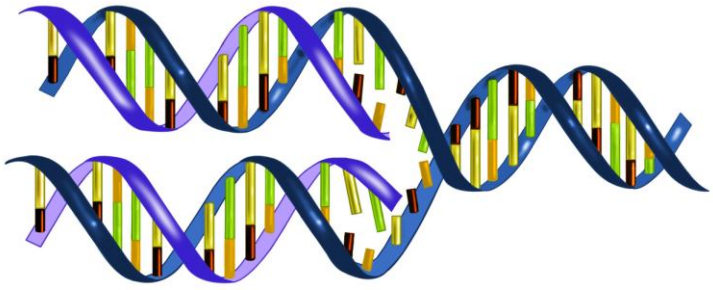
The status of industrial vegetable oils from genetically modified plants



This report was commissioned as a background document for an expert meeting organised by the European Chemicals Agency on the subject of vegetable oils derived from genetically modified organisms (GMOs). The opinions expressed in this report are those of the author and do not necessarily reflect the views or the official position of the European Chemicals Agency.

Contents

Author

Denis J Murphy, Professor of Biotechnology, Head of Biological Research, University of Glamorgan, CF37 4AT, United Kingdom, Email: dmurphy2@glam.ac.uk

Terms and Abbreviations

1st generation GM crops: the vast majority of commercial GM crops grown from the 1990s have modified input traits, such as herbicide tolerance and/or insect resistance, but have unaltered product compositions compared to non-GM counterparts

2nd generation GM crops: newer GM crop varieties are gradually being introduced with modified output traits resulting in different product compositions compared to non-GM counterparts

Amflora™: GM potato variety developed by BASF as a source of high-amylopectin starch for technical applications in the paper, textile and adhesives industries and approved for cultivation in the EU in 2010.

Biofuels: renewable fuels derived from recently living organisms used as alternatives to non-renewable fossil-derived fuels. Existing biofuels include bioethanol from starch/fibre crops such as cereals, and biodiesel from oil crops.

Biodiesel: type of plant-derived biofuel that is produced by transmethylation of refined vegetable oil to form methyl esters that can be used directly or blended with other fuels in vehicle engines. See also SVO.

Breeding: the deliberate manipulation of biological organisms to produce new varieties that are useful to humans.

Cold pressing: method to extract vegetable oils by mechanical pressure used to prepare ‗virgin' oils for edible use. Not normally used to extract industrial oils.

Desaturase: enzyme inserting a double or triple carbon–carbon bond into a lipid acyl chain.

ELISA: enzyme-linked immunosorbent assay. Highly sensitive method for detection of specific proteins in complex mixtures and an approved technique for detection of GM-derived proteins in crop products.

Enogen™: a GM maize variety released in 2011 by Syngenta Seeds Inc with a modified starch content that enabled it use as an improved source of bioethanol.

FAO: Food and Agriculture Organization of the United Nations.

Fat: lipidic substance normally solid at room temperature that is often composed principally of TAGs. Most naturally occurring fats are derived from animals but some vegetable ‗oils' can be classified as solid fats, e.g. cocoa butter.

Functionality: as applied to fatty acids, the way in which a saturated hydrocarbon chain is modified after *de novo* synthesis to create functional groups such as double or triple bonds and epoxy or hydroxyl residues.

Genomics: description and analysis of genomes.

GM: genetic modification or genetic manipulation – the process by which DNA (or occasionally RNA) is transferred into a cell or organism in order to modify its appearance, behaviour, or properties. The transferred DNA may be copied from the same or a different organism. Alternatively, the transferred DNA may be synthetic and hence of non-biological origin. An organism containing introduced DNA is called transgenic or, more popularly, a GMO.

GMO: genetically modified organism – popular term often used to describe a transgenic organism. Scientifically speaking, however, all organisms deliberately bred by humans have been genetically manipulated/modified whether or not they are transgenic.

Input trait: a genetic character that affects how a crop is grown without changing the nature of the harvested product. For example, herbicide tolerance and insect resistance are input traits that assist crop management, but they do not normally alter seed or fruit quality or other so-called output traits that are related to the product(s) obtained from the crop.

Lauric acid: C12 saturated fatty acid, mostly obtained from palm oil and widely used for industrial applications including detergents and cosmetics.

Lecithin: also called gum, a phospholipid-rich fraction obtained during the refining of vegetable oils that has many uses as an emulsifier in a wide range of processed foods.

Lipid: substance soluble in organic solvents, e.g. phospho- and glycolipids, sterols, waxes and triacylglycerols. In plants, lipids function as the matrix of all biological membranes, as

storage reserves, and as hormone-like mediators. Many plant lipids are also major feedstocks for a huge range of industrial chemicals.

Metabolomics: description and analysis of the metabolite composition of a particular cell, tissue or organism.

Mineral oil: also known as petroleum – a complex mixture of hydrocarbons and other minerals obtained from subterranean or offshore deposits. Such oils originate from fossilised plant remains from over 300 million years ago and are therefore non-renewable. In contrast to biofuels, combustion of mineral oil-derived fuels contributes to net global CO_2 emissions.

MSCAs: Member State Competent Authorities.

OECD: Organization for Economic Cooperation and Development.

Oil: see mineral oil or Vegetable oil.

Oleic acid: C18 monounsaturated fatty acid found in most crop oils that has many uses in the food and industrial sectors.

Oleochemicals: group of chemical feedstocks and intermediates derived from renewable, carbon-neutral fats and oils extracted from plants or animals.

Output trait: a genetic character that alters the quality of the crop product itself, *e.g.* by altering its starch, protein, vitamin, or oil composition.

PCR: polymerase chain reaction – a highly sensitive method for detection of DNA sequences in tissues that can unequivocally determine the presence of GM material in suitable biological.

Petrochemicals: group of chemicals derived from non-renewable, fossil-based hydrocarbon feedstocks such as mineral oils and coal.

Petroleum: see Mineral oil.

Plant: oxygenic photosynthetic autotroph, including algae, cyanobacteria, and land plants.

Proteomics: description and analysis of the proteins in a particular cell, tissue or organism.

REACH: REACH is the European Community Regulation on chemicals and their safe use (EC 1907/2006). It deals with the **R**egistration, **E**valuation, **A**uthorisation and Restriction of **Ch**emical substances. The law entered into force on 1 June 2007.

Refined vegetable oil: highly processed vegetable oil that has been extracted and purified by processes such as solvent extraction, distillation, degumming, neutralization, and winterization. Refined oils consist almost entirely of TAGs.

Solvent extraction: most common and efficient method for extraction of vegetable oils from seeds or fruits, see Cold pressing.

SVO: straight vegetable oil, sometimes called pure plant oil (PPO) – a refined, non-methylated vegetable oil used directly or as a blend in engine fuels. See also Biodiesel and WVO.

Trait: genetic character of an organism that is inherited by its progeny. Breeding involves the manipulation of traits in organisms such as crops and livestock.

Transgenic: an organism into which exogenous segments(s) of DNA, containing one or more genes, have been transferred (see GM).

Transgene: exogenous gene transferred to a recipient organism using genetic engineering to create a transgenic organism.

Transgenesis: the process of creating a transgenic, or GM, organism.

TAG: triacylglycerol – an ester of glycerol with three fatty acyl side-chains.

USDA: United States Department of Agriculture.

Vegetable oil: plant-derived, triacylglycerol-based lipidic substance that is normally liquid at room temperature with possible uses in food, feed, or industry. Because they are derived from recent photosynthesis, such oils are renewable and their combustion does not contribute to net global CO_2 emissions.

Virgin oil: relatively unrefined form of edible vegetable oil obtained by cold pressing. Such oils often contain additional components, such as pigments, proteins, DNA, and lecithin, which are not present in more refined oils.

Wax: an ester of two fatty acids that may sometimes have very similar properties to vegetable oils, e.g. jojoba wax.

WVO: waste vegetable oil – used and recycled vegetable oil, e.g. frying oils from restaurants, that can be used directly or as a blend in engine fuels. See also Biodiesel and SVO.

1. Scope of the report

This report aims to address the following five key topics:

A. To explain the technological advantages and industrial relevance behind the genetic modification of plant oils

B. To highlight chemical and biological differences of oils from GMOs and non-GMOs

C. To establish if it is possible to distinguish analytically between oils derived from GMOs and non-GMOs

D. To provide an overview on established or possible hazards (in the context of REACH) regarding vegetable oils obtained from GMOs

E. To establish whether each case should be assessed individually or whether general conclusions can be drawn about differences between oils from GMOs and non-GMOs

Several Appendices provide additional background information on the use of GMO oils for biofuels and cosmetics, and on commercial and regulatory aspects of GM oil crops.

2. Introduction to vegetable oils, their extraction and role of GM technology

This Section provides definitions of basic terms such as ‗vegetable oil' and outlines how such oils are biosynthesised by plants and then extracted and processed for industrial uses.

Vegetable oils, fats, and oleochemicals

Oils and fats are terms used to describe a class of chemicals known as ***lipids***. In its broadest sense, a ‗lipid' refers to a wide range of water-insoluble, hydrophobic, and/or amphipathic compounds. The most abundant plant lipids are acyl (fatty acid-containing) molecules such as phospholipids, glycolipids, and triacylglycerols. Other important lipids include sterols, carotenoids, tocopherols, phytols, and waxes. Lipids are key components of biological membranes and are also used as storage fats and oils by most animals and plants. Plant lipids in the form of vegetable oils have been extracted and processed for various non-food uses by people for well over 7000 years (67).

For the purpose of this report, a ***plant*** is considered to be ‗*any organism capable of oxygenic photosynthesis*'. This definition includes land plants, algae, and cyanobacteria, all of which have been used historically as sources of non-food industrial oil-derived products. Many of these species are being used in research to produce new types of non-food/feed oils for industrial use. For example, researchers in many countries are trying to engineer GM microalgae to serve as large-scale generators of renewable, carbon-neutral oils for non-food use (see Appendix 4).

Oils tend to be liquid at room temperature while ***fats*** are normally solid. Most, but not all, plant lipid products are liquid oils due to their high unsaturated fatty acid content, while most animal lipid products tend to be solid fats that are more enriched in saturated fatty acids. However, there are a few exceptions, such as solid vegetable ‗oils' like cocoa butter or shea butter that can also be classified as fats. Saturated fatty acids do not contain any C=C double bonds whereas unsaturated fatty acids may contain between one and six C=C double bonds (**Fig 1A**). ***Oleochemicals*** can be defined as renewable chemical feedstocks and intermediates derived from plant or animal oils and fats (3, 24, 25, 87). In contrast, ***petrochemicals*** are derived from non-renewable, fossil-based hydrocarbons such as mineral oils and coal.

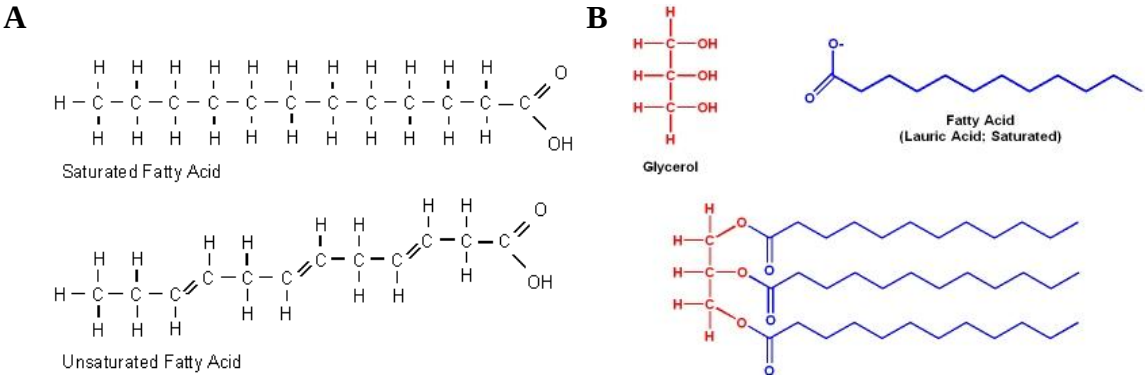


Figure 1. Chemical structures of plant lipid classes. A, fatty acids; B, triacylglycerol

Chemical structures of oils

By far the most common form of biologically derived fats and oils is the ***triacylglycerol*** (TAG) molecule, i.e. a glycerol backbone esterified to three fatty acid (FA) residues (**Fig 1B**). In most cases, the commercial exploitation of fats and oils involves the use of both glycerol and fatty

acids. TAGs are also the second most important source of dietary calories for humans (after starch) and for most of the world's population plant oils are their principal sources of such lipids. Many plant TAGs contain a mixture of fatty acids, with different acyl groups attached to each of the three carbon atoms of the glycerol backbone. In different plant species, storage TAGs can have hugely diverse acyl chain lengths and various acyl modifications such as hydroxylation, epoxidation, conjugation, and triple bonds. The useful properties of many of these fatty acids mean that storage oil composition has become a major target for genetic manipulation of plants.

Biosynthesis of oils in plants

In plants, fatty acids are synthesised from simple acetyl–CoA units (2:0), which are added together to generate increasingly long saturated fatty acid chains from 4:0 right up to 16:0 or 18:0 (see **Fig 2**). In some plants C18 fatty acids can be elongated further as far as C24, but in the major oil crops, the oils are overwhelmingly made up of C16 and C18 fatty acids. Saturated fatty acids can also be desaturated to form unsaturated products such as 18:1 (oleic), 18:2 (linoleic), and 18:3 (linolenic). These three fatty acids are the most common components of most commercial vegetable oils although some plants are able to modify unsaturated fatty acids to produce hydroxy, epoxy, *trans*, and other useful functionalities.

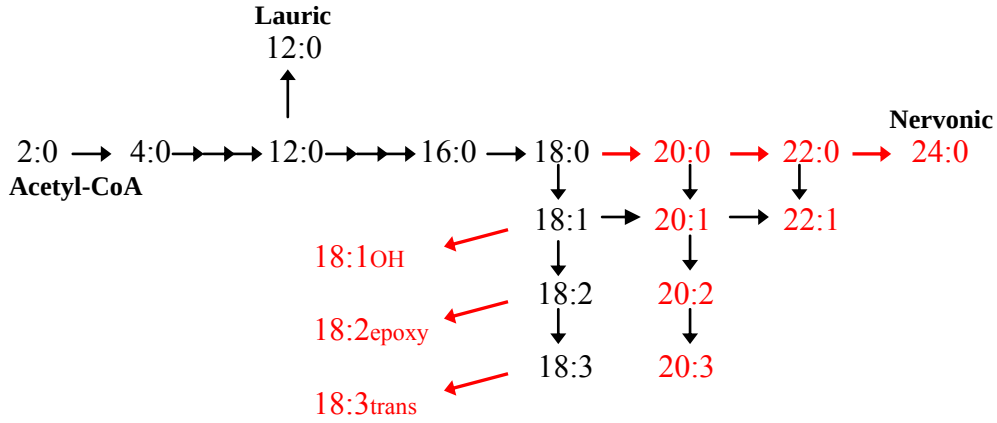


Figure 2. Pathway of fatty acid biosynthesis and modification in plants

One of the key discoveries that made possible the genetic manipulation of fatty acid compositions in plant oils is that chain elongation can be halted to release intermediate length acyl groups, i.e. C4 to C14. This requires the action of one of several specific thioesterases found in the small number of plants that accumulate shorter chain fatty acids in their seed oils. Expression of these thioesterase genes in GM crops leads to premature acyl chain termination and the accumulation of short-chain fatty acids in their seeds. In contrast, some plants are able to elongate storage lipid acyl groups beyond C18 up to C24 by means of additional fatty acid elongases. The expression of exogenous elongase genes in GM oil crops may increase the accumulation of very long-chain fatty acids in seeds.

