DD 2013-100: Determination of the Safety of Cibus Canada Inc. (Incorporated)'s Canola (*Brassica napus* L. (Linnaeus)) Event 5715

This Decision Document has been prepared to explain the regulatory decision reached under Directive 94-08 (Dir9 08) - Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits (/plants/plants-with-nov traits/applicants/directive-94-08/eng/1304475469806/1304475550733), its companion biology document BIO199 - The Biology of *Brassica napus* L. (Linnaeus) (Canola/Rapeseed) (/plants/plants-with-novel-traits/applicants/direc 94-08/biology-documents/brassica-napus-l-/eng/1330729090093/1330729278970) and Section 2.6 - Guidelines f the Assessment of Novel Feeds: Plant Sources (/animals/feeds/regulatory-guidance/rg-1/chapter-2/eng/1329298059609/1329298179464?chap=6) of Chapter 2 of the RG-1 Regulatory Guidance: Feed Registratic Procedures and Labelling Standards.

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 Cibus Canada Inc. (Incorporated), a legal affiliate of Cibus US (United States) LLC (limited liability company). This informa concerns the herbicide tolerant canola event 5715. The CFIA (Canadian Food Inspection Agency) has determined 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 canola varieties currently grown and permitted to be used as livestock feed in Canada.

Taking into account these evaluations, unconfined release into the environment and use as livestock feed of canc event 5715 are therefore authorized by the Plant Biosafety Office of the Plant Health and Biosecurity Directorate and the Animal Feed Division of the Animal Health Directorate, respectively, as of December 3, 2013. Any canola lines derived from canola event 5715 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, and
iii. it is known based on characterization that these plants do not display any additional novel traits and are substantially equivalent to canola varieties that are currently grown and permitted to be used as livestock fe in Canada, in terms of their potential environmental impact and livestock feed safety.

Additionally, with respect to its use as livestock feed, canola event 5715 must meet the restrictions specific to tribenuron-methyl treated feed set out in the authorization.

Canola event 5715 is subject to the same phytosanitary import requirements as unmodified canola varieties. Canc event 5715 is required to meet the requirements of other Canadian legislation, including but not limited to the requirements set out in 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 (plants with nc traits) are critical steps in the potential commercialization of these plant types. Other requirements, such as the evaluation of food safety of novel foods by Health Canada, have been addressed separately from this review.

December 3,

This bulletin was created by the Canadian Food Inspection Agency. For further information, please contact the Plant Biosafety Offi the Animal Feed Division by visiting the Contact Us (/about-the-cfia/contact-us/eng/1299860523723/1299860643049) page.

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I (1). Brief Identification of the Modified Plant

Designation of the Modified Plant	Canola event 5715
Applicant	Cibus Canada Inc. (Incorporated)
Plant Species	Canola (*Brassica napus* L. (Linnaeus))
Novel Trait	Tolerance to tribenuron-methyl and thifensulfuron-m herbicides
Trait Introduction Method	Mutagenesis and conventional breeding
Intended Use of the Modified Plant	Canola event 5715 is intended to be grown for tradit canola human food and livestock feed uses. Canola event 5715 is not intended to be grown outside the normal production area for canola in Canada.

II (2). Background Information

Cibus Canada Inc. (Incorporated) has developed a canola event that is tolerant to the sulfonylurea herbicides tribenuron-methyl and thifensulfuron-methyl. The development of canola event 5715 was accomplished by conventional breeding of two herbicide tolerant mutants, one newly produced and one commercially available. *Brassica napus (B. napus)* carries two complete genomes designated "A" and "C". Each genome has an acetohydroxyacid synthase (*AHAS*) gene (*BnAHAS1* gene in the C genome and *BnAHAS3* in the A genome) codin an AHAS (acetohydroxyacid synthase) enzyme. This enzyme is also referred to as acetolactate synthase (ALS). Tolerance to the sulfonylurea herbicides is achieved in canola event 5715 through the presence of the same single point mutation, known as the PM2 mutation, in both the *BnAHAS1* and *BnAHAS3* genes. This mutation results in modified AHAS (acetohydroxyacid synthase) enzymes carrying a single amino acid substitution which renders the insensitive to tribenuron-methyl and thifensulfuron-methyl. It should be noted that the PM2 mutation confers toler to a range of AHAS (acetohydroxyacid synthase)-inhibiting herbicides commonly referred to as the "group 2" herbicides (i.e. (that is to say) the imidazolinones, pyrimidinylthiobenzoates, sulfonylamino-carbonyltriazolinones, sulfonylureas and triazolopyrimidines). However, the intended use of canola event 5715, as stated by Cibus Canad Inc. (Incorporated), is tolerance to the sulfonylurea herbicides tribenuron-methyl and thifensulfuron-methyl.

Cibus Canada Inc. (Incorporated) has provided information on the identity of canola event 5715; a description of tl breeding history; and information on the modified genes, the resulting proteins and their mode of action and the stability of trait expression. Information was provided for the evaluation of the potential toxicity of the modified proteins to livestock and non-target organisms and potential allergenicity of the modified proteins to humans and livestock. Data were provided for the evaluation of herbicide residues in the feed commodities derived from the cr following the herbicide application of tribenuron-methyl and thifensulfuron-methyl.

