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Nutritional Impact Assessment Report for the Double-Herbicide-Tolerant Soybean (Transformation Event FG72)

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ACRONYMS AND TECHNICAL TERMS

ADF	Acid detergent fibre
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemist
EFSA	European Food Safety Authority
EPA	US-Environmental Potection Agency
2mEPSPS	5-Enolpyruvylshikimate-3-phosphate synthase, confers tolerance to glyphosate herbicide
FAO	Food and Agriculture Organisation of the United Nations
GEMS	Global environment monitoring system
GM	Genetically modified
HPPD	Hydroxyl phenyl-pyruvate-dioxygenase, confers tolerance to isoxaflutole herbicide
LOD	Limit of detection
LOQ	Limit of quantification
NDF	Neutral detergent fibre
OECD	Organisation for Economic Co-operation and Development
SD	Standard deviation
Total Carb.	Total carbohydrates
Total Toc.	Total tocopherols
US, USA	United States, United States of America
WHO	World Health Organisation
% fw	Percent fresh weight
% dm	Percent dry matter
% rel.	Percent fatty acid based on total amount of fatty acids

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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 and feed is derived, is compared with its existing appropriate non-genetically modified counterpart. The goal of this comparison is to ensure that food and 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 and feed derived from its traditional counterpart.

Substantial Equivalence is assessed using data generated from the comparative analysis of the molecular, agronomic and morphological characteristics of the organism and derived products in question, as well as their chemical composition.

Evaluations were conducted to compare the nutritional components found in the double-herbicide-tolerant soybean (transformation event FG72) to its non-transgenic counterpart, Jack, and to other commercial soybean lines currently on the market.

Compositional analyses were performed using the raw agricultural commodity soybean seed grown at 10 field trial sites in the USA in the 2008.

The components, which were selected for compositional and nutritional analyses, comprise the important, basic nutrients of soybean. These are proximate and fibre components, micro-nutrients such as minerals and vitamins, the isoflavones, the anti-nutrients raffinose, stachyose, phytic acid, trypsin inhibitor and the lectins, the total amino acids, and the total fatty acids.

The composition data from all trial sites were statistically analysed with analysis of variance methods using a model with fixed factors for regimen (the transgenic seeds and the non-transgenic comparator) and for site as well as their interaction term. Based on the ANOVA model regimen differences were estimated and presented together with 95% confidence intervals. In cases of interaction between the factors regimen and site, the results of by-site analyses were taken into account.

Statistical evaluation of the composition data showed that for most components no statistical significant differences between FG72 and Jack seeds were detected. Individual t-tests resulted in statistically significant differences for ash, calcium, magnesium, sodium, total tocopherols, raffinose, glycitin, genistin, and the fatty acids. However, these differences have no biological and nutritional relevance, because most mean values calculated for the transgenic and non-transgenic groups are inside the reference ranges calculated from the results of three analysed commercial soybean lines and from literature, and the estimated differences between regimens are very small for most components and very often lower than the natural variation inside the non-transgenic control group.

Based on this statistical evaluation of the analytical data and an assessment of the nutritional impact of the different observations, the soybean seeds from the double-herbicide-tolerant soybean event FG72 are found to be nutritionally equivalent to seeds from the traditional non-transgenic comparator, the variety Jack. There is no impact on the nutritional value of the soybean seeds as a result of the genetic modifications or the treatment with the test herbicides.

Beside the raw agricultural commodity, soybean seed, soybean hulls, meal, toasted meal, protein isolate, crude oil, food grade oil and crude lecithin were analysed for their composition, too. The results obtained were compared to reference ranges from literature.

No differences between the transgenic and non-transgenic soybean products were noticed for most of the analysed components. If slight differences were detected this has no nutritional impact for two reasons:

- The analysed values in the non-transgenic and the transgenic processing products are inside the reference ranges for the commercial product. This is true for the oleic (C18:1) and linoleic (C18:2) contents of the crude and refined oil samples.
- Differences in nutrient levels were only found in one processing product, but not in the raw agricultural commodity, pre- or subsequent processing products. For instance, genistein was found to be different in the meal samples before toasting. But this was not confirmed by the analysis of the soybean seeds and the toasted meal samples.

To enable an assessment of the exposure of humans and animals to the recombinant proteins expressed in double-herbicide-tolerant soybean, the FG72 seed samples and products derived from FG72 seeds were analysed for the 2mEPSPS and HPPD protein contents.

All calculations are based on worst-case scenarios using consumption figures for whole soybeans as pulses and all kinds of oilseeds (with the exception of groundnuts), taking the highest recombinant protein amounts determined in the soybean commodity, and assuming that all commercial soybean seeds taken to produce food or animal feed would be the double-herbicide-tolerant soybean event FG72, and this is not the case because of global commodity trades.

The main soybean product consumed by humans is the vegetable oil. Since the recombinant proteins were not detected in the oil products derived from FG72 seeds, an intake of the 2mEPSPS and HPPD protein is not possible via soybean food grade oil obtained from FG72 seeds or products containing this oil quality. Alternatively, the predicted daily dietary intake calculations were done on the basis of the consumption of whole soybeans as pulses and all kind of oilseeds (with the exception of groundnuts) for different regional diets. Based on these consumption figures, the 2mEPSPS and HPPD protein intake could be at a maximum of 11.0 µg and 6.43 µg per person per day, respectively.

The contribution of double-herbicide-tolerant soybean event FG72 to animal feed was also evaluated. The maximum theoretical concentrations of the 2mEPSPS and HPPD protein are 0.61 µg/g fw (or 6.1×10^{-5} % of the diet) and 0.32 µg/g fw (or 3.2×10^{-5} % of the diet), respectively, if soybean seeds are used to prepare animal feed for swine.

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

Long-term animal toxicity studies are generally applicable for safety assessment of many components in food including pesticides, pharmaceuticals, industrial chemicals and food additives for which human exposure is generally low, since such studies can be carried out utilizing a range of doses (amounts greatly above expected human exposure levels) in order to determine the safe level of exposure for humans. By contrast, traditional toxicological testing is not applicable to the assessment of whole foods that are complex mixtures of constituents, have wide variations in composition and nutritional value, and due to their bulk can only be fed to laboratory animals at low multiples of the amounts that might be present in the human diet. In addition, a diet that consists entirely of a single category of food can cause adverse effects on nutritional status in the animals, potentially masking any other smaller effect of a component or components of the food being tested.

The difficulties of applying traditional toxicological testing and risk assessment procedures to whole foods meant that an alternative approach is required for the safety assessment of GM foods. Expert consultations convened by FAO/WHO and OECD (OECD, 1993; FAO/WHO, 1996) have recommended that the concept of S*ubstantial Equivalence* could be a guiding principle to detect intended and unintended differences between a GM food crop and its non-GM control to address these limitations.

This concept embodies a science-based approach, in which a GM 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 and can be treated in the same manner (FAO/WHO, 2000). No additional safety concerns would be expected. However, when a GM food or food component is not determined to be *substantially equivalent*, the identified differences should be the focus of further nutritional, toxicological and immunological evaluation.

Starting point of the approach is the comparative analysis of the molecular, agronomic and morphological characteristics of the organisms in question, as well as their chemical composition.

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. 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. Consensus documents prepared by OECD (*e.g.* OECD, 2001) provide excellent guidance for the analyses needed and the analyses conducted should be determined on a case-by-case basis and may vary depending on the introduced trait.

S*ubstantial Equivalence* in the chemical composition is established, if the levels and variations of the nutrients and anti-nutrients of the GM crop are within the natural or experimental variability for the respective nutrients and anti-nutrients in the non GM comparator grown under the same regimes and environmental conditions. It should be noted that there are significant differences in composition of conventionally bred varieties and thus the compositional analysis of GM crops must be assessed against the background of natural variability in the conventional counterpart(s). However, modifications that fall outside normal ranges of variation will require further assessment to determine any biological significance.

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.

Statistical analysis of the results is generally performed on the basis of a 95% confidence interval criterion. The application of a randomised trial design enables the analysis of interaction between experimental factors like location, year, climatic conditions, and plant variety (EFSA, 2004).

2 COMPOSITIONAL AND NUTRITIONAL ANALYSES OF SOYBEAN SEEDS

2.1 Field Trial Information for the Production of Soybean Seed

Soybean plants containing the double-herbicide-tolerant soybean event FG72 and representing the non-transgenic comparator, Jack, and 3 commercial soybean lines were tested in the field by MS Technologies, LLC in 2008. Trials were conducted at trial sites that are representative of the commercial production of Group 2 - 3 soybeans in the USA. Table 2.1.1 identifies the study code, trial code and field trial location from which soybean seeds were obtained for composition and recombinant protein analyses.

Table 2.1.1 Source of Soybean Seed for Composition Analysis (Study Code, Trial Code and Field Trial Location)

Study Code	Trial Code	**Field Trial Location:** Nearest Town, County, US State, EPA Region, Principal Field Investigator
HT08SOY002	201	Marcus, Cherokee, Iowa, USA, EPA V, Bill Eby
	202	Iowa Falls, Hardin, Iowa, USA EPA V, Bill Eby
	203	Scranton/Glidden, Carrol, Iowa, USA EPA V, Bill Eby
	204	Perry, Dallas, Iowa, USA EPA V, Bill Eby
	205	Adel, Dallas, Iowa, EPA V, Bill Eby
	206	Winterset, Madison, Iowa, USA, EPA V, Bill Eby
	207	Osborne, Clinton, Missouri, EPA V, Bill Eby
	208	Fithian, Vermillion, Illinois, EPS V, USA, Bill Eby
	209	Sharpsville, Tipton, Indiana, USA, EPA V, Bill Eby
	210	Mediapolis, Boone, Indiana, USA, EPA V, Bill Eby

To compensate for the environmental effects inside a single location, replication and randomization at each site is necessary. The trial design applied at all locations was a randomized complete block design including three plots planted with the non-transgenic variety Jack and six plots planted with the soybean event FG72. Replicate in this report means harvesting samples from replicated plots of a single regimen. Three additional plots were planted with the non-transgenic commercial soybean lines Stine® 2686-6, Stine® 2788 and Stine® 3000-0. At each trial site there were in total 12 plots.

The plants in this study were grown under conditions typical of production practices. Transgenic and non-transgenic plants were treated identically, except for the isoxaflutole and glyphosate treatments of some transgenic plots. Fertilization and normal cultural practices were carried out by MS Technologies, LLC test site personnel.

Treatment with the isoxaflutole (IFT at a target rate of 70 grams ai/hectare) and glyphosate herbicide (at 1060 grams ai/hectare) was done as a foliar spray at about the V4-V5 growth stage. Ammonium sulfate at 2850 grams/hectare was added to the spray mixture. Plots not treated with these herbicides were conventionally treated.

Soybean seed samples were harvested from the center rows of each four-row plot per field trial. The soybean plots were harvested at normal maturity by hand or mechanical means. The samples were stored at ambient temperatures after harvest and shipped ambient to the Bayer CropScience, BioAnalytics, RTP, North Carolina, USA. Within one week of arrival at Bayer CropScience, the seed samples were sub-sampled and transferred to frozen storage. One set of sub-samples were shipped frozen on dry ice via overnight courier to Covance Laboratories, Inc., Madison, Wisconsin, USA, for determination of the composition of the soybean seed. Upon arrival at Covance Laboratories, the samples were transferred to frozen storage. The other set of sub-samples were analysed for their recombinant protein contents at the BioAnalytics Laboratories.

Further detailed information about trial maintenance, herbicide applications, weather data etc. is provided in the field trial report to study HT08SOY002 (Kowite, 2009a).

2.2 Field Trial and Processing Information for Soybean Products

The soybean seeds that were processed to various soybean products were grown in 2008 at the site nearby Adel, in Dallas County, Iowa, USA (EPA region V). The trial was also conducted by MS Technologies, LLC under the study HT08SOY001 (Kowite, 2009b). Grain material from three test plots (Jack, FG72 conventional treated and FG72 treated with glyphosate and IFT) established in this field trial were used for the processing experiment. The treatment of one FG72 plot was done in the same way as described in the previous section (foliar spray at about V4-V5 growth stage; IFT target rate 70 grams ai/hectare; glyphosate target rate 1060 grams ai/hectare; ammonium sulfate added at 2850 grams/hectare). The harvest of the soybean seeds was done on 8th of October 2008. The grain was placed into seed storage in the Adel Regulatory Warehouse of MS Technologies on the day of harvest. Further details from the field trial phase can be found in the report to study HT08SOY001 (Kowite, 2009b).

Approximately 32 kg soybean seeds from Jack and the two FG72 regimens were shipped on 29th of April, 2009 to the processing facility: GLP Technologies, Navasota, Texas, USA. The processing experiment took place between June and July 2009. The non-transgenic soybeans were processed first, followed by the FG72 seeds from conventional treated plants and, at least, the FG72 seeds from glyphosate and IFT treated plants. The three seedlots were processed into hulls, meal, toasted meal, protein isolate, crude oil, refined, bleached and deodorized oil (food grade oil) and crude lecithin. The processed commodities were placed in frozen storage shortly after preparation. The processing experiment is described in the report to study DQ09B002 (Kowite, 2009c).

Sub-samples of the processed samples were shipped in July 2009 in frozen conditions to BioAnalytics Laboratories of Bayer CropScience in RTP, North Carolina, USA. They were transferred to Covance Laboratories, Inc., Madison, Wisconsin, USA, over dry ice by overnight carrier to be analysed for their chemical composition. The results of the compositional analyses are presented in sections 3.1 to 3.7 of this report. The report to study DQ09B009 (Kowite, 2009d) contains further details from the composition analyses of the soybean products. A second set of sub-samples was analysed for the recombinant protein contents at the BioAnalytics Laboratories.

2.3 Components of Interest, Soybean Products to Analyze and Methodology for Compositional Analyses

The components selected for compositional and nutritional analyses of soybean seeds for the assessment of S*ubstantial Equivalence* comprised the important, basic nutrients that are the proximate, fibre components, the micro-nutrients, such as minerals and vitamins, the anti-nutrients raffinose, stachyose, phytic acid, trypsin inhibitors and lectin, the isoflavones, the total amino acids, and the total fatty acids. Table 2.3.1 lists the components analysed in the raw agriculture commodity seeds, as well as the important nutrimental components of the processed soybean products. The analytical program is specified for the kind of soybean product taking into account their different nutritional composition: soybean seeds have relatively high concentration of fat and protein, the hulls have high fibre content and the meals have high protein amounts. Seed, hull and meal samples were analysed for proximate and fibre components. Soybean seeds were analysed for all components except for phosphatides. In the meal samples the isoflavones, anti-nutrients, and the total amino acids were also determined. The protein isolate was analysed for protein, amino acid and trypsin inhibitor and lectin contents. The oil products were analysed for their fatty acid profiles and fat-soluble vitamins. Phosphatides were only determined in crude soybean lecithin.

The analyses performed and the analytical methods used are listed in Table 2.3.2. A brief description of each analytical method is given in the Appendices to the study reports DQ08B009 and DQ09B009 (Mackie, 2009; Kowite, 2009d).

Table 2.3.1 **Components Analysed in Soybean Seeds and Products Derived from Soybean Seeds**

Component	Seed	Hulls	Meal	Toasted Meal	Protein Isolate	Crude Oil	Food Grade Oil	Crude Lecithin
Proximate [a], ADF and NDF	X	X	X	X	X [b]			
Ca, P, K, Mg, Na, Fe	X							
All Vitamins	X							
Fat-soluble Vitamins (A, K, Tocopherols)						X	X	
Isoflavones	X		X	X				
All Anti-nutrients	X		X	X				
Trypsin Inhibitors and Lectin					X			
Total Amino Acids	X		X	X	X			
Total Fatty Acids	X					X	X	
Phosphatides								X

[a] Proximate comprise moisture, crude protein, crude fat, ash and total carbohydrates (calculated)
[b] Protein isolate was only analysed for crude protein content

Table 2.3.2 **Methods Used for Analyses**

Component	Analytical Method
Moisture	AOAC 926.08 and 925.09
Protein	AOAC 955.04 and 979.09 and based on references below [a]
Total Fat	AOAC 960.39 and 948.22
Ash	AOAC 923.03
Carbohydrate	Agric. Handbook No. 74, USDA; Difference between 100 and the sum of crude protein, fat, moisture and ash
ADF	Agric. Handbook No. 379, USDA
NDF	AACC 32.20 + Agric. Handbook No. 379
Ca, P, K, Mg, Na, Fe	AOAC 984.27 and 985.01

AACC American Association of Cereal Chemists International Approved Methods of Analysis (9th edition)
AOAC Association of Official Analytical Chemists International Approved Methods of Analysis (18th edition)
AOCS Official Methods and Recommended Practices of the American Oil Chemists' Society (5th edition)
[a] Bradstreet, R. B., The Kjeldahl Method for Organic Nitrogen, Academic Press: NewYork, (1965); Kalthoff, I. M., and Sandell, E. B., Quantitative Inorganic Analysis, MacMillan: NewYork, (1948).