While chain length is a key determinant of the properties of a fatty acid, the other key property is the way in which the saturated hydrocarbon chains are modified after *de novo* synthesis. This is often referred to as the ***functionality*** of the acyl chain. The most common functionality introduced into an acyl chain is unsaturation, namely presence of one or more double bonds. As shown in **Fig 2** and **Table 2**, other fatty acid functionalities include triple bonds, and hydroxy, epoxy, and oxo groups. Most of the enzymes that introduce such functionalities into fatty acids are derived from mutated versions of fatty acid desaturases. The sequencing of genes encoding many modified desaturases has led to the engineering of new versions of these enzymes that may be able to carry out industrially useful reactions (8, 9, 19, 57).

Seed and fruit oils

Plant oils typically accumulate in the cotyledon or endosperm tissues of seeds (**Fig 3A**). In major oilseed crops, such as rapeseed/canola or sunflower, TAGs can constitute 40–50% of total seed weight. Storage lipids are also found in abundance in certain oil-rich fruits, such as in the mesocarp tissue of olives, avocado, coconut and oil palm (**Fig 3B**).

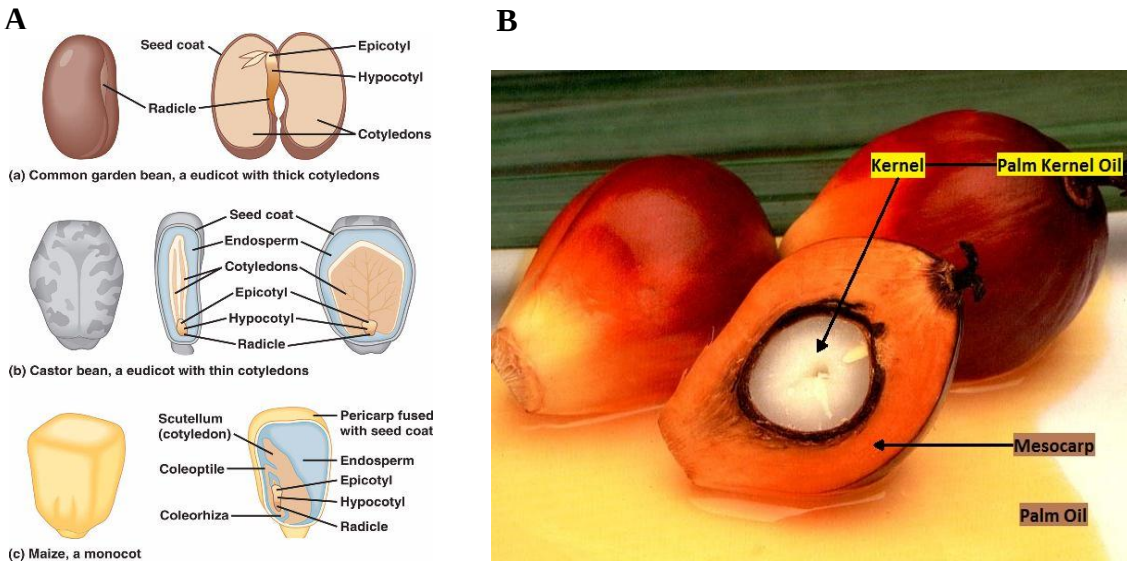


Figure 3. A. structures of oil-bearing seeds; B. oil-rich fruits of oil palm

Storage lipids accumulate in the cytosol of seed or fruit cells as fluid TAG droplets (oil bodies) of about 1-2 μm in diameter. Each oil body is surrounded by a phospholipid monolayer, and often by a layer of specific lipid-binding proteins such as oleosins and caleosins. Oil-rich fruits, such as olive, palm or avocado, accumulate relatively large, TAG-enriched but protein-depleted oil bodies in their fleshy mesocarp (69). In the cells of developing fruits and seeds, oil bodies are formed on the endoplasmic reticulum where acyl–CoAs produced by fatty acid modification enzymes as described above are transferred to a glycerol backbone to form TAGs. Different acylation reactions are catalysed by distinct acyltransferases and these enzymes play important roles in regulating the final acyl composition of storage lipids.

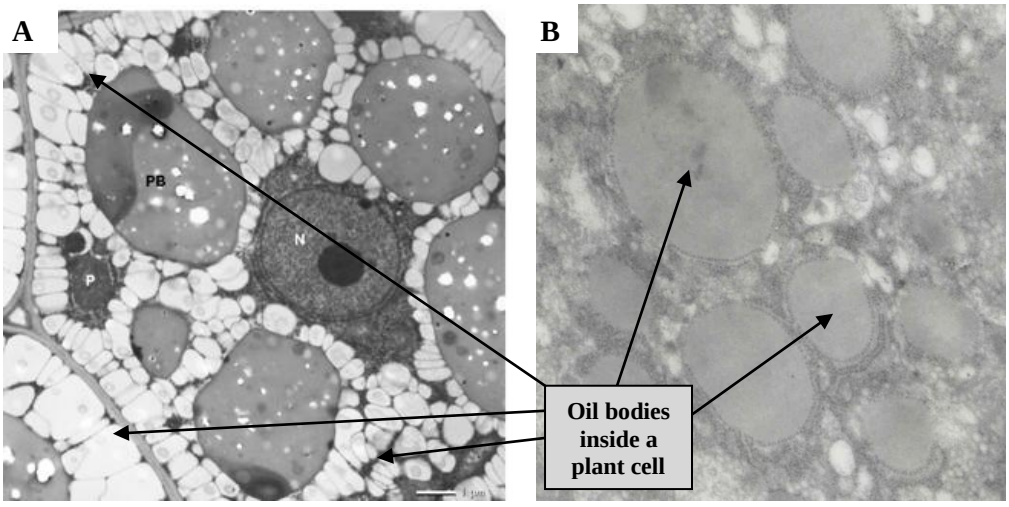


Figure 4. Oil bodies in plant cells at low (A) and high (B) magnification

Extraction of oils from plants

By far the most common method of vegetable oil extraction from plants is via solvents such as hexane, isopropanol, or supercritical CO_2. Typically, the solvent is pumped onto a bed of flaked seeds to extract a crude TAG-rich oil that is largely free of proteins, DNA, or phospholipids (lecithin). The solvent is recovered and reused while the crude oil is processed further to remove remaining impurities. This involves neutralisation (to remove free fatty acids), bleaching (to remove pigments), and deodorisation (to remove volatiles). The result is a highly purified oil fraction consisting almost entirely of TAGs. These highly purified vegetable oils can be used directly to make edible products, such as salad or cooking oils, or blended and/or hydrogenated to make margarines or other spreads.

Alternatively, purified vegetable oils can be used as industrial feedstocks for conversion into oleochemicals or biodiesel (Fig 5A). In many cases, several purified vegetable oils from different sources will be blended together in order to produce mixed oils with appropriate fatty acid compositions for a wide range of purposes, both edible and industrial. With very few exceptions, virtually all industrial vegetable oils are obtained by solvent extraction. Such oils normally contain little or no detectable protein or DNA and their lipid composition cannot be used to diagnose GM origin of the oil (see **Topic B** and Appendix 1).

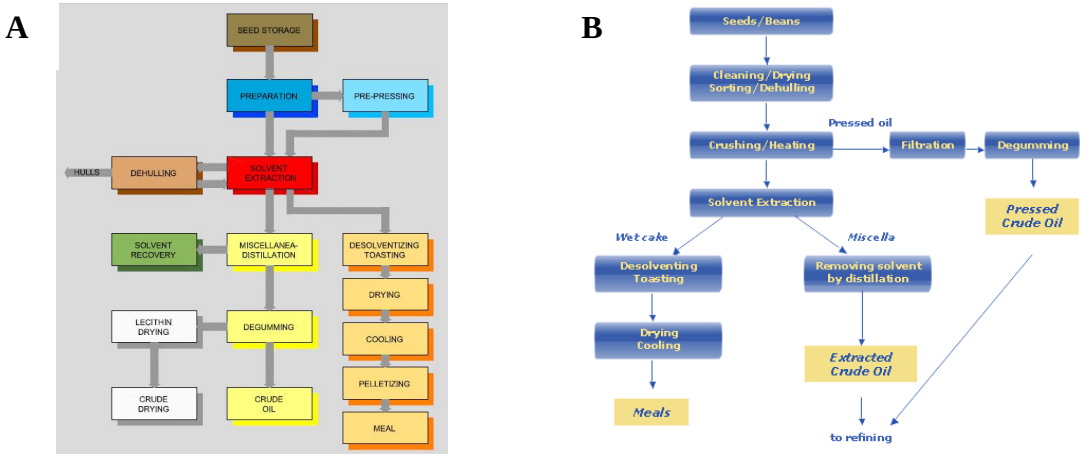


Figure 5. A. conversion of vegetable oils to oleochemicals; B. mechanical extraction of oils

As an alternative to solvent extraction, vegetable oils can be extracted via mechanical methods such as pressing or crushing (Fig 5B). However, these methods are far more expensive and much less efficient than solvent extraction. Today, mechanical methods such as cold pressing are only used in a few highly priced niche markets for edible oils, such as virgin or extra-virgin olive oil. In contrast to purified solvent-extracted oils, cold pressed oils are relatively crude mixtures that may include proteins, DNA, gums, and pigments, in addition to TAGs. Due to their impurity and inefficiency of extraction, cold pressed oils are rarely used in industry. One exception is the occasional inclusion of small amounts of a few cold pressed vegetable oils as ingredients in certain cosmetics, such as skin creams.

GM crops

A GMO (genetically modified organism) is an organism that contains additional DNA inserted via genetic engineering. Most frequently, one or more extra genes are inserted to modify a particular trait to give additional useful properties. In GM crops, two major trait categories have been manipulated for commercial production. By far the most important are the ***input traits*** in which the crop is modified in a way that makes it easier to manage and cultivate. Widely used GM input traits include herbicide tolerance and insect resistance. As discussed in Section 4, over

99.5% of all 1st generation commercialised GM crops grown in 2011 contained either or both of these two input traits. Because input traits only relate to plant growth and management, the products of such crops are the same as those of non-GM versions of the same crop. The essential identity of products such as vegetable oils from GM and non-GM input trait modified plants has been verified by analysis of the full range of metabolites in several recent studies (1, 45).

In contrast to input traits, manipulation of ***output traits*** causes significant alterations in some of the products obtained from a crop. To date, very few GM crops with modified output traits have been commercialised. However, an increasing number of GM output trait-modified crops will be released in the future. Examples include alterations in the oil, protein, starch, or fibre composition of the harvested part of the crop in question. One of the very few GM output trait-modified crops grown in the EU is the Amflora™ potato, which was authorized for cultivation in 2010. Amflora™ potatoes, developed by BASF, are modified to produce pure amylopectin starch for the paper, textile and adhesives industries, and the EC also authorized use of its byproducts as animal feed, but not for human foodstuffs. Over the next decade, existing 1st generation input trait-modified GM crops will be supplemented by a new 2nd generation crops with modified output traits, some designed specifically for industrial use. For example, in 2011 the USDA authorized Syngenta Seeds Inc to market Enogen™ maize, the first GM variety engineered with modified output traits to serve as an improved biofuel feedstock.

Using unmodified vegetable oils as biofuels

The vast majority of vegetable oil-derived biofuel is biodiesel, which has been chemically modified by methylation. However, it is possible to use unmodified or straight vegetable oil (SVO) either directly or as a blend with other fuels in some vehicle engines (49a, 98a). SVO can be sourced from newly refined vegetable oil or from used or waste oil (WVO). An example of WVO would be used frying oils from large-scale food retailers who might collect this waste product and sell it on to a biofuel user. Recently, there have been several high profile instances of the use of SVO or WVO in transport fuels. For example, several airlines including KLM and Finnair have recently announced that they were using WVO in their aviation fuel on some routes (1a). However, in such cases the SVO/WVO was only a small component of the total fuel and these feedstocks are unlikely to become a major form in which vegetable oils are used in the future.

At present, the use of SVO/WVO as vehicle fuel is regulated in different ways by the various EU member states. In some cases it might be used without additional cost while in other cases, as in the UK, it may be taxed similarly to regular diesel fuel (98a). Most of the SVO/WVO in the EU is currently obtained from rapeseed oil sourced from non-GM crops. However, it is also possible to use GM-derived oils as SVO/WVO, e.g. imported Canadian rapeseed or American soybean or maize oils, many of which are already produced from GM crop varieties. While SVO/WVO has a lower carbon footprint to biodiesel, it tends to be expensive and cannot be used below -10ºC, which limits its likely market penetration. Due to the relatively high price of refined SVO and the small quantities and difficulties in collecting WVO, these forms of biofuel make up a very small proportion of the total vegetable oil-derived biofuel used in the EU.

3. Addressing the five key topics

Topic A. What are the technological reasons for genetic modification of plant oils?

GM technology is a powerful tool to modify crops in order to produce a much broader range of industrially useful oils than is currently possible using alternative breeding methods (8, 20, 54, 59, 67, 99). There are many potential markets for renewable, carbon-neutral, „eco-friendly", oil-based chemicals that could be produced by crops as alternatives to non-renewable petroleum products. Unfortunately, however, existing oil crops have restricted fatty acid compositions, which constrains their use as industrial feedstocks (67). These crops mostly accumulate C16 or C18 saturated and unsaturated fatty acids in their storage oils. The major fatty acids comprising the world vegetable oil supply are therefore palmitic, oleic and linoleic acids as shown in **Table 1**.

In contrast to the highly restricted compositions of the major oil crops, surveys of wild plant species have revealed a vast natural diversity of seed oils, many of which have significant potential value, for both nutritional or industrial uses (36). Some measure of this diversity can be seen in **Table 2**, which shows that different plant species can accumulate high levels of a huge range of fatty acids with chain lengths from C8 to C24. In addition, numerous functionalities can occur in these fatty acids, including conjugated and non-conjugated double bonds, triple bonds, hydroxy, epoxy and oxo groups. Cyclopropenoids, cyclopentenoids and even fluoro fatty acids add even more diversity to the list of naturally occurring fatty acid species (37, 59).

Table 1. Percentage fatty acid composition of the „big four" commodity oil crops
Note the predominance of palmitic, oleic and linoleic acids.

