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| <p><b>Data requirements for approval of genetically enhanced plant products.</b></p>  |
| <p>Generalized requirements for typical input and outputs traits. Specific data requirements vary according to factors such as the crop, the nature of the trait (e.g., insect resistance versus herbicide resistance) and the way in which the trait is expressed (e.g., whole plant expression versus tissue specific expression).</p>  |
| <p>Products that show negative reactions in this first tier of tests, or those with more complex modifications (e.g., secondary metabolite modifications for pest resistance) will require additional and different tests which have not been defined yet.</p>  |
| <p><b>Biology</b></p> <p>Thorough knowledge, based on published literature, of the biology of the gene donor organism(s), especially any history of safe food use of the organism, and the presence and nature of any toxins (e.g., solanines in potato), allergens or anti-nutritional substances (e.g., protease inhibitors in soybean).</p>  |
| <p>Thorough knowledge, based on published literature, of the biology of the recipient crop, especially;</p> <ul style="list-style-type: none"> <li>• the presence of and nature of any toxic, allergenic or anti-nutritional substances,</li> <li>• reproductive biology and weediness potential,</li> <li>• ability to out-cross and form fertile offspring with wild or weedy relatives that occur in areas of potential product deployment,</li> <li>• importance as a food source for non-target herbivores, especially any endangered species,</li> <li>• agricultural practices employed in growing the crop and how these may be impacted by the modification (e.g., stacking herbicide resistances),</li> <li>• any secondary effects of changes in agricultural production on wildlife,</li> </ul> <p>Forms the basis for decisions on safety and containment of small scale, contained field testing of genetically enhanced plants (e.g., determining appropriate isolation distances, methods for pollen containment and prevention of seed dissemination).</p> |
| <p>Thorough knowledge, based on the literature, of the uses of the recipient crop including,</p> <ul style="list-style-type: none"> <li>• food and feed uses of the crop in all potential markets,</li> <li>• processed products derived from the crop.</li> </ul> <p>Forms basis for nutritional evaluation.</p>   |
| <p><b>Molecular and biochemical analysis - gene and gene expression product(s)</b></p> <p>Detailed characterization of the gene(s) to be transferred and the expressed protein(s) including;</p> <ul style="list-style-type: none"> <li>• origin of the gene(s),</li> <li>• complete nucleic acid sequence of the isolated gene(s) to be transferred,</li> <li>• deduced amino acid sequence of expressed protein(s),</li> <li>• biochemical function of expressed protein(s).</li> <li>• any anticipated changes (e.g., change of substrate or altered end products) in the functioning of the biochemical pathway(s) in which the protein(s) function.</li> </ul>   |
| <p><b>Molecular analysis - gene control elements</b></p> <p>Detailed characterization of all gene control elements including;</p> <ul style="list-style-type: none"> <li>• origin of control elements,</li> <li>• function of control elements (e.g., tissue specific promoters, transcription enhancers and transcription terminators),</li> <li>• complete nucleic acid sequence of all promoters, terminators, or other control elements (e.g., enhancers).</li> </ul>   |
| <p><b>Molecular analysis - vector</b></p> <p>Complete description of the gene vector system and the potential, if any, for incorporation of unwanted vector DNA into the recipient (e.g., leakiness of <i>Agrobacterium</i> T DNA borders).</p>   |

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| <p>Vector map showing location of key restriction enzyme sites, all genes, control elements and other open reading frames together with table showing details of all genetic elements including;</p> <ul style="list-style-type: none"> <li>• name,</li> <li>• origin,</li> <li>• function,</li> <li>• size.</li> </ul>   |
| Complete nucleic acid sequence of vector DNA.   |
| <p><b>Transformation procedure.</b><br/>Detailed description of the method used for transformation.</p>   |
| Confirmation of the purity of DNA to be delivered to the plant (e.g., by PCR or stained gels).  |
| <p><b>Molecular analysis and molecular stability of enhanced plant</b><br/>Genetic (Southern) analysis of multiple generations of the transformed line(s) confirming stable (i.e., Mendelian) inheritance of the inserted genetic material.</p>   |
| <p>High resolution map (or complete DNA sequence, if necessary) to determine the structure of the inserted DNA and confirm the absence of;</p> <ul style="list-style-type: none"> <li>• any unwanted DNA from the gene donor or vector,</li> <li>• gene fragments that could result in the expression of truncated proteins or fusion proteins [evidence of potential fusion proteins will require further investigation by messenger RNA (mRNA) analysis (e.g., by Northern blotting) or protein analysis (e.g., by Western blotting)].</li> </ul>   |