Table 2.3.2 Methods Used for Analyses (continued)

Component	Analytical Method
Vitamin B1	AOAC 942.23, 953.17 + 957.17
Vitamin B2	AOAC 940.33 + 960.46 and based on the reference below [b]
Folic Acid	AOAC 960.46 + 992.05 and based on the reference below [c]
Vitamin A (β-Carotene)	AOAC 941.15 and HPLC method based on the references below [d]
α, β, γ and δ Tocopherols	HPLC method based on the references below [e]
Vitamin K	AOAC 992.27
Isoflavones	AOAC 2001.10
Raffinose, Stachyose	GC-FID method based on the references below [f]
Phytic Acid	HPLC method based on references below [g]
Trypsin Inhibition	AOCS Ba 12-75
Lectin	Photometric method based on references below [h]
Total Amino Acids	AOAC 982.30
Total Fatty Acids	AOCS Ce 1-62 + Ce1b-89

[b] The US Pharmacopeia, 29th revision, p 1913.

[c] Methods of analysis of infant formula, Infant Formula Council Atlanta, Georgia, Section C-2 (1985).

[d] Quackenbush F.W. Journal of Lipid Chromatography, 10: 643-653 (1987)

[e] Speek, A. J., Schijver, J., and Schreurs, W. H. P., "Vitamin E Composition of Some Seed Oils as Determined by High-Performance Liquid Chromatography with Fluorometric Quantitation," Journal of Food Science, 50(1):121-124, (1985); Cort, W. M., Vincente, T. S., Waysek, E. H., and Williams, B. D., "Vitamin E Content of Feedstuffs Determined by High-Performance Liquid Chromatographic Fluorescence," Journal of Agricultural and Food Chemistry, 31:1330-1333, (1983); McMurray, C. H., Blanchflower, W. J., and Rice, D. A., "Influence of Extraction Techniques on Determination of α-Tocopherol in Animal Feedstuffs," Journal of the Association of Official Analytical Chemists, 63(6):1258-1261, (1980).

[f] Brobst, K.M., "Gas-Liquid Chromatography of Trimethylsilyl Derivatives", Methods in Carbohydrate Chemistry, Vol 6, Academic Press: New York, (1992); Mason, B.S. and Slover, H.T., "A gas chromatographic method for the determination of sugars in foods", J. Agr. Food Chem., 19(3): 551-554, (1971).

[g] Lehrfeld, Jacob, "HPLC Separation and Quantitation of Phytic Acid and Some Inositol Phosphates in Foods: Problem and Solutions," Journal of Agricultural and Food Chemistry, 42:2726-2731, (1994); Lehrfeld, Jacob, "High-Performance Liquid Chromatography Analysis of Phytic Acid on a pH-Stable, Macroporous Polymer Column," Cereal Chemistry, 66(6):510-515, (1989).

[h] Klurfeld, D.M. and Kritchevsky, D., "Isolation and quantification of lectins from vegetable oils", Lipids, 22: 667-668, (1987); Liener, I.E., "The photometric determination of the hemagglutinating activity of soyin and crude soybean extracts", Arch. Biochem. Biophys., 54: 223-231, (1955).

2.4 Statistical Evaluation of the Compositional Analyses of Soybean Seed

The results of the composition analyses of soybean seeds are listed in Appendix 1 to the report of study DQ08B009 (Mackie, 2009). The different regimen and herbicide treatments of regimens are indicated in Table 2.4.1.

Table 2.4.1 <u>Description and Herbicide Treatment of Regimen</u>

Regimen	Description	Herbicide Treatment
A	Non-transgenic Comparator Jack	not sprayed with test herbicides
B	Transgenic FG72	not sprayed with test herbicides
C	Transgenic FG72	sprayed with glyphosate and IFT
D	Stine 2686-6	not sprayed with test herbicides
E	Stine 2788	not sprayed with test herbicides
F	Stine 3000-0	not sprayed with test herbicides

In total 120 soybean seed samples from 10 sites were analysed for 62 components (parameters). The extensive data enabled a sound statistical evaluation. The evaluation and conclusions reached are presented in this and the following sections of the assessment report.

The study data were provided by BCS USA in one excel file after outlier checks were performed for each component according to Grubbs. None of the values were excluded as 'outliers' from analysis. This file was sent to an external biometrician (Dipl. Mathematician Vera Rattemeyer-Matschurat) and transformed into a SAS data set. All further analysis was performed using SAS version 8.2 (WINDOWS XP).

Beta tocopherol and the isoflavone glycitein could not be quantified in any of the samples. The fatty acids C08:0, C10:0, C12:0, C14:0, C14:1, C15:0, C15:1, C16:1, C17:1, C18:3 (gamma), C18:4, C20:2, C20:3, C20:4, C20:5, C22:1, C22:5 and C22:6 were below the limit of quantification (LOQ = 0.02 % fw.) in all soybean seed samples, too. The fatty acid C17:0 was only quantified in 12 of the 120 samples. These components were not further statistically analysed. The data sets of these components (with the exception of margaric acid [C17:0]) are assumed to be equivalent.

Additional components could not be quantified in a number of samples. The sites that were excluded from the statistical analyses are listed in Table 2.4.2.

For calculations values below the limit of quantification (LOQ, fresh conversion) were substituted as follows:

Sodium:	< 0.01 ppm	→	0.01 %mg/kg dm
Vitamin A:	< 0.20 ppm	→	0.20 mg/kg dm
Vitamin K:	< 0.10 ppm	→	0.10 mg/kg dm
Isoflavones:	< 10 ppm	→	10 mg/kg dm
Fatty acids:	< 0.02 %fw	→	0.10 %rel

Table 2.4.2 **Sites Excluded from Statistical Analyses**

Component	Value < LOQ		Sites Excluded from Analysis
	Yes (N)	No (N)	
Sodium	65	55	None
Vitamin A	90	30	None
Vitamin K	22	98	None
β-Tocopherol	120	-	All sites
Daidzein	90	30	None
Glycitein	120	-	All sites
Genistein	60	90	None
C17:0 Margaric Acid	108	12	All sites
C24:0 Lignoceric Acid	58	47	None

Analysis of differences

Descriptive statistics: For each component and each regimen (see Table 2.4.1) mean values, standard deviations, minimum and maximum were calculated per site and over all sites. These data are presented together with the frequencies of non-missing values in Tables 1a to 1g of Appendix A to the statistical report (Rattemeyer, 2009). The commercial lines (regimens D, E and F) were analysed only descriptively.

Over all sites analysis: For each component the data were analysed with analysis of variance (ANOVA) methods using a model with fixed factors REGIMEN (A, B and C) and SITE (for location) as well as their interaction term. Based on the ANOVA model regimen differences (A versus B and A versus C) were estimated and presented together with 95% confidence intervals. Individual regimen comparisons are only valid in cases of significant overall regimen effects (p-value from ANOVA < 0.05) and no regimen*site interactions (p-value from ANOVA ≥ 0.05). The results of the over all analyses are presented in Table 1 of Appendix A to this report and in Tables 2a to 2g of Appendix A to the statistical report (Rattemeyer, 2009).

By site analysis: In cases of interaction between the factors REGIMEN and SITE in the over all sites analyses (p-value ANOVA R*S < 0.05), the results of the by-site analyses are reported. In addition, the statistical analysis was presented on a by-site basis, if the outcomes from the over all sites analyses were different for the comparison of the non-transgenic regimen with the two transgenic regimens. For each component, the analysis was performed on a by-site basis with ANOVA methods including the factor REGIMEN followed by t-tests A versus B and A versus C. Based on the ANOVA, regimen differences and 95% confidence intervals were estimated. Table 2 of Appendix A to this report and Tables 3a-3g of Appendix A to the statistical report (Rattemeyer, 2009) are listing the findings of the statistical tests.

Level of significance: The level of significance is fixed as 0.05 (two-sided). When many hypothesis tests are performed on the same data set, particularly on a related set of outcomes, there is a greatly increased probability of declaring any one of the components falsely as significantly different under the null hypothesis. Results should be interpreted carefully.

More details of the analyses are presented in the statistical report (Rattemeyer, 2009).

2.5 Results of the Statistical Evaluation

Over all sites analysis

For nine components the over all sites analysis is not valid, since significant regimen*site interactions (p-value ANOVA regimen*site interaction < 0.05 in Table 1 of Appendix A) were detected. In consequence, the proof of substantial equivalence must be done on the basis of the by site analysis for those components (see by-site analysis below).

For most of the components no significant differences were found between regimen mean values over all sites (p-value ANOVA regimen effect ≥ 0.05 in Table 1 of Appendix A). Substantial equivalence can be stated between the data sets of the non-transgenic and transgenic groups for these components.

Overall regimen effects (p-value ANOVA regimen effect < 0.05 in Table 1 of Appendix A) were noticed for ash, calcium, magnesium, sodium, vitamin B1, γ-tocopherol, total tocopherols, raffinose, trypsin inhibitor, glycitin, genistin, total isoflavones and most fatty acids so that the individual treatment comparisons had to be evaluated.

The t-tests showed significant differences (p-value in t-test < 0.05 in Table 1 of Appendix A) in both individual treatment comparisons (A versus B and A versus C) for ash, calcium, magnesium, sodium, total tocopherols, raffinose, glycitin, genistin, and all of the indicated fatty acids. The outcome of the t-tests is ambiguous for vitamin B1, γ-tocopherol and total isoflavones, since it is different for the two treatment comparisons (p-values in t-tests < 0.05 only in one comparison A versus B or A versus C; see Table 1 of Appendix A). For these three components, the proof of substantial equivalence will be based in addition on the results of the by site analysis. Although overall regimen effects were detected, no significant differences were found for trypsin inhibitor in the comparisons between regimen A versus B and A versus C.

By-site analysis

Due to regimen*site interactions (p-value ANOVA regimen*site interaction < 0.05 in Table 1 of Appendix A) in the over all sites analysis, regimen comparisons are not valid for carbohydrates, potassium, vitamin A, vitamin K, α-tocopherol, δ-tocopherol, genistein, serine, and lignoceric acid (C24:0). Therefore, the statistical test was performed on a by-site basis. The data evaluation was repeated as a by-site analysis for the components vitamin B1, γ-tocopherol and total isoflavones, because the results of the over all sites analysis were ambiguous.

For all components mentioned above, the results of the by-site analysis (Table 2 of Appendix A) showed that there is no majority of sites at which statistically significant differences occurred so that the data sets can be considered equivalent.

The biological and nutritional relevance of the statistical findings is discussed in the following section.

2.6 Comparison with Reference Ranges from Analyses of Commercial Soybeans and from Literature; Evaluation of the Statistical Analysis

The next seven tables show the comparison of the results pooled from all sites neglecting the environmental effects at the single sites with reference ranges calculated from three commercial soybean lines analysed together with the FG72 and Jack seeds and compiled from a number of reference volumes provided in Appendix B to this report.

Most mean values are inside the reference ranges calculated with the results from the three commercial soybean lines and from the respective soybean literature. Since some samples had no detectable vitamin A and K levels a range including the minimum and maximum result was built for these two nutrients. Both ranges were slightly falling short of the literature ranges. However, it should be taken into account that only few reference values were found for vitamin A and K contents in soybean seeds. The mean value for glycitin and palmitoleic acid were slightly different to the range calculated from the three analysed soybean lines; but they were within the ranges reported in literature.

Statistically significant differences were found for a number of components in the over-all sites analyses. The mean values of all of these components are within the reference ranges from literature, and only the mean values of glycitin and palmitoleic acid (C16:0) were slightly different to the range calculated from the results of the three tested commercial soybean lines.

The estimated differences between regimens are very small for most components. They are very often lower than the variation (SD) inside the non-transgenic control group. This is true for ash, sodium, total tocopherols, raffinose and genistin. In case of the minor fatty acids (contents < 1% rel.) the differences between regimen mean values are ≤ 0.01 % rel. which is the LOQ of the analytical method.

The statistically significant differences in total tocopherol contents are negligible, because no differences were detected for the single tocopherols.

Summarising the statements made above, there is no safety issue related to the consumption of FG72 soybean seed, since the contents of all nutrients and anti-nutrients are comparable to the contents in seeds from other commercial soybean lines even though statistically differences were found between the non-transgenic and transgenic seeds. The relevance of the statistically significant differences found between the non-transgenic control and the transgenic event FG72 is negligible from a biological and nutritional standpoint.

Table 2.6.1 **Proximate and Fibre Components in Soybean Seed of Event FG72 and the Non-Transgenic Counterpart Jack Compared to Commercial Soybean Lines (Reference Ranges)**

Component	Non-Transgenic Not treated with Test Herbicides	Transgenic Not treated with Test Herbicides	Transgenic Treated with Test Herbicides	Range Commercial Soybean Lines [b]	Reference Range [c]
	Mean ± SD	Mean ± SD	Mean ± SD		
Moisture % fw	9.51 ± 0.82	9.65 ± 0.84	9.45 ± 0.83	8.00 – 10.60	5.6 – 12
Protein % dm	38.2 ± 1.1	38.2 ± 0.8	38.1 ± 0.9	35.8 – 40.1	32 – 45.5
Total Fat % dm	19.3 ± 0.9	18.9 ± 1.2	19.2 ± 1.1	15.1 – 21.4	8.1 – 24.7
Ash % dm	5.24 ± 0.31	5.07 ± 0.30	5.06 ± 0.28	4.89 – 5.73	3.9 – 7.0
Total carb. % dm [a]	37.3 ± 1.2	37.9 ± 1.0	37.6 ± 1.2	34.8 – 41.6	29.6 – 50.2
ADF % dm	17.8 ± 1.9	18.1 ± 2.0	17.9 ± 1.8	13.6 – 23.5	7.8 – 18.6
NDF % dm	19.8 ± 2.0	20.3 ± 2.1	20.0 ± 1.5	16.1 – 24.8	5.0 – 21.3

[a] Total carbohydrates calculated as 100% - (protein %dm + fat %dm + ash %dm)

[b] Reference ranges of the 3 analysed commercial soybean lines taken from Table 1a of Appendix A to the statistical report (Rattemeyer, 2009)

[c] Reference ranges from Table 2 of Appendix B

Table 2.6.2 **Minerals in Soybean Seed of Event FG72 and the Non-Transgenic Counterpart Jack Compared to Commercial Soybean Lines (Reference Ranges)**

Component	Based on Dry Matter				
	Non-Transgenic Not treated with Test Herbicides	Transgenic Not treated with Test Herbicides	Transgenic Treated with Test Herbicides	Range Commercial Soybean Lines [b]	Reference Range [b]
	Mean ± SD	Mean ± SD	Mean ± SD		
Calcium (%)	0.282 ± 0.023	0.258 ± 0.024	0.259 ± 0.026	0.212 – 0.347	0.12 – 0.34
Phosphorus (%)	0.626 ± 0.053	0.618 ± 0.062	0.620 ± 0.065	0.499 – 0.651	0.49 – 0.94
Potassium (%)	1.93 ± 0.08	1.85 ± 0.08	1.85 ± 0.09	1.84 – 2.11	1.4 – 2.3
Magnesium (%)	0.241 ± 0.010	0.226 ± 0.012	0.226 ± 0.010	0.197 – 0.263	0.21 – 0.32
Sodium (%)	< 0.010 – 0.020	< 0.010 – 0.040	< 0.010 – 0.040	<0.010 – 0.026	0.002 – 0.02
Iron (mg/kg)	93.3 ± 41.8	82.6 ± 13.3	84.1 ± 18.9	58.8 – 175.0	55.4 - 172

[a] Reference ranges of the 3 analysed commercial soybean lines taken from Table 1b of Appendix A to the statistical report (Rattemeyer, 2009)

[b] Reference ranges from Table 3a of Appendix B

Table 2.6.3 **Vitamins in Soybean Seed of Event FG72 and the Non-Transgenic Counterpart Jack Compared to Commercial Soybean Lines (Reference Ranges)**

Component	Mg/kg Dry Matter				
	Non-Transgenic Not treated with Test Herbicides	Transgenic Not treated with Test Herbicides	Transgenic Treated with Test Herbicides	Range Commercial Soybean Lines [b]	Reference Range [b]
	Mean ± SD	Mean ± SD	Mean ± SD		
Vitamin B1	3.59 ± 0.76	3.44 ± 0.95	3.16 ± 0.91	1.60 – 4.70	1.01 – 16.02
Vitamin B2	4.42 ± 0.88	4.52 ± 0.89	4.80 ± 0.84	3.36 – 6.38	1.9 – 14.5
Folic Acid	2.976 ± 0.353	3.068 ± 0.300	3.122 ± 0.344	2.19 – 4.33	2.4- -4.7
Vitamin A (β-Carotene)	< 0.200 – 0.400	< 0.200 – 0.573	< 0.200 – 0.566	< 0.100	0.26 – 4.37
α-Tocopherol	17.4 ± 3.9	19.0 ± 5.1	20.7 ± 5.8	12.2 – 24.9	2 – 70
β-Tocopherol	< 5.00	< 5.00	< 5.00	< 5.00	0 – 7
γ-Tocopherol	195 ± 16	200 ± 14	198 ± 11	153 – 237	18 – 461
δ-Tocopherol	74.1 ± 7.4	75.2 ± 8.3	74.0 ± 11.1	41.5 – 99.2	31 – 186
Total Tocopherols	286 ± 16	294 ± 14	293 ± 13	225 - 346	120 - 674
Vitamin K	< 0.100 – 0.326	< 0.100 – 0.388	< 0.100 – 0.435	< 0.200 – 0.263	0.38 – 0.51