Canola event 5715 was field tested at one site in the United States (US) in 2009, four sites in the US (United State 2010 and seven sites in Canada in 2011. The locations of the trials in the US (United States) share similar environmental and agronomic conditions to canola production areas in Canada and were considered representati the major Canadian canola growing regions. Two comparator canola lines were also grown during these field trials first was the BN2 parental line, which was used in the development of canola event 5715 and therefore has a simil genetic background to canola event 5715. The second comparator was a commercial control canola line that is tolerant to imidazolinone herbicides but lacks the tribenuron-methyl and thifensulfuron-methyl tolerant trait found canola event 5715. This line was not used in the development of canola event 5715.

Agronomic characteristics of canola event 5715, such as plant stand, plant vigour, date to first flowering, date to 5 flowering, date to final flowering, maturity date, plant height, yield, seed moisture content and total kernel weight v compared to those of the BN2 parental control canola line and the commercial control canola line.

Nutritional components of canola event 5715, such as proximates, acid detergent fibre, neutral detergent fibre, an acids, fatty acids, minerals and glucosinolates, were compared with those of the BN2 parental control canola line the commercial control canola line.

The Plant and Biotechnology Risk Assessment (PBRA) Unit of the Plant Health Science Directorate, CFIA (Canadia Food Inspection Agency), has reviewed the above information, in light of the assessment criteria for determining environmental safety of PNTs (plants with novel traits), as described in Directive 94-08 (Dir94-08) - Assessment Cr for Determining Environmental Safety of Plants with Novel Traits (/plants/plants-with-novel-traits/applicants/directi 94-08/eng/1304475469806/1304475550733). The PBRA (Plant and Biotechnology Risk Assessment) Unit has considered:

- the potential of canola event 5715 to become a weed of agriculture or be invasive of natural habitats;
- the potential for gene flow from canola event 5715 to sexually compatible plants whose hybrid offspring may become more weedy or more invasive;
- the potential for canola event 5715 to become a plant pest;
- the potential impact of canola event 5715 or the gene products on non-target organisms, including humans;
- the potential impact of canola event 5715 on biodiversity.

The Animal Feed Division (AFD), of the Animal Health Directorate, CFIA (Canadian Food Inspection Agency), has a reviewed the above information with respect to the assessment criteria for determining the safety and efficacy of livestock feed, as described in Section 2.6 - Guidelines for the Assessment of Novel Feeds: Plant Sources (/animals/feeds/regulatory-guidance/rg-1/chapter-2/eng/1329298059609/1329298179464?chap=6), of Chapter 2 the RG-1 Regulatory Guidance: Feed Registration Procedures and Labelling Standards.

The AFD (Animal Feed Division) has considered both intended and unintended effects and similarities and differen between canola event 5715 and unmodified canola varieties relative to the safety and efficacy of feed ingredients derived from canola event 5715 for their intended purpose, including:

- the potential impact of canola event 5715 on livestock nutrition; and
- the potential impact of canola event 5715 on animal health and human safety as it relates to the potential tra of residues into foods of animal origin, and worker/bystander exposure to the feed.

The AFD (Animal Feed Division) has also considered whether feeds derived from canola event 5715 meet feed definitions and requirements of feeds as listed in Schedule IV (4) of the *Feeds Regulations*.

Cibus Canada Inc. (Incorporated) has provided the CFIA (Canadian Food Inspection Agency) with a method for th detection and identification of canola event 5715.

III (3). Description of the Novel Trait

1. Development Method

Cibus Canada Inc. (Incorporated) utilized an oligonucleotide-directed mutagenesis approach known as the Rapid Development System™ (***RTDS™***), which included the application of tissue culture techniques that generated plan cells more receptive to mutagenesis. Following treatment of protoplasts of the BN2 parental canola line with the ***R*** (Rapid Trait Development System), a canola event known as BnALS-57 was isolated. Sequencing confirmed that t *BnAHAS1* gene of BnALS-57 contains the PM2 mutation, which confers tolerance to AHAS (acetohydroxyacid synthase)-inhibiting herbicides such as the sulfonylureas and imidazolinones. Although BnALS-57 was isolated following treatment of cells with the ***RTDS*** (Rapid Trait Development System), the PM2 mutation in BnALS-57 is thought to have been created as a result of a spontaneous somaclonal variation that occurred during the tissue cu process, rather than due to the oligonucleotide used in the ***RTDS*** (Rapid Trait Development System). The BnALS-5 event was selected during treatment of calli with the imidazolinone-based herbicide imazethapyr. Canola event BnALS-57 was subsequently crossed with a commercial imidazolinone tolerant canola line, which contains the PM mutation in its BnAHAS3 gene to produce canola event 5715, which is homozygous for the PM2 mutation in both *BnAHAS1* and *BnAHAS3* genes.

