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**Nutritional Impact Assessment Report on
Glufosinate-Tolerant Field Maize
Transformation Event T25**

Code

LLMaize T25

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COMPANY NAMES

On June 3, 2002, Bayer CropScience was formed by the completion of the acquisition of Aventis CropScience S.A. by Bayer AG. From this date, Bayer CropScience is an agricultural business unit of Bayer that is engaged in the research, development and marketing of crop protection products, crop production products and seeds.

The major part of the activities described in this report were undertaken before the acquisition. Consequently, the name of Aventis CropScience GmbH may appear throughout the report and the appendices of this report. However, all inquiries regarding this report and the data contained herein should now be addressed to Bayer CropScience GmbH, Industrial Park Hoechst, D-65926 Frankfurt am Main, Germany.

This report has been approved by the Bayer CropScience management under which the study was brought to completion.



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ACRONYMS AND TECHNICAL TERMS

ALA - Alanine
ARG - Arginine
ASN - Asparagine
ASP - Aspartic Acid
CYS - Cystine
ELISA - Enzyme Linked Immunosorbent Assay
EPA - Environmental Protection Agency
FDA - Food and Drug Administration (Department of the USA)
GLP - Good Laboratory Practice
GLN - Glutamine
GLU - Glutamic Acid
GLY - Glycine
GMO - genetically modified organism
HIS - Histidine
ILE - Isoleucine
LEU - Leucine
Liberty[®] - Trade name of the Glufosinate-ammonium herbicide
LL - LibertyLink[®]
LYS - Lysine
MET - Methionine
PAT - Phosphinothricin-N-acetyltransferase
PHE - Phenylalanine
ppm - parts per million
PRO - Proline
SER - Serine
THR - Threonine
TRY - Tryptophan
TYR - Tyrosine
USA - United States of America
USDA - United States Department of Agriculture
VAL - Valine
%fw - percent fresh weight
%dm - percent dry matter



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SUMMARY

An important component in the safety assessment of foods and food ingredients derived from genetically modified (transgenic) plants is an evaluation of *Substantial Equivalence*. The concept of *Substantial Equivalence* embodies a science-based approach, in which a genetically modified plant, from which food is derived is compared with its existing, appropriate non-genetically modified counterpart. The goal of this comparison is to ensure that food, or feed derived from the genetically modified plants will be compositionally and nutritionally equivalent to that derived from existing non-genetically modified plants and as safe as food or feed derived from its traditional counterpart.

Substantial Equivalence is assessed using data generated from measurements of plant agronomic and phenotypic parameters, nutritional analyses of the raw plant commodity, and animal feeding tests.

Evaluations were conducted to determine, if LibertyLink[®] (LL) Maize transformation event T25 is compositionally and nutritionally equivalent to its non-transgenic counterpart and to other varieties of maize. In this context the agronomic performance of the plants and the composition of the raw commodity maize grain was determined and compared.

Components selected for compositional and nutritional analyses comprise the important, basic nutrients of maize. These are the proximates (moisture, protein, fat, ash, carbohydrates, fibre), the micro-nutrients, such as vitamins and minerals, the anti-nutrient phytic acid, total and free amino acids, and finally total and free fatty acids.

Compositional and nutritional analyses were performed using the raw agricultural commodity grain generated from 15 field trial sites in two different years. This provided a robust data set for a sound statistical evaluation, to establish compositional equivalence of LLMaize event T25 grain and its non-transgenic counterpart.

The statistical evaluation of the analytical data from the grain samples was performed based on a 95% confidence interval and a 20% bio-equivalence range. A range of 20%, which should meet most of the natural variation ranges for the measured components, was suggested in a Report of the TemaNord in 1998 (TemaNord, 1998). The pharmaceutical industry works also with a range of $\pm 20\%$ for the relative treatment difference between product averages as standard equivalence criterion, as laid down in the "Guidance for Industry Concerning Statistical Procedures for Bio-equivalence Studies Using a Standard Two Treatment Crossover Design" by the FDA (FDA, Div. of Bio-equivalence, Office of Generic Drugs, 1997). For the statistical evaluation in this report the same criterion for equivalence of the three "treatment" groups, non-transgenic, transgenic treated with Liberty[®] and transgenic not treated with Liberty[®] was used.

An analysis of variance model with the two factors "site" and "treatment" was conducted with all the data. The interaction between "site" and "treatment" was included in the model in order to determine, if there are interactions between the two factors. For many components interactions were observed.

For most of the proximates, all of the total amino acids, and most fatty acids the comparison between the non-transgenic and the two transgenic treatment groups resulted in a

determination of bio-equivalence. In some cases bio-equivalence between the different treatments was seen at each of the 15 sites.

For the minerals, vitamins, free amino acids, and free fatty acids the evaluation of the data coming from the same site often showed significant differences between these components determined in the transgenic and non-transgenic maize grain.

An additional analysis compared the measured nutritional components against the range of values for maize reported in the cereal chemistry reference guides. Most values are inside the range built by literature. In case there were found slight deviations from the literature range or value, this was observed for both the non-transgenic control group and the two transgenic groups, so it is not caused by the genetic transformation.

The comparison of the measured nutritional components with ranges reported from literature leads to the conclusion: the composition tables in literature show similar values for other maize varieties. There are no significant differences between the LLMaize T25 grains samples and grains from commercial maize varieties currently on the market.

Although statistically significant differences (i.e. non bio-equivalences) were seen between certain components of the non-transgenic and transgenic samples, the differences do not affect a determination that LLMaize event T25 and its non-transgenic counterpart are *substantially equivalent*. The observed differences have no effect on this conclusion, because

- 1) no clear tendency of minor or major findings was observed
- 2) differences were not found in all comparisons (for instance, if differences were observed between the non-transgenic and transgenic Liberty[®] treated sample group, they were not seen between the non-transgenic and the not Liberty[®] treated transgenic sample group)
- 3) the difference was even found between the two transgenic treatment groups
- 4) the difference was not confirmed by the over-all-sites analysis
- 5) the values of all analysed components were inside the literature range
- 6) the detected statistical difference has no nutritional relevance

The observation of the agronomic characters showed that the transgenic LLMaize event T25 plants have the same agronomic behaviour and performance as their non-transgenic counterparts. Although differences were observed, they were not found at all sites and no uniform tendency was seen.

These data and findings lead Bayer CropScience to conclude that LibertyLink[®] Maize event T25 is compositionally and nutritionally equivalent to its non-transgenic counterpart and to current commercial maize varieties. Thus it can be stated that there is “no concern” for the nutritional value of LibertyLink[®] Maize transformation event T25 and its progeny.

1 THE CONCEPT FOR THE SAFETY ASSESSMENT OF GENETICALLY MODIFIED ORGANISMS USED FOR HUMAN FOOD AND ANIMAL FEED

The difficulties of applying traditional toxicological testing and risk assessment procedures to whole foods, meant that an alternative approach was required for the safety assessment of genetically modified foods. Expert consultations convened by FAO/WHO and OECD have recommended that *Substantial Equivalence* be an important component in the safety assessment of foods and food ingredients derived from genetically modified plants intended for human consumption (OECD, 1993; FAO 1996). This concept embodies a science-based approach, in which a genetically modified food is compared with its existing, appropriate counterpart. The approach is not intended to establish absolute safety, which is an unattainable goal for any food. Rather, the goal of this approach is to ensure that the food, and any substances that have been introduced into the food as a result of genetic modification, is as safe as its traditional counterpart. (WHO, 2000)

The determination of the *Substantial Equivalence* between a new transgenic plant or processed product and its traditional counterpart is based on data generated from measurements of agronomic and phenotypic parameters, nutritional analyses and animal feeding tests.

Substantial Equivalence is established, if the levels and variations of the characteristics of the genetically modified organism (GMO) are within the natural range of variation for the respective characteristics in the comparator. A value range of 20% was suggested that should meet most of the natural variation ranges for the measured compounds (TemaNord, 1998). In cases where the average value of the parameter in the GMO differs more than 20% of the average value of the parameter in the traditional comparator, further explanation and evaluation must be made. In these cases bio-equivalence between certain parameters of the GMO and its traditional comparator is not achieved.

The parts, commodities and food or feed products of the GM-crop, which should be analysed, are selected according to their role and importance in human and animal diet. The compositional analysis focuses on the content of critical nutrients and anti-nutrients in the respective plant part, crop commodity, and food or feed product.

To compensate for the potential effect of the environment on the composition of the GM plant, samples from different trial locations have to be taken and analysed. The environmental effect within a single site is minimised by replication of each treatment and application of an appropriate field trial design (randomised block trial design). The agronomic practices followed in growing the GM-plants and their traditional counterparts must be absolutely identical except for a possible treatment with the corresponding herbicide for herbicide-tolerant GM-plants.

The data from each site is evaluated separately and followed by an evaluation over all sites and a comparison with standard values from literature.

A statistical analysis of the results using a 95% confidence interval criterion (MAFF and DH, 1999), is performed to assess the *Substantial Equivalence* of the plants.



Possible results from an evaluation of *Substantial Equivalence* might be:

- 1) the GM crop/food is found to be *substantially equivalent* to its traditional counterpart, which means the crop or food derived from the crop is regarded to be as safe and nutritious as its conventional non-transgenic counterpart,
- 2) the GM crop/food is found to be *substantially equivalent* except for a few clearly defined differences, which are not attributed to the genetic modification, or
- 3) the GM crop/food is not found to be *substantially equivalent* to a conventional non-transgenic counterpart either, because the differences are significant and cannot be defined, which means they may be due to the genetic modification, or, because there is no existing counterpart to compare it with.

2 MAIZE COMMODITIES AND PROCESSING

The economical advantage of maize consists of its different harvested commodities and their manifold uses. Green maize, silo maize, grain maize, maize grain/cob mixes and maize hay are the different products that can be harvested from a maize plant. In warmer climates, the commodity demand increases in order of green maize, silo maize and grain maize. Therefore the cultivation of green maize and silo maize plays a more important role in the industrial countries with cooler climate (France, Germany etc.).

These latter two commodities and the maize hay are used only for animal feed. The silo maize is often mixed with other crops, for instance soybean, fodder beets or by-products from the soybean and sugar beet processing to increase the protein content and digestibility.

The grain maize is used in animal feed, in the human diet usually after processing, in the dry milling, wet milling, brewing and the oil processing industry and for other industrial purposes. In the developing countries human nutrition is usually in the forefront.

Maize is an important crop in human and animal nutrition, because of its high levels of starch, protein, oil and other nutritionally valuable substances that will be described in the further chapters of this report. Maize is considered a basic food in Latin America, South America and parts of Africa and Asia. (Ramstad and Watson, 1999)

3 MAIZE COMPOSITION AND NUTRITION

3.1 Structure and Composition of the Maize Grain

The mature maize grain is composed of three main parts, the bran or pericarp (5-6 %), the endosperm (82-84 %) and the germ (10-12 %).

The endosperm consists of about 90 % starch, 8 % protein, and other, minor compounds. It can be separated into the aleurone layer, a single cell layer lying immediately under the pericarp, and the starchy endosperm. The aleurone itself is rich in proteins and contains a relatively high amount of oil, enzymes and vitamins.

The germ consists of 33% oil, 18% protein, and about 10% of each starch, sugars and ash. Maize grain as well as wheat and millet grains do not have any hulls (Watson, Ramstad, 1999).

There are five general classes of maize - flint, flour, dent, pop, sweet, waxy and pod maize. The maize classes differ from each other in the distribution and location of the horny outer and the floury inner endosperm or in the conversion rate of sugar into starch.

In the horny endosperm the protein matrix surrounding the starch granules is thicker and stays intact during drying, so that the starch granules are compressed into polyhedral shapes. In the floury endosperm the protein shrinks during drying and the starch granules assume a more round shape. The endosperm composition is laid down by genetics.

3.2 Proximates

A percentage breakdown of the proximates shows a relatively homogenous picture for the grains of all seven main cereals: wheat, rye, maize, barley, oat, rice and millet (table 3.2.1). The main fractions are the carbohydrates, followed by the proteins. Cereals are low in crude fat content. The germ is the part of the grain where the highest fat concentration is found. In maize grains more than 80 % of the total fat is located in the germ. This is the reason why maize and wheat germs are processed for their oil.