[a] Reference ranges of the 3 analysed commercial soybean lines taken from Table 1c of Appendix A to the statistical report (Rattemeyer, 2009)

[b] Reference ranges from Tables 3a and 3b of Appendix B

Table 2.6.4 **Isoflavones in Soybean Seeds of Event FG72 and the Non-Transgenic Counterpart Jack Compared to Commercial Soybean Lines (Reference Ranges)**

Component	Mg/kg Dry Matter				
	Non-Transgenic Not treated with Test Herbicides	Transgenic Not treated with Test Herbicides	Transgenic Treated with Test Herbicides	Range Commercial Soybean Lines [b]	Reference Range [b]
	Mean ± SD	Mean ± SD	Mean ± SD		
Daidzein	< 10 – 17.5	< 10 – 15.1	< 10 – 14.6	< 10 – 14.0	5 – 35
Glycitein	< 10	< 10	< 10	< 10	1.1 – 80
Genistein	< 10 – 17.2	< 10 – 15.7	< 10 – 12.2	< 10 – 20.6	0.3 – 46
Daidzin	1035 ± 350	1034 ± 356	994 ± 357	568 – 2530	60.0 – 2454
Glycitin	365 ± 39	414 ± 43	400 ± 56	142 – 315	15.3 – 1070
Genistin	1817 ± 482	1682 ± 465	1640 ± 446	1130 – 3290	144 – 2837
Total Isoflavones	2010 ± 522	1953 ± 507	1891 ± 488	1160 - 3390	679 – 3733

[a] Reference ranges of the 3 analysed commercial soybean lines taken from Table 1e of Appendix A to the statistical report (Rattemeyer, 2009)

[b] Reference range taken from Table 4 of Appendix B

Table 2.6.5 **Anti-nutrients in Soybean Seeds of Event FG72 and the Non-Transgenic Counterpart Jack Compared to Commercial Soybean Lines (Reference Ranges)**

Component	Based on Dry Matter				
	Non-Transgenic Not treated with Test Herbicides	Transgenic Not treated with Test Herbicides	Transgenic Treated with Test Herbicides	Range Commercial Soybean Lines [b]	Reference Range [b]
	Mean ± SD	Mean ± SD	Mean ± SD		
Raffinose (%)	0.361 ± 0.036	0.378 ± 0.053	0.379 ± 0.058	0.290 – 0.504	0.11 – 1.28
Stachyose (%)	2.49 ± 0.24	2.42 ± 0.18	2.50 ± 0.19	2.23 – 2.96	1.21 – 6.30
Phytic Acid (%)	1.40 ± 0.16	1.37 ± 0.23	1.35 ± 0.23	0.96 – 1.50	0.63 – 2.74
Trypsin inhibitor (TIU/mg)	33.0 ± 6.6	30.1 ± 6.1	33.9 ± 5.7	23.5 – 60.1	19.59 – 118
Lectin (HU/mg)	1.74 ± 0.60	1.40 ± 0.50	1.54 ± 0.42	0.46 – 8.63	0.11 - 129

[a] Reference ranges of the 3 analysed commercial soybean lines taken from Table 1d of Appendix A to the statistical report (Rattemeyer, 2009)

[b] Reference range taken from Table 5 of Appendix B

Table 2.6.6 **Total Amino Acids in Soybean Seeds of Event FG72 and the Non-Transgenic Counterpart Jack Compared to Commercial Soybean Lines (Reference Ranges)**

Total Amino Acid	% dry matter				
	Non-Transgenic Not treated with Test Herbicides	Transgenic Not treated with Test Herbicides	Transgenic Treated with Test Herbicides	Range Commercial Soybean Lines [a]	Reference Range [b]
	Mean ± SD	Mean ± SD	Mean ± SD		
Alanine	1.68 ± 0.04	1.68 ± 0.04	1.68 ± 0.04	1.55 – 1.78	1.51 – 2.10
Arginine	2.94 ± 0.10	2.97 ± 0.10	2.95 ± 0.10	2.69 – 3.13	2.17 – 3.40
Aspartic acid	4.40 ± 0.12	4.38 ± 0.12	4.37 ± 0.13	4.06 – 4.67	3.81 – 5.12
Cystine	0.58 ± 0.03	0.58 ± 0.02	0.59 ± 0.03	0.50 – 0.63	0.37 – 0.81
Glutamic acid	6.75 ± 0.21	6.77 ± 0.23	6.74 ± 0.22	6.32 – 7.23	5.84 – 8.20
Glycine	1.68 ± 0.04	1.68 ± 0.04	1.68 ± 0.04	1.53 – 1.76	1.46 – 2.27
Histidine	1.05 ± 0.03	1.05 ± 0.03	1.05 ± 0.03	0.93 – 1.07	0.84 – 1.22
Isoleucine	1.81 ± 0.05	1.80 ± 0.05	1.79 ± 0.05	1.62 – 1.96	1.54 – 2.32
Leucine	2.99 ± 0.08	2.99 ± 0.08	2.98 ± 0.08	2.71 – 3.13	2.2 – 4.0
Lysine	2.48 ± 0.05	2.48 ± 0.06	2.47 ± 0.06	2.34 – 2.64	1.55 – 2.84
Methionine	0.54 ± 0.02	0.54 ± 0.02	0.54 ± 0.02	0.50 – 0.58	0.43 – 0.76
Phenylalanine	1.97 ± 0.05	1.98 ± 0.06	1.96 ± 0.06	1.83 – 2.08	1.60 – 2.39
Proline	1.82 ± 0.07	1.83 ± 0.07	1.82 ± 0.07	1.71 – 1.94	1.69 – 2.33
Serine	1.97 ± 0.07	1.98 ± 0.08	1.99 ± 0.06	1.77 – 2.13	1.11 – 2.48
Threonine	1.55 ± 0.04	1.54 ± 0.04	1.53 ± 0.04	1.44 – 1.62	1.14 – 1.89
Tryptophan	0.45 ± 0.03	0.44 ± 0.03	0.44 ± 0.03	0.39 – 0.54	0.36 – 0.67
Tyrosine	1.40 ± 0.04	1.40 ± 0.04	1.40 ± 0.04	1.32 – 1.48	0.10 – 1.61
Valine	1.89 ± 0.06	1.88 ± 0.05	1.87 ± 0.06	1.66 – 2.03	1.50 – 2.44

[a] Reference ranges of the 3 analysed commercial soybean lines taken from Table 1f of Appendix A to the statistical report (Rattemeyer, 2009)

[b] Reference range taken from Table 6 of Appendix B

Table 2.6.7 **Total Fatty Acids in Soybean Seeds of Event FG72 and the Non-Transgenic Counterpart Jack Compared to Commercial Soybean Lines (Reference Ranges)**

Total Fatty Acid	% relative				
	Non-Transgenic Not treated with Test Herbicides	Transgenic Not treated with Test Herbicides	Transgenic Treated with Test Herbicides	Range Commercial Soybean Lines [c]	Reference Range [d]
	Mean ± SD	Mean ± SD	Mean ± SD		
Saturated					
C16:0 (palmitic)	10.06 ± 0.22	9.34 ± 0.17	9.38 ± 0.23	9.78 – 11.40	7 – 16
C17:0 (margaric) [a]	< 0.10 – 0.12	< 0.10 – 0.11	< 0.10 – 0.11	< 0.10 – 0.12	< 0.10 – 0.15
C18:0 (stearic)	4.28 ± 0.16	4.52 ± 0.19	4.51 ± 0.23	3.49 – 4.81	2 – 5.9
C20:0 (arachidic)	0.31 ± 0.02	0.32 ± 0.02	0.32 ± 0.02	0.25 – 0.35	< 0.10 – 0.48
C22:0 (behenic)	0.32 ± 0.01	0.33 ± 0.01	0.33 ± 0.02	0.25 – 0.35	0.28 – 0.60
C24:0 (lignoceric) [a]	< 0.10 – 0.16	< 0.10 – 0.17	< 0.10 – 0.17	< 0.10 – 0.15	0.15
Sum of Saturated	14.97 [b]	14.51 [b]	14.54 [b]	13.77 – 17.18	9.43 – 23.55
Mono-unsaturated					
C18:1 (oleic)	21.97 ± 1.05	24.65 ± 0.99	24.12 ± 0.90	21.10 – 24.10	14 – 34
C20:1 (eicosenoic)	0.16 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	< 0.10 – 0.18	0.14 – 0.35
Sum of Mono-unsaturated	22.13	24.81	24.29	21.10 – 24.28	14.14 – 34.83
Poly-unsaturated					
C18:2 (linoleic)	54.56 ± 0.90	52.65 ± 0.95	53.08 ± 0.82	51.50 – 55.40	48 – 60
C18:3 (alpha linolenic)	8.27 ± 0.50	7.94 ± 0.45	8.01 ± 0.48	7.59 – 10.30	2 – 10
Sum of Poly-unsaturated	62.83	60.59	61.09	59.09 – 65.70	50 - 70
Sum of all total fatty acids	99.93	99.91	99.92	-	-

[a] Some mean values were not calculated, since fatty acids were not quantified (LOQ <0.10% rel)
[b] Sum of saturated fatty acids calculated excluding ranges for C17:0 and C24:0
[c] Reference ranges of 3 analysed commercial soybean lines taken from Table 1g of Appendix A to the statistical report (Rattemeyer, 2009)
[d] Reference range taken from Table 7 of Appendix B

3 COMPOSITIONAL AND NUTRITIONAL ANALYSES OF SOYBEAN PRODUCTS

Approximately 32 kg soybean seeds from Jack and the two FG72 regimens were processed into hulls, meal, toasted meal, protein isolate, crude oil, refined, bleached and deodorized oil (food grade oil) and crude lecithin. Sub-samples of the processed products were analysed for their chemical composition at Covance Laboratories Inc. The results of the compositional analyses are presented in the following sections of this report. Further details from the composition analyses of the soybean products can be found in the report to study DQ09B009 (Kowite, 2009d).

3.1 Compositional Analyses of Hulls from Soybean Event FG72 and the Respective Conventional Soybean Hulls

The results of the soybean hulls analyses are presented in the Table 3.1.1 below. The transgenic hulls have distinct higher protein and total fat contents at the cost of the total carbohydrates, ADF and NDF contents.

The comparison with reference ranges from literature shows differences for most components. Only the ash content of the tested hull samples was within the reported ranges. The moisture, protein and fat contents of the hull samples are significant higher compared to the reported ranges. Consequently, total carbohydrates and fibre amounts are lower. This can be observed for the transgenic as well as for the non-transgenic hull samples and, is therefore not caused by the genetic modification of the soybean plant.

The reason for the differences between the transgenic and non-transgenic hull samples, but also between the analysed and reported contents might be the incomplete separation between the hull and cotyledon part of the soybean seed. Remaining endosperm and germ tissue of the cotyledons in the hull samples leads to a higher protein and fat content in the hull fraction, and to decreased contents for the other components.

Table 3.1.1 **Proximate and Fibre Components [a] in Hulls of Event FG72 and Non-transgenic Counterpart Jack Compared to Commercial Soybean Lines (Reference Ranges)**

Component	Non-Transgenic Not treated with Test Herbicides	Transgenic Not treated with Test Herbicides	Transgenic Treated with Test Herbicides	Reference Range [c]
Moisture (% fw)	11.1	11.6	11.1	9.0
Protein (% dm)	19.3	23.8	24.6	10.8 - 13.7
Total Fat (% dm)	7.04	10.0	10.4	2.0 - 2.2
Ash (% dm)	4.89	5.07	5.13	4.6 - 9.8
Total Carbohydrates (% dm) [b]	68.7	61.1	59.8	76.0 - 82.6
ADF (% dm)	37.9	30.9	31.0	42.4 - 50.0
NDF (% dm)	51.7	39.7	39.0	59.4 - 67.0

[a] Data from study DQ09B009 (Kowite, 2009d)
[b] Total Carbohydrates calculated as 100% - (crude protein %dm + crude fat %dm + ash %dm)
[c] Reference ranges from Table 8 in Appendix B

3.2 Compositional Analyses of Untoasted Meal from Soybean Event FG72 and the Respective Conventional Untoasted Soybean Meal

The nutrient and anti-nutrient contents determined in the meal samples obtained from FG72 and Jack seeds are comparable (see Tables 3.2.1 – 3.2.3). A major difference was only detected between the genistein contents.

Table 3.2.1 Proximate and Fibre Components [a] in Untoasted Meal of Event FG72 and Non-transgenic Counterpart Jack Compared to Commercial Soybean Lines (Reference Ranges)

Component	Non-Transgenic Not treated with Test Herbicides	Transgenic Not treated with Test Herbicides	Transgenic Treated with Test Herbicides	Reference Range [c]
Moisture (% fw)	11.0	8.83	9.51	6.9 - 10.2
Protein (% dm)	51.6	50.9	51.3	52.8 - 56.7
Total Fat (% dm)	0.734	0.584	0.671	1.0 - 3.3
Ash (% dm)	7.26	7.11	7.17	5.2 - 9.1
Total Carbohydrates (% dm) [b]	40.4	41.5	40.9	31.3 - 41.0
ADF (% dm)	5.10	6.39	6.27	5.2 - 6.9
NDF (% dm)	8.07	9.53	8.00	7.4 - 12.2

[a] Data from study DQ09B009 (Kowite, 2009d)
[b] Total Carbohydrates calculated as 100% - (crude protein %dm + crude fat %dm + ash %dm)
[c] Reference ranges from Table 9 in Appendix B

Table 3.2.2 Isoflavones and Anti-nutrients [a] in Untoasted Meal of Event FG72 and Non-transgenic Counterpart Jack Compared to Commercial Soybean Lines (Reference Ranges)

Component	Based on dry matter			
	Non-Transgenic Not treated with Test Herbicides	Transgenic Not treated with Test Herbicides	Transgenic Treated with Test Herbicides	Reference Range [d]
Daidzein (mg/kg)	48.9	47.1	50.9	5 - 35
Daidzin (mg/kg) [b]	1380	1220	1290	60.0 - 2454
Genistein (mg/kg)	63.1	43.9	47.5	0.3 - 46
Genistin (mg/kg) [b]	2390	1960	2230	144 - 2837
Glycitein (mg/kg)	15.6	20.0	16.5	1.1 - 80
Glycitin (mg/kg) [b]	435	414	412	15.3 - 1070
Total Isoflavones (mg/kg) [c]	2740	2350	2560	679 - 3733
Raffinose (%)	0.796	0.853	0.914	0.11 - 1.28
Stachyose (%)	5.34	5.34	5.07	1.21 - 6.30
Phytic acid (%)	2.07	2.09	2.10	0.63 - 2.74
Trypsin Inhibition (TIU/mg)	7.5	5.46	6.27	23.9 – 52.1
Lectins (HU/mg)	0.116	< 0.10	0.318	2.8 - 2.9 (0.11 - 129 [e])

[a] Data from study DQ09B009 (Kowite, 2009d)
[b] Sum of daidzein, genistin or glycitin glucosides and esters
[c] Sum of aglycones and glucosides/esters reported as aglycone equivalents
[d] Reference ranges from Tables 4, 5 and 10 of Appendix B
[e] Lectin reference range for soybean seeds (Table 7 of Appendix B)

In general, the analysed meal samples show the same nutritional values as commercial soybean meals. Slightly different amounts were found for protein, total fat, trypsin inhibition, daidzein, and some amino acids. But this is the case for the transgenic and the non-transgenic meal samples and, is not caused by the genetic modification of the soybean plant. The determined lectin values are lower than the reange reported for not toasted commercial meal. However, compared to the range reported for soybean seeds there are not different. The genistein value of the meal from Jack seeds is exceeding the reference range, so it might be that the deviation between the transgenic and non-transgenic meal samples is due to the major findings in the non-transgenic sample.

