Decision Document 2012-94 Determination of the Safety of Monsanto Canada Inc.'s Soybean (*Glycine max* (L.) Merr.) Event MON 87708

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October 5, 2012

This Decision Document has been prepared to explain the regulatory decision reached under Directive 94-08 (Dir94-08), entitled "*Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits*", its companion document BIO1996-10, "*The Biology of Glycine max (L.) Merr. (Soybean)*", and Chapter 2.6 of the Regulatory Guidance: Feed Registration Procedures and Labelling Standards entitled "*Guidelines for the Assessment of Novel Feeds: Plant Sources*".

The Canadian Food Inspection Agency (CFIA), specifically the Plant Biosafety Office of the Plant Health and Biosecurity Directorate, the Plant and Biotechnology Risk Assessment Unit of the Plant Health Science Directorate and the Animal Feed Division of the Animal Health Directorate, has evaluated information submitted by Monsanto Canada Inc. This information is in regard to the dicamba tolerant soybean event MON 87708. The CFIA has determined that this plant with a novel trait (PNT) does not present altered environmental risk nor, as a novel feed, does it present livestock feed safety concerns when compared to currently commercialized soybean varieties in Canada.

Taking into account these evaluations, unconfined release into the environment and use as livestock feed of soybean event MON 87708 is therefore authorized by the Plant Biosafety Office of the Plant Health and Biosecurity Directorate and the Animal Feed Division of the Animal Health Directorate as of October 5, 2012. Any soybean lines derived from soybean event MON 87708 may also be released into the environment and used as livestock feed, provided that (i) no inter-specific crosses are performed, (ii) the intended uses are similar, (iii) it is known based on characterization that these plants do not display any additional novel traits and are substantially equivalent to currently grown soybean in Canada, in terms of their potential environmental impact and livestock feed safety, and (iv) the novel gene is expressed at levels similar to those of the authorized line.

The previous version of this document (dated October 5, 2012) indicated that CFIA placed provisional restrictions on dicamba treated forage and hay derived from soybean event MON 87708. As a result of the completion of the regulatory review on dicamba conducted by Pest Management Regulatory Agency of Health Canada (PMRA), the CFIA restrictions were lifted.

Soybean event MON 87708 is subject to the same phytosanitary import requirements as its unmodified counterparts. Soybean event MON 87708 is required to meet the requirements of other jurisdictions; including but not limited to, the *Food & Drugs Act* and the *Pest Control Products Act*.

Please note, that the livestock feed and environmental safety assessments of novel feeds and PNTs are critical steps in the potential commercialization of these plant types. Other requirements, such as the evaluation of food safety by Health Canada, have been addressed separately from this review.

This bulletin is published by the Canadian Food Inspection Agency. For further information, please contact the Plant Biosafety Office or the Animal Feed Division at:

1-800-442-2342
613-225-2342
59 Camelot Drive, Ottawa
Ontario K1A 0Y9

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I. Brief Identification of the Modified Plant

Designation of the Modified Plant	Soybean event MON 87708, OECD Unique Identifier MON-877Ø8-9
Applicant	Monsanto Canada Inc.
Plant Species	Soybean (*Glycine max* (L.) Merr.)
Novel Traits	Tolerance to dicamba (3,6-dichloro-2-methoxybenzoic acid) herbicide
Trait Introduction Method	*Agrobacterium* -mediated transformation
Intended Use of the Modified Plant	Human consumption and livestock feed. Soybean event MON 87708 is not intended to be grown outside the normal production area for soybean in Canada.

II. Background Information

Soybean event MON 87708 was developed by Monsanto Canada Inc. using recombinant DNA technology, resulting in the introduction of the dicamba mono-oxygenase (*dmo*) gene derived from the Gram-negative bacteria *Stenotrophomonas maltophilia*. This bacteria is ubiquitous in the environment. The *dmo* gene encodes a dicamba mono-oxygenase (DMO) enzyme that rapidly inactivates the herbicide dicamba (3,6-dichloro-2-methoxybenzoic acid), thereby conferring tolerance to dicamba. The tolerance of soybean event MON 87708 to dicamba allows for pre-emergence application up to the day of crop emergence and post-emergence in-crop application through the early reproductive growth stages.

Monsanto Canada Inc. has provided data on the identity of soybean event MON 87708, a detailed description of the transformation method, data and information on the gene insertion site, gene copy number and levels of gene expression in the plant and the role of the inserted gene and regulatory sequences. The novel protein was identified and characterized. Data was provided for the evaluation of the potential toxicity of the novel protein to livestock and non-target organisms and potential allergenicity of the novel protein to humans and to livestock. Data were provided for the evaluation of herbicide residues in the feed commodities derived from the crop, following the intended herbicide application.

Soybean event MON 87708 was field tested in the United States (US) at 16 locations and in Canada at 2 locations in the 2008 growing season. Test sites were located within the major soybean producing regions of the US and Canada.