| <p>DNA sequence of the plant genome/insert DNA borders to;</p> <ul style="list-style-type: none"> <li>• confirm absence of DNA re-arrangements that could result in the expression of hybrid (host/insert) fusion proteins,</li> <li>• provide unique line specific probes for monitoring of product(s) in commerce.</li> </ul>   |
| <p><b>Segregation and stability of phenotype</b><br/>Genetic segregation data, over at least 2 –3 generations, confirming stable (i.e., Mendelian) inheritance of phenotypes representative of the introduced trait(s).</p>   |
| <p><b>Gene expression</b><br/>Availability of a validated quantitative assay (usually an immunoassay) for all new proteins expressed in the genetically enhanced line and measurement of the quantity of protein(s) expressed in different tissues of the crop (inbreds and hybrids) grown at several different locations (e.g., 6 sites) during two growing cycles. [Not necessary if no new proteins are produced such as when trait is modified through suppression of gene function (e.g., high oleic soybean)]. Tissues tested include (e.g., corn);</p> <ul style="list-style-type: none"> <li>• young leaf,</li> <li>• stem,</li> <li>• silk,</li> <li>• tassel,</li> <li>• pollen,</li> <li>• grain,</li> <li>• roots,</li> <li>• whole plant,</li> <li>• senescent plant.</li> </ul> <p>Tissues required for safety evaluation can vary depending on reviewing agency.<br/>If a herbicide tolerance trait is present, then a comparison of expression in sprayed versus non-sprayed plants is necessary for EU approval.</p> |
| Where enhancement results from shutting off gene(s) (e.g., gene silencing by co-suppression) mRNA analysis should be used to confirm lack of expression of target gene(s).  |

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| Confirmation that the activity of the expressed protein(s) in the enhanced plant is as expected from biochemical knowledge. Examine elements of biochemical pathway(s) involved to determine if other steps in pathway(s) are effected. Any unexpected effects on the target pathway, or related pathways, would require further investigation.   |
| Confirmation that the size and immunoreactivity of new protein(s) expressed in the enhanced plant are as anticipated (Western blotting).  |
| <p><b>Toxicology and allergenicity</b></p> <p>Detailed review of any safety data concerning gene(s) to be transferred including screening for similarity at the protein sequence (amino acid) level against databases of sequences of;</p> <ul style="list-style-type: none"> <li>• toxins,</li> <li>• food allergens.</li> </ul>   |
| If the gene(s) are isolated from a source that has a confirmed history of causing food allergy, and is to be transferred into a different food crop, then the gene product must be tested (e.g., by RAST or Western) against a statistically appropriate number of sera from patients with a verifiable clinical history of allergy to that food to show that it is not a food allergen. Confirmatory testing may be required including patient skin prick testing and double blind placebo controlled feeding trials with food allergic individuals. Food allergens should not normally be considered for transfer.  |
| <p>Purification of gram quantities of the new protein(s) from the plant or, if not feasible, then production of protein in a microbial expression system (microbially produced protein must be shown to be equivalent to plant protein through functional and/or analytical tests) followed by;</p> <ul style="list-style-type: none"> <li>• <i>in vitro</i> digestibility assay (digestible proteins have less potential to be food allergens),</li> <li>• heat stability (heat labile proteins have less potential to be food allergens in heat processed foods).</li> <li>• acute oral toxicity testing in mice with maximum hazard dose (10 to 100 times what may normally be consumed based on amount measured in plant parts).</li> </ul> <p>Account should be taken of any processing of plant parts that could lead to increased exposure in processed food or feed products.</p>   |
| <p><b>Composition</b></p> <p>Compositional analysis (substantial equivalence) of food and/or feed components (e.g., grain and forage) of the new line relative to a closely related non-modified counterpart grown at several locations (e.g., 6 sites) over two growing cycles, focusing especially on composition of ;</p> <ul style="list-style-type: none"> <li>• proximates (protein, fat, starch, fiber etc),</li> <li>• amino acids,</li> <li>• fatty acids,</li> <li>• carbohydrates,</li> <li>• key vitamins and minerals,</li> <li>• anti-nutrients (analysis based on knowledge of any anti-anti-nutrients in gene donor organisms and enhanced plant (e.g., protease inhibitors in soybeans, glucosinolates in canola)</li> <li>• allergens (analysis based on knowledge of any food allergy associated with the gene donor or recipient plant),</li> <li>• specific components of the biochemical pathway targeted for modification (e.g., linoleic isomer in high oleic soybeans).</li> </ul> <p>Any variations between the genetically enhanced and non-enhanced lines should be within the published ranges for the crop, except where nutritional modifications have been deliberately introduced (e.g., high lysine corn).</p> <p>If a herbicide tolerance trait is present, then a comparison of composition in sprayed versus non-sprayed plants is necessary for EU approval..</p> |