Table 3.2.1 Nutritional Composition of the Main Cereals
(Belitz, Grosch, 1985, pp 514-535)

| | | Wheat | Rye | Maize | Oat | Barley | Rice | Millet |
|-------------|---|-------|------|-------|------|--------|------|--------|
| | % | | | | | | | |
| Moisture | | 13.2 | 13.7 | 12.5 | 11.7 | 13.0 | 13.1 | 12.1 |
| Protein | | 11.7 | 11.6 | 9.2 | 10.6 | 12.6 | 7.4 | 10.6 |
| Fat | | 2.2 | 1.7 | 3.8 | 2.1 | 5.7 | 2.4 | 4.1 |
| Starch | | 59.2 | 52.4 | 62.6 | 52.2 | 40.1 | 70.4 | 64.4 |
| Other CHO# | | 10.1 | 16.6 | 8.4 | 19.6 | 22.8 | 5.0 | 6.3 |
| Crude fibre | | 2.0 | 2.1 | 2.2 | 1.6 | 1.6 | 0.7 | 1.1 |
| Minerals | | 1.5 | 1.9 | 1.3 | 2.25 | 2.85 | 1.2 | 1.6 |

Carbohydrates

3.2.1 Carbohydrates

The main fraction of the carbohydrates is the starch. Starch is stored in the endosperm and serves as the source of energy in the germination process. Characteristic of each cereal is the shape and size of their starch. Maize starch has a particle size of 15-25 μ and a hexagonal shape. It is yellowish white in colour and has a protein taste (Laureys 1999).

The starch fraction consists of two different glucan polymers, the amylose and the amylopectin. They differ in the kind of linkage between the single glucose molecules. Amylose, which makes up 25-30 % of the starch, is an essentially linear molecule of glucose units linked α -(1 4). Amylopectin, comprising 70-75 % of the starch, is a branched molecule with α -(1 6) branch points and linear regions of α -(1 4) linked glucose units. There are two kinds of maize that have been developed to differ in their starch composition. One is waxy maize, in which nearly the entire starch fraction consists of amylopectine. The other is amylose maize with an amylose fraction of up to 80 % (Watson, Ramstad, 1999).

The non-starch fraction of the carbohydrates is, at 8,4 %, far less than the starch fraction. It consists of 1-3 % sugars, mainly as sucrose, glucose and fructose, and the fibre fraction built of hemicelluloses, pentosanes, celluloses, β -glucanes and glucofructanes. The fibre compounds build up the cell walls of cereal cells (Belitz, Grosch, 1985, pp 514-535).

3.2.2 Proteins

The total protein content of the different cereals is quite similar. Differences are found, however, in the amino acid profile and in the classification of proteins according to their solubility (Belitz and Grosch 1985, pp 514-535).

Lysine and methionine are the low level amino acids in cereals. In maize additionally tryptophan and other sulphur-bearing amino acids besides methionine are only found in low levels. The endosperm contains the gluten protein, a mixture of the protein fractions glutelin and prolamin. The prolamin fraction, called zein in maize, is nearly devoid of the essential amino acids lysine and tryptophan. Breeders have been successful in increasing the lysine levels in maize by increasing the glutelin fraction, called zeanin in maize, at the expense of zein (Watson and Ramstad, 1999).

Maize protein has a biological value of 72 %. That means with 100 g maize protein, 72 g of body protein can be built. The higher the biological value of a special protein, the less of it is needed to maintain the body protein balance (Kofrány, Wirths, 1987).

The total protein can be divided in four main protein fractions. In 1907, T.B. Osborne separated wheat proteins into four fractions of solubility, extracting successively the albumins (water fraction), the globulins (saline fraction) and the prolamins (ethanol fraction). The glutelins remain in the residue but are partially soluble in diluted acid and completely soluble after reduction of the disulfide bonds. Table 3.2.2.1 shows the amount of the four protein classes in cereals (Belitz, Grosch, 1985, pp 514-535).

**Table 3.2.2.1 Amount of the Four Protein Classes in Cereals
(Belitz, Grosch, 1985, pp 514-535)**

| Fraction | | Wheat | Rye | Maize | Oat | Barley | Rice | Millet |
|----------|---|-------|------|-------|------|--------|------|--------|
| | % | | | | | | | |
| Albumin | | 14.7 | 44.4 | 4.0 | 12.1 | 20.2 | 10.8 | 18.2 |
| Globulin | | 7.0 | 10.2 | 2.8 | 8.4 | 11.9 | 9.7 | 6.1 |
| Prolamin | | 32.6 | 20.9 | 47.9 | 25.0 | 14.0 | 2.2 | 33.9 |
| Glutelin | | 45.7 | 24.5 | 45.3 | 54.5 | 53.9 | 77.3 | 41.8 |

The prolamin fraction of cereals like wheat, rye and barley contains proteins that have been implicated as a factor in celiac disease. Celiac disease causes an atrophy of the mucosae of the small intestine, resulting in mal-absorption of food. The wheat prolamin (gliadin) seems to be a major problem in celiac disease; gliadin antibodies are commonly found in the immune complexes associated with this disease (NutraMed, 1999)]. Celiac disease may be cured by changing the diet of affected individuals to other foodstuffs such as rice, millet or maize, which do not contain the kind of prolamin proteins responsible for the disease. Therefore these grains are used in baby food or special diets for celiac patients (NutraMed, 1999).

The enzyme fraction of cereals has one interesting compound, the phytase. This enzyme is responsible for the degradation of phytin, one of the natural anti-nutrients of cereals. The phytase is activated, when the cereals cells are injured or during the germination process. From the nutritional aspect, the enzyme activity is desired, because it prevents mal absorption of calcium and trace elements (see chapter anti-nutrients).

3.2.3 Fat

As mentioned before grains of cereals do not contain large oil amounts. The oil is located in the germ and bran fraction. Wheat and maize germ oil belong to the palmitic acid rich oils, although the main fatty acid in cereal oil is linolic acid.

Maize germ oil is processed out of germs coming from the wet-milling process (starch production) and dry-milling process (maize flour production). The oil is recovered from the germs by pressing and/or extraction with the solvent n-hexane.

The detectable fatty acids in maize oil are palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3) and arachidic acid (C20:0). The linoleic and linolenic fatty acids are essential fatty acids, because mammals do not have the respective enzymes to build the cis-double binding at position C12.

3.2.4 Dietary Fibre

The dietary fibre consists of plant compounds that are indigestible for the human digestive system. It is a very inhomogeneous group of organic components that mostly belong to the carbohydrates. Although fibre cannot be digested, it is not an unnecessary food constituent, because it prevents the development of a number of intestine diseases and diseases of the metabolic pathway.

The dietary fibre content of cereals and products thereof is dependent on the milling grade. The higher the milling grade, the higher the fibre content will be.

Maize grains have a fibre content estimated as crude fibre of 2-3 % based on the dry matter.

Cereal fibre consists of hemicelluloses and celluloses (insoluble fibre) and β -glucanes (soluble fibre) (Wahrburg, 1996).

The crude fibre determination is today replaced by a determination of the neutral detergent and acid detergent fibre. The crude fibre method has been discredited, because it underestimates cell wall content due to hydrolysis of hemicelluloses and some celluloses (Watson and Ramstad, 1999). The neutral detergent fibre is the residue that remains after hydrolysis with neutral-detergent-solution and α -amylase. The acid detergent fibre is the residue obtained after treatment with sulphur-acid-detergent-solution. This procedure solubilises all pentosans and other hemi-celluloses, leaving only cellulose and lignin. Another value for the fibre content of food is the total dietary fibre. This value summarises the NDF compounds plus soluble indigestible polysaccharides, like β -glucane and glucofructane. Unlike whole wheat, in which soluble fibre amounts to 11 % of total fibre, the soluble fibre content of maize grains is negligible (Watson and Ramstad, 1999).

3.2.5 Ash

Ash is the residue that remains after the complete combustion of the organic compounds of a food product. It is a sum parameter for the inorganic compounds, the minerals in food, taking into account that some organic carbon may be bound as carbonate and that some inorganic elements, such as sulphur, selenium, iodine, fluorine, and even sodium and chlorine, may be lost during the high-temperature burning.

The estimation of the ash content in cereals enables the classification of flours. It is the same as for the dietary fibre content: The higher the milling grade, the higher the ash content will be, because the minerals are mainly located in the outer layers of the grain.

3.3 Micro-nutrients

3.3.1 Minerals (McDowell, 1992a,b,c)

Unlike other nutrients, mineral elements cannot be synthesised by living organisms, therefore they are all essential in the human and animal diet. The general functions of minerals are structural components of body organs and tissues, constituents of body fluids and tissues, electrolytes, and catalysts in enzyme and hormone systems. Table 3.3.1.1 shows mineral requirements for humans.

The main minerals in maize are the macro-minerals phosphorus, calcium, potassium, sodium and magnesium and the trace elements iron, manganese and zinc. Maize germ is rich in mineral elements. It contains 78 % of the kernel minerals, probably, because they are essential for early growth of the embryo (Watson and Ramstad, 1999).

In human and animal nutrition **calcium (Ca) and phosphorus (P)** are considered together, because they constitute the major part of the mineral content of bone. Ca represents 46 % and P about 29 % of the total body minerals in animals.

Nonskeletal Ca (1 % of the total body Ca) plays an important role in the blood clotting system, as co-factor in many enzymatic reactions (e.g. ATPase), and in hormone regulation. It is required for muscle contraction, membrane permeability, myocardial function, and normal neuromuscular excitability.

Large amounts of P are present in organic combinations such as phosphoproteins, phospholipids, nucleoproteins and mono-saccharide phosphates. P is involved in almost every metabolic reaction providing energy via fission of high-energy phosphate bonds such as in ATP. Many body buffer systems are based on phosphorus combinations.

Deficiency of Ca and P leads to deformation of legs, spine, pelvic and thorax, the so-called classic bone symptoms. The bones become larger, weak in structure, and bend under the weight of the body. They are likely to fracture.

Phosphorus is stored in phytin, a hexaphosphate ester of inositol (see section 3.3.1 anti-nutrients), which is liberated by phytase enzyme to initiate embryo development.

Animal feed is normally analysed for calcium, phosphorus, potassium and sulphur. For most classes of livestock, the two most important mineral supplements are Ca and P.

Potassium (K) is the third most abundant mineral in the body. Beside sodium and chlorine it is one of the three major electrolytes in the body. K is needed to maintain the cation-anion and the acid-base balance in the organism; it regulates the osmotic pressure. It is the major intracellular cation, whereas sodium is the major extracellular cation. K is also important in the transmission of nerve impulses and in the contractility of the muscles.

The first signs of a K deficiency is the loss of appetite followed by depressed growth, muscular weakness, stiffness and paralysis. Potassium is a daily dietary essential. There are no reserves in contrast to bone storage of Ca and P.

Sodium (Na) and Chlorine (Cl) together with potassium maintain the osmotic pressure and regulate acid-base equilibrium. These electrolytes in body fluids are specifically involved at the cellular level in water metabolism, nutrient uptake and transmission of nerve impulses. Most plants and plant products contain relatively small amounts of Na in comparison to animal products. There is only a small volume of analytical data on Cl content in feedstuffs, but omission of salt from most diets results in a Na deficiency before a Cl deficiency. The initial signs of Na (and Cl) deficiency of animals is a craving for salt, demonstrated by the avid licking of wood, soil and sweat from other animals, and by the drinking of water.

Magnesium (Mg) is an active component especially of the enzymes regulating energy metabolism. It is involved in muscle contraction and the transmission of nerve impulses to the muscles. It is important for the integrity of bones and teeth and is involved in protein synthesis. Most cereal grains are fair sources of Mg, varying from 0.13 to 0.22% dry matter. Deficiencies are uncommon, owing to generally adequate concentrations of the element in foods of monogastric animals and humans.

