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**Series on the Safety of Novel Foods and Feeds, No. 11**

**CONSENSUS DOCUMENT ON COMPOSITIONAL CONSIDERATIONS FOR NEW VARIETIES OF  
COTTON (*Gossypium hirsutum* and *Gossypium barbadense*): KEY FOOD AND FEED NUTRIENTS  
AND ANTI-NUTRIENTS**

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No. 2, Consensus Document on Compositional Considerations for New Varieties of Soybean: Key Food and Feed Nutrients and Anti-nutrients (2001)

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OECD Environment, Health and Safety Publications

Series on the Safety of Novel Foods and Feeds

**No. 11**

**Consensus Document on Compositional  
Considerations for New Varieties of Cotton  
(*Gossypium hirsutum* and *Gossypium  
barbadense*): Key Food and Feed Nutrients  
and Anti-Nutrients**

**Environment Directorate**

**Organisation for Economic Co-operation and Development**

**Paris 2004**

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## FOREWORD

The OECD's Task Force for the Safety of Novel Foods and Feeds decided at its first session, in 1999, to focus its work on the development of science-based *consensus documents*, which are mutually acceptable among member countries. These consensus documents contain information for use during the regulatory assessment of a particular food/feed product. In the area of food and feed safety, consensus documents are being published on the nutrients, anti-nutrients or toxicants, information of its use as a food/feed and other relevant information.

This consensus document addresses compositional considerations for new varieties of cotton by identifying the key food and feed nutrients and anti-nutrients. A general description of these components is provided. As well, there is background material on the production, processing and uses of cotton and considerations to be taken when assessing new cotton varieties.

The United States served as the lead country in the preparation of this document.

The Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology has recommended that this document be made available to the public. It is published on the authority of the Secretary-General of the OECD.



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## PREAMBLE

Food and feed products of modern biotechnology are being commercialised and marketed in OECD member countries. The need has been identified for detailed technical work aimed at establishing appropriate approaches to the safety assessment of these products.

At a Workshop held in Aussois, France (OECD 1997), it was recognised that a consistent approach to the establishment of substantial equivalence might be improved through consensus on the appropriate components (*e.g.*, key nutrients, key toxicants and anti-nutritional compounds) on a crop-by-crop basis, which should be considered in the comparison. It is recognised that the components may differ from crop to crop. The Task Force therefore decided to develop consensus documents on compositional data. These data are used to identify similarities and differences following a comparative approach as part of a food and feed safety assessment. They should be useful to the development of guidelines, both national and international and to encourage information sharing among OECD member countries.

These documents are a compilation of current information that is important in food and feed safety assessment. They provide a technical tool for regulatory officials as a general guide and reference source, and also for industry and other interested parties and will complement those of the Working Group on Harmonisation of Regulatory Oversight in Biotechnology. They are mutually acceptable to, but not legally binding on, member countries. They are not intended to be a comprehensive description of all issues considered to be necessary for a safety assessment, but a base set for an individual product that supports the comparative approach. In assessing an individual product, additional components may be required depending on the specific case in question.

In order to ensure that scientific and technical developments are taken into account, member countries have agreed that these consensus documents will be reviewed periodically and updated as necessary. Users of these documents are invited to provide the OECD with new scientific and technical information, and to make proposals for additional areas to be considered.

## THE ROLE OF COMPARATIVE APPROACH AS PART OF A SAFETY ASSESSMENT

In 1990, a joint consultation of the Food and Agriculture Organisation of the United Nations (FAO) and the World Health Organisation (WHO) established that the comparison of a final product with one having an acceptable standard of safety provides an important element of safety assessment (WHO, 1991).

In 1993 the Organisation for Economic Co-operation and Development (OECD) further elaborated this concept and advocated the approach to safety assessment based on substantial equivalence as being the most practical approach to addressing the safety of foods and food components derived through modern biotechnology (as well as other methods of modifying a host genome including tissue culture methods and chemical or radiation induced mutation). In 2000 the Task Force concluded in its report to the G8 that the concept of substantial equivalence will need to be kept under review.

The Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology in 2000 concluded that the safety assessment of genetically modified foods requires an integrated and stepwise, case-by-case approach, which can be aided by a structured series of questions. A comparative approach focusing on the determination of similarities and differences between the genetically modified food and its conventional counterpart aids in the identification of potential safety and nutritional issues and is considered the most appropriate strategy for the safety and nutritional assessment of genetically modified foods. The concept of substantial equivalence was developed as a practical approach to the safety assessment of genetically modified foods. It should be seen as a key step in the safety assessment process although it is not a safety assessment in itself; it does not characterise hazard, rather it is used to structure the safety assessment of a genetically modified food relative to a conventional counterpart. The Consultation concluded that the application of the concept of substantial equivalence contributes to a robust safety assessment framework.

A previous Joint FAO/WHO Expert Consultation on Biotechnology and Food Safety (1996) elaborated on compositional comparison as an important element in the determination of substantial equivalence. A comparison of critical components can be carried out at the level of the food source (*i.e.*, species) or the specific food product. Critical components are determined by identifying key nutrients, key toxicants and anti-nutrients for the food source in question. The comparison of critical components should be between the modified variety and non-modified comparators with an appropriate history of safe use. The data for the non-modified comparator can be the natural ranges published in the literature for commercial varieties or the measured levels in parental or other edible varieties of the species (FAO, 1996). The comparator used to detect unintended effects for all critical components ideally should be the near isogenic non-modified variety grown under identical conditions. While the comparative approach is useful as part of the safety assessment of foods derived from plants developed using recombinant DNA technology, the approach could be applied to foods derived from new plant varieties that have been bred by other techniques.

