Safety, Compositional and Nutritional Aspects of Insect-Protected Corn Lines MON 809 and 810:

Conclusion Based on Studies and Information Evaluated According to FDA's Policy on Foods from New Plant Varieties

Prepared by

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Abbreviations Used in this Summary of the Safety, Compositional, and Nutritional Aspects of Insect-Protected Corn Lines MON 809 and 810

2,4-D	(2,4-dichlorophenoxy)acetic acid
APHIS	Animal Plant Health Inspection Service
bp, Kb	Base pairs, kilobase pairs
B.t.k.	*Bacillus thuringiensis* subsp. *kurstaki*
CaMV	Cauliflower mosaic virus
CFR	Code of federal regulations
CP4 EPSPS	EPSPS from *Agrobacterium* sp. strain CP4
cryIA(b)	Class I (Lepidoptera-specific) crystal protein gene
CTP	Chloroplast transit peptide
DNA	Deoxyribonucleic Acid
E. coli	*Escherichia coli*
E35S	35S promoter with enhancer sequence
ECB	European corn borer
ELISA	Enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
FDA	Food and Drug Administration
FFDCA	Federal Food Drug and Cosmetic Act
FIFRA	Federal Insecticide Fungicide and Rodenticide Act
GLP	Good Laboratory Practice
gox	Gene for glyphosate oxidase
GOX	Glyphosate oxidase
hsp70	Intron sequence from heat-shock protein 70
I-DNA	Integrated-DNA
IPM	Integrated Pest Management
kD	Kilodaltons
N.A.	Not analyzed
N.D.	Not detected
NOS 3'	3' transcriptional termination sequence from nopaline synthase
NPTII	Neomycin phosphotransferase II
nptII	Gene for neomycin phosphotransferase II
ori-pUC	Bacterial origin of replication from the pUC plasmid
subsp.	Subspecies
USDA	United States Department of Agriculture

INFORMATION TO SUPPORT THE HUMAN FOOD/ANIMAL FEED SAFETY OF INSECT-PROTECTED CORN LINES MON 809 and 810

I. INTRODUCTION

A. Subject of Request

Monsanto has developed genetically modified corn plants that control European corn borer (ECB), an economically damaging lepidopteran insect pest. Insect-protected corn lines MON 809 and 810 contain a *cryIA(b)* gene which produces an insect control protein (CryIA(b)) derived from the common soil bacterium *Bacillus thuringiensis* subsp. *kurstaki* (*B.t.k.*). In addition, the 5-enolpyruvylshikimate-3-phosphate synthase gene from *Agrobacterium* sp. strain CP4 (CP4 EPSPS) was introduced into line MON 809 to confer tolerance to glyphosate as a selectable marker in initial laboratory stages of plant cell selection. This line is not tolerant to commercially acceptable levels of glyphosate and therefore would only be marketed as an insect-protected corn product. Monsanto is filing this summary of the safety, compositional and nutritional aspects of lines MON 809 and 810 with FDA based on the studies and information evaluated according to FDA's policy on foods from new plant varieties (FDA, 1992). A detailed description of these corn lines follows in this summary and in the data as submitted and referenced.

B. Application of FDA Food Policy

In its May 29, 1992 statement of policy concerning "Foods Derived from New Plant Varieties," ("Food Policy" or the "Policy"), the Food and Drug Administration ("FDA") provided guidance for determining whether a new plant variety developed with the aid of new genetic techniques is as safe and nutritious as its parental variety (See 57 Fed. Reg. 22965). The Policy is structured around decision trees that are designed to establish whether the new plant variety is materially different in composition, safety or any relevant parameter from its parental variety. The Monsanto Company has carefully followed the guidance in the Policy to assess whether corn modified by the addition of genes producing a lepidopteran insect control protein derived from *Bacillus thuringiensis* subsp. *kurstaki* (*B.t.k.*) and the selectable marker CP4 EPSPS are materially different from corn currently being marketed. To ensure as thorough an assessment as possible, Monsanto has conducted numerous compositional studies and accessed all existing relevant data and information. Upon qualitatively and quantitatively evaluating all of the data available, we have been able to ensure that there are no biologically important compositional differences between insect-protected corn lines MON 809 and 810 and the parental line or other corn varieties grown commercially. This assessment is summarized in this submission in a manner consistent with the Policy. The conclusions are straightforward: except for protection against certain lepidopteran insects imparted by the

CryIA(b) protein in both corn lines and the presence of the CP4 EPSPS selectable marker protein in line MON 809, these lines are not materially different from and are as safe and nutritious as corn varieties now marketed.

C. The CryIA(b) Protein and the Selectable Marker

The use of the CryIA(b) protein in lines MON 809 and 810 is regulated by the EPA as a plant pesticide. The CP4 EPSPS protein present in MON 809 has no insecticidal effect and is currently being evaluated by the EPA as a plant-pesticide inert ingredient. Both the CryIA(b) and CP4 EPSPS proteins have recently received temporary exemptions from the requirement of a tolerance in all raw agricultural commodities of field corn, sweet corn and popcorn.

The CP4 EPSPS protein is equivalent to the CP4 EPSPS protein which was reviewed as part of Monsanto's consultation with the FDA under the "Statement of Policy: Foods Derived from New Plant Varieties" regarding glyphosate tolerant soybeans and cotton. As noted above, our extensive studies and information collected establish that the presence of these proteins and the process used to produce the new plants result in no material difference between lines MON 809 and 810 and the parental line.

The FDA Policy discusses at some length the jurisdictional issues presented upon federal review and consideration of plants modified to express pesticidal substances, (FDA, 1992). The discussion recognizes not only the legal basis for one agency assuming priority of review and decision making on aspects of an issue but also the efficiency inherent in such a process. In fact, FDA's Policy is premised on the practical application of existing statutory provisions in an effort to minimize duplication in the regulation of genetically engineered crops. In particular, the Policy is designed to eliminate repetitive expenditure of federal resources on the same issues and to focus only on those issues appropriate for a decision. This reflects sound regulatory and public health decision making.

The effect of this policy is to allocate the safety review of aspects of lines MON 809 and 810 between the FDA and EPA. The safety of the CryIA(b) and CP4 EPSPS proteins is being thoroughly evaluated by the EPA on the basis of scientific and empirical information submitted to that agency by Monsanto. It is anticipated that evaluation of our submission by the EPA, will shortly result in the registration of the CryIA(b) protein as a plant pesticide and its exemption from the requirement of the tolerance governing such use. A similar exemption is expected for CP4 EPSPS as a plant pesticide formulation inert ingredient. The regulatory and scientific decisions by the EPA, will establish the safety of the CryIA(b) and CP4 EPSPS proteins in these lines . All other issues that pertain to the food and feed safety of corn fall within the compass of the FDA's review and are addressed at length in this submission.

In accordance with this policy, Monsanto will seek the following approvals before commercializing insect-protected corn lines MON 809 and 810:

- A Determination from USDA/APHIS that lines MON 809 and 810, and all progenies derived from crosses between these lines and other corn cultivars, are no longer regulated articles according to 7CFR §340.6. This petition was approved March 11, 1996 (Croon *et al.*, 1996).

- Regulatory approval from the EPA of the CryIA(b) insecticidal protein as expressed in lines 809 and 810 under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). This petition was submitted in January, 1995. In addition, this same petition requests that EPA establish an exemption from the requirement of a tolerance for the CryIA(b) insecticidal protein, under section 408 of the Federal Food Drug and Cosmetic Act (FFDCA). A temporary exemption from the requirement of a tolerance for the CryIA(b) protein in all raw agricultural commodities of field corn, sweet corn and popcorn was issued on April 25, 1996.

- EPA exemption from the requirement of a tolerance for CP4 EPSPS as a plant pesticide formulation inert ingredient. This petition was submitted in May, 1995. A temporary exemption from the requirement of a tolerance for the CP4 EPSPS protein in all raw agricultural commodities of field corn, sweet corn and popcorn was issued on April 25, 1996.

D. Consultations with FDA

The submissions to the EPA and USDA fully support the environmental and non-target safety of these corn lines and the proteins produced. Therefore, we will conclude our consultations with FDA on the safety and wholesomeness of the non-pesticidal components of lines MON 809 and 810, under the Statement of Policy "Foods Derived From New Plant Varieties", published in the Federal Register May 29, 1992 (FDA, 1992). We have held consultations with FDA, starting in March 1995, to define and discuss studies to assess the composition and safety of new corn varieties derived through genetic modification including insect-protected varieties. The concepts and approaches we have employed are derived from and consistent with the guidance presented in the flow charts found in the FDA Food Policy (FDA, 1992). For each question, we have developed answers based on extensive studies or analyses. The thoroughness and detail of these studies are unprecedented for the typical introduction of foods or feeds from a new plant variety. Our data and findings in every case have led us to the conclusion of "no concern", as described in the relevant sections of the following summary. Under these circumstances, following the Agency's Food Policy, the data have provided us with a basis for concluding that lines MON 809 and 810 are as safe and nutritious as corn varieties grown commercially.

II. RATIONALE FOR THE DEVELOPMENT OF INSECT-PROTECTED CORN

A. Rationale for the Development of Insect-Protected Corn

Corn is the largest crop in the United States in terms of planted acreage, total production, and crop value (National Corn Growers Association, 1995). United States production in 1994 was estimated at 256 million metric tons produced on nearly 73 million acres with the majority of national production concentrated across what is known as the "Corn Belt" in the upper Midwest. The European corn borer (ECB), *Ostrinia nubilalis* (Hübner), causes severe economic damage as it feeds on leaf and stalk tissue compromising the structural integrity of the corn plant (Dicke and Guthrie, 1988; Cooperative Extension Service, 1989). This feeding damage leads to plant lodging and yield loss. Chemical insecticides offer limited utility as applications must be made prior to the time the insect bores into the stalk and repeat applications are often necessary. As one of the most important pests of corn in the United States, it is estimated that ECB causes an average five to ten percent crop production loss annually in corn with potentially greater losses in areas of high infestation (Bergman *et al.*, 1985a-f ; Bode and Calvin, 1990; Briggs & Guse 1986; Guthrie *et al.*, 1975; Rice, 1994a-c).

Monsanto has developed genetically modified corn plants that effectively control ECB throughout the growing season. These genetically modified corn plants produce an insect control protein (CryIA(b)) derived from the common soil bacterium *Bacillus thuringiensis* subsp. *kurstaki* (*B.t.k.*). The CryIA(b) protein produced by insect-protected corn lines MON 809 and 810 is identical to CryIA(b) protein found in nature and in commercial microbial formulations (EPA MRID no. 43533203). Microbial formulations containing these insecticidal proteins have been registered by the EPA and commercially available since 1961 (EPA, 1988; Lüthy *et al.*, 1982).

The CryIA(b) and CP4 EPSPS proteins were expressed at low levels in corn leaf, seed, and whole plant tissues from lines MON 809 and 810. Environmental fate studies have shown that the CryIA(b) protein is rapidly degraded in the soil and does not persist in the environment (EPA MRID no. 43533206). In addition, the risk of an uncontrolled introduction of the genes through hybridization or outcrossing to a native species is virtually nonexistent in the United States, as no wild relatives grow with which insect-protected corn can cross (Croon *et al.*, 1995a; Croon *et al.*, 1996). Finally, no differences in agronomic quality, disease, or insect susceptibility other than targeted lepidopteran insect control, were detected between lines MON 809 and 810 and non-transgenic plants (Croon *et al.*, 1996).

Results from field experiments conducted in 1993, 1994 and 1995 throughout the corn growing regions have demonstrated that insect-protected corn is protected season long from the leaf and stalk feeding damage caused by ECB. Growers planting insect-protected corn will not require insecticide applications to control this pest. This reduction in insecticide use will enhance biological control and the implementation of other pest management strategies for other

corn pests not susceptible to the CryIA(b) protein such as spider mites and aphids.

In support of Monsanto's request to the EPA for the registration and exemption from the requirement of a tolerance for the CryIA(b) protein as a plant pesticide, studies demonstrating the safety of this protein to nontarget organisms were conducted. These studies demonstrated that the protein has a limited spectrum of insecticidal activity with no deleterious effect on beneficial nontarget insects, mammals, birds, fish and soil arthropods (EPA MRID nos. 43468001 through 43468005, 43439202, 43439203, 43533205, 43887901, 43887902, and 43941601). These results fully confirm the findings of similar studies conducted with commercially available microbial *B.t.* formulations.

The commercialization of insect-protected lines MON 809 and 810 (and any progenies derived from crosses between lines MON 809 and 810 and traditional corn varieties), following receipt of all required approvals, will represent an efficacious and environmentally compatible addition to the existing options for corn insect pest management. The use of insect-protected corn will provide potential benefits to growers, the general public and the environment, including:

- A more reliable, economical, and less labor intensive means to control targeted lepidopteran insect pests including ECB.
- Insect control without harming non-target species, including humans.
- A means for growers to significantly reduce the amount of chemical insecticides now applied to the crop thereby achieving ECB control in a more environmentally compatible manner than is currently available.
- A reduction in the manufacturing, shipment, and storage of chemical insecticides used in corn.
- A reduction in the exposure to workers to the pesticide and pesticide spray solution.
- A reduction in the number of empty pesticide containers and amount of pesticide spray solution that must be disposed of according to applicable environmental regulations.
- An ideal fit with Integrated Pest Management (IPM) and sustainable agricultural systems.
- A potential reduction in the occurrence of stalk rots associated with ECB damage to the corn ear and associated production of harmful mycotoxins.
- Both large and small growers will benefit from the planting of insect-protected corn as no additional labor, planning, or machinery is required.

In summary, the consistent control afforded by insect-protected corn lines MON 809 and 810 will enable growers to significantly reduce the amount of chemical insecticide now applied to their crop for control of ECB while maintaining yield potential. As a result, they will be able to utilize IPM practices that cannot presently be implemented because of the lack of options other than use of chemical insecticides to control this pest. An increase in the biological and cultural control of non-target corn pests and a more judicious use of chemical insecticides will result in a positive impact on the environment, which will ultimately be advantageous to the grower and the public as well.

B. Benefits of Insect-Protected Corn

Agronomic Benefits of Corn Genetically Modified to Resist European Corn Borer and Other Lepidopteran Pests

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Summary

Insect-protected corn will provide excellent control of an insect (European corn borer) that causes significant decreases in corn yields every year in North America. Results from field experiments with Monsanto's genetically modified corn expressing delta-endotoxin proteins of *Bacillus thuringiensis* var. *kurstaki* (insect-protected corn) revealed a high level of efficacy against European corn borers, *Ostrinia nubilalis* (Hübner), in 1994 (Barrido and Steffey, 1995). The results from field trials in Kansas indicated that Monsanto's transgenic corn also is efficacious against southwestern corn borers, *Diatraea grandiosella* Dyar ([redacted] unpublished data). Southwestern corn borers occur in areas of the western Corn Belt where this pest causes yield reductions. Control of other lepidopteran pests with insect-protected corn may also be possible, and is currently being investigated for armyworms, corn earworms, fall armyworms, and stalk borers in corn.

Currently, corn growers in the eastern Corn Belt treat relatively few acres annually with insecticides to control European corn borers. Corn growers in Colorado, Kansas, and Nebraska treat comparatively more acres to control corn borers. However, yield losses attributable to corn borer damage are appreciable throughout its range. One study in Illinois (Briggs and Guse, 1986) revealed that approximately 10 percent of the corn acres in that state experience a 9- to 15-percent yield loss annually, attributable solely to the damage caused by the second generation of corn borers. [At least two generations of this pest occur annually throughout most of the Corn Belt.] Results from several studies suggest that corn borers cause an estimated 5 to

7.5 percent yield loss annually (first and second generations combined) (Bergman *et al.*, 1985a-f; Bode and Calvin, 1990). These data induce entomologists throughout the United States to consider the European corn borer to be the most under-scouted and under-treated insect that attacks corn. Because European corn borers cause primarily physiological reductions in yield, corn growers are not aware of the significance of their feeding injury during years when infestations are moderate. In addition, efficacy of insecticides applied for control of corn borers is often less than acceptable, particularly for the second generation. Both timing of insecticide applications and placement of the insecticide where corn borer larvae are feeding are difficult. Corn growers frequently are dissatisfied with the level of control of corn borers provided by both chemical and microbial insecticides.

The only other management tactic currently utilized for management of European corn borers is planting of resistant or tolerant corn hybrids (Hudon and Chiang, 1985). Entomologists and corn breeders have attempted for many years to develop hybrids resistant to European corn borers. However, although some hybrids are resistant to first-generation corn borers, none are resistant to second-generation borers (Hudon *et al.*, 1989). Some hybrids also have the ability to tolerate an infestation of corn borers (Steffey *et al.*, 1992). Nevertheless, planting of corn hybrids specifically because they are resistant to European corn borers is not widespread, and tolerant hybrids often do not yield as well when infestations of corn borers are heavy.

Insect-protected corn promises to be a profound breakthrough in corn insect management. Corn growers who plant insect-protected corn will experience yield protection during years when infestations of European corn borers are moderate to large. The potential for substantial reduction or virtual elimination of insecticide use for corn borer control is real. Additionally, the selective activity of the *B.t.k.* endotoxins will not disrupt populations of either beneficial insects or nontarget animals (e.g., birds, fish) (Pilcher and Obrycki, 1994; EPA, 1988). Applications of conventional chemical insecticides often affect nontarget species.

The development of insect-protected corn may become a foundation for corn insect management throughout the United States. Reduced insecticide use and improved yields are the likely outcomes of implementation of this technology. If growing insect-protected corn effectively eliminates all insecticide applications for European corn borers, corn growers would save a conservative $50 million annually. [This figure was derived from an estimate of 5 percent of the acres of corn treated with insecticides for corn borer control and $15 per acre control costs (insecticide + application costs).] The yield protection benefits gained from controlling corn borer infestations are between 1 and 1.5 billion dollars. [This figure was derived from annual estimates of 70 million acres of corn, an average yield of 120 bushels per acre, an average corn price of $2.35 per bushel, and an estimated 5 to 7.5 percent yield loss attributed to corn borer damage.]

The development of insect-protected corn will have a major impact on corn pest

management. The reduction in the use of aerially applied insecticides will preserve many beneficial insects, and the integration of insect-protected corn with other forms of resistance or tolerance will provide solid footing for the development of nonchemical technologies for other major insect pests.

III. MOLECULAR CHARACTERIZATION OF INSECT-PROTECTED CORN LINES MON 809 and 810

Introduction

Insect-protected corn lines MON 809 and 810 were produced with a DNA solution containing the two plasmids PV-ZMBK07 and PV-ZMGT10. The *cryIA(b)* gene was inserted to confer resistance to certain lepidopteran insects while the CP4 EPSPS and *gox* genes produce proteins to confer tolerance to glyphosate, a selective agent used to identify plant cells expressing the *cryIA(b)* gene. In addition to these three genes, a *nptII* gene which produces the enzyme neomycin phosphotransferase II (NPTII) was present in the two vectors under the control of its own bacterial promoter, to enable selection in bacterial systems.

A. The Donor Genes

1. *cryIA(b)* gene

The *cryIA(b)* gene used to produce insect-protected corn lines MON 809 and 810 is a modification of the *cryIA(b)* gene isolated from the DNA of *Bacillus thuringiensis* subsp. *kurstaki* strain HD-1 and is designated *cryIA(b)* according to the nomenclature of Höfte and Whitely (1989). The native full length gene encoding the CryIA(b) protein and its complete nucleotide sequence were described by Fischhoff *et al.* (1987).

2. CP4 EPSPS marker gene

The CP4 EPSPS gene was used as a plant selectable marker to identify the rare corn cells that originally received the introduced *cryIA(b)* gene responsible for insect protection in the initial, laboratory stages of plant cell selection following transformation. The mode of action of glyphosate is the inhibition of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), an enzyme involved in the shikimic acid pathway for aromatic amino acid biosynthesis in plants and microorganisms (Steinruken and Amrhein, 1980). Corn plants tolerant to glyphosate at the laboratory level were produced by stably inserting the CP4 EPSPS and/or *gox* genes into the chromosome of corn.

The CP4 EPSPS gene from *Agrobacterium* sp. strain CP4 is contained in plasmid PV-ZMGT10. The gene for CP4 EPSPS has been completely sequenced and encodes a 47.6 kD protein consisting of a single polypeptide of 455 amino acids. The EPSPS from *Agrobacterium* sp. strain CP4 is naturally

highly tolerant to inhibition by glyphosate and has high catalytic activity, compared to most glyphosate tolerant EPSPSs (Barry *et al.*, 1992; Padgette *et al.*, 1991). Upon glyphosate treatment, the corn cells expressing the CP4 EPSPS are tolerant because the CP4 EPSPS continues to meet the plant's need for aromatic compounds in the presence of glyphosate. The bacterial isolate, CP4, was identified by the American Type Culture Collection as an *Agrobacterium* species. There is no human or animal pathogenicity known from *Agrobacterium* species, nor is the EPSPS gene a determinant of *Agrobacterium* plant pathogenesis.

The plant produced EPSPSs are present in the chloroplast. Therefore, the chloroplast transit peptide coding sequence, CTP2, from *Arabidopsis thaliana* EPSPS (Klee and Rogers, 1987) was fused to the N-terminus of the CP4 EPSPS protein to deliver the CP4 EPSPS to the chloroplasts, the site of EPSPS activity and glyphosate action ([redacted] and Shah, 1988). CTPs are typically cleaved from the "mature" protein following delivery to the plastid (della Cioppa *et al.*, 1986). The deduced amino acid sequence of the CP4 EPSPS protein including CTP2 is given in Figure III.13.

3. *gox* marker gene

The *gox* gene, cloned from *Achromobacter* sp. strain LBAA was also inserted as a selectable marker in plants. The *gox* gene sequence is contained in plasmid PV-ZMGT10. The gene encodes a 46.1 kD protein and was isolated from *Achromobacter* sp. strain LBAA (Barry *et al.*, 1992). The GOX protein is targeted to the plastids with a chloroplast transit peptide sequence, CTP1 derived from the SSU1A gene from *Arabidopsis thaliana* (Timko *et al.*, 1988). GOX degrades glyphosate by converting glyphosate to aminomethyl-phosphonic acid (AMPA) and glyoxylate and has no pesticidal effect. Although the *gox* gene sequence is present in line MON 809 including the CTP, the GOX protein has not been detected by ELISA or western analysis in the seeds or leaves of this corn line. The deduced amino acid sequence of GOX containing CTP1 is shown in Figure III.14.

4. *nptII* bacterial marker gene

The bacterial selectable marker gene, *nptII* , isolated from the prokaryotic transposon, Tn5 (Beck *et al.*, 1982), encodes for the enzyme neomycin phosphotransferase II which confers resistance to the aminoglycoside antibiotics (i.e., kanamycin or neomycin) used for selection of plasmids in *E. coli*. The promoter for this gene is only active in bacterial hosts. The deduced amino acid sequence is given in Figure III.15.

B. Particle Acceleration Transformation System

Plasmid DNA was introduced into the plant tissue by the particle acceleration method described by Klein *et al.* (1987). DNA is precipitated onto microscopic tungsten or gold particles using calcium chloride and spermidine. A drop of the coated particles is then placed onto a plastic macrocarrier, which is accelerated

at a high velocity through a barrel by the explosive force of a gunpowder discharge. The macrocarrier hits a plastic stopping plate which stops the flight of the macrocarrier but allows continued flight of the DNA-coated particles. The particles penetrate the target plant cells, where the DNA is deposited and incorporated into the cell chromosome. The cells are incubated on a tissue culture medium containing 2,4-D which supports callus growth. The introduced DNA contains genes encoding for herbicide tolerance (e.g., the CP4 EPSPS and GOX genes conferring tolerance to glyphosate). The plant cells are grown in the presence of glyphosate and only the transformed cells continue to grow. Plants are regenerated from the tolerant callus tissue, and are assayed for the presence of the expressed CryIA(b) protein product.

Although the DNA solution used for transformation contained genes encoding for glyphosate tolerance (e.g., the CP4 EPSPS and *gox* genes) allowing selection of genetically modified cells on media containing glyphosate, these genes are not present in MON 810 plants. Therefore it is likely that the cell that resulted in the MON 810 line is an "escape" from glyphosate selection (with cells in the vicinity degrading the available glyphosate, for instance, allowing the cell containing the MON 810 insert to survive). In the subsequent phase, plants were regenerated from the callus tissue, in the absence of glyphosate and were assayed for the presence of the expressed CryIA(b) protein product.

C. Construction of the Plasmid Vectors, PV-ZMBK07 and PV-ZMGT10, Utilized for Transformation

Corn lines MON 809 and 810 were produced by transforming the corn genotype Hi-II with a DNA solution containing two plasmids PV-ZMBK07 and PV-ZMGT10. The PV-ZMBK07 plasmid contains the *cryIA(b)* gene and PV-ZMGT10 contains the CP4 EPSPS and *gox* genes. Both plasmids contain the *nptII* gene under the control of a bacterial promoter and an origin of replication from a pUC plasmid, required for selection and replication of the plasmids in bacteria, respectively. A description of the DNA elements in PV-ZMBK07 and PV-ZMGT10 are given in Tables III.1 and III.2, respectively.

1. Plant expression vector - PV-ZMBK07

The plasmid vector PV-ZMBK07 (Figure III.1) contains the *cryIA(b)* gene under the control of the enhanced CaMV 35S promoter (E35S) (Kay *et al.*, 1985 and Odell *et al.*, 1985), which is approximately 0.6 Kb in size. Located between the E35S promoter and the *cryIA(b)* gene is the 0.8 Kb intron from the maize *hsp70* gene (heat-shock protein), present to increase the levels of gene transcription (Rochester *et al.*, 1986). The *hsp70* intron is followed by the 3.46 Kb *cryIA(b)* gene. The *cryIA(b)* gene is joined to the 0.26 Kb nopaline synthase 3' nontranslated sequence, NOS 3', (Fraley *et al.*, 1983) which provides the mRNA polyadenylation signal.

The *cryIA(b)* gene is 3468 nucleotides in length and encodes a full-length *B.t.k.* HD-1 [CryIA(b)] protein of 1156 amino acids (Fischhoff *et al.*,

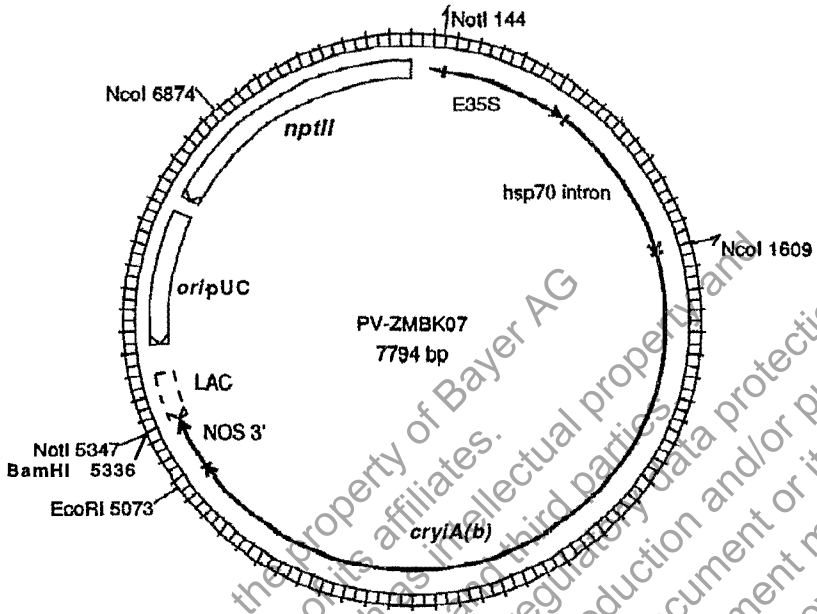


Figure III.1 Plasmid map of PV-ZMBK07. Restriction sites, and their locations in base pairs, used during Southern analyses are shown.

Table III.1 Summary of DNA elements in the plasmid PV-ZMBK07

Genetic Element	Size Kb	Function
E35S	0.61	The cauliflower mosaic virus (CaMV) promoter (Odell *et al.*, 1985) with the duplicated enhancer region (Kay *et al.*, 1985).
*hsp*70	0.80	Intron from the maize *hsp70* gene (heat-shock protein) present to intron increase the level of gene transcription (Rochester *et al.*, 1986).
cryIA(b)	3.46	The gene encodes the nature identical CryIA(b) protein product (Fischhoff *et al.*, 1987).
NOS 3′	0.26	A 3′ nontranslated region of the nopaline synthase gene which terminates transcription and directs polyadenylation (Fraley *et al.*, 1983).
lacZ	0.24	A partial *E. coli lacI* coding sequences, the promoter Plac, and a partial coding sequence for beta-D-galactosidase or *lacZ* protein from pUC119 (Yanisch-Perron *et al.*, 1985).
ori-pUC	0.65	The origin of replication for the pUC plasmids that allows for plasmid replication in *E. coli* (Vieira and Messing, 1987).
nptII	0.79	The gene for the enzyme neomycin phosphotransferase type II. This enzyme confers resistance to aminoglycoside antibiotics and thereby allows for selection of bacteria containing the plasmid (Beck *et al.*, 1982).

1987), which when subjected to trypsin yields an active trypsin-resistant protein product of approximately 600 amino acids *in planta* and *in vitro* ([redacted] 1995). The *cryIA(b)* gene sequence was modified to increase the levels of expression in corn (Perlak *et al.*, 1991). The *cryIA(b)* gene encodes the nature identical CryIA(b) protein product (Fischhoff *et al.*, 1987). The deduced amino acid sequence for the CryIA(b) protein is given in Figure III.12.

The *alpha* region of the *lacZ* gene for beta-galactosidase, present under a bacterial controlled promoter, is present in PV-ZMBK07. This region contained a polylinker (region of multiple cloning sites) which allowed for the cloning of the desired genes within the plasmid vector (Vieira and Messing, 1987). The *lacZ-alpha* region is followed by the 0.65 Kb origin of replication for the pUC plasmids (*ori-pUC*) and which allows for the replication of plasmids in *E. coli* (Vieira and Messing, 1987).

Following the *ori-pUC* region is the gene for the enzyme neomycin phosphotransferase type II (*nptII*). This enzyme confers resistance to aminoglycoside antibiotics (*i.e.*, kanamycin and neomycin) and was used for selection of bacteria during the construction of this plasmid. The coding sequence for the *nptII* gene was derived from the prokaryotic transposon Tn5 (Beck *et al.*, 1982) and is present under its own bacterial promoter.

2. Plant expression vector - PV-ZMGT10

The PV-ZMGT10 plasmid (Figure III.2) contains the *gox* and CP4 EPSPS genes joined to chloroplast transit peptides CTP1 and CTP2, respectively. Both coding regions are under the control of the enhanced CaMV 35S promoter, maize *hsp70* intron and NOS 3' terminator sequences. The PV-ZMGT10 vector contains the same *lacZ-alpha*, *ori-pUC* and *nptII* regions as described above for PV-ZMBK07.

The CP4 EPSPS gene was isolated from *Agrobacterium* sp. strain CP4 (Barry *et al.*, 1992) and has been shown to have the potential to provide high resistance to glyphosate inhibition when introduced into plants ([redacted] 1993). Glyphosate binds to and blocks the activity of its target enzyme, EPSPS, an enzyme of the aromatic amino acid biosynthetic pathway. The CP4 EPSPS protein represents one of many different EPSPSs found in nature (Schulz *et al.*, 1985). CP4 EPSPS is highly tolerant to inhibition by glyphosate and has high catalytic efficiency, compared to most EPSPSs (Barry *et al.*, 1992; [redacted] 1993). Plant cells expressing the CP4 EPSPS protein are tolerant to glyphosate when present in growth medium since the continued action of the tolerant EPSPS enzyme meets the needs for aromatic compounds. The CP4 EPSPS gene is not contained within line MON 810.

The CP4 EPSPS gene in PV-ZMGT10 contains a chloroplast transit

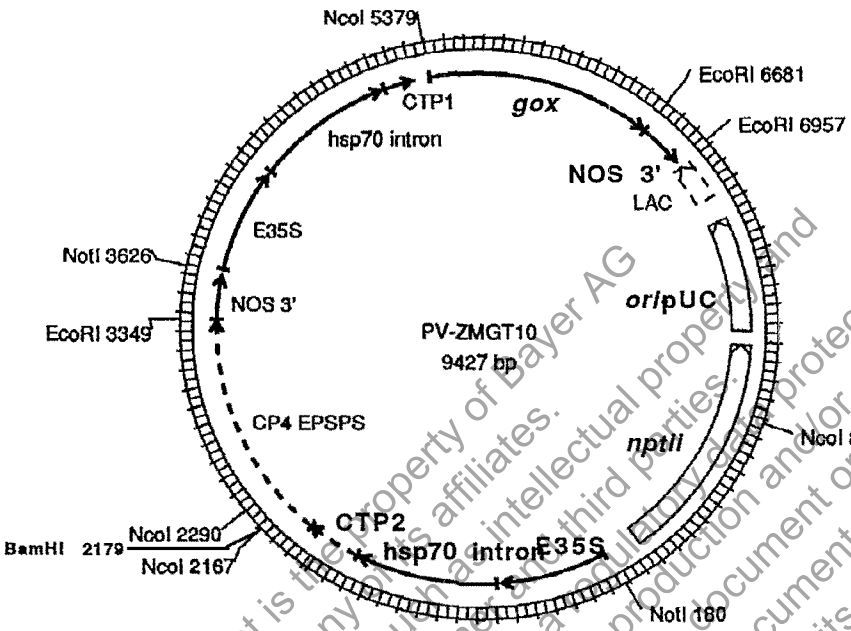


Figure III.2 Plasmid map of PV-ZMGT10. Restriction sites, and their locations in base pairs, used during Southern analyses are shown.

Table III.2 Summary of DNA elements in the plasmid PV-ZMGT10

Genetic Element	Size Kb	Function
E35S	0.61	The cauliflower mosaic virus (CaMV) promoter (Odell *et al.*, 1985) with the duplicated enhancer region (Kay *et al.*, 1985).
*hsp*70	0.80	Intron from the maize *hsp70* gene (heat-shock protein) present to intron increase the level of gene transcription (Rochester *et al.*, 1986).
CTP2	0.31	Chloroplast transit peptide, isolated from *Arabidopsis thaliana* EPSPS (Klee and Rogers, 1987), present to direct the CP4 EPSPS protein to the chloroplast, the site of aromatic amino acid synthesis.
CP4 EPSPS	1.4	The gene for CP4 EPSPS, isolated from *Agrobacterium* sp. strain CP4 ([redacted] *et al.*, 1993) which allows for the selection of transformed cells on glyphosate.
CTP1	0.26	Chloroplast transit peptide, isolated from the small subunit gene of ribulose-1,5-bisphosphate carboxylase (SSU1A) gene from *Arabidopsis thaliana* (Timko *et al.*, 1988), present to direct the GOX protein to the chloroplast, the site of aromatic amino acid synthesis.
gox	1.3	The gene encodes the glyphosate metabolizing enzyme glyphosate oxidoreductase (GOX), isolated from *Achromobacter* sp. (new genus *Ochrobactrum anthropi*) strain LBAA (Hallas *et al.*, 1988; Barry *et al.*, 1992; [redacted] [redacted]., 1994).
NOS 3′	0.26	A 3′ nontranslated region of the nopaline synthase gene which terminates transcription and directs polyadenylation (Fraley *et al.*, 1983).
lacZ	0.24	A partial *E. coli lacI* coding sequences, the promoter Plac, and a partial coding sequence for beta-D-galactosidase or *lacZ* protein from pUC119 (Yanisch-Perron *et al.*, 1985).
ori-pUC	0.65	The origin of replication for the pUC plasmids that allows for plasmid replication in *E. coli* (Vieira and Messing, 1987).
nptII	0.79	The gene for the enzyme neomycin phosphotransferase type II. This enzyme confers resistance to aminoglycoside antibiotics and thereby allows for selection of bacteria containing the plasmid (Beck *et al.*, 1982).

peptide, CTP2, isolated from *Arabidopsis thaliana* EPSPS (Klee and Rogers, 1987) which targets the CP4 EPSPS protein to the chloroplast, the location of EPSPS in plants and the site of aromatic amino acid synthesis ([redacted] and Shah, 1988). The CP4 EPSPS gene with its CTP2 is approximately 1.7 Kb in size. The CP4 EPSPS gene cassette (promoter through 3' termination sequence) is joined to the *gox* cassette.

The *gox* gene that encodes the glyphosate metabolising enzyme glyphosate oxidoreductase (GOX) was cloned from *Achromobacter* sp. (new genus *Ochrobactrum anthropi*) strain LBAA, (Hallas *et al.*, 1988; Barry *et al.*, 1992; [redacted] 1994). The GOX protein is targeted to the plastids with a chloroplast transit peptide sequence, CTP1. The CTP1 was derived from the small subunit gene of ribulose-1,5-bisphosphate carboxylase (SSU1A) gene from *Arabidopsis thaliana* (Timko *et al.*, 1988). The enzyme GOX degrades glyphosate by converting glyphosate to aminomethylphosphonic acid (AMPA) and glyoxylate (Padgette *et al.*, 1994). The *gox* gene is not contained within line MON 810.

D. Genetic Analysis of Insect-Protected Corn Line MON 809

1. Summary

Corn line MON 809 was produced by particle acceleration technology using a DNA solution containing two plasmids, PV-ZMBK07 and PV-ZMGT10. Plasmid PV-ZMBK07 contained the *cryIA(b)* gene and plasmid PV-ZMGT10 contained the CP4 EPSPS and *gox* genes. Corn line MON 809 contains one I-DNA of approximately 23 Kb which includes either complete or partial genes of *cryIA(b)*, CP4 EPSPS and *gox*. The I-DNA contains two *cryIA(b)* genes, one which is the correct size, (3.46 Kb), and one which is smaller (less than 1.0 Kb). There are two CP4 EPSPS genes, both of expected size (1.3 Kb). The single *gox* gene present in corn line MON 809 is not intact. The *nptII* and *ori-p*UC probings showed that the backbone was present in corn line MON 809, but was not the predicted size. Based on these analyses, it was concluded that corn line MON 809 contains a single I-DNA with an intact *cryIA(b)* gene and two CP4 EPSPS genes that are responsible for producing the CryIA(b) and CP4 EPSPS proteins.

Genetic Element	Corn Line MON 809
cryIA(b) gene	1 full length, 1 partial
CP4 EPSPS gene	2 full length
gox gene	1 partial
nptII / *ori-p*UC	present

2. Discussion and results

a. Southern blot results

Two plasmids were utilized during the particle acceleration process to produce the corn line MON 809. Plasmid PV-ZMBK07 contained the *cryIA(b)* gene and plasmid PV-ZMGT10 contained the CP4 EPSPS and *gox* genes. The maps of the two plasmid vectors are presented in Figures III.1 and III.2, along with the locations of the restriction sites utilized for Southern analyses.