| <p><b>Volatiles and exudates</b></p> <ul style="list-style-type: none"> <li>• analysis of volatile substances given off from the enhanced plant compared to non-enhanced control,</li> <li>• analysis of root exudates from the enhanced plant compared to non-enhanced control.</li> </ul> <p>Normally only required by Japanese regulatory agencies.</p>  |

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| <p><b>Herbicide metabolites and residues</b></p> <p>For genetically enhanced plants that are to be sold as tolerant to specific herbicides, metabolism and residue data must usually be generated with the tolerant crop in order to obtain a new label for use of the herbicide on that crop.</p>  |
| <p><b>Plant growth</b></p> <p>Observations based on multiple plantings over at least 2 growing seasons of the genetically enhanced plant growing in different environments confirming that new trait(s) are stable, express the expected phenotype, and have no detrimental effects on plant development (e.g., growth habit, fertility, disease susceptibility, predation by herbivores or tendency to increased weediness) that could be indicative of unexpected effects of the genetic modification.</p>  |
| <p><b>Agronomic performance</b></p> <p>At least one season of observations at multiple sites of agronomic performance (e.g., growth rate, maturity and yield).</p>  |
| <p><b>Environmental risk assessment</b></p> <p>Environmental risk assessment is conducted to evaluate potential routes of exposure of new gene products, or their metabolites, to the environment and significance of those exposures. Some or all of the following hazard and risk considerations are evaluated, depending on the nature of the modification;</p> <ul style="list-style-type: none"> <li>• Can the plant become a weed/pest?</li> <li>• Is the gene product released from any plant parts?</li> <li>• Is the plant a copious producer of wind-borne pollen?</li> <li>• Is the plant naturally self- or cross-pollinated, or both?</li> <li>• Is the pollen transmitted by wind, insects and/or other vectors?</li> <li>• Are sexually compatible, non-target plants nearby?</li> <li>• Can the plant actually transmit the new trait to non-target plants?</li> <li>• What would be the consequences of gene transmission?</li> <li>• Would natural control of the wild plant/weed populations be curtailed?</li> <li>• Will the gene persist or move in the soil?</li> <li>• What is the effect on beneficial soil invertebrates?</li> <li>• Will wildlife feed on moribund insects and what will be the consequence?</li> <li>• What effect would the pollen have on pollinators?</li> <li>• Can animals, birds or bats distribute seed to a weedy relative habitat?</li> <li>• If in an aquatic plant, will these be consumed by aquatic wildlife?</li> <li>• If in an aquatic plant, will proteins be released into water?</li> <li>• Is the gene product expected to reach the estuarine/marine environment in significant concentration?</li> </ul> <p>Tests for impacts on non-target organisms are designed, based on this assessment.</p> |
| <p>Field scale monitoring of non-target populations present at different growth stages of the enhanced plant to confirm absence of negative environmental impacts and/or significant changes in crop management practices..</p>   |
| <p>If the genetically enhanced plant can out-cross with wild or weedy species in the areas where it will be planted, additional field studies will be required to confirm that the fitness of the resulting crosses has not been significantly changed, which could potentially result in new weeds or invasion of natural habitats or species loss. These studies could involve screening collections of wild relatives to show that a trait (e.g., disease resistance) is already present in wild populations or the gene may have to be bred into wild relatives which can then be tested to see if they exhibit altered fitness (e.g., increased seed production on insect resistant plants due to reduced herbivore activity).</p>   |

| <b>Input trait - pest resistant crop<br/>(e.g., plant incorporated protectant such as Bt)</b>   | <b>Output trait - modified food<br/>(e.g., high oleic soybean)</b> |
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| <p><b>Host range</b><br/>Determination of the range of activity of the plant-protectant against target pest(s) and determination of those that can be claimed as controlled on the product label.</p>   |  |
| <p><b>Mode of action</b><br/>Description of the mechanism by which the protein(s) exerts its activity against the target pest(s).</p>   |  |
| <p><b>Environmental fate - soil degradation</b><br/>Determine the rate of disappearance of the protein(s) from different soil types, directly and when incorporated into plant trash.<br/>Soil microbe populations may need to be measured if the gene expression products are active against microbes that play a key role in plant nutrition (e.g., Rhizobium) or breakdown of plant debris (e.g., fungi).</p>  |  |