Iron (Fe) deficiency is one of the most common human deficiency diseases in the world. Fe is present in the blood protein hemoglobin and the muscle protein myoglobin. It is a co-factor for several enzymes such as oxidase, oxygenase and the cytochromes. Fe deficiency leads to a decrease of hemoglobin content and the number of red blood cells (hypochrome anaemia). Signs of a lack of Fe in addition to anaemia and related blood changes, include lower weight gains, listlessness, inability to withstand circulatory strain, laboured breathing after mild exercise, reduced appetite, and decreased resistance to infections.

Manganese (Mn) is the co-factor of the pyruvatcarboxylase and activates together with other 2 valence cations a number of enzymes such as arginase, aminopeptidase, alkaline phosphatase, lecithinase and enolase.

The skeleton is rich in manganese; it is essential for the development of the organic matrix in the bone. Nuts and cereals have higher Mn contents. (Kofrány and Wirths, 1987)

The main manifestations of Mn deficiency are impaired growth, skeletal abnormalities, depressed reproductive function, ataxia of newborn, and defects in lipid and carbohydrate metabolism.

Copper (Cu) is an essential nutrient as well as a toxicant. It is required for cellular respiration, bone formation, proper cardiac function, connective tissue development, myelination of the spinal cord, ceratinisation, and tissue pigmentation. Copper is an essential component of several physiologically important metallo-enzymes, including cytochrome oxidase, lysyl-oxidase, superoxide dismutase, dopamine-beta-hydroxylase and tyrosinase.

The main manifestation of Cu deficiency includes anaemia, diarrhoea, bone disorders, reproductive failure, nerve disorders, cardiovascular disorders, loss of hair pigment, and ceratinisation failure in hair. Considerable variation has been reported in the tolerance by

various species of livestock to chronic Cu toxicosis. The most sensitive to Cu toxicity are the ruminants (Cu content in feed over 20ppm can cause chronic poisoning in sheep), while most non-ruminants have relatively high tolerance for Cu. Chronic Cu toxicity is found in ruminants but not in mono-gastric species and only rarely in humans.

Zinc (Zn) is co-factor or activator of a number of enzymes. It has many biologically significant interactions with hormones. Zn deficiency results in growth retardation, paraceratotic lesions of the skin, disorders of the immune response, dehydrated appearance, diarrhoea and disorder in the vitamin A metabolism. Whole cereal grains are relatively rich in Zn. Most of the Zn is contained in the bran and germ fraction.

Table 3.3.1.1 Requirements of Minerals for Humans

| | Ca | P | K | Na | Cl | Mg | Fe | Mn | Cu | Zn |
|------------------------------|---------|---------|---------|----------|-----------|------------|-------|---------|-------|-------|
| Intake / day | g/d | g/d | g/d | g/d | g/d | g/d | mg/d | mg/d | mg/d | mg/d |
| Adults ^a | 0.8-1.0 | 1.2-1.5 | 2.0-4.0 | 0.5 | | 0.3-0.35 | 10-15 | 2-5 | | 12-15 |
| Infants ^b | - | - | 15-20# | - | - | - | - | - | | - |
| Children ^b | 0.4-0.8 | 0.3-0.8 | 65# | 0.12-0.5 | 0.18-0.75 | 0.04-0.17 | - | 0.3-3.0 | | 5-10 |
| Adults ^b | 0.8-1.2 | 0.8-1.2 | 1.6-2.0 | 0.5 | 0.75 | - | - | 2.0-5.0 | 1.5-3 | 12-15 |
| Lactating ^b | 1.2 | 1.2 | - | - | - | - | - | - | - | 16-19 |
| Adults male ^b | - | - | - | - | - | 0.17-0.35 | 10 | - | - | - |
| Adults female ^b | - | - | - | - | - | 0.28-0.355 | 10-15 | - | - | - |
| Female pregnant ^b | - | - | - | - | - | | 30 | - | - | - |

mg/d

a Wahrburg, 1996

b McDowell 1992

3.3.2 Vitamins

The term vitamins comprises essential organic compounds that the animal organism has to take in with the feed as such or in a form (pro-vitamin), which can be easily transformed to the actual vitamin.

Vitamins are concerning their solubility divided into fat soluble vitamins, these are the vitamins A, E, D and K, and water soluble vitamins (vitamins B1, B2, B6, B12, C, niacin, pantothenic acid, folic acid, biotin).

Cereals, and thus maize, are a rich source for the vitamins B1, B2, niacin and pantothenic acid. Because the vitamins are located mostly in the outer layers of the grain, cereal products from the non milled or unpolished grain are favoured. The oil from cereal germs is a good source of vitamin E.

Vitamin B1 is co-factor of several enzymes especially of decarboxylase- and transketolase reactions in the carbohydrate metabolism. The recommended daily intake is 1,1-1,5 mg/day for an adult. Deficiency diseases are loss of weight, fatigue, depression, muscle weakness and irritability. Beriberi is the name of the vitamin B1 a-avitaminosis.

Vitamin B2 is a constituent of two important co-enzymes of the energy- and protein metabolism. As daily intake 1,5-1,7 mg/day for an adult is recommended. Characteristic signs for a vitamin B2 deficiency are disorders in the mucous membranes of the mouth and tongue, red skin in the area of the eyes, and nose and sensitiveness of the eyes to light. Progressed deficiency leads to depressed growth and anaemia.

Niacin comprises nicotinic acid and nicotinamid. It is a compound of dehydrogenases as NAD and NADP co-enzymes. 15-18 mg/day is the recommended daily intake of niacin. The classic niacin deficiency disease is pellagra, a disease of the skin. It can also lead to diarrhoea and nervous disorders.

Pantothenic acid is part of the co-enzyme A that is important for many reactions of the intermediary metabolism. The daily intake should be about 6 mg/day for an adult. Pantothenic acid deficiency diseases are not known.

Folic acid is involved in the structure of red blood cells and as co-enzyme it is a carrier of C1 bodies in the amino acid metabolism and nucleic acid synthesis (cell division and differentiation). In food, up to 25 % of folic acid is present in its free form and up to 75 % as folate conjugates. Folic acid deficiency results in gastric-intestinal disorders and anaemia. The recommended daily intake of folic acid is 300 µg/day of total folat or 150 µg/g of folat equivalents for adults and 600 µg/day of total folat or 300 µg/day of folat equivalents for pregnant women.

Vitamin E and tocopherols work as anti-oxidative compounds for poly-unsaturated fatty acid in organisms. The supply of vitamin E from food is sufficient, so no deficiency diseases are known. The daily intake of vitamin E should be 12 mg á-tocopherol equivalents/day for an adult (Belitz and Grosch, 1985, pp314-329).

3.4 Anti-nutrients

When compared to other cereals, the anti-nutritional compounds in maize are few. The only two anti-nutrients known are phytic acid and enzyme inhibitors. As already described, the prolamin fraction of maize is free of celiac producing proteins. Mycotoxins can be produced by fungi on maize grown or stored under adverse conditions, however, they are not natural components of maize.

Phytic acid, or inositol hexaphosphoric acid, chelates with calcium, zinc, iron and magnesium in the digestive tract, interfering with absorption and decreasing bio-availability of these nutrients. Phytin is concentrated in the bran fraction. Phytic acid levels in maize grain are typically in the range of 0.5-1.3% based on the dry matter.

Trypsin-inhibitors are proteins with molecular weights between 6-46 kDalton, which form inactive complexes with the proteinase trypsin. They are typical anti-nutritional compounds in soybeans, cereals and potatoes. Proteinase inhibitors are of particular significance in animal nutrition, causing growth depression and pancreatic hypertrophy. Many studies report qualitative rather than quantitative data regarding trypsin-inhibitor levels and activity.

The levels of the trypsin- and chymotrypsin-inhibitors in maize grains are very low and they are subject to heat denaturation (Watson and Ramstad, 1999; Oberdörfer, 2002).

3.5 Standard Composition Tables from Literature for Maize

The tables 1-8 in the appendix A show the compositional tables from literature for maize grain.

4 COMPOSITIONAL AND NUTRITIONAL ANALYSES

4.1 Selection of Parameters of Interest to Analyse

The components selected for compositional and nutritional analyses for the assessment of *Substantial Equivalence*, comprised the important, basic nutrients that were described under section 3 "Maize Composition and Nutrition". These are the proximates, the amino acids, the fatty acids, the micro-nutrients, such as minerals and vitamins, and the anti-nutrient phytic acid. Maize grain samples were not tested for their trypsin inhibition activity since previous studies showed that the activity in conventional maize varieties tested is very low (Oberdörfer, 2002).

4.2 Field Trial Design for the Production of Maize Grains

Grain samples of transgenic LLMaize event T25 for compositional analyses were generated from 15 different field trials performed in 1999 and 2000. The trials were located throughout the main growing areas of the EU northern and southern zone in order to cover different soil and climatic conditions. Table 4.2.1 identifies the trial locations, field trial numbers and their associated studies.

Table 4.2.1 Source of LLMaize Event T25 Grains Samples for Compositional Analyses (Field Locations, Trial Number, Study Designations and Varieties Sown)

| Country, Region, Year | Field Trial number | Study number | Varieties (Non GMO / GMO) |
|------------------------------------|-----------------------|--------------|---------------------------|
| France, La Vienne, 1999 | HP 99 FRA M01 DPP1 | 01 B 005 | Cecilia / LL Moldova |
| France, Le Maine-et-Loire, 1999 | HP 99 FRA M01 CFY1 | 01 B 005 | Torino / LL Kingston |
| France, Pyrénées-Atlantiques, 1999 | HP 99 FRA M01 PRO1 | 01 B 005 | Cecilia / LL Moldova |
| Germany, Bayern, 2000 | NI 00 EUR 01 DEU 0301 | 01 B 006 | Torino / LL Kingston |
| Germany, NRW, 2000 | NI 00 EUR 01 DEU 0501 | 01 B 006 | Torino / LL Kingston |
| Germany, Sachsen, 2000 | NI 00 EUR 01 DEU 0601 | 01 B 006 | Torino / LL Kingston |
| Germany, Niedersachsen, 2000 | NI 00 EUR 01 DEU 0701 | 01 B 006 | Torino / LL Kingston |
| Spain, Valencia, 2000 | NI 00 EUR 01 ESP 0101 | 01 B 006 | Anjou 400 / LL Anjou 400 |
| Spain, Valencia, 2000 | NI 00 EUR 01 ESP 0102 | 01 B 006 | Anjou 400 / LL Anjou 400 |
| Spain, Andalucia, 2000 | NI 00 EUR 01 ESP 0201 | 01 B 006 | Anjou 400 / LL Anjou 400 |
| Spain, Andalucia, 2000 | NI 00 EUR 01 ESP 0202 | 01 B 006 | Anjou 400 / LL Anjou 400 |
| France, L'Indre-et-Loire, 2000 | NI 00 EUR 02 FRA 0101 | 01 B 006 | Torino / LL Kingston |
| France, L'Indre-et-Loire, 2000 | NI 00 EUR 02 FRA 0201 | 01 B 006 | Torino / LL Kingston |
| France, La Haute Garonne, 2000 | NI 00 EUR 02 FRA 0301 | 01 B 006 | Anjou 400 / LL Anjou 400 |
| France, La Haute Garonne, 2000 | NI 00 EUR 02 FRA 0401 | 01 B 006 | Anjou 400 / LL Anjou 400 |

The field trials selected to supply material for study 01 B 005 were part of a Liberty[®] Herbicide, LL Maize event T25 promotion in France. The original objective of the trials was to compare the efficacy of Liberty[®] alone and within a mixture of other herbicides on different weeds in maize.

The underlying trial designs were randomised, complete block with respect to the transgenic plots and consisted of 12 treatment scenarios with a fourfold replication resulting in 48 plots. The size of the plots was 30-32 qm with a length of 10m and a width of 3-3,2 m. Maize seeds of the non-transgenic varieties Cecilia and Torino and of the transgenic varieties LL Moldova and LL Kingstone were planted in 4 rows per plot. The material used in study 01 B 005 came from plots of the treatments designated 4, 7 and 12.