Table 3.2.3 **Total Amino Acid Composition [a] of Untoasted Meal of Event FG72 and Non-transgenic Counterpart Jack Compared to Commercial Soybean Lines (Reference Ranges)**

Total Amino Acid	% Dry matter			
	Non-Transgenic Not treated with Test Herbicides	Transgenic Not treated with Test Herbicides	Transgenic Treated with Test Herbicides	Reference Range [b]
Alanine	2.31	2.26	2.24	2.27
Arginine	4.09	4.06	3.89	3.38 – 4.11
Aspartic Acid	6.07	5.90	5.87	6.07
Cystine	0.731	0.738	0.755	0.66 – 0.83
Glutamic Acid	9.40	9.22	9.15	9.35
Glycine	2.29	2.26	2.23	2.03 – 2.58
Histidine	1.44	1.40	1.39	1.19 – 1.44
Isoleucine	2.53	2.45	2.4	2.15 – 2.89
Leucine	4.09	3.99	3.98	3.65 – 4.22
Lysine	3.34	3.27	3.27	2.99 – 3.53
Methionine	0.730	0.715	0.739	0.58 – 0.79
Phenylalanine	2.76	2.68	2.67	2.33 – 2.58
Proline	2.39	2.39	2.39	2.82
Serine	2.57	2.52	2.54	2.36 – 3.22
Threonine	2.10	2.05	2.03	1.85 – 2.12
Tryptophan	0.592	0.524	0.561	0.66 – 0.74
Tyrosine	1.92	1.82	1.72	1.46 – 2.22
Valine	2.67	2.61	2.60	2.24 – 3.00

[a] Data from study DQ09B009 (Kowite, 2009d)

[b] Reference ranges from Table 11 in Appendix B

3.3 Compositional Analyses of Toasted Meal from Soybean Event FG72 and the Respective Conventional Toasted Soybean Meal

In general, the composition analyses of the meal samples after toasting confirm the findings from analyses of the not toasted meal: the nutrient and anti-nutrient contents in FG72 and Jack meal samples are not different (see Tables 3.3.1 – 3.3.3).

Table 3.3.1 **Proximate and Fibre Components [a] in Toasted Meal of Event FG72 and Non-transgenic Counterpart Jack Compared to Commercial Soybean Lines (Reference Ranges)**

Component	Non-Transgenic Not treated with Test Herbicides	Transgenic Not treated with Test Herbicides	Transgenic Treated with Test Herbicides	Reference Range [c]
Moisture (% fw)	17.6	11.3	11.0	-
Protein (% dm)	51.5	50.2	50.8	52.8 – 56.7
Total Fat (% dm)	0.498	3.20	0.573	1.0 – 3.3
Ash (% dm)	7.18	6.99	7.20	5.2 – 9.1
Total Carbohydrates (% dm) [b]	40.9	39.7	41.5	31.3 – 41.0
ADF (% dm)	5.83	7.08	5.35	5.2 – 6.9
NDF (% dm)	8.75	10.1	8.66	7.4 – 12.2

[a] Data from study DQ09B009 (Kowite, 2009d)
[c] Total Carbohydrates calculated as 100% - (crude protein %dm + crude fat %dm+ ash %dm)
[b] Reference ranges from Table 9 in Appendix B

Table 3.3.2 **Anti-nutrients and Isoflavones [a] in Toasted Meal of Event FG72 and Non-transgenic Counterpart Jack Compared to Commercial Soybean Lines (Reference Ranges)**

Component	Based on Dry Matter			
	Non-Transgenic Not treated with Test Herbicides	Transgenic Not treated with Test Herbicides	Transgenic Treated with Test Herbicides	Reference Range [d]
Daidzein (mg/kg)	58.0	37.2	53.6	5 – 35
Daidzin (mg/kg) [b]	1410	1290	1240	60.0 – 2454
Genistein (mg/kg)	49.6	36.8	58.4	0.3 – 46
Genistin (mg/kg) [b]	2460	2070	2080	144 – 2837
Glycitein (mg/kg)	15.9	16.0	18.5	1.1 – 80
Glycitin (mg/kg) [b]	483	578	404	15.3 – 1070
Total Isoflavones (mg/kg) [c]	2830	2540	2440	679 – 3733
Raffinose (%)	0.811	0.847	0.927	0.11 – 1.28
Stachyose (%)	5.30	5.21	5.22	1.21 – 6.30
Phytic acid (%)	1.94	1.84	1.93	0.63 – 2.74
Trypsin Inhibition (TIU/mg)	< 1.00	1.77	3.02	3.0 – 7.9
Lectins (HU/mg)	< 0.10	< 0.10	< 0.10	< 0.5

[a] Data from study DQ09B009 (Kowite, 2009d)
[b] Sum of daidzein, genistin or glycitin glucosides and esters
[c] Sum of aglycones and glucosides/esters reported as aglycone equivalents
[d] Reference ranges from Tables 4, 5 and 10 of Appendix B

The moisture content of the non-transgenic meal sample and the fat content of the transgenic meal sample derived from not treated FG72 seeds are deviating from all other results determined in meal before and after toasting. The two results might be seen as outliers. The differences between genistein contents were not observed anymore in the meal samples after toasting.

The reason why soybean meal is toasted before it is fed to livestock is to destroy the heat labile anti-nutrients, like trypsin inhibitors and lectins (Liener. 1994). It is expected that the contents of these two anti-nutrients decrease significantly (up to 90%) after heat treatment of the meal. The other anti-nutritional factors like the oligosaccharides and phytin acid, and the bio-active isoflavones are not affected by the toasting, they are known to be heat stable (Liener. 1994).

And in fact, comparing the results between untoasted and toasted meal derived from FG72 and Jack soybean seeds it can be seen that the trypsin inhibitors and lectins are susceptible to heat treatment, and that their activity is clearly decreased after toasting of the three meal samples. The contents of the heat stable components did not change in the meals before and after toasting. The transgenic modification has no influence on the sensitivity of the endogenous trypsin inhibitors and lectins to heat.

Compared to the ranges from literature, the determined protein, two of the fat, two of the trypsin inhibition, daidzein, two of the genistein, and some amino acids contents are again slightly different. But as was said before, this is the case for the transgenic and the non-transgenic toasted meal samples, and is not an unintended effect of the genetic modification of the soybean plant.

Table 3.3.3 **<u>Total Amino Acid Composition [a] of Toasted Meal of Event FG72 and Non-transgenic Counterpart Compared to Commercial Soybean Lines (Reference Ranges)</u>**

Total Amino Acid	% Dry matter			
	Non-Transgenic Not treated with Test Herbicides	Transgenic Not treated with Test Herbicides	Transgenic Treated with Test Herbicides	Reference Ranges [b]
Alanine	2.31	2.27	2.25	2.27
Arginine	4.08	4.00	3.90	3.38 – 4.11
Aspartic Acid	6.04	5.92	5.85	6.07
Cystine	0.738	0.699	0.719	0.66 – 0.83
Glutamic Acid	9.37	9.28	9.13	9.35
Glycine	2.28	2.28	2.24	2.03 – 2.58
Histidine	1.43	1.41	1.38	1.19 – 1.44
Isoleucine	2.51	2.46	2.44	2.15 – 2.89
Leucine	4.08	4.00	3.98	3.65 – 4.22
Lysine	3.29	3.21	3.19	2.99 – 3.53
Methionine	0.738	0.717	0.722	0.58 – 0.79
Phenylalanine	2.77	2.69	2.67	2.33 – 2.58
Proline	2.40	2.36	2.38	2.82
Serine	2.58	2.54	2.55	2.36 – 3.22
Threonine	2.10	2.06	2.04	1.85 – 2.12
Tryptophan	0.562	0.529	0.544	0.66 – 0.74
Tyrosine	1.95	1.75	1.78	1.46 – 2.22
Valine	2.67	2.63	2.60	2.24 – 3.00

[a] Data from study DQ09B009 (Kowite, 2009d)
[b] Reference ranges from Table 11 in Appendix B

3.4 Compositional Analyses of Protein Isolate from Soybean Event FG72 and the Respective Conventional Soybean Protein Isolate

Table 3.4.1 shows the results from protein isolate analyses. The nutrient contents are almost identical in the three samples, and most of the measured values are inside the reported ranges. The moisture content is significantly lower in the transgenic samples compared to the isolate from Jack seeds and to the reference range. The protein content of the transgenic isolates from treated FG72 seeds is slightly exceeding the reported range. Alanine, proline and trypsin inhibition results are falling slightly short of the literature range; but this is again true for all isolate samples.

Table 3.4.1 **Protein, Total Amino Acid and Anti-nutrient Contents [a] of Protein Isolate of Event FG72 and Non-transgenic Counterpart Jack Compared to Commercial Soybean Lines (Reference Ranges)**

Components	Based on Dry Matter			
	Non-Transgenic Not treated with Test Herbicides	Transgenic Not treated with Test Herbicides	Transgenic Treated with Test Herbicides	Reference Ranges [b]
Moisture (% fw)	4.87	1.92	2.10	5.0 – 8.0
Protein (% dm)	93.7	94.1	95.1	72.0 - 93.9
Alanine (% dm)	3.67	3.70	3.66	3.78 - 4.67
Arginine (% dm)	7.95	8.02	7.99	6.67 - 8.24
Aspartic Acid (% dm)	11.5	11.5	11.5	10.20 - 12.74
Cystine (% dm)	1.19	1.14	1.17	1.00 - 1.70
Glutamic Acid (% dm)	19.2	19.6	19.5	18.37 - 22.20
Glycine (% dm)	3.98	4.04	3.99	3.62 - 4.67
Histidine (% dm)	2.53	2.57	2.55	2.30 - 2.87
Isoleucine (% dm)	4.61	4.62	4.63	4.25 - 5.18
Leucine (% dm)	7.39	7.45	7.42	6.78 - 8.46
Lysine (% dm)	5.89	5.89	5.85	5.33 - 6.77
Methionine (% dm)	1.10	1.11	1.16	0.96 - 1.49
Phenylalanine (% dm)	5.15	5.18	5.15	4.59 - 5.71
Proline (% dm)	4.67	4.85	4.74	5.22 - 5.81
Serine (% dm)	4.85	4.91	4.86	4.83 - 6.05
Threonine (% dm)	3.37	3.40	3.35	3.14 - 4.46
Tryptophan (% dm)	0.999	1.03	1.02	0.96 - 1.59
Tyrosine (% dm)	3.55	3.54	3.54	3.38 - 4.14
Valine (% dm)	4.61	4.66	4.63	4.10 - 5.20
Trypsin Inhibition (TIU/mg)	< 1.00	< 1.00	1.60	3.0 – 7.9
Lectin (HU/mg)	0.962	0.472	0.359	< 0.5

[a] Data from study DQ09B009 (Kowite, 2009d)
[b] Reference ranges from Tables 12 and 10 (see ranges for toasted meal/flour) of Appendix B

3.5 Compositional Analyses of Crude Oil from Soybean Event FG72 and the Respective Conventional Soybean Crude Oil

The results of the fatty acid and fat-soluble vitamin analyses of crude soybean oil are given in the Tables 3.5.1 and 3.5.2.

The fatty acid profiles of the non-transgenic and transgenic oil samples are very similar. Small differences were noticed for oleic (C18:1) and linoleic acid (C18:2) contents. However, all determined fatty acid contents lie within the range reported from literature. Fatty acids not listed in the table below were found to be below the limit of quantification (< 0.06 % fresh weight).

Vitamin A and tocopherol contents are comparable between crude oil samples. The vitamin K content in the transgenic crude oil is slightly higher compared to the non-transgenic crude oil sample. The measured values for vitamin K and the tocopherols correspond very well with the reference ranges for food grade soybean oil. The vitamin A contents determined as β-carotene are lower than contents reported for soybean oil, but this was observed for all three crude oil samples.

Table 3.5.1 **Fatty Acid Composition [a] of Crude Oil of Event FG72 and Non-transgenic Counterpart Jack Compared to Commercial Soybean Oil (Reference Ranges)**

Total Fatty Acid	% Relative			
	Non-Transgenic Not treated with Test Herbicides	Transgenic Not treated with Test Herbicides	Transgenic Treated with Test Herbicides	Reference Range [b]
	Mean ± SD	Mean ± SD	Mean ± SD	
Saturated				
C14:0 (myristic)	0.0685	0.0706	< 0.06% fw	< 0.05 – 0.20
C16:0 (palmitic)	10.1	9.36	9.49	6.7 – 14.5
C17:0 (margaric)	0.110	0.107	0.110	< 0.05 – 0.10
C18:0 (stearic)	4.13	4.69	4.49	0.50 – 8.9
C20:0 (arachidic)	0.302	0.332	0.318	0.10 – 0.90
C22:0 (behenic)	0.320	0.339	0.335	< 0.05 – 0.70
C24:0 (lignoceric)	< 0.06% fw	0.106	0.106	< 0.05 – 0.50
Sum of Saturated	15.03	15.00	14.85	7.30 – 25.8
Mono-unsaturated				
C16:1 (palmitoleic)	0.0883	0.0966	0.0899	< 0.05 – 0.50
C18:1 (oleic)	21.5	25.3	23.8	14.3 – 34.0
C20:1 (eicosenoic)	0.152	0.158	0.159	0.14 – 0.35
Sum of Mono-unsaturated	21.74	25.55	24.05	14.44 – 34.85
Poly-unsaturated				
C18:2 (linoleic)	54.8	51.8	53.1	36.5 – 60.0
C18:3 (alpha linolenic)	8.38	7.63	7.92	1.9 – 14.7
Sum of Poly-unsaturated	63.18	59.43	61.02	38.4 – 74.7
Sum of all total fatty acids	99.95	99.98	99.92	-

[a] Data from study DQ09B009 (Kowite, 2009d)
[b] Reference ranges from Table 13 of Appendix B

Table 3.5.2 **Content of Fat-soluble Vitamins [a] in Crude Oil of Event FG72 and Non-transgenic Counterpart Jack Compared to Commercial Soybean Oil (Reference Ranges)**

Fat-soluble Vitamin	Content in mg/kg			
	Non-Transgenic Not treated with Test Herbicides	Transgenic Not treated with Test Herbicides	Transgenic Treated with Test Herbicides	Reference Ranges [b]
Vitamin A (β Carotene)	< 0.600	< 0.600	< 0.600	1.3 – 35
Vitamin K	2.16	3.05	2.80	0.03 – 2.55
α-Tocopherol	66.1	82.7	70.2	9 - 352
β-Tocopherol	10.9	12.2	9.51	0 - 36
γ-Tocopherol	797	820	821	89 - 2307
δ-Tocopherol	299	299	298	154 - 932
Total tocopherols	1170	1210	1200	600 - 3370

[a] Data from study DQ09B009 (Kowite, 2009d)
[b] Reference ranges from Table 14 of Appendix B

3.6 Compositional Analyses of Food Grade Oil from Soybean Event FG72 and the Respective Conventional Food Grade Soybean Oil

The analysis of the refined, bleached and deodorized soybean oil samples (food grade quality) comes to the same conclusion as for the crude oil (see Tables 3.6.1 and 3.6.2) :

- The fatty acid profiles of the non-transgenic and transgenic oil sample are very similar. There were noticed only small differences for oleic (C18:1) and linoleic acid (C18:2).
- Vitamin A and tocopherol contents are comparable between food grade oil samples.
- The vitamin K content in the transgenic oil samples is slightly higher compared to the non-transgenic oil sample.
- The measured values for the fatty acids, vitamin K and the tocopherols correspond very well with the reference ranges for food grade soybean oil.
- The vitamin A contents determined as β-carotene are lower than contents reported for soybean oil, but this was observed for all three oil samples.

Fatty acids not listed in the Table 3.6.1 were found to be below the limit of quantification (< 0.03 % fresh weight).

Table 3.6.1 **Fatty Acid Composition [a] of Food Grade Oil of Event FG72 and Non-transgenic Counterpart Jack Compared to Commercial Soybean Oil (Reference Ranges)**

Total Fatty Acid	% Relative			
	Non-Transgenic Not treated with Test Herbicides	Transgenic Not treated with Test Herbicides	Transgenic Treated with Test Herbicides	Reference Range [b]
	Mean ± SD	Mean ± SD	Mean ± SD	
Saturated				
C14:0 (myristic)	0.0683	0.0648	0.0629	< 0.05 – 0.20
C16:0 (palmitic)	9.97	9.23	9.29	6.7 – 14.5
C17:0 (margaric)	0.108	0.106	0.105	< 0.05 – 0.10
C18:0 (stearic)	4.09	4.66	4.43	0.50 – 8.9
C20:0 (arachidic)	0.303	0.335	0.317	0.10 – 0.90
C22:0 (behenic)	0.318	0.332	0.328	< 0.05 – 0.70
C24:0 (lignoceric)	0.0920	0.100	0.104	< 0.05 – 0.50
Sum of Saturated	14.95	14.83	14.64	7.30 – 25.8
Mono-unsaturated				
C16:1 (palmitoleic)	0.0967	0.0900	0.0921	< 0.05 – 0.50
C18:1 (oleic)	21.6	25.4	24.0	14.3 – 34.0
C20:1 (eicosenoic)	0.156	0.166	0.160	0.14 – 0.35
Sum of Mono-unsaturated	21.85	25.66	24.25	14.44 – 34.85
Poly-unsaturated				
C18:2 (linoleic)	54.9	52.0	53.3	36.5 – 60.0
C18:3 (alpha linolenic)	8.32	7.59	7.88	1.9 – 14.7
Sum of Poly-unsaturated	63.22	59.59	61.18	38.4 – 74.7
Sum of all total fatty acids	100.02	100.08	100.07	-

[a] Data from study DQ09B009 (Kowite, 2009d)
[b] Reference ranges from Table 13 of Appendix B

Table 3.6.2 **Contents of Fat-soluble Vitamins [a] in Food Grade Oil of Event FG72 and Non-transgenic Counterpart Jack Compared to Commercial Soybean Oil (Reference Ranges)**

Fat-soluble Vitamin	Content in mg/kg			
	Non-Transgenic Not treated with Test Herbicides	Transgenic Not treated with Test Herbicides	Transgenic Treated with Test Herbicides	Reference Ranges [b]
Vitamin A (β Carotene)	< 0.300	< 0.300	< 0.300	1.3 – 35
Vitamin K	2.19	2.95	2.62	0.03 – 2.55
α-Tocopherol	72.9	86.6	76.3	9 - 352
β-Tocopherol	10.3	12.1	10.6	0 - 36
γ-Tocopherol	788	765	807	89 - 2307
δ-Tocopherol	299	280	291	154 - 932
Total tocopherols	1170	1140	1180	600 - 3370

[a] Data from study DQ09B009 (Kowite, 2009d)
[b] Reference ranges from Table 14 of Appendix B

3.7 Phospholipid Profile of Crude Lecithin from Soybean Event FG72 and the Respective Conventional Crude Soybean Lecithin

The phospholipid profile of the three crude lecithin samples is similar (see Table 3.7.1). Small differences can be seen between L-α-phosphatidylcholin contents in the crude lecithin from Jack and FG72 seeds. The high L-α-phosphatidylinositol content in the sample from FG72 seeds treated with test herbicides might be an artefact. The content of this component is not different in the other two lecithin samples.