2. Tolerance to Tribenuron-methyl and Thifensulfuron-methyl Herbicides

Sulfonylurea herbicides, including tribenuron-methyl and thifensulfuron-methyl, are active against AHAS (acetohydroxyacid synthase) enzymes. AHAS (acetohydroxyacid synthase) enzymes are found in bacteria, certain other micro-organisms and plants, but not in animals. These enzymes catalyse the first step in the biosynthesis of essential branched chain amino acids isoleucine, leucine and valine. Herbicide-induced AHAS (acetohydroxyacid synthase) inhibition results in a lethal decrease in protein synthesis. Unmodified *B. (Brassica) napus* is not tolerant AHAS (acetohydroxyacid synthase)-inhibiting herbicides.

The introduced single nucleotide PM2 mutation in the *BnAHAS1* and *BnAHAS3* genes of canola event 5715 result a single tryptophan to leucine amino acid substitution at amino acid position 574 (according to amino acid numbe of the *Arabidopsis thaliana* AHAS (acetohydroxyacid synthase) enzyme) in the BnAHAS1 and BnAHAS3 enzymes respectively. This single amino acid substitution alters the binding site of the AHAS (acetohydroxyacid synthase) enzyme, resulting in tolerance to a range of AHAS (acetohydroxyacid synthase)-inhibiting herbicides including the sulfonylurea herbicides tribenuron-methyl and thifensulfuron-methyl.

The expression of the modified BnAHAS1 and BnAHAS3 enzymes is under the control of the native promoters of t *BnAHAS1* and *BnAHAS3* genes respectively, and is believed to be constitutive.

Both the *BnAHAS1* and *BnAHAS3* genes of canola line BnALS-57 and the BN2 parental control canola line were sequenced in their entirety, revealing that the only mutation introduced into these genes in canola line BnALS-57 v the PM2 mutation in the *BnAHAS1* gene. In addition, no changes were detected in promoter regions or locations t would affect expression of the *BnAHAS1* gene. This was confirmed by sequencing a 2500 bp (base pairs) region upstream of the *BnAHAS1* gene.

Since the AHAS (acetohydroxyacid synthase) proteins are involved in the biosynthesis of branched chain amino ac including leucine, isoleucine and valine, amino acid analysis was conducted on the defatted meal from canola eve 5715 seed. The results indicated that the amino acid profile in canola event 5715 is similar to that in the BN2 pare control canola line, therefore it was concluded that the PM2 mutation in the *BnAHAS1* and *BnAHAS3* genes does result in an altered amino acid profile in canola event 5715.

The potential of the modified BnAHAS1 and BnAHAS3 proteins to be allergenic or toxic to livestock and non-targe organisms was evaluated. The weight of evidence indicates that the modified BnAHAS1 and BnAHAS3 proteins ar unlikely to be allergenic. The source of the BnAHAS1 and BnAHAS3 proteins, *B. (Brassica) napus*, is not commonl known to produce allergens, the amino acid sequences of the modified BnAHAS1 and BnAHAS3 proteins lack rele similarities to those of known allergens and the modified BnAHAS1 and BnAHAS3 proteins were shown experimer to be rapidly degraded in simulated gastric fluid and not to be heat stable. It was also concluded that the modifiec BnAHAS1 and BnAHAS3 proteins were unlikely to be toxic to livestock or non-target organisms because they lack mode of action to suggest that they are intrinsically toxic to livestock or non-target organisms, and because their amino acid sequences lack relevant similarities to known toxins. In addition, AHAS (acetohydroxyacid synthase) proteins are ubiquitous in the environment and are not known to be toxic or allergenic. For a more detailed discus of the potential allergenicity and toxicity of the modified BnAHAS1 and BnAHAS3 proteins, see Section V (5), part Potential Impact of Canola Event 5715 on Animal Health and Human Safety as it Relates to the Potential Transfer Residues into Foods of Animal Origin and Worker/Bystander Exposure to the Feed.

3. Stable Integration into the Plant Genome

The PM2 mutation in canola event 5715 was shown by direct sequencing of the mutation site to be stable over thr generations. In addition, Cibus Canada Inc. (Incorporated) reported phenotypic stability of canola event 5715 over breeding generations, based on herbicide tolerance.

IV (4). Criteria for the Environmental Assessment

1. Potential of Canola Event 5715 to Become a Weed of Agriculture or be Invasive of Natural Habitats

Canola (*B. (Brassica) napus*) possesses some of the characteristics that are common to weeds and invasive plants is an annual crop that may persist in unmanaged ecosystems without human intervention. There have been report *B. (Brassica) napus* becoming a weed of agriculture in North America and other parts of the world, however it has become an abundant or problematic weed in Canada, despite being cultivated in Canada for many years. *B. (Bras napus* plants can grow as volunteers in cultivated fields in the seasons following a *B. (Brassica) napus* crop, but th are usually eliminated by soil cultivation or the use of herbicides. According to the information provided by Cibus Canada Inc. (Incorporated), canola event 5715 was determined not to be significantly different from the unmodifie canola varieties in this respect.

