Health Benefits of Organic Food:

Effects of the Environment

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Edited by

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Preface

The last two decades have seen a marked increase in demand for organic produce and a substantial growth of this niche market. This is attributable to public concern regarding the impact of chemical herbicides, pesticides, fertilizers, growth-promoting agents and feed additives in plant and animal production on the integrity and safety of the products. In addition, public concern about the environment is likely to have played a part. Organic food is usually promoted as containing fewer contaminants, more nutrients and as being less likely to cause food poisoning as well as having a positive effect on the environment. Some of these attributes are difficult to quantify and there are also suggestions of potentially harmful consequences of organic production such as increased mycotoxin contamination in products from plants not treated with fungicides. These positions to some extent reflect the views of specific interest groups and need to be presented as part of a wider analysis. Moreover, although the ways in which organic food is produced is largely prescribed by the regulatory authorities, the impact that a wide range of environmental factors might have on any differences between organic and conventional food has not been examined to any extent.

This book is the result of a Workshop. The Workshop brought together a multidisciplinary group of experts from fields including nutrition, animal science, soil science, environmental microbiology, toxicology, consumer science and medicine. The objective of this Workshop was to address three key questions:

1. Are there quantifiable effects of organic rather than conventionally produced food on human health?
2. How might the environment impact on these possible health benefits?
3. How do the public perceive these benefits?

To address these questions, the Workshop examined such factors as the role of certain nutrients (e.g. nitrate and long-chain *n*-3 polyunsaturated fatty

acids) in the prevention and promotion of chronic disease, the potential health benefits of bioactive compounds in plants (e.g. flavonoids), the prevalence of food-borne pesticides and pathogens and how both local and global environmental factors may affect any differences between organic and conventionally produced foods.

Clearly there is much more to learn but we think that this book sheds new and often very revealing light on this complex and sometimes contentious subject.

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1 Organic Farming and Food Systems: Definitions and Key Characteristics

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Introduction

At the point of sale, apart from its labelling, organic food is largely indistinguishable from other foods in many respects. Certification is therefore not of the product, but of the whole production system from soil, via plant and animal, to the consumer. The existence of production and processing standards, certification procedures and, in many countries, a legislative basis gives a clear dividing line between organic and other farming systems, primarily to provide market integrity for the products. The organic farming movement has developed principles and recommendations for farm management from an underpinning recognition of the biological, ecological conception of nature and the importance of the relationships and interactions between organisms – plant and animals, both above ground and within the soil. This comprehensive, integrated view of nature and ethics within organic agriculture is evident in one of the original precepts of organic farming (here in the words of Lady Eve Balfour, 1943): '[T]he health of soil, plant, animal and man is one and indivisible.' In contrast, intensification of conventional agricultural production since the 1940s has been marked by increased use of mechanical and manufactured inputs and increased specialization of production; these changes mean that regulation of the agroecosystem through biological processes is consequently displaced in conventional farming systems (Giller *et al*., 1997) and conventional farmers have become dissociated from food production and sales. In many cases, food is now an industrial product, e.g. dependent upon industrial inputs. None the less 'it is also a socio-cultural symbol and a link between the human being and Nature' (Tozlani, 1998).

In its most developed form, organic farming is both a philosophy and a system of food production. Efforts to ensure short-term viability are tested against long-term environmental sustainability, and attention to the uniqueness

of every operation is considered in relation to ecological, economic and ethical imperatives, with an awareness of local and global implications. Four strong basic unifying principles defined by the International Federation of Organic Agriculture Movements (IFOAM) link the wide range of farming systems and management practices within the organic food system:

- Principle of health should sustain and enhance the health of soil, plant, animal, human and planet as one and indivisible.
- Principle of ecology should be based upon living ecological systems and cycles work with them, emulate them and help sustain them.
- Principle of fairness should build upon relationships that ensure fairness with regard to the common environment and life opportunities.
- Principle of care should be based on a precautionary and responsible manner to protect the health and well-being of the current and future generations and the environment (IFOAM, 2005).

None the less the implementation of these principles across the world in diverse climates leads to a great variety in the types of farming systems which produce organic food products of all types imaginable from vegetables, meat, bread and milk to organic cola and ready meals.

The objectives of environmental, social and economic sustainability lie at the heart of the organic food system and are among the major factors determining the acceptability or otherwise of specific production practices. The term 'organic' is not directly related to the type of inputs used, but refers to the concept of the farm as an organism, first proposed by Steiner (1924), in which all the component parts – soil minerals, organic matter, microorganisms, insects, plants, animals and humans – interact to create a coherent whole. In other European languages terms such as 'biological' and 'ecological' are used for the farming systems described in English as 'organic', reflecting the reliance on ecosystem management rather than external inputs: chemical, organic, biological or otherwise. Detailed descriptions of the principles and practices of organic farming are available (e.g. Lampkin, 1990; Siebeneicher, 1993) and we will not repeat those here. In this chapter, we attempt to draw together information on the key distinguishing features of organic food systems and the extent of organic farming in a European context. We aim to provide a framework for the consideration of how a range of environmental variables affect the composition of organic foods and their differences from foods produced in other systems.

Development of Organic Farming and Food Systems

The roots of organic farming can be traced in the European literature back to the late 19th/early 20th century. The early organic movement focused strongly on issues of human diet, nutrition and health, as well as the promotion of soil fertility through the use of composts and other organic fertilizers. Pesticides did not become a major issue in organic agriculture until the publication of *Silent Spring* generated widespread public concern (Carson, 1963).

Modern organic farming represents a merging of a number of different streams of thought and the history is told differently in different places (Boeringa, 1980; Merrill, 1983; Conford, 1988; Harwood, 1990; Tate, 1994; Heckman, 2006). Of particular interest is the fact that those engaged in developing organic agriculture provided great breadth of vision from a wide variety of backgrounds including philosophers, ecologists and consumers groups, alongside agriculturalists (Michelsen, 2001).

In the English-speaking world, Sir Robert McCarrison's work linking human health and nutrition (McCarrison, 1926), Sir George Stapledon's work with alternate husbandry systems (Stapledon, 1939) and Sir Albert Howard's work on the role of organic matter in soils and composting (Howard, 1943) provided a powerful stimulus to Lady Eve Balfour (Balfour, 1943). In 1939, she began the Haughley Experiment to investigate the links between the way food is produced, food quality and human health; this experiment continued until 1969 (Balfour, 1975). Howard and Balfour's ideas emphasizing the role of a healthy, fertile soil in the production of healthy crops and livestock and the link with human health and nutrition were also pursued in the USA by the Rodale family, who founded the Soil and Health Foundation in 1947 (Merrill, 1983; Harwood, 1990). The Limits to Growth report of the Club of Rome and the energy crisis of 1973 drew attention to the sustainability of resource use (Lockeretz, 1990). During the 1980s and 1990s, other issues increased in importance, in particular nature and biodiversity conservation, animal welfare, social justice issues relating to fair trade with developing countries, and most recently the potential of organic agriculture to contribute to rural development (Anon, 1999). It is inevitable given the historical development of organic farming that it has taken some time for the ideas to fuse into a coherent concept, which is now practised worldwide. The formation of the IFOAM in 1972 gave an international framework for the discussion and codification of internationally recognized principles for organic farming. However, this framework is not fixed; the development of organic agriculture is not complete. The formulation of the principles has changed and will continue to evolve as understanding of the interlocking roles of soil, crops, livestock and natural ecosystems and their link with human health and well-being increases and new technologies, which may be used in agriculture, emerge.

As organic farming has developed, acceptable production practices have been recorded in technical guides and handbooks (Lampkin, 1990; Blake, 1994). Where marketing of produce was locally based and small-scale, production standards might be guaranteed by word of mouth. However, as the market for organic produce expanded, it was necessary to codify commonly accepted production practices. Since the early 1970s, considerable efforts have been put into the development of production standards for organic production in order to create a differentiated market for organic products. Production standards are based on the overarching principles of organic farming but may be expressed in great detail, may be related to local conditions and may indicate recommended, restricted and prohibited practices and inputs (e.g. Soil Association, 2007). Production standards therefore represent a blend of ethics, tradition, experience, scientific knowledge and

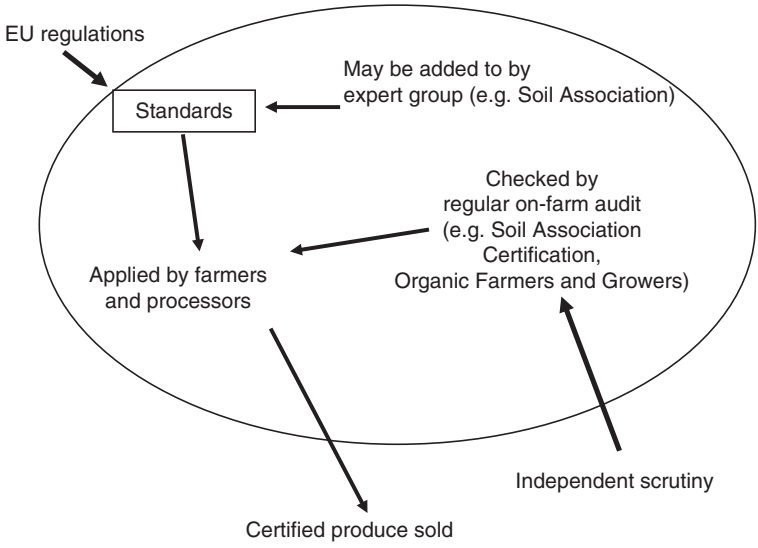

Fig. 1.1. Schematic diagram showing steps in the certification process governing organic food production in the UK.

pragmatism. They are constantly evolving, reflecting the need to respond to the appearance of new technologies (e.g. genetic modification) and new evidence of environmental impact (e.g. the aim to remove Bordeaux mixture as a restricted pesticide for organic farming). Of key importance is that the definition of agriculture is in terms of standards for production and processing, not the certification of the products themselves. It is the way food and fibre is produced, not the end product, which is the focus of organic standards.