Agronomic characteristics of soybean event MON 87708, including early stand count, seedling vigour, days to 50% flowering, plant height, lodging, pod shattering, final stand count, seed moisture, 100 seed weight, seed test weight, yield, susceptibilities to various soybean pests and

pathogens, dormancy/germination, volunteer potential and potential to survive in unmanaged ecosystems, were compared to those of the unmodified control.

Nutritional components of soybean event MON 87708, such as protein, fat, carbohydrates, fibre, ash, moisture, amino acids, fatty acids, vitamins and anti-nutrients were compared with those of the unmodified control.

The Plant and Biotechnology Risk Assessment (PBRA) Unit of the Plant Health Science Directorate, CFIA, has reviewed the above information, in light of the assessment criteria for determining environmental safety of PNTs, as described in Directive 94-08 (Dir94-08), entitled " Assessment Criteria for Determining Environmental Safety of Plants With Novel Traits". The PBRA Unit has considered:

- the potential of soybean event MON 87708 to become a weed of agriculture or be invasive of natural habitats;
- the potential for gene flow from soybean event MON 87708 to sexually compatible plants whose hybrid offspring may become more weedy or more invasive;
- the potential for soybean event MON 87708 to become a plant pest;
- the potential impact of soybean event MON 87708 or the gene products on non-target species, including humans; and
- the potential impact of soybean event MON 87708 on biodiversity.

The Animal Feed Division (AFD), of the Animal Health Directorate, CFIA, has also reviewed the above information with respect to the assessment criteria for determining the safety and efficacy of livestock feed, as described in Chapter 2.6 of the Regulatory Guidance: Feed Registration Procedures and Labelling Standards, entitled "*Guidelines for the Assessment of Novel Feeds: Plant Sources*".

The AFD has considered both the intended and unintended effects of the genetic modification and similarities and differences between the modified plant and the unmodified control relative to the safety and efficacy of feed ingredients derived from soybean event MON 87708 for their intended purpose; including:

- potential impact of soybean event MON 87708 on livestock nutrition and
- potential impact of soybean event MON 87708 on animal health and human safety as it relates to the potential transfer of residues into foods of animal origin, and worker/bystander exposure to the feed.

The AFD has also considered whether feeds derived from soybean event MON 87708 meet feed definitions and requirements of feeds as listed in Schedule IV of the *Feeds Regulations*.

Monsanto Canada Inc. has provided the CFIA with a method for the detection and identification of soybean event MON 87708.

III. Description of the Novel Trait

1. Development Method

Soybean event MON 87708 was developed through *Agrobacterium*-mediated transformation of conventional soybean meristem tissues. Soybean event MON 87708 was developed using a binary vector system containing two T-DNAs. T- DNA I contained the *dmo* expression cassette, which includes the coding region for the DMO protein derived from *S. maltophilia,* along with its regulatory elements. T- DNA II contained a *cp4 epsps* expression cassette, which includes the coding region for the CP4 EPSPS protein derived from *Agrobacterium* sp. strain CP4 along with its regulatory elements. The CP4 EPSPS protein provides tolerance to the herbicide glyphosate and was only used as a selectable marker. The 2 T-DNAs system allowed for insertion T- DNA I (encoding the DMO protein) and T- DNA II (encoding the CP4 EPSPS selectable marker) into two unlinked loci within the genome of the soybean plant. Transformants that contained both T-DNAs were selected then selfed to segregate the unlinked insertions of T- DNA I and T- DNA II. Progeny that contained T- DNA II was eliminated from subsequent breeding, while progeny that only contained T- DNA I was maintained. Soybean event MON 87708 was identified as a successful transformant based on molecular analyses, herbicide efficacy and agronomic evaluations and was thus chosen for further development.

2. Tolerance to Dicamba

Dicamba is a group 4 herbicide that mimics indole-3-acetic acid, a natural plant hormone of the auxin class. Dicamba application causes rapid and uncontrolled growth of the stems, petioles and leaves of sensitive plants. This leads to uncontrolled cell division and growth, resulting in the destruction of vascular tissue and eventually plant death. Dicamba is used for broadleaf weed control on grain crops, pastures and non-crop areas.

Soybean event MON 87708 contains a *dmo* expression cassette that produced a DMO precursor protein. The precursor DMO protein contains a chloroplast transit peptide (CTP) from pea (*Pisum sativum*) to direct transport of the DMO precursor protein to the chloroplast and 27 amino acids from the N-terminal coding region of the pea Rubisco small subunit to stabilize expression of the precursor protein in the plant. It was anticipated that during translocation into chloroplasts the CTP and the additional 27 amino acids would be cleaved resulting in the appropriate amino terminus for the mature DMO protein. However protein analysis revealed that the precursor DMO protein is processed into two forms in MON 87708 soybean tissues; one form that corresponds to the mature DMO protein with the expected N-terminus (designated DMO) and another form that contains the additional 27 amino acids originating from the pea Rubisco small subunit (designated DMO+27). The active form of the DMO enzyme necessary to confer dicamba tolerance is a trimer consisting of three DMO monomers. In soybean event MON 87708 the DMO trimer is comprised of the DMO type, the DMO+27 type or a combination of both types. The DMO trimer rapidly demethylates dicamba rendering it inactive, thereby conferring tolerance to dicamba herbicide.