## SECTION I - BACKGROUND

### A. Production of cotton for food and feed

Cotton is of the *Gossypium* genus that is grown on every major continent and on West Indies and Pacific Basin islands. Cotton is cultivated in areas of intense heat. In the dryer climates irrigation produces high quality cotton. Cotton is considered the most prominent source of textile fibre in the world. It makes up over 40% of the total fibre used in the world (USDA ERS, 2002). It is one of the oldest cultivated crops, dating back to some 5000 years. Documentation of cotton cloth in ancient times has been achieved in Pakistan, Egypt and the south central United States. Explorations from Europe were stimulated during the 15<sup>th</sup> and 16<sup>th</sup> century by a desire to locate more sources of cotton (NCPA, 1999). Natives wearing cotton garments were found in the West Indies and Mexico. There are over 40 species of cotton, but only four are important economically. In the U.S., two primary types of cotton are grown, *Glossypium hirsutum* which has a staple length of 2.5 - 3.2 cm being the dominant variety, and *Gossypium barbadense* with a staple length of 2.5 - 3.8 cm, having limited production (USDA ERS, 2002).

Cotton plant contains a central stem with many branches. There are typically five separate petals per flower and stamens surround the style part of the plant. The ovary of the plant develops into a boll as a dry structure and when dried, splits open along four or five lines. The fibres and seeds are contained within the boll. Each fibre grows as a single cell hair from the epidermis of the coat of the seed. Layers of cellulose form around the cell wall. Cell hairs develop into two lengths, long (lint) and short (fuzz) with the lint being the fibre of choice for textiles.

**Table 1. World cotton production 2001/2002**

	<b>Production<sup>a</sup></b>	<b>% of total</b>
<b>China</b>	5,313	25
<b>USA</b>	4,421	21
<b>India</b>	2,569	12
<b>Pakistan</b>	1,785	8.4
<b>Uzbekistan</b>	1,067	5
<b>Turkey</b>	849	4
<b>Brazil</b>	784	3.7
<b>Australia</b>	675	3.1
<b>Greece</b>	457	2.1
<b>Syria</b>	348	1.6
<b>Egypt</b>	310	1.2
<b>Mali</b>	250	1.1
<b>Other countries</b>	2499	11.7
<b>TOTAL WORLD</b>	21,327	

Source: [http://www.fas.usda.gov/cotton\\_arc.html](http://www.fas.usda.gov/cotton_arc.html)

<sup>a</sup>Thousand tonnes

Only the cotton boll is useful for either textile fibres or for food or feed. The remainder of the plant is left in the field for decomposition as fertilizer. Historically cotton was hand picked, but today in

industrialized countries most is picked with a mechanical harvester. Following picking, the cotton boll is usually mechanically compressed into modules for transport to a processing plant called a cotton gin. The modulated cotton is usually quite high in moisture and must be processed in a timely manner to avoid spoilage. With spindle pickers and stripper harvestors about 15% and 48%, respectively, of the harvested product is a waste product called gin trash. Gin trash consists of stems, leaves, pieces of bolls and sand picked up in the field. Prior to ginning, gin trash is removed from the cotton by cleaning screens, shakers and air equipment. In the ginning process of the cotton boll, the fibre, for textile use, is separated from the seed and compressed into 217.7 kg bales (NCPA, 1999). The separated seed at this point is called fuzzy cottonseed and makes up about 60% of the cotton boll. The resulting cottonseed can either be further processed or be used directly as cattle feed.