The phospholipid contents in the crude lecithin samples from Jack and FG72 seeds are comparable to the levels in commercial crude lecithin products.

Table 3.7.1 **Phospholipid Content [a] in Crude Lecithin of Event FG72 and Non-transgenic Counterpart Jack Compared to Commercial Soybean Lecithin (Reference Ranges)**

Phospholipid Component	% fw			
	Non-Transgenic Not treated with Test Herbicides	Transgenic Not treated with Test Herbicides	Transgenic Treated with Test Herbicides	Reference Range [b]
L-α-Phosphatidic Acid	2.46	2.29	2.54	2 – 14.5
L-α-Phosphatidylcholine	5.97	6.87	7.40	7.5 – 23.5
L-α-Phosphatidylethanolamine	4.23	4.51	4.30	5.3 – 20
L-α-Phosphatidylinositol	4.54	4.98	8.74	5.1 – 21

[a] Data from study DQ09B009 (Kowite, 2009d)
[b] Reference ranges from Table 15 of Appendix B

4 EXPRESSION AND PREDICTED DIETARY INTAKE ASSESSMENT OF 2mEPSPS AND HPPD PROTEINS FROM DOUBLE-HERBICIDE-TOLERANT SOYBEAN EVENT FG72

4.1 Expression of the 2mEPSPS and HPPD Proteins in Soybean Seeds of Event FG72

The same soybean seeds that were analysed for their chemical composition were analysed for the recombinant protein contents (see section 2.1 of this report). The 2mEPSPS and HPPD protein contents in soybean seeds obtained from conventionally and glyphosate + IFT treated plants averaged over all sites are presented in Table 4.1.1.

Table 4.1.1 Quantities of 2mEPSPS and HPPD Protein in Seeds of Soybean Event FG72 as Detected by ELISA[a]

Soybean Seed	2mEPSPS in ng/g fw		2mEPSPS in ng/g dm [c]		2mEPSPS in % total protein [c]	
	Conventional treated	Glyphosate + IFT treated	Conventional treated	Glyphosate + IFT treated	Conventional treated	Glyphosate + IFT treated
Mean + SD over all sites [b]	1360 ± 1080	1180 ± 589	1500 ± 1180	1300 ± 648	4.0×10^{-4}	3.4×10^{-4}
Range over all sites [b]	493 - 5230	458 - 2450	555 - 5730	490 - 2730	$1.4 – 15 \times 10^{-4}$	$1.3 – 7.4 \times 10^{-4}$

Soybean Seed	HPPD in ng/g fw		HPPD in ng/g dm		HPPD in % total protein	
	Conventional treated	Glyphosate + IFT treated	Conventional treated	Glyphosate + IFT treated	Conventional treated	Glyphosate + IFT treated
Mean + SD over all sites [b]	846 ± 183	802 ± 207	936 ± 203	887 ± 233	2.4×10^{-4}	2.3×10^{-4}
Range over all sites [b]	564 – 1130	486 - 1260	622 – 1260	540 – 1400	$1.7 – 3.3 \times 10^{-4}$	$1.4 – 3.7 \times 10^{-4}$

[a] Data from study DQ09B003 (Poe; 2009)
[b] Mean, SD and range values over all sites calculated from mean values per sample
[c] Moisture and total protein contents for individual soybean seed taken from study DQ08B009 (Mackie, 2009)

The 2mEPSPS protein content was between 493 and 5230 ng/g fw in the seeds from conventional treated soybean plants (mean value: 1360 ng/g fw) and between 458 and 2450 ng/g fw in the seeds from soybean plants treated with glyphosate and IFT (mean value: 1180 ng/g fw). Since the moisture and total protein contents of the individual seed samples were available, a conversion of the results based on their dry weight and in % of their total protein was done. The respective dry weight based contents ranged from 555 to 5730 ng/g dm (mean value: 1500 ng/g dm) in the seeds from conventional treated soybean plants and 490 to 2730 ng/g dm (mean value: 1300 ng/g dm) in the seeds from glyphosate and IFT treated soybean plants. The 2mEPSPS protein constitutes between 3.4 - 4.0×10^{-4} % dm of the total seed protein.

The HPPD protein content ranged from 564 to 1130 ng/g fw in the seeds from soybean plants not treated with the test herbicides (mean value: 846 ng/g fw) and from 486 to 1260 ng/g fw in the seeds from soybean plants treated with glyphosate and IFT (mean value: 802 ng/g fw). The respective dry weight based contents are 622 to 1260 ng/g dm (mean value: 936 ng/g dm) and 540 to 1400 ng/g dm (mean value: 887 ng/g dm). The HPPD protein constitutes about 2.4 x 10^{-4} % of the total seed protein.

Details of the protein analyses in soybean seeds are presented in the report to study DQ09B003 (Poe, 2009).

4.2 Expression of the 2mEPSPS and HPPD Proteins in Products derived from FG72 Soybean Seeds

The 2mEPSPS and HPPD protein contents were also determined in the different products derived from the FG72 soybean seeds. Table 4.2.1 lists the 2mEPSPS and HPPD protein contents in ng/g fresh weight, ng/g dry matter and in % of total protein in the respective processed soybean product.

Table 4.2.1 **Quantities of 2mEPSPS Protein in Products derived from Soybean Event FG72 as Detected by ELISA [a]**

Soybean Seed Product	2mEPSPS Protein Content in ng/g Sample fresh weight (Mean ± SD)		2mEPSPS Protein Content in ng/g Sample dry weight [b] (Mean)		2mEPSPS Protein Content in % of total protein [b] (Mean)	
	Conventional treated	Glyphosate + IFT treated	Conventional treated	Glyphosate + IFT treated	Conventional treated	Glyphosate + IFT treated
Hull	552 ± 55.8	501 ± 15.5	624	563	2.63 x 10^{-4}	2.29 x 10^{-4}
Protein Isolate	483 ± 31.0	1020 ± 40.9	493	1042	5.23 x 10^{-5}	11.0 x 10^{-5}
Meal	ND	< 20.0	NA	NA	NA	NA

Soybean Seed Product	HPPD Protein Content in ng/g Sample fresh weight (Mean ± SD)		HPPD Protein Content in ng/g Sample dry weight [b] (Mean)		HPPD Protein Content in % of total protein [b] (Mean)	
	Conventional treated	Glyphosate + IFT treated	Conventional treated	Glyphosate + IFT treated	Conventional treated	Glyphosate + IFT treated
Hull	941 ± 63.5	957 ± 42.1	1064	1077	4.48 x 10^{-4}	4.37 x 10^{-4}
Protein Isolate	627 ± 49.8	1078 ± 16.5	640	1101	6.79 x 10^{-5}	11.6 x 10^{-5}

[a] Data from study DQ09B008 (Robinson, 2009)
ND Not detected
NA Not applicable, since 2mEPSPS was not detected or quantified in the meal sample
[b] Moisture and total protein contents for the soybean seed product taken from study DQ09B009 (Kowite, 2009d)

Both recombinant proteins were not detected (or quantified) in the meal, oil and crude lecithin samples produced from FG72 soybean seeds.

The amount of 2mEPSPS protein was 552 ± 55.8 ng/g fw (or 624 ng/g dm) in the hull samples derived from conventional treated FG72 soybean seeds and 501 ± 15.5 ng/g fw (or 563 ng/g dm) in the hull samples produced from glyphosate and IFT treated FG72 soybean seeds. The respective contents in the protein isolate samples were 483 ± 31.0 ng/g fw (493 ng/g dm) and 1020 ± 40.9 ng/g fw (or 1042 ng/g dm). Based on the total protein contents of the products, the 2mEPSPS protein constitutes between 2.29 and 2.63 x 10^{-4} % of the total protein in soybean hulls and between 5.23 and 11.0 x 10^{-5} % of the total protein in soybean protein isolate.

The amount of HPPD protein in the hull samples was 941 ± 63.5 ng/g fw (or 1064 ng/g dm) derived from conventional treated FG72 soybean seeds and 957 ± 42.1 ng/g fw (or 1077 ng/g dm) derived from glyphosate and IFT treated FG72 soybean seeds. The respective contents in the protein isolate samples were 627 ± 49.8 ng/g fw (640 ng/g dm) and 1078 ± 16.5 ng/g fw (or 1101 ng/g dm). Based on the total protein contents of the products, the HPPD protein constitutes between 4.37 and 4.48 x 10^{-4} % of the total protein in soybean hulls and between 6.79 and 11.6 x 10^{-5} % of the total protein in soybean protein isolate.

Details of the 2mEPSPS and HPPD protein analyses in soybean seed fractions are presented in the report to study DQ09B008 (Robinson, 2009).

4.3 Predicted Dietary Intakes of 2mEPSPS and HPPD Proteins from Double-herbicide-tolerant Soybean Event FG72 Consumed by Humans and Animals

The main product derived from soybean for human consumption is soybean oil. In the course of processing the soybeans to refined vegetable oil of food grade quality, all protein components of the seed are destroyed by the high temperature and pressure of the screw pressing, or separated by extraction with a non-polar solvent and destroyed by the temperature of the solvent recovery. Last traces of protein in the crude oil are removed in the alkali treatment and deodorization steps of oil refining.

This was confirmed by the absence of any detectable 2mEPSPS and HPPD protein amounts in crude and food grade oil produced from soybean event FG72 seeds. Consequently, an intake of these recombinant proteins is not possible via soybean food grade oil or products containing this oil quality.

The raw agricultural commodity soybean is listed in the WHO consumption tables under “Pulses” (GEMS/Food regional diets, FAO/WHO, 2003). The recombinant protein intakes were calculated using the consumption figures for whole soybeans as pulses and, additionally, for all kind of oilseeds except groundnuts. The highest recombinant protein contents were used for the calculation determined in the seeds from plants treated with the test herbicides (see Table 4.1.1).

The predicted 2mEPSPS protein intake via whole soybeans as pulses is between 0.245 and 11.0 µg per person per day for the various regional diets. Based on the consumption of all kinds of oilseeds (except groundnuts), the 2mEPSPS protein intake is between 1.23 and 12.5 µg per person per day. The HPPD protein intake via whole soybeans as pulses for the various regional diets is between 0.126 and 5.67 µg per person per day. Based on the consumption of all kinds of oilseeds (except groundnuts), the HPPD protein intake is between 0.63 and 6.43 µg per person per day.

The scenario for the calculation of the predicted dietary intake of the recombinant proteins is very conservative. It includes three worst-case assumptions. *i.e.* all kinds of oilseeds with the exception of groundnuts consumed by humans would be soybean seeds, all oilseeds on the market would be seeds from soybean event FG72, and the highest amounts of 2mEPSPS and HPPD protein would be expressed in the seeds. Nevertheless, the calculated predicted dietary intakes for the two recombinant proteins are very low. The real per capita daily intake figures for both proteins will be significantly lower. They are expected to be far below 5.0 µg per person per day for both proteins.

Table 4.3.1 Predicted Dietary Intakes for the 2mEPSPS and HPPD Proteins after Consumption of Soybeans from Event FG72 in the Various Regional Diets

	Regional Diets				
	Europe [a]	Latin America	Middle East	Far East	Africa
Consumption of whole soybeans as pulses in gram per person per day	0.1[b]	0.1 [b]	4.5	2.0	0.5
Consumption of all kind of oilseeds (except groundnuts) in gram per person per day	3.1	0.5	5.1	1.2	3.1
Highest 2mEPSPS protein content in FG72 seeds is 2.45 µg/g fw [c]					
Predicted daily intake of 2mEPSPS protein via whole soybeans (µg per person per day)	0.245	0.245	11.0	4.9	1.23
Predicted daily intake of 2mEPSPS protein via all kind of oilseeds (µg per person per day)	7.60	1.23	12.5	2.94	7.60
Highest HPPD protein content in FG72 seeds is 1.26 µg/g fw [c]					
Predicted daily intake of HPPD protein via whole soybeans (µg per person per day)	0.126	0.126	5.67	2.52	0.63
Predicted daily intake of HPPD protein via all kind of oilseeds (µg per person per day)	3.91	0.63	6.43	1.51	3.91

[a] The European diet includes countries with European-type diets, such as Australia, Canada and the USA
[b] Since whole soybean are not consumed in Europe or Latin America a default value of 0.1 g/person x day was assigned.
[c] Highest 2mEPSPS and HPPD contents in soybeans taken from Table 3.1.1

Soybean seeds and processed commodities are also used in animal feed. The EPA residue chemistry test guidelines (EPA, 1996) list seven different plant fractions to be included in feedstuff. These are seeds, aspirated grains fractions, forage, hay, silage, hulls and meal. The maximum theoretical 2mEPSPS and HPPD protein amounts were calculated in the case that FG72 seeds, hulls or meal would be used to prepare the animal diets (see Table 4.3.2). Soybean hulls are not used to prepare diets for swine.

Table 4.3.2 Contribution of Soybean Commodities to Animal Feed (EPA. 1996) and 2mEPSPS and HPPD Protein Amounts in the FG72 Commodities

Agricultural commodity	Maximum contribution to animal diets (%)				Maximum 2mEPSPS protein in µg/g fw	Maximum HPPD protein in µg/g fw
	Beef cattle	Dairy cattle	Poultry	Swine		
Seed	15	15	20	25	2.45 [a]	1.26 [a]
Hulls	20	20	20	-	0.50 [b]	0.96 [b]
Meal	15	15	40	25	< 0.020	ND

[a] Results from 2mEPSPS and HPPD protein analyses of seeds taken from Table 4.1.1
[b] Results from 2mEPSPS and HPPD protein analyses of hulls and meal taken from Table 4.2.1
ND Not Detected

Taking the highest 2mEPSPS and HPPD protein contents determined in seeds, hull and meal from glyphosate and IFT treated FG72 plants (see Table 4.1.1 and 4.2.1) the maximum theoretical amounts of the recombinant proteins and the percentage of the proteins in the livestock diet are presented in Table 4.3.3.

Table 4.3.3 **Calculation of the Maximum Theoretical 2mEPSPS and HPPD Protein Amounts in Animal Diets Produced with FG72 Seeds or Hulls**

Agricultural commodity	2mEPSPS in µg/g animal diet				Percentage of 2mEPSPS in the animal diet			
	Beef cattle	Dairy cattle	Poultry	Swine	Beef cattle	Dairy cattle	Poultry	Swine
Seeds	0.37	0.37	0.49	0.61	3.7×10^{-5}	3.7×10^{-5}	4.9×10^{-5}	6.1×10^{-5}
Hulls	0.10	0.10	0.10	-	1.0×10^{-5}	1.0×10^{-5}	1.0×10^{-5}	-
Agricultural commodity	**HPPD in µg/g animal diet**				**Percentage of HPPD in the animal diet**			
	Beef cattle	Dairy cattle	Poultry	Swine	Beef cattle	Dairy cattle	Poultry	Swine
Seeds	0.19	0.19	0.25	0.32	1.9×10^{-5}	1.9×10^{-5}	2.5×10^{-5}	3.2×10^{-5}
Hulls	0.19	0.19	0.19	-	1.9×10^{-5}	1.9×10^{-5}	1.9×10^{-5}	-

The maximum protein contents in the cattle diets could be 0.37 µg/g (or 3.7×10^{-5} %) 2mEPSPS and 0.19 µg/g (or 1.9×10^{-5} %) HPPD, if prepared with FG72 seeds. The use of FG72 seeds in poultry diets could result in 0.49 µg/g (or 4.9 x 10-5 %) 2mEPSPS and 0.25µg/g (or 2.5 x 10-5 %) HPPD protein. The preparation of swine diets including FG72 seed could lead to 2m EPSPS contents of 0.61 µg/g (or 6.1×10^{-5} %) and HPPD contents of 0.32 µg/g (or 3.2×10^{-5} %). If animal diets are prepared with FG72 hulls the protein amounts could be maximum 0.10 µg/g (or 1.0×10^{-5} %) for 2mEPSPS and 0.19 µg/g (or 1.9×10^{-5} %) for HPPD. Since the 2mEPSPS and HPPD proteins were not detected or quantified in FG72 meal samples, an exposure of livestock animals to the recombinant proteins through this commodity is not possible.