The DMO protein produced in soybean event MON 87708 has an identical sequence to the wild-type DMO protein derived from *S. maltophilia,* except for an additional alanine amino acid at position 2 and a cysteine instead of a tryptophan amino acid at position 112. The DMO+27 protein has the same amino acid differences when compared to the wild-type DMO and contains

the additional 27 amino acids at the N terminal portion of the protein. The differences in the amino acid sequence between the wild-type DMO protein and the DMO and DMO+27 proteins in soybean event MON 87708 are not expected to have an effect on the structure, activity or specificity of the DMO trimer. The identity and functionality of the DMO protein expressed in soybean event MON 87708 was evaluated by examining its molecular weight, immunoreactivity, glycosylation status, N-terminal sequences, tryptic peptide mass maps and enzymatic activity. Based on the results of these studies, the DMO protein expressed in soybean event MON 87708 had the expected composition and function.

DMO protein expression in soybean event MON 87708 is driven by a constitutive promoter. Samples of soybean tissue were collected from plants at various growth stages from five field trial sites in the US in 2008. Average DMO protein expression in micro-grams of protein per gram of dry weight tissue (μg/g dwt) was evaluated by enzyme-linked immunosorbent assay. Expression levels for soybean event MON 87708 were measured as follows: 53 μg/g dwt in forage (R6), 17 μg/g dwt in over season leaf-1 (V3-V4), 31 μg/g dwt in over season leaf-2 (V5-V8), 44 μg/g dwt in overseason leaf-3 (R2-V12), 69 μg/g dwt in over season leaf-4 (R5-V16), 6.1 μg/g dwt in root (R6) and 47 μg/g dwt in seed (R8).

The potential mammalian toxicity and allergenicity of the DMO protein in soybean event MON 87708 was evaluated. The DMO protein lacks sequence similarity to known allergens and protein toxins which have adverse effects to mammals. No adverse effects were observed when the DMO protein from soybean event MON 87708 was ingested by mice at a dose of approximately 140 mg/kg body weight. *In vitro* digestive fate studies have shown that the DMO protein is rapidly degraded in simulated gastric fluid, unlike protein allergens which are normally resistant to digestion. The DMO protein is not glycosylated, unlike many known allergens, providing additional evidence that it does not have the properties of known allergens. These results indicate that the DMO protein expressed in soybean event MON 87708 is unlikely to be toxic or allergenic to mammals.

3. Stable Integration into the Plant Genome

Molecular characterization by Southern blot analysis demonstrated that soybean event MON 87708 contains one intact copy of the *dmo* gene cassette at a single insertion site in the soybean genome. No additional elements, including intact or partial DNA fragments of the *dmo* gene cassette, T-DNA II sequences or backbone sequences from the vector, linked or unlinked to the intact insert, were detected in soybean event MON 87708. Sequencing of the introduced DNA and the flanking genomic DNA confirmed the sequence and organization of the genetic elements and revealed that there was a 128 bp insertion 5' to the insertion site as well as a 35 bp deletion 3' to the insertion site in the flanking genomic DNA. The deletion and insertion had no effect on the functionality of the DNA insert or the plant and such phenomena have been previously observed with *Agrobacterium* transformation.

The stability of the insert within soybean event MON 87708 was demonstrated by Southern blot analysis across five generations. Analysis of the inheritance pattern of the *dmo* gene, DMO protein expression and tolerance to dicamba across multiple segregating generations, confirmed

the stability of the inserted DNA. The results of the analysis are consistent with the finding of a single site of insertion that segregates according to the Mendelian laws of genetics.

IV. Criteria for the Environmental Assessment

1. Potential of Soybean Event MON 87708 to Become a Weed of Agriculture or be Invasive of Natural Habitats

The biology of soybean, as described in the CFIA biology document BIO1996-10, "*The Biology of Glycine max (L.) Merr. (Soybean)*", is such that unmodified plants of this species are not invasive of unmanaged habitats in Canada. Soybean does not possess an intrinsic potential to become weedy in Canada due to traits such as the lack of seed dormancy and the poor competitive ability of seedlings. According to the information provided by Monsanto Canada Inc., soybean event MON 87708 was determined not to be different from unmodified soybean in this respect.