The plants from the plots of treatment 4 were treated with glufosinate-ammonium on two different application dates. The first application was made at the growth stage 13-14 (3-4 leaves unfolded) with a dosage of 2,25 L/ha (450 g active ingredient). The second application, with the same dosage, was made at the growth stage 16-19 (6-9 leaves unfolded).

Grain from the 2 centre rows of each plot was harvested at maturity. Further field trial details are reported in study 01 B 005.

The field trial design used in study 01 B006 was a randomised, complete block design that enabled compensating for internal environmental effects at each trial site. The field trials were sowed and cultivated specifically for the studies NI 00 EUR 01 and NI 00 EUR 02 (GLP field trial parts of study 01 B 006).

Each trial consisted of 3 plots of non-transgenic maize plants, conventionally treated, 3 plots of transgenic maize plants, conventionally treated and 3 plots of transgenic maize plants treated with glufosinate-ammonium, (Liberty[®] Herbicide). The plot size was 60-66 qm with a length of 10m and a width of 6-6,6m. Maize seed of the non-transgenic varieties Anjou 400 and Torino and of the transgenic varieties LL Anjou 400 and LL Kingstone were planted in 8 rows per plot.

The first glufosinate-ammonium application was made at the growth stage 13-14 (3-4 leaves unfolded) with a dosage of 2,25 L/ha (450 g active ingredient). The second application, with the same dosage, was made at the growth stage 16-18 (6-8 leaves unfolded). Grain from the centre rows of each plot was harvested at maturity. Further field trial details are reported in study 01 B 006.

In the trials all plots were planted and cultivated under the same conditions except for the possible glufosinate-ammonium treatment. To compensate the environmental effects inside a single location, replication at each site is necessary. Replicate in this report means harvesting samples from replicated plots of a single treatment.

4.3 Agronomic Performance of LLMaize Event T25 Plants

The observation of the agronomic performance of LLMaize T25 plants was done at 4 different field trials in France. The evaluation of the results has to be done site by site, because the varieties Torino and LL Kingston, more adapted to the North European climate, were sown in the Northern region of France and the 400 and LL Anjou 400, more adapted to the South European climate, were sown Southern France.

For the characters first leaf shape of tip, time of silk emergence, anthocyan coloration of silks, susceptibility to pests and diseases, plant height, diameter of ears, shape of ears, type of grain, anthocyan coloration of glumes and colour of top of grain no differences were seen between the LLMaize event T25 plants and the non-transgenic plants at any trial site.

In the trials FRA 0101 and FRA 0201 the time of anthesis of the non-transgenic plants was slightly earlier (up to 5 days maximum). In these two trials the number of plants in the plots with the conventional maize was higher. For the other two trials this deviation was not observed. Another character that was observed to be different in the four trials was the yield of the two centre rows of each plot. In the trial FRA 0101 the yield of the conventional plot was slightly higher compared with the transgenic plots, whereas in the trial FRA 0401 the transgenic plots had a slightly higher yield. The yield results of the other two trials are in good correspondence.

The ear length of the maize plants from the conventional plots of the trials FRA 0101 and FRA 0201 were a little bit larger than their transgenic counterparts. Again this difference could not be observed in the other two trials.

Since for most characters good correspondence between the LLMaize event T25 plants and their conventional counterparts was found and since an observed difference could not be stated at all sites and, where there was a deviation, it was not uniform at all sites (at one site a larger value and at the other site a lower value) the result of the comparison of the agronomic characters in the context of this study is that the transgenic LLMaize event T25 plants show the same agronomic behaviour and performance as their conventional non-transgenic counterparts.

Table 1 of appendix D summarises the results of the agronomic characters comparison.

4.4 Methodology for Compositional Analyses

Compositional analyses were performed by Institut Fresenius and its sub-contractors Ansynth Service B.V., BLS-Analytik GmbH and VLFS. The methods used and performing laboratory for each compound are listed in table 4.4.1.

Table 4.4.1 Methods Used for Analyses

| Parameter | Laboratory | Method |
|------------------------------------|--------------------|---|
| Moisture | Institut Fresenius | LMBG §35 16.01 1 |
| Total fat | Institut Fresenius | LMBG §35 17.00 4 |
| Protein | Institut Fresenius | LMBG §35 17.00 15 |
| Total dietary fibre | Institut Fresenius | LMBG §35 00.00 18 |
| Ash | Institut Fresenius | AOAC 923.03 / 32.1.05 |
| Total carbohydrates | Calculated | - |
| Available carbohydrates | Calculated | - |
| Minerals-extraction | Institut Fresenius | AOAC 984.27/50.1.15 - ICP* LMBG §35 00.00 19/1 |
| Ca, P, K, Mg, Na, Fe, Mn, Zn | Institut Fresenius | AOAC 984.27/50.1.15 - ICP* DIN EN ISO 11885 |
| Cu | Institut Fresenius | AOAC 984.27/50.1.15 - ICP* EPA method 7211 |
| Chloride | Institut Fresenius | AOAC 983.14 / 33.7.09 |
| Vitamin B1 | BLS* VLFS | SLMB 62/6.2.1* AOAC 942.23/45.1.05 |
| Vitamin B2 | BLS* VLFS | SLMB 62/7.2.3* AOAC 940.33/45.2.06 |
| Niacin | BLS* VLFS | AOAC 961.14 / 45.1.10* AOAC 944.13/45.2.04 |
| Pantothenic acid | BLS* VLFS | AOAC 945.74 / 45.2.05 |
| Folic acid | VLFS | SLMB 62/11.2.1* AOAC 944.12/45.2.03 |
| Tocopherol / Vitamin E activity | BLS* VLFS | SLMB 62/1.2.1* LMBG §35 49.00 5 |
| Phytic acid | Institut Fresenius | AOAC 986.11 / 32.5.18 |
| Total amino acids | Ansynth | SOP Ansynth |
| Free amino acids | Ansynth | SOP Ansynth |
| Total fatty acids | Institut Fresenius | DIN EN ISO 5509 |
| Free fatty acids | Institut Fresenius | DIN EN ISO 5509 |

* specification only valid for study 01 B 005 site DPP1

AOAC Association of Official Analytical Chemists
 DIN European Committee for Standardisation
 EPA Environmental Protection Agency
 LMBG Lebensmittel- und Bedarfsgegenstände Gesetz, §35
 amtliche Sammlung von Analysenmethoden
 SLMB Schweizerisches Lebensmittelbuch

4.5 Compositional Analyses of LLMaize Event T25 Grains versus Non-transgenic Maize Grains

The results of the compositional analyses on maize grains are given in the tables 1-15 of appendix B. The design of the studies and origin of the samples were explained under section 4.2. "Trial Design for the Production of the Maize Grains".

Treatment A in the tables stands for the non-transgenic control conventionally treated, which indicates not sprayed with glufosinate-ammonium (Liberty®).

Treatment B is the transgenic LLMaize event T25 conventionally treated, which indicates not sprayed with glufosinate-ammonium (Liberty®) either.

Treatment C is the transgenic LLMaize event T25 treated with glufosinate-ammonium (Liberty®).

4.6 Statistical Evaluation of the Compositional Analyses of Maize Grains

In total 135 samples from 15 sites taken over two years as part of 2 studies were analysed for a maximum of 92 components. The extensive data enabled a sound statistical evaluation, the results of which demonstrate the *Substantial Equivalence* of the LLMaize Event T25 to its non-transgenic counterparts. The evaluation and conclusions reached are presented in this and the following sections of the assessment report.

Samples from the sites DPP1, CFY1 and PRO1 were measured twice and only mean values of the two measurements were considered for further analysis.

The study data were provided by Institut Fresenius in four different data batches. The data were combined and send to an external biometrician (Dipl. mathematician Vera Rattemeyer-Matschurat). It was then transformed into SAS data sets. All further analysis was performed using SAS version 6.12 (WINDOWS 98).

Outlier checks were performed for each component according to the method of Grubbs. Conspicuous values were checked for possible typing errors or - if necessary - analysed again and corrected. None of the values were excluded from the statistical analysis.

In some cases components were not quantifiable, because the values were below the limit of detection. In those cases, calculations were done using the following, substituted values:

| | | | |
|--------------|-----------------|---|----------------|
| Sodium: | < 100 mg/kg dm | → | 99 mg/kg dm |
| Cystine: | < 0,001 g/kg dm | → | 0,0009 g/kg dm |
| Fatty acids: | < 0,10 % | → | 0,09 % |

Equivalence is established, if the levels and variations of the components in the GMO are within the natural range of variation for the respective characteristics in the comparator. A range of 20% was considered acceptable and should meet most of the natural variation ranges for the measured compounds (Report of the TemaNord, 1998). Based on the "Guidance for Industry Concerning Statistical Procedures for Bio-equivalence Studies Using a Standard Two Treatment Crossover Design" by the FDA (FDA, Div of Bio-equivalence, Office of Generic Drugs, 1997), the pharmaceutical industry works also with a range of \pm

20% for the relative treatment difference between product averages as standard equivalence criterion. For the statistical evaluation of the measured data the same criteria for equivalence of the three groups non-transgenic, transgenic treated with Liberty® and transgenic not treated with Liberty® was used.

To obtain more information about the natural variation of maize components, the reference group (non-transgenic plants, not sprayed with Liberty®) was analysed first. For each component and each site mean values (mean), standard deviation (SD) and the coefficient of variance were calculated. The results are summarised in the tables 1a to 1h of the appendix C. If the coefficient of variance is larger than 20%, this means that the standard equivalence criterion might be too strict due to the high natural variation of the non-transgenic material. In case where the same value for all three replicates of the non-transgenic sample were analysed, the coefficient of variance is 0 (standard deviation = 0).

In addition the variance components 'between sites' and 'within sites' were estimated for each component to determine the reason for the observed variance. If different results were found for a component between the replicates of a single site, then the variance was found 'within sites'. If the results for the single sites were different, the variance was found 'between sites'.

In the next step the analysis of equivalence was performed for each component. As recommended by the "Paper for Consideration by the European Commission: statistically valid data to support applications for safety clearance of crop products under EC regulation on novel foods and novel food ingredients 258/97" (MAFF and DH, 1999) the analysis was made first for each site and then over all sites.

An analysis of variance (ANOVA) was calculated with the factors TREAT (treatment), SITE and the interaction term TREAT*SITE. Significant interaction is indicated with $p < 0.05$. Based on the ANOVA, 2-sided confidence intervals (95%) were calculated pair wise for the treatment differences (LSMEANS statement in SAS-procedure PROC MIXED). Two treatments were considered as equivalent, if the 95%-confidence interval of the difference was within $\pm 20\%$ of the mean value of the respective reference treatment (non-transgenic, not sprayed). In some sites a confidence interval could not be calculated for all components (i.e. all measurements for a treatment were equal). These cases were indicated with 'n.e.' (= not estimable) in the summary table (Table 2a appendix C).

For each parameter the mean, standard deviation and number of results for each treatment site by site and over all sites are listed in the tables 2b to 2i of the appendix C. In the last column(s) the outcome of the comparison between non-transgenic samples and transgenic, not glufosinate-ammonium treated (A vs. B), between non-transgenic samples and transgenic, with glufosinate-ammonium treated (A vs. C) and finally between transgenic, not with glufosinate-ammonium treated samples and transgenic, with glufosinate-ammonium treated (B vs. C) per site and over all sites are presented.

In the tables a "yes" is listed, if the two treatments were considered as equivalent. If the 95%-confidence interval of the difference exceeded the 20% of the mean border of the respective reference treatment (non-transgenic, unsprayed), a "no (-)" is marked and if it fell short of the 20% range, a "no (+)" is marked. If the 95%-confidence interval of the difference is below as well as beyond the bio-equivalence range, "no (+-)" is set.

The analysis of equivalence over all sites shows the result of the comparison between the over all sites pooled data for the respective compound in the transgenic samples and the pooled data package for the same compound in the non-transgenic samples.

For each parameter the confidence intervals are graphically displayed in % of mean of the respective reference. On one page the plots for all three comparisons are presented. This

type of presentation makes it easy to assess whether the confidence interval lies within the pre-specified range of $\pm 20\%$ of the reference mean or not. The plots can be found in the figures 1-8 of appendix C.