The DNAs from MON 818 (non-transgenic control) and MON 809 plants were digested with a variety of restriction enzymes and subjected to Southern blot hybridization analyses to characterize the DNA that was stably transferred during the particle acceleration into the corn genome. Specifically, the insert number (number of integration sites within the corn genome), and the copy number and integrity of each inserted gene were examined.

b. Insert number

NdeI digestion results. The purpose of the NdeI digests was to determine the number of plasmid DNA inserts in the corn line MON 809. The plasmids PV-ZMBK07 and PV-ZMGT10 do not contain a restriction site for NdeI. Thus this enzyme effectively cuts outside any inserted DNA, releasing a fragment containing the inserted DNA. MON 818 control DNA and MON 809 DNA were digested with NdeI and probed with the *cryIA(b)* gene, the CP4 EPSPS gene and the *gox* gene. The results are shown in Figure III.3. No bands were observed when probed with the *cryIA(b)*, CP4 EPSPS or *gox* genes as expected for MON 818 control DNA (lanes 1, 3 and 5). MON 809 DNA produced one band, approximately 23 Kb in size, when probed with: the *cryIA(b)* gene (lane 2), the CP4 EPSPS gene (lane 4) and the *gox* gene (lane 6). The band produced in the *gox* gene probing is very faint and only observed with long exposure times, suggesting that only a portion of the *gox* gene is present in the inserted DNA of corn line MON 809.

c. Insert composition

i. *cryIA(b)* gene integrity. MON 818 and MON 809 DNAs were digested with NcoI/EcoRI to release the *cryIA(b)* gene in MON 809 and the Southern blot probed with the *cryIA(b)* gene. The results are shown in Figure III.4, lanes 1-3. The MON 818 DNA was run alone (lane 1) and mixed with 15 pg of plasmids PV-ZMBK07 and PV-ZMGT10 (lane 2). The MON 818 DNA (lane 1) produces two faint bands, approximately 2.5 Kb and 1.9 Kb in size. These bands are considered to be background bands since they are observed in all three lanes and are not discussed further. The MON 818

DNA mixed with plasmids (lane 2) produced one new 3.46 Kb fragment which corresponds to the expected size of the intact *cryIA(b)* gene (refer to the PV-ZMBK07 plasmid map in Fig. III.1). The MON 809 DNA (lane 3) contains two bands, 3.46 Kb and 1.0 Kb. The 3.46 Kb band is the expected size band for an intact *cryIA(b)* gene, and the 1.0 Kb band represents a partial *cryIA(b)* gene. The NcoI/EcoRI digests, probed with the *cryIA(b)* gene, identified one intact and one partial *cryIA(b)* gene.

ii. CP4 EPSPS gene integrity. MON 818 and MON 809 DNAs were digested with NcoI/EcoRI to release the CP4 EPSPS gene in MON 809 DNA and the Southern blot probed with the CP4 EPSPS gene. The results are shown in Figure III.5, lanes 1-3. The MON 818 DNA was run alone (lane 1) and mixed with 15 pg of plasmids PV-ZMBK07 and PV-ZMGT10 also digested with NcoI/EcoRI (lane 2). The MON 818 DNA (lane 1) showed two bands, approximately 1.37 Kb and 0.80 Kb in size. These two bands, present in all three lanes, are background bands and are therefore not considered further. The MON 818 DNA mixed with the plasmids (lane 2) produced an additional band, 1.06 Kb, which is the expected size of the CP4 EPSPS gene, as predicted from the plasmid map (PV-ZMGT10 in Fig. III.2). The MON 809 DNA (lane 3) also contains a band of 1.06 Kb, the expected size band for the CP4 EPSPS gene. This band contains two expected size CP4 EPSPS genes that are present in corn line MON 809 (data not shown). The NcoI/EcoRI digests, probed with the CP4 EPSPS gene, identified only the expected size CP4 EPSPS gene.

iii. *gox* gene integrity. MON 818 and MON 809 DNAs were digested with NcoI/EcoRI to release the *gox* gene in MON 809 DNA and the Southern blot probed with the *gox* gene. The results are shown in Fig. III.6, lanes 1-3. MON 818 DNA was run alone (lane 1) and mixed with 15 pg of plasmids PV-ZMBK07 and PV-ZMGT10 also digested with NcoI/EcoRI (lane 2). The MON 818 DNA (lane 1) does not show any bands, as expected for the control DNA. The MON 818 DNA mixed with the plasmids (lane 2) produces a 1.3 Kb band, which corresponds to the expected size of the intact *gox* gene, as predicted from the plasmid map (PV-ZMGT10 in Fig. III.2). The MON 809 DNA (lane 3) contains one band of 3.5 Kb. The 3.5 Kb band is faint and was observed only with long exposure times. The faintness of the *gox* band suggests that only a part of the *gox* gene is present. The larger than predicted NcoI/EcoRI fragment size (3.5 Kb rather than 1.3 Kb) indicates a DNA rearrangement has occurred within the *gox* gene. Corn line MON 809 appears to contain a partial *gox* gene.

iv. Backbone integrity. MON 818 and MON 809 DNAs were digested with NotI to release the intact *nptII/ori-p*UC backbone in MON 809 DNA and the Southern blot probed with the *nptII* gene. The results are shown in Figure III.7 (lanes 1 and 2). The digested MON 818 DNA was mixed with 15 pg of PV-ZMBK07 and PV-ZMGT10 also digested with NotI. The MON 818 DNA and plasmid mixture contains two bands of 5.9 Kb and 2.6 Kb (lane 1). The 5.9 Kb band corresponds to the expected size band of the

intact backbone from PV-ZMGT10, the 2.6 Kb band corresponds to the expected size band of the intact backbone from PV-ZMBK07 (refer to Figs. III.1 and III.2). The MON 809 DNA contains a 4.2 Kb band (lane 2) which hybridized to the *nptII* probe.

The Southern blot was stripped and reprobed with the *ori-p*UC genetic region. The MON 818 DNA and plasmid mixture (lane 3) contains three bands of 5.9 Kb, 4.2Kb and 2.6 Kb. The 5.9 Kb band corresponds to the expected size band of the intact backbone from PV-ZMGT10, the 2.6 Kb band corresponds to the expected size band of the intact backbone from PV-ZMBK07 (refer to Figs. III.1 and III.2). The 4.2 Kb band is a background band. The MON 809 DNA contains one band, 4.2 Kb in size (lane 4) which corresponds to the band which hybridized to the *nptII* gene in lane 2. The 4.2 Kb background band (lane 3) co-migrates with the one band which hybridized to the *nptII* and *ori-p*UC probes (lane 4). The 4.2 Kb band hybridized to the *nptII* and *ori-p*UC probes, indicating that the backbone is present but is not the predicted size.

3. Conclusions

The corn line MON 809 was produced by particle acceleration technology with the two plasmids PV-ZMBK07 and PV-ZMGT10 that contained the *cryIA(b)*, CP4 EPSPS, *gox* and *nptII* genes. The I-DNA (23Kb) contains two *cryIA(b)* genes, one which is the correct size, (3.46 Kb), and one which is smaller (less than 1.0 Kb). There are two CP4 EPSPS genes, both of expected size (1.3 Kb). The *gox* gene present in corn line MON 809 is not intact. The *nptII* and *ori-p*UC probings showed that the backbone was present in the corn line MON 809, but was not the predicted size.

Based on these analyses, it was concluded that corn line MON 809 contains a single I-DNA with an intact *cryIA(b)* gene and two CP4 EPSPS genes that are responsible for producing the CryIA(b) and CP4 EPSPS proteins.

Figure III.3 Southern blot analysis of corn line MON 809 DNA: insert number analysis

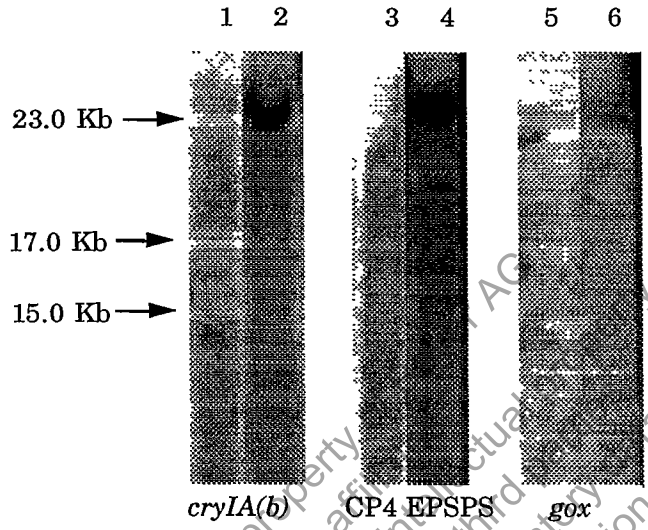


Figure III.3 Southern blot analysis of corn line MON 809 DNA. Lanes 1, 3 and 5 contain 12.5 µg of corn line MON 818 DNA digested with NdeI. Lanes 2, 4 and 6 contain 12.5 µg of corn line MON 809 DNA digested with NdeI. Lanes 1 and 2 were hybridized with the *cryIA(b)* gene. Lanes 3 and 4 were hybridized with the CP4 EPSPS gene. Lanes 5 and 6 were hybridized with the *gox* gene.

→ Symbol denotes sizes obtained from MW markers.

Figure III.4 Southern blot analysis of corn line MON 809 DNA: *cryIA(b)* gene analysis

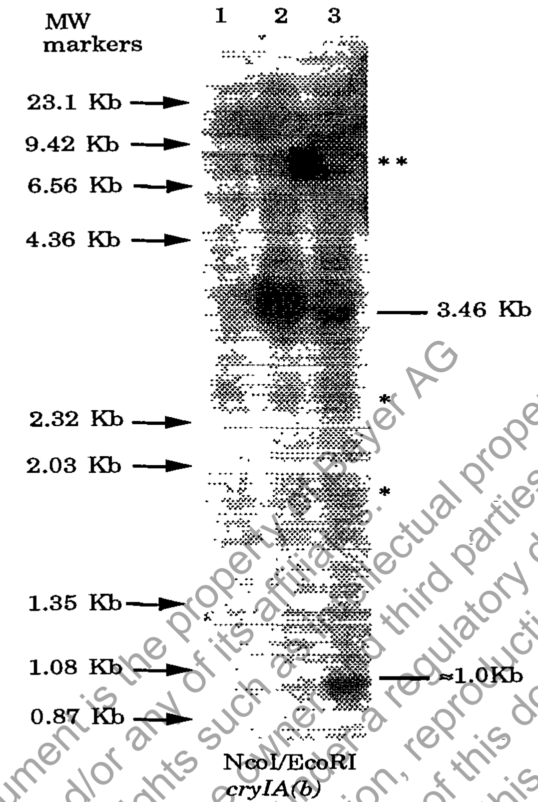


Figure III.4 Southern blot analysis of corn line MON 809 DNA. Lanes 1-3 contain the following DNAs digested with NcoI/EcoRI and probed with the *cryIA(b)* gene: lane 1, MON 818 DNA; lane 2, MON 818 DNA mixed with 15 pg of plasmids PV-ZMBK07 and PV-ZMGT10; lane 3, MON 809 DNA.

⟶	Symbol denotes sizes obtained from MW markers on ethidium stained gel.
—	Symbol denotes sizes obtained from plasmid digests.
*	Symbol denotes background bands (≈2.5 and 1.9 Kb).
≈	Symbol denotes a band size approximated from MW marker and plasmid digests.
* *	Symbol denotes an area of non-specific hybridization. This is supported by the observation that the signal is between two lanes.

Figure III.5 Southern blot analysis of corn line MON 809 DNA: CP4 EPSPS gene analysis

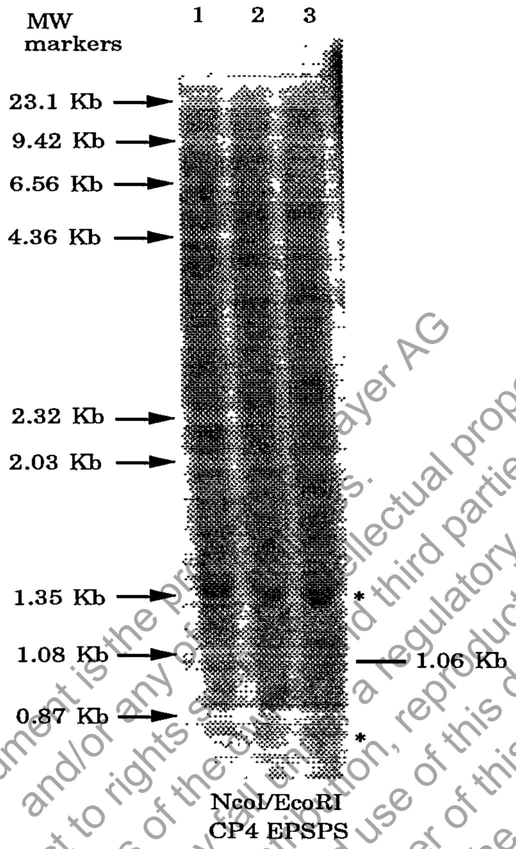


Figure III.5 Southern blot analysis of corn line MON 809 DNA. Lanes 1-3 contain the following DNAs digested with NcoI/EcoRI and probed with the CP4 EPSPS gene: lane 1, MON 818 DNA; lane 2, MON 818 DNA mixed with 15 pg of plasmids PV-ZMBK07 and PV-ZMGT10; lane 3, MON 809 DNA.

→ Symbol denotes sizes obtained from MW markers on ethidium stained gel.
— Symbol denotes sizes obtained from plasmid digests.
* Symbol denotes background bands (≈1.37 and 0.80 Kb).
≈ Symbol denotes a band size approximated from MW marker and plasmid digests.

Figure III.6 Southern blot analysis of corn line MON 809 DNA: *gox* gene analysis

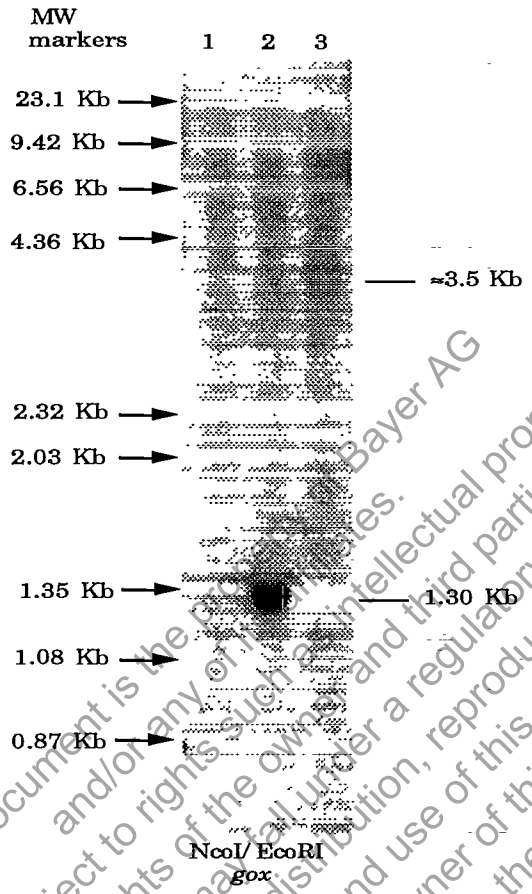


Figure III.6 Southern blot analysis of corn line MON 809 DNA. Lanes 1-3 contain the following DNAs digested with NcoI/EcoRI and probed with the *gox* gene: lane 1, MON 818 DNA; lane 2, MON 818 DNA mixed with 15 pg of plasmids PV-ZMBK07 and PV-ZMGT10; lane 3, MON 809 DNA.

→ Symbol denotes sizes obtained from MW markers on ethidium stained gel.
— Symbol denotes sizes obtained from plasmid digests.
≈ Symbol denotes a band size approximated from MW marker and plasmid digests.

Figure III.7 Southern blot analysis of corn line MON 809 DNA: *nptII* and *ori-p*UC analysis

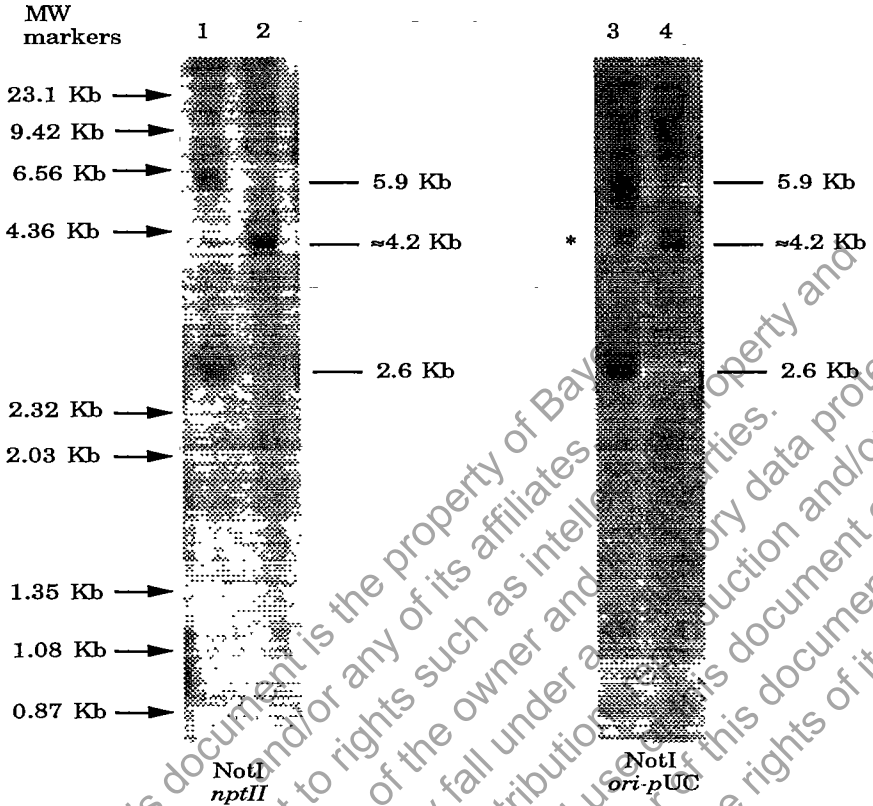


Figure III.7 Southern blot analysis of corn line MON 809 DNA. Lanes 1-4 contain the following DNAs digested with NotI: lanes 1 and 3, MON 818 DNA mixed with 15 pg of plasmids PV-ZMBK07 and PV-ZMGT10; lanes 2 and 4, MON 809 DNA. Lanes 1 and 2 were hybridized with the *nptII* region. Lanes 3 and 4 were hybridized with the *ori-p*UC region.

→ Symbol denotes sizes obtained from MW markers on ethidium stained gel.
— Symbol denotes sizes obtained from plasmid digests.
* Symbol denotes background bands.
≈ Symbol denotes a band size approximated from MW marker and plasmid digests.

E. Genetic Analysis of Insect-Protected Corn Line MON 810

1. Summary

Corn line MON 810 was produced by particle acceleration technology using a DNA solution containing two plasmids, PV-ZMBK07 and PV-ZMGT10. Plasmid PV-ZMBK07 contained the *cryIA(b)* gene and plasmid PV-ZMGT10 contained the CP4 EPSPS and *gox* genes. The DNAs from the control, MON 818, and MON 810 plants were digested with a variety of restriction enzymes and subjected to Southern blot hybridization analyses to characterise the DNA that was transferred during the particle acceleration into the corn genome. Specifically, the insert number (number of integration sites within the corn genome), and the copy number and integrity of each gene was examined.

Molecular analysis of corn line MON 810 established that the line only contains the *cryIA(b)* gene from plasmid PV-ZMBK07. This line does not contain the CP4 EPSPS, *gox*, or *nptII* genes. There is no evidence that any of the DNA contained in plasmid PV-ZMGT10 was inserted. Corn line MON 810 contains one integrated DNA, contained on a 5.5 Kb NdeI fragment, which contains the E35S promoter, maize *hsp70* intron and the *cryIA(b)* gene.

Genetic Element	Corn Line MON 810
cryIA(b) gene	present
CP4 EPSPS gene	not present
gox gene	not present
nptII/*ori-p*UC	not present

2. Discussion and results

a. Insert number

NdeI digestion results. The purpose of the NdeI digests was to determine the number of plasmid DNA inserts in the corn line MON 810. The plasmids PV-ZMBK07 and PV-ZMGT10 do not contain a restriction site for NdeI. Thus this enzyme effectively cleaves outside any inserted DNA, releasing a fragment containing the inserted DNA and adjacent genomic DNA. MON 818 control DNA and MON 810 DNA were digested with NdeI and probed with plasmid PV ZMBK07 DNA. The results are shown in Figure III.8. MON 818 DNA (lane 1), produced one very light, diffused band of approximately 21.0 Kb which is a background band since it is present in both the control MON 818 DNA and the MON 810 DNA. MON 810 DNA produced one band, approximately 5.5 Kb in size (lane 2). This result established that insect-protected corn line MON 810 contains one fragment of integrated DNA. The size of the inserted DNA plus adjacent genomic DNA up to the NdeI restriction sites is approximately 5.5 Kb.

b. Insert composition

i. *cryIA(b)* gene integrity. MON 818 and MON 810 DNAs were digested with NcoI/EcoRI to release the *cryIA(b)* gene and the Southern blot probed with the *cryIA(b)* gene. The results are shown in Figure III.9, lanes 1-3. The positive hybridization control (lane 1) produced one 3.46 Kb fragment which corresponds to the expected size of the *cryIA(b)* gene (refer to the plasmid map in Fig. III.1). Due to the plasmid DNA not being mixed with genomic control DNA the band appears larger than its true molecular weight. The MON 818 DNA (lane 2) does not produce any bands, as expected for the control line. The MON 810 DNA (lane 3) contains one band, approximately 3.1 Kb which corresponds to the *cryIA(b)* gene.

ii. CP4 EPSPS gene integrity. Plasmid DNAs (PV-ZMBK07 and PV-ZMGT10) and insect-protected corn line MON 810 DNA were digested with NcoI/BamHI to release the CP4 EPSPS gene and the Southern blot probed with the CP4 EPSPS gene. The results are shown in Figure III.10, lanes 1 and 2. Approximately 50 pg of a mixture of PV-ZMBK07 and PV-ZMGT10 DNA (lane 1) produced one band, approximately 3.1 Kb in size, which corresponds to the expected size CP4 EPSPS fragment, as predicted from the plasmid map (PV ZMGT10 in Fig. III.2). MON 810 DNA (lane 2) shows no hybridizing fragments to the CP4 EPSPS probe, establishing that insect-protected corn line MON 810 does not contain the CP4 EPSPS gene.

iii. *gox* gene integrity. Plasmid DNAs (PV-ZMBK07 and PV ZMGT10) and insect-protected corn line MON 810 DNA were digested with NcoI/BamHI to release the *gox* gene and the Southern blot probed with the *gox* gene. The results are shown in Figure III.10, lanes 3 and 4. Approximately 50 pg of a mixture of PV-ZMBK07 and PV ZMGT10 DNA (lane 3) produced one band, a NcoI/NcoI fragment, approximately 3.1 Kb, which corresponds to the expected size *gox* fragment, as predicted from the plasmid map (PV-ZMGT10 in Fig. III.2). MON 810 DNA (lane 4) shows no hybridizing fragments to the *gox* probe, establishing that insect-protected corn line MON 810 does not contain the *gox* gene.

iv. Backbone integrity. Plasmid PV-ZMBK07, control line MON 818 and insect-protected corn line MON 810 DNAs were digested with NcoI/EcoRI to release the *nptII/ori-p*UC backbone and the Southern blot probed with the *nptII* gene. The results are shown in Figure III.11 (lanes 1-3). Approximately 50 pg of PV-ZMBK07 DNA produced two bands of 2.5 Kb and 1.8 Kb (lane 1). The 2.5 Kb and 1.8 bands correspond to the expected size fragments of the backbone from vector PV-ZMBK07 (refer to Fig. III.1). The MON 818 DNA alone (lane 2) does not produce any bands, as expected from a non-modified control line. MON 810 DNA (lane 3) shows no bands, establishing that the backbone sequences were not integrated in insect-protected corn line MON 810.

The Southern blot was stripped and reprobed with the *ori-p*UC genetic

region. The PV-ZMBK07 and PV-ZMGT10 DNAs (lane 4) contains one band of 1.8 Kb. The 1.8 Kb band corresponds to the expected size fragment of the backbone from PV-ZMBK07 (refer to Fig. III.1). The MON 818 DNA alone (lane 5) does not produce any bands, as expected for the unmodified control line. MON 810 DNA (lane 6) shows no bands, establishing that the backbone sequences were not integrated in insect-protected corn line MON 810. The lack of observed bands with both *ori-p*UC and *nptII* probes, established that insect-protected corn line MON 810 does not contain any backbone sequences.

3. Conclusions

The insect-protected corn line MON 810 was produced by particle acceleration technology with a DNA solution that contained the *cryIA(b)*, CP4 EPSPS, *gox* and *nptII* genes. Corn line MON 810 contains one integrated DNA contained on a 5.5 Kb NdeI fragment, which contains the E35S promoter, maize *hsp70* intron and the *cryIA(b)* gene. Insect-protected corn line MON 810 does not contain a CP4 EPSPS gene, a *gox* gene or *nptII/ori-pUC* sequences. The continued efficacy of corn line MON 810 confirms that an insecticidally active CryIA(b) protein is produced which provides season long control of European Corn Borer.

Figure III.8 Southern blot analysis of corn line MON 810 DNA: insert number analysis

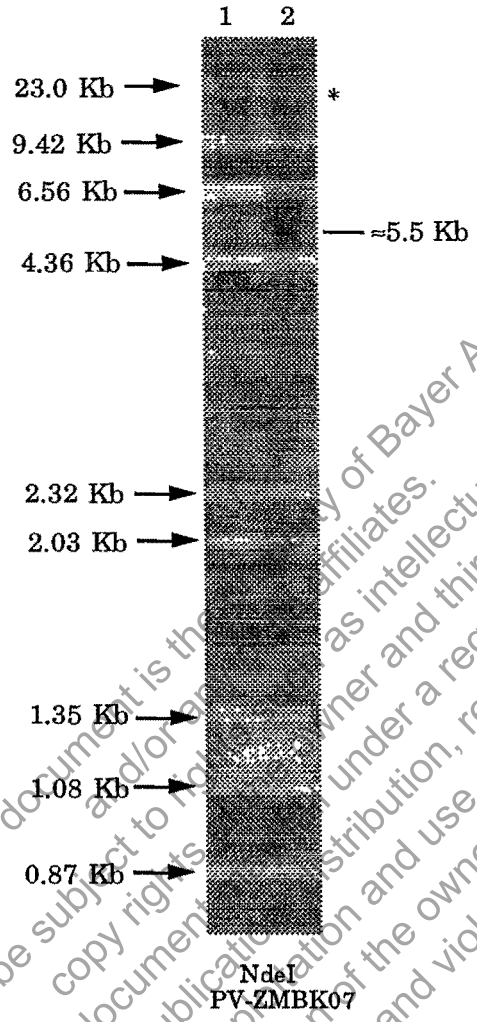


Figure III.8 Southern blot analysis of corn line MON 810 DNA. Lanes 1 and 2 contain the following DNAs digested with NdeI and probed with PV-ZMBK07: lane 1, MON 818 DNA; lane 2, MON 810 DNA.

→ Symbol denotes sizes obtained from MW markers.
≈ Symbol denotes a band size approximated from MW marker and plasmid digests.
* Symbol denotes background bands.

Figure III.9 Southern blot analysis of corn line MON 810 DNA: *cryIA(b)* gene analysis

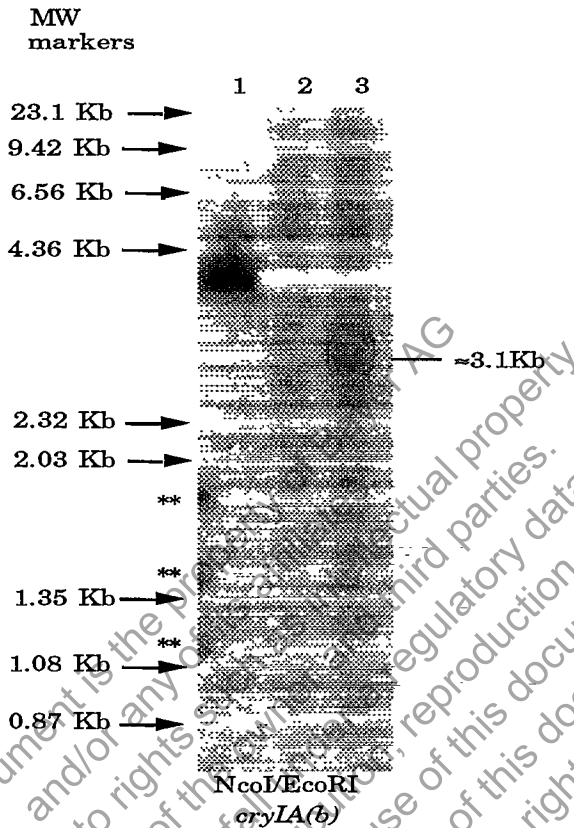


Figure III.9 Southern blot analysis of corn line MON 810 DNA. Lanes 1-3 contain the following DNAs digested with NcoI/EcoRI and probed with the *cryIA(b)* gene: lane 1, ≈50 pg of plasmid PV-ZMBK07; lane 2, MON 818 DNA, lane 3, MON 810 DNA.

→	Symbol denotes sizes obtained from MW markers on ethidium stained gel.
—	Symbol denotes sizes obtained from plasmid digests.
≈	Symbol denotes a band size approximated from MW marker and plasmid digests.
* *	Symbol denotes an area of hybridization in an adjacent lane which only appears to be in lane 1, due to the contents of the lanes migrating at an angle in this portion of the gel.

Figure III.10 Southern blot analysis of corn line MON 810 DNA: CP4 EPSPS and *gox* gene analysis

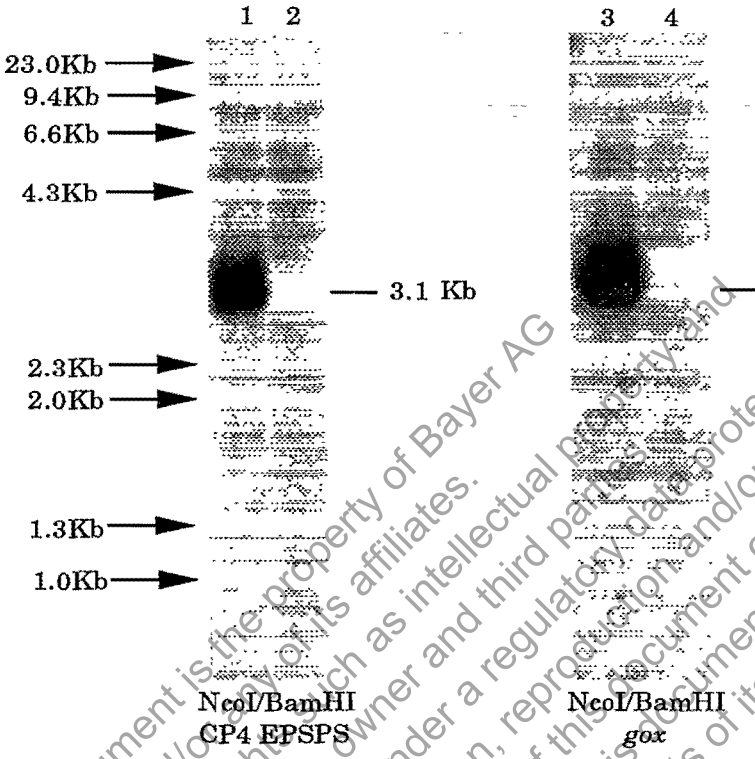


Figure III.10 Southern blot analysis of corn line MON 810 DNA. Lanes 1-4 contain the following DNAs digested with NcoI/BamHI: lanes 1 and 3, ≈50pg of plasmids PV-ZMGT10 and PV-ZMBK07; lanes 2 and 4, MON 810 DNA. Lanes 1 and 2 were hybridized with the CP4 EPSPS gene. Lanes 3 and 4 were hybridized with the *gox* gene.

→ Symbol denotes sizes obtained from MW markers on ethidium stained gel.
— Symbol denotes sizes obtained from plasmid digests.

Figure III.11 Southern blot analysis of corn line MON 810 DNA: *nptII* and *ori-p*UC analysis

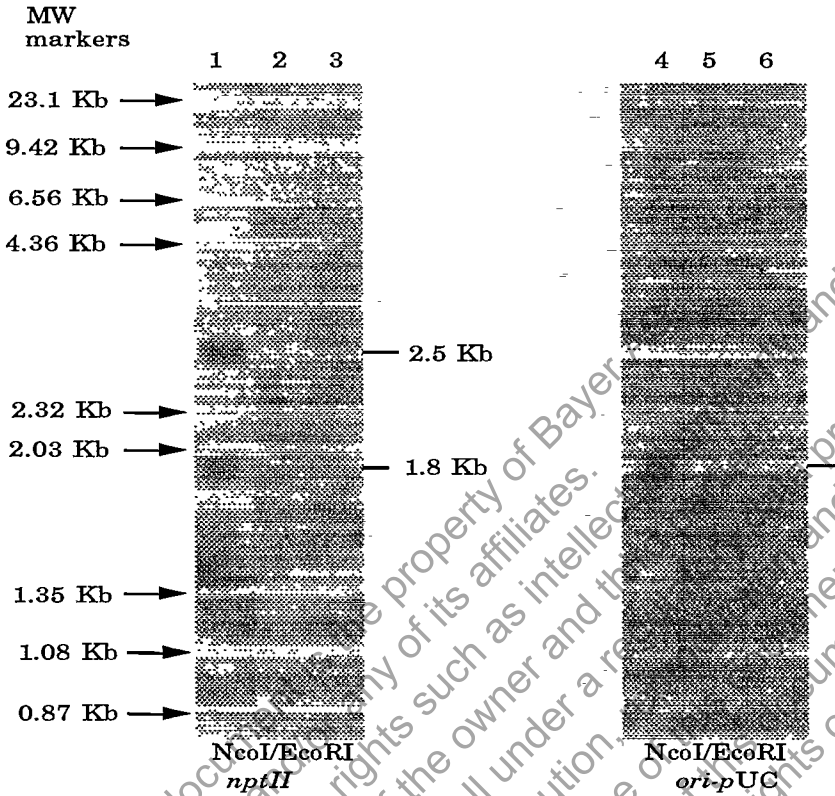


Figure III.11 Southern blot analysis of corn line MON 810 DNA. Lanes 1-6 contain the following DNAs digested with NcoI/EcoRI: lanes 1 and 4, ≈50pg of plasmid PV-ZMBK07; lanes 2 and 5, MON 818 DNA; lanes 3 and 6, MON 810 DNA. Lanes 1-3 were hybridized with the *nptII* region. Lanes 4-6 were hybridized with the *ori-p*UC region.

→ Symbol denotes sizes obtained from MW markers on ethidium stained gel.
— Symbol denotes sizes obtained from plasmid digests.

F. Segregation Data and Stability of Gene Transfer

1. Insect-protected corn line MON 809

Segregation data for the R1 plants (derived from selfing the original transformant, or R0 plant), BC0F1 plants (derived from crossing the R0 with an inbred line), BC0F2 plants (derived from selfing the BC0F1 plants), and BC1F1 plants (derived from crossing the BC0F1 plants to the same inbred used to cross with the R0 plant) are presented in Table III.3. The results in all four cases are consistent with a single active insert segregating according to Mendelian genetics.

Table III.3 Segregation data and analysis of progeny of insect-protected corn line MON 809

Generation	Actual	Expected	Chi Sq
R1[1]	18:9	13.5:13.5	1.000 *
BC0F1[1]	8:2	5:5	3.600 *
BC0F2[1]	38:12	37.5:12.5	0.000 *
BC1F1[1]	47:50	48.5:48.5	0.041 *

[1] Data expressed as number of expressing plants: number of non-expressing plants based on European corn borer feeding assay
* not significant at p = 0.05 (chi square = 3.84, 1 df)

The *cryIA(b)* gene in insect-protected corn line MON 809 was shown to be stable for five generations of crosses to one recurrent parent (B73) and four generations of crosses to a second, unrelated inbred (Mo17) (Table III.4). The Chi square tests for the backcross to B73 and to Mo17 did not deviate from expectations at p=0.05.

Table III.4 Stability of gene transfer based on segregation data for backcross derivatives of insect-protected corn line MON 809 in two unrelated inbred lines (B73 and Mo17).

Generation	Actual	Expected	Chi Sq
BC4F1(B73) [1]	20:18	19:19	0.026 *
BC3F1(Mo17) [1]	19:11	15:15	1.633 *

[1] Data expressed as number of expressing plants: number of non-expressing plants based on CryIA(b) ELISA
* not significant at p = 0.05 (chi square = 3.84, 1 df)

To summarize the segregation and stability data (Tables IV.3 and IV.4), the data are consistent with a single active site of insertion of the cryIA(b) gene into genomic DNA of line MON 809. The stability of this insertion has been demonstrated through five generations of crossing.

2. Insect-protected corn line MON 810

Segregation data for the BC0F1 plants (derived from crossing the R0 with an inbred line), BC1F1 plants (derived from crossing the BC0F1 plants to the same inbred used to cross with the R0 plant), and BC1F2 progeny (derived from crossing individual BC0F2 plants by a non-transgenic tester and analyzing subsequent generation ear to row) are presented in Table III.5. The results are consistent with a single active insert segregating according to Mendelian genetics.

Table III.5 Segregation data and analysis of progeny of insect-protected corn line MON 810

Generation	Actual	Expected	Chi Sq
BC0F1[1]	44:47	45.5:45.5	0.044 *
BC1F1[2]	10:4	7:7	1.786 *
BC1F2 progeny [3]	69:181:77	81.75:163.5:81.75	4.138 #

[1] Data expressed as number of expressing plants: number of non-expressing plants based on European corn borer feeding assay

[2] Data expressed as number of expressing plants: number of non-expressing plants based on CryIA(b) ELISA

[3] Data expressed as number of ear rows with homozygous expressing plants: number of ear rows with segregating plants: number of ear rows with homozygous susceptible plant based on European corn borer feeding assay

* not significant at p = 0.05 (chi square = 3.84, 1 df)

not significant at p = 0.05 (chi square = 5.99, 2 df)

The *cryIA(b)* gene in insect-protected corn line MON 810 has been shown to be stable through seven generations of crosses to one recurrent parent (B73) and six generations of crosses to a second, unrelated inbred (Mo17) (Table III.6). The Chi square tests for the backcross to B73 and to Mo17 did not deviate from expectations at p=0.05.

Table III.6 Stability of gene transfer based on segregation data for backcross derivatives of insect-protected corn line MON 810 in two unrelated inbred lines (B73 and Mo17).

Generation	Actual	Expected	Chi Sq
BC6F1(B73) [1]	8:13	10.5:10.5	0.762 *
BC5F1(Mo17) [1]	11:11	11:11	0.045 *

[1] Data expressed as number of expressing plants: number of non-expressing plants based on CryIA(b) ELISA

* not significant at p = 0.05 (chi square = 3.84, 1 df)

To summarize, the segregation and stability data (Tables III.5 and III.6), the data are consistent with a single active site of insertion of the *cryIA(b)* gene into genomic DNA of line MON 810. The stability of this insertion has been demonstrated through seven generations of crossing.