Crop / Fatty acid[a]	Oil palm[b]	Soybean	Rapeseed/ canola	Sunflower[c]
Palmitic, 16:0	**45**	11	5	6
Stearic, 18:0	5	4	1	5
Oleic, 18:1	**38**	22	**61**	20
Linoleic, 18:2	11	**53**	22	**69**
α-Linolenic, 18:3	0.2	8	10	0.1

[a] Fatty acids are denoted by their carbon chain length followed by the number of double bonds; [b] mesocarp oil; [c] high-linoleic variety. Data from Murphy (67)

In most cases, the plants listed in Table 2 are able to synthesize potential industrial oils because they contain genes that are not present in the major oil crops listed in Table 1. This means that it is normally not possible to use non-GM breeding methods to produce industrial fatty acids such as ricinoleic or nervonic in major oil crop varieties. Another option is to domesticate plants that already make such fatty acids so that they can be grown as major crops. For many decades there have been many attempts to use conventional breeding approaches to improve the agronomic performance of industrial oil crops, such as meadowfoam or castorbean but these have largely failed on a commercial scale (65). More recently, therefore, researchers began to transfer genes to mainstream commercial oil crops via GM technology in order to create new industrial oils.

Over the past 30 years, many genes regulating fatty acid and TAG metabolism have been isolated from various species (see Table 2) and several GM rapeseed and soybean varieties with modified seed oils, are now available for cultivation. In principle (but not yet in commercial practice), genes can be transferred from donor species into crops, such as soybean and rapeseed, to produce fatty acids from C8 to C24 with any functionality (8). Examples of transfer of potentially oil-modifying genes into oilseeds and the levels of the new fatty acids are shown in

Table 10. However, with few exceptions, levels of such fatty acids in transgenic plants remain far from achieving commercial viability (see Appendix 2 and refs 9, 20, 54, 60).

Table 2. Diversity of fatty acyl composition in selected plant oils

Fatty acid		% FA in oil	Plant species	Commercial uses **Non-food uses in bold**
Chain length/ functionality	Common name			
8:0[a]	Caprylic	94%	*Cuphea avigera*	**Fuel,** food
10:0	Capric	95%	*Cuphea koehneana*	**Detergents,** food
12:0	Lauric	94%	*Litsea stocksii*	**Detergents,** food
14:0	Myristic	92%	*Knema globularia*	**Soaps, cosmetics**
16:0	Palmitic	75%	Chinese Tallow *Triadica sebifera*	Food, **soaps**
18:0	Stearic	65%	*Garcinia cornea*	Food, confectionery
16:1	Palmitoleic	40%	Sea Buckthorn *Hippophae rhamnoides*	**Cosmetics**
18:1	Oleic	78%	Olive[b]	Food, **lubricants, inks**
18:1 9c			*Olea europea*	
18:1	Petroselinic	76%	Coriander[b]	**Nylons, detergents**
18:1 6c			*Coriandrum sativum*	
cyclopropene-18:1	Sterculic	50%	*Sterculia foetida*	**Insecticides, herbicides**
18:1 9,10-me, 9c				
18:2	Linoleic	75%	Sunflower *Helianthus annuus*	Food, **coatings**
18:2 9c,12c,				
α-18:3	α-linolenic	60%	Linseed[b]	**Paints, varnishes**
18:3 9c,12c,15c			*Linum usitatissimum*	
γ-18:3	γ-linolenic	25%	Borage[b]	**Therapeutic products**
18:3 6c, 9c,12c			*Borago officianalis*	
OH-18:1	Ricinoleic	90%	Castor[b]	**Plasticizers, lubricants**
18:1 9c,12OH			*Ricinus communis*	
epoxy-18:1	Vernolic	70%	Ironweed	**Resins, coatings**
18:1 9c,12epx			*Vernonia galamensis*	
triple-18:2	Crepenynic	70%	*Crepis alpina*[b]	**Coatings, lubricants**
18:2 9c,12trp				
oxo-18:3	Licanic	78%	Oiticica *Licania rigida*	**Paints, inks**
4-oxo-9c11t13t				
18:3 8t,10t,12c	Calendic	60%	Calendula *Calendula officinalis*	**Paints, coatings**
18:3 9c,11t,13t	α-Eleostearic	70%	Tung *Vernicia fordii*	**Enamels, varnishes, resins, coatings**
18:3 9c,11t,13c	Punicic	70%	Pomegranate *Punica granatum*	**Varnishes, resins, coatings**
20:0	Arachidic	35%	Rambutan *Nephelium lappaceum*[b]	**Lubricants**
20:1	Eicosenoic	67%	Meadowfoam[b] *Limnanthes alba*	**Polymers, cosmetics**
22:0	Behenic	48%	Asian mustard *Brassica tournefortii*	**Lubricants**
22:1	Erucic	56%	Crambe[b] *Crambe abyssinica*	**Polymers, inks**
wax 20:1/22:1	Jojoba wax	95%	Jojoba[b] *Simmondsia chinensis*	**Cosmetics, lubricants**
24:1	Nervonic	24%	Honesty[b] *Lunaria biennis*	**Pharmaceuticals**

[a]c, *cis* double bond; t, *trans* double bond; epx, epoxy group; trp, triple bond; me, methylene
[b]indicates that genes have been isolated for synthesis of these fatty acids. Murphy (67)

Topic B. Highlight the chemical and biological differences of oils generated from genetically modified organisms (GMOs) and non-GMOs

There are ***no chemical or biological differences*** between the fatty acid components of vegetable oils produced from GM or non-GM plants (10, 59, 67). For example, lauric acid or oleic acid molecules from GM plants will be ***identical*** to lauric or oleic molecules derived from non-GM plants (107). In the case of input trait-modified GM plants, their entire oil compositions will be indistinguishable from those produced by non-GM versions of the same crop. For example, oils from the most common GM crops grown at present (maize, soybean, rapeseed, and cotton) have the same acyl compositions as oils from non-GM varieties of these four oilseed crops (62).

It is possible, in principle, to use GM methods to produce new oil compositions in crops via modification of output traits (9, 10, 54, 70). But new oil compositions can also be produced in crops via non-GM breeding methods (60, 67). For example, naturally occurring rapeseed produces an oil enriched in erucic acid (C22:1). During the 1960s, the composition of rapeseed oil was modified by non-GM breeding to produce a new oil with oleic acid (C18:1) as the major component and erucic acid at <1%. Today, all edible rapeseed varieties contain the new high-oleic oil, while the older high-erucic rapeseed varieties are only grown in a few regions to provide specialized industrial oils. Therefore the manipulation of crops to alter their oil content can be achieved using both GM and non-GM methods.

Topic C. Establish if it is possible to distinguish analytically between oils derived from GMOs and non-GMOs

Current analytical techniques do not allow definitive distinctions between the fatty acid compositions of oils from GMOs and non-GMOs (67). As outlined above, individual fatty acids from GM and non-GM crops are chemically identical. If future GM crop varieties were to have different overall oil profiles, it would not be possible to definitively state whether the composition of a particular oil sample was the result of it being derived from a specific type of GM crop. For example, very high oleate (<90% total oil) soybean varieties have been produced by GM methods, but similar oil compositions can be produced by non-GM methods (60, 67). Even if a sample of soybean oil contained high levels of a fatty acid (e.g. lauric) that soybean cannot normally accumulate, the mere presence of lauric acid would not be definitive proof of a GM origin of the oil. This is because the lauric acid might be present in the sample because it had been blended with a non-GM source of lauric, such as palm or coconut oil. Because the lauric acid in each case is chemically identical, there is no way of definitively proving that it originally came from a GM or a non-GM source.

Therefore, while direct analysis of the lipid composition of vegetable oil samples might in some cases give indicative data about a putative GM origin, it would not provide a definitive diagnosis. To give another example, the presence of ricinoleic acid in a rapeseed oil sample might indicate a GM origin because non-GM rapeseed cannot make this oil. However, such an analysis could not rule out the possibility that the ricinoleate came from a non-GM source such as castor oil (67). Therefore, standard lipid analytical techniques such as gas-chromatography (GC), high performance liquid chromatography (HPLC), and GC mass spectroscopy (GC-MS) can only provide inferential data on GM status and will only be useful where the provenance of a batch of oil can be traced with certainty. Because most industrial oilseeds are mixed and blended with other oils at an early stage of processing in order to achieve the desired acyl composition, in the majority of cases it would be virtually impossible to reliably determine the presence of a component of GM origin once an oil had been extracted from the original plant material.

Although fatty acid analysis is of relatively limited value in determining the GM status of a vegetable oil, it may be possible to detect GM components such as proteins or DNA if these are present in the oil. As described in Appendix 1, there are preliminary reports that GM-derived DNA might sometimes be traceable in some refined oils. However, such methods are still under development and may not be suitable for assaying industrial oils.

Topic D. Provide an overview on established or possible hazards (in the context of REACH) regarding vegetable oils obtained from GMOs

Vegetable oils obtained from GMOs are unlikely to provide new hazards to health or safety (58, 59). In the case of input trait-modified GM crops their derived oils will be virtually identical to those from non-GM counterparts. In the case of output trait-modified GM crops their oils might contain fatty acids that are not normally found in a particular crop species, but the same fatty acid will be a naturally occurring constituent of a non-GMO oil (10). It should be stressed that all of the industrial fatty acids that have been proposed as possible targets for GM-based production in oil crops already exist as natural products that occur in various plants or other organisms (8, 11, 25, 58, 59, 65, 67). Many of these fatty acids are listed in Table 2.

As noted above, in the case of oils from GM plants modified to produce different fatty acids, these „new" fatty acids will be physically and chemically identical to existing fatty acids derived from other plants, or from other organisms, or from synthetic (non-biological) sources (57). Therefore there are probably no extra hazards or risks arising from the production of such fatty acids (or their derivatives) simply because they are sourced from GM plant feedstocks. Toxicology data for commercially used fatty acids should already be known as this would be part of existing risk assessment procedures. In terms of ecotoxicological risks, current research and regulation are based largely on an environmental risk analysis (ERA) of the performance of whole plants in agronomic field trials as recently described in EFSA documents and elsewhere (23a, 36a). Such ERA trials have mostly been concerned with products of 1st generation GM crops such as herbicides and insecticides. It seems unlikely that the nature-identical industrial fatty acids that could be present in some future 2nd generation GM crops should merit additional ecotoxicological monitoring.

Any industrial fatty acid or oil could have specific hazards or risks involved in its storage or use, but these will be the same for each particular fatty acid or oil irrespective of its provenance. The use of a particular fatty acid or oil, such as ricinoleic-rich or lauric-rich TAGs, already requires case-by-case risk assessment. But no additional measures should be required whether the fatty acid or oil came from GM rapeseed, non-GM castor, GM or non-GM oil palm, or had a wholly synthetic origin. In all such cases the fatty acid or oil should have identical properties and should therefore merit similar risk assessment procedures. In short, the chemical and physical properties of industrial oils and potential hazards and risk assessment arising from their use are technology-neutral and are already covered by other risk assessment procedures in relevant legislation.

Topic E. Establish whether each case is unique and has to be assessed individually or if it is likely that general conclusions can be drawn on the difference between oils derived from GMO and non-GMOs

The regulation of GM crops and their products is currently focused on crops grown for food and feed markets (23b). The occurrence of several high-profile episodes involving (non-GM) contamination of food/feed chains (e.g. BSE, *E. coli* O157, *Salmonella* etc) has raised public concerns about the potential food safety implications of GM crops, which in turn has shaped the current case-by-case regulatory framework, especially in the EU (62).

As described in more detail in Sections 4 and 5 (see below), almost all commercially grown GM crops over the past two decades have been varieties in which so-called input traits have been modified. The two major input traits (present in over 99% of the global GM crop area in late 2011) are herbicide tolerance and/or insect resistance. Crops modified for these traits still make the same products as their non-GM counterparts and their main benefits are in simplifying crop management by farmers and in some cases in reducing levels of chemical inputs such as insecticides. These are referred to here as 1st generation GM crops. In the next few years these crops will be joined by a 2nd generation of GM crops in which output traits such as oil or starch composition have been modified compared to their non-GM counterparts.

This may require segregation and possibly different forms of regulation for such 2nd generation of GM crops. For example, in terms of their products, the current 1st generation of input trait-modified GM crops are regarded as the same as their non-GM counterparts. This means that their nutritional compositions are virtually identical and is reflected in the way such crops are regulated. However, if an output trait modified GM crop produces a different industrial oil from previous versions of the crop, it may be necessary to assess risks to human/animal health that might result if such a product were to enter food/feed chains (59, 62). For example, scenarios could be created to model possible contamination routes and the amounts of oils or their derivatives that might be present in a particular food/feed product as a result of such contamination.

For any industrial vegetable oil produced in the open environment, whether GM or not, it may be desirable to assess risk of its accidental entry into food/feed chains (23b, 110). A combination of known toxicology data for each plant oil and its assessed likelihood of entry into food/feed chains could then form the basis of regulatory protocols. Such protocols might involve recommendations for management and segregation of any new crops and their products, plus establishment of monitoring procedures in cases where there is a possibility of entry into food/feed chains. Finally, it may be desirable to set upper limits to the permissible presence of such oils in food/feed chains if this is appropriate. As noted above, such a system has functioned well with high-erucic industrial rapeseed oil for over 50 years (60, 62, 81).

These considerations imply that ***a case-by-case approach to the regulation of new vegetable oils may be desirable***. Moreover, because the risk is from the new oil itself, and not from the process by which it was produced in the crop, the regulation of such oils should be the same whether they come from plants that happen to have been bred via GM technology or via any other form of plant breeding. In the case of crops that have GM traits that modify both the oil composition and unrelated characters (such as herbicide tolerance), the risks arising from the new oil should be considered separately from the assessment of the unrelated characters. Within the EU, regulatory procedures covering input traits such as herbicide tolerance that may be the only GM trait in an industrial oil crop are already assessed by EFSA.

4. Implications of first-generation GM crops

The first commercially successful GM crops were developed by agrochemical companies, such as Monsanto and Bayer. The initial targets were single-gene traits for which genes were already available. By the early 1990s, several genes had been identified that altered key input traits in crops. An input trait can be defined as a genetic character that affects how the crop is grown without changing the nature of the harvested product. Examples include herbicide tolerance and insect resistance, which are useful traits for farmers because they simplify the process of crop cultivation and can reduce yield losses due to weeds or insect pests. Because a modified input trait does not affect the product of the crop, e.g. its seed composition, the new GM crop varieties could be harvested and processed in the same way as conventional varieties. In other words there were no additional costs associated with downstream processing of the crop (62).

The input trait strategy was commercially attractive for three main reasons: (i) agrochemical companies could sell a package consisting of their proprietary herbicide + their patented GM seed to farmers, who were obliged to use only this particular herbicide; (ii) the utility patents that applied to GM seeds gave the company stronger ownership rights than for non-GM seeds carrying a similar trait; (iii) because the GM seeds belonged to the company, farmers could not save seed from their crop and had to repurchase new seed and herbicide from the company each year (62). From the early years of GM crop cultivation, herbicide tolerance traits dominated the market and this domination continued into the 2010s, as shown below in **Table 3**. This shows that well >99.9% of current GM crop production comes from the four species: soybean, maize, cotton, and rapeseed. Although all of these crops are technically oilseeds, and some of their oil is used for industrial purposes, by far their main use is as food, feed, or fibre feedstocks. Also, since none of the GM traits involves oil modification, the oil from such input trait modified varieties should be identical in all respects to oil from non-GM varieties of the same crop.