The introduction of clear production standards has also required the introduction of processes of farm certification and inspection, to ensure that the standards are adhered to (Fig. 1.1). Increasing consumer demand has often forced the attention of regional and national governments towards organic farming systems, with the enactment of legislation to regulate the use of the word 'organic' in marketing. In some cases, this may have come to the attention of the departments concerned with consumer affairs or trading standards before a department of agriculture (Hill and Macrae, 1992). This has often led to the legislation of production standards often drawing on those previously developed by organic farming organizations themselves.

Legislation of Organic Food Systems

International agreements (e.g. Codex, 1999) have also been agreed to define organic production and ensure a differentiated market for organic products. In the European Union (EU), Regulation 2092/91 was largely established as a labelling regulation, meant to regulate the internal market for organic pro-

ducts (Commission of the European Communities, 2004). It defines in detail the requirements for agricultural products or foodstuffs describing the organic production standards and the inspection and supervision requirements. While the original regulation covered only plant production and processing, in 1999 (Regulation 1804/99), it was extended substantially to cover animal production. The detailed approach in this regulation was needed as a result of the great diversity of animal production systems throughout the EU and the lack of consensus in the existing rules relating to livestock management developed by organic farming organizations in each country. In addition, Regulation 2092/91 provides for an equivalency regime for organic products imported from third countries, which must adequately demonstrate that they are produced in accordance with production standards and are subject to inspection arrangements equivalent to those applied to organic production in the EU. In March 2000, the European Commission introduced a logo bearing the words 'Organic Farming – EC Control System' (Regulation 331/2000). Since that date, this logo can be used on a voluntary basis by producers whose systems and products have been found to satisfy Regulation 2092/91. Genetically modified (GM) organisms and/or any product derived from such organisms must not be used in organic farming (with the exception of veterinary medicinal products). The presence of GM crops in non-GM farming systems cannot be completely excluded during cultivation, harvest, transport, storage and processing. The main sources of GM admixture are seed impurities, cross-pollination, volunteers and harvesting–storage–processing practices.

Denmark was the first country to introduce public economic support for organic agriculture in 1987 as compensation for economic loss during conversion (Michelsen, 2001). The increasing role of policy support during the 1990s has arisen because of a gradual convergence of policy goals with the underlying objectives of organic agriculture, including environmental protection, animal welfare, resource use sustainability, food quality and safety, financial viability and social justice. Organic farming is also perceived to contribute to reducing problems of overproduction and to rural development. Organic farming offers three potential advantages over other, more targeted policy measures: it addresses all (or most) of these goals simultaneously; it utilizes the market mechanism to support these goals; and it is recognized globally. Increasing consumer demand for organic produce has generated interest within governments of meeting demand for organic food self-sufficiently. In the EU, the agri-environmental measures introduced by Council Regulation 2078/92 included approval for aid for farmers who converted to, or continued with, organic farming measures. Financial support during the conversion period to organic agriculture was therefore provided in some states and countries to provide financial compensation to farmers for any losses incurred during conversion (Hill and Macrae, 1992; Padel and Lampkin, 1994). Within the EU, agri-environment schemes have been applied differently at the country level; where more direct support has been given to organic farming systems, the area under certified organic production has expanded more rapidly (Padel *et al*., 1999). By 2001, organic farming support made up around 15% of the EU

expenditure on agri-environment measures (Haring *et al*., 2004). One of the objectives in the 2003 reform of the Common Agricultural Policy was to promote production that supports environment-friendly, quality products; organic farming is considered to be an important device towards the attainment of this objective (Commission of the European Communities, 2004). Consequently, there is now greater scope for Member States to implement measures to provide support for organic farmers. However, there are still large differences between Member States, for example:

- In Germany, the Federal Organic Farming Scheme (Federal Ministry of Consumer Protection Food and Agriculture, 2003) contains a number of measures targeted at eliminating obstacles to growth of supply and demand in the market for organic food including information, training and advisory activities for organic farmers and those planning conversion, providing suggestions and ideas to foster innovation and competition in food processing, providing consumer information and supporting research and development.
- The devolved administrations of the UK all provide support to organic farming but have slightly different rules in their schemes. In England, the organic entry-level scheme is now a distinct tier in the package of environment stewardship schemes available to all farmers (Defra, 2008). This scheme provides support for those in conversion and those continuing to farm organically in recognition of the role of organic management requirements in delivering effective environmental benefits.
- In autumn 2006, the Spanish Ministry of Agriculture launched a Campaign for Organic Farming with the slogan 'Cultura-Lógica, Agricultura Ecológica, es cultura, es de lógica' co-financed by the EU. The campaign aims to stimulate consumption of organic products, to enhance the knowledge of consumers about organic farming and its products, to promote the EU logo for organic products and to provide information about the EU standards and their monitoring.
- Currently in Greece there is no government support for organic farming.

Governments around the world also provide a range of support measures for organic agriculture. In addition, the FAO provides assistance in establishing appropriate legislative frameworks: tapping market opportunities, improving quality and performance of low-input production systems; and improving value chains.

In many aspects the development of the regulation has followed an evolutionary approach, including several transitional rules within the legislation which facilitated a step-by-step development of organic farming as the number of organic farmers increased (Commission of the European Communities, 2004). A major revision process of the EU regulations governing organic food systems began in 2005; the aim was to replace the current rules by simpler, more transparent ones but which are no less stringent in their requirements for farmers, processors and the certification process. Regulation 834/2007 on organic production and labelling of organic products will come into force on 1 January 2009. The new regulation lays down more explicitly the objectives,

principles and production rules for organic farming while providing flexibility to account for local conditions and stages of development, and it will not prohibit stricter private standards such as those of biodynamic farming from coexisting with organic standards. The regulation will have an extended scope and include both aquaculture and viticulture. The EU logo will become compulsory – along with further certification logos, if appropriate, and an indication of the place of production. IFOAM have expressed their approval of the clearer and more appropriate objectives and principles; however,

> much will now depend on the Implementing Rules, in particular to ensure the criteria for evaluating inputs and for allowing flexibility are sufficiently restrictive. It is vital that the Implementing Rules are adequate both to protect the integrity of organic food and farming, and to ensure a vibrant and successful organic sector.
>
> (IFOAM, 2007)

Land area

Recent years have seen very rapid growth in organic farming and food production, particularly in Europe (until 2002, Fig. 1.2) and the USA, but also in many other regions of the world including China, Latin America and Africa. Worldwide, organic agriculture occupies 31 Mha of certified crop and pasture lands and more than 62 Mha of certified wild-harvested areas (FAO, 2007). In the EU, certified and policy-supported organic production accounted for just 100,000 ha on 6300 holdings in 1995, or less than 0.1% of the total utilizable agricultural area (UAA). In 2005, the organic area within the EU (EU-25) was *c*.4% of the UAA, equivalent to 1.6% of the registered agricultural holdings; both are still increasing slightly (Llorens Abando and Rohner-Thielen, 2007). The land area under organic cultivation in the EU increased at a rate of about 21% per year between 1998 and 2002 (Romer-Thielen, 2005). Geographic trends in organic livestock largely reflect the patterns seen in land area (Llorens Abando and Rohner-Thielen, 2007). Nearly all the expansion in the organically registered land area in Western Europe has taken place since the implementation in 1993 of Regulation 2092/91 defining organic crop production, and the widespread application of policies to support conversion to, and the maintenance of, organic farming as part of the EU's agri-environment programme (Lampkin *et al*., 1999). The former has provided a secure basis for the agri-food sector to respond to the rapidly increasing demand for organic food across Europe. The latter has provided the financial basis to overcome perceived and real barriers to conversion. Similar responses to the development of supportive legislation are seen worldwide.