G. Conclusion

Insect-protected corn lines MON 809 and 810 were produced by particle acceleration technology with a DNA solution containing two plasmids, PV ZMBK07 (which contained the *cryIA(b)* gene) and PV-ZMGT10 (which contained the CP4 EPSPS and *gox* genes). Corn line MON 809 contains one integrated DNA of approximately 23 Kb which includes a complete (3.46 Kb) and a partial (less than 1.0 Kb) *cryIA(b)* gene, two CP4 EPSPS genes, both of expected size (1.3 Kb), and partial *gox* gene. The *nptII* and *ori-p*UC genes are present but not the predicted size.

Corn line MON 810 contains one integrated DNA contained on an approximately 5.5 Kb NdeI fragment which contains a single copy of the E35S promoter, the *hsp70* intron and the *cryIA(b)* gene. The *nptII* gene and backbone sequences of plasmid PV-ZMBK07 were not integrated. This line does not contain the CP4 EPSPS, gox or *nptII* genes, nor the plasmid backbone from plasmid PV-ZMGT10.

The segregation and stability data for both MON 809 and 810 are consistent with the stable introduction at a single site of insertion of the *cryIA(b)* gene into the genomic DNA of corn.

Figure III.12 Deduced amino acid sequence of the CryIA(b) protein.

```
   1  MDNNPNINEC IPYNCLSNPE VEVLGGERIE TGYTPIDISL SLTQFLLSEF
  51  VPGAGFVLGL VDIIWGIFGP SQWDAFLVQI EQLINQRIEE FARNQAISRL
 101  EGLSNLYQIY AESFREWEAD PTNPALREEM RIQFNDMNSA LTTAIPLFAV
 151  QNYQVPLLSV YVQAANLHLS VLRDVSVFGQ RWGFDAATIN SRYNDLTRLI
 201  GNYTDHAVRW YNTGLERVWG PDSRDWIRYN QFRRELTLTV LDIVSLFPNY
 251  DSRTYPIRTV SQLTREIYTN PVLENFDGSF RGSAQGIEGS IRSPHLMDIL
 301  NSITIYTDAH RGEYYWSGHQ IMASPVGFSG PEFTFPLYGT MGNAAPQQRI
 351  VAQLGQGVYR TLSSTLYRRP FNIGINNQQL SVLDGTEFAY GTSSNLPSAV
 401  YRKSGTVDSL DEIPPQNNNV PPRQGFSHRL SHVSMFRSGF SNSSVSIIRA
 451  PMFSWIHRSA EFNNIIPSSQ ITQIPLTKST NLGSGTSVVK GPGFTGGDIL
 501  RRTSPGQIST LRVNITAPLS QRYRVRIRYA STTNLQFHTS IDGRPINQGN
 551  FSATMSSGSN LQSGSFRTVG FTTPFNFSNG SSVFTLSAHV FNSGNEVYID
 601  RIEFVPAEVT FEAEYDLERA QKAVNELFTS SNQIGLKTDV TDYHIDQVSN
 651  LVECLSDEFC LDEKKELSEK VKHAKRLSDE RNLLQDPNFR GINRQLDRGW
 701  RGSTDITIQG GDDVFKENYV TLLGTFDECY PTYLYQKIDE SKLKAYTRYQ
 751  LRGYIEDSQD LEIYLIRYNA KHETVNVPGT GSLWPLSAPS PIGKCAHHSH
 801  HFSLDIDVGC TDLNEDLGVW VIFKIKTQDG HERLGNLEFL EGRAPLVGEA
 851  LARVKRAEKK WRDKREKLEW ETNIVYKEAK ESVDALFVNS QYDRLQADTN
 901  IAMIHAADKR VHSIREAYLP ELSVIPGVNA AIFEELEGRI FTAFSLYDAR
 951  NVIKNGDFNN GLSCWNVKGH VDVEEQNNHR SVLVVPEWEA EVSQEVRVCP
1001  GRGYILRVTA YKEGYGEGCV TIHEIENNTD ELKFSNCVEE EVYPNNTVTC
1051  NDYTATQEEY EGTYTSRNRG YDGAYESNSS VPADYASAYE EKAYTDGRRD
1101  NPCESNRGYG DYTPLPAGYV TKELEYFPET DKVWIEIGET EGTFIVDSVE
1151  LLLMEE
```

Figure III.13 Deduced amino acid sequence of the CP4 EPSPS protein. Sequence includes the CTP2 transit peptide (amino acids 1-76 are the transit peptide).

```
  1  MAQVSRICNG VQNPSLISNL SKSSQRKSPL SVSLKTQQHP RAYPISSSWG
 51  LKKSGMTLIG SELRPLKVMS SVSTACMLHG ASSRPATARK SSGLSGTVRI
101  PGDKSISHRS FMFGGLASGE TRITGLLEGE DVINTGKAMQ AMGARIRKEG
151  DTWIIDGVGN GGLLAPEAPL DFGNAATGCR LTMGLVGVYD FDSTFIGDAS
201  LTKRPMGRVL NPLREMGVQV KSEDGDRLPV TLRGPKTPTP ITYRVPMASA
251  QVKSAVLLAG LNTPGITTVI EPIMTRDHTE KMLQGFGANL TVETDADGVR
301  TIRLEGRGKL TGQVIDVPGD PSSTAFPLVA ALLVPGSDVT ILNVLMNPTR
351  TGLILTLQEM GADIEVINPR LAGGEDVADL RVRSSTLKGV TVPEDRAPSM
401  IDEYPILAVA AAFAEGATVM NGLEELRVKE SDRLSAVANG LKLNGVDCDE
451  GETSLVVRGR PDGKGLGNAS GAAVATHLDH RIAMSFLVMG LVSENPVTVD
501  DATMIATSFP EFMDLMAGLG AKIELSDTKA A
```

Figure III.14 Deduced amino acid sequence of the GOX protein. Sequence includes the CTP1 transit peptide (amino acids 1-88 are the transit peptide).

```
  1  MASSMLSSAT MVASPAQATM VAPFNGLKSS AAFPATRKAN NDITSITSNG
 51  GRVNCMQVWP PIGKKKFETL SYLPDLTDSG GRVNCMQAMA ENHKKVGIAG
101  AGIVGVCTAL MLQRRGFKVT LIDPNPPGEG ASFGNAGCFN GSSVVPMSMP
151  GNLTSVPKWL LDPMGPLSIR FSYFPTIMPW LIRFLLAGRP NKVKEQAKAL
201  RNLIKSTVPL IKSLAEEADA SHLIRHEGHL TVYRGEADFA KDRGGWELRR
251  LNGVRTQILS ADALRDFDPN LSHAFTKGIL IEENGHTINP QGLVTLLFRR
301  FIANGGEFVS ARVIGFETEG RALKGITTTN GVLAVDAAVV AAGAHSKSLA
351  NSLGDDIPLD TERGYHIVIA NPEAAPRIPT TDASGKFIAT PMEMGLRVAG
401  TVEFAGLTAA PNWKRAHVLY THARKLLPAL APASSEERYS KWMGFRPSIP
451  DSLPVIGRAT RTPDVIYAFG HGHLGMTGAP MTATLVSELL AGEKTSIDIS
501  PFAPNRFGIG KSKQTGPAS
```

Figure III.15 Deduced amino acid sequence of the NPTII protein.

```
  1  MIEQDGLHAG SPAAWVERLF GYDWAQQTIG CSDAAVFRLS AQGRPVLFVK
 51  TDLSGALNEL QDEAARLSWL ATTGVPCAAV LDVVTEAGRD WLLLGEVPGQ
101  DLLSSHLAPA EKVSIMADAM RRLHTLDPAT CPFDHQAKHR IERARTRMEA
151  GLVDQDDLDE EHQGLAPAEL FARLKARMPD GEDLVVTHGD ACLPNIMVEN
201  GRFSGFIDCG RLGVADRYQD IALATRDIAE ELGGEWADRF LVLYGIAAPD
251  SQRIAFYRLL DEFF
```

IV. SAFETY OF THE NEW CORN VARIETY

The flow charts presented in the FDA Food Policy (FDA, 1992) were utilized to organize the following summary of the studies conducted on insect-protected corn lines MON 809 and 810 and other information which demonstrate the substantial equivalence of lines MON 809 and 810 to the control line, MON 818, and other corn varieties commercially grown. The pathway leading to "no concern" for lines MON 809 and 810 is highlighted with bold arrows in the flowcharts reproduced below.

A. Safety Assessment of New Varieties: the Host Plant, Corn

1. The history and utilization of modern corn

Corn, *Zea mays* L., the host plant, has a history of safe use. Corn is one of the few major crop species indigenous to the Western Hemisphere and is grown in nearly all areas of the world (Hallauer *et al.*, 1988). The exact origin of modern corn has been debated among botanists for years although evidence exists to support a number of theories based upon teosinte and human involvement (Aldrich *et al.*, 1986; Galinat, 1988; Jugenheimer, 1976, Mangelsdorf, 1974). In the United States, corn is the largest crop in terms of planted acreage, total production, and crop value (National Corn Growers Association, 1995). United States production in 1994 was 256 million metric tons with the majority of national production concentrated across what is known as the "Corn Belt" in the upper midwest. Corn is generally used as an animal feed to provide abundant, high-quality animal products and in a diverse range of human food and large volume industrial products. A discussion of the history and utilization of modern corn is attached in Appendix I.

2. Corn as a food source in the United States

Although an ideal source of energy, little whole kernel or processed corn is consumed by humans worldwide when compared to corn-based food ingredients (Hodge, 1982; Watson, 1988). The low price and ready availability of corn has resulted in the development of large volume food and industrial uses. Corn is an excellent raw material for the manufacture of starch, not only because of price and availability, but also because the starch is easily recovered in high yield and purity (Anderson and Watson, 1982). Nearly one fourth of corn starch is sold as starch products; more than three fourths of the starch is converted to a variety of sweetener and fermentation products including high fructose corn syrup and ethanol (Watson, 1988; National Corn Growers, 1995; Anderson and Watson, 1982; Pollack and White, Appendix II). Additionally, corn oil is commercially processed from the germ and accounts for approximately nine percent of domestic vegetable oil production (Orthoefer and Sinram, 1987). Each of these materials is a component of many foods including bakery and dairy goods, beverages, confections, and meat products. A discussion of corn as a food source in the United States was prepared by Drs. Linda Pollak, Department of Agronomy, and Pamela White, Department of Food Science and Human Nutrition, Iowa State University, and is attached in Appendix II.

3. Corn and animal nutrition in the United States

Animal feeding is by far the largest use of corn in the United States with more than half (50 to 60 percent) of annual production fed to cattle, chickens, and swine (Hodge, 1982; Perry, 1988; U.S. Feed Grains Council, 1992; Watson, 1988; Newcomb, Appendix III). Of an approximately 200 million metric tons of grain, forty to fifty percent is fed to livestock directly as grain. Another 1.5 to 2 million metric tons of by-products of the wet and dry milling industries, primarily corn gluten meal and feed, are fed directly or in formulated feeds (Perry, 1988). In addition to corn grown for grain, approximately 10 to 12% of annual corn acreage is utilized as whole plant corn silage, consumption confined almost entirely to ruminants (Watson, 1988; Perry, 1988). Corn is readily consumed by livestock and because of its high starch-low fiber content is one of the most concentrated sources of energy, containing more total digestible nutrients than any other feed grain. Corn does not normally contain toxins or antinutritional factors (International Food Biotechnology Council, 1990; Watson, 1982). A discussion of corn and animal nutrition in the United States was prepared by Dr. M.D. Newcomb, Assistant Professor of Animal Science at the University of Missouri and is attached in Appendix III.

4. Compositional analysis of insect-protected corn lines MON 809 and 810

The major components of the grain for corn lines MON 809 and 810 and control, MON 818, were analyzed under Good Laboratory Practices (GLP) on grain harvested from 6 U.S. GLP field trials in 1994 (Sanders *et al.*, 1995). The control line, MON 818, is similar in pedigree to lines MON 809 and 810 [(((Hi-IIxB73) selfed)xMo17) selfed] but is not an isogenic control because of the variability in the parental High-Type II line. Proximates (protein, fat, ash, carbohydrates, calories and moisture), amino acid composition and fatty acid profile were performed on the ground grain by published methods (AOAC Methods, 1990). The values reported for the compositional analyses at Corning Hazleton Inc. (Madison, Wisconsin, USA) were expressed as percent dry weight of the sample, correcting for the measured moisture content. The experimental values were compared between corn lines MON 809 and 810 and the control, as well as to published literature ranges (Watson, 1982, 1987; Jugenheimer, 1976) and values reported for a control line with similar genetic background (Sanders and Patzer, 1995). A description of the methods utilized to obtain the data reported below is found in Appendix IV of this summary.

a. Proximate analysis of MON 809 and 810 corn grain

The levels of the major components of corn grain (protein, fat, ash, carbohydrates, calories and moisture) are summarized in Table IV.1. The levels of protein, fat, ash, carbohydrates, calories and moisture were equivalent for lines MON 809 and 810 and the control line, MON 818. The values for both lines were also within the published and reported literature ranges for all components measured.

Table IV.1 Summary of proximate analysis of grain from corn lines MON 809 and 810

Characteristic	MON 818 Mean[b]	MON 818 Range[c]	MON 809 Mean[b]	MON 809 Range[c]	MON 810 Mean[b]	MON 810 Range[c]	Literature Range*	Reported Ranged
Protein	12.8	11.7-13.6	13.1	12.5-13.6	13.1	12.7-13.6	6.0-12.0[e] 9.7-16.1[f]	11.2-12.9
Fat[a]	2.9	2.6-3.2	2.6	2.1-3.2	3.0	2.6-3.3	3.1-5.7[e]	3.8-4.2
Ash[a]	1.5	1.5-1.6	1.5	1.3-1.7	1.6	1.5-1.7	1.1-3.9[e]	1.5-1.8
Carbohydrate[a]	82.7	81.7-83.8	82.8	82.3-83.6	82.4	81.8-82.9	not reported	81.7-83.0
Calories/100g[a]	409	406-410	407	404-410	408	407-410	not reported	412-416
Moisture %	12.0	10.6-14.2	13.2	10.6-14.6	12.4	11.0-14.4	7-23[e]	13.0-15.8

[a]: Percent dry weight of sample.
[b]: Value reported is mean of six samples, one from each field site.
[c]: The range denotes the lowest and highest individual values across sites for each line.
[d]: Sanders and Patzer (1995), range for a control with similar genetic background.
[e]: Watson, 1987.
[f]: Jugenheimer, 1976.

b. Amino acid composition of MON 809 and 810 corn grain

The results of the analysis of the amino acid composition on corn grain samples for both lines MON 809 and 810 and the control MON 818 are presented in Table IV.2. The values for each amino acid (mg/g) were converted to percent of total protein. The values for all amino acids were comparable for both MON 809 an 810 and the control (MON 818) and were typical of the values reported in the literature (Watson, 1982) and for a control corn line with a similar genetic background (Sanders and Patzer, 1995).

Table IV.2 Amino acid composition of corn grain[a]

Amino Acid	MON 818 % of Total Protein Mean[d]	MON 818 Range[e]	MON 809 % of Total Protein Mean[d]	MON 809 Range[e]	MON 810 % of Total Protein Mean[d]	MON 810 Range[e]	Literature Range[b]	Reported Range[c]
							%	%
Nutritionally essential								
Methionine	1.7	1.6-1.7	1.7	1.6-1.8	1.7	1.6-1.9	1.0-2.1	2.0-2.6
Cystine	1.9	1.8-2.0	2.0	2.0-2.1	2.0	1.9-2.1	1.2-1.6	1.9-2.3
Lysine	2.8	2.7-2.9	2.7	2.4-3.0	2.8	2.5-2.9	2.0-3.8	2.9-3.4
Tryptophan	0.6	0.4-0.6	0.6	0.5-0.6	0.6	0.5-0.7	0.5-1.2	0.5-0.6
Threonine	3.8	3.7-3.9	3.5	3.4-3.6	3.9	3.7-4.4	2.9-3.9	4.0-4.2
Isoleucine	3.8	3.6-4.0	3.8	3.5-4.0	3.7	3.3-4.1	2.6-4.0	3.7-3.8
Histidine	2.9	2.8-3.0	2.9	2.7-3.0	3.1	2.9-3.3	2.0-2.8	3.0-3.3
Valine	4.6	4.3-4.8	4.6	4.4-4.9	4.5	4.1-4.9	2.1-5.2	4.5-4.8
Leucine	14.5	13.8-15.0	14.4	13.4-15.2	15.0	14.1-16.7	7.8-15.2	13.6-13.8
Arginine	4.5	4.2-4.7	4.5	4.1-5.0	4.5	4.1-4.7	2.9-5.9	4.4-5.0
Phenylalanine	5.4	5.2-5.6	5.5	5.3-5.7	5.6	5.4-6.1	2.9-5.7	5.2-5.4
Glycine	3.7	3.5-3.8	3.5	3.3-3.9	3.7	3.4-4.0	2.6-4.7	3.9-4.2
Non-essential								
Alanine	7.8	7.5-8.0	8.0	7.7-8.3	8.2	7.8-8.9	6.4-9.9	7.8-8.1
Aspartic acid	6.6	6.3-6.8	6.5	6.3-6.8	7.1	6.4-8.2	5.8-7.2	6.8-7.3
Glutamic acid	21.1	20.1-21.6	21.2	20.4-22.1	21.9	20.4-24.4	12.4-19.6	19.9-20.9
Proline	9.6	9.4-9.8	9.8	9.4-10.3	9.9	9.7-10.5	6.6-10.3	9.0-9.4
Serine	5.2	5.1-5.4	5.2	4.8-5.4	5.5	5.3-5.9	4.2-5.5	5.5-6.0
Tyrosine	4.0	3.9-4.1	4.0	3.9-4.1	4.4	4.1-4.8	2.9-4.7	3.8-4.3

a: Values are expressed as percent of total protein.
b: Watson, 1982. Values are per cent of total protein [10.1% total protein (Nx6.25)].
c: Sanders and Patzer (1995), range for a control with similar genetic background.
d: Value reported is mean of six samples, one from each field site (Sanders *et al.*, 1995).
e: Range denotes the lowest and highest individual values across sites for each line.

c. Fatty acid composition of MON 809 and 810 corn grain

The values for fatty acid composition of corn grain from lines MON 809 and 810 and the control line, MON 818, are summarized in Table IV.3. Results are reported for the fatty acids which gave detectable values in the assay. The fatty acid values were similar between lines MON 809 and 810 and control line, MON 818, and typical of the values previously reported in the literature (Watson, 1982) and for a control corn line with a similar genetic background (Sanders and Patzer, 1995). The fatty acids which were not detectable in the assay were: caprylic, capric, lauric, myristic, myristoleic, pentadecanoic, heptadecanoic, eicosadienoic, eicosatrienoic and arachidonic.

Table IV.3. Fatty acid composition of corn grain[a]

Component	MON 818 Mean[b]	MON 818 Range[c]	MON 809 Mean[b]	MON 809 Range[c]	MON 810 Mean[b]	MON 810 Range[c]	Literature Range[d]	Reported Range[e]
Linoleic (18:2)	63.0	61.8-64.6	62.6	61.9-63.3	62.6	59.5-64.7	35-70	61.7-65.0
Oleic (18:1)	22.8	21.6-23.9	22.9	22.4-23.5	23.2	21.5-25.4	20-46	21.3-23.6
Palmitic (16:0)	10.5	10.2-10.7	10.6	10.5-11.0	10.5	10.2-11.1	7-19	10.2-10.8
Stearic (18:0)	1.8	1.8-1.9	1.9	1.8-2.0	1.9	1.7-2.1	1-3	1.6-2.1
Linolenic (18:3)	0.9	0.8-0.9	1.0	0.9-1.0	0.8	0.7-0.9	0.8-2	0.9-1.1

[a]: Value of fatty acid is % of total lipid. Other fatty acids were below the limit of detection of the assay.
[b]: Values presented are means (six samples for each line).
[c]: Range denotes the lowest and highest individual value across sites for each line.
[d]: Watson, 1982.
[e]: Sanders and Patzer (1995), range for a control with similar genetic background.

d. Inorganic components of MON 809 and 810 corn grain

The values for inorganic components of corn grain from lines MON 809 and 810 and the control line, MON 818, are summarized in Table IV.4. The values for calcium and phosphorus were comparable for both MON 809 and 810 and the control (MON 818) and were typical of the values reported in the literature (Watson, 1982) and for a control corn line with a similar genetic background (Sanders and Patzer, 1995).

Table IV.4. Inorganic components of corn grain[a]

Component	MON 818 Mean[b]	MON 818 Range[c]	MON 809 Mean[b]	MON 809 Range[c]	MON 810 Mean[b]	MON 810 Range[c]	Literature Range[d]	Reported Range[e]
Calcium %	0.0033	0.0029-0.0037	0.0030	0.0027-0.0033	0.0036	0.0033-0.0039	0.01-0.1	0.003-0.004
Phosphorus %	0.348	0.327-0.363	0.334	0.287-0.357	0.358	0.334-0.377	0.26-0.75	0.311-0.363

[a]: Values on a dry weight basis.
[b]: Values presented are means (six samples for each line).
[c]: Range denotes the lowest and highest individual value across sites for each line.
[d]: Watson, 1982.
[e]: Sanders and Patzer (1995), range for a control with similar genetic background.

In summary, compositional data for protein, fat, ash, carbohydrates, calories, moisture, amino acids, fatty acids, calcium and phosphorus for insect-protected corn lines MON 809 and 810 was equivalent to the control line, MON 818, and within the published and reported literature ranges for commercial hybrids. Based on these data, it was concluded that the grain from the corn lines MON 809 and 810 and the control line, MON 818 are similar in composition and representative of corn grain currently in commerce.

e. Compositional analyses of forage from corn lines MON 809 and 810 grown in the 1995 European field trials

The major components of the forage for the representative corn line MON 810 and control, MON 820 were analyzed under Good Laboratory Practices (GLP) on forage plants harvested from three field trials in France. Proximates (protein, fat, ash, carbohydrates, calories and moisture), acid detergent fiber (ADF) and neutral detergent fiber (NDF) were performed on the ground forage plants by published methods (AOAC, 1990; Williams and Norris, 1987). The values reported for the compositional analyses as measured by the AOAC method at Corning Hazleton Inc., Madison, Wisconsin, USA, (Table III.5) were expressed as percent dry weight of the sample, correcting for the measured moisture content.

Table III.5 Compositional analyses on forage samples of corn line 810 from France field trials[a]

Component	MON 820	MON 810
Protein %	4.7-7.4	5.7-8.4
Ash %	2.9-4.4	3.1-3.6
ADF %	25.6-29.2	22.6-27.2
NDF %	39.9-43.3	36.9-41.4
Total fat %	1.4-2.1	1.3-1.7
Carbohydrates, %	88.0-89.1	86.9-89.8
Dry Matter %	26.5-31.3	28.7-32.4

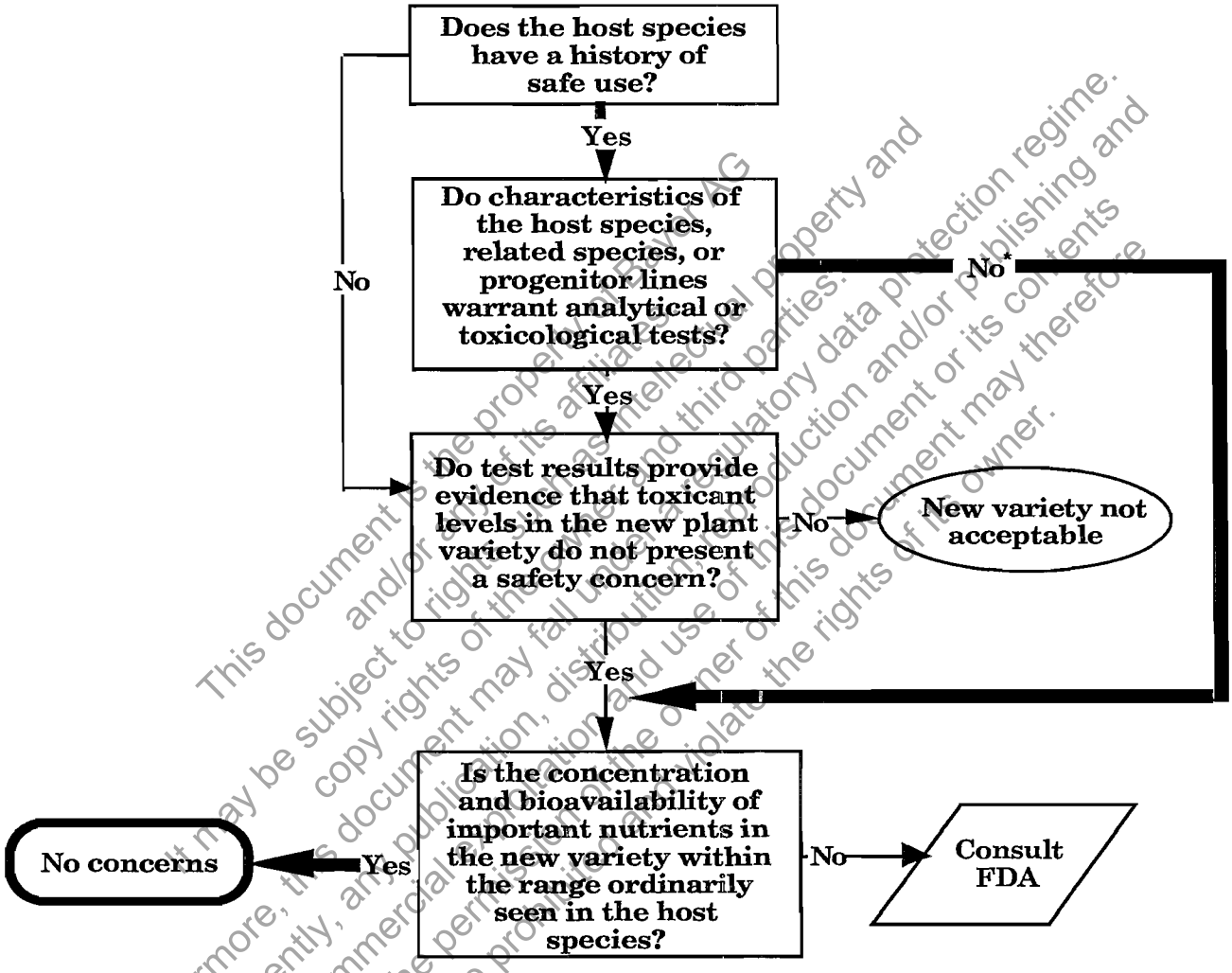
[a]: There were three samples, one from each field site. Values are ranges, the lowest and highest individual value across sites for each line.

In summary, compositional data for protein, fat, ash, acid detergent fiber, neutral detergent fiber, fat, carbohydrates and dry matter for corn line MON 810 was similar to the control line, MON 820. Based on these data, it was concluded that the forage from corn line MON 810 and the control line, MON 820 are similar in composition.

5. Conclusions

The Food Policy recommends that key compositional components of genetically modified plant varieties be assessed prior to commercial introduction. Monsanto has, therefore, performed extensive analytical studies to compare the compositional quality of lines MON 809 and 810 to the control line, MON 818. The absence of unexpected or unintended effects due to the expression of the CryIA(b) and CP4 EPSPS proteins in these lines is demonstrated by the establishment that the host organism, corn, has a safe history of use and extensive compositional analysis of lines MON 809 and 810 with comparison to the control line and published ranges for other corn varieties.

Figure IV.1 Safety Assessment of New Varieties: The Host Plant (taken from FDA Food Policy, Figure 2). The pathway leading to "no concerns" for insect-protected corn lines MON 809 and 810 is highlighted with bold arrows.



* New corn varieties are not typically subjected to extensive analytical tests. However, compositional analyses to verify levels of nutrients to ensure the wholesomeness of insect-protected corn lines MON 809 and 810, were performed as discussed in the Food Policy.

B. Safety Assessment of New Varieties: the Donors

1. Donor organisms

The safety of the donor organisms of the *cryIA(b)*, CP4 EPSPS, *gox* and *nptII* genes was considered. These organisms are not commonly used directly as a food or feed source, however they are ubiquitous in nature and are likely present as contaminates on the food and feed consumed. In addition, *E. coli*, (which contains the *nptII* gene) is present in the digestive systems of humans and animals. In response to the question posed in Figure IV.2, " Safety Assessment of New Varieties: the Donors", the following discussion considers the safety of the donor organisms.

Bacillus thuringiensis subsp. *kurstaki* (B.t.k.), which produces the CryIA(b) insect control protein, is the basis of microbial formulations commercially available for lepidopteran insect control for over 30 years (EPA, 1988; Lüthy *et al.*, 1982). Based on the available scientific data, EPA and other regulatory agencies, worldwide, have determined that use of registered *B.t.k.* products pose no significant risks to human health, non-target organisms or the environment. The protein produced by the gene utilized in the production of lines MON 809 and 810 is identical to that found in nature and in commercial formulations containing the CryIA(b) protein. The results of Monsanto sponsored studies submitted to the Environmental Protection Agency on November 2, 1994 and January 31, 1995, supporting an exemption from the requirement of a tolerance and the registration of the CryIA(b) protein as a plant pesticide, fully confirm the safety of this protein.

The EPSPS from *Agrobacterium* sp. strain CP4 is highly tolerant to inhibition by glyphosate and has high catalytic efficiency, compared to most glyphosate-tolerant EPSPSs (Barry *et al.*, 1992; Padgette *et al.*, 1991). EPSPS is an enzyme of the shikimate pathway for aromatic amino acid biosynthesis in plants (including corn) and microorganisms (Levin and Sprinson, 1964; Steinrüken and Amrhein, 1980); therefore, this enzyme and its activity are not novel in food derived from plant sources. Genes for numerous EPSPSs have been cloned (Padgette *et al.*, 1989, 1991), and active site domains are conserved among the known EPSPSs ([redacted] 1991; 1993). Bacterial EPSPSs have been well-characterized with respect to the 3-dimensional X-ray crystal structure (Stallings *et al.*, 1991) and the detailed kinetic and chemical reaction mechanism (Anderson and Johnson, 1990). CP4 EPSPS thus represents one of many different EPSPSs found in nature.

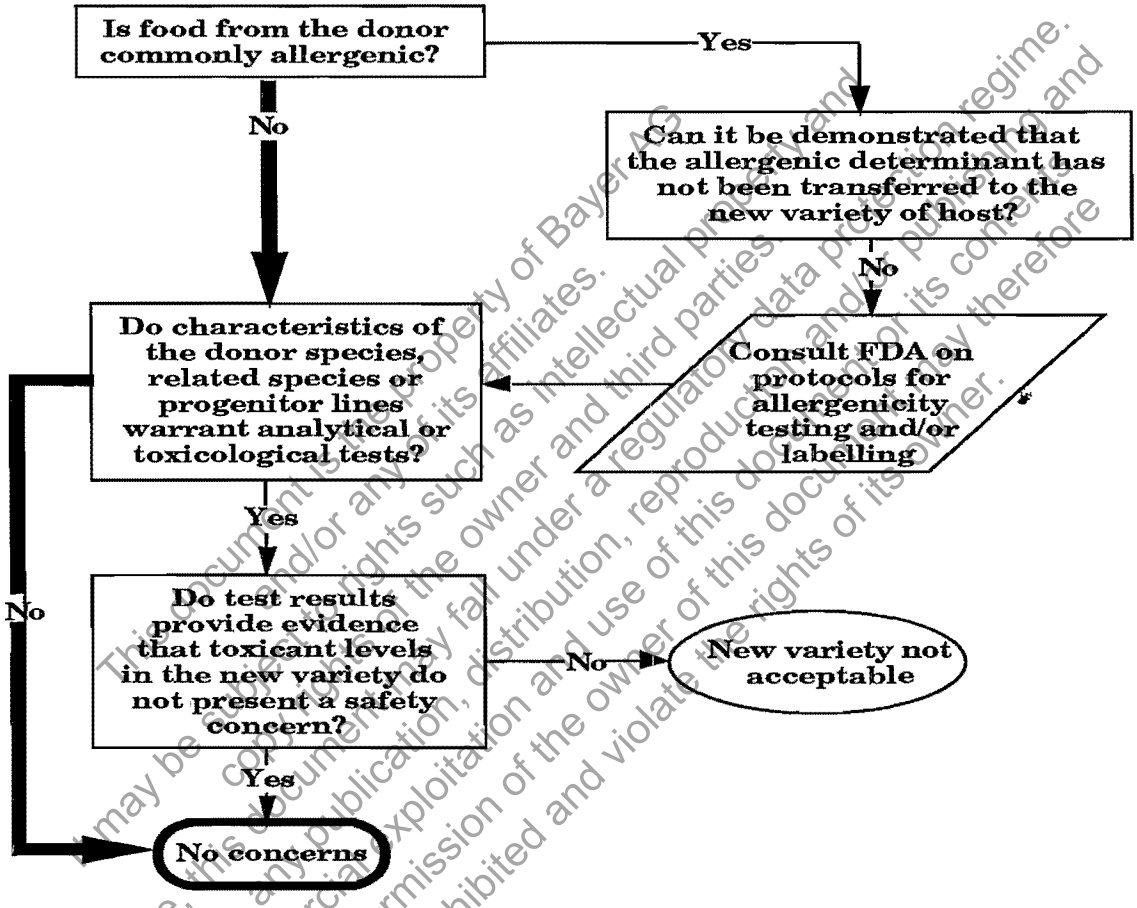
The CP4 EPSPS protein has been reviewed as a part of Monsanto's consultation with the FDA regarding glyphosate-tolerant soybeans and cotton. Based on the description of the data and information presented during the consultations, the new soybean and cotton varieties were not significantly altered from the expression of CP4 EPSPS within the meaning of 21 CFR 170.(f)(2).

The safety of the donor organism of the *gox* gene, *Achromobacter* sp. strain LBAA, was considered. Although *Achromobacter* sp. strain LBAA is not a food source, *Achromobacter* sp. is reported to be one of the most frequently occurring bacteria in the rhizosphere (Joos *et al.*, 1988) and the enzyme has been extensively characterized (Padgette *et al.*, 1994). Since only the *gox* gene was transferred from *Achromobacter* to corn and the sequence of the DNA transferred to the host is completely known, characteristics of this donor species do not warrant further tests.

The *nptII* gene was isolated from kanamycin resistant bacteria that contained the Tn*5* transposon (Berg *et al.*, 1975). This gene has been used as a selectable marker in *E. coli* (Rao and Rogers, 1979), animal and human cells (Blaese, 1993) and plants (Fraley *et al.*, 1983). It was previously determined that as the *nptII* gene is under the control of a bacterial specific promoter and coding region, the NPTII was not expressed in the corn plant (Croon *et al.*, 1995b). The NPTII protein, which has no insecticidal effect, is ubiquitous in the environment and found in microbes present on food and within the human digestive system. The safety of the NPTII protein has been reviewed and discussed broadly because of its wide use as a selectable marker for plant transformation (Nap *et al.*, 1992; Flavell *et al.*, 1992; WHO, 1993; Fuchs *et al.*, 1993a; Fuchs *et al.*, 1993b). In addition, the use of this protein was approved as a processing aid food additive in tomato, canola and cotton, as requested by Calgene, Inc. (FDA, 1994). In addition, the EPA has exempted the NPTII protein and the genetic material necessary for the production of the protein from the requirement of a tolerance in or on all agricultural commodities when used as a plant-pesticide inert ingredient (EPA, 1994a).

In conclusion, the characteristics of the donor organisms, *Bacillus thuringiensis* subsp. *kurstaki*, *Agrobacterium* sp. strain CP4, *Achromobacter* sp. strain LBAA and *E. coli* do not warrant analytical or toxicological tests. Furthermore, only the sequenced and well characterized genes have been derived from these sources. These points, taken with the properties and safety of the CryIA(b) and CP4 EPSPS proteins discussed below (including lack of typical profile for protein allergens), led us to a conclusion of "no concern" for the source of the donor genes as listed on Figure IV.2.

Figure IV.2 Safety Assessment of New Varieties: The Donor (taken from FDA Food Policy, Figure 3). The pathway leading to "no concerns" for insect-protected corn lines MON 809 and 810 is highlighted with bold arrows.



C. Safety Assessment of New Varieties: Proteins Introduced from Donor(s)

FDA's Statement of Policy "Foods Derived From New Plant Varieties", published in the Federal Register May 29, 1992 (FDA, 1992) and the proposed Plant Pesticide Policy of the Environmental Protection Agency (EPA, 1994b), agree that, under FIFRA, the EPA will have regulatory oversight for plant pesticides and selectable markers used in the plant transformation process for the purpose of identifying the cells containing the pesticidal gene. Monsanto has consulted with and submitted studies to the EPA supporting the registration of and exemption from the requirement of a tolerance for the CryIA(b) insect control protein and exemption from the requirement of a tolerance for CP4 EPSPS as a plant pesticide formulation inert ingredient. Both the CryIA(b) and CP4 EPSPS proteins have recently received temporary exemptions from the requirement of a tolerance in all raw agricultural commodities of field corn, sweet corn and popcorn.

Insect-protected corn lines MON 809 and 810 have received Non-regulated Status from the United States Department of Agriculture Animal and Plant Health Inspection Service (Croon *et al.*, 1996). These submissions fully support the environmental and non-target safety of these corn varieties and the proteins produced. The protein expression levels from the introduced genes are provided as well as a demonstration of the lack of allergenic concern for the expressed proteins to address this portion of the FDA flow chart (see figure IV.3).

1. Expression levels of the CryIA(b), CP4 EPSPS, GOX and NPTII proteins

Levels of the expressed proteins were evaluated in young leaf, grain, whole plant and pollen tissues collected from six field locations during the 1994 growing season using Enzyme Linked Immuno-Sorbent Assay (ELISA) (Harlow and Lane, 1988) and western blot methods (Matsudaira, 1987). The six field sites established and conducted under GLP were as follows: Jerseyville, Illinois; Monmouth, Illinois; Johnston, Iowa; Sheldahl, Iowa; Windfall, Indiana; and York, Nebraska (Sanders *et al.*, 1995). The approximate expression levels are shown in the following table.

Table IV.6 Summary of specific protein levels measured in tissues of insect-protected corn lines MON 809 and 810[1]

Corn line	Protein	Leaf	Grain	Whole plant[2,3]	Pollen[2]
		-µg/g fresh weight-			
MON 809					
	CryIA(b)	1.63	0.55	1.23	N.D.[4]
	CP4 EPSPS	21.68	9.41	1.60	N.A.[5]
	GOX	N.D.	N.D.	N.D.	N.A.
	NPTII	N.A.	N.A.	N.A.	N.A.
MON 810					
	CryIA(b)	9.35	0.31	4.15	0.09
	CP4 EPSPS	N.D.	N.D.	N.D.	N.A.
	GOX	N.D.	N.D.	N.D.	N.A.
	NPTII	N.A.	N.A.	N.A.	N.A.

[1]: Values are means calculated from the analyses of six plant samples, one from each of six field sites, unless noted otherwise.
[2]: The mean was calculated from the analyses of plant sample(s) from one site.
[3]: Values are means calculated from the analyses of two replicate plant samples from one site.
[4]: N.D., Not detected
[5]: N.A., Not analyzed

As seen above, expression levels of the CryIA(b) protein in line MON 810 and the CryIA(b) and CP4 EPSPS proteins in MON 809 are low in corn leaf, grain and whole plant tissues. CryIA(b) protein was not detectable in pollen in line MON 809 and just above the limit of detection in line MON 810.