Table 3. Major global transgenic crops and traits in 2010

Crop	**Area, Mha**	**%**
Soybean	73.3	50
Maize	46.8	31
Cotton	21.0	14
Rapeseed/canola	7.0	5
Sugar beet	0.5	<1
Alfalfa	0.1	<1
Others	<0.1	<1
Total	ca148	100

Trait	**Area, Mha**	**%**
Herbicide tolerance	89.3	60
Insect resistance (Bt)	26.3	18
Herbicide tolerance + Insect resistance	32.3	22
Others	<0.1	<0.1
Total	ca148	100

Data adapted from James (42)

Although almost all GM crops grown today contain one or more of the input traits listed in Table 3, several early commercially released GM crops contained modified output traits. Indeed, one of these early GM crops was a rapeseed variety that produced an industrial oil. This GM oilseed crop was released in 1995 in the USA by the small biotech company, Calgene, and was a lauric

acid-rich variety (Laurical™) of rapeseed. Laurical™ was produced by transferring a gene encoding a C12-specific thioesterase from the California Bay plant, *Umbelluria californica*, into rapeseed. The addition of several additional transgenes enabled Calgene breeders to produce Laurical™ seed oil that contained 60% w/w lauric (107). However, even this relatively lauric-rich oil crop was unable to compete commercially with the standard commodity lauric oil, which was (and still is) obtained from the far cheaper and more plentiful (and non-GM) palm kernel oil. Therefore, although the Laurical™ brand of transgenic rapeseed was technically successful, it was a commercial failure and was only grown for a few seasons during the mid-1990s in the southern USA. Since that time there have been no further releases of GM crops specifically modified to produce industrial oils and none of the subsequent 1st generation GM crops released between 1996 and 2009 were modified for output traits such as oil composition.

1st generation GM crops contain one or more GM-derived proteins not present in the original plant and there is a theoretical possibility that such proteins might be allergenic. However, because no proteins are present in refined industrial vegetable oils, such oils would be no different in their allergenicity to non-GM oils. It is also theoretically possible that the presence of a transgene in an organism could lead to unintended phenotypic effects. For example a transgene insertion might alter the expression of an endogenous gene. Such effects might be minor but recent advances in analyzing the extent and nature of gene transcription in different tissues and under many different conditions (the transcriptome) and in determining the identity and levels of thousands of metabolites (the metabolome) have made it possible to detect such changes if they are present in a plant. So far, studies indicate that the overall gene expression and metabolic status of the GM plants analyzed were virtually indistinguishable from their non-GM counterparts. Indeed, in studies with maize and barley, non-GM varieties showed more differences between non-GM forms than with GM forms of each species; also, environmental factors caused more changes than GM influences (1, 45).

The current status of GM versions of the 'big three' vegetable oil crops is as follows:

GM soybean: In 2010, GM herbicide tolerant/insect resistant varieties accounted for >81% of the global soybean crop area, mainly grown in USA, Brazil, and Argentina (28). This is already making it increasingly difficult for importing regions, such as the EU, to exclude such varieties should they wish to maintain a ‗GM-free' policy. At present the EU imports 30 MT soybean seed and 13 MT soybean meal which must have <0.9% GM content to qualify as ‗GM-free' under EU labeling requirements (75). This is now a critical issue for the animal feed sector where organic farmers in particular are facing significant price premiums for increasingly scarce supplies of ‗GM-free' soybean (1c).

GM rapeseed: About 23% of global rapeseed production currently comes from GM varieties, but at present all GM rapeseed stocks are in the form of edible ‗canola'-branded cultivars rather than high-erucic industrial rapeseed. While there is no GM rapeseed grown in the EU at present, 94% of Canadian and 88% of US rapeseed is GM, which complicates the possible import of seed or oil from these major producing countries.

GM oil palm: So far, no GM varieties of oil palm have been produced, but as discussed below in Section 5, research into GM varieties producing a range of additional industrial oils is well underway in Malaysia, and possibly elsewhere (88, 93).

In conclusion: *over 99% of existing '1st generation' GM crops are only modified for output traits that have no impact on the composition of vegetable oils extracted from such crops. Therefore, existing GM-derived vegetable oils are chemically identical to oils from their existing non-GM-derived counterparts.*

5. Implications of second-generation GM crops

In Section 4, we saw that almost all existing GM crops are input trait modified with oils that are identical to those in equivalent non-GM crops. However, as discussed above in **Topic E**, there are compelling reasons to use all available technologies, including GM, to extend the range of fatty acids produced in crops so that more useful industrial (and edible oils) can be obtained. This will involve the development of a new ‗2nd generation' of output trait-modified GM crops that produce new types of oil (or other products such as starch or protein) (56). In the coming years, market drivers such as increased commodity prices (e.g. for fossil-derived oil feedstocks) will accelerate the introduction of GM vegetable oils (8, 9, 20, 54, 60, 69). Other drivers will include wider use of GM technology by developing countries (66, 69, 88).

As discussed below in Appendix 6, 1st generation GM crops are dominated by mainly US-based multinational agrochemical companies, but oil crop production and the development of new GM varieties is increasingly shifting to countries such as Brazil, Argentina, India, South Africa, and Malaysia. For example between 1961 and 2007 global output of the major oilseed crop, soybean, increased from 28.6 to 217.6 MT with a recent shift in production away from the USA to Brazil and Argentina, which together now produce 44% of global output, much of which is exported to Europe (52). New varieties of oil palm could potentially double the yield of this crop and make it even more competitive with mineral oil as a global chemical feedstock (61, 66, 88).

After over a decade of relative stagnation, increasing numbers of 2nd generation GM output trait crops are now being released. Many of these traits, e.g. enhanced nutritional content, are much more relevant to consumers than 1st generation input traits. During the next decade we can expect many more GM crops with improved protein, lipid, starch, and vitamin profiles, and processing or storage properties. Many more GM crop species will be developed and the technology will be used much more in developing countries, often for public-good as well as for commercial applications (69). As the utility and provenance of GM crops is extended, and potential (albeit highly unlikely) risks are addressed (e.g. by eliminating antibiotic resistance genes), it seems likely that the technology will become more acceptable to the general public in a similar manner to the now widespread acceptance of GM technology when used for production of GM-derived therapeutic products such as blood-clotting factors or human insulin (69).

Prospects for the modification of plant oils for non-food/feed use are good as new advanced breeding technologies are increasingly used around the world, including in developing countries that are already major vegetable oil producers. Not all these technologies are GM based, but because the genes required for the biosynthesis of many industrial oils are absent from the best yielding crop production platforms, in many cases GM technology may be the only way to generate new industrial oil compositions. New GM tools include site-specific integration of transgenes, as used in soybean (48), and the stacking of multiple transgenes at a selected genomic site via repeated recombinase-mediated DNA cassette exchanges (49). So-called GM trait stacking is already being applied to the latest batch of 1st generation GM input trait varieties. For example, in August 2011 Dow AgroSciences and MS Technologies submitted the first three-gene multi-herbicide-tolerant variety of soybean (Enlist™) for USDA approval.

GM trait stacking will potentially have even more impact once it is deployed more widely for output traits such as oil modification. Broader approaches include so-called ‗green systems biology' that encompasses the use of genomics, proteomics, and metabolomics for crop manipulation (109). Several gene silencing and mutagenesis technologies are compared in **Table 4**. Although all these methods involve gene disruption, only the final three examples (in red) are classified as GM. In contrast, the first four methods (in blue) are not legally regarded as GM even though they involve the scientific manipulation of DNA sequences using either chemical agents or high-energy radiation sources.

Table 4. Comparison of different gene silencing/mutagenesis technologies

Mutagenic agent	Effect on DNA structure or gene expression	Comments
Ethylmethane sulphonate (EMS)‡	Point mutations, CG to AT transitions	Large range of mutations including gain and loss of gene function High efficiency, 100s of mutations per genome Difficult to locate mutations in genome
Di-epoxy butane (DEB)‡	Point mutations, 6-8 bp deletions	
Gamma-rays or X-rays‡	Chromosome breaks and rearrangements	Large insertions, deletions, and rearrangements Mostly loss of function mutations Medium efficiency Difficult to locate mutations in genome
Fast neutrons‡	Deletions, <1 kb	
T-DNA*	DNA insertion	Insertion of specific DNA sequences Mostly loss of function mutations Low efficiency Easy identification of mutations
Transposons *	DNA insertion/deletion	
RNAi or zinc-finger constructs*	DNA insertion so that transcribed gene product causes gene silencing	Very effective; Targets single genes Causes loss of function Requires knowledge of target gene function

‡ non-GM method; * transgenic (GM) method; data from Murphy (69)

There have been several recent examples of non-GM breeding methods being used to modify seed oil fatty acid content for industrial use, meaning that the oil itself was not the product of GM methods. However, at a later stage of the breeding process, some of these non-GM varieties were crossbred with other varieties of the same crop expressing a GM trait, such as herbicide tolerance, that was unrelated to oil modification. According to current EU and US legislation the resulting plants, which produce industrial oils, are regarded as GMOs although their modified oil composition does not result from GM processes.

For example, Monsanto has recently marketed a very high oleic soybean variety called Vistive™. Due to its high monounsaturate and low polyunsaturate content compared with existing soybean oils, Vistive™ oil is likely to find numerous oleochemical applications such as in lubricant and ink formulations, as well as being a premium quality edible oil. The high oleic acid phenotype of Vistive™ is the result of non-GM breeding but because the breeders subsequently crossed their high oleic line with an older GM herbicide-tolerant line, Vistive™ is now treated as a GMO. Moreover, because high oleic soybean is not necessarily indicative of a GM origin, even within a bag of Vistive™ seed, the only way to diagnose the seeds as of GM origin would be perform protein or DNA analysis. And once the Vistive™ oil has been refined it would be very difficult to infer anything conclusive about its putative GM provenance. This will make it very problematic to attempt to trace Vistive™ oil once it has been refined for industrial (or food) use.

In view of the gradual pace of progress at the R&D stage, it is unlikely that many new industrial oil crops with 2nd generation GM-modified output traits will be widely grown in the immediate future. However, given the recent advances in GM technology and the growing market demands for industrial oils, many more GM industrial oil crops may well be available later in the 2010s. Given the likely increase in the production of and globalized trade in renewable, carbon-neutral oleochemicals in the coming years, therefore, it will become ever more challenging to regulate vegetable oil-derived non-food products on the basis of a possible GM provenance.

6. Concluding remarks

This report provides evidence that over the course of the next decade an increasing volume of GM- and non-GM-derived vegetable oils from a growing range of crops is likely to enter global markets, including the EU. Such vegetable oils will increasingly provide a diverse range of renewable, carbon-neutral, organic feedstocks for use in a wide variety of industrial applications. In the medium term, both GM- and non-GM-derived vegetable oils will be required in ever-increasing quantities to act as substitutes for increasingly scarce and expensive non-renewable, fossil-derived hydrocarbon feedstocks for industrial uses such as oleochemicals ands biofuels.

A key question for this report was whether vegetable oils from GM and non-GM sources can be differentiated in a scientifically meaningful way that would result in their clear separation into two distinctive categories of chemical substance. In approaching this question, the report has examined whether there are any chemical and/or biological differences between vegetable oils derived from GM or non-GM plant sources and if so whether these oils can be distinguished from one another via currently available analytical techniques.

The most important conclusion from this study is that the fatty acid constituents of GM-derived vegetable oils are identical to those that occur naturally in other plants and non-plant organisms. This means that it is not possible to distinguish analytically between a fatty acid, such as lauric acid, that came from a GM crop such as rapeseed from lauric acid produced by a non-GM source such as coconut oil. Although the chemical identity of individual fatty acids present in GM- and non-GM derived vegetable oils will be identical, the composition of a particular refined oil might sometimes enable a putative GM or non-GM origin to be inferred. However, more definite identification techniques based on validated DNA methodologies are not yet available.

All fatty acids likely to be present in future GM-derived vegetable oils are already produced by naturally occurring (non-GM) sources such as plants or other organisms. Therefore the presence of such fatty acids in a given vegetable oil should not create novel categories of risk. However, if a new variety of an existing crop is bred to produce a completely different type of refined oil compared to existing varieties, issues of segregation and entry into food/feed chains may require monitoring on a case-by-case basis. Such procedures should be adopted irrespective of the nature of the kind of breeding approach used (i.e. it should be the same for GM- and non-GM derived oil crops) because the only relevant issue in a risk assessment context is the composition of the oil, not its provenance.

Appendix 1. Methods to detect material of GM origin in plants

Detecting DNA in oils

As noted in **Topic C** of the main document, fatty acid analysis cannot be used to definitively prove a GM origin of a sample of vegetable oil. However, if DNA or protein is present in the oil, the GM origin of these non-lipid components could theoretically be detected. Alternatively, if plant material from which the oil was derived (e.g. seeds) were available this could be tested for GM content. In some cases these plant samples may be highly processed, e.g. by grinding or even toasting, but could still be capable of reliable analysis for GM content. The two principal analytical methods are enzyme-linked immunosorbent assay (ELISA) for detection of GM-derived proteins and polymerase chain reaction (PCR) for detection of transgenic DNA sequences. Both techniques are highly sensitive but both have the drawback that the identity of the target GM protein or DNA must be known in advance. Therefore these methods are less useful in dealing with samples of unknown origin.

ELISA has the advantage of detecting a functional gene product rather than merely the presence of parts of a transgene. Because some ELISA kits have been designed to detect denatured versions of a transgenic protein, they can be used to detect GM material, not only in seeds, but also in highly processed products such as flour or cooked foodstuffs (75). ELISA can detect the presence of a specific GM-derived protein at levels well below the EU threshold of 0.9% that is required for mandatory labeling of an item as GM product. In the case of a GM crop modified to produce a specific industrial oil, reliable ELISA methods could be devised to detect those transgene-derived enzymes, such as hydroxylases or thioesterases, that are responsible for the particular GM phenotype, proving the sample contained such proteins. ELISA kits for GM analysis are already commercially available for most 1st generation traits such as herbicide tolerance and Bt insect resistance and the development of new kits for industrial oil crops could be done quickly and at moderate cost. However, because no proteins are present in refined industrial oils, ELISA cannot be used to detect GM proteins in such oil samples.

The major DNA detection methods are PCR-based and several commercial kits are available (4, 13). The PCR method can be even more sensitive than ELISA but requires the presence of suitably intact DNA in the sample and advance knowledge of which DNA sequence is being sought. Also, PCR normally only detects the presence of a small part of a transgene, and cannot determine whether it is biologically active. At present there are no validated PCR-based methods for detection of DNA in refined industrial oils and several reports of failure to isolate suitably intact DNA from refined oils (18a, 34a, 34b, 42a). However, this is an active field of research and there have been a few recent reports that it might be possible to isolate DNA from some refined oils using some commercial kits (13a, 13b, 18a), and to detect as little as 0.1 fmol DNA in oil samples using thin-film chips (1c, 5). At present, DNA yields tend to be low and highly fragmented so that only very small amplicon lengths (ca 100 bp) can be used and even here the presence of inhibitors means that no PCR products were obtained from several types of refined oil (5, 13a). This means that such methods could give rise to both false positives and false negatives, which makes it unlikely that they would be suitable in their present form to be adopted as officially approved analytical procedures.