The growth of the organically farmed area in the EU (Fig. 1.2) hides great variability within and between countries. Italy, Germany and Spain have the largest areas of organically certified land in the EU, representing between them 46% of organically certified land (Llorens Abando and Rohner-Thielen, 2007), while Austria has the highest proportion of its agricultural area under organic production (11%). These differences are in part a reflection of the

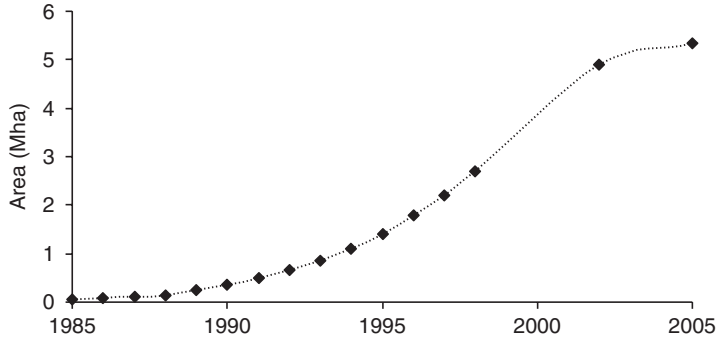

Fig. 1.2. Certified and policy-supported organic and in-conversion land area (Mha) in the European Union (EU-15) 1985–2005. Total UAA in the European Union (EU-15) is approximately 93.3 Mha. (Data taken from Lampkin, 1999; Rohner-Thielen, 2005; Llorens Abando and Rohner-Thielen, 2007.)

intensity of the farming systems previously operating. For example, much of the utilizable agriculture area in Austria is based on upland pastures, which can be relatively easily converted to organic production. The cropping patterns across the EU reflect the dominant locally adapted farming systems so that in 2002 only five Member States (Greece, France, Italy, Cyprus and Portugal) had significant areas under perennial organic crops – mainly fruit trees, olive groves and vineyards (Romer-Thielen, 2005). In general, a larger proportion of mixed farms are managed organically than specialized dairy or arable units. Consequently, long-term pastures and meadows (>5 years duration) used to support livestock are a feature of organic farming systems in all Member States and green forages are also an important component of the area used for annual cropping. The balance between long-term pastures and annual cropping varies, e.g. Italy has 23% of the area of organically certified land under long-term pasture, whereas the UK has more than 70% (Llorens Abando and Rohner-Thielen, 2007). Vegetable production represents only a small proportion of organic cultivation – but the relative share of production does not reflect the proportions of total UAA farmed organically. For example, in 2002, Denmark had the highest share in organic cultivation of fresh vegetables (nearly 14% of the total vegetable cultivation area), closely followed by the UK and Luxembourg; conversely, in Belgium, Greece and Spain, the organic area of fresh vegetables has a share of less than 1% (Romer-Thielen, 2005).

Features of organic food systems in practice

While food production systems involve engagement with both social and ecological systems, only the technical aspects of the differences between organic and conventional food systems are considered briefly below. Organic processing standards prohibit the use of a number of processing aids and additives routinely used in conventional food processing, such as artificial

colours and many preservatives. In several standards, guidelines and publications organic food processing is strongly associated with 'minimal processing' (Beck *et al*., 2006). However, there are frequent discussions regarding the underlying rationales and criteria used to allow some but not other processing methods and additives, especially when new processing technologies or additives have to be assessed for conformity with organic processing standards (Gallmann, 2000). Regulation 2092/91 also requires 'sufficient separation during the harvesting, transportation, processing and packaging of organic food' and traceability to be maintained throughout the processing stages. Hence, organic foods have to be produced on processing lines which are clearly separated in space or time from conventional food processing. There is a lack of detailed consideration of the impacts of organic processing standards on the consequent food processing methods and products, e.g. prohibition of certain flavourings will require redesign of the entire process to enable consumer acceptability to be maintained. The organic food sector is therefore considered to be highly innovative in developing approaches which use natural substances with appropriate technological properties or less critical additives than are used normally, or technologies based on additive-free processes (Beck *et al*., 2006). The focus of this book (and much of the existing literature) is on the differences in organic food arising directly as a result of organic and conventional farming systems rather than food systems taken as a whole; consequently, the distinguishing features of organic food processing will not be discussed further.

Increasing awareness of the environmental impact of conventional farming practice (e.g. Stoate *et al*., 2001) has led to a move towards alternative systems, which explicitly acknowledge the externalities of farming systems (e.g. the impact of pesticides on wildlife or fertilizers on water supplies) and seek to minimize them by changes within the farming system. The organic (biological/ecological) approach is not the only alternative to conventional production systems currently practised or advocated. Lower-input and organic systems are both covered under the headings 'sustainable' and 'alternative' (NRC, 1989; Edwards *et al*., 1990; Francis *et al*., 1990). Conventional agricultural systems often rely on targeted short-term treatments to tackle problems such as application of a soluble fertilizer nutrient or use of herbicides. Reduced use, but not elimination, of these chemical inputs is a key factor distinguishing 'low-input sustainable agriculture' (USA) and 'integrated farming systems' (Europe) from organic farming (e.g. Grubinger, 1992; El Titi, 1995). Organic farming systems use a strategically different approach, which relies on a network of partial longer-term solutions (preventative rather than reactive) operating at the system level (Watson *et al*., 2002). The majority of organic farming in Europe is on mixed farms or livestock units. In these areas crop rotations based on the use of forage legumes offer an important mechanism for nitrogen management in organic farming systems, because they have the potential to support both animal production during the ley phase and a subsequent, exploitative, cropping phase. However, specialized arable, horticultural and livestock organic farming systems also occur. The key characteristics of organic farming include:

- Protecting the long-term fertility of soils by maintaining organic matter levels, fostering soil biological activity and careful mechanical intervention;
- Nitrogen self-sufficiency through the use of legumes and biological nitrogen fixation, as well as effective recycling of organic materials including crop residues and livestock wastes;
- Weed, disease and pest control relying primarily on crop rotations, natural predators, diversity, organic manuring, resistant varieties and limited (preferably minimal) thermal, biological and chemical intervention;
- Supplementing crop nutrients, where necessary, by using nutrient sources which are made available to the plant indirectly by the action of soil microorganisms and chemical reactions in the soil;
- The extensive management of livestock, paying full regard to their evolutionary adaptations, behavioural needs and animal welfare issues with respect to nutrition, housing, health, breeding and rearing;
- Careful attention to the impact of the farming system on the wider environment and the conservation of wildlife and natural habitats (Padel and Lampkin, 1994).

A period of several years, known as the conversion or transition period, is needed to change a farm from conventional to organic management. The length of the conversion period depends on the certification scheme, but under many schemes a period of 2 years in conversion is required before a farm, or part thereof, is certified as organic (Regulation 2092/91). However, there is wide agreement that the development of a fully functioning organic farm takes much longer than 2 years (Voss and Shrader, 1984; Liebhardt *et al*., 1989) and is dependent on the previous management of the farm and the soil and environmental conditions. Martini *et al*. (2004) highlighted the fact that yield changes following conversion to organic farming were as much a result of the farmer's development of skills and understanding as changing processes/systems on the land farmed.

Approaches in cropping

Crop diversification can deliver many agronomic and ecological benefits simultaneously, while maintaining or enhancing the scale and efficiency of production. Such complex systems can have a major influence in limiting diseases, pests and weeds and ensuring a balanced nutrient supply for crops. However, to achieve even some of these benefits requires great attention to management: diversity simply as an end in itself may lead to losses in production and productivity (Altieri, 1999); what is needed is functional diversity. Consequently, in organic farming systems, rotation design is critically important for nutrient cycling and conservation as well as weed, pest and disease control; crop diversity in space and in time is at the heart of a well-designed organic cropping system (Stockdale *et al*., 2001). This is also true for many low-input systems, where legumes are also integrated into rotations to reduce the demand for nitrogen fertilizer (Crews and

Peoples, 2005). Where fixation is the major external source of nitrogen, the balance between nitrogen-fixing and exploitative arable cropping periods is critical in determining not only productivity but also environmental impact. Nitrogen budgets are generally positive for organic systems (Halberg *et al*., 1995), indicating that there is surplus nitrogen, which may be lost by leaching or denitrification, particularly following cultivation and handling of animal manures (Köpke, 1995; Stopes *et al*., 2002). Management of residual nitrogen from legumes, particularly the timing of incorporation and the nitrogen demand of subsequent crops, is critical to minimize crop nitrogen deficiency and nitrate losses (Watson and Philipps, 1997). Organic farming systems rely on the effective recycling of nutrients within the farm system in crop residues and animal manures together with management practices to maintain or improve soil organic matter status; a limited range of external amendments are available to supply nutrients for crops (Watson *et al*., 2002).

Crop health is maintained through complex interactions and feedback among soil, crops, pests and inputs. Crop diversity is also used to maintain crop health, for example, by separating crop hosts so that soil-borne pathogen inoculum is diminished. Crop rotation and soil management have been identified by farmers as a key disease control strategy in organic farming systems (Brenner, 1993). Pest control strategies are largely preventative, rather than reactive. The balance of cropped and uncropped areas, crop species and variety choice and the temporal and spatial pattern of the crop rotation seek to maintain a diverse population of pests and their natural enemies and disrupt the life cycle of pest species. Some external inputs are also permitted to be used as supplementary tools in the control of pests, disease and recalcitrant, particularly perennial, weeds. Weed management strategies involve the whole cropping system with cultural measures providing a form of residual control (Rasmussen and Ascard, 1995; Bond and Grundy, 1998; Bond and Lennartsson, 1999). The aim is to maintain weeds at a manageable level by cultural means to ensure that direct control measures can succeed in preventing crop losses. An integrated approach using a combination of cultural and direct techniques is necessary. Consequently, a higher level of plant diversity is maintained throughout the crops even in monocultures than in their conventional equivalents, where biodiversity is only encouraged at the edges.