The calculation of the maximum theoretical protein amounts or percentages in animal feed is based again on a worst-case scenario, since soybeans used for animal feed production are not solely obtained from the double-herbicide-tolerant soybean event FG72 and the highest amounts of 2mEPSPS and HPPD protein expressed in seeds and hulls were used in the calculations.

5 CONCLUSION FOR THE NUTRITIONAL IMPACT ASSESSMENT OF DOUBLE-HERBICIDE-TOLERANT SOYBEAN (TRANSFORMATION EVENT FG72)

Evaluations were conducted to compare the nutritional components found in the double-herbicide-tolerant soybean (transformation event FG72) to its non-transgenic counterpart, Jack, and to other commercial soybean lines currently on the market.

Compositional analyses were performed using the raw agricultural commodity soybean seed grown at 10 field trial sites in the USA in the 2008.

The components, which were selected for compositional and nutritional analyses, comprise the important, basic nutrients of soybean. These are proximate and fibre components, micro-nutrients such as minerals and vitamins, the isoflavones, the anti-nutrients raffinose, stachyose, phytic acid, trypsin inhibitor and the lectins, the total amino acids, and the total fatty acids.

For 46 of the 62 components no statistical significant differences were detected between FG72 and Jack seeds in the over all site or, in case of site*regimen interaction, in the by-site analyses. Most mean values were inside the reference ranges calculated from the results of three commercial soybean lines and compiled from food composition tables and the soybean literature.

The differences that were detected for a few components (ash, calcium, magnesium, sodium, total tocopherols, raffinose, glycitin, genistin, and eight fatty acids) in the statistical analysis have no biological and nutritional relevance for the following reasons: most mean values calculated for the transgenic groups are all inside the references ranges for commercial soybean lines (the isoflavone glycitin and palmitic acid are the two exeptions), the estimated differences between regimen are very small and often lower than the natural variation inside the non-transgenic control group and, in case of total tocopherol, no significant differences were detected between the single tocopherol mean values and there is no risk of a tocopherol (vitamin E) deficiency, since the total tocopherol mean values of the transgenic groups are in fact slightly higher than the mean value for the non-transgenic control group.

Based on this statistical evaluation of the analytical data and an assessment of the nutritional impact of the different observations, the soybean seeds from the double-herbicide-tolerant soybean event FG72 are found to be nutritionally equivalent to seeds from the traditional non-transgenic comparator, the variety Jack. There is no impact on the nutritional value of the soybean seeds as a result of the genetic modifications or the treatment with the test herbicides

Beside the raw agricultural commodity, soybean seed, soybean hulls, meal, toasted meal, protein isolate, crude oil, food grade oil and crude lecithin were analysed for their composition, too. The results obtained were compared to reference ranges from literature.

No differences between the transgenic and non-transgenic soybean products were noticed for most of the analysed components. If slight differences were detected this has no nutritional impact for two reasons:

- The analysed values in the non-transgenic and the transgenic processing products are inside the reference range for the commercial product. This is true for the oleic (C18:1) and linoleic (C18:2) contents of the crude and refined oil samples.
- Differences in nutrient levels were only found in one processing product, but not in the raw agricultural commodity, pre- or subsequent processing products. For instance, genistein was found to be different in the meal samples before toasting. But this was not confirmed by the analysis of the soybean seeds and the toasted meal samples.

To enable an assessment of the exposure of humans and animals to the recombinant proteins expressed in double-herbicide-tolerant soybean, the FG72 seed samples and products derived from FG72 seeds were analysed for the content of the 2mEPSPS and HPPD protein.

All calculations are based on worst-case scenarios using consumption figures for whole soybeans as pulses and all kinds of oilseeds (with the exception of groundnuts), taking the highest recombinant protein amounts determined in the soybean commodity, and assuming that all commercial soybean seeds taken to produce food or animal feed would be the double-herbicide-tolerant soybean event FG72, and this is not the case because of global commodity trades.

The main soybean product consumed by humans is the vegetable oil. Since the recombinant proteins were not detected in the oil products derived from FG72 seeds, an intake of the 2mEPSPS and HPPD protein is not possible via soybean food grade oil obtained from FG72 seeds or products containing this oil quality. Alternatively, the predicted daily dietary intake calculations were done on the basis of the consumption of whole soybeans as pulses and all kind of oilseeds (with the exception of groundnuts) for different regional diets. Based on these consumption figures, the 2mEPSPS and HPPD protein intake could be at a maximum of 11.0 µg and 6.43 µg per person per day, respectively.

Additionally, the contribution of double-herbicide-tolerant soybean event FG72 to animal feed was evaluated. The maximum theoretical concentrations of 2mEPSPS and HPPD protein are 0.61 µg/g fw or 6.1×10^{-5} % of the diet and 0.32 µg/g fw or 3.2×10^{-5} % of the diet, respectively, if soybean seeds are used to prepare animal feed for swine.

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APPENDIX A

Table 1 **Results from ANOVA**

Parameter	DF	R^2	p-values from ANOVA REGIMEN (R)	SITE (S)	R*S	Regimen	MEAN	95% CI lower bound	95% CI upper bound	p-value t-test
Proximate and Fibre Components										
Moisture	29	0.58	0.499	<.001	0.234	A	9.51	9.28	9.75	
						B	9.65	9.41	9.88	
						C	9.45	9.22	9.69	
						A vs B	-0.14	-0.47	0.20	0.423
						A vs C	0.06	-0.28	0.39	0.728
Protein	29	0.68	0.799	<.001	0.277	A	38.16	37.92	38.39	
						B	38.20	37.97	38.43	
						C	38.09	37.86	38.32	
						A vs B	-0.04	-0.37	0.29	0.794
						A vs C	0.07	-0.26	0.40	0.688
Total Fat	29	0.63	0.064	<.001	0.146	A	19.31	19.02	19.61	
						B	18.85	18.56	19.15	
						C	19.25	18.95	19.54	
						A vs B	0.46	0.05	0.87	0.030
						A vs C	0.07	-0.35	0.48	0.749
Ash	29	0.73	<.001	<.001	0.568	A	5.24	5.17	5.31	
						B	5.07	5.00	5.14	
						C	5.06	4.99	5.13	
						A vs B	0.17	0.07	0.27	0.001
						A vs C	0.18	0.08	0.28	<.001
Carbohydrates	29	0.68	0.027	<.001	0.012	A	37.30	37.00	37.59	
						B	37.88	37.58	38.17	
						C	37.60	37.31	37.90	
						A vs B	-0.58	-1.00	-0.16	0.008
						A vs C	-0.31	-0.73	0.11	0.149
Acid Detergent Fibre	29	0.36	0.832	0.166	0.342	A	17.79	17.13	18.45	
						B	18.07	17.41	18.73	
						C	17.92	17.26	18.58	
						A vs B	-0.28	-1.22	0.65	0.546
						A vs C	-0.13	-1.07	0.80	0.776
Neutral Detergent Fibre	29	0.37	0.500	0.044	0.637	A	19.80	19.15	20.46	
						B	20.34	19.69	21.00	
						C	20.01	19.35	20.66	
						A vs B	-0.54	-1.46	0.38	0.246
						A vs C	-0.20	-1.13	0.72	0.661

Table 1 **Results from ANOVA (continued)**

Parameter	DF	R^2	p-values from ANOVA: REGIMEN (R)	SITE (S)	R*S	Regimen	MEAN	95% CI lower bound	95% CI upper bound	p-value t-test
Minerals										
Calcium	29	0.92	<.001	<.001	0.058	A	0.282	0.279	0.286	
						B	0.258	0.255	0.262	
						C	0.259	0.255	0.262	
						A vs B	0.024	0.019	0.029	<.001
						A vs C	0.024	0.019	0.028	<.001
Phosphorus	29	0.88	0.490	<.001	0.151	A	0.626	0.616	0.635	
						B	0.618	0.609	0.627	
						C	0.620	0.611	0.629	
						A vs B	0.008	-.006	0.021	0.252
						A vs C	0.006	-.007	0.019	0.392
Potassium	29	0.79	<.001	<.001	0.006	A	1.93	1.91	1.95	
						B	1.85	1.83	1.87	
						C	1.85	1.83	1.87	
						A vs B	0.08	0.06	0.11	<.001
						A vs C	0.08	0.06	0.11	<.001
Magnesium	29	0.81	<.001	<.001	0.065	A	0.241	0.238	0.243	
						B	0.226	0.224	0.229	
						C	0.226	0.224	0.228	
						A vs B	0.014	0.011	0.018	<.001
						A vs C	0.015	0.011	0.018	<.001
Sodium	29	0.42	0.010	0.214	0.279	A	0.012	0.009	0.014	
						B	0.015	0.013	0.018	
						C	0.016	0.014	0.019	
						A vs B	-.004	-.007	-.001	0.019
						A vs C	-.005	-.008	-.002	0.004
Iron	29	0.58	0.127	<.001	0.303	A	93.3	85.4	101.3	
						B	82.6	74.6	90.6	
						C	84.1	76.1	92.0	
						A vs B	10.7	-0.5	22.0	0.062
						A vs C	9.3	-2.0	20.5	0.105

Table 1 **Results from ANOVA (continued)**

Parameter	DF	R^2	p-values from ANOVA: REGIMEN (R)	SITE (S)	R*S	Regimen	MEAN	95% CI lower bound	95% CI upper bound	p-value t-test
Vitamins										
Vitamin B1	29	0.76	0.009	<.001	0.072	A	3.59	3.40	3.78	
						B	3.44	3.25	3.63	
						C	3.16	2.96	3.35	
						A vs B	0.15	-0.12	0.42	0.279
						A vs C	0.43	0.16	0.71	0.003
Vitamin B2	29	0.24	0.253	0.588	0.956	A	4.42	4.08	4.76	
						B	4.52	4.18	4.85	
						C	4.80	4.47	5.14	
						A vs B	-0.09	-0.57	0.38	0.694
						A vs C	-0.38	-0.86	0.09	0.113
Folic Acid	29	0.56	0.117	<.001	0.491	A	2.976	2.877	3.075	
						B	3.068	2.969	3.167	
						C	3.122	3.023	3.221	
						A vs B	-.092	-.232	0.048	0.194
						A vs C	-.146	-.286	-.006	0.041
Vitamin A	29	0.97	<.001	<.001	<.001	A	0.217	0.210	0.225	
						B	0.261	0.254	0.269	
						C	0.284	0.276	0.291	
						A vs B	-.044	-.054	-.033	<.001
						A vs C	-.066	-.077	-.056	<.001
Vitamin K	29	0.65	0.261	<.001	0.030	A	0.191	0.171	0.212	
						B	0.203	0.183	0.224	
						C	0.215	0.195	0.236	
						A vs B	-.012	-.041	0.017	0.400
						A vs C	-.024	-.053	0.005	0.103
Alpha Tocopherol	29	0.91	<.001	<.001	0.003	A	17.4	16.7	18.1	
						B	19.0	18.3	19.7	
						C	20.7	20.0	21.4	
						A vs B	-1.6	-2.5	-0.6	0.003
						A vs C	-3.3	-4.3	-2.3	<.001
Gamma Tocopherol	29	0.79	0.038	<.001	0.076	A	194.8	192.1	197.6	
						B	200.0	197.2	202.7	
						C	197.8	195.1	200.6	
						A vs B	-5.1	-9.1	-1.2	0.011
						A vs C	-3.0	-6.9	0.9	0.132
Delta Tocopherol	29	0.89	0.408	<.001	0.014	A	74.1	72.7	75.4	
						B	75.2	73.8	76.5	
						C	74.0	72.7	75.4	
						A vs B	-1.1	-3.0	0.8	0.257
						A vs C	0.0	-1.9	2.0	0.964
Total Tocopherol	29	0.63	0.017	<.001	0.130	A	286.4	282.4	290.3	
						B	294.2	290.3	298.1	
						C	292.5	288.6	296.4	
						A vs B	-7.8	-13.4	-2.3	0.007
						A vs C	-6.1	-11.7	-0.6	0.031

Table 1 **Results from ANOVA (continued)**

Parameter	DF	R^2	p-values from ANOVA: REGIMEN (R)	SITE (S)	R*S	Regimen	MEAN	95% CI lower bound	95% CI upper bound	p-value t-test
Anti-nutrients										
Phytic Acid	29	0.82	0.140	<.001	0.122	A	1.40	1.36	1.44	
						B	1.37	1.34	1.41	
						C	1.35	1.31	1.38	
						A vs B	0.03	-0.03	0.08	0.356
						A vs C	0.06	0.00	0.11	0.049
Raffinose	29	0.76	0.035	<.001	0.106	A	0.361	0.350	0.372	
						B	0.378	0.367	0.389	
						C	0.379	0.368	0.390	
						A vs B	-.018	-.033	-.002	0.027
						A vs C	-.018	-.034	-.003	0.022
Stachyose	29	0.34	0.272	0.048	0.915	A	2.49	2.42	2.56	
						B	2.42	2.35	2.49	
						C	2.50	2.43	2.57	
						A vs B	0.07	-0.04	0.17	0.196
						A vs C	-0.01	-0.11	0.09	0.849
Lectin	29	0.29	0.054	0.739	0.836	A	1.74	1.55	1.94	
						B	1.40	1.20	1.60	
						C	1.54	1.35	1.74	
						A vs B	0.34	0.07	0.62	0.016
						A vs C	0.20	-0.08	0.48	0.155
Trypsin inhibitor	29	0.40	0.041	0.016	0.879	A	33.00	30.83	35.17	
						B	30.07	27.90	32.24	
						C	33.89	31.72	36.06	
						A vs B	2.93	-0.14	6.00	0.061
						A vs C	-0.89	-3.96	2.18	0.564

Table 1 **Results from ANOVA (continued)**

Parameter	DF	R^2	p-values from ANOVA: REGIMEN (R)	SITE (S)	R*S	Regimen	MEAN	95% CI lower bound	95% CI upper bound	p-value t-test
Isoflavones										
Daidzein	29	0.40	0.155	0.113	0.292	A	11.00	10.48	11.52	
						B	10.71	10.19	11.23	
						C	10.28	9.76	10.80	
						A vs B	0.29	-0.44	1.03	0.428
						A vs C	0.72	-0.02	1.45	0.056
Genistein	29	0.75	<.001	<.001	0.010	A	11.48	11.11	11.86	
						B	11.22	10.85	11.60	
						C	10.46	10.09	10.84	
						A vs B	0.26	-0.27	0.79	0.327
						A vs C	1.02	0.49	1.55	<.001
Daidzin	29	0.92	0.320	<.001	0.562	A	1035	991	1079	
						B	1034	990	1078	
						C	994	950	1038	
						A vs B	1	-61	63	0.976
						A vs C	42	-21	104	0.187
Glycitin	29	0.62	<.001	<.001	0.887	A	365	352	379	
						B	414	401	428	
						C	400	386	414	
						A vs B	-49	-69	-30	<.001
						A vs C	-35	-54	-15	<.001
Genistin	29	0.94	<.001	<.001	0.812	A	1817	1767	1867	
						B	1682	1632	1732	
						C	1640	1591	1690	
						A vs B	135	65	205	<.001
						A vs C	177	107	247	<.001
Total Isoflavones	29	0.92	0.030	<.001	0.816	A	2010	1948	2071	
						B	1953	1892	2015	
						C	1891	1829	1952	
						A vs B	56	-31	144	0.201
						A vs C	119	32	206	0.008