GOX protein was not detectable in corn leaf, grain and whole plant tissue when assayed by sensitive and specific ELISA and this result was confirmed by western blot analysis. Since the *nptII* gene is under the control of a bacterial - specific promoter and the lack of expression was previously demonstrated for insect-protected corn line MON 801 (Croon *et al.*, 1995b), further NPTII protein analysis was not performed.

2. Assessment of the allergenic potential of the CryIA(b) and CP4 EPSPS proteins

Introduction of insect-protected corn varieties does not pose an increased risk of allergies. CryIA(b) and EPSPS proteins have a long history of safe use and do not share the biochemical properties common to known allergenic proteins.

Large quantities of a vast variety of proteins are consumed in diets each day. Rarely do any of these tens of thousands of proteins elicit an allergenic response (Taylor, 1992). The most important factor to consider is whether the source of the gene being introduced in to plants is allergenic (FDA, 1992). Neither *Bacillus thuringiensis* nor *Agrobacterium* have histories of causing allergy. In over 30 years of commercial use, there have been no reports of allergenicity to *Bacillus thuringiensis,* including occupational allergy associated with manufacture of products containing *Bacillus thuringiensis* (EPA, 1995). In addition, the biochemical profile of the CryIA(b) and CP4 EPSPS proteins provides a basis for allergenic assessment when compared with known protein allergens. Protein allergens must be stable to the peptic and tryptic digestion and the acid conditions of the digestive system if they are to reach and pass through the intestinal mucosa to elicit an allergenic response. Another significant factor contributing to the allergenicity of proteins is their high concentration in foods that elicit an allergenic response (Taylor, 1992; Taylor *et al.* 1987; and Taylor *et al.*, 1992).

A comparison of the amino acid sequence of an introduced protein with the amino acid sequences of known allergens is a useful first approximation of allergenic potential. The amino acid sequences of most major allergens, including food allergens, have been reported (King *et al.*, 1994) and the list is likely to expand with time. The important IgE binding epitopes of many allergen proteins have been mapped (Elsayed and Apold, 1983; Elsayed *et al.*, 1991; Zhang *et al.*, 1992). The optimal peptide length for binding is between 8 and 12 amino acids (Rothbard and Gefter, 1991). T-cell epitopes of allergenic proteins and peptide fragments appear to be least eight amino acids in length (O'Hehir *et al.*, 1991). Exact conservation of epitope sequences is observed in homologous allergens of disparate species (Astwood *et al.*, 1995). Indeed, conservative substitutions introduced by site-directed mutagenesis reduce epitope efficacy (Smith and Chapman, 1995). Based on this information, an immunologically relevant sequence comparison test for similarity between the amino acid sequence of the introduced protein and known allergens has been defined: a match of at least eight contiguous identical amino acids is required.

We have searched the amino acid sequences of the 219 allergens present in public domain genetic databases (GenBank, EMBL, PIR, and SwissProt) for similarity to the amino acid sequences of CryIA(b) and CP4 EPSPS proteins using the FASTA computer program (Pearson and Lipman, 1988). No biologically significant homology (Doolittle, 1990) and, with the test criteria above, no immunologically significant sequence similarities were observed with allergens. We conclude (1) that the genes introduced into these foods do not encode known allergens, and (2) that none of the introduced proteins share immunologically significant sequences with known allergens.

CryIA(b) and CP4 EPSPS proteins do not possess any of the other characteristics common to protein allergens. The CryIA(b) and CP4 EPSPS proteins were shown to be very labile to digestion by the proteases present in the mammalian digestive system, minimizing any potential for this protein to

be absorbed by the intestinal mucosa, if consumed. *In vitro*, simulated mammalian gastric and intestinal digestive mixtures were established and used to assess the susceptibility of the CryIA(b) and CP4 EPSPS proteins to proteolytic digestion. The method of preparation of the simulated digestion solutions used is described in the United States Pharmacopeia (1989), a frequently cited reference for *in vitro* digestion. *In vitro* studies with simulated digestive solutions are widely used as models of animal digestion. They have been used to investigate the digestibility of plant proteins (Nielson, 1988; Marquez and Lajolo, 1981), animal proteins (Zikakis *et al.*, 1977) and food additives (Tilch and Elias, 1984); to assess protein quality (Akeson and Stahmann, 1964); to study digestion in pigs and poultry (Fuller, 1991); to measure tablet dissolution rates to monitor biodegradation for pharmaceutical applications (Alam *et al.*, 1980); and to investigate the controlled-release of experimental pharmaceuticals (Doherty *et al.*, 1991).

The ability of food allergens to reach and to cross the mucosal membrane of the intestine are likely prerequisites to allergenicity. Clearly, a protein which is stable to the proteolytic and acidic conditions of the digestive tract has an increased probability of reaching the intestinal mucosa. Many allergens exhibit proteolytic stability (King *et al.*, 1967; Kortekangas-Savolainen *et al.*, 1993; Onaderra *et al.*, 1994; Taylor, 1992; Taylor *et al.*, 1987; Metcalfe, 1985), although the majority remain untested directly. Intact proteins are capable of crossing the mucosal membrane of the gut and of entering the circulatory system (Gardner, 1988). Thus, physicochemical properties which favor digestive stability can be used as an important indicator of allergenic potential.

The data from the simulated digestion experiments demonstrated that the CryIA(b) protein degraded rapidly; more than 90% of the initially added CryIA(b) protein degraded after two minutes incubation in the gastric system (EPA MRID no. 43439201). As expected, in the intestinal system, the full length CryIA(b) protein was rapidly converted to the trypsin-resistant core, which was not further degraded. The half-life for CP4 EPSPS was estimated at less than 15 seconds in the gastric system and less than 10 minutes in the intestinal system, based on western blot analysis. To put the rapid degradation of these proteins in the simulated gastric system into perspective, solid food has been estimated to empty from the human stomach by about 50% in two hours, while liquid empties 50% in approximately 25 minutes (Sleisenger and Fordtran, 1989). Therefore, any CryIA(b) or CP4 EPSPS protein consumed would be rapidly degraded in the gastric system.

Finally, most allergens are present as major protein components in the specific food. This is true for the allergens in milk (Baldo, 1984; Lebenthal, 1975; Taylor, 1986; Taylor *et al.*, 1987), soybean (Shibasaki *et al.*, 1980; Burks *et al.*, 1988; Pedersen and Djurtoft, 1989), peanuts (Barnett *et al.*, 1983; Sachs *et al.*, 1981; Barnett and Howden, 1986; Kemp *et al.*, 1985), etc. In contrast to this generality for common allergenic proteins, the CryIA(b) and CP4 EPSPS proteins are present in corn seed at low levels. The low levels of the CryIA(b) and CP4 EPSPS proteins in corn seed, combined with the digestive lability of

these proteins relative to that for known food allergens establishes an extremely low probability of the CryIA(b) or CP4 EPSPS proteins being absorbed via the intestinal mucosa during consumption and triggering production of antibodies including the IgE antibodies responsible for allergenicity.

The CryIA(b) protein expressed in these insect-protected corn plants is identical to the CryIA(b) protein contained in microbial formulations that have been used safely commercially for over 30 years (EPA MRID no. 43533203; EPA, 1988). These microbial formulations have been used on a wide variety of crops, including fresh vegetables, with no reported allergenic responses, establishing a sound basis for the lack of allergenic concern for the CryIA(b) protein.

EPSPS proteins are a diverse set of related proteins typically present in foods and feeds derived from plants (including corn) and microbes. Paired comparisons of the deduced amino acid sequences of CP4 EPSPS (455 amino acids in length) with the corn endogenous EPSPS protein (506 amino acids in length) indicated a 441 amino acid overlap between the two proteins with 70% similarity and 24% identity in amino acid sequence. The amino acid homology of the CP4 EPSPS and the endogenous corn EPSPS further support the lack of allergenic concern.

In summary, the data and analyses described above and summarized in table IV.7 support the conclusion that the CryIA(b) and CP4 EPSPS proteins are not derived from allergenic sources, do not possess immunologically relevant sequence similarity with known allergens and do not possess the characteristics of known protein allergens. Furthermore, these proteins or closely related proteins have a history of use with no allergenic concerns. This information, coupled with the extremely rapid digestion of this protein under *in vitro* digestive conditions that mimic human digestion, established that, using the best methodology available today, there is no reason to believe that either protein should pose any significant allergenic risks for consumption of the products generated from insect-protected corn plants.

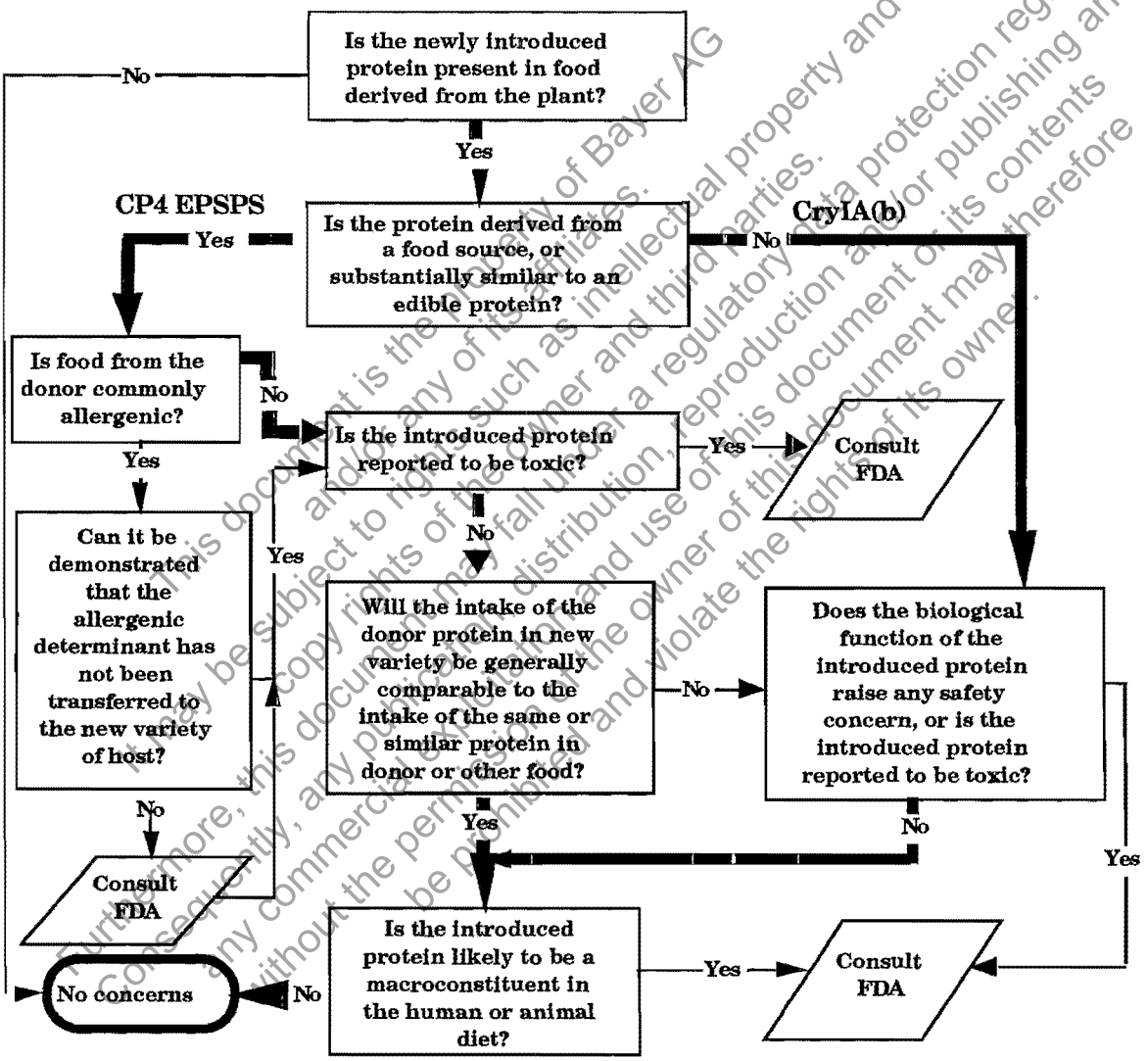
Table IV.7 Characteristics of known allergenic proteins[a]

Characteristic	Allergens	CryIA(b)	CP4 EPSPS
Allergenic source of gene	yes	no	no
Similar sequence to allergens	yes	no	no
Stable to digestion	yes	no	no
Prevalent protein in food	yes	no	no

[a] As described in Taylor (1992) and Taylor *et al.* (1987)

Based upon these data and information we have reached the conclusion of "No Concerns" as listed on Figure IV.3.

Figure IV.3 Safety Assessment of New Varieties: Proteins Introduced from Donor (taken from FDA Food Policy Figure 4). The pathway leading to "no concerns" for insect-protected corn lines MON 809 and 810 is highlighted with bold arrows.



V. Conclusion for the Safety Assessment of Insect-Protected Corn Lines MON 809 and 810

Insect-protected corn lines MON 809 and 810 are not materially different in any meaningful way from corn varieties now being sold except for the ability to resist feeding by certain lepidopteran insect pests including ECB. The results of extensive analyses demonstrate that the levels of the important corn seed and plant components (protein, fat, carbohydrate, ash, fiber, fatty acids, amino acids, and moisture) in lines MON 809 and 810 are comparable to the parental control line, MON 818 and are within the published literature ranges.

Both the CryIA(b) and CP4 EPSPS proteins are present in insect-protected corn line MON 809 at very low levels whereas only the CryIA(b) protein is present in line MON 810. The CryIA(b) protein is the basis of microbial formulations commercially available for lepidopteran insect control for over 30 years in the United States. The CP4 EPSPS protein is related to EPSPSs already found in foods derived from plants, microbes and fungi. The CryIA(b) and CP4 EPSPS proteins are rapidly degraded in simulated digestive fluids and lack other properties associated with allergenic proteins.

These data led to a conclusion of "no concerns" for every criterion in the flow charts outlined in the Food Policy. Corn modified to be resistant to lepidopteran insect pests is not materially different in composition, safety, wholesomeness, or any relevant parameter from corn now grown, marketed, and consumed. Sales and consumption of corn grain derived from these corn lines and all progenies derived from crosses with these lines and introduced varieties would be fully consistent with the Agency's Food Policy, the Federal Food, Drug, and Cosmetic Act, and current practices for the development and introduction of new corn varieties.

VI. List of Supporting Studies and Submissions

A. Summary Assessments Previously Submitted to the FDA

Croon, K.A., P. R. Sanders, P. J. Keck, B.G. Hammond, J.D. Astwood, and R.L. Fuchs. 1995. Safety, Compositional and Nutritional Aspects of Insect-Protected Corn Line MON 801: Conclusion Based on Studies and Information Evaluated According to FDA's Policy on Foods from New Plant Varieties. Submitted to the FDA on September 15, 1995.

B. Studies Submitted to the EPA to Support the Registration and Exemption from the Requirement of a Tolerance of the CryIA(b) Protein as a Plant Pesticide

PRODUCT ANALYSIS

[redacted] 1995. "Molecular Characterization of Insect Protected Corn Line MON 810", MSL 14204, an unpublished study conducted by Monsanto Company. EPA MRID no. 43665501.

[redacted] 1995. "Evaluation of Insect Protected Corn Lines in 1994 U.S. Field Test Locations", Study Number 94-01-39-01, an unpublished study conducted by Monsanto Company. EPA MRID no. 43665502.

[redacted] 1995. "Assessment of the Equivalence of B.t.k. HD-1 Protein Produced in Several Insect Protected Corn Lines and Escherichia coli", Study Number 95-01-39-01, an unpublished study conducted by Monsanto Company. EPA MRID no. 43665503.

[redacted] "Molecular Characterization of Insect Protected Corn Line MON80100", MSL-13924, an unpublished study conducted by Monsanto Company. EPA MRID no. 43533201.

[redacted] "Evaluation of European Corn Borer Resistant Corn Lines in 1993 U.S. Field Test Locations", Study Number 93-01-39-02, an unpublished study conducted by Monsanto Company. EPA MRID no. 43533202.

[redacted] "Compositional Comparison of *Bacillus thuringiensis* subsp. *kurstaki* HD-1 Protein Produced in ECB Resistant Corn and the Commercial Microbial Product, DIPEL®", Study Number 94-01-39-12, an unpublished study conducted by Monsanto Company. EPA MRID no. 43533203.

[redacted] "Assessment of the Equivalence of the *Bacillus thuringiensis* subsp. *kurstaki* HD-1 Protein Produced in *Escherichia coli* and European Corn Borer Resistant Corn", Study Number 94-01-39-09, an unpublished study conducted by Monsanto Company. EPA MRID no. 43533204.

AVIAN DIETARY TESTING

[redacted] "A Dietary Toxicity Study with MON 80187 Meal in the Northern Bobwhite", Project Number 139-387, an unpublished study conducted by [redacted] under contract to Monsanto Company. EPA MRID no. 43533205.

ENVIRONMENTAL FATE

[redacted] "Aerobic Soil Degradation of *Bacillus thuringiensis* var. *kurstaki* HD-1 Protein", Study Number 94-01-39-13, an unpublished study conducted by Monsanto Company. EPA MRID no. 43533206.

TOXICOLOGY

[redacted] 1992. "Acute Oral Toxicity Study of *Btk* HD-1 Tryptic Core Protein in Albino Mice". Study Number 92069, an unpublished study conducted by [redacted]. EPA MRID no. 43468001.

[redacted] 1994. "Assessment of the *In Vitro* Digestive Fate of *Bacillus thuringiensis* subsp. *kurstaki* HD-1 Protein", Study Number 93-01-39-04, an unpublished study conducted by Monsanto Company. EPA MRID no. 43439201.

NONTARGET ORGANISM TESTING

[redacted] 1994. "Stability of the CryIA(b) Insecticidal Protein of *Bacillus thuringiensis* var. *kurstaki* (*B.t.k.* HD-1) in Sucrose and Honey Solutions Under Non-Refrigerated Temperature Conditions", Study Number IRC-91-ANA-11, an unpublished study conducted by Monsanto Company. EPA MRID no. 43468002.

[redacted] 1994. "Evaluation of the Dietary Effects of Purified *B.t.k.* Endotoxin Proteins on Honey Bee Larvae", Study Number IRC-91-ANA-13, an unpublished study conducted by Monsanto Company and California Agricultural Research, Inc. EPA MRID no. 43439202.

[redacted] 1994. "Evaluation of the Dietary Effects of Purified *B.t.k.* Endotoxin Proteins on Honey Bee Adults", Study Number IRC-91-ANA-12, an unpublished study conducted by Monsanto Company and California Agricultural Research, Inc. EPA MRID no. 43439203.

[redacted] 1992. "Activated *Btk* HD-1 Protein: A Dietary Toxicity Study with Green Lacewing Larvae", Study Number WL-92-155, an unpublished study conducted by Monsanto Company and Wildlife International Ltd. EPA MRID no. 43468003.

[redacted] 1992. "Activated *Btk* HD-1 Protein: A Dietary Toxicity Study with Parasitic Hymenoptera (*Brachymeria intermedia*)", Study Number WL-92-157, an unpublished study conducted by Monsanto Company and Wildlife International Ltd. EPA MRID no. 43468004.

[redacted] 1992. "Activated *Btk* HD-1 Protein: A Dietary Toxicity Study with Ladybird Beetles", Study Number WL-92-156, an unpublished study conducted by Monsanto Company and Wildlife International Ltd. EPA MRID no. 43468005.

[redacted] 1995. "CryIA(b) Insecticidal Protein: An Acute Toxicity Study with the Earthworm in an Artificial Soil Substrate", Study Number WL-95-281, an unpublished study conducted by Monsanto Company and Wildlife International Ltd. EPA MRID no. 43887902.

[redacted] 1996. "Effect of the *Bacillus thuringiensis* Insecticidal Proteins CryIA(b), CryIA(c), CryIIA, and CryIIIA on *Folsomia candida* and *Xenylla grisea* (Insecta: Collembola)", an unpublished study conducted by Monsanto Company. EPA MRID no. 43941601.

[redacted] 1995. "Evaluation of the European Corn Borer Resistant Corn Line MON 801 as a Feed Ingredient for Catfish", Study Number 94-01-39-16, an unpublished study conducted by Monsanto Company. EPA MRID no. 43887901.

C. Studies submitted to the EPA to Support the Exemption from the Requirement of a Tolerance for the CP4 EPSPS Protein as a Pesticide Inert Ingredient

PRODUCT ANALYSIS

[redacted] "Characterization of Microbially-Expressed Protein: CP4 EPSPS", Study Number 92-01-30-14, an unpublished study conducted by Monsanto Company. EPA MRID no. 43643301.

[redacted]
"Assessment of the Equivalence of CP4 EPSPS Protein Produced in *Escherichia coli* and European Corn Borer Resistant Corn", Study Number 94-01-39-10, an unpublished study conducted by Monsanto Company. EPA MRID no. 43643302.

[redacted] 1993. "Purification, Cloning, and Characterization of a Highly Glyphosate-tolerant 5-Enolpyruvylshikimate-3-phosphate Synthase from *Agrobacterium* sp. strain CP4". MSL-12738, an unpublished study conducted by Monsanto Company. EPA MRID no. 43807601.

[redacted] 1993. "Production of CP4 EPSPS Synthase in a 100 Liter Recombinant *Escherichia coli* Fermentation". MSL-12389, an unpublished study conducted by Monsanto Company. EPA MRID no. 43807602.

[redacted] 1993. "The Purification of Recombinant *Escherichia coli* CP4 5-Enolpyruvalshikimate-3-phosphate Synthase for Equivalence Studies". MSL-12574, an unpublished study conducted by Monsanto Company. EPA MRID no. 43807603.

TOXICOLOGY

[redacted] "Preparation and Confirmation of Doses for an Acute Mouse Feeding Study with CP4 EPSPS", Monsanto Study 92-01-30-12, an unpublished study conducted by [redacted] EPA MRID no. 43691901.

[redacted] "Acute Oral Toxicity Study of CP4 EPSPS in Albino Mice", [redacted] Study Nymber 92223, an unpublished study conducted by [redacted] EPA MRID no. 43643303.

[redacted] "Assessment of the *in vitro* digestive fate of CP4 EPSP synthase, Monsanto Study 92-01-30-15, an unpublished study conducted by Monsanto Company. EPA MRID no. 43643304.

D. Petitions Previously Submitted to the USDA in Support of a Determination of Nonregulated Status.

[redacted] 1995. Petition for Determination of Nonregulated Status: Insect Protected Corn (*Zea mays* L.) with the *cryIA(b)* Gene from *Bacillus thuringiensis* subsp. *kurstaki*. USDA Petition no. 95-093-01p.

[redacted] 1996. Petition for Determination of Nonregulated Status: Additional YieldGard™ Corn (*Zea mays* L.) Lines with the *cryIA(b)* Gene from *Bacillus thuringiensis* subsp. *kurstaki*. USDA Petition no. 96-017-01p.

VII. References

Akeson, W.R. and M.A. Stahmann. 1964. A pepsin pancreatin digest index of protein quality evaluation. J. Nutrition 83:257-261.

Alam, A.S., L.M. Hagerman, and A.R. Imondi. 1980. Bioavailability of sulpiride tablet and capsule in dogs. Arch. Int. Pharmacodyn. 247:180-189.

Aldrich, S.R., W.O Scott, and R.G. Hoeft. 1986. Modern Corn Production, Third Edition. A & L Publications, Inc., Champaign, Illinois, USA.

Anderson. K.S. and K.A. Johnson. 1990. Kinetic and structural analysis of enzyme intermediates: Lessons from EPSP synthase. Chem. Rev. 90:1131-1149.

Anderson, R.A. and S.A. Watson. 1982. The corn milling industry. pp. 31 - 78. *In* CRC Handbook of Processing and Utilization in Agriculture, Wolff, I.A. (ed.), Volume II: Part 1, Plant Products. CRC Press, Inc., Boca Raton, Florida.

Astwood, J.D., S.S. Mohapatra, H. Ni, and R.D. Hill. 1995. Pollen allergen homologues in barley and other crop species. Clin. Exp. Allergy 25:150-157.

Baldo, B.A. 1984. Milk allergies. Austral. J. Dairy Technology 39:120-128.

Barnett, D. and M.E.H. Howden. 1986. Partial characterization of an allergenic glycoprotein from peanut (*Arachis hypogaea* L.). Biochem. Biophys. Acta 882:97-105.

Barnett, D., B.A. Baldo, and H.E.H. Howden. 1983. Multiplicity of allergens in peanuts. J. Allergy Clin. Immunol. 72:61-68.

Barrido, R. and K. Steffey. 1995. The use of Bt-corn to control European corn borers: 1994 results and future expectations. pp. 28-32. *In* Proc. 1995 Illinois Agric. Pestic. Conf., Coop. Ext. Serv., Univ. of Illinois, Urbana-Champaign.

Barry, G., G. Kishore, S. Padgette, M. Taylor, K. Kolacz, M. Weldon, D. Re, D. Eichholtz, K. Fincher, L. Hallas. 1992. Inhibitors of amino acid biosynthesis: strategies for imparting glyphosate tolerance to crop plants. pp. 139 - 145. *In* Biosynthesis and Molecular Regulation of Amino Acids in Plants, Singh *et al.* (eds.), American Society of Plant Physiologists.

[redacted] 1994. Cloning and Expression in *Escherichia coli* of the Glyphosate-to-Aminomethylphosphonic acid degrading activity from *Achromobacter* sp. Strain LBAA. Report Number MSL-13245, an unpublished technical report by Monsanto Company.

Beck, E., G. Ludwig, E.A. Auerswald, B. Reiss, and H. Schaller. 1982. Nucleotide Sequence and Exact Localization of the Neomycin Phosphotransferase Gene from Transposon Tn5. Gene 19:327-336.

Berg, D.E., J. Davies, B. Allet, and J.D. Rochaix. 1975. Transposition of R factor genes to bacteriophage lambda. Proc. Natl. Acad. Sci. USA, 72:3628-3632.

Bergman, P.W., R.A. Davis, S.N. Fertig, F. Kuchler, R. McDowell, C. Osteen, A.L. Padula, K.L. Smith & R.F. Torla. 1985a. Pesticide assessment of field corn and soybeans: Corn Belt states. National Agric. Pestic. Impact Assessment Program, U.S. Dep. Agric., Washington, D.C. 26 pp.

Bergman, P.W., R.A. Davis, S.N. Fertig, F. Kuchler, R. McDowell, C. Osteen, A.L. Padula, K.L. Smith & R.F. Torla. 1985b. Pesticide assessment of field corn and soybeans: Delta states. National Agric. Pestic. Impact Assessment Program, U.S. Dep. Agric., Washington, D.C. 28 pp.

Bergman, P.W., R.A. Davis, S.N. Fertig, F. Kuchler, R. McDowell, C. Osteen, A.L. Padula, K.L. Smith & R.F. Torla. 1985c. Pesticide assessment of field corn and soybeans: Lake states. National Agric. Pestic. Impact Assessment Program, U.S. Dep. Agric., Washington, D.C. 22 pp.

Bergman, P.W., R.A. Davis, S.N. Fertig, F. Kuchler, R. McDowell, C. Osteen, A.L. Padula, K.L. Smith & R.F. Torla. 1985d. Pesticide assessment of field corn and soybeans: Northeastern states. National Agric. Pestic. Impact Assessment Program, U.S. Dep. Agric., Washington, D.C. 28 pp.

Bergman, P.W., R.A. Davis, S.N. Fertig, F. Kuchler, R. McDowell, C. Osteen, A.L. Padula, K.L. Smith & R.F. Torla. 1985e. Pesticide assessment of field corn and soybeans: Northern Plains states. National Agric. Pestic. Impact Assessment Program, U.S. Dep. Agric., Washington, D.C. 22 pp.

Bergman, P.W., R.A. Davis, S.N. Fertig, F. Kuchler, R. McDowell, C. Osteen, A.L. Padula, K.L. Smith & R.F. Torla. 1985f. Pesticide assessment of field corn and soybeans: Southeastern states. National Agric. Pestic. Impact Assessment Program, U.S. Dep. Agric., Washington, D.C. 26 pp.

Blaese, R.M. 1993. Development of gene therapy for immunodeficiency: adenosine deaminase deficiency. Pediatric Research, 33:S49-S53.

Bode, W.M. and D.D. Calvin. 1990. Yield-loss relationships and economic injury levels for European corn borer (Lepidoptera: Pyralidae) populations infesting Pennsylvania field corn. J. Econ. Entomology. 83(4):1595-1603.

Briggs, S.P. & C.A. Guse. 1986. Forty years of European corn borer data: what have we learned? pp. 169-173. *In* Thirty-Eighth Illinois Custom Spray Operators Training Manual, Coop. Ext. Serv., Univ. of Illinois, Urbana-Champaign.

Burks, A.W., Jr., J.R. Brooks, and H.A. Sampson. 1988. Allergenicity of major component proteins of soybean determined by enzyme-linked immunosorbent assay (ELISA) and immunoblotting in children with atopic dermatitis and positive soy challenges. J. Allergy Clin. Immunol. 81:1135-1142.

Cooperative Extension Service. 1989. European Corn Borer Development and Management. North Central Regional Extension Publication No. 327. Extension Service, U.S.D.A., Washington, D.C.

Croon, K.A., P.R. Sanders, P.J. Keck, T.C. Lee, and G.B. Parker. 1995a. Petition for Determination of Nonregulated Status: Insect Protected Corn (*Zea mays* L.) with the *cryIA(b)* Gene from *Bacillus thuringiensis* subsp. *kurstaki*. USDA Petition no. 95-093-01p.

Croon, K.A., P. R. Sanders, P. J. Keck, B.G. Hammond, J.D. Astwood, and R.L. Fuchs. 1995b. Safety, Compositional and Nutritional Aspects of Insect-Protected Corn Line MON 801: Conclusion Based on Studies and Information Evaluated According to FDA's Policy on Foods from New Plant Varieties. Submitted to the FDA on September 15, 1995.

[redacted] 1996. Petition for Determination of Nonregulated Status: Additional YieldGard™ Corn (*Zea mays* L.) Lines with the *cryIA(b)* Gene from *Bacillus thuringiensis* subsp. *kurstaki*. USDA Petition no. 96-017-01p.

della-Cioppa, G., C.S. Bauer, B.K. Klein, D.M. Shah, R.T. Fraley, and G.M. Kishore. 1986. Translocation of the Precursor of 5-Enolpyruvylshikimate-3 phosphate Synthase into Chloroplasts of Higher Plants *in vitro*. Proc. Natl., Acad. Sci. USA 83:6873-6877.

Dicke, F.F. and W.D. Guthrie. 1988. The Most Important Corn Insects. *In* Sprague, G.F. and Dudley, J.W., Editors. Corn and Corn Improvement, Third Edition. Number 18 in the series Agronomy. American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of America, Inc., Madison, Wisconsin.

Doherty, A.M., J.S. Kalternbronn, J.P. Hudspeth, J.T. Repine, W.H. Roark, I. Sircar, F.J. Tinney, C.J. Connolly, J.C. Hodges, M.D. Taylor, B.L. Batley, M.J. Ryan, A.D. Essenburg, S.T. Rapundalo, R.E. Weishaar, C. Humblet, and E.A. Lunney. 1991. New inhibitors of human renin that contain novel replacements at the P_2 site. J. Med. Chem. 34:1258-1271.

Doolittle, R.F. 1990. Searching through sequence databases. Methods in Enzymology 183: 99-110.

EPA. 1988. Guidance for the reregistration of pesticide products containing *Bacillus thuringiensis* as an active ingredient. NTIS PB 89-164198.

EPA. 1994a. Neomycin phosphotransferase II; tolerance exemption. Federal Register 59:49351-49353.

EPA. 1994b. Proposed policy; plant pesticides subject to the federal insecticide, fungicide, and rodenticide act and the federal food, drug, and cosmetic act. Federal Register 59:60496-60518.

EPA. 1995. Plant pesticide *Bacillus thuringiensis* CryIIIA delta-endotoxin and the genetic material necessary for its production; tolerance exemption. PP3F4273/R2132.

Elsayed, S. and J. Apold. 1983. Immunochemical analysis of cod fish allergen M: locations of the immunoglobulin binding sites as demonstrated by native and synthetic peptides. Allergy 38:449-459.

Elsayed, S., J. Apold, E. Holen, H. Vik, E. Florvaag, and T. Dybendal. 1991. The structural requirements of epitopes with IgE binding capacity demonstrated by three major allergens from fish, egg and tree pollen. Scand. J. Clin. Lab. Invest. 51: 17-31.

FDA. 1992. Statement of Policy: Foods Derived from New Plant Varieties. Federal Register 57:22984-23005.

FDA. 1994. Secondary Direct Food Additives Permitted in Food for Human Consumption; Food Additives Permitted in Feed and Drinking Water of Animals; Aminoglycoside 3' Phosphotransferase II. Federal Register 59:26700-26711.

Fischoff, D.A., K.S. Bowdish, F.J. Perlak, P.G. Marrone, S.M. McCormick, J.G. Niedermeyer, D.A. Dean, K. Kusano-Kretzmer, E.J. Mayer, D.E. Rochester, S.G. Rogers, and R.T. Fraley. 1987. Insect Tolerant Transgenic Tomato Plants. Biotechnology 5:807-813.

Flavell, R.B., E. Dart, R.L. Fuchs, and R.T. Fraley. 1992. Selectable marker genes: Safe for plants? Bio/Technol., 10:141-144.

Fraley, R.T., S.G. Rogers, R.B. Horsch, P.R. Sanders, J.S. Flick, S.P. Adams, M.L. Bittner, L.A. Brand, C.L. Fink, J.S. Fry, G.R. Galluppi, S.B. Goldberg, N.L. Hoffmann, and S.C. Woo. 1983. Expression of bacterial genes in plant cells. Proc. Natl. Acad. Sci. USA, 80:4803-4807.

Fuchs, R.L., R.A. Heeren, M.E. Gustafson, G.J. Rogan, D.E. Bartnicki, R.M. Leimgruber, R.F. Finn, A. Hershman, and S.A. Berberich. 1993a. Purification and characterization of microbially expressed neomycin phosphotransferase II (NPTII) protein and its equivalence to the plant expressed protein. Bio/Technology, 11:1537-1542.

Fuchs, R.L., J.E. Ream, B.G. Hammond, N.W. Naylor, R.M. Leimgruber, and S.A. Berberich. 1993b. Safety assessment of the neomycin phosphotransferase II (NPTII) protein. Bio/Technology, 11:1543-1547.

Fuller, M.F. ed. 1991. *In Vitro* Digestion for Pigs and Poultry, C.A.B. International, Wallingford, UK.

Galinat, W.C. 1988. The Origin of Corn. pp. 1 - 31. *In* Sprague, G.F. and Dudley, J.W., Editors. Corn and Corn Improvement, Third Edition. Number 18 in the series Agronomy. American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of America, Inc., Madison, Wisconsin, USA.

Gardner, M.L.G. 1988. Gastrointestinal absorption of intact proteins. Ann. Rev. Nutr. 8:329-50.

Guthrie, W.D., W.A. Russell, F.L. Neumann, G.L. Reed, and R.L. Grindeland. 1975. Yield losses in maize caused by different levels of infestation of second-brood European corn borers. Iowa State Journal of Research. Vol. 50, no. 2.

Hallas, L. E., E.M. Hahn, and C. Korndorfer. 1988. Characterization of Microbial Traits Associated with Glyphosate Biodegradation in Industrial Activated Sludge. J. Industrial Microbiol. 3:377-385.

Hallauer, A.R., Russell, W.A., and Lamkey, K.R. 1988. Corn Breeding. pp. 463-564. *In* Sprague, G.F. and Dudley, J.W., Editors. Corn and Corn Improvement, Third Edition. Number 18 in the series Agronomy. American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of America, Inc., Madison, Wisconsin, USA.

Harlow, E., and D. Lane. 1988. Immunoassay. Antibodies: A Laboratory Manual. Chapter 14:553-612.

[redacted] 1993. Characterization of Microbially-Expressed Protein: CP4 EPSPS. Study Number 92-01-30-14, MSL-12901, an unpublished study conducted by Monsanto Company. EPA MRID no. 43643301.

Hodge, J.E. 1982. Food and Feed Uses of Corn. pp. 79 - 87. *In* CRC Handbook of Processing and Utilization in Agriculture, Wolff, I.A. (ed.), Volume II: Part 1, Plant Products. CRC Press, Inc., Boca Raton, Florida.

Höfte, H. and H.R. Whiteley. 1989. Insecticidal Crystal Proteins of *Bacillus thuringiensis*. Microbiol. Reviews 53:242-255.

Hudon, M. and M.S. Chiang. 1985. Resistance and tolerance of maize germplasm to the European corn borer *Ostrinia nubilalis* (Hübner) and its maturity in Quebec. Maydica 30: 329-337.

Hudon, M., E.J. LeRoux and D.G. Harcourt. 1989. Seventy years of European corn borer (*Ostrinia nubilalis*) research in North America. Agric. Zool. Rev. 3: 53-96.

International Food Biotechnology Council. 1990. Biotechnologies and food: assuring the safety of foods produced by genetic modification. Regulatory Toxicology and Pharmacology 12:S1-S196.

Joos, S., B. Lambert, F. Leyns, A. De Roeck, and J. Swing. 1988. Inventory of natural rhizobacterial populations from different crop plants. pp. 139-142. *In* The Release of Genetically-engineered Micro-organisms. M, Sussman, C.H. Collins, F.A. Skinner, and D.E. Stewart-Tull, eds. Academic Press, London.

Jugenheimer, R.W. 1976. Corn Improvement, Seed Production, and Uses. John Wiley & Sons, Inc. New York, New York, USA.

Kay, R., A. Chan, M. Daly, and J. McPherson. 1985. Duplication of CaMV 35S Promoter Sequences Creates a Strong Enhancer for Plant Genes. Science 236:1299-1302.

Kemp, A.S., C.M. Mellis, D. Barnett, E. Sharota, and J. Simpson. 1985. Skin test, RAST and clinical reactions to peanut allergens in children. Clin. Allergy, 15:73-78.

King, T.P., D. Hoffman, H. Lowenstein, D.G. Marsh, T.A.E. Platts-Mills, and W. Thomas. 1994. Allergen nomenclature. Int. Arch. Allergy Immunol. 105: 224-233.

King, T.P., P.S. Norman, and J.J. Connell. 1967. Isolation and characterization of allergens from ragweed pollen, IV. Biochemistry 6:1992-2000.

Kishore, G. and D. Shah. 1988. Amino acid biosynthesis inhibitors as herbicides. Ann. Rev. Biochem. 57:627-663.

Klee, H.J. and S.G. Rogers. 1987. Cloning of an *Arabidopsis* Gene Encoding 5-enolpyruvylshikimate-3-phosphate Synthase: Sequence Analysis and Manipulation to Obtain Glyphosate-Tolerant Plants. Mol. Gen. Genet. 210: 437-442.

Klein, T.M., E.D. Wolf, R. Wu, and J.C. Sanford. 1987. High velocity microprojectiles for delivering nucleic acids into living cells. Nature 327:70-73.