The CFIA evaluated data submitted by Monsanto Canada Inc. on the reproductive biology and life history traits of soybean event MON 87708. This event was field tested in the US at 16 locations and in Canada at 2 locations in the 2008 growing season. The US locations share similar environmental and agronomic conditions to South western Ontario and were considered to be representative of major Canadian soybean growing regions. During the field trials, soybean event MON 87708 was compared to the unmodified control. Commercial soybean varieties were also grown in these trials to provide a reference range for typical soybean behaviour. Phenotypic and agronomic traits were evaluated, covering a broad range of characteristics that encompass the entire life cycle of the soybean plant. The traits included early stand count, seedling vigour, days to 50% flowering, plant height, lodging, pod shattering, final stand count, seed moisture, 100 seed weight, seed test weight and yield. For the majority of agronomic traits, no statistically significant differences between soybean event MON 87708 and the unmodified control were observed. For the few instances where statistically significant differences were observed, there were few consistent trends in the data across locations that would indicate that the differences were due to the genetic modification. For example, there was indication of a trend occurring in which soybean event MON 87708 had a higher plant height and a lower 100 seed weight than those of the unmodified control. However, the values for soybean event MON 87708 were within the reference range established for commercial soybean varieties grown in the same field trials and did not impact final yield. Therefore, the statistical analysis of these observations showed no biologically meaningful differences between soybean event MON 87708 and the unmodified control, and supports a conclusion of phenotypic equivalence to commercial soybean varieties.

Monsanto Canada Inc. provided information on the dormancy and germination of soybean event MON 87708 seed under six different temperature regimes. The following seed germination characteristics were evaluated: percent germinated seed, percent viable hard seed, percent dead seed and percent viable swollen seed. Soybean event MON 87708 was compared to the unmodified control. Eight commercial soybean varieties were also included to provide a reference range of comparative values for each germination characteristic. Seed lots were produced at 3 sites in the US. No statistically significant differences were detected between soybean event MON 87708 and the unmodified control with respect to percentages of dead seed,

viable firm swollen seed and viable hard seed (viable hard seed is associated with dormancy). Statistically significant differences were observed between soybean event MON 87708 and the unmodified control for 2 temperature regimes for percent germinated seed and percent dead seed in the individual and combined site analysis. However the values were within the reference range established for commercial soybean varieties and therefore not considered to be biologically meaningful.

Volunteer potential was assessed at 4 locations in the US. Soybean event MON 87708 seeds were seeded in the fall of 2008. No volunteers were detected the following spring.

The susceptibility of soybean event MON 87708 to various abiotic stressors was evaluated in the field at the same locations as the agronomic characteristic studies. The stressors observed included: cold stress, soil compaction, soil crusting, drought, flood, frost, hail, heat stress, moisture stress, mineral toxicity, nutrient deficiency and wind. No qualitative differences were observed for 193 out of 194 observations between soybean event MON 87708 and the unmodified control. A single qualitative difference was observed for wind damage. The observed wind damage rating for soybean event MON 87708 was outside the reference range established for commercial varieties, however the difference between soybean event MON 87708 and the unmodified control was not consistently detected across collection times or sites. These results support that the detected difference is not associated with the novel trait and is not biologically meaningful.

The susceptibility of soybean event MON 87708 to various soybean pests and pathogens was evaluated in the field at the same locations as the agronomic characteristic studies (further detail provided below in Section 3: Altered Plant Pest Potential of Soybean Event MON 87708). No trend in increase or decrease of susceptibility to pests and pathogens was observed in soybean event MON 87708 compared to the unmodified control.

No competitive advantage was conferred to plants of soybean event MON 87708, other than that conferred by tolerance to dicamba, as the reproductive characteristics, growth characteristics and tolerance to abiotic and biotic stresses of soybean event MON 87708 were comparable to those of commercial soybean varieties. Tolerance to dicamba provides a competitive advantage only when this herbicide is used, and will not, in and of itself, make a dicamba-tolerant soybean weedier or more invasive of natural habitats than dicamba-sensitive soybean. Dicamba-tolerant soybean volunteers will not be controlled in subsequent crops if dicamba is used as the only weed control tool. However, control of dicamba-tolerant soybean as a volunteer weed in other crops or in fallow ground can readily be achieved by the use of other classes of herbicides, or by mechanical means.

The novel trait has no intended or observed effects on weediness or invasiveness. The CFIA has therefore concluded that soybean event MON 87708 has no altered weed or invasiveness potential in Canada compared to currently grown soybean varieties.

The CFIA considers the changes in usual agronomic practices that may arise from volunteer plants with novel herbicide tolerances. Similarly, the CFIA considers the potential that continued application of the same herbicide in subsequent rotations may lead to increased selection

pressure for herbicide resistant weed populations. In order to address these issues, an herbicide stewardship plan which includes integrated weed management strategies should be implemented. These plans may include a recommendation to rotate or combine weed control products with alternate modes of action and to employ other weed control practices.

Monsanto Canada Inc. has submitted an herbicide tolerance stewardship plan to the CFIA, which was determined to be satisfactory when evaluated by the PBRA Unit.