## **B. Processing of fuzzy cottonseed**

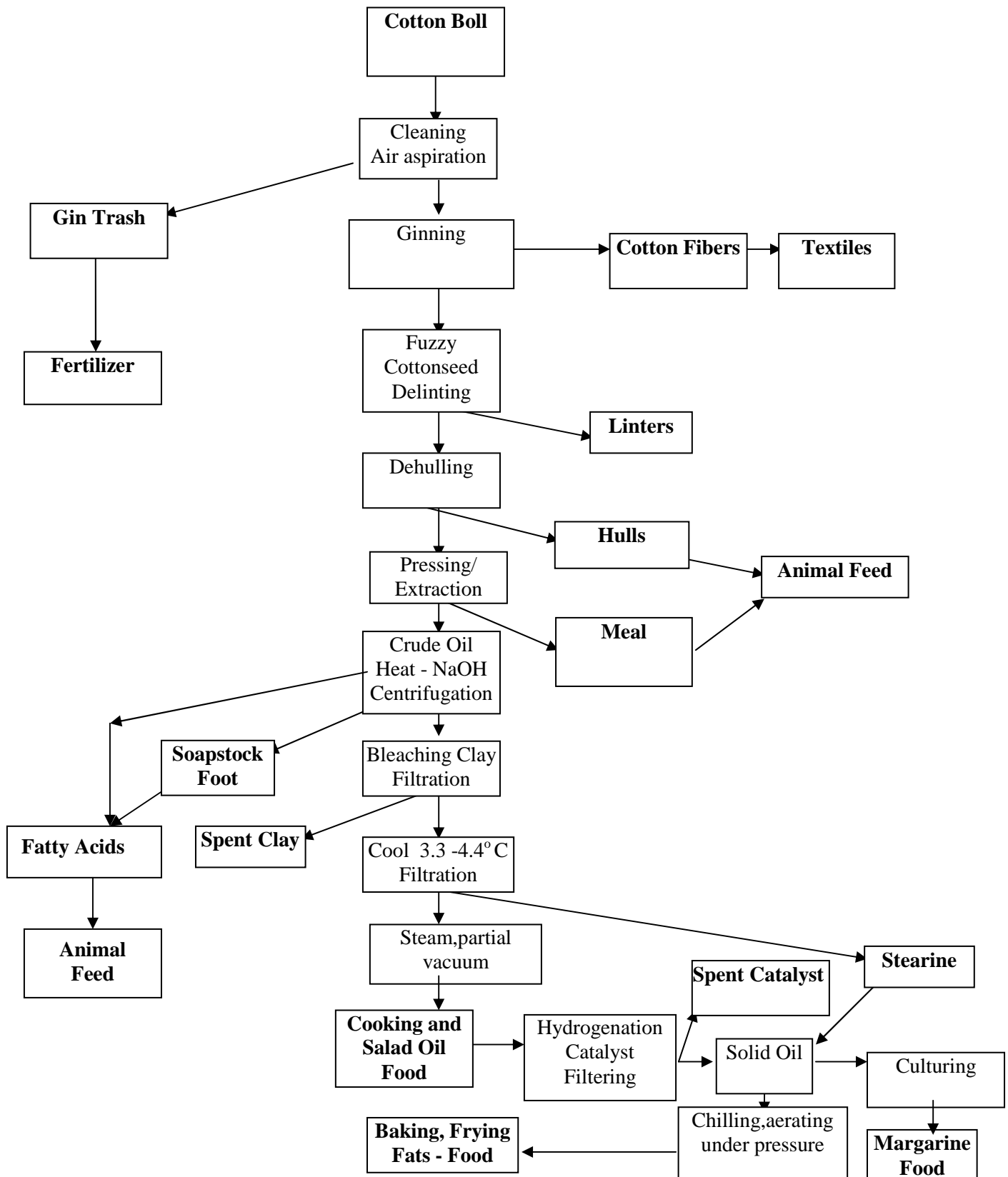
Fuzzy cottonseed is processed into four major products: oil, meal, hulls, and linters. Cherry and Leffler (1984) list typical yields as 45% meal, 26 % hulls, 16 % oil, 9% linters and 4% lost in processing. Upon arrival of fuzzy cottonseed at the processing plant, fuzzy cottonseed is delinted, by a machine which has a series of fine circular saws that cuts off the fibres, producing linters that are used for human food (NCPA, 1999). Linters are highly processed (alkaline pH, high temperature) to remove non-cellulose components. Linters are a major source of cellulose for chemical and food use. The delinted cottonseed is then dehulled by machines equipped with knife blades cutting the hulls away from the seed. Separators sift out the seeds from the hull. Hulls are used in animal feed. The resulting dehulled cottonseed (meats) are processed through a series of iron rollers to produce flakes. The flakes are cooked, reducing the moisture level. The flaked cooked seed moves to the presser to remove the oil. Modern high-pressure screw presses are employed but solvent extraction is also commonly included for maximum efficiency. Oil is pumped, filtered and stored in tanks. Oil goes for further processing for human consumption. The flake remnants are collected, cooled and ground into meal. The process is 96 - 97% efficient in removing oil, but can leave 3-4% of the oil in the meal. The meal is used for animal feed.

## **C. Processing of cottonseed oil**

Cottonseed oil requires further processing for food use. Sodium hydroxide is added after heating and forms soapstock or foot that is removed by centrifugation. Both soapstock and crude oil are used to produce fatty acids. To get clear oil, bleaching clay is added and combines with coloring material that can be separated from the oil by filtration. Stearine, a component of cottonseed oil, is further removed from the oil by reducing the temperature to 3.3 - 4.4 °C, at which point the stearine crystalizes, lending itself to separation by filtration. All cottonseed oil is further treated with steam under a partial vacuum to remove off-flavors. This produces a very highly refined and quality product. Because of its superior flavor stability, most of the pure oil is used as cooking or salad oil.

The stearine that was separated by solidification is used in margarine and shortening products. For the pure oil to be used in shortening and margarine, it must be solidified by hydrogenation in the presence of a catalyst. Following hydrogenation, the product is again filtered to remove the catalyst. To make margarine, the solidified oil is mixed with cultured pasteurized skim milk, salt and minor ingredients. Shortening is prepared by chilling and aerating the solidified oil under pressure.

Figure 1 – Processing of Cotton



**D. Appropriate comparators for testing new varieties**

This paper suggests parameters that cotton developers should measure. Measurement data from the new variety should ideally be compared to those obtained from the near isogenic non-modified variety. A developer can also compare values obtained from new varieties with literature values present in this paper. Critical components include key nutrients, toxicants, and antinutrients for the food source in question. Key nutrients are those components in a particular product, which may have a substantial impact in the overall diet. These may be major constituents (fats, proteins, and structural and non-structural carbohydrates) or minor compounds (vitamins and minerals). Key toxicants are those toxicologically significant compounds known to be inherently present in the species, *i.e.*, compounds whose toxic potency and levels may impact human and animal health. Similarly, the levels of known anti-nutrients and allergens should be considered. As part of the comparative approach, selected secondary plant metabolites, for which characteristic levels in the species are known, are analysed as further indicators of the absence of unintended effects of the genetic modification on the metabolism.

**E. Traditional characteristics screened by cotton developers**

Phenotypic characteristics provide important information related to the suitability of new varieties for commercial distribution. Selecting new varieties is initially based on parent data. Plant breeders developing new varieties of cotton evaluate many parameters at different stages in the developmental process. In the early stages of growth, breeders evaluate stand count and seedling vigor. As the plant matures, pesticide resistance and disease data is evaluated, *e.g.*, root rot, leaf spots, blight, bollworm/tobacco budworm, cotton aphid, and *Verticillium* and *Fusarium* wilt (U.Ga. 2002; TAM, 2002). The harvested cottonseed is measured for yield, staple length and strength (Bourland, 2002). In some cases, plants are modified for specific increases in certain components, and the plant breeder would be expected to analyse for such components.

