CROP LIFE INTERNATIONAL POSITION PAPER –METHODOLOGIES TO INFORM RISK ASSESSMENT

INTRODUCTION

Crop Life member companies are committed to ensuring that genetically modified (GM) crops are as safe as conventional crops for food and feed. The principles for assessing the safety of GM crops have been defined by Codex and the Organisation for Economic Co-operation and Development (OECD) (Codex Alimentarius, 2003, 2004; OECD, 2015) and have been applied by applicants and regulators globally in the risk assessments of commercialized GM products over the past 20 years. During this time, methodologies employed to inform risk assessments have evolved and today numerous scientific methods can be used to generate the key data needed to demonstrate the safety of GM crops.

CLI POSITION

Following internationally agreed principles defined by Codex and OECD for risk assessment of GM crops, the safety of GM crops can be assessed using different scientific methodologies to generate the quality data necessary to inform on the specific endpoints of the risk assessment. The evolution of methodologies that inform the risk assessment are the result of continuous advances in analytical techniques or product-specific safety assessment needs and diverse methods for risk assessment are widely accepted by the scientific community globally. Overall, data generated using one methodology are not always "better" than another in terms of suitability to assess GM crop safety (as long as the method is properly developed and validated); therefore the focus should be on risk assessment endpoints, open to the inevitable evolution and need for different and valid technologies, and not prescriptive technical methodology. Endpoint assessment focus allows for method flexibility as new methods emerge and enables data packages to continue to meet the data requirements. Additionally, less prescriptive methodology requirements enable regulatory agencies to adapt the assessments of products in an efficient, case-by-case manner following a weight-of-evidence approach to risk assessment.

RATIONALE FOR POSITION

Methods that are used to measure and assess the safety of new products may vary from country to country and may depend on available equipment, capability of the individual laboratory, and product specific needs (OECD 2007). The OECD repeatedly reports that scientific data for risk assessment are often obtained from a variety of sources and testing procedures and that multiple methods may be used for the risk assessment of products, provided they are appropriately developed and validated (OECD 1998, 2005, 2007, 2015). Numerous international and national agencies recognize the need for a flexible approach to risk assessment with a focus on the endpoints (AOAC[1]; AOCS[2]; ICCVAM[3]; ECVAM[4]; JaCVAM[5];

[1] Association of Official Analytical Chemists

ICH[6]). The Codex Alimentarius "*Principles for the Risk Analysis of Foods Derived from Modern Biotechnology*" comments specifically on the need for flexibility and evolution, stating "risk analysis methodology and its application should be consistent with new scientific knowledge and other information relevant to risk analysis." (Codex Alimentarius, 2003). The following are examples of alternative, validated methodologies for several endpoints that inform the risk assessment of GM crops.

MOLECULAR CHARACTERIZATION

As described by Codex Alimentarius (2004), molecular characterization of GM products includes (A) the characterization and description of the inserted genetic materials; (B) the number of insertion sites; and (C) the organisation of the inserted genetic material at each insertion site including copy number and sequence data of the inserted material and of the surrounding region. Historically this characterization was achieved utilizing Southern blot analysis. The advent and commercial availability of next generation sequencing (NGS) systems such as SOLiD and Ion Torrent from Life Technologies, Genome Analyzer, HiSeq 2000, and MiSeq from Illumina, or 454 LifeSciences from Rosche, makes molecular characterization by NGS possible. Sequence-based molecular characterization is similar in principle to Southern blot analysis and both methods accurately characterize the molecular endpoints listed above (Kovalic et al., 2012 and Zastrow-Hayes et al., 2015, Pauwels et al., 2015). Just as either Southern blots or NGS can be used for molecular characterization of single events, multiple methods are equally appropriate to apply for molecular bridging of a stacked GM crop to its parental singles events. For stacked trait GM crops, the maintenance of the inserted DNA(s) during conventional breeding is assessed. The Southern blot technique can measure this endpoint by hybridization of restriction enzyme digested DNA from the stacked trait product with probes designed for each inserted DNA. Similar conclusion can be obtained by other technologies, for example PCR amplification of the inserted DNA(s) followed by sequencing. Multiple methods are able to demonstrate the maintenance of the inserted DNA(s), stacked through traditional breeding, in the stacked GM product.