| <p><b>Effects on non-target organisms</b><br/>Description of environments in which the protectant may be found and identification of non-target organisms that may consequently be exposed, followed by testing of representative non-target organisms exposed to purified protein and/or consumed plant parts (e.g., pollen) administered by the most appropriate route (usually oral). Representative organisms typically include;</p> <ul style="list-style-type: none"> <li>• soil invertebrates - earthworm, springtail/Collembola</li> <li>• freshwater aquatic invertebrate - Daphnia</li> <li>• terrestrial invertebrates - at least 3 beneficial species (e.g., ladybug, lacewing, parasitic wasp)</li> <li>• endangered or threatened species - surrogate (e.g., monarch butterfly),</li> <li>• pollinators - honey bee (adults and larvae),</li> <li>• freshwater fish - rainbow trout,</li> <li>• avian - bobwhite quail,</li> <li>• wild mammal - mouse.</li> </ul> <p>Additional species and routes of exposure may be added depending on the findings of the environmental risk assessment (e.g., expression of a plant-protectant in rice would trigger more studies on aquatic species).</p> |  |

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| <p><b>Insect resistance management</b><br/>Development of an insect resistance management (IRM) plan to delay the onset of pest resistance to the plant-protectant. IRM plans typically comprise recommendations for the management of the crop (e.g., planting of refuges) and provisions for monitoring for resistance and restorative actions. Additional studies may be required to support the IRM plan.</p> |  |
| <p><b>Nutritional Quality</b><br/>Animal feeding study using whole food (e.g., 42 day chick feeding study with modified grain) to confirm nutritional quality over the growth phase of the animal.</p>  |  |
|   | <p>Genetically enhanced plants with altered nutritional properties require additional nutritional studies. Any significant impact on the human diet must be evaluated based on predicted dietary exposure (calculated from typical consumption patterns). Adjustments may have to be made for regional differences in diet. Any deficiencies must be accounted for (e.g., by blending of products, supplementation or labeling).</p>   |
|   | <p>Where quality changes are directed at a specific processed fraction (e.g., high lysine soybean meal) then the composition of the appropriate fraction from the enhanced plant should be analyzed to confirm the intended change.</p>  |
|   | <p>If compositional analysis reveals additional products of the target biochemical pathway(s) beyond those predicted, then the impact of those products on the human or animal diet must be evaluated. Data on predicted dietary exposure for these components should be developed if the information is not available.</p>  |
|   | <p>Additional nutritional studies may be conducted, as deemed appropriate for marketing purposes, using appropriate feed-stuff (e.g., processed meal, forage) to measure growth rate and basic parameters of health for some or all of the following domestic animals;</p> <ul style="list-style-type: none"> <li>• pigs,</li> <li>• sheep,</li> <li>• dairy cows,</li> <li>• beef cows,</li> <li>• broiler chickens</li> <li>• laying chickens.</li> </ul> <p>Studies may include analysis of meat quality and milk and egg production.</p> |

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| <p><b>Approvals</b><br/> Before sales in the U.S. the applicant must receive;</p> <ul style="list-style-type: none"> <li>• an EPA registration for the plant-protectant and associated nucleic acid under the Federal Insecticide Fungicide and Rodenticide Act and an exemption from the requirement for a tolerance under the Federal Food Drug and Cosmetics Act,</li> <li>• a certificate of de-regulation from the federal Plant Pest Act (FPPA) from USDA,</li> <li>• a letter of completed consultation from the FDA.</li> </ul>   | <p><b>Approvals</b><br/> Before sales in the U.S. the applicant must receive;</p> <ul style="list-style-type: none"> <li>• a certificate of de-regulation from the FPPA from USDA</li> <li>• a letter of completed consultation from the FDA.</li> </ul> |
| <p>Unless the crop is identity preserved and can be contained and channeled into specific domestic markets, approvals are also required in key importing countries including;</p> <ul style="list-style-type: none"> <li>• Canada <ul style="list-style-type: none"> <li>➤ Novel Food approval from Health Canada,</li> <li>➤ Environmental Safety approval under Directive 95-03, and Feed Safety approval under Directive 94-08, from the Canadian Food Inspection Agency - Plant Products Division.</li> </ul> </li> <li>• Japan <ul style="list-style-type: none"> <li>➤ Novel Food approval from Ministry of Health</li> <li>➤ Environmental Safety and Feed Safety from Ministry of Agriculture, Food and Fisheries.</li> </ul> </li> <li>• European Union <ul style="list-style-type: none"> <li>➤ Novel food approval from the E.U. Commission under Regulation 258/97 E.U. ,</li> <li>➤ Approval from the E.U. Commission to place on the market (includes use as animal feed) under Directive 90/220 E.E.C.</li> </ul> </li> </ul> <p>Other countries requiring approvals for import and/or marketing include; Argentina, Australia, Brazil, Bulgaria, China, India, Hungary, Mexico, New Zealand , Philippines, Romania, Russia, South Africa, South Korea, Switzerland, Turkey.</p> |  |