## SECTION II- NUTRIENTS IN WHOLE COTTONSEED AND COTTONSEED PRODUCTS

## A. Cottonseed

Fuzzy or whole cottonseed is the linted cottonseed remaining after the ginning process to remove cotton fibres for textile production (NCPA, 2002). However, cottonseed is sometimes delinted and not further processed. Also there are varieties, notably Pima that have no linters. These products currently make up only a small percentage of cottonseed available for livestock feeding. Not much data is available on the delinted cottonseed or the Pima varieties except that it contains more gossypol than other varieties (Kirk and Higginbotham, 1999). Arana *et al.* (2000) indicated that they found lower neutral detergent fibre and acid detergent fibre levels in the delinted products than for whole cottonseed. The nutrient composition of whole cottonseed is shown in Tables 2 - 5.

Table 2 – Proximate analysis of cottonseed<sup>a</sup>

Reference		USDA <sup>b</sup>	Ensminger <sup>c</sup>	NCPA <sup>d</sup>	NRC <sup>e</sup>	Commercial range <sup>f</sup>	Range of all reported values
Moisture	% of fw <sup>g</sup>	4.7	9.0	8.4	8.0 - 9.9	4.0-8.7	4.0 – 9.9
Protein	% of dw <sup>g</sup>	34.2	24.0	22.5	23.0 -24.4	21.8 – 28.2	21.8 – 34.2
Total fat	% of dw	36.3		29.5	17.2 – 23.1	15.4-23.8	15.4 – 36.3
Ash	% of dw	4.8 <sup>d</sup>		3.8	4.2 – 5.0	3.8-4.9	3.8 – 5.0
Neutral detergent fibre (total fibre)	% of dw			47.2	40.0 – 50.3	42.1 – 54.8	40.0 – 54.8
Acid detergent fibre (cellulose)	% of dw			38.8	29.0 – 40.1	35.5 – 37.7	29.0 – 40.1
Crude fibre	% of dw		21.4		20.8 – 24.0	15.4 - 28.2	15.4 – 28.2
Total dietary fibre	% of dw	5.77				5.77	5.77
Nonfibrous Carbohydrates <sup>h</sup>	% of dw				23.0	45.6 - 53.6	23.0 – 53.6

<sup>a</sup>: Proximate analysis of cotton usually includes acid detergent fibre (ADF) and neutral detergent fibre (NDF). The terms ADF and NDF are commonly used in the feed industry and values for comparison are available. Crude fibre, though not the preferred constituent, is still used by some. For food use, however, the concept of dietary fibre is preferred, although different definitions and methods of analysis are being used (see: USA Panel on the Definition of Dietary Fibre (NRC, 2001b)). The value for total dietary fibre from Souci *et al.* (1989) is obtained using a modification of the analytical method recommended by the Association of Official Analytical Chemists (AOAC). Total Dietary Fibre determined this way includes lignin and non-starch polysaccharides (including cellulose, hemicellulose and pectin).

<sup>b</sup>: USDA ARS (2004) Cottonseed kernels roasted; Dry weight data was converted from g/100g edible portion using the stated moisture content; Possibly including modified-varieties

<sup>c</sup>: Ensminger *et al.* (1990)

<sup>d</sup>: NCPA (1999)

<sup>e</sup>: Values taken from, NRC (1982), NRC (1989), NRC (1994), NRC (2000) and NRC (2001a); Possibly including modified-varieties

<sup>f</sup>: Commercial range on non-modified controls, compiled from data from acid delinted cottonseed, Monsanto (2000), and Bayer (2002)

<sup>g</sup>: fw = fresh weight; dw = dry weight

<sup>h</sup>: Nonfibrous carbohydrate = 100 – (%NDF + %CP + % Fat + % Ash)

Table 3 - Levels of minerals and vitamins in cottonseed<sup>a</sup>

Reference	USDA <sup>b,c</sup>		NRC <sup>c,d</sup>	NCPA <sup>c,e</sup>	Commercial range <sup>f</sup>	Range of all reported values
Na	mg/100g	26.2	10 - 290	8.0	5.4 - 300	5.4 - 300
K	mg/100g	1417	1210 - 1240	1140	1080 - 1250	1080-1417
Ca	mg/100g	105	160 - 170	140	120 - 330	105-330
P	mg/100g	839	600 - 750	560	610 - 860	560-860
Mg	mg/100g	461	320 - 380	350	370 - 490	320-490
Fe	mg/100g	5.7	9.4 - 16.0	5.0	4.2 - 7.2	4.2-16.0
Cu	mg/100g	1.3	.7 - 5.4	.7	.4 - 1.0	.4 - 5.4
Se	mg/100g		.00- .01			.00 - .01
Zn	mg/100g	6.3	3.7 - 3.8	3.3	2.7 - 5.1	2.7-6.3
Mn	mg/100g	2.3	1.0 - 1.3		1.1 - 1.8	1.0 - 2.3
Vit. A	mg/kg RE <sup>g</sup>	442				442
Vit. B1 (Thiamin)	mg/kg	7.5				7.5
Vit. B2 (Riboflavin)	mg/kg	2.6				2.6
Vit. B6 (Pyridoxine)	mg/kg	7.8				7.8
Vit. C (Ascorbic acid)	mg/kg	90				90
Vit. E	mg ATE <sup>h</sup>	30				30
Folate, total	mcg/100g	2.0				2.0
Niacin (Nicotinic acid)	mg/100g	3.0				3.0