The CFIA (Canadian Food Inspection Agency) evaluated data submitted by Cibus Canada Inc. (Incorporated) on th reproductive biology and life history traits of canola event 5715. Field studies were conducted in the US (United States) at four locations in 2010 and in Canada at seven locations in 2011. It was determined that the US (United States) locations share similar environmental and agronomic conditions to the canola growing regions of Manitoba Saskatchewan and were considered to be representative of major Canadian canola growing regions. Canola even 5715 was compared to the BN2 parental control canola line and the commercial control canola line during the 201 field trials and to the commercial control canola line during the 2011 field trials. Commercially grown canola varieti were also included in these trials to establish ranges of comparative values that are representative of currently gro canola varieties. Phenotypic and agronomic traits were evaluated, covering a broad range of characteristics that

encompass the entire life cycle of the canola plant. The traits included plant stand, plant vigour, date to first flowe date to 50% flowering, date to final flowering, maturity date, plant height, yield, seed moisture content and total k weight. Instances of statistically significant differences were observed between canola event 5715 and the BN2 parental control canola line for date to first flowering, date to final flowering, plant height and yield. For date to fina flowering and yield, the values for canola event 5715 were within the reference range established for the commerc grown canola varieties grown in the same field trials. For date to first flowering and plant height, the values for can event 5715 were outside of the reference range of the commercially grown canola varieties but the differences we small in magnitude and not considered biologically meaningful. Similarly, instances of statistically significant differences were observed between canola event 5715 and the commercial control canola line for date to first flowering, date to final flowering, plant height, yield, plant vigour and maturity date. For date to final flowering and maturity date, the values for canola event 5715 were within the reference range established for the commercially grown canola varieties grown in the same field trials. For date to first flowering, plant height, yield and plant vigour values for canola event 5715 were outside of the reference range of the commercially grown canola varieties but, i most instances, the differences were small in magnitude and not considered biologically meaningful. There was o indication of a trend occurring in which canola event 5715 had lower yield than that of the commercial control can line, however the difference was not observed across all sites and is not expected to affect canola's weediness or invasiveness potential. Therefore, the statistical analysis of these observations showed no biologically meaningful differences between canola event 5715 and the BN2 parental control canola line or the commercial control canola and supports a conclusion of phenotypic and agronomic equivalence to currently grown canola varieties.

Cibus Canada Inc. (Incorporated) evaluated the germination of canola event 5715 seed under two temperature regimes. During the germination test conducted at 15 to 25°C (Celsius), canola event 5715 had lower percentage normal germinated seed and higher percentages of dead seed and abnormal germinated seed compared to the B parental control canola line. However, during the germination test conducted at 10°C (Celsius), no differences wer observed between canola event 5715 and the BN2 parental control canola line for the percentages of germinated and dead seed. The poorer germination of canola event 5715 at the higher temperature is not indicative of increas weediness. No dormant or hard seed was identified in canola event 5715 or in the BN2 parental control canola lin either temperature regime. Therefore the introduction of the novel trait did not impact the germination of the canol seed and did not increase the dormancy of the canola seed.

Canola event 5715 was exposed to excessive moisture, high heat and drought in the field during the agronomic characteristic studies conducted in Canada in 2011. No trend in increased or decreased susceptibility to these ab stressors was observed in canola event 5715 compared to the commercial control canola line.

The susceptibility of canola event 5715 to canola pests and pathogens was evaluated in the field at the same loca as the 2011 agronomic characteristic studies (further detail provided below in Section IV (4), part 3: Potential for Canola Event 5715 to Become a Plant Pest). No trend in increased or decreased susceptibility to pests or pathoge was observed in canola event 5715 compared to the commercial control canola line.

No competitive advantage was conferred to plants of canola event 5715, other than that conferred by tolerance to tribenuron-methyl and thifensulfuron-methyl herbicides, as the reproductive characteristics, growth characteristics tolerance to abiotic and biotic stresses of canola event 5715 were comparable to those of the BN2 parental contro canola line. Tolerance to tribenuron-methyl and thifensulfuron-methyl herbicides provides a competitive advantage only when these herbicides are used, and will not, in and of itself, make a plant tolerant to these herbicides weedi more invasive of natural habitats. Canola event 5715 plants growing as volunteers will not be controlled if certain sulfonylurea or imidazolinone herbicides are used as the only weed control tools. In addition, other AHAS (acetohydroxyacid synthase)-inhibitors from the group 2 herbicides may not provide effective volunteer control. However, control of canola event 5715 as a volunteer weed in subsequent crops or in fallow ground can be achiev 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 (Canadian Food Inspection Agency) has therefore concluded that canola event 5715 has no altered weediness or invasiveness potential in Canada compared to currently grown canola varieties.

The CFIA (Canadian Food Inspection Agency) considers the changes in usual agronomic practices that may arise volunteer plants with novel herbicide tolerances. Similarly, the CFIA (Canadian Food Inspection Agency) considers potential that continued application of the same herbicide in subsequent rotations may lead to increased selectior pressure for herbicide resistant weed populations. In order to address these issues, an herbicide stewardship plar 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.

