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**Canadian Food Inspection Agency,
Plant Health and Production Division,
Plant Biosafety Office**

Regulatory Directive Dir94-11: The Biology of *Zea mays* L. (Corn/Maize)

A companion document to the Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits

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Part A. General Information

A1.0 Background

Since 1988, Agriculture and Agri-Food Canada has been regulating the field testing in Canada of agricultural and horticultural crop plants with novel traits (PNT's). "Plants with novel traits" are defined as a plant variety/genotype possessing characteristics that demonstrate neither familiarity nor substantial equivalence to those present in a distinct, stable population of a cultivated species of seed in Canada and that have been intentionally selected, created or introduced into a population of that species through a specific genetic change. "Familiarity" is defined as the knowledge of the characteristics of a plant species and experience with the use of that plant species in Canada. "Substantial equivalence" is defined as the equivalence of a novel trait within a particular plant species, in terms of its specific use and safety to the environment and human health, to those in that same species, that are in use and generally considered as safe in Canada, based on valid scientific rationale.

The PNT's can either be derived from recombinant DNA technologies, or from traditional plant breeding. Regulated field testing is necessary when the PNT's have traits of concern, i.e., the traits themselves, their presence in a particular plant species or their use are: 1) considered unfamiliar when compared with products already in the market; 2) not considered substantially equivalent to similar, familiar plant types already in use, and regarded as safe.

Before PNT's may be authorized for unconfined release, they must be assessed for

environmental safety. Regulatory guidelines entitled *Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits* have been developed to define criteria and information requirements that must be considered in the environmental assessment of PNT's to ensure environmental safety, in the absence of confinement conditions.

A2.0 Scope

The present document is a companion document to the regulatory guidelines *Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits*. It is intended to provide background information on the biology of *Zea mays* L., its centres of origin, its related species, and the potential for gene introgression from *Zea mays* into relatives, and details of the life forms with which it interacts.

Such species-specific information will serve as a guide for addressing some information requirements of Part D of the regulatory guidelines. Specifically, it will be used to determine whether there are significantly different/altered interactions with other life forms, resulting from the PNT's novel gene products, which could potentially cause the PNT to become a weed of agriculture, become invasive of natural habitats, or be otherwise harmful to the environment.

The conclusions drawn in this document about the biology of *Zea mays* only relate to plants of this species with no novel traits. Novel traits of concern might confer new characteristics to the plant, that could impact on the environment pursuant to their unconfined release.

Part B. The Biology of *Zea mays*

B1.0 General Description, Cultivation, Use as a Crop Plant and Hybrid Production

Zea is a genus of the family Graminae (Poaceae), commonly known as the grass family. Maize (*Zea mays* L.) is a tall, monoecious annual grass with overlapping sheaths and broad conspicuously distichous blades. Plants have staminate spikelets in long spike-like racemes that form large spreading terminal panicles (tassels) and pistillate inflorescences in the leaf axils, in which the spikelets occur in 8 to 16 rows, approximately 30 long, on a thickened, almost woody axis (cob). The whole structure (ear) is enclosed in numerous large foliaceous bracts and a mass of long styles (silks) protrude from the tip as a mass of silky threads (Hitchcock and Chase, 1971).

Pollen is produced entirely in the staminate inflorescence and eggs, entirely in the pistillate inflorescence. Maize is wind pollinated and both self and cross pollination are usually possible. Shed pollen usually remains viable for 10 to 30 minutes, but can remain viable for longer durations under favorable conditions (Coe et al., 1988).

Cultivated maize is presumed to have been derived from teosinte (*Z. mexicana*) and is thought to have been introduced into the old world in the sixteenth century. Maize is cultivated worldwide and represents a staple food for a significant proportion of the world's population. No significant native toxins are reported to be associated with the genus *Zea* (International Food Biotechnology Council, 1990).