Absolute yield levels under organic management are lower than those of conventional systems; most studies have been carried out with temperate crops where arable crops yields around 60–80% of those of conventional systems (Stockdale *et al*., 2001). Yields of forage crops are often similar with no difference in feed quality (Stockdale *et al*., 2001). In developing countries, the UNDP (1992) concluded that organic farming methods seem able to provide similar outputs, with less external resources, supplying a similar income per labour day as high-input conventional approaches. Studies commonly show large increases where local farmers adopt organic farming systems reaching levels similar to those of high-input systems (FAO, 2007). Direct comparisons of yields are difficult because of the differences in

the farming systems adopted under high-input or organic management. Absolute yields are, however, subject to considerable variability due to a number of factors, including variety selection and plant breeding, soil type, climate, rotation design and nutrient management, length of time under organic management, as well as management ability and developments in scientific knowledge and technology. A significant number of critical voices raise concerns that organic agriculture is not capable of meeting the world's growing food needs due to lower productivity per unit area (e.g. Borlaug, 2000; Trewavas, 2002). However, recent work using the International Food Policy Research Institute's International Model for Policy Analysis of Agricultural Commodities and Trade (IMPACT) and extensive farming systems data showed that even at high levels of conversion to organic agriculture (up to 50%) in Europe and North America, there would be relatively little impact on the availability of food, and price changes would be limited. In the case of sub-Saharan Africa, a conversion of up to 50% would likely increase food availability and decrease food import dependency, with negligible changes in prices and no changes in current malnutrition rates (FAO, 2007).

Approaches for livestock management

Regulation 1804/99 effectively ruled out landless livestock production systems emphasizing that 'organic stockfarming is a land-related activity', with livestock as the intermediary between the utilization of home-grown feed and the return of nutrients as manure. This consequently leads to the greatest distinction between livestock conventionally reared very intensively, such as pigs and poultry, and their organic counterparts (e.g. Millet *et al*., 2005). Within organic systems partnerships between poultry producers and arable farmers are increasingly common, solving a waste disposal problem for one and a nutrient deficiency for the other. A comprehensive review of organic dairy production was recently completed by Nicholas *et al*. (2004) and it is clear that uptake of organic farming systems in most European countries has been greater in regions with less intensive grazing livestock systems, such as dairy, beef and sheep production.

The emphasis of organic livestock production is to optimize production systems to avoid animal health problems and to guarantee species-specific animal welfare standards. Organic systems achieve higher animal welfare standards than conventional systems, mainly due to reduced tethering and/or caging of animals and the use of bedding (Hörning, 1998; Sundrum *et al*., 1999). The requirement to retain as closed a herd/flock as possible reduces the risk of buying in disease, and encourages selection on the basis of farm needs as well as the development of greater resistance to the spectrum of disease present. Reviews of animal health and welfare in organic livestock systems have recently been completed (Lund and Algers, 2003; Kijlstra and Eijick, 2006); consequently, these aspects will not be considered further here.

Organic farming aims to apply species-specific husbandry with consideration for the animal's ability to express its natural behaviour, by preventing intentional and unintentional mutilation of animals and by applying species-specific feeding regimes. Sound nutrition, taking account of the physiological adaptations of livestock to different types of feedstuffs, underpins health, vitality and productivity. The basic features of organic livestock feeding have been described by Kamphues (1998), emphasizing the integrated nature of feed production and manure use. Ruminant diets must be forage-based with organically produced feed; zero grazing is not permitted. Due to a prohibition of solvent-extracted feeds, there is greater reliance on home-produced forage legumes, such as peas and beans. For monogastric animals transitional rules still apply, allowing a small proportion of non-organic feeds while the supply chain develops. The use of pure nutrients and feed additives is limited by organic production standards to 'natural' ingredients and levels are determined by an animal's minimum needs rather than maximum production levels (e.g. antibiotic production enhancers are not acceptable). Maximum stocking densities are set both in housing and grazing situations (Regulation 2092/91); these are lower than conventional stocking rates and are now related to the return of nitrogen (<170 kg N/ha) to the land. Organic systems also promote the use of appropriate breeds, suited to the system of production, which ideally are locally adapted. The judicious use of breed is seen as an important strategy to improve disease resistance, reduce metabolic problems and retain genetic diversity. In addition, opportunities may be presented to exploit possibilities to market specialist breeds and meats in order to improve producer returns (Marchini and Santucci, 1998).

With greater emphasis on management approaches, the performance of an organic livestock system will be highly dependent on the level of stockmanship applied. Value judgements may be difficult when dealing with complex biological systems, particularly at the limits of current knowledge. With less emphasis on a prescriptive, blueprint approach, observation of the farming system, and of the behavior and performance of the animals within that system, plays an important role in the development and refinement of an organic livestock system (Boehncke, 1998).

Impacts of organic farming systems

The Commission of the European Communities (2004) explicitly recognizes a dual role for organic food systems within society: first, providing food products in response to the demand of consumers – this role should therefore be constrained by market rules, rewarded by the market and hence be financed by consumers; and second, providing public goods, as a result of farm management practices, primarily environmental, but also rural development benefits and improved animal welfare – this role is external to the operation of markets and should be driven by society.

So far in this chapter we have largely focused on the first of these roles. However, much of the policy support for organic farming is predicated by its

second role, i.e. the delivery of a range of public benefits not rewarded by the market. The recognition that agricultural systems must take a multifunctional role in support of the provision of a broad range of ecosystem goods and services is now well established in Europe. Organic food and farming systems provide a good example of such integration, but by no means the only one. A large number of studies have considered the relative importance and value of the benefits of organic farming systems for biodiversity (e.g. Bengtsson *et al*., 2005; Hole *et al*., 2005), animal welfare (Lund and Algers, 2003) and impacts on air, water and soil quality, especially in relation to mitigation of, and adaptation to, climate change (e.g. Shepherd *et al*., 2003). The Commission of the European Communities (2004) considers that some of these are well established (Box 1.1); however, it does not currently accept that organic farming gives additional benefits with regard to food safety or nutritional quality. A number of reviews of food quality of organic and conventional products have been written (e.g. Woese *et al*., 1997; Brandt and Molgaard, 2001; Bourn and Prescott, 2002). All highlight the technical difficulties associated with such comparisons (different methods of sampling variations in other factors not directly related to the production system) and the lack of robust information currently available to allow the hypotheses that enhance the nutritional value of plant foods to be tested.

Box 1.1. The main benefits of organic farming (Commission of the European Communities 2004, Annex).

- *Pesticides: research indicates that organic farming has, on average, a greater effect on the improvement of the landscape, wildlife conservation and faunal and floral diversity than non-organic farming systems. Restricting the use of pesticides, as is the case in organic farming, also improves water quality and fewer pesticide residues are found in food products*.
- *Plant nutrients: organic farming usually results in lower nitrate-leaching rates than those achieved on average in integrated or non-organic agriculture, as shown by studies on autumn nitrogen residues in the soil of almost all relevant crops*.
- *Soil protection: management practices broadly used by organic farmers, such as growing catch crops to reduce nitrate leaching, wider and more varied crop rotations, and mixed grazing to reduce mono-specific overgrazing, all help to protect the soil. Although the organic matter content of soil is highly site-specific, it is usually higher on organic compared to non-organic farms*.
- *Biodiversity and nature protection: organic farming contributes to the reservation of species and natural habitats by means of its reduced inputs, its high share of grassland within holdings and its greater use of indigenous breeds and plant varieties*.
- *Animal welfare: organic farming may have a positive impact on animal welfare since the standards for organic farming include several requirements in this area that go further than the statutory provisions*.

With regard to *food safety* it is, in general, not possible to claim that all organic food is more or less safe than non-organic food.

Comparison of organic and conventional farming and food systems are fraught with difficulties – there is no clear definition of what conventional is, and even within the legally defined organic systems there is significant variation in practices. Watson *et al*. (2008) provide a useful review of the research approaches applied in the study of organic farming systems; they highlight the need to ground all future comparative work in improved understanding of all farming systems and their environments. In fact, it can be argued that what is needed is not a narrow comparison of organic and conventional systems with regard to one or other aspect or outcome. Instead there is a need for a holistic view of food systems beyond productivity to include the integrated evaluation of environmental, social and health impacts; only when food systems are viewed in this way might we be able to move forward towards meeting the food-related Millennium Goals (FAO, 2007). More remains to be determined about the links between health and nutrition for both humans and animals. There is currently a lack of methodologies available which work across the whole food system and link production systems to product quality and onwards into livestock and human health and well-being (Watson *et al*., 2008). Science continues to advance and it is important that we continue to evaluate the importance of environmental and management factors in controlling the composition of our diet.