Cibus Canada Inc. (Incorporated) has submitted an herbicide tolerance stewardship plan to the CFIA (Canadian F Inspection Agency), addressing the considerations associated with the use of sulfonylurea herbicides tribenuron-methyl and thifensulfuron-methyl, which was determined to be satisfactory when evaluated by the PBRA (Plant an Biotechnology Risk Assessment) Unit. Cibus Canada Inc. (Incorporated) 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 canola event 5715. Cibus Canada Inc. (Incorporated) will provide an efficient mechanis growers to report agronomic problems to the company, which will facilitate the ongoing monitoring of canola even 5715. Cibus Canada Inc. (Incorporated) will monitor grower implementation to determine the effectiveness of the stewardship plan and make any changes to the plan as appropriate.

2. Potential for Gene Flow from Canola Event 5715 to Sexually Compatible Pla Whose Hybrid Offspring May Become More Weedy of More Invasive

Successful interspecific and intergeneric crosses between *B. (Brassica) napus* and some related species have bee reported in the scientific literature (see biology document BIO1994-09 - The Biology of *Brassica napus* L. (Linnaeu (Canola/Rapeseed) (/plants/plants-with-novel-traits/applicants/directive-94-08/biology-documents/brassica-napus /eng/1330729090093/1330729278970) for more information). However, many of these crosses have required exte human intervention and the rates of natural hybridization between *B. (Brassica) napus* and weedy relatives resultin fertile offspring appear to be very low. *Sinapsis arvensis* is considered the worst of the weedy relatives of *B. (Brass napus* in Western Canada. Hybrids between both species can be produced under field conditions, however at ver frequency. Additionally, backcrossing of the hybrids to *S. (Sinapsis) arvensis* failed to produce viable progeny. Therefore, the likelihood of introgression of traits from *B. (Brassica) napus* to *S. (Sinapsis) arvensis* appears to be v low. In crosses with other wild related species (i.e. (that is to say) *Raphanus raphanistrum* and *Erucastrum gallicum* viable hybrid seed was produced.

Stable gene transfer from *B. (Brassica) napus* is most likely with *Brassica* crops such as *B. (Brassica) juncea* and *B (Brassica) rapa*. Any hybrids resulting from outcrossing between canola event 5715 and *B. (Brassica) rapa* or *B. (Brassica) juncea* could be controlled by herbicides other than AHAS (acetohydroxyacid synthase)-inhibitors from t group 2 herbicides, or by mechanical means.

If tribenuron-methyl and thifensulfuron-methyl or other AHAS (acetohydroxyacid synthase)-inhibiting tolerant individuals arose through interspecific or intergeneric hybridization, the novel trait would confer no competitive advantage to these plants unless challenged by AHAS (acetohydroxyacid synthase)-inhibitors from the group 2 herbicides. This would only occur in managed ecosystems where AHAS (acetohydroxyacid synthase)-inhibiting herbicides are used for weed control. In the case of canola event 5715 volunteers, these herbicide tolerant individ should they arise, could be controlled using mechanical means or herbicides other than AHAS (acetohydroxyacid synthase)-inhibitors. These hybrids, if they developed, could potentially result in the loss of these herbicides as a t to control these species. This, however, can be avoided by the use of sound crop management practices. Cibus Canada Inc. (Incorporated)'s herbicide tolerance stewardship plan includes recommendations to minimize and manage outcrossing to related species.

This information led the CFIA (Canadian Food Inspection Agency) to conclude that gene flow from canola event 57 to related species in Canada is possible but would not result in increased weediness or invasiveness of the resulti progeny.

3. Potential for Canola Event 5715 to Become a Plant Pest

Canola is not considered a plant pest in Canada and the tribenuron-methyl and thifensulfuron-methyl herbicide tolerance trait introduced into canola event 5715 is unrelated to plant pest potential (e.g. (for example) the potenti the plant to harbour new or increased populations of pathogens or pests).

The susceptibility of canola event 5715 to canola pests and pathogens was evaluated in the field at the same loca as the 2011 agronomic characteristic studies. The stressors observed included low incidence of diamondback mo and blackleg. Canola event 5715 did not display any increased or decreased susceptibility to diamondback moth blackleg compared to the commercial control canola line.

The CFIA (Canadian Food Inspection Agency) has therefore concluded that canola event 5715 does not display ar altered plant pest potential compared to currently grown canola varieties.

4. Potential Impact of Canola Event 5715 or Its Gene Products on Non-Target Organisms, Including Humans

The tribenuron-methyl and thifensulfuron-methyl herbicide tolerance trait introduced into canola event 5715 is unrelated to a potential impact on non-target organisms.