Cultivation and Use. Maize has been cultivated by the indigenous peoples of North America, including Canada, for thousands of years. The modern era of maize hybrid production in Canada began in the U.S. where research conducted in the early part of this century proved that hybrid maize could produce a yield superior to open-pollinated varieties (Sprague and Eberhart, 1977). Gradually, hybrid-derived varieties replaced the open-pollinated types in Canada in the 1930's and 1940's. In Canada, maize is grown primarily in Ontario and Quebec. Farmers must purchase new supplies of hybrid seed each season.

Maize is planted when soil temperatures are warm (greater than or equal to 10° C) usually early to mid-May in southern Ontario (OMAF, 1994) and Quebec (MAPAQ, 1984). Optimum yields occur when the appropriate hybrid maturity and population density are chosen. In addition, exogenous sources of nitrogen fertilizer are generally applied and weed and insect control measures are generally recommended. Choice of the appropriate hybrid for the intended growing area helps to ensure that the crop will mature before frost halts the growth of the plant at the end of the season; hybrids are categorized according to the amount of "heat units" that will be required for maturity. The maize-growing areas of Canada are illustrated on maps that indicate the number of heat units that they receive (e.g., OMAF, 1994; MAPAQ, 1984). Therefore, a hybrid developed for a specific heat unit zone, will not mature in (cooler) areas that receive fewer "heat units". Traditional cultivation practices in maize often result in bare soil which is susceptible to erosion by wind or water; increasingly, "no till" maize

is being grown in an effort to reduce this soil loss. For more information on specific cultivation practices, please contact the appropriate Canadian provincial agricultural authorities.

In 1993, there were more than 24 million ha planted to the crop in North America. Maize grown in North America is predominantly of the yellow dent type, a commodity crop largely used to feed domestic animals, either as grain or silage. The remainder of the crop is exported or processed by wet or dry milling to yield products such as high fructose maize syrup and starch or oil, grits and flour. These processed products are used extensively in the food industry, for example, maize starch serves as a raw material for an array of processed foods, and in industrial manufacturing processes. Since the early 1980's a significant amount of grain has also been used for fuel ethanol production. The by-products from these processes are often used in animal feeds. For a full discussion of the uses of maize see Watson (1988).

Hybrid Production. The production of hybrid seed requires the development and maintenance of inbred lines and subsequent controlled crosses to produce commercial seed. Self pollination is essential for inbred development while controlled cross pollination is mandatory for hybrid seed production. Mechanisms have been developed to ensure the correct form of pollination for each process and to prevent genetic contamination of seed stocks (Wych, 1988).

In breeding nurseries, receptive ear shoots are protected from unwanted pollination by ear shoot bags that cover the silks. Pollen is contained and collected in bags that cover the tassels. Controlled hand pollinations are then made by exposing the ear shoot on the selected female parent and covering it with the bag containing pollen from the selected male parent.

Breeder or foundation seed is produced from self pollinated seed after the eighth or ninth generation of inbreeding. A high degree of self pollination is assured by planting in blocks that are isolated by a distance of at least 200 meters from any other contaminating source of pollen.

Hybrid seed production is accomplished by interplanting rows of the male and female inbred parents (e.g., one row of male to four female rows). Hybrid seed production requires isolation similar to that for foundation seed. Self pollination of the female parent is prevented through detasseling prior to pollen shed or by the use of male sterile females.

Genetic conformity of inbreds and hybrids is monitored and assured through grow-outs of representative seed lots and laboratory screening using such criteria as isozyme profiles.

B2.0 The Centres of Origin of the Species

It is generally agreed that teosinte (*Z. mexicana*) is an ancestor of maize, although opinions vary as to whether maize is a domesticated version of teosinte, (Galinat, 1988).