The extremely high sensitivity of PCR methods can be a drawback as they can detect tiny amounts of GM-derived DNA (<0.1 fmol) that may result from contamination that is several orders of magnitude below the 0.9% EU threshold. Current PCR-based methods are not suitable for precise quantitative determination of DNA sequences so a positive analysis would have limited value for monitoring or regulation. Given that most industrial vegetable oil feedstocks are bulk shipped as seed or oil in tankers in a globalized trade network, it would be virtually impossible to guarantee that a single GM seed or a gram of oil was not present in a tankerload of millions of seeds of thousands of tonnes of oil. Therefore, simply detecting the adventitious presence of GM-derived DNA sequences via PCR may not be very informative or useful.

Even if it eventually becomes technically feasible, the successful detection of GM DNA in oil samples will still require advanced knowledge of identity of the particular transgene in question (16, 17). Even today there are many different transgenic DNA sequences in various GM oil crops and the number is likely to increase significantly in the future as more GM crop varieties are produced around the world. It is impossible to set up an analytical method in a cost-effective manner that can assay for all the hundreds of known and unknown GMOs that may be in circulation at a given time. This is becoming an increasing challenge as an many more countries, especially in Asia and South America, develop homegrown GM technologies and release their own GM crops, many of which will then exported into global trade networks.

There is at present very limited scope for monitoring industrial raw materials such as refined vegetable oils for the presence of GM-derived material.

Even if DNA detection methods improve in the future, the expansion of GM technology and use of additional transgenes will make it almost impossible to assay samples for unknown DNA sequences.

Appendix 2. Technical challenges to producing GM industrial oil crops

Transgenesis is the transfer of genes into an organism using recombinant DNA methods. Transgenes can be derived from any biological organism or may be completely synthetic. Prior to transgenesis, the only way to incorporate new genes into an organism was to set up a sexual cross with a compatible species containing the gene of interest. However, this approach is relatively time consuming and is limited to species with which the plant can be successfully crossed. Thanks to advances in tissue culture-assisted wide crossing this list is steadily growing, but it will always be restricted to relatively close relatives of the target plant. Therefore transgenesis has been seen as a way to enhance variation in plants far beyond the capacity of methods previously available to plant breeders (69).

The transfer of genes into cells that could be successfully regenerated into viable adult plants was first achieved in the early 1980s. It was made possible by advances in DNA manipulation and in the culturing and regeneration of plant cells and tissues. The core technologies of DNA manipulation are based on the use of enzymes to cleave and ligate small sections of DNA. This can create new DNA combinations that can be inserted into a vector for eventual transfer into a plant cell. Many of these methods were originally developed in the 1970s using microbial systems and then extended to higher plants. It is also necessary to know the identity of and to have isolated copies of the genes that are to be transferred to a recipient plant. Initially, researchers only had access to a few relatively well-studied genes that regulated genetically simple traits. The small number of available transgene candidates has greatly limited the scope of GM technology in its early decades (62, 69).

Gene transfer into a recipient organism can be achieved either by physical propulsion (biolistics) or via biological vectors. It is then necessary to select what is often a small number of transformed cells and to eliminate non-transformants. Sometimes this has involved use of antibiotic resistance genes. In certain cases, use of such genes has been controversial and has complicated regulatory processes in some jurisdictions, although alternative less problematic selection methods are now available (62). The next challenge is to regenerate transgenic plants in which the transgene function in the appropriate manner. This requires a great deal of tissue culture expertise and selection to eliminate chimeric or other undesirable progeny. The rate of success in producing suitable transformants can be low and the entire process is often time consuming and labour intensive. However, in comparison with other biotechnologies such as wide crossing or mutagenesis, transgenesis can be relatively fast, although its subsequent progress may be delayed (sometimes considerably) by the often complex and lengthy regulatory procedures that are not applied to other crop biotechnologies (62, 69).

The as-yet unrealized challenge for biotechnologists has been to produce a wide range of industrially useful fatty acids in GM plants at sufficiently high levels for commercial viability (70, 99). At present, most of these fatty acids only accumulate at relatively low levels in transgenic species (see **Table 5**). In vast the majority of cases, most of which are not shown in the Table, extremely low levels (<5%) of the desired fatty acids were produced in GM seeds – as in the example of <1% in coriander shown in Table 5. The main reason for these disappointingly low oil yields is that simply transferring the relevant hydroxylase or desaturase gene into a plant does not mean that the corresponding hydroxy or polyunsaturated fatty acid will necessarily accumulate at high levels in the storage oil of the recipient plant. Indeed, despite over 20 years of often-ingenious efforts by molecular biologists, yields of such fatty acids in most transgenic plants mostly remain low (9, 60, 67).

Sometimes, as with the transgenic Laurical™ rapeseed (see Section 4), desired levels of useful fatty acid can be increased by transferring additional acyltransferase genes to ensure that the fatty acids are efficiently assembled onto triacylglycerols (107). But it is not always possible to predict which additional enzymes/genes may be required to accumulate a given fatty acid. More

recently, the role of diacylglycerol acyltransferase (DGAT) in ensuring accumulation of fatty acids has been reported by several groups (9, 67). Evidence from several plant species suggests that the type-2 acyl-coenzyme A:diacylglycerol acyltransferase (DGAT2) can sometimes stimulate the accumulation of exotic fatty acids in storage triacylglycerols (46, 92).

Table 5. List of some GM oilseeds modified to produce potential industrial oils.

Fatty acid[a]	Donor species	% FA in donor species	Recipient GM oilseed	% FA in recipient species
Lauric 12:0	California Bay	65	Rapeseed (Laurical™)	60
Petroselinic 18:1 6c	Coriander	80	Arabidopsis	<1
Ricinoleic 18:1-OH	Castor bean	90	Arabidopsis	26
Vernolic 18:1 9c,12OH	*Crepis palaestina*	60	Arabidopsis	15
Crepylinic 18:2 9c,12trp	*Crepis alpina*	70	Arabidopsis	25
α-Eleostearic 18:3 9c,11t,13t	*Mormordica charantia*	65	Soybean	17
Calendic 18:3 8t,10t,12c	*Calendula officinalis*	60	Arabidopsis	20

[a]c, *cis* double bond; t, *trans* double bond; trp, triple bond; data from Murphy (67)

A further problem in obtaining high levels of desirable fatty acids is that in some crop species, such as rapeseed, novel acyl groups are not always channeled to storage lipids. Instead, some of the fatty acids may accumulate on membrane lipids. This ectopic accumulation of fatty acids in membrane lipids can trigger a regulatory response, which results in their removal from membranes and subsequent breakdown via the ß-oxidation and glyoxylate cycle pathways (60). This is one reason why some transgenic plants are unable to accumulate high levels of desirable fatty acids. It is also an important reminder of the complexity of metabolic regulation in plants and the difficulties of manipulating this process via the insertion of one, or a few, transgenes (67). Indeed, despite considerable advances over recent decades, a lack of fundamental scientific knowledge about the mechanisms and regulation of acyl incorporation into TAGs is still delaying the development of GM crops expressing high levels of useful fatty acids.

Although GM oil modification has mainly focused on the introduction of new fatty acids, it has also been used to downregulate existing genes in order to decrease levels of unwanted fatty acids. Most frequently this has involved technologies such as antisense or RNAi to suppress genes encoding oleate and/or linoleate desaturases in order to reduce levels of oxidation-prone α-linolenate in seed oils used for industrial applications. These approaches involve the insertion of various versions of the gene in question, such as a backwards (or antisense) copy or a partial version of its corresponding RNA. In the latter case, the strategy is called RNA interference, or RNAi. These inserted DNA or RNA segments interfere with expression of the target gene, either partially or completely suppressing its activity. In one recent study, a combined antisense-hairpin RNAi approach was used successfully to modify the seed oil of the model species *Arabidopsis thaliana*, which is a relative of the oil crop, rapeseed (72).

Downregulation of oleate and/or linoleate desaturases will also normally result in an increase in levels of oleate in seed oils. High-oleate oils are desirable for both edible and many industrial applications and have been successfully developed in several major oil crops by non-transgenic

methods (60). However, several companies have also developed high-oleate, low-polyunsaturate oils using various GM strategies. For example, high-oleate GM lines have been developed for soybean (90% oleate), rapeseed (89% oleate), and cottonseed (78% oleate) (61).

The acyl composition of storage triacylglycerols is determined by complex interactions between acyltransferases, acyl exchange and acyl modification enzymes. Therefore oil composition in plants tends to behave as a ‗quantitative trait', i.e. a genetic character determined by several (often many) genes (107). Unfortunately, for efforts to modify oil composition by genetic engineering, quantitative traits are much more difficult, time-consuming and expensive to manipulate than simple monogenic traits. This is a major factor behind the slow pace of progress in producing GM oil crops compared to the sometimes optimistic projections from researchers in the past (57, 58). However, recent progress in GM technology will doubtless facilitate the manipulation of such complex traits during the coming decade.

The manipulation of many complex traits such as oil composition using GM methods will require the insertion of several, and perhaps over a dozen, genes. With conventional insertion technology and using similar or identical regulatory sequences (promoters, terminators *etc*) for each transgene, there is a risk of interference between the various transgenes leading to gene silencing (69). This has led to development of methods including co-transformation and use of polycistronic transgenes to enable multiple gene insertion into plants. It is possible to transform separate explants with different transgenes to produce a series of transgenic lines, each expressing a different transgene. These transgenic lines are then crossed to create progeny carrying multiple transgenes. Although this strategy has been used successfully, it is relatively lengthy and labour intensive and the various transgenes will be dispersed in various genomic locations where they may be subject to different position effects.

Co-transformation involves the simultaneous addition of several transgenes either as different T-DNAs in a single *Agrobacterium* strain or in different strains that are inoculated together onto explants. Co-introduced transgenes tend to integrate into the same chromosomal position, which makes it much easier to detect and remove unwanted position effects at an early stage. Co-transformation has been used to create ‗stacked traits', such as crops expressing both herbicide tolerance and insect resistance transgenes. It was also used to engineer the ‗golden rice' varieties that accumulate ß-carotene in their grains. In future an increasing proportion of transgenic crops, including oilseeds, is likely to contain ‗stacked traits' created via co-transformation (69).

Although GM technologies are always improving, it should be stressed once again that GM is not the only option for the manipulation of plants to produce industrial oils. Broadly speaking, there are three breeding approaches that can be used:

(i) advanced non-GM breeding of existing oil crops including use of mutagenesis and wide crosses for gene manipulation

(ii) GM methods to bring new genes into existing crops (but with the realization that most oil modifications will be complex traits involving the addition of several key genes, not all of which may be identified at present)

(iii) domestication of new oil crops using advanced molecular breeding methods such as marker-assisted selection (67).

The choice of which approach to adopt will vary from case to case, but all of them have the potential to deliver useful results for the production of renewable crop-derived industrial oils in the future.

Appendix 3. Global vegetable oil production and use

Annual global vegetable oil production (edible and non-edible) has increased over 6-fold from 25 MT in 1975 to 152 MT in 2011. In 2010, global oil production was split between food and non-food (i.e. feed, fuel, and oleochemicals) use in the ratio 77.5 to 22.5. In the past decade there has been a particularly pronounced increase in global biofuel use with biodiesel production increasing from <0.1 MT in 1992 to 1 MT in 2000 and to 13 MT in 2009 (35). About 80% of EU biofuel comes from vegetable oil-based biodiesel. Recent trends in non-food/industrial vegetable oil consumption are shown in more detail in **Table 6**.

Table 6. Global consumption of nine major vegetable oils between 1997/98 and 2009/10
Note the steady rise in % consumption of non-food oil

Year	Total, MT	Food	Non-food	% non-food
1997/98	73.8	66.4	7.4	10.0
1998/99	78.6	70.7	7.9	10.0
1999/00	82.9	74.4	8.5	10.2
2000/01	88.8	78.7	10.1	11.4
2001/02	91.2	80.3	10.9	11.9
2002/03	95.3	83.1	12.2	12.8
2003/04	100.8	87.0	13.8	13.7
2004/05	108.2	91.5	16.7	15.4
2005/06	115.0	94.4	20.6	17.9
2006/07	119.7	96.0	23.7	19.8
2007/08	125.8	99.1	26.7	21.2
2008/09	130.3	101.9	28.4	21.8
2009/10	138.4	107.3	31.1	22.5

Data adapted from Gunstone (35)

The geographical distribution of vegetable oil consumption is shown in **Table 7**. The highest ratio of non-food to food use is found in SE Asia (mainly due to the large palm oil-based oleochemical industry in Malaysia), and in the EU (with an oleochemical industry partially based on imported palm oil, plus expanding biodiesel production mainly sourced from local and imported rapeseed oil). There is also significant non-food vegetable oil use in USA and Japan, both of which have mature oleochemical industries, and growing use of biodiesel in countries such as the USA, Brazil and Argentina, where it is mostly produced from soybean oil for either for local use and/or for export (35).

Table 7. Consumption (MT) of the major vegetable oils in 2009/10 by country/region

Region	Total	Food	Non-food				
			Total	Palm	Rape	Soya	Other
SE Asia	15.9	8.2	7.8	5.0	0.0	0.1	2.7
Middle East	4.8	4.4	0.4	0.2	0.0	0.1	0.1
EU-27	23.9	13.0	10.9	2.1	6.8	1.1	0.9
China	26.8	24.4	2.4	2.0	0.0	0.0	0.4
India	15.8	14.9	0.9	0.6	0.0	0.0	0.3
USA	11.2						
Other	40.0						
Total	138.4	107.3	31.1	12.0	7.0		

Data adapted from Gunstone (35)

As shown below in **Table 8**, global vegetable oil production is very much dominated by the ‗big three' crops, namely oil palm, soybean, and rapeseed, which together account for over 80% of total production. Although the EU produces most of its own rapeseed, almost two thirds (64.4%) of global vegetable oil production comes from just two crops, oil palm and soybean, both of which are almost completely sourced from outside the EU. This means that future oleochemical supplies in the EU could largely depend on the import of seed and/or oil from other regions of the world where attitudes to, and regulation of, GM technology may be significantly different from norms that currently prevail in the EU.

Table 8. Global production of nine major vegetable oils in 2009/10

Oil crop	Production MT	%
Palm mesocarp	45.86	32.8
Palm kernel	5.50	3.9
Soybean	38.76	27.7
Rapeseed	22.35	16.0
Sunflower	11.66	8.3
Peanut	4.67	3.3
Cottonseed	4.66	3.3
Coconut	3.62	2.6
Olive	2.92	2.1
Total	139.99	100.0

Data adapted from Gunstone (35)

Plant-derived fatty acids can be converted to methyl esters, alcohols, and amines from which intermediate chemicals such as alcohol sulfates, alcohol ethoxylates, alcohol ether sulfates, quaternary ammonium salts, monoacylglycerols, diacylglycerols, tailored TAGs, and glycoesters may be produced. In 2010, global production of fatty acids was 8.0 MT of which 1.4 MT was in the EU. Global production of detergent alcohols 3.4 MT of which 0.7 MT was in the EU (104). Detergent alcohols are used mainly to produce surface-active compounds for personal care and for cleaning processes. In the EU, other major oleochemical intermediates include fatty esters (0.4 MT), lubricant esters (0.09 MT), and solvent replacers (0.015 MT). These chemicals are used in paints, inks, lubricants, and production of polyols to make polyurethanes. Oleochemicals are also used to produce many fine chemicals, cosmetics, pharmaceuticals, coatings, textiles, polymer, paper, fuel and rubber additives, biodegradable polymers, and offshore drilling muds.