AHAS (acetohydroxyacid synthase) proteins do not confer resistance to agricultural pests and are commonly foun a wide variety of plants and micro-organisms with a history of safe use. The modified BnAHAS1 and BnAHAS3 enzymes in canola event 5715 contain the same amino acid substitution that allows them to remain active in pres of tribenuron-methyl and thifensulfuron-methyl herbicides. The modified BnAHAS3 protein has been present in commercial canola varieties for years without any reports of adverse effects on animals and humans. Detailed characterization of the BnAHAS1 and BnAHAS3 proteins expressed in canola event 5715 confirmed that these

proteins do not display any characteristic of potential toxins or allergens (see Section III (3), part 2: Tolerance to Tribenuron-methyl and Thifensulfuron-methyl Herbicides). Therefore, no negative impacts resulting from exposure organisms to the modified BnAHAS1 and BnAHAS3 proteins expressed in canola event 5715 are expected.

Composition analyses showed that the levels of key nutrients and anti-nutrients in grain from canola event 5715 a comparable to those in the commercial control canola line (see Section V (5), part 1: Potential Impact of Canola Ev 5715 on Livestock Nutrition). Therefore, it is very unlikely that the introduction of the novel trait may have caused unintended changes to the composition of canola event 5715 tissues that would negatively impact organisms interacting with canola event 5715.

Field evaluations of canola event 5715 did not show any increased resistance to pests or pathogens compared to commercial control canola line (see Section IV (4), part 3: Potential for Canola Event 5715 to Become a Plant Pest)

Collectively, these information elements indicate that the interactions between canola event 5715 and the populati of animals and microorganisms interacting with canola crops will be similar compared to currently grown canola varieties.

The CFIA (Canadian Food Inspection Agency) has therefore determined that the unconfined release of canola ever 5715 in Canada will not result in altered impacts on non-target organisms, including humans, compared to current grown canola varieties.

5. Potential Impact of Canola Event 5715 on Biodiversity

Canola event 5715 expresses no novel phenotypic characteristics that would extend its range beyond the current geographic range of canola production in Canada. Canola event 5715 is unlikely to cause adverse effects on non-target organisms and does not display increased weediness, invasiveness or plant pest potential. Canola (*B. (Brass napus*) can outcross to *B. (Brassica) rapa* and *B. (Brassica) juncea*, and potentially to wild relatives, under natural conditions in Canada. However, the consequences of the transfer of the tribenuron-methyl and thifensulfuron-met tolerance trait are minimal as the novel trait does not confer any selective advantage in the absence of these herbicides, and tribenuron-methyl and thifensulfuron-methyl tolerant hybrids can be controlled by herbicides with other modes of action and cultivation. It is therefore unlikely that canola event 5715 will have any direct effects on biodiversity, in comparison to the effects that would be expected from the cultivation of the canola varieties that a currently grown in Canada.

Canola event 5715 has tolerance to tribenuron-methyl and thifensulfuron-methyl herbicides. The use of these herbicides in cropping systems has the intended effect of reducing local weed populations within agro-ecosystem This may result in a reduction in local weed species biodiversity, and may have effects on other trophic levels whic 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 (plants with novel traits), canola event 5715 or the cultivation of canola. It is therefore unlikely that canola event 5715 will have any indirect effects on biodiversity, in comparison to the effects would be expected from cultivation of currently grown canola varieties.

The CFIA (Canadian Food Inspection Agency) has concluded that the modified genes and their corresponding nov trait do not confer to canola event 5715 any characteristic that would result in unintended environmental effects following unconfined release. The CFIA (Canadian Food Inspection Agency) has therefore concluded that the pote impact on biodiversity of canola event 5715 is unlikely to be different from that of the canola varieties that are curr grown in Canada.

V (5). Criteria for the Livestock Feed Assessment

The AFD (Animal Feed Division) considered nutrient and anti-nutrient profiles; the safety of feed ingredients derive from canola event 5715, including the presence of gene products, residues and metabolites in terms of animal hea and human safety as it relates to the potential transfer of residues into foods of animal origin and worker/bystande exposure to the feed; and whether feeds derived from canola event 5715 meet the definitions and requirements of feeds as listed in Schedule IV (4) of the *Feeds Regulations*.

1. Potential Impact of Canola Event 5715 on Livestock Nutrition

Nutrient and Anti-nutrient Composition

The nutritional equivalence of canola event 5715 was compared to the commercial control canola line from replica trials conducted at five of the Canadian locations in 2011. Seeds were planted in a randomized complete design w three plots of each canola line. In addition, the nutritional equivalence of canola event 5715 to the BN2 parental co canola line was compared from a replicated trial in US (United States) during the 2009 and 2011 growing seasons. Seed samples were collected from each replicated plot for compositional analyses. Seed and meal samples were analysed for protein, fat, crude fibre, ash, acid detergent fibre (ADF) and neutral detergent fibre (NDF), amino acids

(meal only), calcium, phosphorous and glucosinolates. Fatty acids were analysed in the extracted oil as recommer the OECD (Organisation for Economic Co-operation and Development) consensus document for new varieties of canola (OECD (Organisation for Economic Co-operation and Development) 2011 - PDF (Portable Document Forma (548 kb (kilobytes)) (http://www.oecd.org/science/biotrack/49343153.pdf)). Anti-nutrients (phytic acid and trypsin inhibitor) were also measured in seeds of canola event 5715 and the commercial control canola line. Composition was analysed statistically using analysis of variance and statistically significant differences among canola lines we identified ($P<0.05$). The biological relevance of any statistically significant differences observed between canola ev 5715 and the parental and commercial control canola lines was evaluated by comparing the results to the range o published literature values (OECD (Organisation for Economic Co-operation and Development) 2011).