Teosinte is an ancient wild grass found in Mexico and Guatemala. Because it has differentiated into various races, species and plant habits, taxonomic classification is still a matter of controversy. Doebley and Iltis (1980) and Iltis and Doebley (1980) classified the annual teosintes into two subspecies of *Z. mays*: ssp. *mexicana* (including races Chalco, Central Plateau and Nobogame) and ssp. *parviglumis*-var. *parviglumis* (race Balsas) and var. *huehuetenangensis* (race Huehuetenango) and the species *Z. luxurians* (race Guatemala). The perennial teosintes from Jalisco, Mexico are separated into two more species according to ploidy, *Z. perennis* and *Z. diploperennis*.

B3.0 Summary of the Ecology of *Z. mays* and its Progenitors

During its domestication from teosinte, maize has gained many agronomically significant attributes but it has lost the ability to survive in the wild. It has become so domesticated that seeds cannot be separated from the cob and disseminated without human intervention. Maize seeds show poor dormancy, especially in the northern ranges of the crop's distribution. Plants occasionally grow in uncultivated fields and by roadsides or occur as volunteers in cultivated crops in the year following cultivation of a maize crop. However, maize is incapable of sustained reproduction outside of domestic cultivation and is non-invasive of natural habitats (Gould, 1968). Some *Zea* species are successful wild plants in Central America, but they have no pronounced weedy tendencies (Galinat, 1988).

Part C. *Zea mays* Related Species

C1.0 Inter-Species/Genus Hybridization

Maize and other species and subspecies of teosinte are sexually compatible and can produce fertile hybrids (Wilkes, 1977). Related *Zea* species are geographically restricted and occur only in Mexico and Guatemala. The closest known relative of *Zea* is *Tripsacum*, a genus of eleven species, widely distributed between 42°N and 24°S latitude (de Wet et al., 1981). Three species occur in the United States, two of which, *Tripsacum floridanum* (Florida Gamagrass) and *Tripsacum lanceolatum* (Mexican Gamagrass), are confined to the southernmost states of the United States. Only one, *Tripsacum dactyloides* (Eastern Gamagrass), has a distribution that includes the northern (U.S.) maize belt (Gould, 1968).

C2.0 Potential for Introgression of Genetic Information from *Z. mays* into Relatives

An examination of the literature prior to 1980 would lead to the conclusion that there is constant gene flow between maize and teosinte, and that the weedy teosinte (*Z. mays* ssp. *mexicana*) is a hybrid of the two sub-species, and functions as a genetic bridge between the two (de Wet and Harlan, 1972; de Wet, 1975; Galinat, 1973).

However, this premise has been re-evaluated using techniques of gene mapping, which failed to show any evidence of recent introgression between maize and teosinte (Smith et al., 1985). Moreover, *Z. mays* ssp. *mexicana* seems not to be a hybrid of the wild and cultivated forms of *Zea* and therefore probably does not serve as a genetic bridge; physical similarities being due to parallel adaptation to the same habitat (Doebley, 1984).

There is evidence of highly restricted gene flow between *Zea* spp. that apparently occurs predominantly from teosinte into maize (Doebley et al, 1987).

Tripsacum and *Zea* have different chromosome numbers ($n = 9$ versus $n = 10$). Crosses between *Z. mays* and *T. dactyloides* can be made, but only through human intervention and, even then, only with extreme difficulty. Moreover, the progeny are frequently sterile or genetically unstable (Manglesdorf, 1974). The process of transferring *Tripsacum* germplasm into maize is technically difficult. The transmission rate of the single extra *Tripsacum* chromosome added to the genome is so low and the rate of maize *Tripsacum* crossing over so reduced, as to practically exclude the general use of experimentally-introduced *Tripsacum* germplasm in maize improvement (Galinat, 1988).

C3.0 Occurrence of *Z. mays* and Related Species in Canada

Of the *Zea* species, only *Z. mays* is common to Canada and is known only from cultivation (Gould, 1968). *T. dactyloides* occurs from Massachusetts to Michigan in the United States (Hitchcock and Chase, 1971), but it has not been reported in Canada.