<sup>a</sup>: Values are expressed on a dry weight-basis

<sup>b</sup>: USDA ARS (2004) Cottonseed kernels roasted; Values calculated from given values on total weight-basis, using reported moisture content of 4.65 %

<sup>c</sup>: Possibly including modified-varieties

<sup>d</sup>: Values taken from NRC (1982), NRC (2000) and NRC (2001a)

<sup>e</sup>: NCPA (1999)

<sup>f</sup>: Monsanto (2000)

<sup>g</sup>: RE (Retinol Equivalent)

<sup>h</sup>: One mg ATE (Alpha tocopherol equivalent) equals 1.1 international units of vitamin E



**Table 4 - Amino acid composition of cottonseed in % of d.w. <sup>a</sup>**

Reference	USDA <sup>bc</sup>	NRC <sup>c,d</sup>	Commercial Range <sup>e</sup>	Range of all reported values
Methionine	.53	.40	.35 - .54	.35 - .54
Cystine	.86	.41	.38 - .48	.38 - .86
Lysine	1.65	1.02	1.01 - 1.33	1.01 - 1.65
Tryptophan	.49	.30	.23 - .36	.23 - .49
Threonine	1.21	.81	.74 - .96	.74 - 1.21
Isoleucine	1.17	.75	.71 - .88	.71 - 1.17
Histidine	1.03	.73	.62 - .82	.62 - 1.03
Valine	1.67	1.10	1.01 - 1.28	1.01 - 1.67
Leucine	2.23	1.38	1.27 - 1.65	1.27 - 2.23
Arginine	4.40	2.71	2.38 - 3.23	2.38 - 4.40
Phenylalanine	2.03	1.25	1.13 - 1.45	1.13 - 2.03
Glycine	1.58		.93- 1.19	.93 - 1.58
Alanine	1.51		.85- 1.13	.85 - 1.51
Aspartic acid	3.55		2.09 - 2.66	2.09 - 3.55
Glutamic acid	8.16		4.33 - 5.28	4.33 - 8.16
Proline	1.39		.82 - 1.14	.82 - 1.39
Serine	1.63		.94 - 1.32	.94 - 1.63
Tyrosine	1.17		.48 - .79	.48 - 1.17

<sup>a</sup>: Data is presented on a dry weight basis

<sup>b</sup>: USDA ARS (2004) Cottonseed kernels roasted.

<sup>c</sup>: Possibly including modified-varieties

<sup>d</sup>: NRC (1994, 1998 and 2001a); Values from NRC (1994 and 1998) were calculated from given values on total weight basis; Values from NRC (2001a) were calculated from reported % of crude protein, using given crude protein content on a dry basis.

<sup>e</sup>: Bayer (2002) and Monsanto (2000).

**Table 5 - Fatty acid composition of cottonseed in % of d.w. <sup>a</sup>**

Reference	USDA <sup>b</sup>	Monsanto <sup>c</sup>	Monsanto <sup>d</sup>	Range
14:0 Myristic	.36	.35	.32	.32 - .36
16:0 Palmitic	8.84	9.41	8.88	8.84- 9.41
16:1 Palmitoleic	.27	.24	.21	.21- .27
18:0 Stearic	.89	.88	.88	.88 - .89
18:1 incl. Oleic	6.93	6.09	5.13	5.13- 6.93
18:2 incl. Linoleic	18.74	20.12	16.01	16.01 - 20.12
18:3 incl. Linolenic	.07	.07	.07	.07

<sup>a</sup>: Data is presented on a dry weight basis

<sup>b</sup>: USDA ARS (2004) Cottonseed kernels roasted; Possibly including modified-varieties; Data converted from g/100g edible portion to % d.w. using stated moisture content of 4.65%

<sup>c</sup>: Monsanto (1994) Nontransgenic parent variety ; Values converted from % total lipid to % d.w. using mean lipid level in cottonseed of 39.2%

<sup>d</sup>: Monsanto (1995) Nontransgenic parent variety ; Values converted from % total lipid to % d.w. using mean lipid level in cottonseed of 33.5%

## B. Oil

Cottonseed oil was the first oilseed oil produced in the United States (White, 2000). The crude oil contains about 2% nonglyceride materials, which are mostly removed during processing. Included in these materials are terpenoid phytoalexin, cyclopropanoid fatty acids (CPFA), phospholipids, sterols, resins, carbohydrates and related pigments. The most notable terpenoid phytoalexin is gossypol (Hanson,

2000). The toxic effects of gossypol and CPFAs will be discussed later in this document. Processing of the oil as described above removes most of the gossypol. Also the deodorization step removes most of the CPFAs. Cottonseed oil is a pure source of fatty acids. The fatty acid composition of refined cottonseed oil is shown in Table 6.