No statistically significant differences were observed between meal from canola event 5715 and meal from the commercial control canola line for protein, fat, crude fibre, ash, ADF (acid detergent fibre), NDF (neutral detergent fibre), calcium and phosphorous. Total glucosinolate levels were significantly different between meal from canola e 5715 and the commercial control canola line, however the levels in meal from both canola event 5715 and the commercial control canola line were less than the limit of 30 µmoles/gram established for oil-free meal (OECD (Organisation for Economic Co-operation and Development) 2011 - PDF (Portable Document Format) (548 kb (kilobytes)) (http://www.oecd.org/science/biotrack/49343153.pdf)). There were statistically significant differences observed between canola event 5715 and the BN2 parental control canola line for levels of protein (seed and mea (seed), crude fibre (seed), ADF (acid detergent fibre) and total glucosinolates (seed and meal), however the mean values for both canola types were within the natural variation found in the published literature (OECD (Organisatior Economic Co-operation and Development) 2011), and the differences were not considered biologically relevant. N statistically significant differences were identified in amino acid levels of meal from canola event 5715 when comp to that of the commercial control canola line. Levels of the amino acids arginine, glutamine, histidine, proline, tyros and tryptophan were significantly different in meal from canola event 5715 when compared to those in the BN2 parental control canola line, however the means were within published literature values (OECD (Organisation for Economic Co-operation and Development) 2011), and therefore the differences were not considered biologically relevant. For extracted oil, statistically significant differences were observed between canola event 5715 and the commercial control canola line for levels of linoleic, linolenic, eicosadienoic and erucic acids. Erucic acid values fo both canola event 5715 and the commercial control canola line oil were below the limit of 2% for canola quality (O (Organisation for Economic Co-operation and Development) 2011). In comparing canola event 5715 to the BN2 parental control canola line oil, statistical significant differences were observed for the levels of stearic, oleic, linole linolenic, and arachidic fatty acids. All mean values were however within the range of the published literature value (OECD (Organisation for Economic Co-operation and Development) 2011)) and the differences were not considere biologically relevant. No statistically significant differences were observed between seeds of canola event 5715 an the commercial control canola line for levels of the anti-nutrients: phytic acid and trypsin inhibitor.

Conclusion

It was concluded that, based on the evidence provided by Cibus Canada Inc. (Incorporated), the nutritional composition of canola event canola event 5715 is similar to commercially grown canola varieties.

2. Potential Impact of Canola event 5715 on Animal Health and Human Safety it Relates to the Potential Transfer of Residues into Foods of Animal Origin, ar Worker/Bystander Exposure to the Feed

Canola event 5715 is tolerant to sulfonylurea herbicides tribenuron-methyl and thifensulfuron-methyl due to produ of modified BnAHAS1 and BnAHAS3 enzymes having a PM2 mutation. The assessment of canola event 5715 evaluated the impact of the following potential hazards relative to the safety of feed ingredients derived from this event:

- the presence of novel proteins BnAHAS1 and BnAHAS3; and
- the chemical pesticide residue profile.

Novel BnAHAS1 and BnAHAS3 proteins

The potential allergenicity and toxicity of the modified BnAHAS1 and BnAHAS3 proteins to livestock were evaluate With respect to their potential allergenicity, no single experimental method yields decisive evidence, thus a weight evidence approach was taken, taking into account information obtained with various test methods. The source of BnAHAS1 and BnAHAS3 proteins, *B. (Brassica) napus*, is not commonly known to produce allergens, the BnAHAS and BnAHAS3 proteins in canola event 5715 are modified forms of canola AHAS (acetohydroxyacid synthase) pro which are not known to be allergenic, and a bioinformatics evaluation of the both AHAS (acetohydroxyacid syntha protein amino acid sequences confirmed the lack of relevant similarities between the modified BnAHAS1 and BnAHAS3 proteins and known allergens. The weight of evidence thus indicates that the modified BnAHAS1 and BnAHAS3 proteins are unlikely to be allergenic.

In terms of their potential toxicity to livestock, the BnAHAS1 and BnAHAS3 proteins lack a mode of action that suggests they are intrinsically toxic to livestock, and a bioinformatics evaluation of the AHAS (acetohydroxyacid synthase) protein amino acid sequences confirmed the lack of relevant similarities between the modified BnAHAS and BnAHAS3 proteins and known toxins. This information indicates that the BnAHAS1 and BnAHAS3 proteins ar unlikely to be toxic to livestock.

