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# Corn Producing Aryloxyalkanoate Dioxygenase-1 (AAD-1) - DAS-40278-9

## Novel Food Decision

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Issued by Health Canada's Food Directorate

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Health Canada has notified Dow Agrosciences Canada, Inc. that it has no objection to the food use of herbicide tolerant corn event DAS-40278-9. The Department conducted a comprehensive assessment of this corn event according to its *Guidelines for the Safety Assessment of Novel Foods*. These guidelines are based upon internationally accepted principles for establishing the safety of foods with novel traits.

The following provides a summary of the notification from Dow Agrosciences Canada, Inc. and the evaluation by Health Canada and contains no confidential business information.

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### 1. Introduction

DAS-40278-9 corn produces aryloxyalkanoate dioxygenase-1 (AAD-1), an enzyme which confers resistance to 2,4-dichlorophenoxyacetic acid (2,4-D) and certain aryloxyphenoxypropionate (AOPP, or "fop") herbicides. The herbicide tolerance was achieved through transformation of a conventional corn variety.

The assessment performed by Food Directorate evaluators determined: how DAS-40278-9 corn was developed; how the composition and nutritional quality of DAS-40278-9 corn compared to non-modified varieties; and what the potential is for DAS-40278-9 corn to be toxic or cause allergic reactions. Dow Agrosciences has provided data that demonstrates DAS-40278-9 corn is as safe and of the same nutritional quality as traditional corn varieties used as food in Canada.

The Food Program has a legislated responsibility for pre-market assessment of novel foods and novel food ingredients as detailed in Division 28 of Part B of the *Food and Drug Regulations (Novel Foods)*. Foods derived from DAS-40278-9 corn are considered novel foods under the following part of the definition of novel foods:

- "c) a food that is derived from a plant, animal or microorganism that has been genetically modified such that
  - (i) the plant, animal or microorganism exhibits characteristics that were not previously observed in that plant, animal or microorganism.

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This Novel Food Information document has been prepared to summarize the opinion regarding the subject product provided by the Food Directorate, Health Products and Food Branch, Health Canada. This opinion is based upon the comprehensive review of information submitted by the petitioner according to the *Guidelines for the Safety Assessment of Novel Foods*.

## 2. Development of the modified plant

DAS-40278-9 was genetically modified using *whiskers*-mediated transformation. In this method, embryonic plant cells in liquid suspension cultures are combined with the purified DNA expression cassette fragment and silicon carbide fibres, termed “whisker fibres”. Agitating the mixture promotes cellular uptake of the DNA. The cells were recovered on non-selective media followed by growth in media containing the AOPP herbicide R-haloxyfop in order to select directly for expression of the *aad-1* transgene. The surviving cells were grown into regenerated whole plants. The transferred DNA consisted of a linear plasmid fragment bearing an expression cassette for *aad-1* and regulatory elements to control expression.

## 3. Characterization of the Modified Plant

Southern blot analysis of DAS-40278-9 was presented to demonstrate that an intact copy of the gene cassette was inserted at a single locus in the corn genome. The integrity of the *aad-1* gene and regulatory elements was shown, and the absence of plasmid backbone DNA in DAS-40278-9 was confirmed. The stability and inheritance of the inserted cassette was also evaluated in several generations of DAS-40278-9 by Southern blot analysis. The results demonstrated the apparent molecular stability of the inserted cassette following a traditional breeding program. As well, the genetic segregation data showed that the AAD-1 trait was inherited and expressed in the expected Mendelian manner.

The identity of the AAD-1 protein was confirmed using mass spectrometry (MS) fingerprinting, N-terminal and tryptic peptide sequencing, electrophoresis, and immunological staining. Glycosylation analysis showed that AAD-1 was not post-translationally modified with sugars.

The expression levels of AAD-1 in plant tissues were determined using a validated double sandwich enzyme-linked immunosorbent assay (ELISA) on greenhouse-grown samples. All tissues of the transgenic plants tested positive for AAD-1 in this assay. The portion of the plant that enters the food supply is the grain, which is processed into refined corn oil, starch, and high fructose syrup. Grain harvested from DAS-40278-9 plants grown to maturity contained on average 4.81 ng AAD-1/mg of tissue (dry weight; range: 1.07 to 9.10 ng/mg). The level of protein appears to be in a similar range in plants treated with different combinations of the herbicides quizalofop and 2,4-D.