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2 The Health Benefits of *n*-3 Fatty Acids and Their Concentrations in Organic and Conventional Animal-derived Foods

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Introduction

The amount and composition of dietary fat is recognized to be an important determinant of the risk of many common chronic diseases such as cardiovascular disease (CVD), the metabolic syndrome and diabetes, and certain cancers. A significant body of consistent evidence indicates that a decrease in the ratio of dietary saturated fat to unsaturated (polyunsaturated + monounsaturated) fat and an increase in the intake of long-chain (carbon chain ≥ 20) *n*-3 polyunsaturated fatty acids (LC *n*-3 PUFA) found in fish oils have positive health benefits. Earlier research conducted in the 1960s through the 1980s has largely focused on the benefits of LC *n*-3 PUFA on cardiovascular health, but more recent work is focussing on the anti-inflammatory properties of these fatty acids and their role in cognitive development and in reducing the incidence of age-related cognitive decline. Furthermore, there is much current interest in the 'health attributes' of the shorter-chain *n*-3 PUFA, alpha-linolenic acid (αLNA), and its ability to act as a precursor for LC *n*-3 PUFA synthesis in humans.

Current dietary recommendations suggest a minimum intake of LC *n*-3 PUFA of 450 mg/day in the UK (SACN/COT, 2004) with recent estimates suggesting that, for up to 75% of the population, intakes are likely to be <100 mg/day. In the UK, meats (in particular poultry) and eggs contribute approximately 20% of total LC *n*-3 PUFA intakes in the general population and are the almost exclusive source in non-fish consumers. As a result, there is a widespread interest in factors determining the *n*-3 PUFA content

of commonly consumed foods including milk, meat and other animal products. In this chapter the main factors determining the *n*-3 PUFA content of animal-derived products will be considered, with a particular focus on organic versus conventional farming practices.

Dietary Fat: The Basics

Fat is an essential nutrient, which contributes approximately 30–45% of food energy in Western diets. In the UK, fat provides on average 35.5% of total energy intake, with mean intakes of 61 and 87 g/day in adult females and males, respectively (Henderson *et al*., 2003). Over 95% of dietary fat is in the form of triglycerides (TG; glycerol + three fatty acids), with cholesterol and phospholipids being the other main components. There are approximately 21 different fatty acids found in appreciable amounts in foods, a selection of which is shown in Table 2.1.

The nature of the fatty acid mix in a particular oil or solid fat source determines both its physical properties and its impact on health. Fatty acids may be broadly categorized as saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), containing zero, one, and two or more double bonds, respectively. A PUFA can be further characterized as either an *n*-3 PUFA or *n*-6 PUFA, with

Table 2.1. An example of fatty acids commonly found in the diet.

Fatty acid	Structural title
Saturated	
Lauric	C12:0
Myristic	C14:0
Palmitic	C16:0
Stearic	C18:0
Monounsaturated	
Oleic	C18:1–*cis* (*n*-9)
Elaidic	C18:1–*trans* (*n*-9)
Polyunsaturated	
Linoleic	C18:2–*cis* (*n*-6)
Alpha-linolenic	C18:3–*cis* (*n*-3)
Arachidonic	C20:4–*cis* (*n*-6)
Eicosapentaenoic	C20:5–*cis* (*n*-3)
Docosapentaenoic	C22:5–*cis* (*n*-3)
Docosahexaenoic	C22:6–*cis* (*n*-3)

The structural title provides information regarding the number of carbon atoms and double bonds in the fatty acids, with, for example, C12:0 containing 12 carbons and no double bond, and C22:6 (*n*-3) containing 22 carbons, 6 double bonds, with the first double bond at carbon 3 from the methyl end. The *cis* and *trans* refer to the configuration of the double bond.

3 and 6 referring to the position of the first double bond relative to the methyl end of the molecule. The fatty acid composition of commonly consumed oils is shown in Table 2.2. The three main *n*-3 PUFA present in the diet are αLNA, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA; see Fig. 2.1).

Table 2.2. The fatty acid profile of selected fat sources.[a]

Food	SFA	C12:0–C16:0	MUFA	PUFA	TFA
Coconut oil	86.5	70.4	6.0	1.5	Trace
Olive oil	14.3	10.1	73.0	8.2	<1.0
Palm oil	47.8	42.8	37.1	10.4	Tr
Rapeseed oil	6.6	4.2	59.2	29.3	<1.0
Soybean oil	15.6	10.8	21.2	58.8	<1.0
Sunflower oil	12.0	6.3	20.5	63.3	<1.0
Lard	40.6	25.8	43.0	9.8	Tr
Palm olein oil	45.1	41.4	42.3	9.6	<1.0
Partially hydrogenated rapeseed oil	11.5	5.0	79.0	8.3	19
High oleic sunflower[b]	10.0	3.4	81.0	9.0	<1.0

Values are given as a percentage of total fatty acids present.
SFA, saturated fatty acids, C12:0–C16:0 are the saturated fatty acids of chain length 12 carbons through to 16 carbons; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids, TFA, *trans* fatty acids.
[a]The values given in the table are average values, with the fatty acid profile of the native oils varying depending on a number of factors, including climate, geography, state of ripeness, etc. For the hydrogenated oils, the fatty acid composition of the end product can vary greatly depending on the hydrogenation conditions.
[b]Trisun.

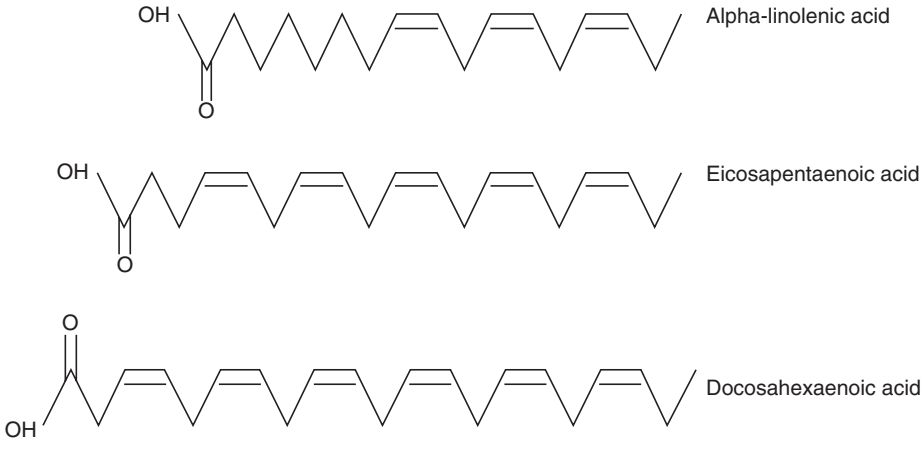

Fig. 2.1. Structure of *n*-3 polyunsaturated fatty acids (PUFA).

The Structure and Dietary Sources of *n*-3 Polyunsaturated Fatty Acids

Eicosapentaenoic acid and docosahexaenoic acid

EPA and DHA represent the main LC *n*-3 PUFA in the diet, with these fatty acids containing 20 and 22 carbon atoms and 5 and 6 double bonds, respectively (Fig. 2.1). Oily fish such as herring, mackerel, sardines, salmon and trout are the richest dietary sources of these LC *n*-3 PUFA, providing 1–3 g/100 g fish (Calder, 2004). As is the case with humans, fish do not have the enzymatic 'machinery' required to synthesize these fatty acids to any extent. EPA and DHA are originally synthesized by marine microalgae, which ultimately are the source of these fatty acids in the larger fish species. In farmed fish, such as farmed salmon or trout, the EPA + DHA composition of the fish is dependent on the provision of dietary fish oils. In the current era of concern regarding the sustainability of world fish oil stocks, there is much interest in the use, and timing of use (during the growth cycle of the fish), of alternative oil sources in fish farming, while still providing a product with 'adequate' EPA + DHA content.

In white fish such as cod, haddock and plaice considerably lower levels of EPA + DHA are present, with these species providing approximately 100–300 mg EPA + DHA per 100 g of consumed product (Calder, 2004). In these white fish, a considerable proportion of the oil is stored in the liver. The liver of white fish species, in particular cod and halibut, is used to provide much of the oil for human consumption. On average, commercially available fish oil capsules provide between 200 and 500 mg EPA + DHA per capsule. Meat and eggs contain typically up to 50 mg EPA + DHA per 100 g product, and, as will be described below and discussed in Chapter 3 (this volume), these levels are highly variable and dependent on the composition of the diet of the animal.

Alpha-linolenic acid

The shorter-chain *n*-3 PUFA, αLNA, is the most abundant *n*-3 PUFA in Western diet. αLNA is an 18-carbon fatty acid containing three double bonds (Fig. 2.1). The richest dietary sources of αLNA include vegetable oils such as flaxseed (linseed) oil, rapeseed (canola) oil and soybean oil; certain nuts such as walnuts; and green leafy vegetables. In forages for ruminant animals, grass and other plant species such as clovers provide a rich dietary source, which ultimately significantly increases both αLNA and EPA + DHA content of animal products, as discussed below and in Chapter 3 (this volume).