Kortekangas-Savolainen, O., J. Savolainen, and R. Einarsson. 1993. Gastrointestinal stability of baker's yeast allergens: an in vitro study. Clin. Exp. Allergy 23:587-590.

Lebenthal, E. 1975. Cows' milk protein allergy. Pediatr. Clinics N. Am., 22:827-833.

[redacted] 1995. Assessment of the Equivalence of the *Bacillus thuringiensis* subsp. *kurstaki* HD-1 Protein Produced in *Escherichia coli* and European Corn Borer Resistant Corn. Study Number 94-01-39-09, an unpublished study conducted by Monsanto Company.

Levin, J.G. and D.B. Sprinson. 1964. The enzymatic formation and isolation of 3-enolpyruvylshikimate-5-phosphate. J. Biol. Chem. 239:1142-1150.

Lüthy, P., J.L. Cordier, and H.M. Fischer. 1982. *Bacillus thuringiensis* as a bacterial insecticide: Basic considerations and application. *In* Microbial and Viral Pesticides (E. Kurstak, ed.), pp. 35-71, Marcel Dekker, Inc., New York.

Mangelsdorf, P.C. 1974. Corn - Its Origin, Evolution, and Improvement. Harvard University Press. Cambridge, Massachusetts, USA.

Marquez, U.M.L. and F.M. Lajolo. 1981. Composition and digestibility of albumin, globulins, and glutelins from *Phaseolus vulgaris*", J. Agric. Food Chem. 29:1068-1074.

Matsudaira, P. 1987. Sequence from picomole quantities of proteins electroblotted onto polyvinylidene difluoride membranes. J. Biol. Chem. 262:10035-10038.

Metcalfe, D.D. 1985. Food allergens. Clin. Rev. Allergy 3:331-349.

Nap, J.-P., J. Bijvoet, and W.J. Stikema. 1992. Biosafety of kanamycin-resistant transgenic plants. Transgenic Research 1:239-249.

National Corn Growers Association. 1995. The World of Corn. St. Louis, MO.

Nielson, S.S. 1988. Degradation of bean proteins by endogenous and exogenous proteases - a review. Cereal Chem., 65:435-442.

Odell, J.T., F. Mag, and H.-H. Chua. 1985. Identification of DNA Sequences Required for Activity of the Cauliflower Mosaic Virus 35S Promoter. Nature 313:810-812.

O'Hehir, R.E., R.D. Garman, J.L. Greenstein, and J.R. Lamb. 1991. The specificity and regulation of T-cell responsiveness to allergens. Ann. Rev. of Immunology 9:67-95.

Onaderra, M., R.I. Monsalve, J.M. Mancheno, M. Villalba, A. Martinez Del Pozo, G. Gavilanes, and R. Rodriguez. 1994. Food mustard allergen interaction with phospholipid vesicles. Eur. J. of Biochem. 225:609-615.

Orthoefer, F.T. and R.D. Sinram. 1987. Corn oil: composition, processing, and utilization. pp. 535-551. *In* Corn Chemistry and Technology. Watson, S.A. and R.E. Ramstad, Eds. American Association of Cereal Chemists, Inc., St. Paul, Minnesota.

[redacted] 1993. Purification, Cloning, and Characterization of a Highly Glyphosate-tolerant EPSP synthase from *Agrobacterium* sp. strain CP4. Monsanto Technical Report MSL-12738, St. Louis.

Padgette, S.R., G. della-Cioppa, D.M. Shah, R.T. Fraley, and G.M. Kishore. 1989. Selective herbicide tolerance through protein engineering. Cell Culture and Somatic Cell Genetics of Plants 6:441-476.

Padgette, S.R., D.B. Re, C.S. Gasser, D.A. Eichholtz, R.B. Frazier, C.M. Hironaka, E.B. Levine, D.M. Shah, R.T. Fraley, and G.M. Kishore. 1991. Site-directed mutagenesis of a conserved region of the 5-enolpyruvylshikimate-3-phosphate synthase active site. J. Biol. Chem. 266:22364-22369.

[redacted] 1994. Characterization of Glyphosate Oxidoreductase. Monsanto Technical Report MSL-13234, St. Louis.

Pearson, W. and D. Lipman. 1988. Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA 85:2444-2448.

Pedersen, H.S. and R. Djurtoft. 1989. Antigenic and allergenic properties of acidic and basic peptide chains from glycinin. Food Agric. Immunol. 1:101-109.

Perlak, F.J., R.L. Fuchs, D.A. Dean, S.L. McPherson, and D.A. Fischoff. 1991. Modification of the Coding Sequence Enhances Plant Expression of Insect Control Protein Genes. Proc. Natl. Acad. Sci., USA 88:3324-3328.

Perry, T.W. 1988. Corn as a Livestock Feed. pp. 941-963. *In* Sprague, G.F. and Dudley, J.W., Editors. Corn and Corn Improvement, Third Edition. Number 18 in the series Agronomy. American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of America, Inc., Madison, Wisconsin.

Pilcher, C.D. and J.J. Obrycki. 1994. The presence of predators of European corn borer on transgenic *Bt* corn versus isogenic non*Bt* corn, pp. 259-260. *In* Proc. Integrated Crop Management Conf., Iowa State Univ. Ext., Ames.

Rao, N.R. and S.G. Rogers. 1979. Plasmid PKC7: a vector containing ten restriction endonuclease sites suitable for cloning DNA segments. Gene, 7:79-82.

Rice, M.E. 1994a. Aerial application of insecticides for second generation European corn borer control. 1993. p. 206. *In* Arthropod Management Tests: 1994. Entomological Society of America, Lanham, Maryland.

Rice, M.E. 1994b. Second generation European corn borer control with aerial application of insecticides. 1992. pp. 205-206. *In* Arthropod Management Tests: 1994. Entomological Society of America, Lanham, Maryland.

Rice, M.E. 1994c. Aerial application of insecticides for control of second generation European corn borer. 1991. p. 204-205. *In* Arthropod Management Tests: 1994. Entomological Society of America, Lanham, Maryland.

Rochester, D.E., J.A. Winer, and D.M. Shah. 1986. The Structure and Expression of Maize Genes Encoding the Major Heat Shock Protein, hsp70. EMBO J. 5:451-458.

Rothbard, J.B. and M.L. Gefter. 1991. Interactions between immunogenic peptides and MHC proteins. Ann. Rev. Immunol. 9:527-565.

Sachs, M.I., R.T. Jones, and J.W. Unginger. 1981. Isolation and partial characterization of major peanut allergen. J. Allergy Clin. Immunol. 67:27-34.

[redacted] 1995. Evaluation of Insect Protected Corn Lines in 1994 U.S. Field Test Locations. Study Number 94-01-39-01, MSL-14179, an unpublished study conducted by Monsanto Company. EPA MRID no. 43665502.

[redacted] 1995. "Compositional Analyses of MON 801 Grain and Silage from the 1993 and 1994 Corn Field Trials", Study Number 94-01-39-08, an unpublished study conducted by Monsanto Company.

Schulz, A., A. Krüper, and N. Amrhein. 1985. Differential sensitivity of bacterial 5-enolpyruvylshikimate-3-phosphate synthases to the herbicide glyphosate. FEMS Microbiology Letters 28:297-301.

Shibasaki, M., S. Suzuki, S. Tajima, H. Nemoto, and T. Kuroume. 1980. Allergenicity of major component proteins of soybean. Int. Arch. Allergy. Appl. Immunol. 61:441-448.

Sleisenger, M.H. and M.D. Fordtran. 1989. Gastrointestinal Disease, Volume 1. *In* Pathophysiology Diagnosis Management, 4th, ed. W.B. Saunders Company, Philadelphia, PA.

Smith, A.M. and M.D. Chapman. 1995. Reduction of IgE antibody binding to r*Der p* 2 variants generated by site-directed mutagenesis. Paper 42, presented at the International Symposium on Molecular Biology of Allergens and the Atopic Immune Response, Quebec, P.Q. Feb 18-22.

Stallings, W.C., S.S. Abdel-Meguid, L.W. Lim, H.S. Shieh, H.E. Dayringer, N.K. Leimgruber, R.A. Stegeman, K.S. Anderson, J.A. Sikorski, S.R. Padgette, and G.M. Kishore. 1991. Structure and topological symmetry of the glyphosate target 5-enolpyruvylshikimate-3-phosphate synthase: A distinctive protein fold. Proc. Natl. Acad Sci. USA 88:5046-5050.

Steffey, K.L., M.E. Gray, T.A. Royer and R.A. Weinzierl. 1992. Insect pest management for field and forage crops, pp. 3-50. *In* Illinois Pest Control Handbook, Univ. of Illinois Coop. Ext. Serv., Champaign-Urbana.

Steinrüken, H.C. and N. Amrhein. 1980. The herbicide glyphosate is a potent inhibitor of 5-enolpyruvyl-shikimate-3-phosphate synthase. Biochem. Biophys. Res. Comm. 94:1207-1212.

Taylor, S.L. 1986. Immunologic and allergic properties of cows' milk proteins in humans. J. Food Protect. 49:239-250.

Taylor, S.L. 1992. Chemistry and detection of food allergens. Food Technol., 39:146-152.

Taylor, S.L., R.F. Lemanske Jr., R.K. Bush, and W.W. Busse. 1987. Food allergens: Structure and immunologic properties", Ann. Allergy, 59:93-99.

Taylor, S.L., J.A. Nordlee, and R.K. Bush. 1992. Food allergies. *In* Food Safety Assessment, Finley,J.W., S.F. Robinson, and D.J. Armstrong, eds. ACC Symposium Series 484, American Chemical Society, Washington, D.C.

Tilch, C. and P.S. Elias. 1984. Investigation of the mutagenicity of ethylphenylglycidat", Mutation Research 138:1-8.

Timko, M.P. L. Herdies, E. de Alameida, A.R. Cashmore, J. Leemans, and E. Kreffers. 1988. Genetic Engineering of Nuclear-encoded Components of the Photosynthetic Apparatus of *Arabidopsis* in The Impact of Chemistry on Biotechnology - A Multidisciplinary Discussion. ACS Books, Washington, DC. pp. 279-295.

U.S. Feed Grains Council. 1992. Feed Grain Facts. Washington, D.C.

Vieira, J. and J. Messing. 1987. Production of Single Stranded Plasmid DNA. Meth. Enzymol. 153:3-11.

WHO. 1993. Health Aspects of Marker Genes in Genetically Modified Plants. World Health Organization. Geneva. 35p.

Watson, Stanley A. Corn: Amazing Maize. General Properties. 1982. pg 3-29. *In* CRC Handbook of Processing and Utilization in Agriculture, Volume II: Part 1 Plant Products. I.A. Wolff (ed). CRC Press, Inc., Florida.

Watson, S.A. 1987. Structure and composition. pp. 53-82. *In* Corn Chemistry and Technology. Watson, S.A. and R.E. Ramstad, Eds. American Association of Cereal Chemists, Inc., St. Paul, Minnesota.

Watson, S.A. 1988. Corn Marketing, processing, and utilization. pp. 881-940. *In* Corn and Corn Improvement, Third Edition. Sprague, G.F. and J.W. Dudley, Eds. Number 18 in the series Agronomy. American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of America, Inc., Madison, Wisconsin.

Yanisch-Perron, C., J. Vieira and J. Messing. 1985. Improved M13 Phage Cloning Vectors and Host Strains: Nucleotide Sequences of the M13 mp18 and pUC19 Vectors. Gene 33:103-119.

Zhang, L., E. Olsen, F.T. Kisil, R.D. Hill, A.H. Sehon, and S.S. Mohapatra. 1992. Mapping of antibody binding epitopes of a recombinant *Poa p* IX allergen. Mol. Immun. 29:1383-1389.

Zikakis, J.P., S.J. Rzucidlo, and N.O. Biasotto. 1977. Persistence of bovine milk xanthine oxidase activity after gastric digestion *in vivo* and *in vitro*. J. Dairy Sciences 60:533-541.

Appendix I

The History and Utilization of Modern Corn

THE HISTORY AND UTILIZATION OF MODERN CORN

Origins of Modern Corn

The exact origin of modern corn has been debated among botanists for years. However, most agree that the initial steps in the origin of corn (*Zea mays* L.) were taken in the general area of Mexico and Central America more than 8,000 years ago (Gould, 1968; Galinat, 1988; Jugenheimer, 1976). Suggested hypotheses for the ancestry of corn include four principal theories: (1) that cultivated corn originated from pod corn, a form in which the individual kernels are enclosed in floral bracts as they are in other cereals and in the majority of grasses; (2) that corn originated from its closest relative, teosinte (*Zea mexicana* (Schrad.) Kuntze), by direct selection; (3) corn, teosinte and *Tripsacum* have descended along independent lines from a common unknown ancestor; (4) the tripartite theory that (a) cultivated corn originated from pod corn, (b) teosinte derived from a cross of corn and *Tripsacum*, and (c) the majority of modern corn varieties evolved as a product of corn intercrossing with teosinte or *Tripsacum* or both (Mangelsdorf, 1974). Although there exists evidence to support each of the above theories, cultivated corn is presumed to have been derived from teosinte, under human involvement (Aldrich *et al*., 1986; Galinat, 1988; Jugenheimer, 1976).

Crosses between corn and teosinte and *Tripsacum* have been successful. Corn and teosinte are sexually compatible and intercrossing and gene exchange between teosinte and corn occurs freely producing fertile offspring. In contrast, crosses between corn and *Tripsacum* have been made, but only with human intervention and extreme difficulty, and the offspring of the cross show varying levels of sterility (Galinat, 1988; Mangelsdorf, 1974; Russell and Hallauer, 1980).

Taxonomy

Zea mays L. is known as maize throughout most of the world and as corn in the United States. Corn is a member of the family Graminae (Poaceae), commonly known as the grass family. Corn is a tall, monecious 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 axils of the leaves, in which the spikelets occur in 8 to 16 rows, on a thickened, almost woody axis (cob). The complete 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. Fully developed ears typically bear 750 to 1000 kernels each, are 6 to 12 in. long and 1.5 to 3 in. in diameter. Corn has the largest seed of all the cereal grains (Hitchcock and Chase, 1951; Watson, 1982).

Modern Corn Production

The second major transformation of modern corn production occurred as hybridization became the fundamental concept used in the breeding and production of corn in the United States. Prior to and including the early years of the twentieth century, corn varieties were developed by simple mass selection of open-pollinated cultivars (Hallauer *et al.*, 1988; Watson, 1982). In 1906, New York scientist George H. Schull concluded that although there was a marked loss of vigor upon the inbreeding of inbred corn strains developed by self pollination, some of the resulting hybrids from the crossing of these inbreds were superior to the parental lines (Hayes, 1963; USDA, 1961; Wallace and Brown, 1988). In fact, by the early 1920s hybrid crosses and their resulting vigor were clearly demonstrating improved yields over open-pollinated corn varieties. In 1934, approximately 1% of United States corn acreage was planted with hybrid seed (Wych, 1988). By 1943, the hybrid corn acreage was 52%, and by 1961 this acreage reached 96% (USDA, 1961; Wennblom, 1976). Today, essentially 100% of United States corn acres are planted with hybrid corn varieties.

In this period, corn grain yields in the U.S. increased from approximately 1.3 metric ton per hectare in 1930 to 7.5 metric ton per hectare in 1985 (Hallauer *et al.*, 1988; Olson and Sander, 1988). Most of the improvement in corn hybrids has been achieved to date by the use of conventional breeding methods. Large and consistent genetic gains have been made in resistance to disease, insects, herbicides and adverse environmental conditions such as heat and drought tolerance (Coe *et al.*, 1988; Duvick, 1984). Contributing to these yield gains were increased use of commercial fertilizers, more effective pest control, higher plant densities, and improved crop management (Watson, 1988).

Scope of Corn Production

Corn is widely cultivated on every continent except Antarctica and represents a staple food for a significant portion of the world's population. Much of this progress has been attributed to the fact that corn has proved to be a flexible species amenable to selection so that types have been developed to many areas where it was not grown or was relatively unimportant (Hallauer *et al.*, 1988). An example of this is the rapid expansion of corn in parts of Europe and Asia made possible by the development of earlier maturity, better adapted cultivars. In France, for example, the hectarage has increased more than five times from the 1930's to the present time. France is the leading corn producer in the European Union, producing approximately one half of total production (USDA, 1992). Estimated worldwide production in 1993-94 was 456 million metric tons with almost two thirds of the total production originating in three locations: the United States (35.3%), China (22.4%), and the European Union (6.2%) (National Corn Growers Association, 1994; USDA, 1993). Due to the large corn harvest in the United States in 1994, the United States portion of estimated 1994 worldwide production increased substantially to 46.3% (National Corn Growers Association, 1995).

Within the United States, corn is the largest crop in terms of planted acreage, total production, and crop value. United States production in 1993 was 161 million metric tons or 6.34 billion bushels with Illinois (20%), Iowa (14%), Nebraska (12%), and Indiana (11%) accounting for over fifty percent of the corn grown in the U.S. (National Corn Growers Association, 1994). A combination of ideal weather and soil conditions for growth are the reasons that the majority of national production is concentrated across what is known as the "Corn Belt" in the upper midwest. In 1994, estimated corn production in the United States rose significantly to 256 million metric tons or 10.1 billion bushels (National Corn Growers Association, 1995).

The Seed Corn Industry

The complete acceptance by U.S. farmers of hybrid corn varieties has provided the basis for the hybrid seed corn industry. Many firms have become involved in the production and sale of hybrid seed corn ranging from small, privately owned companies which may produce a few thousand bushels of seed to large publically owned companies which produce large volumes of seed and distribute it through extensive sales organizations (Wych, 1988). Small privately owned companies usually depend on research conducted by public institutions, or on that conducted by private firms that produce and sell foundation seed stocks. Smaller companies usually purchase foundation seed, produce their supplies of hybrid seed, and then sell it directly to farmers in their local areas. Large companies usually carry on their own research and development programs, produce their own foundation seed stocks, produce the commercial seed, and distribute it through their own farmer-dealer sales organization. These farmer-dealers purchase the hybrid seed corn, and in turn, sell it to neighboring farmer customers.

Currently, the domestic hybrid seed corn industry has grown to a gross annual sales volume of over $1.5 billion excluding foreign market sales (Wych, 1988). The industry has seen both attrition in the number of companies and great variation in the relative growth rate of individual companies, often the result of mergers or acquisitions. The many relatively small regional operations within the industry have a collective market share of 30%, while the industry's eight largest companies have a total estimated 70% share of the U.S. hybrid seed corn market (Doane Marketing Research, 1994; Wych,1988).

Corn Utilization

Although an ideal source of food energy, little whole kernel or processed corn is directly consumed worldwide when compared to corn-based food items (Hodge, 1982; Watson, 1988). Corn, like other cereals, probably became established as a food crop because it provided a storable form of food energy and was amenable to domestication. Today, areas of Central and South America,

Central and South Africa, and parts of Asia, where subsistence agriculture is the norm, utilize corn as a staple food. However, many people in developed parts of these areas and developed nations favor corn-based food ingredients (Watson, 1988).

The low price and ready availability of corn has resulted in the development of large volume food and industrial uses in developed nations. Corn is an excellent raw material for the manufacture of starch, the major product recovered from the wet milling process. This is not only because of price and availability, but also because the starch is easily recovered in high yield and purity, and because the by-products have significant monetary value (Anderson and Watson, 1982). Starch, in purified form, is recovered in yields of 67 to 69% of corn dry weight from the wet milling process with a recovery efficiency of 93 to 96% of the total contained starch (Anderson and Stanley, 1982; Watson, 1988). Nearly one fourth of corn starch is sold as starch products; more than three fourths of the starch is converted to a variety of sweetener and fermentation products. The production from starch of High Fructose Corn Syrup (HFCS) expanded dramatically in the 1970's in response to high sugar prices and today has replaced more costly sugar in most nutritive drinks and snack foods (Watson, 1988).

A significant volume of corn continues to be fermented to alcohol, both beverage and fuel. By 1986, 60% of all alcohol was produced by wet millers, 83% of which was fuel alcohol (Watson, 1988). The process for fuel ethanol production is similar to that used for beverage alcohol except that since flavor is not important, lower quality corn can be used, the starch conversion conducted with lower cost commercial enzymes, and distillation done at higher efficiency. However, the fuel alcohol process remains uncompetitive with petroleum at this time without government support (Watson, 1988).

Among the remaining fractions, corn oil is commercially produced from the germ isolated by the wet milling process. Because of this process supply restriction combined with strong demand, corn oil usually commands a slightly higher price than the other two major U.S. vegetable oils, soybean and cottonseed. In the U.S., corn oil comprises approximately four percent of domestic oil production with one half of production utilized directly as household oil while the remainder is processed into margarines (Anderson and Watson, 1982; Watson, 1988).

Each of these materials are a component of many foods including bakery and dairy goods, beverages, confections, and meat products. Industrial uses include paper products, textiles, cosmetics, and biodegradable materials. Approximately fifteen to twenty percent of annual corn production in the United States is utilized for food and industrial use (National Corn Growers Association, 1994; 1995).

Animal feeding is by far the largest use of corn in the United States with more than half (50 to 60 percent) of annual production fed to cattle, chickens, and swine (U.S. Feed Grains Council, 1992; Hodge, 1982; Perry, 1988; Watson, 1988). Of an approximately 200 million metric tons grain, forty to fifty percent is fed to livestock directly as grain. Another 1.5 to 2 million metric tons of by-products of the wet and dry milling industries, primarily corn gluten meal and feed, are fed directly or in formulated feeds. An additional 130 million metric tons of whole plant silage is produced and consumed almost entirely by ruminants. Corn is readily consumed by livestock and because of its high starch-low fiber content is one of the most concentrated sources of energy, containing more total digestible nutrients than any other feed grain. Corn does not normally contain toxins or antinutritional factors (International Food Biotechnology Council, 1990; Watson, 1982). It is, however, the lowest in protein of all feed grains and is generally supplemented with soybean meal to provide the required protein (amino acids), minerals, and vitamins for efficient livestock growth (Perry, 1988; Watson, 1988).

Export Value

With exception of several metric tons of hybrid corn seed which is utilized to plant the following years crop, the remaining percentage of United States corn production is exported. In 1992 - 93, for example, 42.1 million metric tons (mmt) of corn or approximately a quarter of the U.S. corn harvest was exported. The top United States export customers for corn included Japan (14.1 mmt), Tawain (5.3 mmt) and Russia (2.8 mmt) (National Corn Growers Association, 1994). The export market for corn appears to be growing, although large variations in the purchasing patterns of Eastern European countries including Russia since the late 1980s greatly influenced this trend. In contrast, Japan and other nations in the Pacific Rim region (Tawain, Korea) have remained a consistent purchaser of between one quarter and one third of U.S. corn exports (National Corn Growers Association, 1995). Leading corn export competitors include China (5.5 mmt), Argentina (4.2 mmt) and France (7.0 mmt) (U.S. Feed Grains Council, 1992). Additionally, the United States exports large quantities of corn gluten meal, corn gluten feed, corn starch, corn oil, and high fructose corn syrup (HFCS) and glucose (U.S. Feed Grains Council, 1992; Watson, 1988).

REFERENCES

Aldrich, S.R., Scott, W.O., and Hoeft, R.G. 1986. Modern Corn Production, Third Edition. A & L Publications, Inc., Champaign, Illinois, USA.

Anderson, R.A. and S.A. Watson. 1982. The Corn Milling Industry. pp. 31 - 78. *In* CRC Handbook of Processing and Utilization in Agriculture, Wolff, I.A. (ed.), Volume II: Part 1, Plant Products. CRC Press, Inc., Boca Raton, Florida, USA.

Coe, E.H., M.G. Neuffer, and D.A. Hoisington. 1988. The Genetics of Corn. pp. 81 - 258. *In* Spraugue, G.F. and Dudley, J.W., Editors. Corn and Corn Improvement, Third Edition. Number 18 in the series Agronomy. American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of America, Inc., Madison, Wisconsin, USA.

Doane Marketing Research. 1994. U.S. Farm Seed Corn Seed Study. 1994. St. Louis, Missouri, USA.

Duvick, D.N. 1984. Genetic Contributions to Yield Gains of U.S. Maize Hybrids, 1930 - 1980. p. 15 - 47. *In* W.R. Fehr (ed.) Genetic Contributions to Yield Gains of Five Major Crop Plants. Crop Science Society of America, Inc., Madison, Wisconsin, USA.

Galinat, W.C. 1988. The Origin of Corn. pp. 1 - 31. *In* Spraugue, G.F. and Dudley, J.W., Editors. Corn and Corn Improvement, Third Edition. Number 18 in the series Agronomy. American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of America, Inc., Madison, Wisconsin, USA.

Gould, F.W. 1968. Grass Systematics. McGraw Hill, New York, USA.

Hallauer, A.R., Russell, W.A., and Lamkey, K.R. 1988. Corn Breeding. pp. 463 - 564. *In* Spraugue, G.F. and Dudley, J.W., Editors. Corn and Corn Improvement, Third Edition. Number 18 in the series Agronomy. American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of America, Inc., Madison, Wisconsin, USA.

Hayes, H.K. 1963. A Professor's Story of Hybrid Corn. Burgess Publishing Company, Minneapolis, Minnesota, USA.

Hitchcock, A.S. and A. Chase. 1951. Manual of Grasses of the United States. U.S. Government Printing Office, Washington, D.C., USA.

Hodge, J.E. 1982. Food and Feed Uses of Corn. pp. 79 - 87. *In* CRC Handbook of Processing and Utilization in Agriculture, Wolff, I.A. (ed.), Volume II: Part 1, Plant Products. CRC Press, Inc., Boca Raton, FL, USA.

International Food Biotechnology Council. 1990. Biotechnologies and food: assuring the safety of foods produced by genetic modification. Regulatory Toxicology and Pharmacology 12: S1-S196.

Jugenheimer, R.W. 1976. Corn Improvement, Seed Production, and Uses. John Wiley & Sons, Inc. New York, New York, USA.

Mangelsdorf, P.C. 1974. Corn - Its Origin, Evolution, and Improvement. Harvard University Press. Cambridge, Massachusetts, USA.

National Corn Growers Association. 1994. The World of Corn. St. Louis, Missouri, USA.

National Corn Growers Association. 1995. The World of Corn. St. Louis, Missouri, USA.

Olson, R.A. and D.H. Sander. 1988. Corn Production. pp. 639 - 686. *In* Spraugue, G.F. and Dudley, J.W., Editors. Corn and Corn Improvement, Third Edition. Number 18 in the series Agronomy. American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of America, Inc., Madison, Wisconsin, USA.

Perry, T.W. 1988. Corn as a Livestock Feed. pp. 941 - 963. *In* Spraugue, G.F. and Dudley, J.W., Editors. Corn and Corn Improvement, Third Edition. Number 18 in the series Agronomy. American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of America, Inc., Madison, Wisconsin, USA.

Russell, W.A. and A.R. Hallauer. 1980. Corn. pp. 299 - 312. *In* Fehr, W.R. and Hadley, H.H., Editors. Hybridization of Crop Plants. American Society of Agronomy and Crop Science Society of America, Publishers, Madison, Wisconsin, USA.

USDA. 1993. World Agriculture Supply and Demand Estimates. Economic Research Service, Foreign Agricultural Service. Washington, D.C., USA.

USDA. 1992. Agricultural Statistics. United States Government Printing Office, Washington, D.C., USA.

USDA. 1961. Seeds. The Yearbook of Agriculture. United States Government Printing Office, Washington, D.C, USA..

U.S. Feed Grains Council. 1992. Feed Grains Facts. Washington, D.C. USA.

Wallace, H.A. and W.L. Brown. 1988. Corn and Its Early Fathers, Revised Edition. Iowa State University Press, Ames, Iowa, USA.

Watson, S.A. 1982. Corn: Amazing Maize. General Properties. pp. 3 - 29. *In* CRC Handbook of Processing and Utilization in Agriculture, Wolff, I.A. (ed.), Volume II: Part 1, Plant Products. CRC Press, Inc., Boca Raton, Florida, USA.

Watson, S.A. 1988. Corn Marketing, Processing, and Utilization. pp. 881 - 940. *In* Sprague, G.F. and Dudley, J.W., Editors. Corn and Corn Improvement, Third Edition. Number 18 in the series Agronomy. American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of America, Inc., Madison, Wisconsin, USA.

Wennblom, R.D. 1976. How Corn Became our Greatest Crop. Farm Journal. Farm Journal Inc., Philadelphia, Pennsylvania, USA.

Wych, R.D. 1988. Production of Hybrid Seed Corn. pp. 565 - 607. *In* Sprague, G.F. and Dudley, J.W., Editors. Corn and Corn Improvement, Third Edition. Number 18 in the series Agronomy. American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of America, Inc., Madison, Wisconsin, USA.

Appendix II

Corn as a Food Source in the United States

APPENDIX II. CORN AS A FOOD SOURCE IN THE UNITED STATES

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HISTORICAL PERSPECTIVE

Corn (*Zea mays* L.) is known only as a cultivated species, and cannot survive without human intervention (Galinat 1977). The center of origin was likely Mexico or Guatemala (Goodman 1978), with domestication occurring about 5000 B.C. (Benz 1994). The native peoples of the Americas domesticated and improved corn after realizing its potential for food, feed, and fuel (Hallauer 1981). Corn was widely distributed in North and South America before the Christian era (Kempton 1936).

Corn Usage by Native Americans and European Settlers

Corn was a minor crop in eastern North America for about 800 years after its initial appearance in the eastern woodlands about the first century B.C. (Hastorf and Johannessen 1994), with A.D. 985 the earliest date it was identified in New England (Bendremer et al 1994). Between A.D. 750 and 1000, there were many social changes among native peoples. This was accompanied by rapid increases in the amount of corn produced and consumed until corn suddenly (perhaps within two to three generations) became a dietary staple with many groups. Reasons for its rapid acceptance include its extraordinary genetic flexibility and variablity giving breeding an interactive quality for humans, and its inability to survive without human care. Therefore, corn in native American cultures had a long-term capacity for carrying cultural meaning, and became a cuisine and a staple food (Hastorf and Johannessen 1994).

By the time European settlers came to America, corn was the main cultivated crop (Kastner 1980). Corn formed the subsistence base of the prehistoric Pueblo cultures of the U.S. Southwest (Galinat and Gunnerson 1963), and the Coronado expedition found corn growing there in 1540 with high yields. The De Soto expedition found cornfields commonplace from Florida to North Carolina and westward beyond the Mississippi River in 1540. The expeditions made by French Jesuits westward to the Great Lakes and down the Mississippi found corn everywhere. Regions westward into Montana were also dotted with cornfields. Some plantings in New York and the upper Ohio Valley covered hundreds of acres (Weatherwax 1954).

Most of corn's food value is concentrated in the grain. The simplest and probably the first method of cooking corn was to place it on hot stones, ashes, or heat it in sand. Methods of crushing or grinding corn between stones or in mortars had simple and early origins. The grain was often ground dry and raw, but sometimes first softened by parching, boiling or treatment with alkali. It was discovered that if wood ashes or quicklime were placed in the water in which corn was boiled, the pericarp of the grains became soft and could be easily removed, and the food value of the remaining grain was not impaired. The finished product was lye hominy, which is still eaten today, especially in the South (Weatherwax 1954). Hominy was often dried for storage, later to be cooked again or ground for other uses such as hominy grits (Fussell 1992). From Texas southward, the hominy was ground to a dough, masa, from which tortillas were made by baking a thin disc on a hot griddle or which was used to make a drink, atole. Masa could be preserved for carrying on journeys when wrapped in corn husks, then made into atole by simply stirring in water. Corn meal was often simply boiled in water and eaten as porridge or gruel. This was especially adaptable to the flint varieties common in New England that did not make a fine meal. The commonest and most dependable food in the entire corn-growing region of North America was corn bread. The dry meal moistened with water or the dough produced by the wet-grinding process was kneaded, shaped into a loaf, then boiled, baked, or both. Other ingredients were sometimes added for flavoring or to improve texture. In the Southwest, corn and other seeds were often parched and ground, then taken as a lightweight and nutritious food on long journeys. The meal was eaten directly or mixed with water (Weatherwax 1954). The Pueblo people made a paper bread, piki, which has been called the original cornflakes (Fussell 1992).

The English colonists learned early that corn was a better crop for settlers than crops brought from Europe. They learned methods of cultivation and preparation from the Indians, which were described by Kalm (1974) during his travels in North America in 1748. Corn insured not only a reliable food supply but also a surplus and means of exchange (Earle 1898). In 1608, colonists in Virginia bought corn from the Indians that tided them over their second winter. By 1616, corn was so abundant in the colony that the Indians were buying it from the colonists. The taste acquired for corn in the various forms in which it was prepared, no less than its great economic advantages, made it an important element of the diet for rich and poor alike (Gray 1933).

Corn Usage During Settlement and Industrialization of the U.S.

Corn became the food staple in every colony from Maine to Georgia (Kirkland 1932). As pioneers pushed westward it practically fed the people

during the time they were creating farms from the forest (Hedrick 1933). Many pioneer farmers moved frequently, because corn was grown year after year in the same fields without fertilization, and it was cheaper to clear new land than to fertilize (Hardeman 1981). The climate of much of the U.S. was ideal for corn, and its ease of cultivation hastened settlement (Faulkner 1924). For 240 years after the Jamestown settlement, nearly 90% of Americans depended on farming for their livelihood, and probably more than 90% of them depended mainly on corn for their survival (Hardeman 1981). Pioneers milled corn with Indian metates, European mortars and pestles, and hominy blocks. Revolving stone mills soon were operated on a commercial scale, with energy usually supplied by water (Hardeman 1981). Because whole grain cornmeal rapidly became rancid and could not be stored very long, there were many small mills supplying cornmeal for local villages and towns. The invention of degerminators in the early 1900's allowed the production of low-fat cornmeal that led to large-scale milling (Serna-Saldivar et al 1994). Pioneer farmers west of the Appalachian mountains made corn into whiskey, which was the form in which it could profitably be packed across the mountains to eastern markets (Humphrey 1931). American whiskey consumption during the period 1790-1840 has been estimated at five gallons per person per year (Rupp 1987). In the Southern plantation system, most of the food products consumed were produced on the plantation. Corn, especially, was the staple item of food that furnished meal and hominy to the people and feed for hogs (Humphrey 1931). It is estimated that each person and each hog in the South ate, on the average, thirteen bushels of corn per year, or two pounds per day (Hardeman 1981).

The value of corn meal as a constituent of the army ration was demonstrated during the Civil War. The larger portion of the bread used by the Southern army was made from corn, while at the same time it furnished a large part of the food supply of the Federal forces (Snow 1891).

In 1917 it was stated that "Corn is the principal source of food supply of the American people, but outside of the South very little of the corn is directly consumed by man" (Finch 1917). In 1930 human consumption accounted for about one-twentieth of the output, and owed its position in the American economy to its qualities for feeding animals and producing meat and dairy products (Durand 1930).

Early Sweet Corn Usage

Although the sugary mutation of corn, known today as sweet corn, frequently arises spontaneously (Galinat 1971), the Indians of the United

States did not think much of sweet corn as green corn, preferring starchy varieties eaten raw in the "milk" stage or cooked when slighly starchy (Weatherwax 1954). Sweet corn was valued for the pleasant taste of the parched grain. The first colonial sweet corn was a variety called Papoon, introduced to New England by soldiers returning from an expedition against the Iroquoian confederation of tribes from New York in 1779 (Galinat 1971), although this account has been debated (Carter 1948, Erwin, 1951). Papoon and other varieties obtained from the Iroqui were grown privately until the establishment of the seed industry in the 1820's when they became forerunners of the hundreds of named sweet corn varieties that have since developed. Erwin (1947) put the date of general use of sweet corn in the U.S. at about 1800. Drying or pickling was once the primary means of preserving sweet or green corn (Hardeman 1981), but was gradually replaced by canning after Isaac Winslow was issued a patent in 1862 for an "Improved Process of Preserving Green Corn" (Fussell 1992).

Early Popcorn Usage

Popcorn was known to the Indians in many places (Weatherwax 1954). Colonial popcorn was eaten for breakfast with milk and maple sugar. By the mid-nineteenth century popcorn was no longer a mealtime staple, but a popular snack food (Rupp 1987). Before 1880, popcorn was only grown as a garden crop (Erwin 1949). The development of popcorn into a commercialized industry in the U.S. occurred in the 1880's. By 1912 commercial production was 19,000 acres (7700 hectares) (Eldredge and Thomas 1959). In the late 1970's, popcorn was not only a snack eaten with salt and butter, but many special flavorings and forms became popular, increasing its usage (Rupp 1987).

Early Corn Manufacturing

The wet milling process can be traced back 3,000 years when it was used in Egypt to derive starch from potatoes, wheat and other sources (Anderson and Watson 1982). In 1842 Thomas Kingsford developed and patented a manufacturing process in which crude corn starch was purified by alkaline treatment. Cornstarch was first produced industrially in the U.S. in 1844 (Whistler 1984). The industry grew slowly until 1860, eventually becoming large and complex (Anderson and Watson 1982). Industrial conversion of cornstarch to sugar began in 1865 (Fussell 1992). In the early 1900's cornflakes, invented by J.H. Kellogg, became a popular food for breakfast, starting the breakfast food industry. Corn oil became an important cooking oil in 1910 when a process to refine it for cooking was invented (Rupp 1987).

Corn Breeding

Great diversity was found among thousands of populations of maize that were collected in North and South America between 1950-1960 by the Rockefeller Foundation (Gay 1984). These populations were classified into more than 200 races (Goodman and Brown 1988). Breeding by indigenous Americans before European settlement resulted in the development of most of these races (Russell 1991). Most commercial maize grown in the U.S. belongs to the Corn Belt Dent race, derived from crosses of varieties belonging to the Northeastern Flint and Southern Dent races beginning about 1850, followed by subsequent selection (Anderson and Brown 1952). These crosses provided the germplasm sources from which the inbred parental lines were developed that produced the first double-cross hybrids used in the U.S. (Hallauer et al 1988).

Corn breeding to develop hybrids began in the early 1900's with the work of East (1908) and Shull (1909). Public corn breeding programs were soon established, followed by corn breeding as a commercial industry. Farmer acceptance of hybrid seed became commonplace in the 1930's, and during the 1960's single crosses became the predominant type of hybrid, replacing double crosses (Russell 1991). The most widely used breeding method to develop inbred lines for use as parents of hybrids is pedigree selection. Pedigree selection was practiced on native open-pollinated corn populations in the early days of corn breeding. Now, pairs of elite inbred lines are crossed that complement each other, the cross is self pollinated, and lines are selected by selfing in the crossed population to find lines that are more elite than their parents (Hallauer et al 1988). Because corn is naturally cross pollinated, commercial hybrid seed is produced by removing the male flowers (tassels) of the inbred line used as a pollen parent before pollen shedding and before silks emerge on the female flowers (ears) of the inbred line used as a seed parent. This is a very costly operation for the seed producer, and is usually done with manual labor in combination with mechanical devices or genetics (Craig 1977). The high corn yields obtained by farmers in the U.S. result from selecting the best seed, planting procedure, tillage method, fertilization practices, weed and pest control, and harvesting and marketing procedures (Larson and Hanway 1977).