**Table 6 - Relative fatty acid composition of refined cottonseed oil (% of total fatty acids)**

Reference	USDA <sup>a,b</sup>	NCPA <sup>c</sup>	White <sup>d</sup>	Monsanto <sup>e</sup>	Bayer <sup>f</sup>	Range
14:0 Myristic	.8	.8	.9	.8 – 2.4	.6	.6 – 2.4
16:0 Palmitic	23.8	24.4	24.7	24.3 – 28.1	21.1	21.1 – 28.1
16:1 Palmitoleic	.8	.4	.7	.4 – 1.0	.6	.4 – 1.0
18:0 Stearic	2.4	2.2	2.3	2.1 – 3.1	2.9	2.1 – 3.1
18:1 Oleic	17.8	17.2	17.6	12.9 – 20.1	14.9	12.9 – 20.1
18:2 Linoleic	54.0	55.0	53.3	46.0 – 57.1	58.2	46.0 – 58.2
18:3 Linolenic	.2	.3	.3	.1 – .3	.2	.1 – .3

<sup>a</sup>: USDA ARS (2004); Cottonseed kernels roasted. Possibly including modified-varieties

<sup>b</sup>: Values converted from g/100g oil to % of total fatty acids

<sup>c</sup>: NCPA (1999)

<sup>d</sup>: White (2000)

<sup>e</sup>: Monsanto (2000) Nontransgenic commercial varieties

<sup>f</sup>: Bayer (2002) Nontransgenic parent variety

### C. Meal, linters and hulls

Cottonseed meal, hulls and linters are by-products of the cottonseed oil industry. Of these cottonseed meal is the most abundant and is produced by pressing and solvent extraction. It is produced with and without hulls. The most common is a 41% crude protein product but some official feed definitions require a minimum of 36% crude protein for all cake and meal cottonseed products. In order to be sold as a low gossypol product, the gossypol content is limited to 0.04% (400 ppm) (AAFCO, 2003). Linters are composed of almost pure cellulose. The highest quality linters are purified in a chemical treatment of digesting, bleaching, washing and drying (NCPA, 1999). Hulls are very high in indigestible fibre. The proximate analysis, mineral content and amino acid content of meal and hulls are shown in Tables 7 through 9, respectively.

**Table 7 - Proximate analysis of meal and hulls in % of d.w.<sup>a</sup>**

Reference	Meal <sup>b</sup>		Hulls <sup>c</sup>
	Mechanical	Solvent	Range
Moisture	7.7 – 9.2	8.0 – 10.9	10.0 - 11.0
Protein	41.7 – 46.1	41.7 – 48.9	4.2 - 6.2
Fat	3.9 – 11.4	0.8 – 3.5	2.5
Crude Fibre	11.4 - 12.6	11.2 – 12.7	47.8 - 48.6
NDF <sup>d</sup>	28 – 32.3	20.8 – 30.8	89.0
ADF <sup>e</sup>	18.1	17.3 – 19.9	64.9
Ash	6.0 – 7.2	6.2 – 7.5	2.8

<sup>a</sup>: Data is presented on a dry weight basis

<sup>b</sup>: NRC (1998, 2000, 2001a); NCPA (1999); Tanksley (1990); Values from NRC (1998) were converted from an 'as fed' basis to a dry matter basis; Meal was prepared by mechanical extraction or by solvent extraction

<sup>c</sup>: NRC (2001a), NCPA (1999)

<sup>d</sup>: Neutral detergent fiber

<sup>e</sup>: Acid detergent fiber

**Table 8 - Levels of minerals in hulls and meal<sup>a</sup>**

Reference		Meal <sup>b</sup> :		Hulls <sup>c</sup> :
		Mechanical	Solvent	Range
Na	mg/100g	0.7 - 40	30 - 140	150 - 180
K	mg/100g	1240 - 1680	1200 - 1720	1130 - 1160
Ca	mg/100g	160 - 230	160 - 222	150 - 180
P	mg/100g	760 - 1140	760 - 1200	120 - 150
Mg	mg/100g	350 - 650	350 - 660	80 - 170
Fe	mg/100g	1070 - 1600	1260 - 1620	301 - 680
Cu	mg/100g	109 - 539	260 - 440	50 - 360
Zn	mg/100g	377 - 628	610 - 740	99 - 170

<sup>a</sup>: Data is presented on a dry weight basis

<sup>b</sup>: USDA ARS (2004), NRC (2000 and 2001a) Data possibly contains modified varieties; Tanksley, 1990. Meal was prepared by mechanical extraction or by solvent extraction

<sup>c</sup>: NRC (2001a), NCPA (1999); Data possibly contains modified varieties

**Table 9 – Amino acid composition of cottonseed meal in % of meal d.w. <sup>a,b</sup>**

Reference	Meal – Mechanically Extracted	Meal – Solvent Extracted
Amino acids	%	%
Methionine	.62 - .73	.62 - .74
Cystine	.64 - .78	.69 - .90
Lysine	1.57 – 1.79	1.85- 2.01
Tryptophan	.51 - .57	.53 - .56
Threonine	1.44 – 1.52	1.45 -1.58
Isoleucine	1.27 – 1.56	1.29 -1.59
Histidine	1.15 – 1.45	1.27-1.50
Valine	1.80 – 2.05	1.83 - 2.20
Leucine	2.50 – 2.74	2.62 - 2.67
Arginine	4.40 – 4.63	4.71 - 4.96
Phenylalanine	2.14 – 2.35	2.21 - 2.38
Glycine	1.83	1.87
Tyrosine	1.01	1.27
Serine	1.84	2.01

<sup>a</sup>: Data is presented as a % of dry weight

<sup>b</sup>: NCPA (1999), NRC (1982, 1998 and 2001a). Data possibly includes modified-varieties. Values from NRC (1998) were converted from an 'as fed' basis to a dry matter basis; Meal was prepared by mechanical extraction or by solvent extraction