4.7 Results of the Statistical Evaluation

Proximates (tables 1a, 2b and figure 1 in the appendix C)

In all sites the components are homogenous in the reference group (non-transgenic, not Liberty[®] sprayed), that is, the coefficient of variance is less than 20%. This means that the defined range of 20% as standard equivalence criterion corresponds to the natural variation for the proximates in the non-transgenic material.

Table 4.7.1 gives a summary of the equivalence evaluation for the proximates. Equivalence between the three treatments can be assumed for the components moisture, fat, protein, ash, total and available carbohydrates. For all of these compounds equivalence is stated in most of the 15 comparisons in the site-by-site analysis. In case of fat, total carbohydrates and available carbohydrates equivalence could be stated between all treatments at each of the 15 sites.

Table 4.7.1 Results of the Equivalence Evaluation for Proximates

| Summary Equivalence * | A vs B | | | | A vs C | | | | B vs C | | | |
|-------------------------|--------|--------|--------|----------|--------|--------|--------|----------|--------|--------|--------|----------|
| | Yes | No (+) | No (-) | No (+ -) | Yes | No (+) | No (-) | No (+ -) | Yes | No (+) | No (-) | No (+ -) |
| Moisture | 15 | - | - | - | 14 | - | 1 | 1 | 14 | - | 1 | - |
| Fat | 15 | - | - | - | 15 | - | - | - | 15 | - | - | - |
| Protein | 10 | 2 | 2 | 1 | 11 | 2 | 2 | - | 13 | 1 | 1 | - |
| Total Dietary fibre | 12 | - | 3 | - | 9 | - | 6 | - | 8 | 1 | 6 | - |
| Ash | 12 | 2 | 1 | - | 12 | 2 | 1 | - | 12 | 2 | 1 | - |
| Total carbohydrates | 15 | - | - | - | 15 | - | - | - | 15 | - | - | - |
| Available carbohydrates | 15 | - | - | - | 15 | - | - | - | 15 | - | - | - |

* N of sites with equivalence = "yes" etc.

A = non-transgenic, control samples

B = transgenic, not Liberty[®] sprayed samples

C = transgenic, Liberty[®] sprayed samples

Only for the compound total dietary fibre was there a slight tendency of major findings observed for the transgenic, Liberty[®] treated samples. The maximum deviation from the control mean was calculated with - 42,1 %. But this observation was also made between the two transgenic sample groups. There the difference is - 32,% of the mean for treatment B (transgenic, not Liberty[®] treated samples).

In the over-all-sites analysis bio-equivalence was established for total dietary fibre between all treatments. But the over all sites assessment is critical, since the p-value is <0.05 and interactions between site and treatment exist.

Minerals (tables 1b, 2c and figure 2 in the appendix C)

Since the sodium values are below the limit of detection at all sites and all samples, this component is not analysed further. For this compound bio-equivalence is established between all different treatments at every single site.

For all other components in the reference group (non-transgenic) the coefficient of variance is more than 20% within at least one site. For these components - with exception of chloride - the variance within sites is greater than 40%. This indicates no homogeneity within the sites and between sites. The natural variation for the minerals in the non-transgenic material exceeds the defined range of 20% for the standard equivalence criterion. As a consequence, the majority of the comparisons result in no bio-equivalence between the different treatments. Nevertheless the evaluation was done based on the $\pm 20\%$ range for the relative treatment difference as standard equivalence criterion.

Table 4.7.2 Results of the Equivalence Evaluation for Minerals

| Summary Equivalence * | A vs B | | | | A vs C | | | | B vs C | | | |
|-----------------------|--------|--------|--------|----------|--------|--------|--------|----------|--------|--------|--------|----------|
| | Yes | No (+) | No (-) | No (+ -) | Yes | No (+) | No (-) | No (+ -) | Yes | No (+) | No (-) | No (+ -) |
| Calcium | 3 | 4 | 3 | 5 | 2 | 6 | 4 | 3 | 4 | 4 | 4 | 3 |
| Phosphorus | 2 | 3 | 4 | 6 | 2 | - | 7 | 6 | 2 | 1 | 8 | 4 |
| Potassium | 5 | 4 | 6 | - | 6 | 1 | 7 | 1 | 4 | 1 | 7 | 3 |
| Magnesium | 4 | 2 | 3 | 6 | 3 | - | 6 | 6 | 3 | - | 6 | 6 |
| Iron | 1 | 5 | 4 | 5 | 2 | 1 | 6 | 6 | 2 | - | 6 | 7 |
| Manganese | - | 5 | 3 | 7 | 1 | 3 | 6 | 5 | 2 | - | 6 | 7 |
| Copper | - | 3 | 9 | 3 | - | 3 | 8 | 4 | 2 | 2 | 6 | 5 |
| Zinc | 1 | 1 | 3 | 10 | 1 | 1 | 8 | 5 | 1 | - | 7 | 7 |
| Chloride | 7# | 3 | - | 5 | 7# | 7 | - | 1 | 6# | 5 | 1 | 3 |

* N of sites with equivalence = "yes" etc.

A = non-transgenic, control samples

B = transgenic, not Liberty[®] sprayed samples

C = transgenic, Liberty[®] sprayed samples

at 5 sites the measurements for the three treatments were equal.

In the site-by-site analysis the comparison between the treatments A vs. B showed no uniform tendency of minor or major findings. Only the copper contents were found to be slightly higher in the transgenic, not Liberty[®] treated samples.

In contrast, major findings for most minerals, except calcium, and minor findings for chloride, were noticed comparing the treatment A against treatment C. This observation is also true for the comparison between the two transgenic sample groups. The Liberty[®] treated transgenic samples had slightly higher mineral and lower chloride contents than the samples from the untreated maize plants. The reason for these findings may be due to chance or to the herbicide treatment. The treatment of the plants with Liberty[®] could lead to a more successful depression of weeds or to a less stronger negative effect on the mycorrhiza flora of the soil in the plots than the treatment with conventional herbicides. Mycorrhiza fungi built a symbiosis with plants enabling them to absorb more soil nutrients such as minerals. However, the statistically significant differences in the mineral contents are not caused by the genetic modification of the maize plants. If they were, both transgenic treatment groups would have altered mineral contents compared to the non-transgenic control group

For the components phosphorus, potassium, magnesium, manganese, zinc and chloride an over-all-sites analysis is valid (p-value of interaction > 0.05). Evaluating the data pooled from

all 15 sites bio-equivalence between all three treatments was stated for phosphorus, potassium, magnesium, manganese, zinc and chloride.

For the components calcium, iron and copper the over-all-sites assessment is critical, because the p-values are <0.05. Nevertheless the comparison of data pooled over all sites results in bio-equivalence for calcium and iron. For copper, major findings for the transgenic sample groups were even detectable in the over all sites evaluation.

The deviation in the copper contents between the 95%-confidence interval of the differences was – 25,9 % of the mean value in the comparison between A vs B and -38,8 % of the mean value in the comparison between A vs C. But even between the two transgenic sample groups significant differences of –21,8 % of the mean value for the untreated transgenic sample group was found.

Vitamins (tables 1c, 2d and figure 3 in the appendix C)

With the exception of niacin and folic acid, for all other vitamins in the reference group (non-transgenic, unsprayed) the coefficient of variance is more than 20% within at least one site. But the variance within sites is only for vitamin B1 and beta tocopherol greater than 40%. The non homogeneity for beta-tocopherol results mainly from the small range of the values in all sites: 0.01 to 0.05 mg/100g dm).

The site-by-site analysis of the results for the vitamins shows, in most of the comparisons no bio-equivalence. However, again no uniform tendency of minor or major findings can be observed. Only for alpha-tocopherol and alpha-tocotrienol were slight minor findings for the transgenic samples detected. In the case of alpha-tocotrienol the minor findings were also observed for the Liberty® treated transgenic samples in the comparison B vs C.

In the over-all-sites analysis, equivalence between the three treatments can be assumed for the components vitamin B1, alpha-, beta- and gamma-tocopherol, alpha-tocotrienol and vitamin E (activity). The respective p-values of interaction for these compounds were greater than 0.05. This means the minor findings in the alpha-tocopherol and alpha-tocotrienol contents for the transgenic samples were not detected in the over-all-sites evaluation of the data.

Table 4.7.3 Results of the Equivalence Evaluation for Vitamins

| Summary Equivalence * | A vs B | | | | A vs C | | | | B vs C | | | |
|-----------------------|--------|--------|--------|----------|--------|--------|--------|----------|--------|--------|--------|----------|
| | Yes | No (+) | No (-) | No (+ -) | Yes | No (+) | No (-) | No (+ -) | Yes | No (+) | No (-) | No (+ -) |
| Vitamin B1 | 1 | 4 | 3 | 7 | 2 | 2 | 6 | 5 | 3 | 1 | 4 | 7 |
| Vitamin B2 | 6 | 6 | 3 | - | 4 | 5 | 4 | 2 | 8 | 4 | 3 | - |
| Niacin | 9 | 2 | 4 | - | 6 | 5 | 4 | - | 9 | 4 | 2 | - |
| Pantothenic acid | 4 | 5 | 5 | 1 | 5 | 6 | 3 | 1 | 6 | 5 | 3 | 1 |
| Folic acid | 3 | 4 | 5 | 3 | 5 | 3 | 5 | 2 | 2 | 5 | 6 | 2 |
| Alpha tocopherol | 3 | 5 | 1 | 5 | 1 | 7 | 4 | 2 | 4 | 2 | 2 | 6 |
| Beta tocopherol | 3 # | 2 | 3 | 6 | 4 # | 1 | 2 | 7 | 3 # | 2 | - | 9 |
| Gamma Tocopherol | 6 | 3 | 4 | 1 | 6 | 4 | 4 | - | 7 | 3 | 3 | 1 |
| Delta tocopherol | - | 3 | 5 | 6 | - | 3 | 3 | 8 | 1 | 4 | 2 | 7 |
| Alpha tocotrienol | 4 | 5 | 1 | 4 | 4 | 5 | - | 5 | 4 | 5 | 1 | 4 |
| Vitamin E activity | 9 | 2 | 2 | 2 | 7 | 4 | 3 | 1 | 10 | 3 | - | 2 |

* N of sites with equivalence = "yes" etc.

A = non-transgenic, control samples

B = transgenic, not Liberty® sprayed samples

C = transgenic, Liberty® sprayed samples

at 3 sites the measurements for the three treatments were equal.



Anti-nutrient (Tables 1d, 2e and Figure 4 in the appendix C)

In 7 of the 15 tested sites of the reference group (non-transgenic, unsprayed) the coefficient of variance is greater than 20%. The variance within sites indicates 42% non homogeneity. The natural range of the phytic acid in the control group is greater than the 20% bio-equivalence range. The majority of the comparisons result, therefore, in no equivalence between the different treatments.

A slight tendency of major findings for phytic acid in the transgenic samples compared to the non-transgenic samples was detected in the site-by-site analysis. The over-all-sites analysis of the data results in bio-equivalence between all three treatments, but this kind of evaluation is critical, since an interaction between site and treatment was noticed.

Table 4.7.4 Results of the Equivalence Evaluation for Phytic acid

| Summary Equivalence * | A vs B | | | | A vs C | | | | B vs C | | | |
|-----------------------|--------|--------|----------|----------|--------|--------|----------|----------|--------|--------|--------|----------|
| | Yes | No (+) | No (-) | No (+ -) | Yes | No (+) | No (-) | No (+ -) | Yes | No (+) | No (-) | No (+ -) |
| Phytic acid | 1 | 3 | 7 | 4 | - | 2 | 8 | 5 | 1 | 3 | 3 | 8 |

- * N of sites with equivalence = "yes" etc.
- A = non-transgenic, control samples
- B = transgenic, not Liberty® sprayed samples
- C = transgenic, Liberty® sprayed samples

Total amino acids (table 1e, 2f and figure 5 of appendix C)

In all sites all total amino acids are homogenous in the reference group (non-transgenic, unsprayed). The coefficient of variance is less than 40% in all cases.