Monsanto Canada Inc. will make this stewardship plan readily available to growers and agriculture extension personnel, in both private and public sectors, to promote careful management practices for soybean event MON 87708. Monsanto Canada Inc. will provide an efficient mechanism for growers to report agronomic problems to the company, which will facilitate the ongoing monitoring of dicamba-tolerant soybean. Monsanto Canada Inc. will monitor grower compliance to determine the effectiveness of the stewardship plan and make any changes to the plan as appropriate.

2. Potential for Gene Flow from Soybean Event MON 87708 to Sexually Compatible Plants Whose Hybrid Offspring May Become More Weedy or More Invasive

Natural hybridization between cultivated soybean and the wild annual species *Glycine soja* can occur. However, *G. soja* is not naturalized in North America, and although this species is occasionally grown in research plots, there are no reports of its escape to unmanaged habitats nor of it becoming a weed in Canadian agro-ecosystems. The biology of soybean, as described in the CFIA biology document BIO1996-10, shows that soybeans exhibit a high degree of self-fertilization. Cross pollination is usually less than one percent, suggesting that any pollen flow from cultivated soybeans to related species is minimal.

This information, together with the fact that the novel trait has no intended effects on soybean reproductive biology, led the CFIA to conclude that there is minimal potential for gene flow from soybean event MON 87708 to related species in Canada.

3. Altered Plant Pest Potential of Soybean Event MON 87708

Soybean is not considered a plant pest in Canada and the novel trait is unrelated to plant pest potential (i.e. the potential for the plant to harbour new or increased populations of pathogens or pests).

The susceptibility of soybean event MON 87708 to various soybean pests and pathogens was evaluated in the field at the same locations as the agronomic characteristic studies (See 1. Potential of Soybean Event MON 87708 to Become a Weed of Agriculture or be Invasive of Natural Habitats). The stressors observed included: armyworms, aphids, bean leaf beetles, blister beetles, cabbage loopers, corn rootworm beetles, cutworms, fall armyworms, grasshoppers, green cloverworms, Japanese beetles, leaf hoppers, seedcorn maggots, soybean loopers, spider mites, stink bugs, thistle caterpillars, thrips, yellow woollybears caterpillars, *Alternaria* (leaf spot), anthracnose, Asian rust, bacterial blight, brown stem rot, *Cercospora*, charcoal rot, downy

mildew, frogeye leaf spot, *Phytophthora*, pod and stem blight, powdery mildew, *Pythium*, *Rhizoctonia*, *Septoria* brown spot, soybean cyst nematode, soybean mosaic virus, soybean rust, stem canker, sudden death syndrome, white mould and yellow mosaic virus. Evaluations of soybean event MON 87708 did not show any increase or decrease in susceptibility to disease stressors compared to the unmodified control and commercial soybean varieties grown at the same locations for any of the 215 observations. A total of six statistically significant differences involving four taxa (aphids, blister beetles, Japanese beetles and leafhoppers) were observed between soybean event MON 87708 and the unmodified control out of 95 observations for the susceptibility to pest insects. The mean damage ratings were within the reference range established for commercial soybean varieties for four of the six detected differences. The remaining two ratings were outside of the reference range established for commercial varieties, however the differences between soybean event MON 87708 and the unmodified control were not consistently detected across collection times or sites. These results support that the detected differences in susceptibility to pest insects are not associated with the novel trait and are not biologically meaningful.

The CFIA therefore concludes that soybean event MON 87708 does not display any altered plant pest potential compared to currently grown soybean varieties.

4. Potential Impact of Soybean Event MON 87708 on Non-Target Organisms

Detailed characterization of the novel DMO protein expressed in soybean event MON 87708 led to the conclusion that it does not display any characteristics of a potential toxin or allergen (see III: Description of the Novel Trait). The DMO protein shares homologies across all levels of protein structure (i.e., primary, secondary, tertiary) with a wide variety of oxygenases present in bacteria and plants widely prevalent in the environment. Animals and humans are extensively exposed to these structural homologs without any reports of adverse effects. Therefore, no negative impacts resulting from exposure of organisms to the DMO protein expressed in soybean event MON 87708 are expected.

Composition analyses showed that the levels of key nutrients and anti-nutrients in soybean event MON 87708 grain are comparable to those in commercial soybean varieties. Therefore, it is very unlikely that the genetic transformation may have caused unintended changes to the composition of soybean event MON 87708 tissues that would negatively impact organisms interacting with soybean event MON 87708.

Soybean event MON 87708 and the unmodified control were compared for key characteristics of their symbiotic association with the nitrogen-fixing soil bacteria *Bradyrhizobium japonicum*. No statistically significant differences were observed for nodule number, shoot total nitrogen (percent and mass) and biomass of nodules, shoot material and root material. Therefore, the symbiotic relationship between *B. japonicum* and soybean has not been altered by the genetic modification in soybean event MON 87708.