CURRENT CORN CONSUMPTION RATES

Corn is the leading cereal in the U.S. (FAO 1990). Corn foods have a unique and distinctive flavor contributing to their popularity, and products

from corn are found in almost everything we eat (Serna-Saldivar et al 1994). Per-capita consumption of corn or food derived from corn (meat and dairy products) comes to more than three pounds a day (Kahn 1984).

In the U.S., corn is generally used as animal feed (usually 80% of production) and to produce cornmeal, flour, grits, starches, sweeteners, cooking oil, industrial alcohol, alcoholic beverages, tortillas, snacks, breakfast food, and other products (usually more than 25 million tons per year) (Serna-Saldivar et al 1994). Alkaline-cooking of corn to produce dry masa flour, tortillas, and snack foods like corn and tortilla chips, is becoming more important as the popularity of Mexican foods increases (Gould 1988).

Most corn consumed in the U.S. is first processed by either wet or dry milling. A billion bushels of corn are processed annually by approximately 20 corn wet milling operations in the United States (Anonymous 1993b, Watson, 1988). Relatively pure starch, protein, fiber, and germ are products of wet milling. Further processing of the starch creates corn sweeteners, dextrins and ethanol (Munro 1994). The germs are processed into oil. Dry milling produces endosperm fractions that differ in particle size. These fractions include grits, meal, and flour. Germs are again processed into oil (Serna-Saldivar et al 1994). The corn bran can be saved and processed for further use (Burge and Duensing 1989).

Alkaline Cooked Products

Snack foods based on corn are sold in bags in the grocery stores but many are also consumed in restaurants and from snack bars. Corn chips are made from fried masa, while tortilla chips are made from masa that is first baked, then fried (Serna-Saldivar et al 1994). Corn snacks are easier to process and more profitable than potato chips (Gould 1988). Snack Food's 26th Annual State of the Industry Report (Mitchell 1994) reported an overall pound volume increase of 5.3% for corn and tortilla chips in 1993. Corn and tortilla chips represent 5% of the nearly $42 billion U.S. snack food market, compared to 6.2% for potato chips.

With the rapid increase in consumption of Mexican foods during the 1980's (Gould, 1988), industrial tortilla production has become important in the U.S. Dry masa flour has also become more available in different types to produce products such as white and yellow table tortillas, restaurant style tortilla chips, corn and tortilla chips, and tamales (Serna-Saldivar et al 1994).

Industrial Dry-Milled Products

Less than 5% of total corn produced is dry milled, which is about 160 million bushels (Anonymous 1991) or 4.1 tons (Faga 1988, Serna-Saldivar et al 1994). In 1977, brewing accounted for 29%, food uses in the U.S. for almost 18%, and about 7.6% was used for a corn-soy-milk or corn-soy blend food item under contract with U.S. AID for distribution to other countries. The balance was accounted for by industrial uses, pet foods and animal feed. Beverage alcohol uses consumed about 83 million bushels of corn in 1993, while cereals and other products consumed about 118 million bushels of corn in 1993 (Anonymous 1994). Consumption of ready-to-eat breakfast cereals has steadily increased in the U.S., with an estimated per capita consumption of 4.1 kg in 1985 (Fast 1987), and, in 1988, 128 million kg of extruded snacks made of cornmeal or grits were sold in the form of curls, puffs, and balls (Faga, 1988). Total and per capita civilian consumption for corn food products in the U.S. for selected years are given in Table 1.

Specialty Products

A hybrid developed from the large-kerneled corn race Cuzco from Peru is used to produce the popular parched corn snack CORNNUTS®. The pericarp is removed by alkali cooking, then the kernel is dried, tempered, deep-fat fried, and flavored (Serna-Saldivar et al 1994).

Popcorn

Americans eat 9.7 billion quarts of popcorn per year (Rupp 1987). The U.S. popcorn industry has grown during the past 40 years except for a couple of leveling-off periods. Sale of unpopped popcorn has shown continuous growth on a year-to-year basis during this time. Popcorn consumption grew in the 1980's due to the development of microwave technology and popcorn products that could be easily prepared in the microwave oven (Ziegler and Ashman 1994).

Sweet Corn

The most important direct consumption of corn as food is as sweet corn (Huelsen 1954). Sweet corn is consumed fresh (0.7 million tons, average 1975 to 1977), and processed (2.33 million tons, average 1975 to 1977) by canning and freezing. Frozen corn is marketed both as cut kernels and as corn-on-the-cob (Hodge 1982). Per capita utilization of cut sweet corn was 9.0 pounds in

1992, up from 6.6 pounds in 1983. Canned corn comsumption was 11.9 pounds in 1992, versus 11.6 pounds in 1983 (Anonymous 1993). A low estimate of sweet corn's farm value ranks as sixth among vegetables produced for fresh market consumption (Tracy 1994).

SOURCE OF CORN-BASED FOOD ITEMS

In the previous section, the use of corn as a basic food and of ground or fractionated corn in food products was discussed. The use of corn in the human diet, however, extends far beyond these products recognizable as corn. For instance, besides the production of corn into cornmeal, flour, grits, tortillas, snacks and breakfast cereal, corn can undergo other processing to produce many ingredients used in foods throughout our U.S. food supply. Most of the corn used domestically as food is industrially processed by either wet or dry milling (Anderson and Watson 1982, Serna-Saldivar et al 1994).

Wet Milling of Corn

The Process. The raw material used for wet milling is shelled corn, generally U.S. No. 2 yellow dent corn, but also some specialty corn-types such as waxy and high-amylose corn that are contract-grown and identity preserved. Before the corn enters the plant, it is inspected for U.S. Grade factors, freedom from aflatoxin, insect, and rodent infestation (Freeman, 1973). Accepted corn is thoroughly cleaned by screening to remove pieces of cob, chaff, sand and other undesirable foreign material (Watson 1984). Dust and light chaff are removed by aspiration. The wet milling process begins with the corn being steeped to soften the kernel for optimum milling conditions (Watson 1988). The steep water is then drawn off and the softened kernels are passed through an attrition (cracking) mill to liberate the germs. The germ fraction, containing approximately 50% oil on a dry-weight basis, is separated from the denser components by floatation, and then washed and dried in preparation for oil recovery.

The remaining portion is finely milled corn endosperm containing starch and gluten (protein), and fibrous hulls (pericarps). The starch and gluten particles are separated from the fiber by passing the slurry over a series of screens. The starch and gluten are then separated, and the starch is washed and dried to be used as starch or converted into other products.

The Products. The milling process separates each bushel of corn into 31.5 pounds of starch, 12.4 pounds of 21% protein feed, 3 pounds of 60%

gluten meal, and 1.5 pounds of corn oil (Anonymous 1993b). The starch can be further converted into 33 pounds of sweeteners, 2.6 gallons of ethanol, or 17 pounds of carbon dioxide. The oil can make 2 pounds of margarine.

Starch. The primary product of wet milling is starch, a polymer made up of thousands of glucose units. About 60% of the starch is converted to various sweeteners, while the other 40% is consumed directly in foods and used for other industrial purposes. Much of the starch is chemically modified to provide specific functions for food and industrial uses. Either normal or modified corn starches are found in many food products, as well as in other edible materials such as drugs, as noted in Table 2. The major purpose for adding starch to foods is to provide thickness and/or texture, such as in baby foods, chocolate drinks, and puddings. When starch is heated in the presence of water, it gelatinizes to form a viscous paste, having properties of viscosity, adhesion, water retention, film formation and other physical properties which make it desirable in many food products (Wurzburg 1978, Anderson and Watson 1982, Smith and Bell 1986, Orthoefer, 1987). Starch also serves as an inert material that can absorb moisture in baking powders and on chewing gum, and can help keep powdered sugar properly dispersed (Orthoefer 1987).

Corn Syrups. Since the mid-1800s, the corn wet milling industry has produced corn syrups known originally as, simply, glucose syrup. Corn syrups are used in a wide array of foods and drugs (Table 2). The syrup types can be divided into regular corn syrups, high fructose corn syrups and maltodextrins.

The manufacture of corn syrup involves heating under pressure to 140C - 160C, a 35 to 40% starch slurry with hydrochloric acid (Anderson and Watson 1982, Watson 1988). This process breaks down the long starch chains into smaller molecules to form a syrup containing glucose, maltose, and other glucose polymers of up to 12 to 15 glucose units. This product is a regular corn syrup that is used in foods to provide sweetness, viscosity, hygroscopisity (absorbance of moisture), freezing point depression, flavor and color enhancement. Regular corn syrup is less sweet than sucrose, so is added in greater quantities to foods than is sucrose (Watson 1988). Corn syrup can provide a desirable texture without excessive sweetness (Newton 1975, Long 1978).

The starch can be completely hydrolyzed to glucose by the application of enzymes (generally, alpha-amylase and glucoamylase enzymes). The product is clarified and refined, and then is further processed to crystalline dextrose, liquid dextrose or high-dextrose corn syrup (Hebeda 1987).

Another enzyme (glucose isomerase) can be used to convert the refined dextrose syrup to high-fructose corn syrups (HFCS, Hebeda 1987). The glucose molecules undergo a chemical shift to form fructose molecules. A syrup containing approximately 55% fructose competes favorably in the market place with hydrolyzed sucrose solutions, which are similar to these HFCS in amounts of fructose and glucose (Watson 1988). Alternatively, the fructose can be separated, dried and sold as a pure cystalline product. The properties of HFCS are similar to those of regular corn syrup except that HFCS is much sweeter. Crystalline fructose is 1.8 times sweeter than sucrose and 2.4 times sweeter than crystalline glucose (Hanover 1982). The HFCS just mentioned is similar to sucrose in sweetness, although exact comparisons are difficult because sweetness intensities of sugars change with conditions such as temperature, moistness, acidity and concentration. None-the-less, HFCS has found application in a wide variety of food systems (Table 2, Young and Long 1982). A major marketing goal was to gain acceptance of HFCS in all carbonated beverages. In 1982, major bottlers accepted HFCS for 100% of their sweeteners (Morris 1984). This act gave HFCS a guaranteed place in the food industry.

Maltodextrins are starch hydrolysis products that have not been broken down to compounds as small as glucose and maltose. Commercial products are manufactured by hydrolysis of regular corn starch or waxy maize starch by acid, acid-enzyme, or enzyme-enzyme techniques (Hebeda 1987). Maltodextrins are the least hygroscopic of the corn sweeteners because they have a low sugar (mono- and disaccharide) content. They still contribute viscosity, mouthfeel and body to food products as a result of the higher-molecular weight saccharides they contain. Because of their low monosaccharide content, maltodextrins do not exhibit any sweetness, thus they can be added to food products solely for their contributions to texture.

<u>Ethanol.</u> Ethanol is produced in the United States by two major procedures, fermentation and chemical synthesis. Currently, over one billion gallons of ethanol are produced annually in the U.S., with approximately 95% derived from corn starch (Bothast 1994). All beverage alcohol is produced in the United States by the fermentation of starch and sugar or from grains and molasses that are high in starch and sugar content, respectively (Otey and Doane 1984). Ethanol produced by fermentation of starch hydrolyzates is regarded as legally equivalent to grain alcohol and may be used in beverages. It also qualifies for tax-free status when blended with gasoline at a level of 10% for use as a fuel in motor vehicles (Watson 1984).

Oil. Corn oil is produced commercially from corn germ isolated by wet or dry milling; thus, it is actually a by-product of the corn milling industry. Also, the corn kernel has a relatively low oil content (4.4% dry weight basis, Watson 1984). None-the-less, the U.S. corn crop is so large that the total amount of corn oil produced is enormous (Weber 1987a).

Oil is recoved from corn germ by expelling, solvent extraction, or by a combination of the two processes (Reiners and Gooding 1970, Orthoefer 1987, Watson 1988). The immediate product of these processes is a crude corn oil composed of 95% triacylglycerols plus minor components including free fatty acids, waxes, phospholipids, pigments, and odorous compounds, which must be removed by refining, bleaching and deodorization to produce a good quality edible oil (Watson 1988, P. White 1992). Corn oil, like other vegetable oils, has no cholesterol and generally contains no protein (Tattric and Yaguchi 1973).

Corn oil is a widely accepted food oil because of its bland flavor and high smoke point during heating (Watson 1988). Also, because of the presence of natural antioxidants, especially tocopherols, and a low level of linolenic acid, it has good flavor stability during storage and cooking (Weber 1987a). The corn oil market is divided into three major categories (Sculeva 1992). These include 1) margarines, which are partially hydrogenated oil blends made into stick margarines, spreads or general frying fats used in the food industry, 2) salad and cooking oils, which are predominantly used for retail salad oils, salad dressings, mayonnaises and for general frying in the snack food areas (cooking oils), and in pharmaceuticals, and 3) baking and frying fats, which are hydrogenated oils used as a shortening in the baking industry or for heavy duty deep frying applications in the fast food and institutional markets.

Dry Milling of Corn

The Process. Corn is dry milled by one of two general processes, nondegerming and degerming. The nondegerming system stone-grinds the corn, preferably a white dent, to produce hominy grits and whole meals rich in bran and germ. This traditional process is used widely in Africa, Latin America, and Asia, and by some small mills in the U.S. (Hill et al 1991). In the U.S., most corn is degerminated during dry milling (Serna-Saldivar 1994). The degerminating process, often called the tempering degerminating system (TD), aims to make as complete a separation of the corn parts as possible to 1) retain the maximum amount of horny endosperm portion as discrete pieces,

2) remove the maximum amount of germ and pericarp to give a low-fat, low-fiber product, and 3) recover the maximum number of intact germs (Alexander, 1987; Watson 1988). The corn type most frequently used by millers is U.S. No. 2 yellow dent. The TD system produces highly refined grits, meals, and flours. Before purchase, the corn is inspected for freedom from aflatoxin, insects, mold, and some physical attributes of the corn kernels (Watson, 1987b). High-quality corn is purchased for food production and care is taken to avoid contamination during the dry milling process. The TD process involves cleaning, tempering by water addition, degerming, drying, cooling, grading, aspirating, grinding, sifting, and packaging (Watson 1988).

The Products. The nondegerming process results in whole cornmeals having a rich flavor because of the high oil content arising from the presence of the germ. But, as just mentioned, the storage life and flavor stability of whole corn meal are poor. Typical products and yields of the dry-milled fractions of degermed corn include: flaking grits 12%, coarse grits 15%, regular grits 23%, coarse meal 3%, dusted meal 3%, flour 4%, oil 1%, and hominy feed 35% (Brekke 1970). The major by-products of the dry-milling industry are corn germ and bran. Generally, the oil is recovered from the germ and processed and used as described above under wet-milled products. Many different foods are prepared from corn dry-milling products and are described.

Germ residue is blended with other fractions and corn cleanings to produce a commodity feed called hominy feed. Solvent-extracted germ can also be processed to a germ flour, which has become a useful, nutritious food ingredient (Watson 1988). Corn bran recently has increased in popularity as a dietary fiber source and can be found in many prepared foods (Burge and Duensing 1989). Dry milled corn bran has many desirable properties, such as low calorie content, high dietary fiber, and high moisture retention. These combined properties make it useful in creating low calorie - high fiber foods. Flaking grits are large endosperm chunks obtained after corn degerming and are used almost exclusively in the manufacture of cornflakes, the most popular ready-to-eat breakfast cereal in the world. In general, breakfast cereals commonly use corn alone or in combination with other cereals and ingredients. Corn grits are smaller endosperm chunks than flaking grits, widely utilized by the snack, breakfast cereal, and brewing industries. Corn grits are also eaten as a breakfast side dish (Serna-Saldivar et al 1994). Corn grits, meal, or flours are cooked into a dough that is processed into flakes, shreds, granules, puffs or collets, then toasted to improve flavor, aroma, and texture (Serna-Saldivar et al 1994). Corn meal is used to produce a variety of products such as corn bread, muffins, pancakes, cornsticks,

fritters, and hush puppies. Corn flour is used as an ingredient in pancakes, muffins, doughnuts, breadings, batters, ready-to-eat breakfast foods, snacks, and as a binder in processed meats (Serna-Saldivar et al 1994). The brewing industry has traditionally been the largest user of corn dry-milled products, using them as a source of carbohydrates in the production of beer (Anderson and Watson 1982). Whole grain corn and corn grits are used to produce many distilled alcoholic beverages. Bourbon whiskey is produced from distillation of a fermented mash containing a minumum of 51% corn (Serna-Saldivar et al 1994).

COMPOSITION OF CORN GRAIN

Moisture, Carbohydrate, Protein, Fat and Ash

Pollination is a critical time in corn production fields. After fertilization, the seed grows rapidly by cell division and synthesis of starch, storage protein, and oil within the cells (Kieselbach 1949). The kernel goes through maturity stages denoted by "milk", "dough", and "dent" that refer to consistency of the crushed kernel. As solids accumulate, moisture declines from 60 to 70% to approximately 30% at maturity, which occurs at about 50 to 60 days after pollination (Hillson and Penny 1965). At maturity, kernel dry matter is about 72% starch, 9 to 10% protein, and 4.0 to 5.0% oil. The kernel is 82% endosperm and 12% germ, and wrapped in a cellulosic covering called the pericarp. All free oil is in the germ, and all starch is in the endosperm. Starch occurs in microscopic granules, surrounded by the storage proteins zein and glutelin (Watson 1982). Proximate analysis of corn grain from numerous sources and from corn purchased on the open market during 1980-1984 in Illinois, Iowa, and Indiana is shown in Table 3.

Analysis of Key Amino Acids

Individual samples of corn vary considerably in amino acid composition due to genetics influencing the ratio of zein to glutelin. Because whole corn in commercial channels is blended, amino acid composition of commodity corn is fairly constant. Amino acid analysis of whole dent corn is shown in Table 4.

Fatty acids

About 85% of the lipids in corn are in the germ, which has a lipid concentration of 30 to 38%. Most lipids are free, with bound lipids (mostly to the starch) constituting 0.3 to 0.9% of the kernel. Triacylglycerols are 75 to

92% of the total lipids and comprise C16, C18, and C20 fatty acids containing 0, 1, 2, and 3 double bonds. The ratio among them varies a great deal with the genetic background, but standard hybrids contain a range of 4.0 to 5.0% total oil. Typical fatty acid compositions of dent corn grain (as % of triacylglycerol fatty acids) is shown in Table 5.

Composition and Breeding

Starch, protein, and oil components of corn are the predominant fractions of commercial and human nutrition interest. Although all are modifiable by traditional breeding, and are not particularly expensive to monitor, corn breeders have focused on agronomic properties including yield, maturity, and standability. Nutrient composition is not normally analyzed as a routine procedure of traditional corn breeding with the exception of specialty corn products.

NUTRITIVE VALUES OF CORN

Nutritive values of corn and corn products are available from several reliable sources (Pennington 1994, USDA 1993). Values for corn and some typical corn products are listed in Table 6. Fatty acid values for corn oil were mentioned above.

How Corn and Corn Products fit within Dietary Guidelines

Public health organizations in the U.S. are in general agreement that Americans could significantly reduce health risks from coronary heart disease, cancer, obesity and other chronic diseases by making fundamental changes in their eating habits (NRC 1989, J. White 1992). The most recent national dietary advice from the Committee on Diet and Health of the National Academy of Sciences (1989) recommends that Americans:

1. Reduce total fat consumption to 30% or fewer of total calories; reduce saturated fatty acid intake to less than 10% of calories and cholesterol intake to less than 300 mg daily.

2. Daily, eat five or more servings of a combination of fruits and vegetable, especially green and yellow vegetables and citrus fruits.

3. Maintain protein intake at moderate levels (less than twice the RDA). This amount is about 15% of total calories.

4. Balance food intake and physical activity to maintain appropriate body weight.

5. Do not consume alcohol. For those who drink alcohol, less than two drinks per day (one ounce pure alcohol) is recommended.

6. Limit total daily intake of salt (NaCl) to 6 grams or less.

7. Maintain adequate calcium intake (approximately 800 mg per day).

8. Avoid taking dietary supplements in excess of the RDA in any one day.

9. Maintain an optimal intake of fluoride, particularly during the years of primary and secondary tooth formation and growth.

By implication, the diet should then contain at least 55% carbohydrates (30% fat + 15% protein + 55% carbohydrate = 100%), most of which should be complex carbohydrates (NRC 1989). Although an increase in complex, rather than simple carbohydrates (sugars) was recommended, it is important to note that sugars have not been demonstrated to affect health risks other than to increase the incidence of dental caries (Glinsmann et al 1986). The guidelines from the NRC are very similar to recommendations by The American Heart Association (AHA, American Heart Association 1986) and The American Cancer Society (ACA, American Cancer Society 1984). The AHA specifies that 50 to 55% of calories come from carbohydrates, with emphasis on increased complex carbohydrates, and the ACS specifies avoiding obesity and moderating intake of salt-cured, smoked and nitrate-cured foods.

A special panel of the National Academy of Sciences recommends a fiber intake of between 20 to 35 grams per day (NRC 1989). The ACA also recommends eating more high-fiber foods such as fruits, vegetables, and whole grain cereals. These dietary recommendations are reflected in the "Food Guide Pyramid" also published by the NRC. Suggested numbers of servings for various foods include: 6-11 servings of bread, cereal, rice and pasta; 3-5 servings of vegetables; 2-4 servings of fruits; 2-3 servings (3-ounces each) of meat, poultry, fish, dry beans, eggs and nuts; 2-3 servings of milk, yogurt and cheese; and sparing use of fats and oils (about 2 tablespoons total) and sweets. Also published, is a detailed listing of Recommended Dietary Allowances (RDA) for most nutrients, designed for the maintenance of good nutrition of practically all healthy people in the United States (NRC 1989). These recommendations are divided into amounts per age and sex. The amounts of vitamins and minerals recommended for males and females

between the ages of 25 and 50 years are listed in Table 6 for comparison with these nutrients reported in corn and corn products. The only macronutrient with a specific recommendation is protein.

The proteins in corn have a relatively high percentage of the nutritionally essential sulfur-bearing amino acids, methionine and cystine, but, like most cereals, are limited in their quantity of lysine and tryptophan (Wright 1987, Table 4). None-the-less, when eaten with a complementary protein source such as legumes, or with a small amount of complete protein generally supplied from animal sources, corn protein becomes entirely utilizable.

Corn may be readily incorporated into the recommended diet in a number of different ways. As a fresh, frozen or canned product, sweet corn is eaten as a vegetable, thus contributing to the daily servings set up in the "Food Guide Pyramid". As shown in Table 6, the fresh, cooked product provides macronutrients, vitamins and minerals that fit well within the NRC dietary guidelines. One serving provides a moderate number of calories and protein, a good amount of carbohydrate (mostly complex), only 1.1 g of fat, which is less than 15% saturated (Weber 1987a), and contains no cholesterol. The serving of yellow corn contains vitamin A desired from yellow or green vegetables, and contributes to the requirements for vitamins and minerals. In addition, only a minimal amount of sodium is present.

Other corn products, such as corn flakes or products made from corn meal and usually derived from U.S. dent corn, generally fit into the category of a cereal. Once again, these products provide basic macronutrients well within the NRC guidelines. Significant changes do occur when raw corn is cooked into tortillas (Rooney and Serna-Saldivar 1987). These losses occur in one of two ways: 1) by the physical loss of some components such as germ and pericarp and 2) by their chemical loss, destruction, or transformation (Bressani 1958). These combined losses for thiamine, riboflavin, niacin, fat and crude fiber average 60, 52, 32, 44, and 46%, respectively. On the other hand, calcium content is increased significantly during lime processing of the corn to produce flour for traditional tortilla preparation.

Since popcorn is just another type of corn, it should not be surprising that its nutrient composition is quite similar to that of regular corn (Table 6). Popcorn does, however, have a greater protein composition, likely because of its smaller kernel size and greater endosperm density ([redacted] 1995). Popcorn also is a good source of total dietary fiber.

After carbohydrates, proteins and fats, dietary fiber is the chemical component found in the greatest amounts in corn (FAO 1992). Today, we recognize that dietary fiber is extremely important to human health and that there are several different types of fiber, each of which is important. Some total dietary fiber values are listed in Table 6, but because dietary fiber is difficult to measure and because the definition of fiber has changed over recent years, values are still missing for many products. Other sources report a total dietary fiber in corn of about 12.2% to 12.8% on a dry weight basis, with about 11% being insoluble fiber and the rest being soluble fiber (Bressani et al 1989). Corn flakes was reported to have 12.8% (dry weight basis) total plant fiber, with 7.5% being soluble and 5.3% being insoluble fiber (Chen and Anderson 1981), but no value was listed by the USDA. Canned sweet corn was reported to have 17.2% total plant fiber, including 9.5% soluble and the rest being insoluble fiber (Chen and Anderson 1981). When converted to a dry weight basis, the value given in Table 6 for raw sweet corn is 13.3%. Corn tortillas were listed as having about 10.9% total dietary fiber, of which 9.5% was insoluble and 1.4% was soluble fiber (FAO 1992), whereas the value given in Table 6 is 6.5% dietary fiber, when converted to a dry weight basis.

Corn contains colored pigments called carotenoids. Two general classes of carotenoids, namely carotenes and xanthophylls, are primarily responsible for the yellow color of corn grain (Weber 1987b). The carotenes also provide the vitamin A for corn and corn products (Table 6). Carotenoids are believed to provide antioxidant protection to lipid-rich tissues (Jacob 1994). Vitamin E (tocopherol), present in corn in useful amounts, also serves as a natural antioxidant in the diet (Weber 1987b). Selenium is the most recent trace mineral recognized to be essential in the diet. It also serves as an antioxidant in biological functions ([redacted] 1985).

Corn contains some phytic acid (Graf 1983). This natural plant antioxidant, which is common but not typically measured, constitutes 1 - 3% by weight of many cereals and legumes and typically accounts for 50 - 90% of the total seed phosphorus (Lolas et al 1976). All of the antioxidant properties of phytic acid likely are derived from its relatively high binding affinity for iron, which can stabilize oils and lipid-containing foods against rancidity (Graf and Eaton 1990). Unfortunately, this chelating property interferes with the bioavailability of certain minerals in the diet, especially calcium, magnesium, zinc and iron.

Antinutrients in Corn

Mycotoxins are metabolites produced by fungi that grow on corn kernels produced or stored under adverse conditions (Shotwell 1977, Watson 1987b). They are not a natural component of sound corn (Wright 1987). When they are present at toxic levels in corn fed to animals, various disease symptoms may develop. Of the mycotoxins, aflatoxins and fumonisins are the most serious threat to animal and human nutrition because they possess acute and subclinical toxicity and carcinogenicity. Aflatoxins are highly stable to the level of heat generally encountered in the processing of grain. In a laboratory wet-milling procedure, all of the toxin was concentrated in the steep liquor, fiber, gluten, and germ, in that order (Yahl et al 1971). The Food and Drug Administration established a 20-ppb action level for aflatoxin in human food in about 1979 (Wright 1987). Murphy et al (1993) surveyed the fumonisin content of Iowa, Wisconsin and Illinois corn from the 1988 to 1991 crop years and found concentrations of three fumonisins ranging from 0 to 37.9 ppm in the corn, with average values of about 0.3 to 3.0 ppm. Values for corn from the 1992, 1993 and 1994 crops had average levels of less than 0.4 ppm for each fumonisin (Murphy et al 1995). The available data on the toxicity and carcinogenicity of the fumonisins and the fact that one of the fumonisins was found to be heat stable (Alberts et al 1990) suggest that these compounds could be a potential risk to human health (Hopkins and Murphy 1995); however, further work on this topic is necessary. At present, there are no federal guidelines on limits of fumonisins in human foods (Murphy 1995).

Corn contains only low levels of the natural antinutrients trypsin and chymotrypsin inhibitors (Wright 1987), neither of which is considered nutritionally significant. The presence of phytic acid in corn and its binding action with calcium and other minerals was noted above. No known allergens have ever been confirmed to be present in corn (Taylor 1995). Sulfites added to corn during wet milling are present at levels too low to be of concern to people who are sensitive to the compound.

CONCLUSIONS

Corn has become an integral part of the U.S. diet, and is found in many different products throughout the marketplace. Corn is eaten whole, ground, fractionated, and popped and its parts appear in foods as both recognizable and unrecognizable corn ingredients. The high productivity of modern corn hybrids, the food flavor of corn and the ease of processing corn into many foods and functional ingredients will likely result in even more corn usage for human consumption. Recent efforts to modify kernel components will provide an even wider diversity of corn products in the future.

Traditional breeding and variety testing of corn has generally focused on agronomic improvement. Nutritional and compositional analyses, when done, generally only include analyses for starch, protein, fat, and ash/fiber. Much less information is available regarding more detailed composition of corn and its minor constituents, such as discussed in this paper.

REFERENCES

Alberts, J.F., Gelderblom, W.C.A., Thiel, P.G., Marasas, W.F.O., Van Schalkwyk, D.J., and Behrend, Y. Effects of temperature and incubation period on production of fumonisin B_1 by *"Fusarium moniliforme"*. Appl. Environ. Microbiol., 56:1729, 1990.

Alexander, R.J. Corn dry milling: process, products and applications. In: *Corn: Chemistry and Technology.* S.A. Watson and P.R. Ramstad, Eds. Am. Assoc. of Cereal Chemists, St. Paul, MN, 1987.

American Cancer Society. *Dietary Recommendations*, 1984.

American Heart Association. Dietary guidelines for healthy American adults. *Circulation* 70:1, 1986.

Anderson, E., and Brown, W.L. Origin of Corn Belt maize and its genetic significance. In: *Heterosis.* J.W. Gowen Ed. Iowa State College Press, Ames, 1952.

Anderson, R.A., and Watson, S.A. The corn milling industry. In: *CRC Handbook of Processing and Utilization in Agriculture, Volume II: Part 1 Plant Products.* I.A. Wolff, Ed. CRC Press, Inc., Boca Raton, FL, p 30, 1982.

Anonymous. *1991 Corn Annual.* Corn Refiners Association, Inc., Washington, D.C., 1991.

Anonymous. *Agricultural Statistics.* Economic, Statistical and Cooperative Service, USDA, Washington, D.C., 1993.

Anonymous. Corn, touching our lives everyday. Illinois Corn Marketing Board, 1993b.

Anonymous. *1994 Corn Annual.* Corn Refiners Association, Inc., Washington, D.C., 1994.

Bendremer, J.C.M, and Dewar, R.E. The advent of prehistoric maize in New England. In: *Corn and Culture in the Prehistoric New World.* S. Johannessen and C.A. Hastorf, Eds. Westview Press, Boulder, CO, 1994.

Benz, B.F. Reconstructing the racial phylogeny of Mexican maize: Where do we stand? In: *Corn and Culture in the Prehistoric New World.* S. Johannessen and C.A. Hastorf, Eds. Westview Press, Boulder, CO, 1994.

Bothast, R.J. Genetically engineered microorganisms for the conversion of multiple substrates to ethanol. In: *Corn Utilization Conference V, Proceedings.* National Corn Growers Association, 1994.

Brekke, O.L. Corn dry milling industry. In: *Corn Culture, Processing, Products.* G.E. Inglett, Ed. Avi Publishing Co., Inc., Westport, CT.

Bressani, R., Breuner, M. and Ortez, M.A. Contenido de filra ácido y neutro-detergente y de minerales menores en maíz y sec tortilla. Arch. Latinoam. Nutr. 39: 382, 1989.

Bressani, R., Paz y Paz, R., and Scrimshaw, N.S. Corn nutrient losses: chemical changes in corn during the preparation of tortillas. J. Agric. Food Chem. 6:770-774, 1958.

Burge, R.M, and Duensing, W.J. Processing and dietary fiber ingredient application of corn bran. Cereal Foods World 34:535, 1989.

Carter, G.F. Sweet corn among the Indians. Georg. Rev. 38:206, 1948.

Chen, W-J.L. and Anderson, J.W. Soluble and insoluble plant fiber in selected cereals and vegetables. Am. J. of Clin. Nutr. 34:1077, 1981.

Craig, W.F. Production of hybrid seed corn. In: *Corn and Corn Improvement, 2nd Edition.* G.F. Sprague, Ed. American Soc. Agronomy, Madison, WI, 1977.

Durand, E.D. American Industry and Commerce. Ginn and Co., Boston, 1930.

Earle, A.M. Indian corn in colonial times. Chatauquan 26:586, 1898.

East, E.M. Inbreeding in corn. In: *Connecticut Agric. Exp. Sta. Rep.*, 1907.

Eldredge, J.C., and Thomas, W.I. *Popcorn: Its Production, Processing and Utilization.* Iowa State Univ. of Science and Tech. Bull. p127, Ames, 1959.

Erwin, A.T. Sweet corn not an important Indian food plant in the pre-Columbian period. J. Amer. Soc. Agron. 39:117, 1947.

Erwin, A.T. The origin and history of popcorn, *Zea mays* L. var. indurata (Sturt.) Bailey mut. everta (Sturt) Erwin. Agron. J. 41:53, 1949.

Erwin, A.T. Sweet corn: mutant or historic species? Econ. Bot. 5:302, 1951.

Faga, B. Dry milling: a variety of products. *1988 Corn Annual.* Corn Refiners Association, Inc., Washington, D.C., 1991.

Fast, R.B. Breakfast cereals: processed grains for human consumption. Cereal Foods World 32:241, 1987.

Faulkner, H.U. *American Economic History.* Harper & Bros., New York, 1924.

Finch, V.C., and Baker, O.E. *Geography of the World's Agriculture.* U.S. Dept. Agric., Office of the Secretary, Washington, D.C., 1917.

Food Agriculture Organization (FAO). *Production Yearbook: FAO Statistics.* FAO Vol. 43, 1990.

Food Agriculture Organization (FAO). *Production Yearbook: FAO Statistics.* FAO Vol. 45, 1992.

Freeman, J.E. Quality factors affecting value of corn for wet milling. Trans. ASAE 16:671, 1973.

Fussell, B. *The Story of Corn.* Alfred A. Knopf, New York, 1992.

Galinat, W.C. The evolution of sweet corn. Univ. Mass. Agr. Expt. Sta. Res. Bull. 591, 1971.

Galinat, W.C. The origin of corn. In: *Corn and Corn Improvement, 2nd Edition.* G.F. Sprague, Ed. American Soc. Agronomy, Madison, WI, 1977.

Galinat, W.C., and Gunnerson, J.H. Spread of eight-rowed maize from the prehistoric Southwest. Bot. Museum Leaflets Harvard Univ. 20:117, 1963.

Gay, J.P. *Fabuleux Maïs.* Assoc. Générale des Producteurs de Maïs, Pau, France, 1984.

Glinsman, W.H., Irausquin, H. and Park, Y.K. Evaluation of health aspects of sugars contained in carbohydrate sweeteners. Report of sugars task force. Health and Human Services, Food and Drug Administration, Center for Food Safety and Applied Nutrition, 1986.

Goodman, M.M. Chapter 1. History and origin of corn. In: *Melhoramento e Producão do Milho no Brasil.* E. Paterniani, Ed. Fundacão Cargill, Piracicaba, Brasil, 1978.

Goodman, M.M., and Brown, W.L. Races of Corn. In: *Corn and Corn Improvement, 3rd Edition.* G.F. Sprague and J.W. Dudley, Eds. American Society of Agronomy, Madison, WI, 1988.

Gould, W.A. Changes and trends in the snack food industry. Cereal Foods World 30:219, 1988.

Graf, E. Applications of phytic acid. *J. of Am. Oil Chem. Soc.* 60:1861, 1983.

Graf, E. and Eaton, J.W. Antioxidant functions of phytic acid. *Free Radical Biol. Med.* 8:61, 1990.

Gray, L.C. History of agriculture in the southern United States to 1860. Carnegie Inst. of Washington Publ. no. 430, 1933.

Hallauer, A.R. *Quantitative Genetics in Maize Breeding.* Iowa State Univ. Press, Ames, 1981.

Hallauer, A.R., W.A. Russell, and Lamkey, K.R. Corn breeding. In: *Corn and Corn Improvement,* 3rd Edition. G.F. Sprague and J.W. Dudley, Eds. American Society of Agronomy, Madison, WI, 1988.

Hanover, L.M. Functionality of corn derived sweeteners in formulated foods. In: *Chemistry of foods and beverages: recent developments.* G. Charalambous and G.E. Inglett, Eds. Academic Press, Orlando, FL p. 211, 1982.

Hardeman, N.P. *Shucks, Schocks and Hominy Blocks.* Louisiana State Univ. Press, Baton Rouge, 1981.

Hastorf, C.A., and Johannessen, S. Becoming corn-eaters in prehistoric America. In: *Corn and Culture in the Prehistoric New World.* S. Johannessen and C.A. Hastorf, Eds. Westview Press, Boulder, CO, 1994.

Hebeda, R.E. Corn sweetness. In: *Corn: Chemistry and Technology.* S.A. Watson and P.E. Ramsted, Eds., American Association of Cereal Chemists, Inc. St. Paul, MN p. 501, 1987.

Hedrick, U.P. *A History of Agriculture for the State of New York.* Printed for the New York State Agric. Soc., 1933.

Hill, L., Paulsen, M., Bouzaher, A., Patterson, M., Bender, K., and Kirleis, A. *Economic Evaluation of Quality Characteristics in the Dry Milling of Corn.* North Central Reg. Res. Bull. 330, 1991.

Hillson, M.T., and Penny, L.H. Dry matter accumulation and moisture loss during maturation of corn grain. Agron. J. 57:150, 1965.

Hodge, J.E. Food and feed use of corn. In: *CRC Handbook of Processing and Utilization in Agriculture Volume II: Part I Plant Products.* I.A. Wolff Ed. CRC Press, Inc., Boca Raton, FL, 1982.

Hopkins, E.C. and Murphy, P.A. Fumonisins: mycotoxins produced by *"Fusarium"* species. Natural protectants against natural toxicants, Vol. 1. W.R. Bidlack and S.T. Omaye, Eds. Technomic Pub. Co. 1:61, 1995.

Huelsen, W.A. *Sweet Corn.* Interscience, New York, 1954.

Humphrey, E.F. *An Economic History of the United States.* The Century Co., New York, 1931.

Jacob, R.A. Nutrition, health and antioxidants. Inform 5:1271, 1994.

Kahn Jr., E.J. The staffs of life. I-The golden thread. The New Yorker, June 18:46, 1984.

Kalm, P. Description of maize (Translated from Swedish by M. Oxholm and S.S. Chase). Econ. Bot. 28:105, 1974.

Kastner, J. The conundrum of corn. American Heritage (Aug./Sept.):14, 1980.

Kempton, J.H. Maize as a measure of Indian skill. In: *The University of New Mexico Bulletin.* University Press, Albuquerque, 1936.

Kieselbach, T.A. *The Structure and Reproduction of Corn.* Univ. Nebraska Res. Bull. 161, Lincoln, 1949.

Kirkland, E.C. *A History of American Economic Life.* F.S. Crofts & Co., New York, 1932.

Larson, W.E., and J.J. Hanway. Corn production. In: *Corn and Corn Improvement, 2nd Edition.* G.F. Sprague, Ed. American Soc. Agronomy, Madison, WI, 1977.

Lolas, G.M., Palamidis, N. and Markakis, P. The phytic acid-total phosphorus relationship in barley, oats, soybeans, and wheat. *Cereal Chem.* 53:867, 1976.

Long, J.E. Conventional Corn Syrups. In: *Products of the Corn Refining Industry in Food*, Semin. Proc. Corn Refiners Association, Washington DC p. 35, 1978.

