hybrids to a conventional comparator: univariate and multivariate assessments. *J Agric Food Chem*, *62*(39), 9597-9608. https://doi.org/10.1021/jf5019609