Ecological evaluations confirmed that the abundance of pest and beneficial arthropods in soybean event MON 87708 plots was similar to that in plots of commercial soybean varieties grown at the same locations. A total of 151 observations were made for arthropod abundance

involving the following pest and beneficial arthropods: aphids, bean leaf beetles, grape colaspis, garden leafhoppers, green cloverworms, Japanese beetles, potato leafhoppers, stink bugs tarnished plant bugs, velvet bean caterpillars, wollybear caterpillars, *Araneae* (spiders), big-eyed bug, carabid beetle, lacewing, ladybird beetle, micro-parasitic hymenoptera, Nabis sp., Orius sp., *Opiliones* (harvestman) and *Syrphidae* (hoverfly larvae). No statistically significant differences were detected between soybean event MON 87708 and the unmodified control for 142 out of 151 observations, including 74 observations of pest arthropod and 77 observations of beneficial arthropods. Although seven differences were detected out of 74 pest arthropod observations involving green cloverworms, Japanese beetles and stink bugs, these differences were not consistently detected across collection times or sites. A total of two differences were detected out of 77 beneficial arthropod observations involving *Araneae* and *Nabis* spp. The mean abundance value for *Nabis* sp. was within the range for commercial soybean varieties. The mean abundance value for *Araneae* was outside of the range for commercial varieties, however the difference between soybean event MON 87708 and the unmodified control was not consistently detected across collection times or sites. These results support that the detected differences in pest and beneficial arthropod abundance are not associated with the novel trait and are not biologically meaningful. In addition, soybean event MON 87708 did not display any increase in resistance to pest insects or pathogens compared to commercial soybean varieties (further detail provided above in Section 3: Altered Plant Pest Potential of Soybean Event MON 87708).

Collectively, these information elements indicate that the interactions between soybean event MON 87708 and the populations of animals and microorganisms interacting with soybean, including beneficial arthropods and symbiotic bacteria, will be similar compared to currently grown soybean varieties.

The CFIA has therefore determined that the unconfined release of soybean event MON 87708 will not result in altered impacts on non-target organisms, including humans, compared to currently grown soybean varieties.

5. Potential Impact of Soybean Event MON 87708 on Biodiversity

Soybean event MON 87708 expresses no novel phenotypic characteristics that would extend its range beyond the current geographic range of soybean production in Canada. Soybean's only sexually compatible wild relative in Canada (*G. soja*) does not occur in unmanaged habitats, and the possibility of soybean outcrossing to *G. soja* is very low. Soybean event MON 87708 is unlikely to cause adverse effects on non-target organisms and does not display increased weediness, invasiveness or plant pest potential. It is therefore unlikely that soybean event MON 87708 will have any direct effects on biodiversity, in comparison to the effects that would be expected from the cultivation of soybean varieties currently grown in Canada.

Soybean event MON 87708 has tolerance to the broadleaf herbicide dicamba. The use of this herbicide in cropping systems has the intended effect of reducing local weed populations within agroecosystems. This may result in a reduction in local weed species biodiversity, and may have effects on other trophic levels which utilize these weed species. It must be noted however that the goal of reduction in weed biodiversity in agricultural fields is not unique to the use of PNTs, soybean event MON 87708 or the cultivation of soybean. It is therefore unlikely that soybean

event MON 87708 will have any indirect effects on biodiversity, in comparison to the effects that would be expected from cultivation of soybean varieties currently grown in Canada.

The CFIA has concluded that the novel gene and its corresponding trait do not confer to soybean event MON 87708 any characteristic that would result in unintended environmental effects following unconfined release. The CFIA has therefore concluded that the impact on biodiversity of soybean event MON 87708 is unlikely to be different from that of soybean varieties currently grown in Canada.

V. Criteria for the Livestock Feed Assessment

The AFD considered nutrient and anti-nutrient profiles; the safety of feed ingredients derived from soybean event MON 87708, including the presence of gene products, residues, and metabolites in terms of animal health, human safety as it relates to the potential transfer of residues into foods of animal origin, and worker/bystander exposure to the feed; and whether feeds derived from soybean event MON 87708 meet the definitions and requirements of feeds as listed in Schedule IV of the *Feeds Regulations.*

1. Potential Impact of soybean event MON87708 on Livestock Nutrition

Nutrient and anti-nutrient composition:

The nutritional equivalence of soybean event MON 87708 to the unmodified control (A3525) and 20 conventional commercial soybean varieties was determined from five replicated field sites in the US during the 2008 growing season. Seed were planted in a randomized complete block design with three plots per each of soybean event MON 87708, unmodified control and conventional soybean varieties. Seed and forage samples were harvested from all plots and shipped on dry-ice (forage) or ambient temperature (seeds) to Monsanto Co. (St. Louis. MO.). A sub-sample for compositional analysis was obtained from each tissue sample collected. Sub-samples were ground and stored in freezer set at -20°C until composition analysis were conducted.