The livestock exposure to the modified BnAHAS1 and BnAHAS3 proteins is expected to be negligible as AHAS (acetohydroxyacid synthase) proteins are not expressed at high levels in wild type canola and there were no exogenous promoters introduced or changes to the endogenous promoter in canola event 5715, which might resu altered protein expression.

Chemical pesticide residue profile

The safety of herbicide residues and metabolites in canola event 5715, following application of herbicides, was als evaluated as part of the feed safety assessment.

It was determined that potential thifensulfuron-methyl in livestock feed produced from canola event 5715 would n present levels of concern to livestock, nor humans, via the potential transfer into foods of animal origin, when comparing the estimated exposure to established legal residue limits in Canada and the US (United States).

Herbicide tolerant canola for use with tribenuron-methyl has not at this time been the subject of any prior AFD (An Feed Division) Decisions. The safety of tribenuron-methyl residues in feed ingredients after the application of tribenuron-methyl to canola event 5715 has not been fully assessed, as complete residue data were not available demonstrate that when tribenuron-methyl was used as proposed, residues in livestock commodities would not pr levels of concern to livestock or humans, in comparing the estimated exposure to established legal residue limits i Canada and the US (United States). The AFD (Animal Feed Division) has therefore placed a provisional restriction feed commodities derived from canola event 5715 following the application of tribenuron-methyl, until an authoriz for tribenuron-methyl application on canola event 5715 has been granted by Health Canada's Pest Management Regulatory Agency (PMRA).

Conclusion

It was concluded, based on the evidence provided by Cibus Canada Inc. (Incorporated), that the novel BnAHAS1 BnAHAS3 protein-based herbicide tolerance trait will not confer to canola event 5715 any characteristic that woul raise concerns regarding the safety of canola event 5715. Feed ingredients derived from canola event 5715, witho the application tribenuron-methyl, are considered to meet present ingredient definitions for canola.

VI (6). New Information Requirements

If at any time Cibus Canada Inc. (Incorporated) becomes aware of any information regarding risk to the environme including risk to human or animal health, which could result from release of canola event 5715 in Canada or elsew Cibus Canada Inc. (Incorporated) is required to immediately provide such information to the CFIA (Canadian Food Inspection Agency). On the basis of such new information, the CFIA (Canadian Food Inspection Agency) will re-evaluate the potential impact of canola event 5715 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 canola eve 5715.

VII (7). Regulatory Decision

Based on the review of the data and information submitted by Cibus Canada Inc. (Incorporated) and other relevan information, the Plant and Biotechnology Risk Assessment Unit of the Plant Health Science Directorate, CFIA (Canadian Food Inspection Agency) has determined that canola event 5715 does not present altered environment risk when compared to canola varieties that are currently grown in Canada.

Based on the review of the data and information submitted by Cibus Canada Inc. (Incorporated) and other relevan information, the AFD (Animal Feed Division) of the Animal Health Directorate, CFIA (Canadian Food Inspection Agency), has concluded that the novel BnAHAS1 and BnAHAS3 protein-based herbicide tolerance trait will not co to canola event 5715 any characteristic that would raise concerns regarding the safety or nutritional composition canola event 5715. Grain canola, its byproducts and canola oil are currently listed in IV (4) of the *Feeds Regulation* and are, therefore approved for use in livestock feeds in Canada. Canola event 5715 has been found to be as safe and as nutritious as currently and historically grown canola varieties. Canola event 5715 and its products are considered to meet present ingredient definitions and are approved for use as livestock feed ingredients in Canad

Please note that the safety of tribenuron-methyl residues in feed ingredients after the application of tribenuron-me to canola event 5715 was not fully assessed. Any feed ingredient(s) derived from the combination of canola event and tribenuron-methyl may not be manufactured or sold in Canada, or imported into Canada, until an authorizatio

tribenuron-methyl application on canola event 5715 has been granted by PMRA (Pest Management Regulatory Agency).

Unconfined release into the environment and use as livestock feed of canola event 5715 is therefore authorized b the Plant Biosafety Office of the Plant Health and Biosecurity Directorate and the Animal Feed Division of the Ani Health Directorate as of December 3, 2013. Any canola lines derived from canola event 5715 may also be releas into the environment and used as livestock feed, provided that

i. no inter-specific crosses are performed,
ii. the intended uses are similar, and
iii. it is known based on characterization that these plants do not display any additional novel traits and are substantially equivalent to canola varieties that are currently grown and permitted to be used as livestock f in Canada, in terms of their potential environmental impact and livestock feed safety.

Additionally, with respect to its use as livestock feed, canola event 5715 must meet the restrictions specific to tribenuron-methyl treated feed set out in this authorization.

Canola event 5715 is subject to the same phytosanitary import requirements as unmodified canola varieties. Canc event 5715 is required to meet the requirements of other Canadian legislation, including but not limited to the requirements set out in 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 canc event 5715. The food safety decisions (http://www.hc-sc.gc.ca/fn-an/gmf-agm/appro/index-eng.php) are available the Health Canada web site.

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