Table 1 **Results from ANOVA (continued)**

Parameter	DF	R²	p-values from ANOVA: REGIMEN (R)	SITE (S)	R*S	Regimen	MEAN	95% CI lower bound	95% CI upper bound	p-value t-test
Total Amino Acids										
Alanine	29	0.45	0.901	<.001	0.644	A	1.68	1.67	1.69	
						B	1.68	1.67	1.69	
						C	1.68	1.67	1.69	
						A vs B	0.00	-0.01	0.02	0.693
						A vs C	0.00	-0.01	0.02	0.693
Arginine	29	0.55	0.344	<.001	0.487	A	2.94	2.91	2.97	
						B	2.97	2.94	3.00	
						C	2.95	2.92	2.98	
						A vs B	-0.03	-0.07	0.01	0.153
						A vs C	-0.01	-0.05	0.03	0.631
Aspartic acid	29	0.54	0.555	<.001	0.450	A	4.40	4.36	4.43	
						B	4.38	4.34	4.42	
						C	4.37	4.33	4.41	
						A vs B	0.02	-0.04	0.07	0.523
						A vs C	0.03	-0.02	0.08	0.283
Cystine	29	0.56	0.476	<.001	0.245	A	0.58	0.57	0.59	
						B	0.58	0.57	0.59	
						C	0.59	0.58	0.59	
						A vs B	0.00	-0.01	0.01	0.951
						A vs C	-0.01	-0.02	0.01	0.307
Glutamic acid	29	0.55	0.812	<.001	0.409	A	6.75	6.68	6.81	
						B	6.77	6.71	6.84	
						C	6.74	6.68	6.81	
						A vs B	-0.02	-0.11	0.07	0.618
						A vs C	0.00	-0.09	0.10	0.919
Glycine	29	0.55	0.960	<.001	0.575	A	1.68	1.67	1.69	
						B	1.68	1.67	1.69	
						C	1.68	1.67	1.69	
						A vs B	0.00	-0.01	0.02	0.871
						A vs C	0.00	-0.01	0.02	0.776
Histidine	29	0.52	0.963	<.001	0.720	A	1.05	1.04	1.06	
						B	1.05	1.04	1.06	
						C	1.05	1.04	1.05	
						A vs B	-0.00	-0.01	0.01	0.991
						A vs C	0.00	-0.01	0.01	0.816
Isoleucine	29	0.32	0.379	0.052	0.977	A	1.81	1.79	1.83	
						B	1.80	1.78	1.82	
						C	1.79	1.77	1.81	
						A vs B	0.01	-0.01	0.04	0.373
						A vs C	0.02	-0.01	0.04	0.171
Leucine	29	0.51	0.671	<.001	0.575	A	2.99	2.96	3.01	
						B	2.99	2.97	3.01	
						C	2.98	2.95	3.00	
						A vs B	-0.00	-0.04	0.03	0.923
						A vs C	0.01	-0.02	0.05	0.473
Lysine	29	0.49	0.943	<.001	0.731	A	2.48	2.46	2.50	
						B	2.48	2.46	2.50	
						C	2.47	2.46	2.49	
						A vs B	-0.00	-0.03	0.03	0.980
						A vs C	0.00	-0.02	0.03	0.778

Table 1 **Results from ANOVA (continued)**

Parameter	DF	R^2	p-values from ANOVA: REGIMEN (R)	SITE (S)	R*S	Regimen	MEAN	95% CI lower bound	95% CI upper bound	p-value t-test
Total Amino Acids (continued)										
Methionine	29	0.46	0.916	<.001	0.461	A	0.54	0.53	0.55	
						B	0.54	0.53	0.55	
						C	0.54	0.54	0.55	
						A vs B	0.00	-0.01	0.01	0.891
						A vs C	-0.00	-0.01	0.01	0.785
Phenylalanine	29	0.47	0.264	<.001	0.603	A	1.97	1.95	1.99	
						B	1.98	1.96	1.99	
						C	1.96	1.94	1.97	
						A vs B	-0.00	-0.03	0.02	0.777
						A vs C	0.02	-0.01	0.04	0.211
Proline	29	0.51	0.753	<.001	0.291	A	1.82	1.80	1.84	
						B	1.83	1.81	1.85	
						C	1.83	1.80	1.85	
						A vs B	-0.01	-0.04	0.02	0.484
						A vs C	-0.01	-0.04	0.02	0.557
Serine	29	0.56	0.546	<.001	0.047	A	1.97	1.95	1.99	
						B	1.98	1.96	2.00	
						C	1.99	1.97	2.01	
						A vs B	-0.01	-0.04	0.02	0.497
						A vs C	-0.02	-0.05	0.01	0.278
Threonine	29	0.59	0.254	<.001	0.156	A	1.55	1.53	1.56	
						B	1.54	1.53	1.56	
						C	1.53	1.52	1.54	
						A vs B	0.00	-0.02	0.02	0.908
						A vs C	0.01	-0.00	0.03	0.138
Tryptophan	29	0.34	0.119	0.551	0.445	A	0.45	0.44	0.46	
						B	0.44	0.43	0.45	
						C	0.44	0.43	0.45	
						A vs B	0.01	-0.00	0.03	0.057
						A vs C	0.01	-0.00	0.03	0.100
Tyrosine	29	0.50	0.582	<.001	0.225	A	1.40	1.39	1.42	
						B	1.40	1.39	1.41	
						C	1.40	1.38	1.41	
						A vs B	0.00	-0.01	0.02	0.629
						A vs C	0.01	-0.01	0.03	0.300
Valine	29	0.39	0.609	0.007	0.861	A	1.89	1.87	1.91	
						B	1.88	1.86	1.90	
						C	1.87	1.85	1.89	
						A vs B	0.01	-0.02	0.04	0.520
						A vs C	0.01	-0.01	0.04	0.329

Table 1 **Results from ANOVA (continued)**

Parameter	DF	R²	p-values from ANOVA REGIMEN (R)	SITE (S)	R*S	Regimen	MEAN	95% CI lower bound	upper bound	p-value t-test
Total Fatty Acids										
C16:0 Palmitic	29	0.87	<.001	<.001	0.376	A	10.06	10.00	10.12	
						B	9.34	9.28	9.40	
						C	9.38	9.32	9.45	
						A vs B	0.72	0.63	0.81	<.001
						A vs C	0.67	0.59	0.76	<.001
C18:0 Stearic	29	0.86	<.001	<.001	0.358	A	4.28	4.24	4.31	
						B	4.52	4.48	4.56	
						C	4.51	4.47	4.54	
						A vs B	-0.24	-0.29	-0.19	<.001
						A vs C	-0.23	-0.28	-0.18	<.001
C18:1 Oleic	29	0.90	<.001	<.001	0.153	A	21.97	21.76	22.19	
						B	24.65	24.43	24.87	
						C	24.12	23.91	24.34	
						A vs B	-2.68	-2.98	-2.37	<.001
						A vs C	-2.15	-2.46	-1.84	<.001
C18:2 Linoleic	29	0.86	<.001	<.001	0.230	A	54.56	54.36	54.75	
						B	52.65	52.46	52.85	
						C	53.08	52.88	53.28	
						A vs B	1.90	1.62	2.18	<.001
						A vs C	1.48	1.20	1.76	<.001
C18:3 Linolenic	29	0.88	<.001	<.001	0.608	A	8.27	8.20	8.35	
						B	7.94	7.86	8.02	
						C	8.01	7.93	8.08	
						A vs B	0.33	0.22	0.44	<.001
						A vs C	0.27	0.16	0.38	<.001
C20:0 Arachidic	29	0.82	<.001	<.001	0.067	A	0.312	0.309	0.316	
						B	0.324	0.321	0.327	
						C	0.324	0.321	0.327	
						A vs B	-.012	-.016	-.007	<.001
						A vs C	-.012	-.016	-.007	<.001
C20:1 Eicosenoic	29	0.81	0.003	<.001	0.454	A	0.161	0.159	0.163	
						B	0.165	0.162	0.167	
						C	0.166	0.164	0.168	
						A vs B	-.004	-.007	-.001	0.017
						A vs C	-.005	-.009	-.002	<.001
C22:0 Behenic	29	0.54	0.001	<.001	0.462	A	0.319	0.315	0.323	
						B	0.330	0.326	0.335	
						C	0.327	0.322	0.331	
						A vs B	-.011	-.017	-.005	<.001
						A vs C	-.007	-.013	-.001	0.016
C24:0 Lignoceric	29	0.80	0.019	<.001	0.033	A	0.113	0.109	0.118	
						B	0.119	0.114	0.123	
						C	0.122	0.118	0.127	
						A vs B	-.005	-.012	0.001	0.088
						A vs C	-.009	-.016	-.003	0.005

Table 2 **Results from T-tests - By-site Analysis**

Summary t-test procedures *)	A vs B		A vs C	
	significant	not significant	significant	not significant
Carbohydrates	2	8	1	9
Potassium	4	6	5	5
Vitamin B1	1	9	1	9
Vitamin A #)	3	2 (5)	4	1 (5)
Vitamin K	1	9	1	9
Alpha Tocopherol	2	8	3	7
Gamma Tocopherol	1	9	2	8
Delta Tocopherol	1	9	4	6
Genistein #)	-	6 (4)	1	5 (4)
Total Isoflavones	1	9	2	8
Serine	3	7	-	10
C24:0 Lignoceric	2	8	1	9

*) N of sites with significant ($p < 0.05$) and not significant ($p \geq 0.05$) treatment differences
A = non-transgenic seed from the control Jack
B = transgenic seed from the not glyphosate treated FG72 plants
C = transgenic. seed from the not glyphosate treated FG72 plants
#) 'not significant' was also assumed if all samples of a site were equal or below the limit of quantification for the two respective treatments (N of sites in brackets)

APPENDIX B

Table 1: Sources of Reference Composition Data

Abbreviation	**Literature Source: Author(s). Year.** [a]
BG	Belitz H.-D., Grosch W. 1985.
Codex	FAO/WHO Food Standards. Codex Alimentarius. 2001.
CRC (1983)	CRC. 1983.
CRC (1989)	CRC. 1989.
Ensminger	Ensminger M.E., et al. 1990.
FSK	Scherz H., Senser F. 1994.
ILSI	ILSI. 2007.
Hui	Hui Y.H. 1992.
Kakade	Kakade M.L., et al. 1972.
Kellems	Kellems R.O., Church D.C. 1998.
Liener	Liener I.E. 1994.
Macgregor	Macgregor C.A. 1994.
Nasner	Nasner A. 1985.
NCDA	North Carolina Feed Report. 1984-1985.
Novak + Haslberger	Novak W.K., Haslberger A.G. 2000.
Nut Beef	Nutrient Requirements of Beef Cattle. 1984.
Nut Cow	Nutrient Requirements of Dairy Cattle. 1978.
OECD	OECD. 2001.
Pardun	Pardun H. 1989.
Red Book	Feed Industry Red Book. 1993.
Sotirhos	Sotirhos N. *et al.* 1986.
Stephan	Stephan A. 1999.
TAS	Douglas J.S. 1996.
USCA	USCA. 1982.
USDA	USDA. 2001.
USDA-IOWA	USDA-IOWA. 2001.

[a] Detailed literature source see References (pages 37-39)

Table 2: Reference Ranges for Proximate and Fibre Compounds in Soybean Seeds

Parameter	Unit	OECD	ILSI	FSK Range	BG	Ens-minger	USDA	USCA	Nut Beef	Nut Cow	Red Book	Mac-gregor	Range
Moisture	% fw	5.6 - 11.5	-	7.5 - 10.1	ND	8	8.5	8	8	9	12	8	5.6 - 12
Protein	% dm	32 - 43.6	33.2 - 45.5	33.6 - 40.7	39.0	41.7	39.9	42.8	42.8	42.8	40	41.3	32 - 45.5
Crude fat	% dm	15.5 - 24.7	8.1 - 23.6	17.6 - 23.7	19.6	18.7	21.8	18.8	18.8	18.8	18.5	18-22	8.1 - 24.7
Ash	% dm	4.5 - 6.4	3.9 - 7.0	4.2 - 5.8	5.5	5.5	5.3	5.5	5.5	5.5	5.0	ND	3.9 - 7.0
Total Carbo-hydrates [a]	% dm	31.7 - 31.8	29.6 - 50.2	37.8 - 38.9	35.5	ND	33.0	32.9	ND	ND	ND	ND	29.6 - 50.2
ADF	% dm	9.0 - 11.1	7.8 - 18.6	ND	ND	11.0	ND	10.0	10.0	10.0	11	11.3	7.8 - 18.6
NDF	% dm	10.0 - 14.9	8.5 - 21.3	ND	ND	ND	ND	ND	ND	ND	5	13.0	5.0 - 21.3

ND No data

[a] Total carbohydrates calculated as: 100% - (protein %dm + fat %dm + ash %dm)

Table 3a: Reference Ranges for Minerals and Vitamins in Soybean Seeds

Parameter	Unit	ILSI	FSK Range	BG	Ens-minger	USDA	USCA	Nut Beef	Nut Cat	Red Book	Range
Calcium	% dm	0.12 - 0.31	0.22 - 0.34	0.21	0.27	0.30	0.27	0.27	0.27	0.27	0.12 - 0.34
Phosphorus	% dm	0.51 - 0.94	0.52 - 0.71	0.49	0.65	0.77	0.65	0.65	0.65	0.64	0.49 - 0.94
Potassium	% dm	1.87 - 2.32	1.8 - 2.1	1.4	1.80	1.80	1.82	1.82	1.82	1.6	1.4 - 2.3
Magnesium	% dm	0.22 - 0.31	0.23 - 0.32	0.21	0.29	0.31	0.29	0.29	0.29	0.28	0.21 - 0.32
Sodium	% dm	ND	0.0043 - 0.0077	0.0033	ND	0.0022	0.02	0.02	0.02	0.02	0.002 - 0.02
Iron	mg/kg dm	55.36 - 109.5	71.4 - 111	71	97.8	172	91.0	91.0	91.0	75.0	55.4 - 172
Vitamin B1	mg/kg dm	1.01 - 2.54	3.24 - 16.02	8.2	12.3	9.56	10.6	ND	ND	ND	1.01 - 16.02
Vitamin B2	mg/kg dm	1.90 - 3.21	2.7 - 14.5	4.3	3.2	9.51	3.1	ND	ND	ND	1.9 - 14.5
Folic Acid	mg/kg dm	2.39 - 4.71	2.49 - 2.78	ND	ND	4.10	3.9	ND	ND	ND	2.4 - 4.7
Vitamin A	mg/kg dm	ND	3.72 – 4.37	ND	0.98	0.26	1.1	ND	ND	ND	0.26 – 4.37
Vitamin K	mg/kg dm	ND	0.38 – 0.51	ND	ND	ND	ND	ND	ND	ND	0.38 – 0.51

ND No data

Table 3b: Reference Ranges for Tocopherols in Soybean Seeds

Parameter	Unit	Codex	FSK	Range
α-Tocopherol	mg/kg dm	2 – 70 [a]	7	2 – 70
β-Tocopherol	mg/kg dm	0 – 7 [a]	ND	0 – 7
γ-Tocopherol	mg/kg dm	18 – 461 [a]	90	18 – 461
δ-Tocopherol	mg/kg dm	31 – 186 [a]	71	31 – 186
Total Tocopherol	mg/kg dm	120 - 674 [a]	167.7	120 - 674

ND No data

[a] For conversion of tocopherol levels from mg/kg oil into mg/kg seed dry matter use factor F=0.20, since the average oil content in soybean seeds is about 20% dm.

Table 4: Reference Ranges for Isoflavones in Soybean Seeds

Parameter	Unit	OECD	ILSI	CRC (1989)	USDA-IOWA (US food + commodity quality)	TAS	Range
Daidzein	mg/kg	ND	ND	6	ND	5 - 35	5 - 35
Daidzin [a]	mg/kg	ND	ND	581	ND	13 - 1244	13 - 1244
Total Daidzin [b]	mg/kg aglycone	202 - 2060	60.0 - 2454	ND	466 - 522	206 - 2060	60.0 - 2454
Genistein	mg/kg	ND	ND	14	ND	0.3- 46	0.3 - 46
Genistin [a]	mg/kg	ND	ND	1644	ND	16 - 2105	16 - 2105
Total Genistin [b]	mg/kg aglycone	315 - 2680	144 - 2837	ND	738 - 917	430 - 2040	144 - 2837
Glycitein	mg/kg	ND	ND	1	ND	1.1 - 80	1.1 - 80
Glycitin [a]	mg/kg	ND	ND	338		53 - 285	53 - 338
Total Glycitin [b]	mg/kg aglycone	109 - 1070	15.3 - 310	ND	109 - 121	82 - 107	15.3 - 1070
Total Isoflavones [b]	mg/kg aglycone	440 - 9100 [a]	679 - 3733	2600 [a]	1284 - 1534	995 - 1636	679 - 3733

ND No data
[a] Result not converted to aglycone
[b] Amounts given as aglycone(s) after hydrolysis of the isoflavone glucosides and esters

Table 5: Reference Ranges for Anti-nutrients in Soybean Seed

Parameter	Unit	OECD	ILSI	Kakade	Hui	FSK	CRC (1989)	Range
Raffinose	%dm	0.11 - 1.02	0.21 - 0.66	ND	1.25	1.10 - 1.28	1.10	0.11 - 1.28
Stachyose	%dm	1.48 - 4.66	1.21 - 3.50	ND	6.30	3.70 - 3.75	3.70	1.21 - 6.30
Phytic acid	%dm	1.0 - 2.74	0.63 - 1.96	ND	1.0 - 1.5	1.0 - 1.5	ND	0.63 - 2.74
Trypsin inhibition	TIU/g dm	ND	19 590 - 118 680	40 000 - 73 600 [a]	ND	ND	ND	19 590 - 118 680
Lectins	HU/mg dm	ND	0.11 - 9.04	14.8 - 129 [b]	ND	ND	ND	0.11 - 129

ND No data
[a] Value reported (100-184 TIU/mg protein) converted to TIU/g seed based on an average protein content of 40% seed dm; f = 400.
[b] Value reported (37 - 323 HU/mg protein) converted to HU/mg seed based on an average protein content of 40% seed dm, f = 0.4.