Table 9. Recent trends in global consumption (MT) of two major oil crops

Year	All plant oils			Rapeseed			Palm		
	Total	Food	Non-food	Total	Food	Non-food	Total	Food	Non-food
96/97	73.8	65.2	8.6	10.5	9.5	1.0	17.6	14.1	3.5
02/03	96.1	83.3	12.8	12.2	11.0	1.2	27.6	22.0	5.6
09/10	138.4	107.3	31.1	22.2	15.2	7.0	45.3	33.3	12.0

Data adapted from Gunstone (35)

The two most important oil crops for the EU oleochemical industry are oil palm and rapeseed. Almost all the industrial oil palm consumed in the EU is imported from SE Asia while the bulk of industrial rapeseed is grown locally. Recent trends in these two major crops are shown in **Table 9**. The 7-fold increase in industrial rapeseed consumption is mainly due to subsidy-led biofuel use in the EU where the proportion of rapeseed oil used for biodiesel increased from 10% in 2002/03 to 31% in 2009/10. In contrast, while there have been substantial increases in palm oil use for biodiesel in SE Asia (much of which is then exported to the EU), the bulk of the

6.6 MT increase in industrial palm oil consumption has been due to expansion of oleochemical production, especially in Malaysia. In contrast with Europe, most biofuel in the Americas currently comes from bioethanol (mainly from maize in USA and sugar cane in Brazil), although some modest production of biodiesel from soybean oil is beginning in the USA (mostly for local markets) and in Brazil and Argentina (mostly for export) (35).

Over recent decades several EU-funded initiatives have explored the potential of new oleochemical crops such as crambe and high-oil cultivars of oats (10). Similar research programs have been carried out in individual EU countries and elsewhere, most notably in the USA and Canada (50). Following the EC/USDA-funded EPOBIO project, it was agreed that it was important to develop *"a general purpose non-food GM oilseed crop as a platform to produce novel fatty acids for industrial applications"* (10). For data on current and possible future trends for industrial oil crops see USDA (104, 105) and OECD/FAO (74) while Rabobank (79) reviews the global grains and oilseeds sector.

Appendix 4. Biofuels from vegetable oils

In 2000, global biofuel production was <13 MT, but this had almost quintupled to 60 MT by 2010 (68). Although the vast majority of global biofuel production comes from starch-derived bioethanol, about 8 MT is sourced from vegetable oil-derived biodiesel, half of which is produced in the EU. Major drivers for the increased production of biofuels include policies aimed at developing alternative, renewable, and carbon-neutral energy sources (especially in the EU), and to ensure national independence from perceived over-reliance on imported fossil fuels (especially in the USA). During the 2000s, most advanced economies formulated national and/or regional plans to develop and promote biofuels such as biodiesel and bioethanol. For example, in the USA, a National Biofuels Action Plan (71) was drawn up by the Department of Energy and a target of a ‗billion ton' annual production of biofuel feedstock was proposed (76).

Much biofuel production in the USA is focused on starch or cellulosic feedstocks and their conversion to bioethanol or biohydrogen (38, 43, 80, 84). In contrast, the major source of biofuels in the EU is vegetable oil-derived biodiesel. The EU adopted a biofuels directive in 2003 with the aim of replacing 2% of petrol and diesel for transport by biofuels by 2005, and 5.75% by 2010. The Commission also proposed reinforcing the legislative framework, with a 10% minimum for the market share of biofuels in 2020 (21), followed by further proposals in 2008 (23, 27). The Fuel Quality Directive 2009 also requires Member States to reduce life cycle greenhouse gas emissions of transport fuels by 6% per cent by 2020, which has indirectly affected biofuels markets. In the UK, the Renewable Transport Fuel Obligations (RTFO) Order mandates that 5% of total transport fuel should originate from renewable sources by 2013.

The production of biodiesel from vegetable oils in different regions of the world is shown in **Table 10**. The EU total of 9.55 MT accounts for about half of global biodiesel production and EU biodiesel production increased over ten-fold between 1998 and 2009 (29). As discussed above, during the mid-2000s pressure to meet biofuel targets led to subsidies that also favoured the increasing import of palm biodiesel in the EU (34). This led to large increases in palm oil prices that impacted on food markets in Asia where palm oil is a major dietary component. There are now signs of a slowdown in biodiesel production in the EU, partly due to a backlash against the use of food crops, such as rapeseed and oil palm, for biofuel. Such concerns are especially acute in developing countries where food insecurity caused by diversion of food crops to non-food use is an increasingly serious issue (26, 31, 102).

Table 10. Global production (MT) of biodiesel since 2006

Year / Producer	2006	2007	2008	2009	2010*
Germany	2.55	2.93	2.67	2.50	2.73
France	0.74	0.87	1.82	2.00	2.20
Other EU	1.56	2.15	4.00	4.92	4.62
EU total	**4.85**	**5.95**	**7.49**	**8.42**	**9.55**
USA	1.13	1.70	2.69	1.80	2.10
Argentina	0.05	0.18	0.74	1.16	1.60
Brazil	0.06	0.36	1.03	1.40	2.00
Other countries	1.03	1.33	2.37	2.94	3.91
Global Total	**7.12**	**9.52**	**14.32**	**15.72**	**19.16**

*forecast; data from USDA (104)

The contrasting biofuel policies in the USA and EU are discussed by Tyner (101) who criticizes the current US policy of selectively favouring bioethanol technologies. The policy of favouring biofuels has become increasingly controversial within Europe since the food price rises of 2007-08 (73) and is much discussed by groups such as the European Biodiesel Board (www.ebb-

eu.org), European Oleochemicals & Allied Products Group (30), International Energy Agency (41), and others (33). One result of the EU biofuel policy has been a huge increase in production of rapeseed oil for non-food use, from 1.3 MT in 2002/03 to 6.7 MT in 2009/10 (50). The vast majority of this industrial rapeseed oil is used for biodiesel production, mostly within the EU. The greatly increased global production total of 60 Mt of biofuels in 2010, which was a factor in driving up the prices of many staple food crops, was still only sufficient to cover a mere 2% of the annual global transport fuel requirement (68). By 2008, international bodies such as FAO and several NGOs were expressing significant concern about the policy of encouraging the use of potential food crops such as rapeseed, oil palm, and maize to produce transport fuels (FAO, 2008). Specific concerns included the diversion of prime cropland away from food production, increased conversion of rainforest and other pristine habitats to grow biofuel crops, and a questioning of the overall energy balance (based on life-cycle analysis) involved in producing high input-requiring biofuel crops (12, 14, 18, 34, 63, 64, 100, 106).

The capacity of biofuels to contribute to overall transportation fuels is analyzed below in **Table 11**. The take-home message is that crop-derived biofuels can only ever produce a tiny fraction of transport fuel requirements. In view of the problems involved in using food-competitive crops as biofuel feedstocks, there has been growing interest in developing novel crops or other biological production platforms that can generate environmentally sustainable biofuels using terrestrial or aquatic areas that cannot be used to grow food crops (111). There has also been interest in breeding or engineering improved versions of existing oil crops (11, 19, 83, 85) and/or in developing new plant-based feedstocks for biodiesel production (63, 69, 86). One example of a new biodiesel oil crop is *Jatropha curcas*, whose oil-rich nuts can potentially yield 1.5-2.5 T/ha biodiesel. Unfortunately, while Jatropha has been successfully grown on land that is unsuitable for food crops, its oil yields and other performance traits have been disappointing and will require considerably more R&D in the future (7).

Table 11. Potential usage of biofuels in the transportation sector

Consumption scenario	Consumption	Arable land needed for 100% biofuel substitution (million ha)	Arable land needed for 10% biofuel substitution (million ha)
Global transport fuel, 2007 (>95% fossil derived)	2.4 billion T	800-1200 Mha	80-120 Mha
Global transport fuel consumption, 2020 estimate	3.4 billion T	1700 Mha*	170 Mha
EU transport fuel, 2007	0.5 billion T	167-250 Mha (>100% arable area)	17-25 Mha (14-21% arable area)

EU or USA policy target	Biofuel production¶	Arable land required (million ha)	% present arable area in region	% total global arable area
EU transport biofuel, 2010	17 MT	5.5-8.7 Mha	4.5-7.2†	0.3-0.5
EU transport biofuel, 2020§	37 MT	12-19 Mha	10-16†	0.9-1.2
USA transport biofuel, 2017	4100 MT	1 Mha	570‡	72
USA transport biofuel, 2025	9200 MT	1-2 Mha	570-1140‡	72-145

* assuming 50% future increase in biofuel yields; ¶ production figures in MT are minimal values based on 2007 data – but according to industry estimates, fuel consumption may increase >50% by 2030 with a commensurate increase in land required for biofuels; † total EU arable area is 120 Mha; ‡ total USA arable area is 175 Mha; § according to Woods (111), achieving the 2020 biofuels target would require c.600 Mha land using conventional technologies and c.250 Mha with „next generation" technologies. Estimates are based on a global arable land area of 1380 Mha. Data from Murphy (63).

In several cases, GM approaches are being used to develop new varieties of existing oil crops engineered for improved biofuel production (44, 51, 91, 98, 103). In 2011, the first GM crop variety engineered for improved biofuel characteristics was approved for commercial use in the USA. This GM maize variety, called Enogen™, has a modified α-amylase gene that enhances dry grind ethanol production in a way that can be easily integrated into the existing infrastructure of ethanol production facilities, resulting in significant cost savings and a much reduced environmental footprint (103). In other cases, the possibility of manipulating vegetable oil crops to enhance their utility for biodiesel production is being investigated (68).

For example, palm oil-derived biodiesel has a relatively high saturated fatty acid content, which can limit its use (or require blending with other oils) as a fuel under cool conditions, such as Northern Hemisphere winters. The high oleate oil palm varieties currently under development would address this problem (88). It may also be possible to engineer vegetable oils with high C8 compositions that could be converted to high-octane gasoline for applications where diesel fuels are not suitable. As shown in Table 2, the non-crop oilseed plant, *Cuphea avigera*, already produces oil containing 94% C8 acyl residues. Also, GM rapeseed has been produced with 60% C12, instead of 90% C18 as in the conventional crop (107). Therefore if there is a commercial requirement for C8 oils, GM technology could in principle to supply such oils.

In other cases, new non-crop sources of biofuels are being investigated, one of the most promising of which is microalgae (6, 40, 90, 97, 108). Although microalgae are single-celled aquatic eukaryotes capable of photosynthesis, the term as used popularly can sometimes include non-algal photosynthetic prokaryotes such as cyanobacteria. Some species of microalgae accumulate as much as 50% of their mass as storage lipids similar to those found in oil crops. Some algae also grow well in brackish or salty water and can therefore be cultivated without using scarce cropland. Microalgae can grow much faster than crops and, providing high-oil varieties can be cultured on a sufficiently large scale, they could potentially generate huge amounts of biodiesel.

It is estimated that if microalgae could be grown on a large scale without yield losses, they could produce 10-50 times more biodiesel per hectare than existing crops such as rapeseed or oil palm. If microalgae were grown in shallow ponds over an area of 0.2 Mha, one billion tonnes of biodiesel could be produced each year. Therefore an area of 3.8 Mha could potentially generate sufficient biodiesel to replace all petroleum-based transport fuels currently used in the USA. This is only 2% of the current arable acreage in the USA and a mere 0.4% of the total land area of the country (63, 68). Although they will require large land areas for economic cultivation, many microalgae grow well in sunny, warm habitats such as shallow ponds. It may therefore be possible to set up ponds as microalgal bioreactors in sunny but arid and little-used areas such as the Colorado Desert of the western USA.

Many technical challenges will have to be overcome before microalgae can provide a realistic alternative to fossil fuels. Particular problems are maintaining oil yields as cultures are scaled up and contamination by other algae and pathogens. As well as improved bioreactor design, several GM and mutagenesis approaches are being used to improve oil yields. One strategy is to transfer the genes responsible for oil secretion from slow-growing species such as *Botryococcus braunii* to more productive microalgae such as *Chlamydomonas* spp. If microalgae can be engineered to secrete oil, this will collect on the surface and could be continuously harvested rather than the current less efficient method of periodically collecting algal cells and extracting the oil from them. Further into the future, microalgae and bacteria may be harnessed to produce gaseous fuels, such as biogas and biohydrogen. For example, in anaerobic conditions *Chlamydomonas* can produce hydrogen in the light and this might be enhanced by manipulation of photosystem II activity. Microalgal biofuel production has considerable promise but will probably take several decades to become economically feasible (63, 68). However, it is likely that future commercial oil production from microalgae will involve some form of GM technology.

Appendix 5. Crop-derived, lipid-based biopolymers and waxes

As an alternative to modifying TAG-based storage lipids in oil crops, fatty acid biosynthetic pathways can be diverted to the production of other useful carbon-based compounds. For example, there is great interest in using GM crops for large-scale production of biodegradable polymers such as polyhydroxyalkanoates (PHAs). Such biopolymers could potentially substitute for conventional plastics that are currently derived from non-renewable petroleum feedstocks (2, 55, 77). Almost all conventional plastics are currently made from petroleum-derived chemicals such as adipic acid and vinyl chloride but the manufacturing processes are relatively energy intensive and frequently produce undesirable byproducts that can be costly and difficult to dispose of. Finally, many petroleum-based polymer products are difficult to recycle and take decades before they break down in landfill sites. An attractive alternative to conventional plastics is renewable, biodegradable biopolymers that are less energy-intensive to manufacture.

Some soil bacteria, such as *Ralstonia eutrophus* or *Alcaligenes latus*, can accumulate up to 80% of their mass as non-toxic biodegradable PHA polymers. The PHAs accumulate as tiny semi-solid granules in bacterial cells. The PHAs are made up of *β*-hydroxyalkanoate subunits that are synthesized from acetyl-CoA via a relatively short pathway involving as few as three enzymes for the most common PHA, polyhydroxybutyrate (96). Currently, PHAs are made industrially via a bioreactor process but cost as much as ten-fold more to produce than conventional plastics. Following the development of GM technologies in the 1980s, it was realized that the three bacterial genes could also be transferred into crops, which would then hopefully accumulate PHAs on an agricultural scale. Plant-derived PHAs had the prospect of being much cheaper and capable of production on a much larger scale that might make them competitive with petroleum-derived plastics. Providing the PHAs accumulate in plastids, it was possible to obtain modest yields of polymers in leaves or seeds (39, 94). GM oil crops such as rapeseed or oil palm (61) have been most the common production platforms for biopolymers but there has been a recent report of PHA accumulation in the biomass crop, switchgrass or *Panicum virgatum* (95).

A major and as yet unresolved technical hurdle is how to extract biopolymer granules from plant tissues in an efficient and cost-effective manner. Over recent years, there have been several patent filings for PHA extraction from plants based on non-halogenated solvent extraction at high-temperature, plus procedures that allow simultaneous extraction of PHAs and oils from oilseed crops using differential solvent extraction (55). However, none of these methods are particularly environmentally friendly, which may be a major issue for a product, such as a biopolymer, whose major raison d'être is based on its environmental credentials. To date, none of these methods have been published for commercial-scale extraction from plants and further progress on PHAs may be delayed until this challenge can be overcome (67).