Forage and seed samples were analysed for proximate (protein, crude fat, ash and moisture), acid detergent fibre (ADF) and neutral detergent fibre (NDF). Seed samples were further analysed for amino acids, fatty acids, vitamin E, isoflavones (diadzein, genistein and glycitein) and anti-nutrients (lectin, phytic acid, trypsin inhibitor, raffinose and stachyose) as recommended by the OECD consensus document for new varieties of soybean (OECD, 2001)[footnote 1]. Composition data was analysed statistically using a mixed model analysis of variance, and statistical differences among treatments were identified ($P< 0.05$). The biological relevance of any differences in nutrient levels between soybean event MON 87708 and the control was assessed within the 99% tolerance interval calculated from the 20 conventional soybean varieties. Statistically significant differences between soybean event MON 87708 and the control were also assessed within the context of the natural variability of conventional soybean composition data published in scientific literature, OECD (2001)[footnote 2] and in the ILSI Crop Composition Database (ILSI 2009)[footnote 3].

Except for ADF, no statistically significant differences were observed between soybean event MON 87708 and the control forage for the proximate and neutral detergent fibre (NDF). The ADF content in soybean event MON 87708 was within the natural variation of the conventional soybean varieties. Statistically significant differences were observed between soybean event MON 87708 and the unmodified control seed for crude protein, crude fibre, ash, ADF and NDF, however the means of soybean event MON 87708 were within the range of the nutrients of the conventional soybean varieties and those in published literature and ILSI database and was not considered biologically relevant. Amino acids; arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, phenylalanine, proline, tyrosine and valine, were statistically significantly lower in soybean event MON 87708 than the control but the values were with the range or natural variation of the conventional soybean varieties and therefore the differences were not considered biologically relevant. Vitamin E, palmitic, oleic, linoleic, linoleic, arachidic and behenic acids in soybean event MON 87708 seed were statistically significantly different from the unmodified control. The mean values were however within the range of conventional soybean varieties and those found in the ILSI database. Except for diadzein, no statistically significant differences were observed between soybean event MON 87708 and the unmodified control for genistein and glycitein. The isoflavone, diadzein, in soybean event MON 87708 was statistically significantly higher than the control soybeans but the mean values were within the natural variation of the conventional soybean varieties and therefore not biologically relevant. The anti-nutrients, phytic acid, raffinose and stachyose, were statistically significantly lower in soybean event MON 87708 than the unmodified control crop, however the mean values were within the natural variation for the conventional soybean varieties and ILSI database values.

Broiler chicken performance

800 birds were assigned to 80 pens (10 birds/pen), to examine the health and performance effects of feeding soybean event MON 87708 meal compared to an unmodified control (A3525) and six conventional soybean varieties for 42 days. Soybean meal for each diet treatment was sampled prior to being used in the diets for nutrient, mycotoxins and pesticides analyses. The treatment diets were formulated to contain the same energy levels and the same amount of soybean meal for the starter (32.5%) and grower/finisher diets (30%). From day 0-21, birds were fed the starter diets and then switched onto the grower/finisher diets for the remaining of the study. Water and feed were provided to the birds on a continual basis. Data on bird mortality, feed intake, body weights, gains and feed efficiencies were collected. On day 43 and 44 of the trial, birds were selected from each pen and processed to determine carcass yield data. Breast and thigh meat samples were also collected and analysed for protein, fat and moisture content. Chick mortality ranged from 0-2% from day 7-42, and was not related to the dietary treatments. No statistically significant differences were observed between birds on diets from soybean event MON 87708, the unmodified control and the conventional varieties for average daily gains, feed intake and feed efficiencies. Except for breast weight, carcass yields and meat nutrient analysis were not different for broilers fed soybean event MON 87708 meal compared to those fed the unmodified control or the conventional soybean meal. The breast weight of broilers on soybean event MON 87708 diets was within the natural variation observed for those on the conventional soybean diets and therefore the difference was not considered biologically relevant.

Conclusion

It was concluded based on the evidence provided by Monsanto that the nutritional composition of soybean event MON 87708 is similar to the conventional soybean varieties. No biologically relevant differences were observed on the performance and health of birds consuming diets containing soybean event MON 87708 meal.

2. Potential Impact of Soybean Event MON 87708 on Animal Health and Human Safety as it Relates to the Potential Transfer of Residues into Foods of Animal Origin, and Worker/Bystander Exposure to the Feed

Soybean event MON 87708 is tolerant to dicamba as a result of the insertion of a *dmo* gene from the bacterium *S. maltophilia*, encoding the DMO and DMO+27 proteins subunits . The active form enzyme necessary to confer dicamba tolerance is a trimer consisting of different combinations of the protein subunits. The assessment of soybean event MON 87708 evaluated the impact of the following potential hazards relative to the safety of feed ingredients derived from these events:

- The presence of the novel DMO protein
- The chemical pesticide residue profile

Novel DMO protein:

The two subunits DMO and DMO+27 of the DMO protein share no significant biologically relevant sequence homology with any known toxins or allergens, and the enzyme itself lacks a mode of action that suggests that it is intrinsically toxic. The protein complex is also heat labile and rapidly degraded under conditions similar to those encountered in the gastrointestinal tract. No signs of toxicity were demonstrated in single-dose oral toxicity studies in mice using purified DMO protein, from soybean event MON 87708 seed, at doses up to 140 mg/kg-bw. These factors support the lack of intrinsic toxicity of the DMO protein.