## SECTION III- ANTI-NUTRIENTS IN COTTON

**A. Gossypol**

Cotton contains a number of terpenoid phytoalexins. Phytoalexins are antibiotics that, in cotton, accumulate in the pigment glands. They play a critical role in their resistance to potential pathogens that attack cotton. Terpenoid phytoalexins common to cotton include gossypol, hemigossypol, desoxyhemigossypol, 2,7-dihydroxy cadalene, hemigossypolone and heliocides H1 and H2 (Stipanovic, 1994). Gossypol is the most notable of the terpenoid phytoalexins and was first isolated from the pigment glands in cottonseed. It is particularly toxic to non-ruminants and has male anti-fertility properties. Gossypol is either free or bound. Free gossypol is the toxic compound. Sudweeks (2002) reported a gossypol toxicity incident where large amounts of cottonseed meal were fed, estimated to be 24 mg gossypol per head per day. Based on a review of the data, Sudweeks (2002) has suggested that 18 mg of free gossypol (equivalent to 0.1% free gossypol) is the maximum that should be fed to dairy cows. Bailey *et al.* (2000) and Ziehr *et al.* (2000) have shown that gossypol exists as two isomers, (+) and (-). The (-) isomer is the more toxic one. However, researchers are also investigating gossypol as an anti viral and anti carcinogenic drug (NIH, 2002; Reidenberg, 2003). Typical total and free gossypol levels reported for cottonseed are shown in Table 10.

**B. Cyclopropenoid fatty acids**

Cotton contains several cyclopropenoid fatty acids (CPFA) that are associated with the oil. Those identified that can be measured are malvalic, sterculic and dihydrosterculic acids (Wood *et al.*, 1994). These CPFAs elevate the melting point of fats in animals fed whole cottonseed and cottonseed meal. The mechanism of action appears to be inhibition of desaturation of saturated fatty acids. In chickens, egg yolk discoloration and reduced hatchability are two detrimental effects, and consequently, the industry limits the use of cottonseed meal and cottonseed oil in poultry diets (Phelps *et al.*, 1965). CPFAs have also been implicated in a high incidence of liver cancer in trout fed whole cottonseed (Hendricks *et al.*, 1980), although it is known that aflatoxin, a common mycotoxin contaminant of cotton, also causes liver cancer in rainbow trout (Park and Price, 2001). Typical levels for these CPFAs in cottonseed are shown in Table 10.

**Table 10 - Levels of gossypol (% of d.w.)<sup>a</sup> and cyclopropenoid fatty acids (% of fatty acids) in whole cottonseed, cottonseed meal and cottonseed oil**

Reference	Whole Cottonseed <sup>b,c,e,f,g</sup>	Cottonseed oil <sup>b,g</sup> (Refined)	Cottonseed meal <sup>b,c,d</sup>
Gossypol (total)	.51-1.43	.00 - .09	.93 - 1.43
Gossypol (free)	.47-.70	ND <sup>h</sup>	.02-1.77
Malvalic acid	.17-61	.22 - 1.44	
Sterculic acid	.13-.70	.08 - .58	
Dihydrosterculic acid	.11-.50	.00 - .22	

<sup>a</sup> Dry weight; <sup>b</sup>Monsanto (2000); <sup>c</sup>Martin (1990); <sup>d</sup>Tanksley (1990) Converted values to a dry matter basis.; <sup>e</sup>Arana *et al.* (2000) Converted values assuming a 91% dry matter; <sup>f</sup>Bayer (2002); <sup>g</sup>Berberich *et al.* (1996); <sup>h</sup>ND = non-detectable

**C. Other Compounds**

The leaves of cotton contain flavonoids, tannins and anthocyanin. Some of the leaves are harvested with the cotton bolls and these are removed during the ginning process. Under exceptional circumstances, *e.g.*, drought conditions, cotton plants in the form of gin trash or cotton stubble are sometimes used for cattle feed. However, because of this limited exposure, flavonoids, tannins and anthocyanin are not considered key anti-nutrients/natural toxicants.

## SECTION IV- FOOD USE

### A. Identification of key cotton products consumed by humans

Cottonseed oil is the primary cotton product used for human consumption. Cottonseed oil ranks a distant third behind soybean and corn oil for human consumption making up only 5-6% of the total U.S. domestic fat and oil supply (NCPA, 1999). Crude cottonseed oil contains about 2% of non-glyceride materials such as gossypol and CPFAs, most of which are removed in processing as previously discussed (White, 2000). About 56% of the oil is used for salad or cooking oil, 36% is used for baking and frying fats, and the remaining 8% goes into margarine and other uses. Cottonseed oil is one of the most unsaturated oils, ranking with canola, corn, soybean, safflower and sunflower seed oils. Its mild, nut like taste makes it highly desirable for use as a salad oil.

The processed linter pulp product is used in food mainly in the production of casings for bologna, sausages, and frankfurters. However, the total amount of linters used is very small. Cotton fibre is also used in ice cream and salad dressings to increase viscosity (NCPA, 1999).

A food grade cottonseed flour product is mixed with corn flour, torula yeast and fortified with niacin, riboflavin, vitamin A and iron and is given to children throughout Central America in their first year of age to combat protein deficiency. Similar products have been marketed in other Latin American countries and India (Franck, 1989; Ensminger *et al.*, 1994). However, the product may be prone to contamination with aflatoxin making it unsuitable for human consumption (FDA, 1998). Another cottonseed flour product is used as a color additive for foods with restrictions as to its arsenic, lead and gossypol content (FDA, 2002).

### B. Identification of key products and suggested analysis for new varieties

For human nutrition, it is important to assess the fatty acid composition of the oil. Cottonseed oil should also be assessed for its tocopherol content. Tocopherol (vitamin E) is an important micronutrient and antioxidant that prolongs the shelf life of the oil and food products containing the oil. It is also important to measure the levels of gossypol and CPFAs (sterculic, malvalic and dihydrosterculic acids) either in cottonseed or the cottonseed oil. Because other cottonseed products are used to some extent in human food, the proximate analysis of cottonseed is recommended. Table 11, below, lists the key products and suggested analysis for new varieties.