Wax esters are rarely found in plant oils, but the desert shrub, jojoba (*Simmondsia chinensis*) can accumulate as much as 95% of its seed oil as a long chain C20:1/C22:1 wax. This liquid wax performs especially well in high-temperature and high-pressure applications such as jet engine lubricants and as hydraulic fluids. Jojoba wax is also highly prized in the cosmetics industry. Because jojoba plants are difficult to cultivate, grow slowly, and have low oil yields, GM methods have been used to engineer wax accumulation in at least one mainstream oilseed crop. Co-expression of genes encoding a jojoba acyl-CoA reductase and β-ketoacyl-CoA synthase from *Lunaria annua* in the model plant *Arabidopsis* resulted in seed oils with wax levels of up to 70% w/w (47). Following this success, expression of jojoba acyl-CoA reductase in high-erucic rapeseed resulted in the synthesis of a small amount of wax ester (53). However, wax ester yields in this GM rapeseed were insufficient for commercial production and there have been no subsequent reports of GM wax ester crops. It is possible that part of the reason for the failure by Monsanto (the owner of the technology) to pursue this project after the early 2000s was the relatively small market for specialty jojoba-like oils in comparison with the relatively high R&D and regulatory costs required to bring the product to market.

Appendix 6. Socioeconomic dimensions of GM industrial oil crops

From its beginning in the 1970s, there was opposition to all forms of GM technology, including the use of microbial, animal, and plant systems. During the 1990s, however, the objections became focused more on GM crops (and to a lesser extent on animals). In contrast, GM microorganisms are now widely used in manufacture of a dozens of recombinant pharmaceutical products including insulin, vaccines, and specialized therapeutic proteins (69). Initially, the opposition to GMOs was based mainly on the principle that genes should not be inserted into any higher organisms, either on ethical grounds or due to the perceived risks that potentially dangerous new life forms might be created. However, once 1st generation GM crops were released on a large scale in the mid-late 1990s, much of the opposition was as much concerned with the **provenance** of the technology as its perceived risks in regard to environmental impacts or food safety.

In particular, campaign groups stressed that almost all transgenic crop varieties were patented and owned by a few large multinational companies. This led to concerns that global food supplies might in the future be controlled by a few large companies for their own interests (62). Moreover, consumers in Europe saw little or no benefit from 1st-generation GM crops developed mainly in the USA with modified input traits designed to facilitate crop management rather than to produce better or cheaper food. Public disquiet was exacerbated in 1999 by claims that transgenic potatoes might have adverse effects on laboratory rats. Although subsequent research has shown that many original claims about the dangers of GM technology were unjustified, public disquiet remains and since 1999 most GM crop products have been effectively excluded from EU markets. This was partially due to pressure from anti-GM campaigning groups, but their success was facilitated by consumer uncertainty about the risk/benefit equation of GM technology and concerns about its provenance. This has framed a political context that can make it difficult for governments to openly endorse GM crop products, whatever the scientific facts.

Table 12. Private sector firms and R&D expenditures by type of activity in 2006

Market sector	**Number of companies**	**Agricultural R&D, $ billion**	**% total R&D spending**
_Big six‘ agrochemical/ agbiotech companies*	6	2.03 + 1.57 (chemicals + seed/biotech)	66
Other agrochemical companies	122	0.62	11
Other seed companies	82	0.63	12
Other agbiotech companies	45	0.17	3
Fertilizer companies	No data	0.45	8
Total	255	5.47	100

*The _Big six‘ are BASF, Bayer, Syngenta, Dupont, Dow and Monsanto; data adapted from Piesse & Thirtle (78)

Ownership of GM technology was consolidated in the 1990s, when a spate of acquisitions and mergers resulted in most small agbiotech startup companies being taken over by a few large multinational agrochemical companies. For example Calgene and many other biotech and seed companies were acquired by Monsanto while other large agrochemical multinationals, such as Dow Chemical, DuPont, BASF, and Syngenta, acquired many remaining smaller independent agbiotech companies. As shown above in **Table 12**, by 2006 the _big six‘ companies controlled

66% of R&D spending on crop improvement. This concentration is even more marked in existing commercial GM crop varieties, where > 90% of seed sales come from Monsanto. By the 2010s, only a few small agbiotech companies remained and these were mostly either service providers or firms working in small high-risk niche areas such as biopharming where the multinationals were less active.

One of the challenges for the future acceptability of GM crops will be to broaden their provenance beyond a few multinational companies and to involve the public sector in GM-related R&D to a much greater extent than at present (62, 89). Ironically, one of the barriers to such a broadening of the R&D and ownership base of GM crops may be the complex, lengthy, expensive, and unpredictable nature of the current mechanisms for regulation of GM crops in the EU (15, 82). These make it more difficult for smaller companies and public sector researchers to develop improved form of GM technology in Europe compared to many other parts of the world.

Many of those objecting to GM technology say that it is unnecessary and has not been used to address key crop traits such as yield, drought or salinity. Indeed, as discussed above, after two decades of commercial use >99.5% of GM technology involves just two agronomic traits in only four crops. While it is true that the impact of GM technology has been very significant in the four crops where it has been widely applied, its impact has been relatively modest in terms of the bigger picture of global agriculture and the dozens of agronomic traits involved in crop performance. This also applies to the manipulation of crop composition, e.g. to produce industrial oils.

However, GM technology is still developing and new tools such as genomics are just beginning to have a significant impact. Therefore GM technology may be used in much wider applications in the next few decades, as discussed in Section 5. Even today, the overall socio-economic context of GM crops may be gradually changing away from the initial strong rejectionism of the late 1990s towards a more nuanced perspective that takes into account changes in the nature and provenance of the technology (22). To quote from a recent EU-wide survey of attitudes to GM foods reported by Gaskell et al (32):

"*The latest Eurobarometer survey ... conducted in February 2010, points to a new era in the relations between science and society. While entrenched views about GM food are still evident, the crisis of confidence in technology and regulation that characterised the 1990s ... is no longer the dominant perspective. In 2010 we see a greater focus on technologies themselves: are they safe? Are they useful? And are there 'technolite' alternatives with more acceptable ethical-moral implications? Europeans are also increasingly concerned about energy and sustainability. There is no rejection of the impetus towards innovation: Europeans are in favour of appropriate regulation to balance the market, and wish to be involved in decisions about new technologies when social values are at stake.*"

References

1a. Aviatime.com (2011) Finnair in the forefront of flying on biofuel, http://www.aviatime.com/finnair-in-the-forefront-of-flying-on-biofuel.html

1b. Backus GBC et al (2008) EU policy on GMOs; a quick scan of the economic consequences, Report 2008-070, LEI Wageningen University Research Centre, The Hague

1c. Bai et al (2011) Rapid detection of eight vegetable oils on optical thin-film biosensor chips, *Food Control* 22 1624-1628

1. Barros E, Lezar S, Anttonen MJ, van Dijk JP, Rohlig RM, Kok EJ, Engel KH (2010) Comparison of two GM maize varieties with a near-isogenic non-GM variety using transcriptomics, proteomics and metabolomics, *Plant Biotechnology Journal* 8, 436–451

2. van Beilen JB, Poirier Y (2007) Prospects for biopolymer production in plants. *Advances in Biochemical Engineering and Biotechnology* 107,133-151

3. Biermann U, Bornscheuer U, Meier MAR, Metzger JO, Schäfer HJ (2011) Oils and Fats as Renewable Raw Materials in Chemistry. *Angewandte Chemie International Edition* 50, 3854 -3871

4. Bogani P, Minunni M, Spiriti MM, Zavaglia M, Tombelli S, Buiatti M, Mascini M (2009) Transgenes monitoring in an industrial soybean processing chain by DNA-based conventional approaches and biosensors. *Food Chem* 113, 658-664

5. Borg K (1975) Physiopathological effects of rapeseed oil: a review. *Acta Med Scand Suppl* 585, 5-13

6. Briggs M (2004) *Widescale biodiesel production from algae*, University of New Hampshire, http://www.unh.edu/p2/biodiesel/article_alge.html

7. Brittaine R, Lutaladio N (2010) Jatropha: a smallholder bioenergy crop. The potential for pro-poor development, *Integrated Crop Management* 8-2010

8. Cahoon EB, Kinney AJ (2005) Production of vegetable oils with novel properties: using genomic tools to probe and manipulate fatty acid metabolism. *Eur J Lipid Sci Technol* 107, 239-243

9. Cahoon EB, Shockey JM, Dietrich CR, Gidda SK, Mullen RT, Dyer JM (2007) Engineering oilseeds for sustainable production of industrial and nutritional feedstocks: solving bottlenecks in fatty acid flux. *Current Biology* 10, 236-244

10. Carlsson AS, Clayton D, Salentijn E, Toonen M (2007) *Oil crop platforms for industrial uses*, Outputs from the EPOBIO project, Cpl Press, http://epobio.net/pdfs/0704OilCropsReport.pdf

11. Chhetri AB, Tango MS, Budge SM, Watts KC, Islam MR (2008) Non-Edible Plant Oils as New Sources for Biodiesel Production, *Int J Mol Sci* 9, 169-180

12. Christian Aid (2009) Growing pains: the possibilities and problems of biofuels, www.christianaid.org.uk

13. Costa J, Mafra I, Amaral JS, Oliveira MBPP (2008) Transgenes monitoring in an industrial soybean oil processing by conventional and real-time polymerase chain reaction, In 9º Encontro de Química dos Alimentos, Angra do Heroísmo, http://bibliotecadigital.ipb.pt/handle/10198/5109

13a. Costa J, Mafra I, Amaral JS, Oliveira MBPP (2010a) Monitoring genetically modified soybean along the industrial soybean oil extraction and refining processes by polymerase chain reaction techniques, *Food Research International* 43, 301–306

13b. Costa J, Mafra I, Amaral JS, Oliveira MBPP (2010b) Detection of genetically modified soybean DNA in refined vegetable oils, *Eur Food Res Technol* 230, 915–923

14. Davis SC, Anderson-Teixeira KJ, DeLucia EH (2009) Life-cycle analysis and the ecology of biofuels, *Trends in Plant Science* 14, 140-146

15. Davison J (2010) GM plants: Science, politics and EC regulations. *Plant Science* 178, 94–98

16. Davison J, Bertheau Y (2007) EU regulations on the traceability and detection of GMOs: difficulties in interpretation, implementation and compliance, *CAB Review: Perspect. Agric. Vet. Sci. Nutr. Nat. Resources* 2, 1–14

17. Davison J, Bertheau Y (2008) The theory and the practice of European traceability regulations for GM food and feed, *Cereal Foods World* 53, 186–196

18. Doornbosch R, Steenblik R (2007) Biofuels: is the cure worse than the disease? 20th meeting of the OECD round table on sustainable development, Paris, September 2007, http://www.oecd.org/dataoecd/15/46/39348696.pdf

18a. Doveri S, Lee D (2007) Development of sensitive crop-specific polymerase chain reaction assays using 5S DNA: Applications in food traceability, *J. Agric. Food Chem.* 55, 4640-4644

19. Durrett TP, Benning C, Ohlrogge J (2008) Plant triacylglycerols as feedstocks for the production of biofuels, *Plant Journal* 54, 593-607

20. Dyer JM, Mullen RT (2008) Engineering plant oils as high-value industrial feedstocks for biorefining: the need for underpinning cell biology research, *Physiol Plantarum* 132: 11–22

21. EC (2003) Directive 2003/30/EC of the European Parliament and of the Council of 8 May 2003 on the promotion of the use of biofuels or other renewable fuels for transport, May 2003

22. EC (2011) Report from the Commission to the European Parliament and the Council on socio-economic implications of GMO cultivation on the basis of Member States‘ contributions, as requested by the Conclusions of the Environment Council of December 2008, SANCO/10715/2011 (POOL/E1/2011/10715/10715SWP-EN.doc)

23. EC (2008): Proposal for a directive of the European Parliament and of the Council on the promotion of the use of energy from renewable sources, COM (2008) 19 final, January 2008

23a. EFSA (2010b) Guidance on the environmental risk assessment of genetically modified plants, Panel on Genetically Modified Organisms, EFSA Journal 2010-8, 1879 http://www.efsa.europa.eu/en/scdocs/doc/1879.pdf

23b. EFSA (2010b) Guidance for risk assessment of food and feed from genetically modified plants, Panel on Genetically Modified Organisms, *EFSA Journal* 2011-9, 2150 http://www.efsa.europa.eu/en/efsajournal/doc/2150.pdf

24. Ehran SZ (2005) *Industrial uses of vegetable oils*, AOCS Press, Urbana

25. Ehran SZ, Adhvaryu A (2005) Non-food lipids, In Murphy DJ, ed, *Plant Lipids: biology, utilisation and manipulation*, Blackwell, Oxford

26. Ericksen P, Thornton P, Notenbaert A, Cramer L, Jones P, Herrero M (2011) Mapping hotspots of climate change and food insecurity in the global tropics. CCAFS Report no. 5, CGIAR Research Program on Climate Change, Agriculture and Food Security (CCAFS). Copenhagen, www.ccafs.cgiar.org

27. EurActive (2008) EU agrees 10% 'green fuel' target in renewables deal, Available at: http://www.euractiv.com/en/transport/eu-agrees-10-green-fuel-target-renewables-deal/article-177812

28. EuropaBio (2011) *Pocket guide to GM crops and policies*, EuropaBio, Brussels, www.europabio.org

29. European Biodiesel Board (2009) *The EU biodiesel industry*, http://www.ebb-eu.org/stats.php

30. European Oleochemicals & Allied Products Group (2006) Challenges and potential of biomass & biofuels for the European Oleochemical Industry, Public Hearing 060601

31. FAO (2008) Biofuels: prospects, risks and opportunities, FAO, Rome Italy

32. Gaskell G, Stares S, Allansdottir A, Allum N, Castro P, Esmer Y et al (2010) Europeans and biotechnology in 2010. Winds of change? A report to the European Commission Directorate-General for Research, EUR 24537 EN, European Commission Publications Office, Brussels

33. Gilbertson T, Holland N, Semino S, Smith K (2007) Paving the way for Agrofuels, EU policy, sustainability criteria, and climate calculations, 25 September 2007, http://www.tni.org/detail_pub.phtml?&know_id=202

34. de Gorter (2010) Why the EU biodiesel import tariff on US „splash and dash" exports will make no difference, *Biofuels* 1:247-250

34a. Gryson N, Messens K, Dewettinck K (2004) Influence of different oil-refining parameters and sampling size on the detection of genetically modified DNA in soybean oil, *Journal American Oil Chemists Society* 81, 231-234

34b. Gryson N, Ronsse F, Messens K, De Loose M, Verleyen T, Dewettinck K (2002) Detection of DNA during the refining of soybean oil, *Journal American Oil Chemists Society* 79, 171-174

35. Gunstone F (2011) Non-food markets, Lipid Library website, http://lipidlibrary.aocs.org/market/nonfood.htm

36. Gunstone, FD, Harwood JL (2007) Occurrence and characterization of oils and fats, in, Gunstone, FD, Harwood JL, Dijkstra AJ (eds.), *The Lipid Handbook*, 3rd Ed, pp 37-141, Taylor & Francis, London