Mitchell, D.P. Snack food's 26th annual state of the industry report. Snack Food 83:41, 1994.

Morris, C.E. Industry efforts upgrade HFCS. *Food Eng.* 7:55, 1984.

Munro, E.M. Corn refining: a classic value added success story. Cereal Foods World 39:552, 1994.

Murphy, P.A., Hendrich, S., Hopmans, E.C., Lu, Z., Buseman, G. and Munkvold, G. Effect of processing on the fumonisin content of corn in *"Fumonisins in Foods,"* L. Jackson, Ed. American Chemical Society monograph, in press, 1995.

[redacted] Iowa State University. Personal communication, 1995.

Murphy, P.A., Rice, L.G. and Ross, P.F. Fumonisin B_1, B_2 and B_3 content of Iowa, Wisconsin, and Illinois Corn and Corn Screenings. *J. of Agric. Food Chem.* 41:263, 1993.

National Research Council (NRC). *Diet and Health: Implications for reducing chronic disease risk.* Report of the Committee on Diet and Health, Food and Nutrition Board, Commission on Life Sciences. National Academy Press, Washington DC 1989.

Newton, J.M. Nutritive Sweetening from Corn at 16th Annual Symposium: Fabricated Foods-The role of Cereals and Oilseeds. Central States Section of American Association of Cereal Chemists, St. Louis, MO, 1975.

[redacted] Nutritional implications of selenium. *Monsanto Nutrition Update, Vol. 3.* March. Monsanto Chemistry Company, St. Louis, MO, 1985.

Orthoefer, F.T. Corn starch modification and uses. In: *Corn: Chemistry and Technology*. S.A. Watson and P.E. Ramsted, Eds., American Association of Cereal Chemists, Inc. St. Paul, MN p. 479, 1987.

Otey, F.H. and Doane, W.M. Chemicals from starch. In: *Starch: Chemistry and Technology, 2nd Edition.* R.L. Whistler, J.N. BeMiller and E.F. Paschall, Eds. Academic Press, Inc., Orlando, FL p. 389, 1984.

Pennington, J.A.T. Bowes and Church's Food values of portions commonly used. 16th Edn., 1994.

Reiners, R.A. and Gooding, C.W. Corn oil. In: *Corn: Culture, processing, products.* G.E. Inglett, Ed. AVI Publishing Co., Westport, CT. p. 241, 1970.

Rooney, L.W. and Serna-Salvidar, S.O. Food uses of whole corn and dry-milled fractions. In: *Corn: Chemistry and Technology*. S.A. Watson and P.E. Ramsted, Eds., American Association of Cereal Chemists, Inc. St. Paul, MN p. 399, 1987.

Rupp, R. *Blue Corn & Square Tomatoes.* Storey Communications, Inc., Pownal, VT, 1987.

Russell, W.A. Genetic improvement of maize yields. Adv. Agron. 46:245, 1991.

Serna-Saldivar, S.O., Gomez, M.H., and Rooney, L.W. Food uses of regular and specialty corns and their dry-milled fractions. In: *Specialty Corns.* A.R. Hallauer, Ed. pp. 263, CRC Press, Inc., Boca Raton, FL, 1994.

Sculeva, J.J. Industry outlook for use of corn oils current uses and new opportunities. In: *Corn Utilization Conference IV, Proceedings*, National Corn Growers Association, 1992.

Shotwell, O.L. Mycotoxins: corn related problems. Cereal Foods World 22:524, 1977.

Shull, G.H. A pure-line method in corn breeding. Am. Breeders' Assoc. Rep. 5:51, 1909.

Smith, P.S., and Bell, H. New starches for food applications. Cereal Foods World 31:724, 1986.

Snow, B.W. Indian corn industry in the United States. In: *U.S. Dept. Agric. Report on the Use of Maize (Indian Corn) in Europe and on the Possibilities of its Extension.* Dept. Report no. 49, Washington, D.C., 1891.

Tattric, N.H., and Yaguchi, M. Protein content of various processed edible oils. J. Inst. Can. Sci. Technol. Aliment. 6:289, 1973.

[redacted] University of Nebraska. Personal communication, 1995.

Tracy, W.F. Sweet corn. In: *Specialty Corns.* A.R. Hallauer, Ed. CRC Press, Inc., Boca Raton, FL, 1994.

United States Department of Agriculture. USDA handbook No. 8 via Telnet, Mar 3, 1993.

Watson, S.A. Corn marketing, processing, and utilization. In: *Corn and Corn Improvement, 3rd Edition.* G.F. Sprague and J.W. Dudley, Eds. American Society of Agronomy, Madison, WI, p 881, 1988.

Watson, S.A. Corn: Amazing maize. General Properties. In: *CRC Handbook of Processing and Utilization in Agriculture Volume II: Part 1 Plant Products.* I.A. Wolff Ed. CRC Press, Inc., Boca Raton, FL, 1982.

Watson, S., Corn and Sorghum Starches: Production. In: *Starch Chemistry and Technology, 2nd Edn.* R.L. Whistler, J.N. BeMiller and E.F. Paschall, Eds. Academic Press, Inc., Orlando, FL, p. 417, 1984.

Watson, S.A. Structure and composition. In: *Corn: Chemistry and Technology.* S.A. Watson and P.R. Ramstad, Eds. Am. Assoc. of Cereal Chemists, St. Paul, MN, 1987a.

Watson, S.A. Measurement and Maintenance of Quality. In: *Corn: Chemistry and Technology.* S.A. Watson and P.R. Ramstad, Eds., Am. Assoc. of Cereal Chem. St. Paul, MN, p. 125, 1987b.

Weatherwax, P. *Indian Corn in Old America.* The Macmillan Co., New York, 1954.

Weber, E. Lipids of the kernel. In: *Corn: Chemistry and Technology.* S.A. Watson and P.E. Ramstad, Eds., American Association of Cereal Chemists, Inc. St. Paul, MN, p. 311, 1987a.

Weber, E.J. Carotenoids and tocols of corn grain determined by HPLC. J. of Am. Oil Chem. Soc. 64:1129, 1987b.

Whistler, R.L. History and future expectation of starch use. In: *Starch: Chemistry and Technology.* R.L. Whistler, J.N. BeMiller, and E.F. Paschell, Eds. Academic Press, Orlando, FL, 1984.

White, J.R. Calorie control and nutrition management with products from corn. In: *Corn Utilization Conference IV, Proceedings*, National Corn Growers Association, 1992.

White, P.J. Fatty acids in oilseeds. In: *Fatty acids in food and their health implications.* C.K. Chow, Ed. Marcel Dekker, Inc., New York, NY, p. 237, 1992.

Wright, K.N. Nutritional Properties and feeding value of corn and its by-products. In: *Corn: Chemistry and Technology.* S.A. Watson and P.E. Ramsted, Eds., American Association of Cereal Chemists, Inc. St. Paul, MN, p. 447, 1987.

Wurzberg, O.B., Starch, modified starch and dextrin, In: *Products of the Corn Refining Industry*, Seminar. Proc., Corn Refiners Association, Washington DC, p 23, 1978.

Yahl, K.R., Watson, S.A., Smith, R.J., and Bavabolok, R. Laboratory wet-milling of corn containing high levels of aflatocen and a survey of commercial wet-milling products. *Cereal Chem.* 48: 385, 1971.

Young, L.S., and Long, J.E. Manufacture use and nutritional aspects of 90% high fructose corn sweeteners. In: *Chemistry of foods and beverages: recent developments.* G. Charalambous and G.E. Inglett, Eds. Academic Press, Orlando, FL, p. 195, 1982.

[redacted] Iowa State University. Personal Communication, 1995.

Ziegler, K.E., and Ashman, B. Popcorn. In: *Specialty Corns.* A.R. Hallauer, Ed. CRC Press, Inc., Boca Raton, FL, 1994.

Table 1. Total and per capita civilian consumption of corn food products for the United States in selected years (from Anonymous, 1993).

Year	Total consumed (million bushels)	Flour and meal per capita (lb.)	Hominy and grits per capita (lb.)	Syrup per capita (lb.)	Sugar per capita (lb.)	Starch per capita (lb.)
1978	436	6.8	3.1	29	3.8	2.5
1983	528	8.4	3	49.2	3.5	3.3
1990	728	14	3.4	68.7	4.5	4.3
1992	762	n.a.	n.a.	72.8	4.7	4.4

n.a. not available

Table 2. Food Uses of Corn-Based Products[a]

STARCHES

Corn Starch

Antibiotics
Aspirin
Baby foods
Bakery products (bread, rolls, cakes, pies, crackers and cookies)
Baking powder
Beverages, brewed (beer, ale, etc.)
Chewing gum
Chocolate drink
Confectionery
Cosmetics
Desserts (puddings, custards, etc.)
Drugs and pharmaceuticals
Flours, prepared (including prepared mixes)
Foods and drug coatings
Gravies and sauces
Meat products
Mixes, prepared (pancake, waffle, cake, candy, etc.)
Mustard, prepared
Pie filling
Precooked frozen meals
Salad dressing
Soaps and cleaners
Soups
Sugar, powdered
Surimi fish analog
Vegetables, canned
Yeast

SYRUPS

Corn Syrup

Baby foods
Bakery products (bread, rolls, biscuits, doughnuts, pies, cakes, cookies, pretzels, etc.)
Beverages, brewed (beer, ale, etc.)
Beverages, carbonated
Breakfast foods
Catsup, chili sauce, tomato sauce
Cereals, prepared
Cheese spreads and foods
Chewing gum
Chocolate products
Coffee whiteners
Condensed milk, sweetened
Confectionery
Cordials and liqueurs
Desserts
Eggs, frozen or dried
Extracts and flavors
Frosting and icings
Fruit butters and juices
Fruits (canned, candied, fillings, frozen, etc.)
Fruit drinks
Ice cream, water ices and sherbets
Jams, jellies, marmalades and preserves
Licorice
Malted products
Marshmallows and related products
Meat products (sausage, etc.)
Medicinal preparations (drugs, pharmaceuticals)
Mixes, prepared (cakes, infant foods, pie fillings, pudding powders, ice cream, etc.)
Peanut butter
Pickles and pickle products
Rice and coffee polish
Salad dressing
Sauces (seasoning specialty, etc.)
Seafood, frozen
Soups, dehydrated
Syrups (table, chocolate, cocoa, fruit, medicinal, soda fountain, cordials, etc.)
Toppings
Vinegar

High Fructose Corn Syrup

Bakery products
Canned fruits
Canned juices
Condensed soups
Condiments
Confectionery products
Dry cereals
Frozen desserts
Jams, jellies and preserves
Low calorie yogurt
Marinades
Microwave entrees
Salad dressings
Table syrup
Wine

Maltodextrins

Bakery mixes
Beverage powders
Condiments
Dehydrated foods
Dry soup mixes
Gum confections
Icings and glazes
Instant breakfast foods
Instant tea
Low calorie sweeteners
Marshmallows
Nougats
Pan coatings
Sauce and gravy mix
Snack foods

Table 2. Food Uses of Corn-Based Products[a] continued

(Glucose) DEXTROSE

Dextrose

Antibiotics
Baby Foods
Bakery products (biscuits, bread, crackers, fillings, icings, macaroons, pretzels, cookies, wafers, etc.)
Berries, canned and frozen
Beverages, brewed (beer, ale, etc.)
Beverages, carbonated
Breakfast foods
Caramel color
Cheese foods and spreads
Chewing gum
Chocolate products
Citrus juices
Coloring, pure food mix
Condensed milk
Confectionery
Cordials, liqueurs and brandy
Cream, frozen
Dairy products
Desserts
Dietetic preparations
Distillation products
Doughnuts (cake, yeast)
Drugs (fermentation process)
Eggs, frozen or dried
Fish, pickled
Flavoring extracts
Food acids (citric, etc.)
Fruits and vegetables (canned)
Fruits (candied glace, frozen)
Fruit juices
Gelatin desserts
Ice cream, water ice and sherbets
Infant and invalid feeding
Jams, Jellies, marmalades and preserves
Lactic Acid
Meat products (bacon, bologna, hams, sausage, frankfurters, mincemeat)
Medicinal preparations (intravenous injections, pills, tablets, drugs, etc.)
Mixes, prepared (cake, icings and frosting, infant foods, pie fillings, toppings, etc.)
Peanut butter
Peas, canned
Pectin, fruit
Pickles and pickle products
Powders (ice cream, prepared dessert, pudding, summer drink powders, etc.)
Prepared mixes
Sauces (catsup, tomato, etc.)
Seasoning mixes, dry
Sorbitol (in candies, toothpaste, etc.)
Soups, dehydrated
Spices and mustard preparations
Syrups (table, fountain, medicinal, etc.)
Vinegar
Wine

Ethanol

Alcoholic beverages
Industrial alcohol
Motor fuel extender

OIL

Corn Oil, Refined

Carriers for vitamins and other medicinal preparations in capsule form
Cooking oil
Margarine
Mayonnaise
Potato chips
Salad dressing
Sauces, seasoning
Shortening
Soups

[a]Information from Corn Refiners' Association, Inc.

Table 3 Proximate analysis of corn grain from numerous sources and from corn purchased on the open market during 1980-1984 in Illinois, Iowa, and Indiana (from Watson, 1987)

Characteristic	Range	Average
Moisture (%, wet basis)	7-23	16
Starch (%, dry basis)	61-78	71.7
Protein (%, dry basis)	6-12	9.5
Fat (%, dry basis)	3.1-5.7	4.3
Ash (oxide) (%, dry basis)	1.1-3.9	1.4
Fiber (neutral detergent residue)(%, dry basis)	8.3-11.9	9.5

Table 4. Amino acid analysis of whole dent corn.

	Values from the literature[a]			Values from current analysis[b]		
		% of total protein[c]			% of total protein[d]	
Amino acid	% of dry matter	Range	Average	% of dry matter	Range	Average
Nutritionally essential						
Methionine	0.19	1.0-2.1	1.9	0.19	1.8-2.9	2.2
Cystine	0.15	1.2-1.6	1.5	0.22	2.3-3.1	2.5
Lysine	0.25	2.0-3.8	2.5	0.29	3.0-3.9	3.3
Tryptophan	0.10	0.5-1.2	1.0	0.06	0.5-0.7	0.7
Threonine	0.39	2.9-3.9	3.8	0.32	3.2-4.5	3.7
Isoleucine	0.42	2.6-4.0	4.2	0.31	3.1-4.4	3.6
Histidine	0.22	2.0-2.8	2.1	0.26	2.8-3.7	3.0
Valine	0.48	2.1-5.2	4.7	0.44	4.5-6.0	5.0
Leucine	1.14	7.8-15.2	11.2	1.12	11.2-15.8	12.8
Arginine	0.59	2.9-5.9	5.8	0.41	4.1-5.8	4.7
Phenylalanine	0.50	2.9-5.7	4.9	0.45	4.5-6.2	5.2
Glycine	0.38	2.6-4.7	3.7	0.34	3.7-4.7	3.9
Nonessential						
Alanine	0.78	6.4-9.9	7.8	0.67	6.8-9.0	7.7
Aspartic acid	0.68	5.8-7.2	6.8	0.59	5.9-8.1	6.8
Glutamic acid	1.77	12.4-19.6	17.7	1.62	16.7-23.0	18.5
Proline	0.84	6.6-10.3	8.4	0.80	8.1-11.3	9.2
Serine	0.46	4.2-5.5	4.6	0.40	4.0-5.6	4.6
Tyrosine	0.47	2.9-4.7	4.7	0.26	2.5-3.9	3.0

[a] from Watson (1982).
[b] unpublished 1994 data from Iowa Gold Catalog Corn Test Entries, Iowa State University Grain Quality Laboratory.
[c] 10.1% total protein (N x 6.25), dry basis.
[d] 8.74% total protein (N x 6.25), dry basis

Table 5. Typical fatty acid compositions of dent corn grain (as % of triacylglycerol fatty acids) (from Watson 1982).

Triacylglycerol	Range	Average
Palmitic acid, 16:0	7-19	11.5
Palmitoleic acid, 16:1	1	1
Stearic acid, 18:0	1-3	2
Oleic acid, 18:1	20-46	24.1
Linoleic acid, 18:2	35-70	61 9
Linolenic acid, 18:3	0.8-2	0.7
Arachidic acid, 20:2	0.1-2	0.2

Table 6. Nutrient composition of corn and typical corn products[a]

Nutrient	Units	Recommended dietary allowances[b]		Product					
		men age 25-50 years	women age 25-50 years	sweet corn, raw	yellow dent corn	cornmeal,[c] yellow, degermed, enriched	corn flakes, Kellogg's	popcorn, air popped	corn[d] Tortilla, enriched
Macronutrients									
weight	g	NL	NL	100	100	100	100	100	30
Kcal		NL	NL	86	365	366	389	382	67
H_2O	g	NL	NL	76	10.4	11.6	2.6	4.1	13.6
protein	g	63	50	3.2	9.4	8.5	8.1	12.0	2.1
carbohydrate	g	NL	NL	19.0	74.3	77.7	86.1	77.9	12.8
fat	g	NL	NL	1.2	4.7	1.7	0.3	4.2	1.1
polyunsaturated fatty acids	g	NL	NL	0.56	2.16	0.71	NL[e]	1.90	NL
saturated fatty acids	g	NL	NL	0.18	0.67	0.23	NL	0.57	NL
cholesterol	mg	NL	NL	0	0	0	NL	0	NL
Total dietary fiber	g	NL	NL	3.2	NL	5.2[f]	NL	15.1	1.0
Minerals									
Sodium	mg	NL	NL	15.2	35	3	1,238	4	53
Potassium	mg	NL	NL	270	287	162	92	301	52
Calcium	mg	800	800	2	7	5	3	10	42
Phosphorus	mg	800	800	89	210	84	63	300	55
Magnesium	mg	350	280	37	127	40	12	131	20
Iron	mg	10	15	0.5	2.7	4.1	6.3	2.7	1.4
Zinc	mg	15	12	0.45	2.21	0.72	0.28	3.44	0.43
Copper	mg	NL	NL	0.05	0.31	0.08	0.07	0.42	0.90
Manganese	mg	NL	NL	0.16	0.49	0.11	0.08	0.94	0.123
Iodine	µg	150	150	NL	NL	NL	NL	NL	NL
Selenium	µg	70	55	NL	NL	NL	NL	NL	NL

Vitamins									
A	RE	1,000	800	28[g]	NL	41[g]	1,324	20	NL
C	mg	60	60	6.8	0	0	53	0	0
thiamin	mg	1.5	1.1	0.20	0.39	0.72	1.3	0.20	0.20
riboflavin	mg	1.7	1.3	0.06	0.20	0.41	1.5	0.28	.14
niacin	mg	19	15	1.7	3.63	5.03	17.6	1.95	1.5
B-6	mg	2.0	1.6	0.06	0.62	0.26	1.8	0.25	.09
B-12	µg	2.0	2.0	0	0	0	NL	0	0
folate	µg	200	180	45.8	NL	48	353	23	6
pantothenic acid	mg	NL	NL	0.76	0.42	0.31	0.18	0.42	.06
D	µg	5	5	NL	NL	NL	NL	NL	NL
E	α-Toc	10	8	NL	.49	NL	NL	NL	NL
K	µg	80	65	NL	NL	NL	NL	NL	NL

[a] Values from USDA 1993 unless specified
[b] NRC 1989
[c] Unenriched cornmeal contains 1.1 mg iron, 0.14 mg thiamin, 0.05 mg riboflavin, and 0.1 mg niacin
[d] Values from Pennington 1994
[e] NL = not listed
[f] A value of 11.0 g total dietary fiber is listed for whole-grain cornmeal
[g] For yellow varieties: white varieties contain only a trace of vitamin A. RE = retinol equivalents, 1 retinol equivalent = 1 µg retinol or 6 µg β-carotene

Appendix III

Corn and Animal Nutrition in the United States

Corn and Animal Nutrition in the United States

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Feed costs are the major component in livestock and milk production cost according to the University of Missouri MIR records (Plain, 1993a,b, and Bailey, 1995). In many species, grains make up a large portion of the energy contribution to the diet as well as a significant portion of essential amino acids or nitrogen for the animal. As a feedstuff for nonruminant animals, corn provides energy, and fat as well as both nonessential and essential amino acids. However, corn itself is not essential in nonruminant diets and its use will readily move, based on price, in and out of least-cost diet formulations according to the cost of corn and other energy containing feedstuffs such as sorghum grain, wheat, barley, fat, as well as other lesser recognized ingredients. Corn is generally the grain of choice in most United States swine and poultry rations because of its value as a nutrient and its relatively low cost. Corn is price sensitive in ruminant rations as well. As with nonruminant species, corn is readily substituted on a price basis with other coarse grains. Ruminants, however, can rely on fibrous ingredients for energy that nonruminants cannot utilize, and in fact high concentrate feedstuff use can depress digestibility of high fiber feedstuffs. Coarse grains are often useful as an energy source in situations where voluntary dry matter intake of the animal is insufficient to supply energy at a level that supports optimal performance. This is often the case with the high producing dairy cow and the finishing steer and heifer in a feedlot situation. High grain diets can be beneficial in feedlot situations to promote desirable marbling characteristics in beef and lamb carcasses. Corn silage can be used successfully in ruminant rations. When harvested at the proper stage of maturity and when correctly handled during harvest and the ensiling process, corn silage provides energy as well as structural carbohydrates needed by the ruminant.

[1]The assistance of [redacted], extension associate, and [redacted], assistant professor, in writing the beef and dairy feeding sections is gratefully acknowledged.

Background

While its origin in the Americas dates back some 7,000 to 10,000 years, corn has wide distribution today over all continents. Corn is produced in many areas of the world, according to Anderson and Watson (1982). North America is responsible for more than 40% of the total world corn production (Anon., 1992a) as shown in Table 1.0. Corn is the predominant grain utilized in the U.S. as well as exported from the U.S. As can be seen in Table 2.0, corn use for all purposes constitutes about 71% of domestic grain uses and 57% of grain exports when averaged over a 15 year period. During this period, corn exported equaled 35.5% of the corn used for domestic purposes.

The USDA Crop Production report (Anon. 1992c) lists grain production numbers as well as beginning and year ending inventory of grain, and the amount of grain used for food, seed, and industrial purposes along with import and export figures. From these numbers, they can, by difference, estimate a value listed as "feed and residual." This number is somewhat interesting in that it indicates corn disappearance but does not mean that it found its way to useful purposes in livestock. The USDA (Anon., 1974) put forth a set of statistics to help identify the actual livestock uses of grain by building an index of expectations of feed consumption based on animal inventory. These indexes are referred to as "grain consuming animal units (GCAU), and roughage consuming animal units (RCAU)." A GCAU was arbitrarily assigned as the grain fed to an average dairy cow during the years 1969 to 1971 or 4,293 lbs. The University of Missouri "Food and Agricultural Policy Research Institute" (FAPRI; Adams and Brown, 1991) also calculate a GCAU. Unlike the USDA numbers, FAPRI calculates GCAU's allowing for changes in market weights of hogs, and cattle as well as changes in feed efficiency assumptions. The USDA calculations assume today's livestock production efficiency is essentially unchanged since 1971. Figure 1 illustrates estimated corn utilization as calculated by both FAPRI (FAPRI, 1995) and USDA (Anon., 1985) between the years of 1970 and 1984 which is the most recently calculated data provided in USDA literature. Figure 2 illustrates FAPRI (Adams and Brown, 1991) estimates of percent of corn consumption by species between 1961 and 1989. FAPRI (Adams and Brown, 1991) also summarized USDA reported uses of corn for various purposes. These use estimates from 1961 to 1989 are illustrated in Figure 3, and clearly show that, over the time frame, feed represents the largest use of corn (64.1%), followed by exported grain (26.2%), and finally food, seed and industrial uses (9.7%). From these same data, it is evident, that use has changed dramatically, over the time frame. Exports and industrial use made up almost 50% of use throughout the 1980's while exports and industrial use made up less than 25% of use throughout the 1960's (FAPRI, 1991).

The industrial uses of corn are outlined well by Anderson and Watson (1982). Corn wet milling makes up a large portion of industrial use of corn. Corn wet milling is a complex process that is well described by Anderson and Watson (1982). The ultimate product goal of this process is to produce corn starch, corn oil, and corn sweeteners such as high fructose corn syrup and/or glucose. The U.S. Feed Grains council (Anon.,1992b) presents data that support the importance of the wet milling industry as a major exporter of U.S. value added agriproducts. A portion of all of the following products produced during or with the end- products of the wet milling process are exported from the U.S.: corn gluten meal; corn gluten feed; corn starch; corn oil; high fructose corn syrup; and glucose (Table 3.0).

Wet milling of grain leaves approximately one-third of the starting material as a by-product. By-products obtained from this process include: corn gluten feed, corn gluten meal, corn germ meal, corn starch molasses, steep liquor or condensed fermented corn extractive, corn bran, and a feedgrade or industrial grade hydrolyzed corn oil. While many of these products will enter the livestock feed chain, the products of major significance are the corn gluten meal and feed. Shroder and Heimen (1970) suggest that most corn gluten feed is utilized in ruminant rations, and to a lesser degree in swine rations, and that the major use of corn gluten meal is in poultry rations. This is due to the carotenoid pigments concentrated in the product and the desirability of carotene pigments in layer and broiler production.

Corn is also harvested as silage. Corn silage is regarded highly by dairymen as a very palatable product for dairy cattle as well as supplying high energy content. Table 4.0 shows USDA estimates of corn acreage and the split between that harvested as grain or silage (Anon., 1994). For this 3 year period, 89.2% of the corn acreage was harvested as grain.

Corn competes in most U.S. livestock rations with other grains such as wheat, rye, oats, barley, and sorghum grain. An appreciation of the importance of corn to U.S. livestock production is illustrated by comparing corn use relative to total grain use in livestock and poultry feeds (Table 5.0). Over this 15 year time frame, corn accounted for about 73.4 % of grains fed to livestock and poultry (Anon., 1992a).

The amount of corn fed in some processed form is somewhat hard to estimate. The vast majority of grain fed to livestock will be processed to assure that the flinty shell is broken to allow microbial or enzymatic action to be exerted on the internal components of the grain. For most monogastric species, processing generally consists of grinding through a hammermill or a roller mill. In ruminant rations, processing can significantly alter the manner

in which ruminal bacteria can attack the grain. As such various processing practices such crimping, kibbling, or flaking can be used to target specific feeding situations and goals. No real effort has been made from recognized sources to track amounts of processed grain fed to livestock. Table 6.0 from the U.S. Dept. Of Commerce (Anon., 1982) illustrates the breakdown of grain containing feeds sold during 1982 vs. the amount sold in 1977. Complete feeds contain an unknown amount of corn or other grain-based components.

Corn is generally one of the products considered to have the least incidence of any type of antinutritional factors or other quantity limiting factors to consider in formulation of rations. In many quality assurance programs, corn is evaluated for test weight, presence of foreign materials, broken grain, and the presence of molds which might be an indicator of potential mycotoxin presence. Test weight is considered because it is a commonly held thought that light test weight corn has a lower feeding value for livestock than does normal test weight grain. However, feeding data (Johnston, 1994) suggests that when used on a weight versus a volume basis there may not be as large of a difference in feeding value for pigs than is commonly assumed. It is also a commonly held thought that light test weight corn has a higher incidence of mycotoxin contamination. This may be due to increased physical damage normally associated with light test weight corn and the correlation between damage and aflatoxin presence in grain (Zuber et al., 1987).

The USDA regulates grading standards of U.S. grains. The grading standards for corn are given in Table 7.0 (Anon. 1988). Number 2 corn is the generally accepted feed-grade standard. Number 3 to 5 grade corn should be considered high risk for mold growth during prolonged or inappropriate storage conditions due to the high moisture content and the high percentage of damaged grain in the mix (Zuber et al., 1987). As with all feed ingredients, corn adulterated as defined under section 406 of the Federal Food, Drug, and Cosmetic Act (AAFCO, 1994) is not suitable for livestock feed.

Like most grains, corn is subject to numerous mold growth problems. The topics of the fumonisin and aflatoxin in feed have been reviewed by Guzman and Casteel, 1994 and Zuber et al., 1987. Romer labs presented an outline of mold and mycotoxin issues which outlines the following classical mold promoting environmental issues and the likely mycotoxin that the mold could produce:

1) Dry, hot conditions around tasseling followed by wet conditions: Aspergillus sp.; capable of producing the entire aflatoxin family of toxins. Classic sublethal indicators of presence in feed: reduced growth rate and feed refusal. Residues in tissue or milk are of concern to humans due to its potent carcinogenic activity.

2) Cool and damp growing season: Fusaria sp., capable of producing deoxynivalenol (DON or Zearalenone); Fumonisin; and subclasses or other unidentified toxins. Classic sublethal indicators of mold presence in feed:

DON: vomiting, inappetence, estrogenic characteristics may cause inappropriate reproductive signs and/or reproduction failures.

Fumonisin: newer class of toxins thought to be the cause of pulmonary edema in pigs, immunosuppression in several species, and leukoencephalomalacia in horses.

Corn has been selected historically to have specific characteristics that might be advantageous to the potential end users of the product. Just a brief list of the phenotypes that have been successfully selected for include: high oil varieties; high and low protein varieties; high lysine; and altered amylopectin containing varieties (Sprague and Dudley, 1988). Of the possible phenotypes, only the high amylopectin (waxy corn) and flint varieties are recognized separately from standard grades of corn under the USDA grading standards (Anon. 1988). These varieties of corn and the genetic basis to select them are well described by Sprague and Dudley, 1988. The value of these hybrid varieties have historically been determined by the end user and the value that is achieved in the marketplace.

Corn Uses in Livestock Rations

Swine:

Corn is a major constituent of U.S. swine feed. Corn supplies readily available energy and protein to support growth and maintenance of the animal. Tables 8.0, 9.0 and 10.0 list the major nutrients supplied by whole corn and from by-products of industrial corn processing. The metabolizable energy for swine is 3420 kcal/kg (NRC, 1988b). Only fat exceeds corn in energy density and generally cost and physical handling properties dictate that corn is used preferentially as a calorie source in swine rations. The amino acid content of corn is listed as well in the

Table 9.0. The pig does not require intact protein in its diet. Rather, the pig requires the constituents of proteins which are amino acids. Of the 20 amino acids normally considered as constituents of protein growth in the pig, 10 must be supplied in the diet (essential amino acids). The remaining 10 amino acids can be generated by transamination in the animal's own tissues as required for protein synthesis. These 10 amino acids may be referred to as nonessential amino acids. For the body to produce these nonessential amino acids, excess quantities of one may be converted to another (transamination). Unlike ruminants, swine and other monogastric species cannot utilize nonprotein nitrogen sources such as urea. The 10 essential amino acids either cannot be synthesized from amino groups taken from excesses of other amino acids, or they cannot be synthesized in sufficient quantities for metabolic and growth requirements.

Corn has a complementary amino acid profile with soybean meal to adequately supply the nutritional requirements of the pig (NRC, 1988b). For example, three amino acids critical to growth yet are provided in marginal amounts in oilseed-grain-based diets are lysine, methionine, and cystine. Often methionine and cystine are evaluated together since methionine can be converted to cystine. Soybean meal has an exceptionally high lysine content (3.12%), but the methionine and cystine content is somewhat low at 1.41%. The lysine content of corn is fairly low at 0.25%, however, the methionine and cystine content is 0.40%. While the corn methionine and cystine level is low relative to soybean meal, the ratio of methionine and cystine to lysine in corn is 1.6, while the ratio in soybean meal is 0.45. This complementary ratio of amino acids allows for the final ration to supply the amount of each essential amino acid required for growth and maintenance in the pig without excessive supplementation of total crude protein. While it is beyond the scope of this discussion, the concept of "ideal protein" deals with the ratio of essential amino acids required to satisfy the demands of tissue maintenance and growth. Diets based on blends of corn and soybean meal can be formulated to equal or exceed the ratios of the "ideal protein" concept.

Pigs, and other monogastric species, rely on dietary source for supply of what have been termed essential fatty acids. These include linoleic and arachidonic acids. Arachidonic acid can be produced from linoleic acid if it is present in excess relative to the animal's requirement. It is extremely difficult to produce an essential fatty acid deficiency lesion due to the extremely low requirement by the pig and in fact the

requirement has not been determined with any degree of precision. Corn has about 2.2% by weight linoleic acid.

A growing percentage of U.S. hogs are fed diets that have been pelleted. It is unclear why pelleting improves feed efficiency, however, swine fed pelleted rations typically have improved feed efficiency by 4 to 6% relative to the same diet in meal form. This improvement has been attributed by some to having less feed wastage of pelleted rations and by others to having improved digestion (metabolizable energy) when processed such that the starch components are somewhat gelatinized. A review (Newcomb, 1994) of the literature finds both explanations for the improved feed efficiency to be supported in some studies but neither is supported in the majority studies.

Poultry:

Poultry includes three distinct production units: 1) broiler production; 2) laying hen or egg production; and 3) turkey production. These three production units are similar in that they metabolize amino acids and energy in the same manner, but they all require differing amounts of each essential amino acid as well as energy. Unlike swine, the sulfur amino acids (i.e., methionine and cystine) are first limiting in typical U.S. diets for poultry. This is predominantly due to the high demand for sulfur amino acids to support feather growth. Lysine becomes second limiting in most grain oilseed meal diets used in the U.S. Poultry have large energy demands to support the growth rate or lay rates achieved today. Table 11.0 lists relative nutrient requirements as listed by NRC 1994. It should be noted the metabolizable energy of corn is slightly different from that of swine at 3350 vs. 3240 kcal/kg respectively. Poultry rations range widely in forms, which include pellets, crumbles, and meal.

The linoleic acid requirement of poultry has been estimated by Balnave (1970) to be about 1% of the diet. Since corn contains about 2.2% of linoleic acid a diet needs only contain 45% corn to satisfy the essential fatty acid (EFA) demands of the bird without any other components contributing to the EFA content of the diet.

Beef Cattle:

Ruminants are significantly different in their nutritional requirements relative to monogastric species due to the bacterial fermentation that takes place in the rumen. Ruminants can utilize a wide variety of roughage products because the ruminal bacteria digest

the roughage and convert it into bacterial proteins and volatile fatty acids. The bacterial protein is then transported to the true stomach of the cow where digestion of the bacterial proteins occurs much like that of the monogastric species. The volatile fatty acids produced from the bacterial fermentation process are absorbed across the rumen mucosa into the blood stream to be utilized as an energy source by the animal. This process of conversion from a plant source to a bacterial source and finally to an animal source is less efficient than a simpler conversion found in monogastric species. However, the animal is capable of converting high fiber feedstuffs into animal protein which is poorly done in most monogastric species.

The ruminant is not reliant on dietary additions of specific essential amino acids as was discussed for the monogastric species. While similar post absorption transamination of amino acids occurs metabolically, the bacterial proteins produced in the rumen are responsible for supplying essential amino acids for absorption and use by the animal. The rumen microflora has the remarkable ability to utilize a variety of nitrogenous substrates to synthesize the desired bacterial proteins. Corn can supply a small amount of the nitrogen needed by the bacteria, but its major contribution is as a readily available energy source for bacteria fermentation. Corn protein is only about 40% degradable by the rumen microflora (NRC, 1984). Other nitrogen sources commonly fed to ruminants include soybean meal, other oilseed meals, as well as several nonprotein nitrogen sources such as urea, uric acid, or biuret.

The classification of beef cattle can be broken down into discussions of breeding herd feeding, backgrounding/stocker calf feeding and finishing cattle feeding. While this discussion will be fairly simplistic in its approach, it is useful to understand the goals and the common feeding practices used in each production phase. The nutritional goal for the breeding female is to provide minimal harvested inputs in order to achieve a 450 lb. calf or better every year. The intent is to allow the animal to harvest forage in place of mechanical harvesting and to provide grain or protein sources only when adequate energy and protein are not available from the forage base. As a rule, this translates into feeding grain to the female for between one and four months depending on the weather and region of the country.

The goal of backgrounding/stocker calf production is to grow a weaned calf from 400 and 500 lbs. to between 700 and 900 lbs. utilizing

low cost roughage feed resources. In many production scenarios, weaned calves will be purchased and will be kept on low cost forages throughout the winter seasons. As cool weather grasses emerge, calves will generally compensate for previously low growth rates by exceeding the growth rates that would have been expected had they been provided high quality forages the entire time. Backgrounding generally consists of feeding calves in a drylot situation some type of harvested forage product to achieve growth with minimal inputs. Often, these cattle will be fed some type of concentrated grain ration prior to shipping to the feedyard as a preconditioning program.

The goal of the feedlot phase of cattle production is to achieve a carcass that will have the following characteristics in the USDA grading system: carcass wt., 550 to 900 lbs.; quality grade of low choice or better; yield grade three or less; total age <24 months. This goal is generally achieved by feeding a very high grain-based diet along with a small amount of roughage and a protein, a vitamin, and mineral supplement. Like monogastric species, grain choice is highly dependent upon price. Cattle will perform well with most coarse grains available in the U.S.. The grain which provides the best energy:cost ratio at a given time will be selected.

Unlike monogastric species, the ruminant consuming high grain diets can be subject to numerous metabolic problems. Acidosis can be caused by changing diets too quickly toward a high grain ration. In this case, introduction of large amounts of highly available starch to the rumen causes inappropriate lactic acid production via the glycolysis cycle by ruminal bacteria. Without proper diet introduction, a cycle of acidosis followed by gorge feeding and again subsequent acidosis can develop. Proper feed introduction includes the gradual increase in grains to allow adaptation of the rumen microflora to species capable of handling high starch loads without producing large lactic acid loads.

Dairy Cattle:

The previous discussion regarding beef cattle nutrition relates well to dairy calves and heifers with some slight differences. Dairy calves are removed from their dams within two to three days postpartum. Most are removed immediately after birth and bottle fed colostrum in an effort to control disease. New born calves are fed waste milk or a commercially available milk replacer. At four to five days of age, calves are offered a coarse calf starter grain mix. Coarsely rolled corn and oats are blended with protein to formulate most calf starters.

The coarse grain helps stimulate intake and rumen development as the calf grows.

At six to eight weeks of age, calves are weaned to an all dry feed diet consisting of calf starter/grower and dry hay. The goal in developing dairyreplacement heifers is to achieve 1.8 to 2.0 lb average daily gain. This growth rate allows heifers to reach breeding weights (breed specific) at 14 to 16 months old and first calving at 24 to 26 months of age. During the development phase from weaning to calving, the diet is predominantly forage with moderate grain supplementation based on forage quality. Forages used are predominantly grass or grass legumes pasture and/or hay. In some systems, corn silage may be used as a forage source. Corn silage feeding is usually associated with periods of low pasture availability (ie. winter months, drought). Most developing heifers are fed 5 to 7 pounds daily of a 12 to 14 percent protein ration to supplement the forage base. Bred heifers are generally grouped and fed with mature non-lactating (dry) cows three to four weeks prepartum. During this period dry cows and springing heifers are fed a transition diet in order to adapt rumen microflora to higher grain intakes.

At calving, the lactating dairy cows are fed based on level of milk production. During early lactation (the first 30 to 60 days), energy lost through milk production exceeds energy intake resulting in a negative energy balance and loss of body weight. Excessive body weight losses early postpartum adversely affects reproductive performance of lactating cows. Therefore, nutritional management of early lactation cows is designed to optimize intake of an energy dense diet in an effort to minimize excessive body weight loss. Energy density of the diet is increased by increasing the grain portion of the diet and reducing the forage component of the diet. This shift in the forage:concentrate ratio is changed to include a maximum of 60 percent of grain concentrate.