The site-by-site analysis for all total amino acids showed equivalence of the three treatments in most of the comparisons within all sites.

Table 4.7.5 Results of the Equivalence Evaluation for Total Amino Acids

| Summary Equivalence * | A vs B | | | | A vs C | | | | B vs C | | | |
|----------------------------|--------|--------|--------|----------|--------|--------|--------|----------|--------|--------|--------|----------|
| | Yes | No (+) | No (-) | No (+ -) | Yes | No (+) | No (-) | No (+ -) | Yes | No (+) | No (-) | No (+ -) |
| Alanine | 12 | 2 | 1 | - | 11 | 2 | 2 | - | 13 | 1 | 1 | - |
| Arginine | 15 | - | - | - | 14 | - | 1 | - | 15 | - | - | - |
| Aspartic acid + Asparagine | 13 | 1 | 1 | - | 13 | - | 2 | - | 14 | - | 1 | - |
| Cystine | 11 | 3 | 1 | - | 13 | 1 | 1 | - | 14 | - | 1 | - |
| Glutamic acid + Glutamine | 11 | 2 | 2 | - | 12 | 1 | 2 | - | 12 | 2 | 1 | - |
| Glycine | 15 | - | - | - | 15 | - | - | - | 14 | - | 1 | - |
| Histidine | 14 | 1 | - | - | 13 | 1 | 1 | - | 14 | - | 1 | - |
| Isoleucine | 13 | 1 | 1 | - | 13 | 1 | 1 | - | 13 | 1 | 1 | - |
| Leucine | 10 | 2 | 2 | 1 | 11 | 2 | 1 | 1 | 12 | 2 | 1 | - |
| Lysine | 14 | 1 | - | - | 14 | - | 1 | - | 13 | - | 2 | - |
| Methionine | 12 | - | 3 | - | 13 | - | 2 | - | 15 | - | - | - |
| Phenylalanine | 13 | 1 | 1 | - | 12 | 1 | 1 | 1 | 13 | 1 | 1 | - |
| Proline | 13 | 1 | 1 | - | 12 | 1 | 2 | - | 13 | 1 | 1 | - |
| Serine | 13 | 1 | 1 | - | 12 | 1 | 2 | - | 14 | - | 1 | - |
| Threonine | 14 | 1 | - | - | 14 | - | 1 | - | 14 | - | 1 | - |
| Tryptophan | 13 | 1 | 1 | - | 10 | 2 | 3 | - | 13 | 1 | 1 | - |
| Tyrosine | 13 | 1 | 1 | - | 13 | - | 2 | - | 14 | - | 1 | - |
| Valine | 13 | 1 | 1 | - | 14 | - | 1 | - | 14 | - | 1 | - |

* N of sites with equivalence = "yes" etc.

A = non-transgenic, control samples

B = transgenic, not Liberty® sprayed samples

C = transgenic, Liberty® sprayed samples

Free amino acids (table 1f, 2g and figure 6 of appendix C)

Free cystine values are below the limit of detection in all sites and all samples. This component is not analysed further.

The results for the other free amino acids are quite different from the results of the total amount of amino acids. With the exception of asparagine, tryptophan and the sum of the free amino acids, for all other the components in the reference group (non-transgenic, unsprayed) the coefficient of variance is more than 20% at least within one site. But the variance within sites is less 12% for all components.

In the site-by-site analysis the results for these components are ambiguous. But a clear tendency of minor or major findings for the transgenic samples was not noticed. Slight minor findings or major findings in only one of the comparisons with the control samples was found for free histidine, free tyrosine, and free isoleucine. But in the evaluation with the other transgenic sample group this observation could not be stated. Only the values of free proline were slightly higher in the samples of both transgenic treatments compared to the control group.

The overall comparisons is only valid for free alanine (p-value of interaction = 0,17). For free alanine equivalence of the three treatment can be assumed. The over-all-site assessment for free proline results in equivalence between the three treatment groups and the findings of the site-by-site evaluation were not confirmed, but this analysis is critical, since an interaction between site and treatment exists.

Table 4.7.6 Results of the Equivalence Evaluation for Free Amino Acids

| Summary Equivalence * | A vs B | | | | A vs C | | | | B vs C | | | |
|-------------------------|--------|----------|----------|----------|--------|----------|----------|----------|--------|--------|--------|----------|
| | Yes | No (+) | No (-) | No (+ -) | Yes | No (+) | No (-) | No (+ -) | Yes | No (+) | No (-) | No (+ -) |
| Alanine | - | 5 | 7 | 3 | - | 4 | 7 | 4 | 3 | 2 | 4 | 6 |
| Arginine | 4 | 4 | 4 | 3 | 5 | 5 | 3 | 2 | 4 | 4 | 3 | 4 |
| Asparagine | 2 | 5 | 3 | 2 | 3 | 5 | 3 | 1 | 7 | 1 | 1 | 3 |
| Aspartic acid | 2 | 4 | 6 | 3 | 1 | 4 | 5 | 5 | 5 | 1 | 4 | 5 |
| Cystine | | | | | | | | | | | | |
| Glutamic acid | 1 | 4 | 2 | 8 | 1 | 5 | 3 | 6 | 1 | 1 | 3 | 10 |
| Glutamine | - | 6 | 4 | 2 | - | 5 | 4 | 3 | - | 1 | 4 | 7 |
| Glycine | 2 | 6 | 5 | 2 | - | 7 | 7 | 1 | 5 | 4 | 4 | 2 |
| Histidine | 3 | 9 | 3 | - | 2 | 7 | 4 | 2 | 5 | 3 | 3 | 4 |
| Isoleucine | - | 6 | 5 | 4 | - | 8 | 4 | 3 | 1 | 4 | 5 | 5 |
| Leucine | 2 | 5 | 5 | 3 | 2 | 5 | 6 | 2 | 3 | 3 | 3 | 6 |
| Lysine | 3 | 6 | 3 | 3 | 2 | 5 | 3 | 5 | 3 | 4 | 5 | 3 |
| Methionine | 1 | 5 | 6 | 3 | 1 | 6 | 5 | 3 | 1 | 4 | 4 | 6 |
| Phenylalanine | 1 | 5 | 5 | 4 | 1 | 5 | 7 | 2 | 3 | 3 | 5 | 4 |
| Proline | 5 | 1 | 6 | 3 | 3 | 2 | 6 | 4 | 3 | 3 | 5 | 4 |
| Serine | 1 | 6 | 7 | | 1 | 7 | 6 | 1 | 4 | 4 | 2 | 5 |
| Threonine | 2 | 4 | 6 | 3 | - | 6 | 7 | 2 | 1 | 4 | 5 | 5 |
| Tryptophan | 8 | 3 | 4 | - | 5 | 6 | 3 | 1 | 8 | 3 | 2 | 2 |
| Tyrosine | 3 | 6 | 2 | 4 | 4 | 5 | 4 | 2 | 5 | 2 | 4 | 4 |
| Valine | 1 | 5 | 4 | 5 | 1 | 6 | 7 | 1 | 2 | 4 | 5 | 4 |
| Sum of free amino acids | 5 | 5 | 4 | 1 | 8 | 4 | 3 | - | 7 | 3 | 3 | 2 |

* N of sites with equivalence = "yes" etc.

A = non-transgenic, control samples

B = transgenic, not Liberty® sprayed samples

C = transgenic, Liberty® sprayed samples

Total fatty acids (table 1g, 2h and figure 7 in the appendix C)

Values for C14:0 (Tetradecanoic) and C17:0 (Heptadecanoic) are below the limit of detection in nearly all sites and all samples. These components are not analysed further. The components C22:0 (Docosanoic) and C24:0 (Tetracosanoic) are only detectable in the sites DPP1, CFY1 and PPO1. Only these sites are analysed. For the equivalence analysis of the fatty acid C16:1 (Hexadecenoic) the only sites considered were those, in which more than one third of the values were measurable.

Coefficients of variance of more than 20% occurred in the reference group (non-transgenic, unsprayed) only for small number of sites and components:

C16:1: PRO1, DEU0301, ESP0201, FRA0201
 C20:1: DEU0601, ESP0201, FRA0201

Equivalence of the three treatments in the site-by-site analysis can be assumed for palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linolic acid (C18:2), linolenic acid (C18:3) and arachidic acid (C20:0). In these cases equivalence is stated in most of the comparisons. For myristic acid (C14:0) and margaric acid (C17:0) that were not detectable in any of the control and transgenic samples, and for behenic acid (C22:0) and lignoceric acid (C24:0), that were not detectable at 12 of the 15 sites, this is also true. Additionally, the mean differences in the behenic acid (C22:0) and lignoceric acid (C24:0) values between those treatments with quantifiable amounts are less or equal than 0.02% in all sites. So the difference is irrelevant.

The site-by-site analysis for palmitoleic acid (C16:1) (with data from 12 sites) and eicosenoic acid (C20:1) resulted in no bio-equivalence in most of the comparisons between the different treatments, but a uniform tendency for the deviation was not noticed.

In the over-all-sites analysis valid only for the fatty acids C18:0, C18:3, C20:0, and C20:1 (p-value of interaction > 0.05 and detectable values from 15 sites available), equivalence of the three treatments can be assumed.

Table 4.7.7 Results of the Equivalence Evaluation for Total Fatty Acids

| Summary Equivalence * | A vs B | | | | A vs C | | | | B vs C | | | |
|-------------------------------|-----------------|--------|--------|----------|-----------------|--------|--------|----------|-----------------|--------|--------|----------|
| | Yes | No (+) | No (-) | No (+ -) | Yes | No (+) | No (-) | No (+ -) | Yes | No (+) | No (-) | No (+ -) |
| Palmitic C16:0 | 15 | - | - | - | 15 | - | - | - | 15 | - | - | - |
| Palmitoleic C16:1 | 5 ^a | 3 | 2 | 5 | 4 ^a | 4 | 2 | 5 | 3 ^a | 3 | 2 | 7 |
| Stearic C18:0 | 14 | 1 | - | - | 14 | 1 | - | - | 15 | - | - | - |
| Oleic C18:1 | 15 | - | - | - | 15 | - | - | - | 15 | - | - | - |
| Linoleic C18:2 | 15 | - | - | - | 15 | - | - | - | 15 | - | - | - |
| Linolenic C18:3 | 15 | - | - | - | 15 | - | - | - | 15 | - | - | - |
| Arachidic C20:0 | 9 | 4 | 1 | 1 | 10 | 2 | 2 | 1 | 11 | - | 2 | 2 |
| Eicosenoic C20:1 | 7 | 3 | 4 | 1 | 5 | 4 | 2 | 4 | 6 | 2 | 4 | 3 |
| Behenic C22:0 | 12 ^a | 2 | - | 1 | 12 ^a | 1 | 1 | 1 | 12 ^a | - | 3 | - |
| Lignoceric C24:0 ^b | 13 ^a | 2 | - | - | 13 ^a | - | 2 | - | 12 ^b | - | 3 | - |

* N of sites with equivalence = "yes" etc.
 A = non-transgenic, control samples
 B = transgenic, not Liberty[®] sprayed samples
 C = transgenic, Liberty[®] sprayed samples
^a including sites with no detectable amounts for the fatty acid

Free fatty acids (table 1h, 2i and figure 8 in the appendix C)

The components C14:0 (Tetradecanoic), C16:1 (Hexadecenoic), C20:0 (Eicosanoic), C20:1 (Eicosenoic), C22:0 (Docosanoic) and C24:0 (Tetracosanoic) are only detectable in the sites DPP1, CFY1 and PRO1. The fatty acid C17:0 (Heptadecanoic) is only detectable in site DPP1. Only the sites with detectable amounts for the respective free fatty acid were included in the statistical analysis. For sites with free fatty acid values below the limit of quantification for all three treatments bio-equivalence between the non-transgenic and transgenic samples is determined.

For the equivalence analysis of the fatty acid C18:3 (Octadecatrienoic), only those sites were considered, in which more than one third of the values were measurable.