## 4. Product Information

DAS-40278-9 corn differs from traditional corn by the addition of the coding sequence of *aad-1* derived from *Sphingobium herbicidovorans*, a Gram negative soil bacterium that is not known to be a human pathogen. The *aad-1* gene in DAS-40278-9 is identical to its counterpart in *S. herbicidovorans* except that codons were modified to optimize gene expression in plants without altering the amino acid sequence, and an additional alanine codon was inserted at position 2 in order to facilitate cloning. Gene regulatory elements are included in the inserted DNA which control the expression and stability of the AAD-1 enzyme. Expression of AAD-1 imparts resistance to 2,4-dichlorophenoxyacetic acid (2,4-D) and certain aryloxyphenoxypropionate (AOPP, or “fop”) herbicides by catalyzing their degradation into herbicidally-inactive phenols.

## 5. Dietary Exposure

DAS 402378-9 corn is expected to be used in similar applications as traditional corn varieties by the food industry. The introduction of DAS-40278-9 corn will only replace a fraction of current corn varieties and is not anticipated to result in a change in the dietary intake of corn and corn-derived products.

## 6. Nutrition

Nutrient composition trials of DAS-40278-9 and a control corn line of the same genetic background were conducted at six sites located in the U.S. and Canada. The data was collected using an appropriate study design and accepted analytical methods.

The study included nutritional composition data on all corn matrices suggested in the OECD document ENV/JM/MONO(2002)25 on corn. Grain samples were analysed for key components including nutrients, anti-nutrients and secondary metabolites including the following: proximates (protein, fat, ash, moisture, cholesterol, carbohydrate); fibre (acid detergent fibre, neutral detergent fibre, total dietary fibre); minerals (calcium, chromium, copper, iodine, iron, magnesium, manganese, molybdenum, phosphorus, potassium, selenium, sodium, zinc); amino acids; fatty acids (C8-C22); vitamins (A, B1, B2, B5, B6, B12, C, D, E (alpha tocopherol), niacin, folic acid); secondary metabolites (coumaric acid, ferulic acid, furfural, inositol); and anti-nutrients (phytic acid, raffinose, trypsin inhibitor). Proximates, fibres and minerals (calcium and phosphorus) were also analyzed in corn forage.

The results from the compositional analysis showed that overall several analytes (32 of 80) in DAS-40278-9 corn grain were statistically different from the control in combined-site analyses; however, for most of these analytes (21 of 32) significant differences were not found consistently across all treatment groups or across individual sites. All remaining statistically significant differences were observed among the amino acids. However, the data from individual sites failed to produce similar results as no statistically significant adjusted p-values were noted for any amino acid from all 6 sites. These results indicated that the variation found in the levels of amino acids between different sites was higher than the variation that existed between DAS-40278-9 corn and the control variety within a site. Furthermore, the levels of all analytes from combined-site and individual site analyses were within the reported literature ranges.

Suitable insect, weed and disease control practices were applied to produce an agronomically acceptable crop. The temperatures and rainfall ranges data showed that no extreme cases were observed at any site. Samples for compositional analysis were collected at the appropriate growth stage of the plant.

#### 7. Chemistry/Toxicology

In terms of chemical safety considerations, it is expected that DAS-40278-9 corn would be no different from conventional corn, assuming that the ability of DAS-40278-9 corn to uptake heavy metals and its susceptibility to mycotoxin-producing fungi have not been altered relative to conventional corn.

The novel *aad-1* gene present in DAS-40278-9 was not derived from a pathogen. The AAD-1 protein is not considered likely to be a toxin based on acute toxicity studies in mice which were administered the protein at levels orders of magnitude greater than the hypothetical intake level from this crop in the human diet. Furthermore, AAD-1 bears no biologically significant sequence similarity to any known toxin.

AAAD-1 is also not considered to be an allergen as it does not share characteristics with food allergens. The amino acid sequence of the protein does not bear similarity to any known allergens. AAD-1 is quickly degraded in simulated gastric fluid as well as at high temperatures similar to those that would be used during the industrial milling process of corn commodities. This suggests that an intact protein would not likely be consumed. Given that corn is not a priority allergen in Canada, it is unlikely that DAS-40278-9 corn containing a small amount of AAD-1 protein would pose an allergenic risk to human health.

#### 8. Conclusion

Health Canada's review of the information presented in support of the food use of DAS-40278-9 corn does not raise concerns related to food safety. Health Canada is of the opinion that food derived from DAS-40278-9 is as safe and nutritious as food from current commercial corn varieties.

Health Canada's opinion deals only with the food use of DAS-40278-9 corn. Issues related to its use as animal feed have been addressed separately through existing regulatory processes in the Canadian Food Inspection Agency.

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