Limiting the amount of grain concentrate in the diet dry matter is an attempt to prevent ruminal acidosis. As mentioned earlier in the beef cattle section, pregastric fermentation significantly alters feedstuffs. The primary by-products of microbial fermentation are the volatile fatty acids. A shift in substrates for (starch vs fiber) and rate of (quickly vs slowly fermented) fermentation can change animal performance. For example, rumen fermentation and specifically fiber fermentation are optimized when ruminal pH is maintained between 6.0 to 6.5. Excessive dietary starch consumed during a short period of time (ie. grain fed in milking parlor during milking) can lower rumen pH below

5.7 and result in ruminal acidosis. Therefore, minimum fiber levels for lactating dairy cows have been established at 19% acid detergent fiber and 25% neutral detergent fiber by NRC, 1988a. These fiber levels help in minimizing abnormal and unhealthy rumen fermentation patterns.

Balancing the carbohydrate fraction to allow maximum energy intake while optimizing microbial fermentation in the rumen is critical in optimizing performance of dairy cattle. In addition to energy fermentation, protein utilization relative to energy digestion must also be balanced. Nocek and Russel (1988) described this relationship between carbohydrate and protein fermentation. In their review, they also described an optimal range for non-structural carbohydrates (NSC) in order to optimize milk production. Milk production was optimized with NSC ranging from 30 to 42 percent of the diet dry matter. In order to maintain adequate NSC in dairy cow diets, grains are used to supply starch and sugars.

With pregastric fermentation, dairy cows can also utilize fibrous by-product feeds. According to Weigel (1994), one billion bushels of corn are processed by corn refiners, dry millers, and distillers. Corn processors generate by-products such as corn gluten feed, corn gluten meal, corn distillers grains, and corn hominy. Fibrous by-product feeds can be beneficial in manipulating the NSC in the diet. In addition to adding digestible fiber to the diet, these corn by-product feeds can be used to improve other aspects of the diet based on individual feed characteristics. For example, corn gluten meal and dried corn distillers grains contain a high proportion of rumen escape or by-pass protein (NRC, 1988a). Corn gluten feed contains a more soluble rumen degradable protein. Corn hominy feed can be used to reduce starch but increase energy density due to its fiber and fat content relative to corn grain. Corn and corn by-product feeds are basic ingredients used throughout the dairy feed industry.

Corn silage can play a role in providing the nutrients required by both dairy and beef cattle. When properly ensiled, both the aerial portion of the plant as well as the grain contribute nutritionally. The stage of maturity of the plant at harvest impacts the proximate analysis of the silage produced (NRC, 1988a). For the purposes of this discussion only the most commonly used silage has been included in tables 8.0, 9.0, and 10.0. Corn silage is frequently used as a feedstuff in dairy rations in the Midwestern U.S. due to its value as a high quality feedstuff.

Summary

Corn is an important feedstuff in U.S. livestock production either as whole grain, as a by-product from the corn milling industry, or as silage. Corn is a highly concentrated energy source for all livestock species. In addition, corn can supply a significant portion of protein and amino acids required for animal growth and maintenance. Corn by-products and silage are useful in ruminant rations to help regulate rumen microflora metabolism as well as to provide a source of rumen bypass protein. The value of corn to the livestock industry is based on the energy and protein or amino acid content of the grain. This value is in a dynamic relationship based on costs of other energy containing feedstuffs and other amino acid or protein sources available to the feed manufacturer.

Table 1.0. Major Corn Producing Countries and Continents, Production in million metric tons (adapted from Anon. 1992a)			
Continent or Country	1989	1990	1991
North America (total)	210.3	225.9	214.4
U.S.A.*	191.1	201.5	189.9
Canada*	6.4	7.2	7.3
Mexico*	9.8	14.1	14.5
South America (total)	32.4	36.6	44.4
Brazil*	21.8	23.7	28.5
Argentina*	5.2	7.6	10.5
Eastern and Western Europe (total)	57.8	43.1	62.0
France*	13.4	9.5	12.9
Romania*	9.0	6.1	10.5
Yugoslavia*	9.4	6.7	11.6
Soviet Union	15.2	9.9	9.0
China (mainland)	78.9	96.8	98.8
India	9.4	9.1	8.4
Others	56.9	54.7	45.4
World (total)	460.9	476.1	482.5

*Major corn producing countries on the continent

Table 2.0. U.S. Grain Use in Million Metric Tons (adapted Anon. 1992a).

	Total Grain Use		Corn Use	
Year	Domestic	Exports	Domestic	Exports
1977	162.6	89.1	109.7	48.3
1978	180.6	95.1	124.3	53.8
1979	185.4	111.8	132.4	61.1
1980	171.9	115.5	124.5	60.9
1981	179.4	111.5	126.7	50.8
1982	195	96.8	138.2	46.4
1983	183	98.1	122.3	48
1984	197.9	97.6	131.9	47.1
1985	202	63.5	134.1	31.2
1986	217	76.9	150	37.9
1987	217.3	98.9	153.8	43.7
1988	187.7	103.7	133.2	51.6
1989	204	106.8	146.2	60.3
1990	219.7	83.8	153.6	43.9
1991	178.9	37.3	161.5	39.4

Table 3.0. U.S. Exports of Wet Milling Products (Anon. 1992)

1000 Metric Tons

Ingredient	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991
Corn Gluten Meal	-	-	-	-	161	176	205	125	386	532	477
Corn Gluten Feed	2796	3433	3665	2107	3855	4141	4073	1639	5310	5450	5632
Corn Starch	29.8	27.1	34.5	32.8	34.5	60.5	47.4	57.5	58.7	47.9	-
Corn Oil	88	106	136	122	153	119	166	171	184	219	-
HFCS & Glucose	.86	1.11	1.28	.53	.56	1.04	5.08	15.4	47.1	84.9	

Table 4.0. USDA Crop Production Estimates-Corn 1991-1993 (Anon. 1994).

Year	Acres Planted X1000	Acres Grain Harvest X1000	Acres Silage Harvest X1000	Yield/AC Bushels GrainX1000	Yield/AC Tons Silage X1000	Total Bu Grain Bill Bu	Total Tons Silage X1000
1991	75,951	68,847	6,101	108.6	13.2	7.475	80,543
1992	79,340	72,162	6,009	131.4	14.5	9.482	86,849
1993	73,323	62,991	6,846	100.7	12	6.344	82,052

Table 5.0. Energy Feed Use In Livestock Production (Million Metric Tons) Adapted from Anon. 1992a

Year	Corn	Coarse Grains[a]	% Corn Use
1977	94.9	122.5	77.5
1978	108.8	138.3	78.7
1979	116.2	157	74.0
1980	107.7	143.6	75
1981	108	146.3	73.8
1982	116.4	163.7	71.1
1983	98.6	147.3	67.8
1984	104.7	155.1	67.5
1985	104.7	161	65.0
1986	118.8	171.1	69.4
1987	122.1	167.6	72.9
1988	100.3	123.4	81.3
1989	111.7	141.5	78.9
1990	118.8	150.3	79.7
1991	124.7	145.4	85.8
Average	111.7	152.1	73.4

aIncludes: corn, sorghum grain, oats, barley, wheat, and rye.

Table 6.0. The Census of Commercial Feed Sales 1000 Metric Tons (Anon. 1982)		
Feed Type	1982	1977
Complete Poultry Feed	16369.7	13833.5
Complete Dairy Feed	8380.1	8055.4
Complete Swine Feed	2563.7	3068.4
Complete Beef Cattle Feed	3081.6	2658.4
Horse, Mules, Ducks, etc.	1863	1611.3
Ground, Crimped, Chopped Rolled or Pulverized Grain	586.3	540.8

Table 7.0. USDA Corn Grading Standards (Anon. 1988).

Grade	Min. Test Wt Lb/Bu	Maximum Moisture, %	Max. Broken or Foreign, %	Heat Damage %	Total Damage %
1	56	14	2	.1	3
2	54	15	3	.2	5
3	52	17.5	4	.5	7
4	49	20	5	1	10
5	46	23	7	3	15

Table 8.0. Proximate composition of Corn and Corn By-products[1] (adapted from Fonnesbeck and Lloyd, 1984 and NRC, 1994).

Item		DM, %	Protein, %	ADF, %	Cellulose, %	Lignin, %	Crude Fiber,%	Ether Extract, %	Ash, %	Linoleic Acid %
Corn	Avg	88	10.4	3.7	2.3	1.1	2.6	4.4	1.6	2.20
	N	562	544	5	4	3	90	102	88	
	Range	81.8-94.2	6.7-14.1	*-7.5	1.6-3.0	.2-2	*-5.4	2-6.8	.1-2.5	
Corn Gluten Feed	Avg	90.7	25.7	10.8	8.0	.6	7.5	3.0	6.9	*
	N	23	22	1	1	1	11	11	3	
	Range	88.2-92.2	20.5-30.9				2.9-12.1	.9-5.1	3.8-10.1	
Corn Gluten Meal	Avg	90.3	67.6	4.7	2.4	1.5	2.0	2.2	2.3	*
	N	12	12	2	1	2	6	11	4	
	Range	87.8-92.8	63.5-71.7	3.5-5.9		.1-3.1	.5-3.5	.3-4.1	*-4.6	
Corn Hominy Grits By-Product	Avg	90.2	11.8	7.4	3.9	1.5	5.5	6.3	3.0	3.28
	N	23	18	6	3	4	16	15	11	
	Range	88.2-92.2	10.6-12.9	*-15.4	3.2-4.6	.3-2.7	2.6-8.4	4.5-8.1	2.3-3.7	
Corn Silage	Avg	26.7	8.3	32.5	No	4.7	21.7	3.2	5.2	No
	N	6	6	2	Data	2	8	6	5	Data
	Range	15.4-38	7.2-9.4	27.2-37.8		3.6-5.8	5.7-37.7	2.6-3.8	2.2-8.2	
Corn Opaque-2	Avg	88.8	11.2	No	No	No	2.9	5 0	2.0	No
	N	48	69	Data	Data	Data	56	56	57	Data
	Range	81.2-96.5	7.7-14.8				2.4-3.4	3-7.2	*-4.0	
Corn Waxy	Avg	89.1	10.1	1.1	No	No	2.1	3.1	1.5	No
	N	12	12	1	Data	Data	7	10	10	Data
	Range	86.2-92.1	8.6-11.6				.9-3.3	2.4-3.8	.9-2.1	
Corn Flint	Avg	88.2	11.2	No	No	No	2.1	4.3	1.6	No
	N	9	5	Data	Data	Data	5	5	5	Data
	Range	85.1-91.3	7.8-14.6				2.0-2.2	2.5-6.1	1-2.2	

[1]Abbreviations used: DM=dry matter; ADF=acid detergent fiber; AVG=average; N=number of observations; *=Negligible amount.
All values on a expressed on a dry basis. Range calculated as: AVG+/-(2.5*Standard Deviation).
Values for linoleic acid have no estimate of variability.

Table 9.0. Essential amino acid content of Corn and Corn By-Products[1] (adapted from Fonnesbeck and Lloyd, 1984).

Item		ARG	HIS	ILE	LEU	LYS	MET	CYS	PHE	TYR	THR	TRP	VAL
Corn	Avg N Range	.49 433 .37-.61	.31 430 .23-.39	.40 428 .27-.53	1.35 428 .87-1.83	.34 437 .23-.45	.20 434 .09-.31	.13 428 .02-.24	.52 427 .33-.71	.35 425 .13-.57	.41 430 .29-.53	.10 19 .06-.14	.54 429 .4-.68
Corn Gluten Feed	Avg N	.87 1	.68 1	.98 1	2.44 1	.71 1	.41 1	.47 1	.90 1	.81 1	.87 1	.17 1	1.22 1
Corn Gluten Meal	Avg N	2.31 1	1.55 1	2.54 1	11.58 1	1.14 1	1.97 1	1.12 1	4.48 1	3.67 1	2.5 1	.33 1	3.45 1
Corn Hominy Grits By-Product	Avg N Range	.55 10 .43-.67	.24 6 .13-.35	.44 6 .40-.49	.97 5 .51-1.31	.40 5 .27-.53	.17 10 .08-.26	.15 8 .10-.21	.37 6 .16-.58	.52 4 .36-.68	.44 7 .40-.48	.14 11 *-.28	.57 6 .45-.69
Corn Silage	Avg N	.97 1	.21 1	.25 1	.93 1	.43 1	.44 1	No Data	.36 1	.30 1	.36 1	No Data	.45 1
Corn Opaque-2	Avg N Range	.74 21 .14-1.3	.33 18 .14-.52	.31 21 .11-.51	.88 21 .28-1.5	.45 27 .27-.63	.16 20 *-.31	.21 18 *-.41	.44 20 .17-.71	.37 20 .09-.66	.39 21 .17-.61	.10 18 *-.20	.51 21 .17-.85
Corn Waxy	Avg N Range	.41 2 .06-.76	.22 2 .2-.24	.41 2 .24-.59	1.72 2 *-3.78	.16 2 *-.33	.14 2 *-.31	.28 1	.72 1	.38 1	.31 1	.No Data	.46 2 .42-.50
Corn Flint	Avg N	No Data	No Data	No Data	No Data	.30 1	.20 1	No Data	No Data	No Data	No Data	.10 1	No Data

[1]Abbreviations used: DM=dry matter; AVG=average; N=number of observations; *=Negligible amount.
All values on a expressed on a dry basis. Range calculated as: AVG+/-(2.5*Standard Deviation).

Table 10.0. Energy estimates of Corn and Corn By-products[1] (adapted from Fonnesbeck and Lloyd, 1984, and NRC, 1982).

Item		Cattle			Poultry	Swine	
		NEm Mcal/kg	NEg Mcal/kg	NEl Mcal/kg	ME Mcal/kg	ME Mcal/kg	TDN,%
Corn	AVG N Range	2.16 1	1.48 1	2.05 **	3.93 14 3.4-5.0	3.78 9 3.1-4.4	87
Corn Gluten Feed	AVG N	1.9 **	1.26 **	1.80 **	1.59 **	2.90 **	83
Corn Gluten Meal	AVG N	2.13 **	1.46 **	2.00 **	No Data	3.75 **	89
Corn Hominy Feed (Grits By-Product)	AVG N Range	2.4 **	1.68 **	2.22 **	3.31 8 2.4-4.2	3.69 9 3.4-4.0	91
Corn Silage	AVG N	1.52 **	.92 **	1.51 **	No Data	No Data	69
Corn Opaque-2	AVG N	1.98 **	1.33 **	1.87 **	3.74 2	3.81 1	89
Corn Waxy	AVG N	1.97 **	1.32 **	1.86 **	No Data	3.68 **	No Data
Corn Flint	AVG N	1.99 **	1.33 **	1.88 **	No Data	3.70 **	No Data

1Abbreviations Used: NEm=net energy of maintenance; NEg= net energy of gain; NEl= net energy of lactation; ME=metabolizable energy; TDN-total digestible nutrients; ** Estimates made by Fonnesbeck and Lloyd, 1984.

Table 11.0 Selected Requirements of Swine and Poultry (Adapted from NRC, 1988b and 1994).

Nutrient	Layers[1]	Broilers 0-3 wks	Broilers 3-6 wks	Broilers 6-8 wks	Turkeys 0-3 wks	Turkeys 15-18 wks Toms 14-16 wks Hens	Swine 5-10 kg	Swine 50-110 kg
Crude Protein,%[2]	15	23	20	18	28	14	20	13
Histidine, %	.17	.35	.32	.27	.58	.20	.31	.18
Isoleucine, %	.65	.80	.73	.62	1.1	.45	.65	.38
Leucine, %	.82	1.2	1.09	.93	1.9	.8	.85	.50
Lysine, %	.69	1.10	1.00	.85	1.6	.65	1.15	.60
Methionine+Cystine,%	.58	.90	.72	.60	1.05	.45	.58	.34
PHE+TYR, %	.83	1.34	1.22	1.04	1.80	.90	.94	.55
Threonine, %	.47	.80	.74	.68	1.0	.5	.68	.40
Tryptophan, %	.16	.20	.18	.16	.26	.13	.17	.10
Valine, %	.70	.90	.82	.70	1.2	.60	.68	.40
Linoleic Acid, %	1.0	1.0	1.0	1.0	1.0	.8	.1	.1
M.E.[3],kcal/kg	2900	3200	3200	3200	2800	3300	3240	3275

[1]Assumes 100 g/d intake.

[2]Not required per se, but needed to supply amino nitrogen for synthesis of nonessential amino acids.

[3]M.E. = Metabolizable energy, These values are typical dietary concentrations which may vary due to dietary formulations.

Fig. 1 Corn Use By Species (1970-1984)
(adapted: FAPRI, 1995 and Anon. 1985)

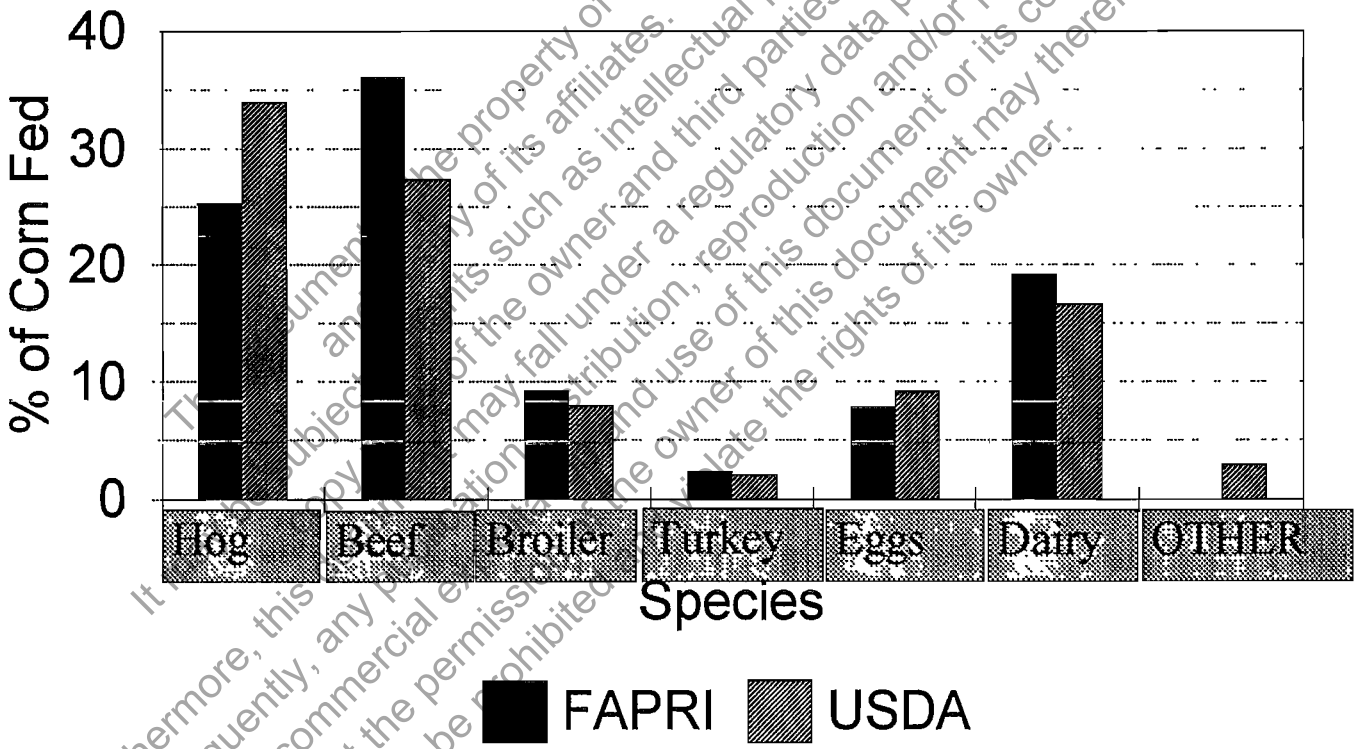


Fig. 2 Livestock Corn Consumption

(adapted from Adams and Brown, 1991)

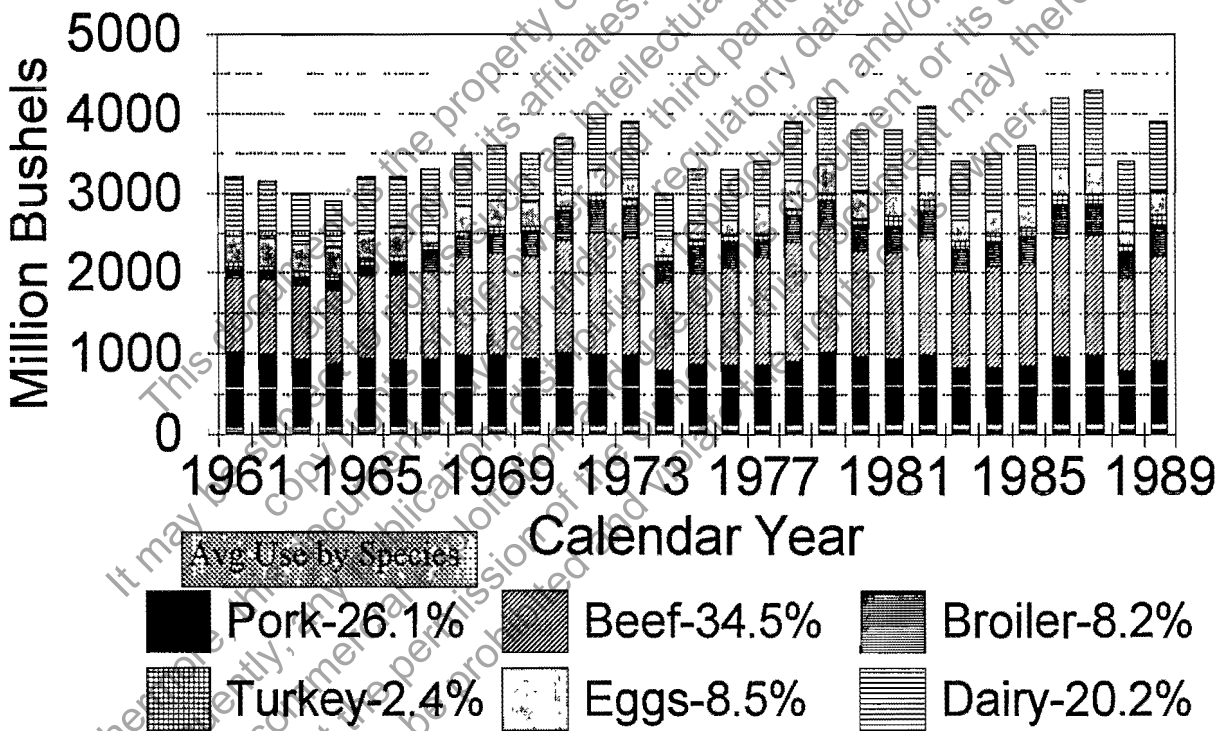
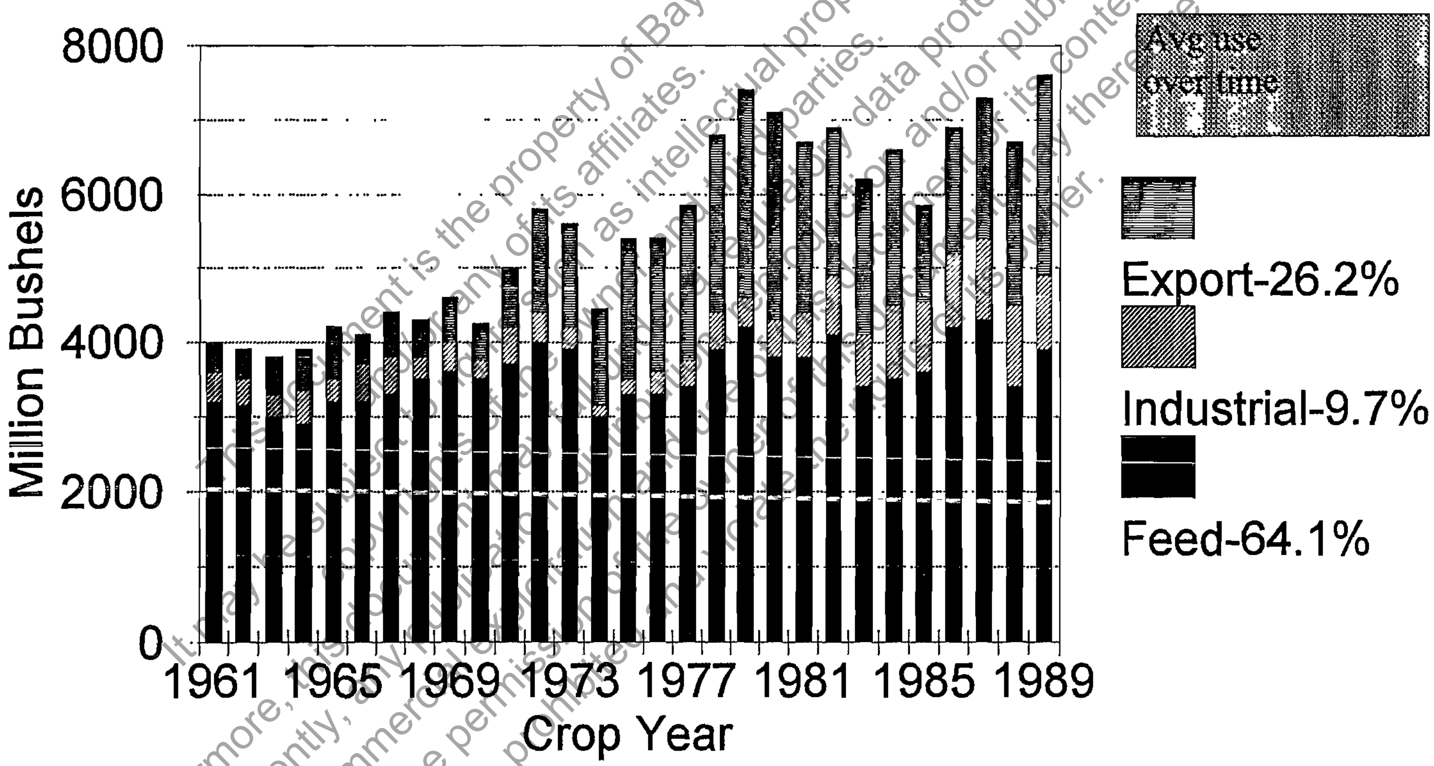


Fig. 3 Corn Utilization

(Adams and Brown, 1991)



References

AAFCO. 1994. Official Publication of the American Association of Feed Control Officials.

Adams, G.M., and D.S. Brown. 1991. Analysis of the link between aggregate livestock production and corn feed use. Proceedings of the Southern Agricultural economics Association. Dallas, Texas.

Anderson, R.A. and S.A. Watson. 1982. The Corn Milling Industry. In CRC Handbook of Processing and Utilization in Agriculture, Vol. II Part 1. Plant Products. I.A. Wolff ed pp. 31-61.

Anonymous. 1974. Livestock-Feed Relationships National and State. Stat. Bull. No. 530. Economic Research Service. U.S. Department of Agriculture. U.S. Government Printing Office. Washington, DC.

Anonymous. 1982. Census of Manufactures. Grain Mill Products. MC82-I-20D. Bureau of the Census. Department of Commerce. U.S. Government Printing Office. Washington, DC.

Anonymous. 1985. Feed Outlook and Situation Yearbook. FdS-298. Economic Research Service. U.S. Department of Agriculture. U.S. Government Printing Office. Washington, DC.

Anonymous. 1988. Official United States Standards for Grain United States Standards for Corn. Federal Grain Inspection Service, U.S. Department of Agriculture. U.S. Government Printing Office. Washington, DC.

Anonymous. 1992a. Agricultural Statistics. U.S. Department of Agriculture. U.S. Government Printing Office. Washington, DC.

Anonymous. 1992b. Feed Grain Facts. U.S. Feed Grains Council. Washington, DC.

Anonymous. 1992c. Crop Production. Economic Research Service. U.S. Department of Agriculture. U.S. Government Printing Office. Washington, DC.

Anonymous. 1994. Crop Production. Economic Research Service. U.S. Department of Agriculture. U.S. Government Printing Office. Washington, DC.

Bailey, K. 1995. 1993 Dairy Enterprise Business Earnings and Costs with Projections for 1995. FM95-1. University of Missouri, Columbia.

Balnave, D. 1970. Essential Fatty Acids in Poultry Nutrition. World's Poult. Sci. J. 26:442.

FAPRI. 1995. FAPRI 1995 Baseline Briefing Paper. Center for National Food and Agricultural Policy, University of Missouri and The Center for Agricultural and Rural Development, Iowa State University, CNFAP #4-95.

Fonnesbeck,P.V., and H. Lloyd. 1984. IFI Table of Feed Composition. International Feedstuffs Institute, Utah Agriculture Experiment Station, Logan.

Guzman, R.E. and S.W. Casteel. 1994. Fumonisin mycotoxins: Their origins and effects on livestock. The Professional Animal Scientist 10:124.

Johnston, L.J. 1994. Effect of harvest year, moisture content and bulk density of corn of performance of growing-finishing pigs. J. Anim. Sci. 72:(Suppl.2) 57.

Newcomb, M.D. 1994. Pelleting-What are the advantages. Mizzou Pork Pages. University of Missouri Quarterly Swine Management Newsletter.

NRC. 1982. United States-Canadian Tables of Feed Composition (3rd Ed). National Academy Press. Washington, DC.

NRC. 1984. Nutrient Requirements of Beef Cattle (6th Ed). National Academy Press. Washington, DC.

NRC. 1988a. Nutrient Requirements of Dairy Cattle (6th Ed). National Academy Press. Washington, DC.

NRC. 1988b. Nutrient Requirements of Swine (9th Ed). National Academy Press. Washington, DC.

NRC. 1994. Nutrient Requirements of Poultry (9th Ed). National Academy Press. Washington, DC.

Nocek, J.E, and J.B. Russell. 1988. Protein and Energy as an Integrated System. Relationship of Ruminal Protein and Carbohydrate Availability to Microbial Synthesis and Milk Production. J. Dairy Sci. 71: 2070.

Plain, R. 1993a. 1992 Missouri Hog Returns. Farm Management Newsletter. FM93-5. University of Missouri, Columbia.

Plain, R. 1993b. 1992 Missouri MIR Cow-Calf Returns. Farm Management Newsletter. FM93-4. University of Missouri, Columbia.

Shroder, J.D. and V. Heimen. 1970. Feed products from corn processing. In Corn:

Culture, Processing, Products. G.E. Inglett ed. Chapter 12.

Sprague, C.F. and J.W. Dudley eds.1988. Production of Hybrid Seed Corn. In Corn and Corn Improvement. Am. Soc. Of Agronomy, Crop Science Soc. of America, Soil Science of America. Madison, Wisconsin.

Weigel, J.1994. The Wet Milling Industry. In Alternative feeds for dairy and beef cattle. E.R. Jordan ed. Proceeding of the National Invitational Symposium. Texas Agricultural Extension Service.

Zuber, M.S., E.B. Lillejoj, and B.L. Renfro, eds. 1987. Aflatoxin in Maize: A Proceedings of the Workshop. CIMMYT, Mexico, D.F.

APPENDIX IV

Summary of the Materials and Methods for the Proximate Analysis, Amino Acid Composition, Fatty Acid Analyses, and Quantification of Inorganic Components of Grain and Silage Samples.

MATERIALS AND METHODS

A. Test System

The panel of analytical biochemical methods employed in this study was considered the test system. These are standard published analytical methods which are currently used to evaluate nutritional quality and composition parameters in corn for commercial purposes.

B. Proximate Analysis of Grain and Silage Samples

The same methods were used for both grain and silage (green chop) samples except for fat, as described below.

Moisture (M100). The sample was dried in a vacuum oven at 100°C and dried to a constant weight (approximately 5 hours) (AOAC methods 926.08 and 925.09, 1990). The moisture loss was determined gravimetrically. There was no analytical reference substance for these analyses.

Protein (PGEN). Protein and other organic nitrogen in the sample was converted to ammonia by digesting the sample with sulfuric acid containing a mercury catalyst mixture. The acid digest was made alkaline, and the ammonia was distilled and titrated with standard acid. The percent nitrogen was determined and converted to protein using the factor 6.25 (AOAC methods 955.04C and 979.09, 1990; Bradstreet, R.B. 1965; Kalthoff and Sandell, 1948). There was no analytical reference substance for these analyses.

Fat (FAAH). The forage sample was hydrolyzed in a water bath using hydrochloric acid. The fat was extracted using ether and hexane. The extract was washed with a dilute alkali solution and filtered through a sodium sulfate column. The remaining extract was evaporated, dried and weighed (AOAC methods 922.06 and 954.02, 1990). This method was used for the silage samples. There was no analytical reference substance for these analyses.

Fat (FSOX). The grain sample was weighed into a cellulose thimble containing sand or sodium sulfate. The thimble was dried to remove excess moisture. Pentane was dripped through the sample to remove the fat. The extract was evaporated, dried and weighed (AOAC methods 960.39). This method was used for the grain samples. There was no analytical reference substance for these analyses.

Ash (ASHM). Volatile organic matter was driven off when the sample was ignited at 550°C in an electric furnace. The residue was quantitated gravimetrically and calculated to determine percent ash (AOAC method 923.03, 1990). There was no analytical reference substance for this analysis.

Calories (CALC). Calories were calculated using the Atwater factors with the fresh weight-derived data and the following equation (USDA Agricultural Handbook No. 8, 1975):

calories (kcal/100g) = (4 * % protein) + (9 * % fat) + (4 * % carbohydrates)

There was no analytical reference substance for these analyses.

Carbohydrates (CHO). Carbohydrates were calculated by difference using the fresh weight-derived data and the following equation (USDA Agricultural Handbook No. 8, 1975):

% carbohydrates = 100% - (% protein + % fat + % ash + % moisture)

There was no analytical reference substance for these analyses.

Crude Fiber (CFIB). Crude fiber is the loss on ignition of dried residue remaining after digestion of the samples with 1.25% sulfuric acid and 1.25% sodium hydroxide solutions under specfic conditions (AOAC method 962.09, 1990). There was no reference substance for this method.

C. Amino Acid Composition (TAAP)

Grain samples were hydrolyzed with hydrochloric acid, and adjusted to pH 2.2. The individual amino acids were quantitated using an automated amino acid analyzer. This assay was based on previously published references (AOAC method 982.30, 1990). The reference substances used for these analyses were: K18 (Beckman, lot #A304008), L-Tryptophan (Sigma Chemical, lot #60H0635 and 52H0717), Cysteic Acid Monohydrate (Sigma Chemical, lot #50H2616), Methionine Sulfone (Sigma Chemical, lot #49F0113).

D. Fatty Acid Analyses (FAC)

The lipid in the grain samples was extracted, saponified with 0.5N sodium hydroxide in methanol, and methylated with 14% boron trifluoride:methanol. The resulting methyl esters were extracted with heptane containing an internal standard. The methyl esters of the fatty acids were analyzed by gas chromatography using external standards for quantitation (AOCS method Ce 1-62, 1981). The reference substances are listed in the study data files.

E. Inorganic Components

Calcium (CAA). The grain and silage samples were dried, pre-charred, and ashed overnight at 500° to 550°C. The samples were treated with nitric acid, dried, reashed and solubilized in 4% hydrochloric acid. The amount of calcium was determined at a wavelength of 422.7 nm by comparison of test sample to the signal of the standard solution. All solutions contain 1% lanthanum and 5% hydrochloric acid (AOAC method 965.09, 968.08 and 985.35). The reference substance for this method was 1000 ppm calcium solution (Fisher, lot #940982-24).

Phosphorus (PTA). The grain and silage samples were dried, pre-charred, and ashed overnight at 500° to 550°C. The samples were treated with nitric acid, dried, reashed and solubilized in 4% hydrochloric acid. The amount of phosphorus was determined colorimetrically at a wavelength of 420 nm by comparison of test sample to the signal of the standard solution, each reacted with molybdovanadate solution (AOAC method 965.17 and 962.11). The reference substance for this method was 10,000 ppm phosphorus solution (SPEX, lot #E-87P).

Literature Cited

AOAC Method 982.30. 1990. *In* Official Methods of Analysis, 15th Edition. Association of Official Analytical Chemists, Arlington, Virginia, modified.

AOAC Method 965.17, 962.11. 1990. *In* Official Methods of Analysis, 15th Edition. Association of Official Analytical Chemists, Arlington, Virginia, modified.

AOAC Method 965.09, 968.08, 985.35. 1990. *In* Official Methods of Analysis, 15th Edition. Association of Official Analytical Chemists, Arlington, Virginia, modified.

AOAC Method 962.09. 1990. *In* Official Methods of Analysis, 15th Edition. Association of Official Analytical Chemists, Arlington, Virginia, modified.

AOAC Method 960.39. 1990. *In* Official Methods of Analysis, 15th Edition. Association of Official Analytical Chemists, Arlington, Virginia, modified.

AOAC Method 955.04C, 979.09. 1990. *In* Official Methods of Analysis, 15th Edition. Association of Official Analytical Chemists, Arlington, Virginia, modified.

AOAC Method 926.08, 925.09. 1990. *In* Official Methods of Analysis, 15th Edition. Association of Official Analytical Chemists, Arlington, Virginia, modified.

AOAC Method 923.03. 1990. *In* Official Methods of Analysis, 15th Edition. Association of Official Analytical Chemists, Arlington, Virginia, modified.

AOAC Method 922.06, 954.02. 1990. *In* Official Methods of Analysis, 15th Edition. Association of Official Analytical Chemists, Arlington, Virginia, modified.

Bradstreet, R.B. 1965. The Kjelhahl Method for Organic Nitrogen, Academic Press, New York, New York, modified.

Determination of High Performance Liquid Chromatography. 1988. *Journal of the Association of Official Analytical Chemists* 71(1), methods 26.052-26.060, modified.

Determination by One Dimensional Thin Layer Chromatography. 1988. *Journal of the Association of Official Analytical Chemists* 71(1), method 26.031, modified.

Determination by Two Dimensional Thin Layer Chromatography. 1988. *Journal of the Association of Official Analytical Chemists* 71(1), method 26.074, modified.

[redacted]. 1995. 1994 Large Scale Production of European Corn Borer Resistant and Control Corn Plants. Monsanto Technical Report MSL-14271, St. Louis.

Kalthoff, I.M. and Sandell, E.B. 1948. Quantitative Inorganic Analysis, MacMillan, New York, modified.

Official and Tentative Methods of the American Oil Chemists Society. 1981. Method Ce 1-62, American Oil Chemists Society, Champaign, Illinois, modified.

Proceeding of the 3rd International Congress of Food Science and Technology, 1979. Pages 705-711, modified.

SAS Institute Inc. 1990. SAS/STAT® User's Guide, Version 6, Fourth Edition, Volumes 1 and 2; SAS Procedures Guide®, Version 6, Third Edition; Cary NC.

USDA Agriculture Handbook No. 8. 1975. Composition of Foods. *In* Agricultural Hadnbook No. 8. United States Department of Agriculture, Washington, D.C. pp. 159-165.