αLNA is classified as an essential fatty acid, with current UK dietary recommendations suggesting a minimum intake of 0.2% of dietary energy. Data from the most recent UK adult National Diet and Nutrition Survey (NDNS; Henderson *et al*., 2003) indicate population intakes well in excess

of this, with average intakes of 1% of dietary energy in both males and females. The other essential fatty acid in the diet is the *n*-6 PUFA, linoleic acid; and the clinical manifestations of combined essential fatty acid deficiency, which include dermatitis, poor wound healing and failure to grow in infants and children, are thought to be attributable to inadequate *n*-6 PUFA rather than *n*-3 PUFA status as tissues are depleted of these fatty acids more rapidly.

n-3 PUFA deficiency in humans is specifically associated with visual acuity and peripheral neuropathy, which is consistent with the defects in visual and central nervous function evident in *n*-3 PUFA-deficient animals (Salem *et al*., 2001). Although αLNA is classified as essential, no unique function has been identified for this fatty acid except to serve as a substrate for LC *n*-3 PUFA synthesis. The fact that DHA is the predominant fatty acid present in the eye and brain tissues (making up 20–40% of total fatty acids), the primary tissues affected by αLNA deficiency, with negligible concentrations of αLNA present, is consistent with this theory.

Bioconversion of Alpha-Linolenic Acid to Eicosapentaenoic Acid and Docosahexaenoic Acid

Through a process of elongation and desaturation, mammals possess a limited and variable capacity to synthesize EPA and DHA from αLNA (Fig. 2.2). This metabolic process occurs predominantly in the liver, but also takes

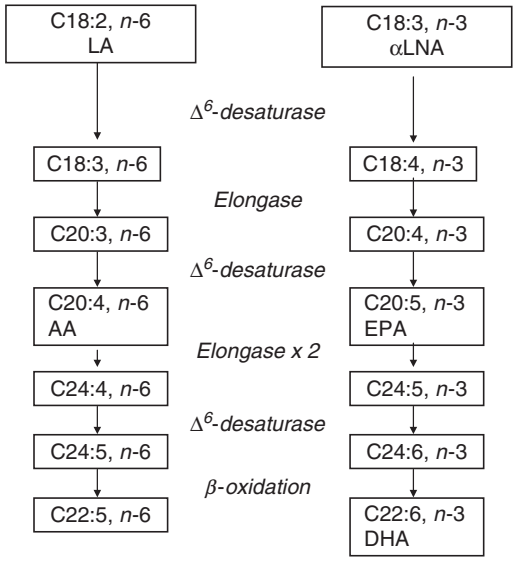

Fig. 2.2. Biosynthesis of long-chain polyunsaturated fatty acids (PUFA) from linoleic acid and alpha-linolenic acid. AA, Arachidonic acid; LA, linoleic acid; αLNA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

place in other tissues, in particular the brain, retina and the heart. In general, the biosynthesis of EPA + DHA from αLNA is considered a relatively inefficient process. Although estimates vary greatly, it has been suggested that in healthy individuals ~5–10% of αLNA is converted to EPA and ~2–5% to DHA (Davis and Kris-Etherton, 2003; Burdge and Calder, 2005; Burdge, 2006).

As depicted in Fig. 2.2, the bioconversion of both LA and αLNA to the longer-chain metabolites uses a common series of desaturation and elongation enzymes and therefore competition for synthesis of the longer-chain metabolites occurs. Although there is some controversy over the degree of influence of dietary *n*-6:*n*-3 PUFA ratio on the synthesis of EPA + DHA from αLNA, it is likely that an excessive *n*-6 PUFA intake inhibits the αLNA to LC *n*-3 PUFA biosynthetic process (Minihane *et al*., 2005) and that the dramatic increase in use of *n*-6 PUFA-enriched vegetable oils over the last 50 years (Ailhaud *et al*., 2006) may at least in part contribute to a low *n*-3 PUFA status. However, a greater influence is undoubtedly exerted by the absolute amounts of dietary *n*-3 PUFA, with increased αLNA and EPA + DHA, increasing and decreasing the biosynthetic processes, respectively (Burdge *et al*., 2003; Finnegan *et al*., 2003).

In addition to dietary fat composition, a number of studies with both rodents and humans conducted by Burdge and co-workers over the last decade have highlighted the significant impact of gender and menopausal status on the biosynthetic capacity (Burdge, 2006; Childs *et al*., 2008). In a series of tracer studies using isotopically labelled αLNA investigating the partitioning of αLNA between storage, β-oxidation and LC *n*-3 PUFA synthesis, conversion rates of αLNA to EPA of 4% and 21% were evident for young men and women, respectively, with αLNA to DHA conversion rates of <0.02% and 9%. Associated lower β-oxidation of αLNA was evident in the female subgroup (22% versus 33%; Burdge and Wootton, 2002; Burdge *et al*., 2002; Burdge *et al*., 2003). The upregulation of the biosynthetic process in premenopausal women is thought to serve an evolutionary role, ensuring an adequate provision of DHA for the foetus and neonate.

Investigations of the impact of oral contraception and hormone replacement therapy in women, sex hormone treatment in transsexuals and studies in rodents indicate that differences in sex hormone status mediate differences in the rate of conversion of αLNA to LC *n*-3 PUFA which results in the higher plasma and tissue LC *n*-3 PUFA levels evident in women (as reviewed by Childs *et al*., 2008). The presence of EPA and DHA in the tissue of vegetarians and vegans with little or no dietary EPA or DHA is indicative of the ability of αLNA to provide a source of these fatty acids. However, the inefficiency of the synthesis of EPA, and in particular DHA from αLNA, is highlighted in the fact that these groups, in particular vegans, often present with tissue EPA + DHA levels several fold lower than omnivores (Davis and Kris-Etherton, 2003). This finding indicates that increased EPA + DHA intakes should remain the focus of public health strategies to improve population *n*-3 PUFA status.

Eicosapentaenoic Acid and Docosahexaenoic Acid and Chronic Disease: The Evidence

Although some controversy exists, the general consensus is that αLNA has little independent benefits with respect to chronic disease prevention (Burdge and Calder, 2006; Wang *et al*., 2006), with any benefits due to its ability to act as a substrate for EPA and DHA synthesis. Therefore, the associations with chronic disease risk will focus on the evidence regarding the cardioprotective and anti-inflammatory effects of EPA + DHA and their role in the prevention of age-related cognitive decline. Evidence derived from epidemiological studies generally describes the benefits of EPA + DHA intakes in the 0–500 mg/day range, with intervention trials often focusing on the effects of doses >2.5 g EPA + DHA per day, consumed in the form of fish oil supplements, on biomarkers of disease. As a result, there is a distinct lack of evidence regarding the health benefits of intakes of EPA + DHA in the range of 500 mg to 2.5 g/day, intakes which could be achieved through the consumption of two to four portions of oily fish per week. Furthermore, little evidence on the benefits of EPA + DHA derived from non-fish sources (such as meats or fortified foods) is available. Given the low consumption of fish, and in particular oily fish, in the UK, these foods hold enormous potential as a means of increasing current EPA + DHA intakes, as will be discussed.

Eicosapentaenoic acid and docosahexaenoic acid and cardiovascular disease

Epidemiological evidence

Undoubtedly the most widely investigated health effect of EPA + DHA is their beneficial effects on CVD risk and pathology, which was first postulated and investigated in the pioneering experiments conducted by Hugh Sinclair in the 1950s (e.g. Sinclair, 1953). Subsequent observations in Greenland Inuits noted that despite the high fat intake (about 80% total energy intake) the Greenland Inuits had tenfold lower incidence of CVD mortality than their Danish counterparts (Bang *et al*., 1976). This led to the hypothesis that the protective effects were in large part attributable to LC *n*-3 PUFA intakes of 5–15 g/day derived from seal meat and whale blubber. The lower mortality rates in Inuits were largely attributed to the anti-thrombotic action of these fatty acids.

Subsequent studies among other populations which also consume diets rich in LC *n-3* PUFA, such as Alaskans and the Japanese, also present with low incidences of coronary heart disease (CHD; Hamazaki *et al*., 1988; Newman *et al*., 1993). The majority (Kromhout *et al*., 1985; Daviglus *et al*., 1997; Hu *et al*., 2002) but not all (Ascherio *et al*., 1995) studies assessing the impact of EPA and DHA intakes more relevant to Western populations (<1 g EPA + DHA per day) have provided evidence of cardioprotective properties. For example in the Nurses Health Cohort, which involved over 80,000 participants, a relative risk (RR) for CHD of 0.66 was evident in those who consumed fish more than five

times per week compared with women who rarely ate fish (<1 per month; Hu *et al*., 2002). Similarly, in the same study women with a higher intake of *n*-3 PUFA had a lower risk of CHD with a RR of 1.0, 0.93, 0.78, 0.68 and 0.67 across the quintiles of intake. In contrast, in the Health Professionals Study increasing intake from one to two servings to five to six servings per week was not associated with any significant reduction in CHD risk (Ascherio *et al*., 1995). In 2004, the available epidemiological data from 11 eligible studies, involving over 220,000 participants, were summarized in a meta-analysis (He *et al*., 2004). The study found that for every 20 g/day increase in fish consumption, a 7% decrease in CHD mortality risk was observed, and that eating fish once per week significantly reduced CHD mortality rates.