Coefficients of variance of more than 20% occurred in the reference group (non-transgenic, unsprayed) only for small number of sites and components:

| | | | |
|--------|------------|--------|------------|
| C14:0: | DPP1, PRO1 | C20:1: | CFY1, PRO1 |
| C16:1: | PRO1 | C22:0: | CFY1, PRO1 |
| C18:3: | CFY1 | C24:0: | CFY1, PRO1 |

Equivalence of the three treatments can be assumed for the free fatty acids C16:0, C18:0, C18:1 and C18:2. In these cases equivalence is stated in most of the comparisons. Many of the comparisons for the fatty acids C18:3 result in no equivalence between the three treatments. Nevertheless a uniform tendency in the deviation is not detectable. Equivalence of the three treatments from the sites, where amounts of the respective fatty acid were detected, cannot be assumed for C14:0, C16:1; C20:0, C22:0 and C24:0. 16:1. These results should be considered carefully, however, because only values from three sites are available. For C17:0 only results from site DPP1 are available. The mean differences between the treatments are less than or equal to 0.02% in this site.

Table 4.7.8 Results of the Equivalence Evaluation for Free Fatty Acids

| Summary Equivalence * | A vs B | | | | A vs C | | | | B vs C | | | |
|-----------------------|-----------------|--------|--------|----------|-----------------|--------|--------|----------|-----------------|--------|--------|----------|
| | Yes | No (+) | No (-) | No (+ -) | Yes | No (+) | No (-) | No (+ -) | Yes | No (+) | No (-) | No (+ -) |
| Myristic C 14:0 | 12 ^a | 1 | - | 2 | 12 ^a | 1 | - | 2 | 12 ^a | - | - | 3 |
| Palmitic C16:0 | 11 | 4 | - | - | 8 | 5 | 2 | - | 10 | 3 | 2 | - |
| Palmitoleic C16:1 | 13 ^a | 1 | - | 1 | 12 ^a | 2 | - | 1 | 13 ^a | - | - | 2 |
| Margaric C17:0 | 14 ^a | 1 | - | - | 15 ^a | - | - | - | 14 ^a | - | 1 | - |
| Stearic C18:0 | 12 | 2 | 1 | - | 11 | 3 | 1 | - | 13 | 1 | 1 | - |
| Oleic C18:1 | 15 | - | - | - | 15 | - | - | - | 15 | - | - | - |
| Linoleic C18:2 | 15 | - | - | - | 15 | - | - | - | 15 | - | - | - |
| Linolenic C18:3 | 7 | 2 | 5 | - | 7 | 5 | 2 | - | 8 | 4 | 2 | - |
| Arachidic C20:0 | 13 ^a | - | 1 | 1 | 13 ^a | 1 | - | 1 | 12 ^a | 2 | - | 1 |
| Eicosenoic C20:1 | 12 ^a | - | - | 3 | 12 ^a | 1 | - | 2 | 12 ^a | - | - | 3 |
| Behenic C22:0 | 12 ^a | 2 | - | 1 | 12 ^a | 1 | - | 2 | 12 ^a | 1 | - | 2 |
| Lignoceric C24:0 | 12 ^a | 2 | 1 | - | 12 ^a | 2 | - | 1 | 12 ^a | 1 | - | 2 |

* N of sites with equivalence = "yes" etc.

A = non-transgenic, control samples

B = transgenic, not Liberty[®] sprayed samples

C = transgenic, Liberty[®] sprayed samples

^a including sites with no detectable amounts for the free fatty acid

4.8 Comparison of the Analysed Data with Reference Ranges Reported from Literature

The next six tables show the comparison of the results pooled from all sites, neglecting the environmental effects at the single site, with standard values from literature. The values reported from literature must be examined critically, because no information is readily available about the kind of maize varieties tested, the analytical methods used, the number of samples tested and the statistical evaluation of the results achieved. Therefore the comparison with literature values is always of secondary importance.

Nevertheless this comparison is valuable to establish, that LL Maize event T25 provides the same nutritional value as maize currently being consumed.

Table 4.8.1 lists the results for the proximates. Most values are inside the range built by literature. The moisture and total dietary fibre contents are slightly higher than the literature range or value respective. The moisture content of agricultural material is very much influenced by the climate during the field trial and the post-harvest treatment. This is the reason why all results have to be converted to the content based on their dry weight. For total dietary fibre only one reference value was found (Scherz H. and Senser F., 1994). This makes the comparison between the analytical results and the literature value difficult. It might be that the analysed values are inside the natural range for this compound in maize grains.

Table 4.8.1 Proximates in LL Maize Event T25 Grains, Non-transgenic Counterpart and Commercial Maize Varieties (Standard Values)

| | Non-Transgenic | Transgenic Not sprayed | Transgenic Sprayed | Standard-Values ^a |
|--|----------------|------------------------|--------------------|------------------------------|
| Moisture %fw | 27,41 | 27,47 | 27,14 | 7 - 23 |
| Fat %dm | 4,91 | 4,99 | 4,97 | 3.1 - 5.7 |
| Protein %dm | 9,28 | 9,27 | 9,39 | 6 - 12 |
| TDF %dm | 10,77 | 10,93 | 11,30 | 10.5 ^d |
| Ash %dm | 1,51 | 1,48 | 1,47 | 1.1 - 3.9 |
| Total Carbohydrates %dm ^b | 84,30 | 84,27 | 84,17 | 82.9 - 84.9 |
| Available Carbohydrates %dm ^c | 73,52 | 73,34 | 72,87 | 61 - 78 |

^a Standard values from table 2 of appendix A.

^b Total Carbohydrates calculated as 100% - (protein %dm + fat %dm + ash %dm)

^c Available Carbohydrates calculated as 100% - (protein %dm + fat %dm + TDF %dm + ash %dm)

^d value from Scherz and Senser, 1994

Table 4.8.2 summarises the results of the mineral, vitamin and phytic acid analyses. Most contents fall into the ranges reported for grain from commercial maize varieties. Clear minor findings for calcium and small major findings for folic acid were found through all different treatments. This has no influence on the equivalence statement made in the previous section.

Slight major findings in some sample groups, were noticed for magnesium and alpha-tocotrienol. However, for these two components the equivalence between the transgenic and non-transgenic maize grains was stated in the statistical analysis over-all-sites (section 4.7, minerals and vitamins). For beta- and delta-tocopherol no literature values were found.

Table 4.8.2 Minerals, Vitamins and Phytic Acid in LLMaize Event T25 Grains, Non-transgenic Counterpart and Commercial Maize Varieties (Standard Values)

| Parameter | On dry matter basis | | | |
|----------------------------|---------------------|------------------------|--------------------|------------------------------|
| | Non-Transgenic | Transgenic Not sprayed | Transgenic Sprayed | Standard-Values ^a |
| Calcium mg/kg | 45,6 | 43,4 | 44,9 | 100 - 1000 |
| Phosphorus mg/kg | 2783 | 2769 | 2992 | 2300 - 7500 |
| Potassium mg/kg | 3428 | 3492 | 3679 | 3200 - 7200 |
| Magnesium mg/kg | 989 | 991 | 1056 | 900 - 1000 |
| Sodium mg/kg | <100 | <100 | <100 | 0 - 1500 |
| Iron mg/kg | 22,9 | 22,6 | 25,3 | 1 - 274 |
| Manganese mg/kg | 4,8 | 4,7 | 5,1 | 0,7 - 54 |
| Copper mg/kg | 1,5 | 1,8 | 2,0 | 0,8 - 10 |
| Zinc mg/kg | 16,9 | 17,2 | 18,5 | 10 - 30 |
| Chlorine g/100g | 0,04 | 0,03 | 0,04 | 0,014 - 0,06 |
| Vitamin B1 mg/100g | 0,31 | 0,32 | 0,33 | 0,23 - 0,86 |
| Vitamin B2 mg/100g | 0,17 | 0,16 | 0,17 | 0,025 - 0,56 |
| Niacin mg/100g | 2,21 | 2,25 | 2,21 | 0,93 - 7,0 |
| Pantothenic acid mg/100g | 0,79 | 0,80 | 0,78 | 0,35 - 1,4 |
| Folic acid µg/100g | 49 | 48 | 48 | 23 - 46 |
| Alpha-Tocopherol mg/100g | 0,63 | 0,60 | 0,59 | 0,23 - 2,17 ^c |
| Beta-Tocopherol mg/100g | 0,02 | 0,02 | 0,02 | NF |
| Gamma.-Tocopherol mg/100g | 4,63 | 4,66 | 4,58 | 1,71 - 7,54 ^c |
| Delta-Tocopherol mg/100g | 0,16 | 0,18 | 0,17 | NF |
| Alpha-Tocotrienol mg/100g | 0,65 | 0,62 | 0,60 | 0,03 - 0,64 ^c |
| Vitamin E activity mg/100g | 1,96 | 1,93 | 1,90 | 0,3 - 3,1 |
| Phytic Acid mg/100g | 477 | 517 | 532 | 450 - 1260 |

^a Standard values from table 3, 7 and 8 of appendix A (Conversion factors from mg/kg dm to mg/100g dm f = 0,1 and from mg/kg dm to µg/100g dm f = 100).

^b values were not obtained by calculation of the mean, since all results are below LOQ.

^c value from Scherz and Senser, 1994

In table 4.8.3 the results for the total amino acids are given. Although the comparison of results from the non-transgenic maize grain samples with the results from transgenic samples showed no significant differences, slight minor findings are found, if compared with the literature values for some amino acids.

The cereal chemistry reference guidelines and other sources for composition tables do not list any free amino acid values. Therefore a comparison with the analysed results is not possible.

Table 4.8.3 Total Amino Acids in LL Maize Event T25 Grains, Non-transgenic Counterpart and Commercial Maize Varieties (Standard Values)

| Parameter | g/kg Dry matter | | | Standard-Values ^a |
|-----------|-----------------|------------------------|--------------------|------------------------------|
| | Non-Transgenic | Transgenic Not sprayed | Transgenic Sprayed | |
| Ala | 6,90 | 6,90 | 6,98 | 7,3 - 9,6 |
| Arg | 3,95 | 3,94 | 3,99 | 2,2 - 6,5 |
| Asp + Asn | 6,20 | 6,23 | 6,30 | 6,0 - 7,3 |
| Cys | 1,77 | 1,80 | 1,83 | 0,8 - 3,2 |
| Glu + Gln | 17,05 | 17,08 | 17,24 | 17,7 - 21,7 |
| Gly | 3,41 | 3,39 | 3,43 | 3,7 - 5,1 |
| His | 2,26 | 2,26 | 2,28 | 1,5 - 3,8 |
| Ile | 2,94 | 2,96 | 2,96 | 3,4 - 7,1 |
| Leu | 11,11 | 11,18 | 11,24 | 10,3 - 24,3 |
| Lys | 2,77 | 2,73 | 2,81 | 0,5 - 5,5 |
| Met | 1,70 | 1,76 | 1,79 | 1,0 - 4,6 |
| Phe | 4,50 | 4,49 | 4,51 | 3,6 - 5,9 |
| Pro | 7,79 | 7,79 | 7,76 | 8,3 - 13,7 |
| Ser | 4,50 | 4,52 | 4,56 | 4,5 - 6,1 |
| Thr | 3,38 | 3,38 | 3,41 | 3,4 - 5,9 |
| Try | 0,81 | 0,81 | 0,82 | 0,5 - 1,3 |
| Tyr | 2,79 | 2,82 | 2,86 | 2,2 - 7,9 |
| Val | 4,10 | 4,11 | 4,12 | 4,5 - 8,5 |

a Standard values from table 4 of appendix A. (Conversion factor from % dm to g/kg dm f = 10)

Table 4.8.4 Free Amino acids in LLMaize Event T25 Grains and Non-transgenic Counterpart

| Parameter | g/kg Dry matter | | |
|-----------|-----------------|------------------------|--------------------|
| | Non-Transgenic | Transgenic Not sprayed | Transgenic Sprayed |
| Ala | 0,221 | 0,221 | 0,232 |
| Arg | 0,167 | 0,171 | 0,173 |
| Asn | 0,447 | 1,456 | 0,455 |
| Asp | 0,364 | 0,373 | 0,379 |
| Cys | <0,001 | <0,001 | <0,001 |
| Glu | 0,175 | 0,156 | 0,155 |
| Gln | 0,139 | 0,143 | 0,148 |
| Gly | 0,048 | 0,050 | 0,049 |
| His | 0,050 | 0,048 | 0,049 |
| Ile | 0,041 | 0,041 | 0,042 |
| Leu | 0,079 | 0,083 | 0,083 |
| Lys | 0,170 | 0,169 | 0,172 |
| Met | 0,026 | 0,027 | 0,026 |
| Phe | 0,061 | 0,062 | 0,062 |
| Pro | 0,658 | 0,670 | 0,675 |
| Ser | 0,115 | 0,118 | 0,121 |
| Thr | 0,067 | 0,069 | 0,068 |
| Try | 0,021 | 0,022 | 0,021 |
| Tyr | 0,074 | 0,075 | 0,075 |
| Val | 0,063 | 0,065 | 0,066 |
| Sum | 2,87 | 2,90 | 2,93 |

a mean values not calculated, since below LOQ.