PROTEIN EXPRESSION

In the characterization of a GM crop, protein expression is performed to determine margins of exposure to exposed organisms. Quantifying the amount of the expressed introduced protein in plant tissues can be achieved by a variety of methods, accepted by the scientific community, including Enzyme Linked Immunosorbent Assay (ELISA), western blot, Mass Spectrometry-based methods, and micro-bead based immunoassays (Grothaus et al., 2006, Fantozzi et al., 2007, Hu et al., 2011, Shan, 2011, Bushey et al., 2014). To date, ELISAs have been used to assess the expression levels of the expressed introduced protein in support of the majority of regulatory assessments of GM crops and this analytical method has been widely adopted by academia and private-sector product developers. However, because proteins are diverse with various characteristics, there are examples of GM crops, approved for commercial use, in which protein expression levels were assessed by western blot due to product specific needs (USDA, 2012; EC, 2015). Western blot methods, like ELISA

[2] American Oil Chemists' Society
[3] Interagency Coordinating Committee on the Validation of Alternative Methods
[4] European Centre for the Validation of Alternative Methods
[5] **Japanese Center for the Validation of Alternative Methods**
[6] International Conference on Harmonization

methods, have been validated following internationally accepted good laboratory practices (GLP). Regardless of the analytical method, these studies have provided the protein expression data that inform risk assessments.

PROTEIN CHARACTERIZATION

To demonstrate the intended effect of the plant modification was achieved, characterization of the physicochemical and functional properties of the plant produced introduced protein must be completed (Codex Alimentarius, 2004).

In cases where the weight of evidence regarding the introduced protein indicates potential uncertainties (for example no history of safe use), oral toxicity studies (such as acute toxicology studies with mice) may be needed (Codex Alimentarius, 2004). These studies often require large quantities of the introduced protein in excess of what can be efficiently isolated from plant sources and therefore are typically conducted using a surrogate protein over-expressed in *E. coli* or another expression system. This material must be shown to be biochemically, structurally and functionally equivalent to that produced in plant (Codex Alimentarius, 2004). To determine equivalence, several types of analyses are conducted (e.g., for immunoreactivity, sequence and molecular weight, and glycosylation status) to assess whether the heterologously produced protein is equivalent to the plant-produced protein to assure that results from toxicological studies using the heterologously produced protein are relevant to the GM crop safety assessment. For example, comparing the molecular size of plant-produced protein and heterologously produced protein may be assessed using a number of different, analytical methods, including SDS-PAGE, western blot, and intact mass analysis by mass spectrometry (Gao, et al. 2006; Raybould, et al. 2013; Wang, et al. 2015). Analytical methods frequently evolve towards methods that are faster and/or require less sample for analysis, and provide equivalent data sets that can be employed in the protein equivalence assessment.

TOXICOLOGICAL ASSESSMENT

While not explicitly required by Codex, for decades, toxicology studies have been conducted with GM proteins and crops on a case-by-case basis to satisfy regulatory requirements. These studies have used different validated methods to demonstrate lack of hazard and results from these studies have been accepted by regulatory agencies (OECD 1998; Shah, 2007). For example, Koch et al. (2015) discuss methodological differences in the large number of toxicology studies with Cry proteins, all of which showed an absence of toxicity in rodents.

Additionally, a number of different rodent species may be used to conduct toxicology testing in support of GM crop product applications, and within these rodent species, several strains are suitable for use (OECD 1998; Parasuraman, 2011). Lastly, different validated assays and instrumentation may be used to generate data for the same toxicological endpoint. Specific examples include common clinical chemistry and hematology variables. Multiple validated kits and analyzers are commercially available and accepted by regulatory agencies. In accordance with internationally established guidelines, method flexibility does not require that the data be obtained using a specific assay or instrument (OECD 1998).

COMPOSITIONAL ASSESSMENT

Another key component of the risk assessment is demonstrating that the GM product is compositionally and nutritionally equivalent to its conventional comparator and that any compositional change (intended or otherwise) did not negatively impact nutritional safety. In compositional analyses of grain and forage there may be multiple validated analytical methods using different techniques for the measurement of the same analyte. Organizations such as AOAC and AOCS publish numerous methods that are internationally accepted by the scientific community and regulators. For example, AOAC has three methods for measuring protein in grain, using combustion, Kjeldahl, or Near-Infrared Spectrophotometry techniques (AOAC 2005 a, b, and c). AOCS has three different methods for fat (oil) in grain, using butt-tube extraction with petroleum ether, Soxhlet extraction with hexane, or supercritical fluid extraction (AOAC 2009 a, b, and c). A new method for soybean lectin was recently implemented as an AOCS official method based on ELISA-type immunoassay. The historical lectin method (Leiner 1955) depended on hemagglutination in rabbit blood that was much more difficult and unreliable to source than the new immunoassay. These techniques are all internationally recognized as equally valid for measurement of the analytes in question, and reflect continuing development and refinement of analytical methods and technologies.

CONCLUSION

Scientific advancements and product specific factors will continue to drive new method developments to inform risk assessment studies. Numerous validated methods have been and will continue to be used across the scientific community, product developers and independent testing organizations. The above examples demonstrate the acceptance and importance for regulatory authorities to continue to recognize the validity of different methodologies, without being exhaustive, for use in informing risk assessments. Focusing on the endpoints, and not the methodological approach, enables efficient and effective safety assessments of GM crop products using data generated from multiple rigorous methods that are scientifically defensible.

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