36a. Hilbeck A, Meier M, Römbke J, Jänsch S, Teichmann H, Tappeser B (2011) Environmental risk assessment of genetically modified plants - concepts and controversies. *Environmental Sciences Europe* 23:13

37. Hill K (2000) Fats and oils as oleochemical raw materials. *Pure Appl Chem* 72, 1255–1264

38. Hoffer T (2010) Farewell to Fossil Fuels? *Science* 329, 1292-1295

39. Houmiel KL, Slater S, Broyles D, Casagrande L, Colburn S, Gonzalez K *et al*. (1999) Poly(*β*-hydroxybutyrate) production in oilseed leukoplasts of *Brassica napus*. *Planta* 209, 547–550

40. Hu Q, Sommerfeld M, Jarvis E, Ghirardi M, Posewitz M, Seibert M et al (2008) Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances, *Plant Journal* 54, 621-639

41. International Energy Agency (2009) *Key World Energy Statistics*, Paris, www.iea.org

42. James C (2010) Global status of commercialized biotech/GM crops: 2010. *ISAAA Brief* No. 42, ISAAA, Ithaca, NY, http://www.isaaa.org

42a. Jinxia A, Qingzhang L, Xuejun G, Yanbo, Y Lu L, Minghui Z (2011) A multiplex nested PCR assay for the simultaneous detection of genetically modified soybean, maize and rice in highly processed products, *Food Control* 22, 1617-1623

43. Kausch A, Hague J, Oliver M, Li Y, Daniell H, Mascia P et al (2010) Transgenic perennial biofuel feedstocks and strategies for bioconfinement. *Biofuels* 1, 163-176

44. Knothe G (2008) ―Designer" biodiesel: optimizing fatty ester composition to improve fuel properties. *Energy and Fuels* 22, 1358-1364

45. Kogel KH, Voll LM, Schäfer P, Jansen C, Wu Y, Langen G et al (2010) Transcriptome and metabolome profiling of field-grown transgenic barley lack induced differences but show cultivar-specific variances. *Proceedings of the National Academy of Sciences USA* 107, 6198-6203

46. Kroon JT, Wei W, Simon WJ, Slabas AR (2006) Identification and functional expression of a type 2 acyl-CoA:diacylglycerol acyltransferase (DGAT2) in developing castor bean seeds which has high homology to the major triglyceride biosynthetic enzyme of fungi and animals. *Phytochemistry* 67, 2541–2549

47. Lardizabal KD, Metz JG, Sakamoto T, Hutton WC, Pollard MR, Lassner MW (2000) Purification of a jojoba embryo wax synthase, cloning of its cDNA, and production of high levels of wax in seeds of transgenic Arabidopsis. *Plant Physiology* 122, 645-655

48. Li Z, Xing A, Moon BP, McCardell RP, Mills K, Falco SC (2009) Site-specific integration of transgenes in soybean via recombinase-mediated DNA cassette exchange. *Plant Physiol* 151, 1087-1095

49. Li Z, Moon BP, Xing A, Liu ZB, McCardell RP, Damude HG et al (2010) Stacking multiple transgenes at a selected genomic site via repeated recombinase-mediated DNA cassette exchanges. *Plant Physiol* 154, 622-631

49a. Lopez GP, Laan T (2008) Biofuels – at what cost? Global Studies Initiative, Geneva, ISBN 978-1-894784-22-1

50. Lu C et al (2010) New frontiers in oilseed biotechnology: meeting the global demand for vegetable oils for food, feed, biofuel, and industrial applications, *Curr Opinion Biotechnol* 22, 252-259

51. Lynd LR, Laser MS, Bransby D, Dale BE, Davison B, Hamilton R et al (2008) How biotech can transform biofuels, *Nature Biotechnology* 26, 169-173

52. Masuda T, Goldsmith PD (2009) World soybean production: area harvested, yield, and long-term projections, *International Food and Agribusiness Management Review* 12, 143-162

53. Metz JG, Pollard MR, Anderson L, Hayes TR, Lassner MW (2000) Purification of a jojoba embryo fatty acyl-Coenzyme A reductase and expression of its cDNA in high erucic acid rapeseed. *Plant Physiology* 122, 635-644

54. McKeon TA (2005) Genetic Modification of Seed Oils for Industrial Applications, In: *Industrial Uses of Vegetable Oils*, Erhan SZ, ed, AOCS Press, Urbana

55. Mooney BP (2009) The second green revolution? Production of plant-based biodegradable plastics. *Biochemical Journal* 418, 219-232

56. Moschini G (2006) Pharmaceutical and industrial traits in genetically modified crops: coexistence with conventional agriculture. *American J Agric Economics* 88, 1184–92

57. Murphy DJ (1991) Designer oilseed crops. Genetic engineering of new oilseed crops for edible and non- edible applications. *Agro-Industry Hi-Tech* 5, 5–10

58. Murphy DJ (1992) Modifying oilseed crops for non-edible products. *Trends in Biotechnol* 10, 84–87.

59. Murphy DJ (1994) Designer Oil Crops, Murphy DJ, ed, VCH Press, Weinheim

60. Murphy DJ (2006) Molecular breeding strategies for the modification of lipid composition, *In Vitro Cellular and Developmental Biology-Plant* 42, 89-99

61. Murphy DJ (2007a) Future prospects for oil palm in the 21st century: Biological and related challenges, *European Journal of Lipid Science and Technology* 109, 296-306

62. Murphy DJ (2007b) *Plant Breeding and Biotechnology: Societal Context and the Future of Agriculture*, Cambridge University Press, Cambridge

63. Murphy DJ (2008) Future prospects for Biofuels, *Chemistry Today* 26, 44-48

64. Murphy DJ (2009a) Global oil yields: have we got it serious wrong? *Inform* 20, 499-500

65. Murphy DJ (2009b) Industrial oil crops, when will they finally deliver on their promise? *Inform* 20, 749-751

66. Murphy DJ (2009c) Oil palm: future prospects for yield and quality improvements, *Lipid Technology* 21, 257-260

67. Murphy DJ (2010) Manipulation of oil crops for industrial applications, In: Singh BP, ed, *Industrial Crops and Uses*, CABI Press, UK, pp 183-206, http://bookshop.cabi.org/default.aspx?site=191&page=2633&pid=2210

68. Murphy DJ (2011a) Oil crops: a potential source of biofuel, in: *Technological Innovations in Major World Oil Crops, Volume 2: Perspectives*, Springer, Berlin, *in press*

69. Murphy DJ (2011b) *Plants, Biotechnology and Agriculture*, CABI Press, Wallingford *http://bookshop.cabi.org/?page=2633&pid=2263&site=191*

70. Napier JA (2007) The production of unusual fatty acids in transgenic plants. *Annual Review of Plant Biology* 58, 295-319

71. National Biofuels Action Plan (2007) US Department of Energy, http://www.biofuelspostureplan.govtools.us/documents/NationalBiofuelsActionPlanWorkshopSummaryReportFinal-5-30-07.pdf

72. Nguyen T, Shanklin J (2009) Altering Arabidopsis Oilseed Composition by a Combined Antisense-Hairpin RNAi Gene Suppression Approach, *Journal of the American Oil Chemists' Society* 86, 41-49

73. Nuffield Council on Bioethics (2011) *Biofuels: ethical issues*, Nuffield Press, Abingdon

74. OECD/FAO (2011) *Oilseeds and oilseed products*, In: OECD-FAO Agricultural Outlook 2011-2020, pp 107- 118 FAO, Rome

75. Partridge M, Murphy DJ (2004) Detection of genetically modified soya in a range of organic and health food products. Implications for the accurate labelling of foodstuffs derived from potential GM crops. *British Food Journal* 106, 166-180

76. Perlack RD, Wright LL, Turhollow AF, Graham RL, Stokes BJ, Erbach DC (2005) *Biomass as Feedstock for a Bioenergy and Bioproducts Industry: The Technical Feasibility of a Billion-Ton Annual Supply*. Department of Energy/GO-102005-2135, ORNL, 2005, http://www1.eere.energy.gov/biomass/pdfs/final_billionton_vision_report2.pdf

77. Philip S, Keshavarz T, Roy I (2007) Polyhydroxyalkanoates: biodegradable polymers with a range of applications. *Journal of Chemical Technology and Biotechnology* 82, 233–247

78. Piesse J, Thirtle C (2010) Agricultural R&D, technology and productivity, *Philosophical Transactions of the Royal Society B* 365, 3035-3047

79. Rabobank (2007) *World map grains & oilseeds*, Utrecht

80. Ragauskas AJ, Williams CK, Davison BH, Britovsek G, Cairney J, Eckert CA et al (2006) The path

forward for biofuels and biomaterials. *Science* 311, 484

81. Ramessar K, Capell T, Twyman RM, Christou P (2010) Going to ridiculous lengths—European coexistence regulations for GM crops, *Nature Biotechnology* 28, 133-136

82. Ramessar K, Capell T, Twyman RM, Quemada H, Christou P (2008) Trace and traceability—a call for regulatory harmony, *Nature Biotechnology* 26, 975–978

83. Razon LF (2009) Alternative crops for biodiesel feedstock. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources* 4, No. 056, 1-15

84. Regalbuto JR (2009) Cellulosic Biofuels—Got Gasoline? *Science* 325, 822-824

85. Rosillo-Calle F, Pelkmans L, Walter A (2009) A global overview of vegetable oils with reference to biodiesel, A Report for the IEA Bioenergy, Task 40, Imperial College, London

86. Ruane J, Sonnino A, Agostini (2010) Bioenergy and the potential contribution of agricultural biotechnologies in developing countries, *Biomass and Bioenergy* 34, 1427-1439

87. Rupilius W, Ahmad S (2008) The Changing World of Oleochemicals. *Palm Oil Devel* 44, 15-28

88. Sambanthamurthi R, Siti Nor Akmar A, Ahmad Parveez GK (2002) Genetic manipulation of the oil palm – challenges and prospects. *The Planter* 78, 547-562

89. Sappington TW, Ostlie KR, DiFonzo C, Hibbard BE, Krupke CH, Porter P (2010) Conducting public-sector research on commercialized transgenic seed. In search of a paradigm that works. *GM Crops* 1, 1-4

90. Schenk MP, Thomas-Hall SR, Stephens E, Marx UE, Mussgnug JH, Posten C, et al. (2008) Second generation biofuels: high-efficiency microalgae for biodiesel production. *BioEnergy Research* 1, 20-43

91. Sexton S, Zilberman D, Rajagopal D, Hochman G (2009) The Role of Biotechnology in a Sustainable Biofuel Future, *AgBioForum* 12, 130-140

92. Shockey JM, Gidda SK, Chapital DC, Kuan JC, Dhanoa PK et al (2006) Tung tree DGAT1 and DGAT2 have nonredundant functions in triacylglycerol biosynthesis and are localized to different subdomains of the endoplasmic reticulum. *Plant Cell* 18, 2294–2313

93. Siti Nor Akmar A, Sambanthamurthi R, Ahmad Parveez GK (2001) Genetic modification of oil palm for producing novel oils. Proceedings of the PIPOC 2001 International Palm Oil Congress, pp 18-30, MPOB, Kuala Lumpur

94. Snell KD, Peoples OP (2002) Polyhydroxyalkanoate polymers and their production in transgenic plants, *Metabolic Engineering* 4, 29-40

95. Somleva MN, Snell KD, Beaulieu JJ, Peoples OP, Garrison BR, Patterson NA (2008) Production of polyhydroxybutyrate in switchgrass, a value-added co-product in an important lignocellulosic biomass crop. *Plant Biotechnology Journal* 6, 663–678

96. Steinbüchel A, Füchtenbusch B (1998) Bacterial and other biological systems for polyester production. *Trends in Biotechnology* 16, 419–427

97. Stephens E, Ross IL, Mussgnug JH, Wagner LD, Borowitzka MA, Posten C et al (2010) Future prospects of microalgal biofuel production systems, *Trends in Plant Science* 15, 554-564

98. Sticklen MB (2006) Plant genetic engineering to improve biomass characteristics for biofuels, *Curr Opinion Biotechnol* 17, 315-319

98a. SVO (2011) Straight vegetable oil, the carbon neutral fuel, www.vegoilmotoring.com

99. Thelen JJ, Ohlrogge JB (2002) Metabolic Engineering of Fatty Acid Biosynthesis in Plants, *Metabolic Engineering* 4, 12-21

100. Tilman D Socolow R, Foley JA, Hill J, Larson E, Lynd L et al (2009) Beneficial biofuels – the food, energy, and environment trilemma, *Science* 325, 270-271

101. Tyner WE (2010) Comparison of the US and EU approaches to stimulating biofuels, *Biofuels* 1, 19-21

102. UK Foresight (2011) The Future of Food and Farming, Final Project Report, Government Office for Science, London, www.bis.gov.uk/foresight

103. Urbanchuk JM, Kowalski DJ, Dale B Kim S (2009) Corn Amylase: Improving the Efficiency and Environmental Footprint of Corn to Ethanol through Plant Biotechnology. *AgBioForum* 12, 149-154

104. USDA (2010) Oilseeds: World markets and trade, http://www.fas.usda.gov/psdonline/circulars/oilseeds.pdf

105. USDA (2011) USDA Issues Oil Crops Outlook, *Oil Mill Gazetteer* 116, 2-15, http://usda.mannlib.cornell.edu/usda/current/OCS/OCS-05-12-2011.pdf

106. de Vries SC, van der Ven GWJ, Ittersum MK, Giller KE (2010) Resource use efficiency and environmental performance of nine major biofuel crops, processed by first-generation conversion techniques, *Biomass and Bioenergy* 34, 588-601

107. Voelker TA, Hayes TR, Cranmer AM, Turner JC, Davies HM (1996) Genetic engineering of a quantitative trait: metabolic and genetic parameters influencing the accumulation of laurate in rapeseed. *Plant Journal* 9, 229–241

108. Waltz E (2009) Biotech's green gold? *Nature Biotechnology* 27, 15-18

109. Weckwerth W (2011) Green systems biology—From single genomes, proteomes and metabolomes to ecosystems research and biotechnology, *Journal of Proteomics* Epub ahead of print

110. Wolt JD, Keese P, Raybould A, Fitzpatrick JW, Burachik M, Gray A, Olin SS, Schiemann J, Sears M, Wu F (2009) Problem formulation in the environmental risk assessment for genetically modified plants. *Transgenic Research* 19:425-436

111. Woods J (2007) Sustainable Biofuel Systems: opportunities and threats, *International Biofuels Opportunities*, Royal Society, London, http://royalsoc.ac.uk/downloaddoc.asp?id=4170

Figure citations

Front cover: Oilseed rape field, England; DNA molecule, courtesy of National Institute of General Medical Sciences, USA.
Fig 1: Lipid structures, A. courtesy of JS Carter, University of Cincinnati; B. courtesy of M Blaber, Florida State University.
Fig 2: Fatty acid pathway, Murphy laboratory.
Fig 3: Seed structures, A. courtesy of MJ Farabee, Maricopa College, Phoenix; B. oil palm, courtesy of Jitu Saririch, Malaysia.
Fig 4: Oil bodies, Murphy laboratory.
Fig 5: Oleochemical flowchart, courtesy of Bernardini Srl, Italy; mechanical extraction, courtesy of Fediol, Brazil.