Furthermore, the lack of performance effects or significant health observations in the broiler study using soybean meal derived from soybean event MON 87708 supports the safety of the DMO protein.

Chemical pesticide residue profile

The safety of herbicide residues of dicamba and metabolites in soybean event MON 87708 , following application of the herbicide, was also evaluated as part of the feed safety assessment.

It was determined that dicamba and its metabolites (investigated as dicamba equivalent residues of dicamba + DCSA + DCGA +5-hydroxy dicamba) in livestock feed produced from soybean event MON 87708, did not present levels of concern to livestock, nor humans via the potential transfer into foods of animal origin.

The previous version of this document indicated that the CFIA placed provisional CFIA restrictions on dicamba treated forage and hay derived from soybean event MON 87708. Monsanto provided documentary evidence to show that PMRA considered forage and hay dicamba residues in their completed assessment & decision, which took place after the CFIA authorization of October 5, 2012. As a result of the completion of the regulatory review on dicamba (conducted by PMRA), the CFIA restrictions were lifted.

Conclusions

Feed ingredients derived from soybean event MON 87708 as outlined above, are considered to meet present ingredient definitions for soybean and as such are approved for use as livestock feed in Canada.

VI. New Information Requirements

If at any time Monsanto Canada Inc. becomes aware of any information regarding risk to the environment, including risk to human or animal health, which could result from release of soybean event MON 87708 in Canada or elsewhere, Monsanto Canada Inc. is required to immediately provide such information to the CFIA. On the basis of such new information, the CFIA will re-evaluate the potential impact of soybean event MON 87708 on the environment, livestock and human health, and may re-evaluate its decision with respect to the livestock feed use and environmental release authorizations of soybean event MON 87708.

VII. Regulatory Decision

Based on the review of the data and information submitted by Monsanto Canada Inc. and other relevant information, the Plant and Biotechnology Risk Assessment Unit of the Plant Health Science Directorate, CFIA has determined that soybean event MON 87708 does not present altered environmental risk when compared to soybean varieties currently grown in Canada.

Based on the review of submitted data and information by Monsanto Canada Inc., including comparisons of soybean event MON 87708 with its unmodified soybean counterpart, the unmodified control, the Animal Feed Division of the Animal Health Directorate, CFIA, has concluded that the novel DMO enzyme-based herbicide tolerance trait will not confer to soybean event MON 87708 any characteristic that would raise any concerns regarding the safety or nutritional composition of soybean event MON 87708. Grain soybean, soybean oil, and soybean byproducts are currently listed in Schedule IV of the *Feeds Regulations* and are approved for use in livestock feeds in Canada. Feed ingredients from soybean event MON 87708 have been assessed and found to be as safe as and as nutritious as traditional soybean varieties.

Unconfined release into the environment and use as livestock feed of soybean event MON 87708 is therefore authorized by the Plant Biosafety Office of the Plant Health and Biosecurity Directorate and the Animal Feed Division of the Animal Health Directorate as of October 5 , 2012. Any soybean lines derived from soybean event MON 87708 may also be released into the environment and used as livestock feed, provided that (i) no inter-specific

crosses are performed, (ii) the intended uses are similar, (iii) it is known based on characterization that these plants do not display any additional novel traits and are substantially equivalent to currently grown soybean varieties in Canada, in terms of their potential environmental impact and livestock feed safety, and (iv) the novel gene is expressed at levels similar to those in the authorized line.

Soybean event MON 87708 is subject to the same phytosanitary import requirements as its unmodified counterparts and is required to meet the requirements of other jurisdictions; including but not limited to, the *Food & Drugs Act*, and the *Pest Control Products Act*.

Please refer to Health Canada's Decisions on Novel Foods for a description of the food safety assessment of soybean event MON 87708.

Footnotes

Footnote 1

OCDE 2001, *Consensus document on compositional consideration for new varieties of soybean: Key food and feed nutrient and anti-nutrients*, ENV/JM/MONO (2001)15, Series on the Safety of Novel Foods and Feeds No. 2, Organisation de coopération et de développement économiques, Paris, France.

Return to footnote 1 referrer

Footnote 2

OCDE 2001, *Consensus document on compositional consideration for new varieties of soybean: Key food and feed nutrient and anti-nutrients*, ENV/JM/MONO (2001)15, Series on the Safety of Novel Foods and Feeds No. 2, Organisation de coopération et de développement économiques, Paris, France.

Return to footnote 2 referrer

Footnote 3

ILSI 2009, Crop Composition Database, version 3.0. International Life Science Institute http://www.cropcompostion.org/

Return to footnote 3 referrer

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