Intervention trials

In addition to epidemiological evidence, a number of intervention studies have examined the impact of increased EPA + DHA intakes on CVD mortality and incidence (Burr *et al*., 1989; GISSI, 1999; Burr *et al*., 2003; Yokoyama *et al*., 2007), the results of which have been summarized in meta-analyses (Bucher *et al*., 2002; Hooper *et al*., 2006). For example in the GISSI-Prevenzione I Study, which is the largest intervention study to test the efficacy of fish oil LC *n*-3 PUFA for secondary prevention of CHD in individuals who have suffered a myocardial infarction (MI), the consumption of 850 mg of EPA + DHA for 3.5 years resulted in a 20%, 30% and 45% reduction in total, cardiovascular and sudden deaths, respectively (GISSI, 1999).

Mechanisms underlying the cardiovascular benefits of EPA and DHA

Evidences from the above-mentioned epidemiological and interventionstudies, which generally assess the cardioprotective effects of doses of EPA + DHA up to 1 g/day, are indicative that at these doses the benefits are largely due to an anti-arrhythmic effect of the fatty acids, reducing the likelihood and extent of post-MI ventricular fibrillation, thereby increasing survival rates following a heart attack. Many studies have not observed any benefits on cardiovascular or MI incidence at these levels of LC *n*-3 PUFA intake. For example, in the GISSI trial, no significant impact of intervention on non-fatal cardiovascular events was observed (GISSI, 1999).

At intakes of EPA + DHA of 2.5–5 g/day, intakes generally achieved through use of fish oil supplements, a range of additional cardioprotective benefits have been observed in human intervention studies. These benefits include modest reductions in blood pressure, anti-inflammatory and anti-thrombotic effects, and positive effects on endothelial function and the plasma lipid profile (Harris, 1997; Minihane *et al*., 2000; Mori *et al*., 2000; Nestel, 2000; Woodman *et al*., 2003; Buckley *et al*., 2004; Calder, 2006; Mori, 2006), benefits which would be predicted to increase MI survival and also reduce the incidence of CHD.

The anti-inflammatory actions of EPA and DHA, in addition to being protective against CVD, have also been shown to be associated with a reduction in the severity of symptoms for a number of autoimmune diseases including psoriasis, asthma and inflammatory bowel diseases, with the most

consistent evidence available for rheumatoid arthritis (Calder, 2006; Goldberg and Katz, 2007). At intakes of 2 g or more per day, positive effects on pain, number of tender joints, duration of morning stiffness and reduced need for non-steroidal anti-inflammatory drugs have been repeatedly reported (Goldberg and Katz, 2007). The anti-inflammatory impact of doses <2 g/day, and the ability of lower doses to maintain an inflammatory 'status quo' once a lowering of the inflammatory state has been achieved using higher doses, is currently uncertain.

The impact of long-chain n-3 PUFA on the plasma lipid profile

Perhaps the most widely investigated cardiovascular biomarker in fish oil intervention trials is the blood lipid profile. Numerous studies indicate an anti-atherogenic effect of EPA + DHA on the plasma lipid profile, including a reduction in fasting and non-fasting TG, a high density lipoprotein (HDL, 'good') cholesterol-raising effect and an increase in low density lipoprotein (LDL) size (Harris, 1997; Minihane *et al*., 2000; Buckley *et al*., 2004; Balk *et al*., 2006). In a study conducted in individuals at risk of diabetes, the consumption of fish oils providing 3 g EPA + DHA per day for 6 weeks resulted in a respective 35%, 23% and 26% reduction in plasma fasting TG, postprandial TG and total LDL in the small dense atherogenic LDL-3 form (Minihane *et al*., 2000). As with inflammation, the impact of lower, more dietary achievable intakes (<2 g EPA + DHA per day) on blood lipids has not been widely investigated. Studies which have used these doses report a lack of a significant effect; this may be, in part, likely attributable to the low numbers of study participants, with questionably insufficient numbers to detect the significance of more modest changes (Valdini *et al*., 1990; Brown and Roberts, 1991). In a recently completed study in our laboratory, significant 8% and 11% reductions in fasting plasma TG levels were evident following the consumption of, respectively, 700 mg and 1.8 g EPA + DHA per day for 8 weeks. There was also a significant effect of gender on response, with evidence of greater TG lowering in males (Caslake *et al*., 2008). The recognition of the TG-lowering capacity of LC *n*-3 PUFA has led to recommendations by expert bodies (e.g. American Heart Association) for the use of EPA and DHA for TG lowering in clinical practice (Lichtenstein *et al*., 2006).

Furthermore, although in its relative infancy, there is also current interest in the relative effect of EPA versus DHA on cardiovascular risk. Evidence to date is suggestive that both fatty acids are associated with cardioprotective benefits, with some differences in biopotencies depending on the cardiovascular biomarkers of interest. For example, the limited evidence available indicates that DHA is marginally more effective at TG lowering than EPA (Woodman *et al*., 2002; Buckley *et al*., 2004). However, further studies are needed with separate feeding of EPA and DHA in order to make firm conclusions.

With the recent interest in the application of plant bioengineering as a potential means of producing plant sources of EPA and DHA, there is a great interest in the outcome of investigations to test which particular fatty acid is more potent and therefore should be the focus for introduction into plant material.

Eicosapentaenoic acid, docosahexaenoic acid and age-related cognitive decline

In addition to cardiovascular health, the role of LC *n*-3 PUFA in cognitive development and decline is being increasingly recognized. DHA and arachidonic acid are essential for the development of the central nervous system in mammals and an adequate supply of these fatty acids is particularly important during the growth spurt of the human brain which occurs during the last trimester of pregnancy and the first post-natal months. In the central nervous system, DHA is a key determinant of neuronal integrity and in particular synaptic fluidity and neurotransmitter action. As a result, specific recommendations have been developed regarding DHA intake during pregnancy and lactation and its content in milk formulas (SACN/COT 2004; Koletzko *et al*., 2007).

In recent years the potential role of EPA + DHA in protecting against age-related cognitive decline, dementia and Alzheimer's disease (AD) has been recognized, with evidence to date largely derived from epidemiological studies (Connor and Connor, 2007). In a case-control study, Tully and co-workers observed significantly lower LC *n*-3 PUFA and cholesterol ester levels in serum of AD patients compared with matched controls (Tully *et al*., 2003). In three recently published large prospective trials, namely the Framingham Heart Study, the Zupthen Elderly Study and the Atherosclerosis Risk in Communities Study, with 5–11 years follow-up, intakes of EPA + DHA in the 200–400 mg/day range were shown to have a beneficial effect of the rate of age-related cognitive decline as assessed by the Mini Mental State Examination (MMSE) and a range of other neuropsychological tests (Schaefer *et al*., 2006; Beydoun *et al*., 2007; van Gelder *et al*., 2007). There is currently a paucity of information from human intervention trials regarding the benefits of LC *n*-3 PUFA with respect to cognitive decline, although a number are currently ongoing. In a study which included 204 AD patients, consumption of 1.7 g DHA + 0.6 g EPA per day was found to have had no impact on cognitive performance in the group as a whole compared with the placebo group (Freund-Levi *et al*., 2006). However, in a subgroup (n = 32) with mild cognitive dysfunction at baseline, a significant reduction in MMSE decline rate was evident following fish oil supplementation.

In summary, the evidence to date is suggestive of a protective effect of modest intakes (200 mg to 1 g/day) of dietary LC *n*-3 PUFA against the development of age-related cognitive decline and a potentially positive effect of EPA + DHA supplementation in preventing, or at least delaying, the progression of mild cognitive impairment to the more advanced dementias such as AD. More evidence is needed from long-term (>1 year) intervention trials in humans in order to draw more definite conclusions.

Summary of impact of *n*-3 PUFA on health

In conclusion, EPA and DHA are the key bioactive *n*-3 PUFA. Increasing intakes of αLNA will undoubtedly improve EPA, and to a much lesser extent,

Table 2.3. Recommended daily intakes of EPA + DHA for adults in various countries.

Country	Recommended intake of EPA + DHA (mg/person/day)	Reference
Various	500[a]	WHO/FAO (2003)
UK	450	SACN/COT (2004)
Various	500	ISSFAL (2004)
USA	270[b]	Institute of Medicine (2005)
Belgium	680[b]	Belgian Health Council (2007)

[a]Estimated from recommendation to eat 1–2 portions of fish/week.
[b]Estimated from original recommendation expressed as a percentage of energy intake assuming an intake of 8.3 MJ/day.