**Table 11 - Suggested nutritional and compositional parameters to be analysed in cottonseed matrices for human food**

<b>Parameter</b>	<b>Oil</b>	<b>Cottonseed</b>
Proximates <sup>a</sup>		X
Tocopherol (Vitamin E) <sup>b</sup>	X	X
Fatty acids	X	X
Gossypol (Total and free)	X	X
Malvalic acid	X	X
Sterculic acid	X	X
Dihydrosterculic acid	X	X

<sup>a</sup> Proximates include protein, fat, ash, total dietary fiber, carbohydrate (calculated) and moisture.

<sup>b</sup> One IU of vitamin E is the activity of 1 mg of DL-alpha – tocopherol.

## SECTION V - FEED USE

### A. Identification of key cottonseed products consumed by animals

Cottonseed meal is an excellent source of protein for ruminant animals. It is the most valuable animal product of cottonseed, making up over a third of the value. The presence of free gossypol, its lower content and digestibility of the limiting amino acid lysine and its low energy digestibility limits its use primarily for ruminant feed. However, recent research indicates it can also be used in non-ruminant feed, but the level has to be less than 50% of the total protein (Tanksley, 1990). High quality proteins, such as soybean meal or fish meal are necessary to include in the diet with the cottonseed meal in order to obtain the best performance for swine (Dove, 1997). It has also been suggested that ferrous sulphate be added in a 1:1 ratio of the free gossypol content. Solvent extracted meals tend to contain the least amount of gossypol (< .05%) (Tanksley, 1990). Improvements in the efficacy of removing the oil from cottonseed have produced a less valuable meal product because of lowered oil content of the meal, which means it is a poorer source of energy. For ruminant animals, proximate analysis is important to delineate its nutrient value. For non-ruminant animals, amino acid content is important in addition to the proximate analysis. It is limiting in the amino acid lysine.

Whole cottonseed is a very important dairy feed, and a lesser important beef and sheep feed. It is added to dairy feed as a concentrated source of protein, fat and energy at levels of up to 15% of the total diet or at a total dietary amount of 1.8 - 3.2 kg per head per day. Higher levels usually decrease feed intake. The important nutritional parameters are proximates, amino acids and fatty acids. The minerals, calcium and phosphorus are also important. The level of gossypol and to some extent, CPFAs, limits the level of cottonseed that can be added to dairy cow feeds.

Cottonseed hulls are very palatable for ruminant animals and are commonly used in combination with limited amounts of corn silage or hay. Fuzzy cottonseed hulls are preferred over delinted cottonseed hulls. They are also preferred in starter rations for newly weaned calves. Ration texture and palatability appear to be improved by the inclusion of hulls in the diet.

### B. Identification of key products and suggested analysis for new varieties

Proximate analyses are commonly conducted on animal feedstuffs, including the amounts of nitrogen, ether extract, ash, and crude fibre. Carbohydrates are measured as starch or nitrogen free extract. Nitrogen free extract includes starch, sugars, some cellulose, hemicellulose and lignin, and is calculated using the equation:  $100 - CP\% - EE\% - ash\% - CF\%$ . Crude protein is calculated by multiplying the nitrogen content by 6.25, a conversion factor based on the average amount of nitrogen in protein. Fat is considered to be acid-ether-extractable material (Ensminger *et al.*, 1990). In the case of ruminants and swine, the traditional analysis for crude fibre is considered obsolete and has been replaced by analyses for acid detergent fibre and neutral detergent fibre. For amino acids, the ten essential amino acids plus glycine, cystine, tyrosine, serine and proline are the key nutrients. Linoleic is the fatty acid of key importance for the meal, while the relative fatty acid spectrum is more important for the oil.



Other minerals such as selenium are also important, but the amount in plants has been shown to reflect the amount of the mineral in the soil. Nutritionists incorporate supplemental sources of calcium, phosphorus, sodium chloride, magnesium, iron, zinc, copper, manganese, iodine and selenium as needed to balance diets. Again, nutritionists supplement swine diets with vitamins A, D, E, K, B12, riboflavin, niacin and pantothenic acid (NRC, 1998); and ruminant diets with vitamins A, D, E, and K (NRC, 2000 and 2001a).

In considering the anti-nutrients and natural toxins in cottonseed and cottonseed products, gossypol, malvalic, sterculic and dihydrosterculic acids are significant to the animal feed.

When one considers the cottonseed products that might be fed to animals, their nutrient content would not be expected to change if the content of whole cottonseed is not changed. Hence, only the whole cottonseed and cottonseed meal are suggested to be analysed (Table 12). However, for amino acids and fatty acids, either whole cottonseed or cottonseed meal would yield equivalent results.

**Table 12 - Suggested nutritional and compositional parameters to be analysed in cotton matrices for animal feed**

<b>Parameter</b>	<b>Cottonseed</b>	<b>Meal</b>
Proximates <sup>a</sup>	X	X
Amino acids <sup>b</sup>	X	
Fatty acids <sup>c</sup>	X	
Calcium	X	X
Phosphorus	X	X
Gossypol (Total and free)	X	X
Sterculic acid	X	X
Dihydrosterculic acid	X	X
Malvalic acid	X	X

<sup>a</sup> Proximates include protein, fat, ash, neutral detergent fiber, acid detergent fiber and moisture. <sup>b</sup> See paragraph 22 for the key amino acids to be measured. <sup>c</sup> See paragraph 22 for the key fatty acid to be measured.

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