In the last two tables (table 4.8.5 and 4.8.6) the total and free fatty acid results are summarised. For total fatty acid values beyond the 1% level the findings for the non-transgenic and transgenic samples are in good correspondence with the range reported in literature. For values below 1%, relative comparison with literature values is difficult. In the case of arachidic acid, slightly higher results were found in the reference tables. For behenic, lignoceric and eicosanoic acid, values were not listed in the composition tables, because the respective fatty acid was not detected. For the free fatty acid, no literature values were available. For these compounds only the mean from the overall-sites pooled data is shown.

Table 4.8.5 Total Fatty acids in LLMaize Event T25 Grains, Non-transgenic Counterpart and Commercial Maize Varieties (Standard Values)

| Fatty Acid | | % Relative | | | Std. Values ^a |
|------------------------------|-------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| | | Non-transgenic | Transgenic Not sprayed | Transgenic Sprayed | |
| Saturated | Myristic C 14:0 | < 0,1 ^b | < 0,1 ^b | < 0,1 ^b | 0,0 - 0,3 |
| | Palmitic C16:0 | 12,69 | 12,53 | 12,51 | 7 - 19 |
| | Margaric C17:0 | < 0,1 ^b | < 0,1 ^b | < 0,1 ^b | NF |
| | Stearic C18:0 | 1,71 | 1,69 | 1,70 | 1,0 - 3,3 |
| | Arachidic C20:0 | 0,37 | 0,37 | 0,37 | 0,1 - 2,1 |
| | Behenic C22:0 | <0,1 - 0,17 ^c | <0,1 - 0,14 ^c | <0,1 - 0,24 ^c | NF |
| | Lignoceric C24:0 | <0,1 - 0,19 ^c | <0,1 - 0,19 ^c | <0,1 - 0,20 ^c | NF |
| Total Saturated ^d | | 14,77 | 14,59 | 14,58 | - |
| Mono-unsaturated | Palmitoleic C16:1 | 0,17 | 0,16 | 0,16 | 0,0 - 1,1 |
| | Oleic C18:1 | 26,31 | 26,53 | 26,40 | 20 - 46 |
| | Eicosenoic C20:1 | 0,28 | 0,28 | 0,27 | NF |
| Total Mono-uns. | | 26,76 | 26,97 | 26,83 | - |
| Poly-unsaturated | Linoleic C18:2 | 57,08 | 57,06 | 57,22 | 35 - 70 |
| | Linolenic C18:3 | 1,36 | 1,33 | 1,32 | 0,8 - 2,0 |
| Total Poly-uns. | | 58,44 | 58,39 | 58,54 | - |
| Grand Total | | 99,97 | 99,95 | 99,95 | - |

NF not found

a Standard values from table 6 of appendix A.

b values were not obtained by calculation of the mean, since all results are below LOQ.

c C22:0 (Docosanoic) and C24:0 (Tetracosanoic) are only detectable in the sites DPP1, CFY1 and PPO1

d Total saturated fatty acid values calculated with C22:0 and C24:0 <0,10%.

Table 4.8.6 Free Fatty acids in LLMaize event T25 Grains and Non-transgenic Counterpart

| Fatty Acid | | % Relative | | |
|------------------------------|------------------|--------------------------|--------------------------|--------------------------|
| | | Non-transgenic | Transgenic Not sprayed | Transgenic Sprayed |
| Saturated | Myristic C 14:0 | <0,1 – 0,26 ^a | <0,1 – 0,26 ^a | <0,1 – 0,32 ^a |
| | Palmitic C16:0 | 19,45 | 18,95 | 19,02 |
| | Margaric C17:0 | <0,1- 0,16 ^a | <0,1 - 0,1 ^a | <0,1 - 0,14 ^a |
| | StearicC18:0 | 1,52 | 1,48 | 1,48 |
| | Arachidic C20:0 | <0,1- 2,06 ^a | <0,1- 2,00 ^a | <0,1- 1,99 ^a |
| | Behenic C22:0 | <0,1 – 0,74 ^a | <0,1 – 0,21 ^a | <0,1 – 0,86 ^a |
| | Lignoceric C24:0 | <0,1 – 0,89 ^a | <0,1 – 0,69 ^a | <0,1 – 0,54 ^a |
| Total Saturated ^d | | 20,97 | 20,43 | 20,5 |
| Mono-unsaturated | Palmitoleic 16:1 | <0,1- 0,64 ^a | <0,1- 0,32 ^a | <0,1- 0,33 ^a |
| | Oleic C18:1 | 21,90 | 21,94 | 21,95 |
| | Eicosenoic 20:1 | <0,1 – 0,30 ^a | <0,1 – 0,22 ^a | <0,1 – 0,30 ^a |
| Total Mono-uns. ^b | | 21,9 | 21,94 | 21,95 |
| Poly-unsaturated | Linoleic C18:2 | 54,86 | 55,51 | 55,44 |
| | Linolenic C18:3 | 1,91 | 1,86 | 1,84 |
| Total Poly-uns. | | 56,77 | 57,37 | 57,28 |
| Grand Total | | 99,64 | 99,74 | 99,73 |

a C14:0, C16:1, C20:0, C20:1, C22:0 and C24:0 are only detectable in the sites DPP1, CFY1 and PPO1, and C17:0 is only detectable in the site DPP1.

b Total fatty acid values calculated with C14:0, C16:1, C17:0, C20:0, C20:1, C22:0 and C24:0 <0,10%.

4.9 Evaluation of Compositional and Nutritional Equivalence between LLMaize event T25 and the Non-transgenic Counterparts

Bio-equivalence – meaning no statistically significant differences – was found between the non-transgenic and the two transgenic sample groups for most proximate components (except for total dietary fibre), for all total amino acids and for most total fatty acids [except palmitoleic acid (C16:1) and eicosenoic acid (C20:1)].

For some components this could be stated for each comparison at every single site.

For total dietary fibre, the minerals, the vitamins, phytic acid, free amino acids, total palmitoleic acid (C16:1) and eicosenoic acid (C20:1), and free fatty acids, statistically significant differences were observed. Nevertheless, these statistically significant differences have no impact on the compositional and nutritional equivalence statement, because

1. no clear tendency of minor or major findings could be observed
2. the difference was not found in all comparisons (differences were observed for instance in the comparison between control and transgenic Liberty[®] treated sample group, but not in the comparison between control and the not Liberty[®] treated transgenic sample group)
3. the difference was found between transgenic sample groups
4. the difference was not confirmed by the over-all-sites analysis
5. all analysed values were inside the literature range
6. the detected statistical difference has no nutritional relevance

Statistically significant differences that were found at most of the single sites with a slight tendency for major or minor findings were only found for 3 of the 92 tested compounds. These are copper, phytic acid and free proline.

In case of copper, the major findings in both transgenic samples groups were confirmed in the over-all-sites assessment (p values <0.05; so this statement is critical). For the two other components, the over-all-site assessment resulted in bio-equivalence, but as for copper this statement is critical, since an interaction between site and treatment was noticed.

In evaluating the **copper** results, a tendency for major findings was seen for both transgenic sample groups and was confirmed in the over-all-sites assessment (p values <0.05; so not valid). However, the nutritional impact of the observed differences is negligible, since all measured values are inside the range reported from literature. The absolute difference between the mean of the non-transgenic samples and the transgenic samples is 0,5 mg/kg, which, if compared to the literature range of 0,8-10 mg/kg dm, is very small.

The maximum deviation between the **phytic acid** means for the transgenic and the non-transgenic samples was calculated to be 55 mg/100g dm. However, nutritional impact of this observation is not important, since the analysed results do not exceed the reported ranges given by the respective cereal chemistry reference guides. On the contrary, all measured values are at the lower border of the literature range.

For **free proline**, major findings were calculated for the transgenic samples. The nutritional effect of this finding is also not relevant, because the content of total proline, which is 10 fold higher than the free proline amount, was found to be in bio-equivalence between all treatments. The absolute difference in the free proline amounts is, at 0,017 g/kg, very low and proline is not an essential amino acid.

In summary, there was not one compound that was found to exceed or fall short of the bio-equivalence range of 20% at every site in all three comparisons. The statistically significant differences that were found for three components in some of the comparisons have no influence on the compositional and nutritional equivalence statement and no impact on the nutritional value of LLMaize T25 grains, as was explained above. All analysed components are in the range of nutrients determined for commercial maize varieties currently on the market.

Based on this statistical evaluation of the analytical data and an assessment of the nutritional impact of the different observations, the grains from LibertyLink[®] Maize event T25 are found to be compositionally and nutritionally equivalent to their traditional non-transgenic counterpart. There is no impact on the nutritional value of the maize grains caused by the genetic transformation.

5 CONCLUSION FOR THE NUTRITIONAL IMPACT ASSESSMENT OF LLMAIZE EVENT T25

Evaluations were conducted to demonstrate that LibertyLink[®] Maize event T25 is compositionally and nutritionally equivalent to its non-transgenic counterpart and to other varieties of maize. In this context the agronomic performance of LLMaize event T25 plants was compared to plants of the non-transgenic counterpart and the raw agricultural commodity grain was analysed for its composition.

Compositional and nutritional analyses from maize grains generated from 15 field trials in two different growing seasons in Europe were performed, which provide a robust data set for a sound statistical evaluation to enable proof of *Substantial Equivalence* between the LLMaize event T25 grains, from Liberty[®] treated and non Liberty[®] treated plants, and their non-transgenic counterparts.

The components, which were selected for compositional and nutritional analyses, comprise the important, basic nutrients of maize. These are proximates, micro-nutrients such as vitamins and minerals, the anti-nutrient phytic acid, amino acids, and fatty acids.

The conclusion of the compositional analyses of LibertyLink[®] Maize T25 grains compared to their non-transgenic counterpart varieties and the comparison between the analysed values with reference data from the respective cereal chemistry reference guidelines is:

- there is not one compound, which was found to exceed or fall short of the bio-equivalence range of 20% in all three comparisons within all sites and over all sites.
- the statistically significant differences that were found for three compounds (copper, phytic acid and free proline) in some of the comparisons have no influence on the compositional and nutritional equivalence statement.
- the observed differences have no impact on the nutritional value of LLMaize T25 grains.
- all analysed compounds are in the range of nutrients determined for commercial maize varieties currently on the market.

The observation of the agronomic characters showed that the transgenic LLMaize event T25 plants have the same agronomic behaviour and performance as their conventional non-transgenic counterparts. If a difference was observed, it was not seen at all sites or the deviation had no uniform tendency at all sites.

Based on the statistical evaluation of the analytical data, the assessment of the nutritional impact of the different observations and the outcome of the agronomic performance, the LibertyLink[®] Maize event T25 is found to be compositionally and nutritionally equivalent to its traditional non-transgenic counterpart and to other current commercial maize varieties. There is no impact on the nutritional value of the maize grains caused by the genetic transformation.

These data and findings lead Bayer CropScience to a conclusion of “no concern” for the safety and nutritional value of LLMaize T25 and its progeny.

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