C4.0 Summary of the Ecology of Relatives of *Z. mays*

With the possible exception of *T. dactyloides*, relatives of *Z. mays* do not occur in Canada. *T. dactyloides* is not weedy nor invasive of natural habitats and is extremely unlikely to form fertile hybrids with cultivated *Z. mays*. *T. dactyloides* is not regarded as a noxious weed.

In the absence of sexually compatible species related to *Z. mays* in Canada, there is no potential for interspecific gene flow to occur.

Part D. Potential Interactions of *Z. mays* with Other Life Forms

Table 1 is intended to be used to guide applicants in their considerations of potential impacts of the release of the PNT on non-target organisms.

The intention is not to require comparison data between the PNT and its *Z. mays* counterpart (s) for all interactions. Depending on the novel traits, applicants might decide to submit data for only some of the interactions. Sound scientific rationale will be required to justify the decision that data would be useless or irrelevant for the remaining interactions. For example, the applicant might choose not to provide data on the potential for gene transfer from the PNT to related species if it can be clearly shown that the novel trait will not affect reproductive characteristics of *Z. mays*, either directly or indirectly.

Some of the life forms are listed as categories (i.e., pollinators, mycorrhizal fungi, animal browsers, birds, soil microbes, and soil insects). When, because of the novel traits, a concern is perceived for these specific categories, applicants will be required to provide detailed information on interactions with indicator species in each category.

Where the impact of the PNT on another life form (target or non-target organism) is significant, secondary effects may need to be considered.

Table 1: Potential interactions of *Z. mays* with other life forms during its life cycle (information requirements may be waived if valid scientific rationale is provided).

Other life forms <i>Common Name</i>	Interaction with <i>Z. mays</i> Pathogen; Symbiont or Beneficial Organism; Consumer; Gene Transfer
<i>Gibberella zeae</i> Gibberella stalk and ear rot	Pathogen
<i>Fusarium</i> spp. Fusarium seedling blight, root, stalk and kernel rot	Pathogen
<i>Pythium</i> spp. Pythium seedling blight and root rot	Pathogen
<i>Rhizoctonia</i> spp. Rhizoctonia seedling blight and root rot	Pathogen
<i>Setosphaeria turcicum</i> Northern corn leaf blight	Pathogen
<i>Ustilago maydis</i> Corn smut	Pathogen
<i>Colletotrichum graminicola</i> Anthracnose stalk rot and leaf blight	Pathogen
<i>Puccinia sorghi</i> Leaf rust	Pathogen
<i>Sphacelotheca reiliana</i> Head smut	Pathogen
<i>Erwinia stewartii</i> Bacterial leaf blight	Pathogen
<i>Pseudomonas syringae</i> Holcus spot	Pathogen
<i>Ostrinia nubilalis</i> European corn borer	Consumer
<i>Diabrotica</i> spp. Corn rootworm	Consumer
<i>Diabrotica</i> spp. Corn rootworm	Consumer
<i>Elaterid</i> spp. Wireworm	Consumer
<i>Pseudaletia unipuncta</i> Armyworm	Consumer
<i>Agrotis ipsilon</i> Black cutworm	Consumer
<i>Papaipema nebris</i> Stalk borer	Consumer
<i>Heliothis zea</i> Corn earworm	Consumer
<i>Chaetocnema pulicaria</i>	

Corn flea beetle	Consumer
<i>Rhopalosiphum maidis</i>	
Corn leaf aphid	Consumer
<i>Nitidulid</i> spp.	
Sap beetles	Consumer
<i>Delia patura</i>	
Seedcorn maggot	Consumer
Beneficial insects	Symbiont or Beneficial Organisms
Birds	
e.g., crows	Consumer
Animal browsers	
e.g., deer, raccoon	Consumer
Soil microbes	Symbiont or Beneficial Organisms; Consumer
Earthworms	Symbiont or Beneficial Organisms; Consumer
Soil insects	Symbiont or Beneficial Organisms; Consumer
Other <i>Zea mays</i>	Gene Transfer

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