Table 6: Reference Ranges for Amino Acids in Soybean Seeds

Parameter	Unit	OECD	ILSI	FSK	USDA	USCA	Red Book	Range
Alanine	% dm	ND	1.51 - 2.10	1.67	1.88	ND	ND	1.51 - 2.10
Arginine	% dm	2.45 - 3.1	2.29 - 3.40	2.17 - 2.24	3.09	3.11	2.95	2.17 - 3.40
Aspartic acid	% dm	ND	3.81 - 5.12	4.36	5.01	ND	ND	3.81 - 5.12
Cystine	% dm	0.45 - 0.67	0.37 - 0.81	0.56 - 0.73	0.64	0.60	0.61	0.37 - 0.81
Glutamic acid	% dm	ND	5.84 - 8.20	7.09	7.72	ND	ND	5.84 - 8.20
Glycine	% dm	ND	1.46 - 2.00	1.55	1.84	1.66	2.27	1.46 - 2.27
Histidine	% dm	1.0 - 1.22	0.88 - 1.18	0.84 - 0.98	1.08	1.06	1.14	0.84 - 1.22
Isoleucine	% dm	1.76 - 1.98	1.54 -2.08	1.71 - 2.20	1.93	2.32	2.27	1.54 - 2.32
Leucine	% dm	2.2 - 4.0	2.59 - 3.62	3.10	3.24	3.28	3.07	2.2 - 4.0
Lysine	% dm	2.5 - 2.66	2.29 -2.84	1.55 - 2.59	2.65	2.67	2.56	1.55 - 2.84
Methionine	% dm	0.5 - 0.67	0.43 -0.68	0.53 - 0.76	0.54	0.59	0.61	0.43 - 0.76
Phenylalanine	% dm	1.6 - 2.08	1.63 -2.35	1.98 - 2.39	2.08	2.22	2.16	1.60 - 2.39
Proline	% dm	ND	1.69 - 2.28	1.99	2.33	ND	2.16	1.69 - 2.33
Serine	% dm	ND	1.11 - 2.48	1.85	2.31	2.33	2.39	1.11 - 2.48
Threonine	% dm	1.4 - 1.89	1.14 - 1.86	1.46 - 1.85	1.73	1.81	1.59	1.14 - 1.89
Tryptophan	% dm	0.51 - 0.67	0.36 - 0.50	0.43 - 0.57	0.58	0.59	0.63	0.36- 0.67
Tyrosine	% dm	ND	0.10 - 1.61	1.28 - 1.48	1.51	1.12	1.36	0.10 - 1.61
Valine	% dm	1.5 - 2.44	1.60 - 2.20	1.54 - 2.16	1.99	2.25	2.05	1.50 - 2.44

ND No data

Table 7: Reference Ranges for Fatty Acids in Soybean Seeds

Fatty acids	OECD	OECD	ILSI	FSK	FSK	USDA	USDA	Range
	% dm	% rel [a]	% rel.	% dm	% rel [b]	% dm	% rel [b]	% rel
Saturated								
Lauric acid C12:0	ND	ND	< 0.10 - 0.13	0.011	0.06	ND	ND	< 0.10 – 0.13
Myristic acid C14:0	ND	ND	< 0.10 - 0.24	0.039	0.17	0.055	0.27	< 0.10 – 0.27
Palmitic acid C16:0	1.44 - 2.31	7 - 12	9.55 - 15.77	1.89	10.05	2.31	11.41	7 - 16
Margaric acid C17:0	ND	ND	< 0.10 - 0.15	ND	ND	ND	ND	< 0.10 - 0.15
Stearic acid C18:0	0.54 - 0.91	2 - 5	2.70 - 5.88	0.67	3.55	0.78	3.84	2 - 5.9
Arachidic acid C20:0	0.04 - 0.7	<1.0	0.16 - 0.48	ND	ND	ND	ND	< 0.10 – 0.48
Behenic acid C22:0	ND	ND	0.28 - 0.60	ND	ND	ND	ND	0.28 - 0.60
Lignoceric acid C24:0	ND	ND	0.15	ND	ND	ND	ND	0.15
Mono-unsaturated								
Palmitoleic acid C16:1	ND	ND	< 0.10 - 0.19	0.091	0.48	0.060	0.30	< 0.10 - 0.48
Oleic acid C18:1	3.15 - 8.82	19 - 34	14.3 - 32.2	4.35	23.14	4.75	23.46	14 - 34
Gadoleic acid C20:1	ND	ND	0.14 - 0.35	0.039	0.17	ND	ND	0.14 – 0.35
Erucic acid C22:1	ND	ND	ND	ND	ND	ND	ND	ND
Poly-unsaturated								
Linoleic acid C18:2	6.48 - 11.6	48 - 60	42.3 - 58.8	10.71	56.97	10.85	53.58	48 - 60
Linolenic acid C18:3	0.72 - 2.16	2 - 10	3.00 - 12.52	1.02	5.43	1.45	7.16	2 - 10

ND No data
[a] Fatty acid profile in soybean oil
[b] Converted from % dm values

Table 8: Reference Ranges for Proximate and Fibre Compounds in Soybean Hulls

Parameter	Unit	OECD	Ensminger	Kellems	Nut Beef	USCA	Nut Cow	CRC (1983)	Range
Moisture	%	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0
Protein	% dm	10.8	11.9	12.1	12.1	12.1	12.0	13.7	10.8-13.7
Crude fat	% dm	2.0	2.2	ND	2.1	2.1	ND	ND	2.0-2.2
Ash	% dm	4.6	5.1	ND	5.1	5.1	ND	ND	4.6-9.8
Total Carbo-hydrates [a]	% dm	82.6	80.9	ND	80.7	80.7	ND	ND	76.0-82.6
Crude fibre	% dm	ND	39.8	40.0	40.1	40.1	39.0	39.0	39.0-40.1
ADF	%dm	42.4	46.6	50.0	50.0	50.0	46.0	ND	42.4-50.0
NDF	%dm	59.4	65.3	67.0	ND	ND	ND	ND	59.4-67.0

ND No data found

[a] Total carbohydrates calculated as: 100% - (protein %dm + fat %dm + ash %dm)

Table 9: Reference Ranges for Proximate and Fibre Compounds in Soybean Meal

Parameter	Unit	OECD 44% protein	OECD 49% protein	Ensminger 44% protein	Ensminger 49% protein	NCDA 41% protein	NCDA 44% protein	Kellems 44% protein	Kellems 51% protein	USDA 44% protein	USDA 49% protein	USCA 44% protein	Range ≤ 44%fw protein	Range ≥ 49%fw protein
Moisture	% fw	-	-	11	10	-	-	11.0	10.2	6.9	6.9	11.0	6.9 - 11.0	6.9 - 10.2
Protein	% dm	43.8 - 49.9	52.8 - 56.3	49.9	54.4	41.0	44.0	49.4	56.7	48.2	52.8	49.9	41.0 - 49.9	52.8 - 56.7
Crude fat	% dm	0.55 - 3.00	1.00 - 3.30	1.7	1.3	4.0	0.5	ND	ND	2.57	2.57	1.5	0.5 - 4.0	1.0 - 3.3
Ash	% dm	5.6 - 7.2	5.2 - 9.1	7.2	6.8	6.0	6.0	ND	ND	6.0	6.0	7.3	5.6 - 7.3	5.2 - 9.1
Total Carbo-hydrates [a]	% dm	39.9 - 50.1	31.3 - 41.0	41.2	37.5	ND	ND	ND	ND	43.1	38.5	41.3	39.9 - 50.1	31.3 - 41.0
Crude fibre	% dm	4.3 - 7.2	3.14 - 4.10	7.0	4.1	6.0	6.0	7.9	3.1	ND	ND	7.0	4.3 - 7.9	3.1 - 4.1
ADF	% dm	8.9 - 11.9	5.2 - 6.7	10.0	6.9	ND	ND	ND	ND	ND	ND	ND	8.9 - 11.9	5.2 - 6.9
NDF	% dm	12.3 - 18.9	7.4 - 12.2	14.0	7.3	ND	ND	ND	ND	ND	ND	ND	12.3 - 18.9	7.4 - 12.2

ND No data

[a] Total carbohydrates calculated as: 100% - (protein %dm + fat %dm + ash %dm)

Table 10: Reference Ranges for Anti-nutrients in Soybean Meal [a]

Parameter	Unit	Liener	ASA	Novak + Haslberger	Range
Raw soybean meal/flour					
Trypsin inhibition	TIU/g dm	52 100	23 900	43 000	23 900 – 52 100
Lectins	HU/mg	ND	2.9 [b]	2.8 [b]	2.8 - 2.9
Toasted soybean meal/ flour					
Trypsin inhibition	TIU/g dm	3 200 – 7 900	3 100	3 000	3 000 - 7900
Lectins	HU/mg	ND	0.02 [b]	< 0.5	< 0.5

ND No data

[a] Stachyose, raffinose, phytic acid and isoflavone levels in toasted soybean meal are not different from the levels in soybean seeds and not toasted soybean meal (Liener, 1994).

[b] Values converted from HU/mg protein to HU/mg seed based on an average protein content of 40% seed dm, f = 0.4.

Table 11: Reference Ranges for Amino Acids in Soybean Meal

Parameter	Unit	OECD 44%fw protein	Kellems 45,8%fw protein	USCA 44%fw protein	USDA 44%fw protein	CRC (1983) 44%fw protein	CRC (1983) 49%fw protein	Range
Alanine	% dm	ND	ND	ND	2.27	ND	ND	2.27
Arginine	% dm	3.49-3.78	3.60	3.38	3.75	3.71	4.11	3.38-4.11
Aspartic acid	% dm	ND	ND	ND	6.07	ND	ND	6.07
Cystine	% dm	0.66-0.75	0.75	0.83	0.78	0.79	0.78	0.66-0.83
Glutamic acid	% dm	ND	ND	ND	9.35	ND	ND	9.35
Glycine	% dm	ND	2.36	2.03	2.23	2.58	2.56	2.03-2.58
Histidine	% dm	1.21-1.32	1.24	1.19	1.30	1.35	1.44	1.19-1.44
Isoleucine	% dm	2.15-2.78	2.81	2.27	2.34	2.70	2.89	2.15-2.89
Leucine	% dm	3.66-3.92	3.82	3.65	3.93	3.93	4.22	3.65-4.22
Lysine	% dm	2.99-3.22	3.26	2.99	3.21	3.29	3.53	2.99-3.53
Methionine	% dm	0.60-0.69	0.67	0.58	0.65	0.79	0.78	0.58-0.79
Phenylalanine	% dm	2.35-3.0	2.47	2.36	2.52	2.58	2.33	2.33-2.58
Proline	% dm	ND	ND	ND	2.82	ND	ND	2.82
Serine	% dm	ND	ND	2.36	2.80	2.61	3.22	2.36-3.22
Threonine	% dm	1.89-2.03	1.91	1.85	2.10	2.03	2.12	1.85-2.12
Tryptophan	% dm	0.66-0.75	0.67	0.71	0.70	0.70	0.74	0.66-0.74
Tyrosine	% dm	ND	1.57	1.48	1.83	1.46	2.22	1.46-2.22
Valine	% dm	2.24-2.67	2.70	2.25	2.51	2.58	3.00	2.24-3.00

ND No data

Table 12: Reference Ranges for Moisture, Protein and Amino Acids in Soy Protein Isolate

Parameter	Unit	OECD Protein Isolate 80 % protein	USDA Protein Conc. > 70 % protein	USDA Protein Isolate 80 % protein	CRC (1983) Protein Conc.	CRC (1983) Protein isolate	Range
Moisture	% fw	-	8.0	4.98	5.8	5.4	5.0 - 8.0
Protein	% dm	80	91.9	84.9	72.0	93.9	72.0 - 93.9
Alanine	% dm	ND	ND	3.78	4.67	4.33	3.78 - 4.67
Arginine	% dm	6.67	8.00	7.02	7.96	8.24	6.67 - 8.24
Aspartic acid	% dm	ND	ND	10.20	12.74	12.58	10.20 - 12.74
Cystine	% dm	1.05	1.00	1.10	1.70	1.37	1.00 - 1.70
Glutamic acid	% dm	ND	ND	18.37	21.02	22.20	18.37 - 22.20
Glycine	% dm	ND	3.62	3.79	4.67	4.33	3.62 - 4.67
Histidine	% dm	2.3	2.63	2.42	2.87	2.85	2.30 - 2.87
Isoleucine	% dm	4.25	5.02	4.48	5.10	5.18	4.25 - 5.18
Leucine	% dm	6.78	6.90	7.14	8.28	8.46	6.78 - 8.46
Lysine	% dm	5.33	6.12	5.61	6.69	6.77	5.33 - 6.77
Methionine	% dm	1.13	0.96	1.19	1.49	1.37	0.96 - 1.49
Phenylalanine	% dm	4.59	4.71	4.83	5.52	5.71	4.59 - 5.71
Proline	% dm	ND	ND	5.22	5.52	5.81	5.22 - 5.81
Serine	% dm	ND	5.66	4.83	6.05	5.28	4.83 - 6.05
Threonine	% dm	3.14	3.64	3.30	4.46	3.91	3.14 - 4.46
Tryptophan	% dm	1.12	0.96	1.17	1.59	1.48	0.96 - 1.59
Tyrosine	% dm	ND	3.38	3.39	4.14	4.12	3.38 - 4.14
Valine	% dm	4.1	4.77	4.31	5.20	4.97	4.10 - 5.20

ND No data

Table 13: Reference Ranges for Fatty Acids in Soybean Oil

Fatty acids	OECD	Codex	FSK	BG	USDA	Range
	% rel	% rel	% rel	% rel	% rel	% rel
Saturated						
Myristic acid C14:0	ND	<0.05 - 0.2	ND	ND	0.10	<0.05 - 0.2
Palmitic acid C16:0	7 - 12	8.0 - 13.5	6.7 - 14.5	10	10.3	6.7 - 14.5
Margaric acid C17:0	ND	<0.05 - 0.1	ND	ND	ND	<0.05 - 0.1
Stearic acid C18:0	2 - 5	2.0 - 5.4	0.5 - 8.9	3.5	3.8	0.5 - 8.9
Arachidic acid C20:0	<1.0	0.1 - 0.6	0.1 - 0.9	0.5	ND	0.1 - 0.9
Behenic acid C22:0	ND	<0.05 - 0.7	ND	0	ND	<0.05 - 0.7
Lignoceric acid C24:0	ND	<0.05 - 0.5	ND	ND	ND	<0.05 - 0.5
Mono-unsaturated						
Palmitoleic acid C16:1	ND	<0.05 - 0.2	ND	ND	0.2	<0.05 - 0.5
Oleic acid C18:1	19 - 34	17.0 - 30.0	14.3 - 28.7	21	22.8	14.3 - 34.0
Gadoleic acid C20:1	ND	<0.05 - 0.5	ND	0.5	0.2	<0.05 - 0.5
Poly-unsaturated						
Linoleic acid C18:2	48 - 60	48.0 - 59.0	36.5 - 57.8	56	51.0	36.5 - 60.0
Linolenic acid C18:3	2 - 10	4.5 - 11.0	1.9 - 14.7	8	6.8	1.9 - 14.7

ND No data

Table 14: Reference Ranges for Fat-Soluble Vitamins in Soybean Oil

Vitamin	Unit	Codex	FSK	BG	Range for Seed	Range
Vitamin A (β-Carotene)	mg/kg	ND	35	ND	0.26 – 4.37	1.3 – 35
Vitamin K	mg/kg	ND	0.03	ND	0.38 – 0.51	0.03 – 2.55
α-Tocopherol	mg/kg	9 – 352	75.0 – 95.3	179	-	9 – 352
β-Tocopherol	mg/kg	ND – 36	9.00 – 13.1	28	-	ND – 36
γ-Tocopherol	mg/kg	89 – 2307	662 – 699	604	-	89 – 2307
δ-Tocopherol	mg/kg	154 – 932	191 – 288	371	-	154 – 932
Total Tocopherol	mg/kg	600 - 3370	937 - 1095	ND	-	600 - 3370

[a] Vitamin A and K ranges calculated including the ranges for seeds. For conversion of vitamin A and K levels from mg/kg dm in seeds into mg/kg oil use factor F=5, since the average oil content in soybean seeds is about 20% dm.

Table 15: Reference Ranges for Phosphatides in Soybean Lecithin

Parameter	Unit	Pardun (crude)	Nasner (crude)	Nasner (refined)	Sotirhos Sample I (refined)	Sotirhos Sample II (refined)	Stephan (crude)	Range (only crude)
Total phospholipids	%	45 - 60	56 [a]	86	ND	ND	64.9	45 – 64.9
Phosphatidylcholine	%	23.5	13 - 16	20 - 23	21.2	20.2	7.5 – 21.9	7.5 - 23.5
Phosphatidylethanolamine	%	20	14 - 17	21 – 24	16.8	12.9	5.3 – 20	5.3 - 20
Phosphatidylinositol	%	14	11 - 14	18 – 22	9.4	9.0	5.1 – 21	5.1 - 21
Phosphatidic acid	%	ND	3 - 8	6 – 12	ND	ND	2.0 – 14.5	2 – 14.5
Phosphatidylserin	%	4	ND	-	ND	ND	0 – 0.3	0 - 4
Other phospholipids	%	14.5	5 - 10	8 - 13	0.5	1.1	3.6	3.6 - 14.5

ND No data
[a] Crude lecithin consists of about 56% phospholipids, 6% glycolipids and 38.5 - 45% neutral lipids