DHA status. However, the efficiency of conversion is poor, particularly in males, and there is little indication that the changes in αLNA status achieved have major health benefits. There is substantial evidence, however, to indicate a beneficial effect of EPA and DHA intakes on cardiovascular and cognitive health, which has led many researchers to suggest that these LC *n*-3 PUFA should be referred to as essential fatty acids (EFA) in contrast to αLNA, which has no recognized independent role in mammalian metabolism.

Recommended daily intakes of EPA and DHA

In the review of dietary factors affecting CHD, COMA (Department of Health, 1994) recommended that in the UK, intake of LC *n*-3 PUFA should be increased to 200 mg/person/day from the estimated then current intake of about 100 mg/person/day. The subsequent review of SACN/COT (2004) concluded that the population recommendation of COMA (Department of Health, 1994) should be increased from 200 to 450 mg/day which is consistent with the consumption of two portions of fish per week, with one of these being oil-rich. No consideration was given to the role of EPA/DHA in arresting cognitive decline due to the paucity of data in this area at that time.

Table 2.3 summarizes the recommended daily intake of EPA + DHA from various sources. Current recommendations range from 270 mg/day in the USA (Institute of Medicine, 2005) to almost 700 mg/day in Belgium (Belgian Health Council, 2007). However, both of these are calculated from the original recommendation which was expressed as a percentage of energy intake assuming an intake of 8.3 MJ/day; this may be an underestimate for the USA at least.

Current Intakes of LC *n*-3 Fatty Acids

For the UK, some estimates of EPA + DHA intake have been made over the last 15 years but have produced variable numbers. An intake of 308 mg/day

may be calculated from the report of Gregory *et al*. (1990) while Sanders and Roshanai (1992) and Sanders and Reddy (1992) reported intakes of 600 and 500 mg/day, respectively. A much lower value of 100 mg/day was assumed by the Department of Health (1994). Some of the variability in estimated mean intake is likely to be due to the use of different dietary assessment methods which provide variable information regarding key food types, in particular fish, as well as variable and often scant information on EPA and DHA concentrations in foods. Based on the recommendation of SACN/COT (2004) that canned tuna should be excluded from the oil-rich fish food category, it is also likely that some of these studies substantially overestimated EPA + DHA intake.

A recent study (Givens and Gibbs, 2006) re-evaluated EPA + DHA intake for UK adults using calculations based on intakes of fish, meat and eggs according to the data of SACN/COT (2004), NDNS (Henderson *et al*., 2002) and BEIS (2005), respectively. This followed the principle adopted by SACN/COT (2004) of recognizing the NDNS data (Henderson *et al*., 2002) as being the most appropriate current estimate of consumption by adults except where there is strong evidence that other or adjusted values should be used. Based on the food intakes, together with reported values for the concentrations of LC *n*-3 fatty acids in these foods (for details see Givens and Gibbs, 2006), Table 2.4 provides mean estimates of daily intake of LC *n*-3 fatty acids in UK

Table 2.4. Estimated mean intakes of EPA and DHA by adults in the UK. (From Givens and Gibbs, 2006.)

Food category	Intake of food category (g/person/week)[a]	Intake of EPA + DHA (mg/person/day)
Fish		
White fish	104	39
Shellfish	27	14
Oil-rich fish	50	131
Other fish	36	14
Total fish	217	199
Meat		
Beef and veal	249	4.1
Sheep meat	51	2.1
Pork	63	1.3
Bacon and ham	105	1.2
Poultry	374	26.7
Sausages	68	0.3
Other products	216	1.3
Total meat	1126	37.0
Eggs	194	8.8
Total intake		244

[a]Intake of fish, meat and eggs based on data of SACN/COT (2004), NDNS (Henderson *et al.* 2002) and BEIS (2005), respectively.

adults. It is of concern that the mean intake is only about 54% of the SACN/COT (2004) target of 450 mg/day. Of the total intake of 244 mg/person/day, approximately 54% is provided by oil-rich fish but, notably, poultry meat provides most (73%) of the contribution made by all meats. It is, however, critical to realize that, as reported by SACN/COT (2004), only about 27% of the adult population consumes any oil-rich fish and thus, for the vast majority of the adult population, the daily intake will be at best a little in excess of 100 mg, with almost half of this provided by non-fish-derived foods. The contribution made by poultry meat may indeed be higher than this if the consumption data for poultry meat reported by AVEC (2005) are a more accurate reflection of reality than those of the NDNS (Henderson *et al*., 2002).

A key assumption in this analysis is, however, that the LC *n*-3 fatty acid content of poultry meat purchased by the public is similar to that observed in research studies. It is likely that much of the LC *n*-3 fatty acids found in poultry meat from birds which did not have fish oil in their diets is due to the diet containing fishmeal which has some residual fish oil. In 2004, some 48,000 t of fishmeal (25% of total use) was used in the UK for poultry diets (Fishmeal Information Network, 2007) although this has probably declined somewhat since. There are now considerable amounts of poultry meat imported into the UK both from other EU Member States and from other parts of the world. Whether this will have similar background concentrations of LC *n*-3 fatty acids is not known, but a study is currently under way in the authors' laboratory to analyse a range of poultry meat products at retail to obtain new data.

A number of studies have taken place recently to estimate EPA + DHA intake in various countries. A summary of these is given in Table 2.5. A large variation in mean intake is apparent between studies and countries but many

Table 2.5. Recent estimated daily intakes of EPA + DHA in various countries.

Country	Details	Intake of EPA + DHA (mg/person/day)	Reference
UK	Adults, 19–64 years, mean	244	Givens and Gibbs (2006)
UK	Females, 19–24 years, mean	109	Gibbs *et al*. (2007)
Belgium	Females, 18–39 years, mean	209	Sioen *et al*. (2006)
Belgium	Females, 18–39 years, median	50	Sioen *et al*. (2006)
Belgium	Children 4–6.5 years, mean	75	Sioen *et al*. (2007)
France	Women 45–63 years	344	Astorg *et al*. (2004)
Australia	Adults	143	Howe *et al*. (2006)
North America	Adults	200	Vermunt and Zock (2007)
Mid-Europe	Adults	250	Vermunt and Zock (2007)
Northern Europe	Adults	590	Vermunt and Zock (2007)
Japan	Adults	950	Vermunt and Zock (2007)

values are considerably below the recommended target intakes. Some of the variation may be due to the different methods to collect food consumption data with some (e.g. Howe *et al*., 2006) being based on 24 h recall and some (e.g. Givens and Gibbs, 2006) on 7-day weighed intakes. Two other points are of note. First, in agreement with the UK study in adults (Givens and Gibbs, 2006), a study with Belgian women (Sioen *et al*., 2006) showed that the majority of the population consumes considerably less than the mean value. The lack of normality in the distribution of EPA + DHA intake across many populations was recently demonstrated by Sioen (2007), and highlights the dangers of interpreting mean values derived from non-normally distributed population data. Second, intakes of EPA + DHA appear to be generally lower in young people and children. Gibbs *et al*. (2007) showed that in the UK at least, there is a trend towards increased consumption of oily fish with increasing age, rendering young people particularly vulnerable to suboptimal intakes of LC *n*-3 PUFA. The data for Belgian children (Sioen *et al*., 2007) support this view. Low intakes of EPA + DHA in the young are due to low or zero consumption of oil-rich fish, a habit which young people may carry forward into middle and later life. In the analysis of Gibbs *et al*. (2007), it is interesting to note that in young women (19–24 years) intake of canned tuna was considerable, possibly in the mistaken belief that this was a good source of fish oils.

In most studies, the primary source of EPA and DHA is fish and seafood and thus variation in intake is a function of variation in consumption of these foods. In the studies of Astorg *et al*. (2004) and Howe *et al*. (2006), meat, poultry and eggs were reported to contribute substantially to intake of docosapentaenoic acid (DPA), another LC *n*-3 fatty acid. Howe *et al*. (2006) indicated that DPA contributed 29% of the total LC *n*-3 fatty acids consumed. These data highlight the need to better understand the physiological effects of dietary DPA relative to EPA and DHA.

From the foregoing it is clear that, for many parts of the population, an increase in intake of EPA and DHA would be beneficial. Clearly an option would be to encourage the consumption of more oily fish. However, given that young people appear to consume only small amounts if any, education in this area needs to start at a very young age and be built into an increased awareness of diet and health in general.

n-3 Fatty Acid Content of Organic and Conventionally Produced Foods

A number of reviews (Woese *et al*., 1997; Worthington, 1998; Williams, 2002; Magkos *et al*., 2003; Williamson, 2007) have tried to compare the nutritional quality of organic versus traditionally produced foods. Williamson (2007), referring to the work of Ellis *et al*. (2006), points out that milk produced organically may have a higher concentration of *n*-3 fatty acids than that produced from conventional systems although the increased *n*-3 was primarily due to the contribution from αLNA. This raises the possibility that animal products produced organically may help to deal with the current dietary *n*-3

fatty acid inadequacy. To our knowledge there has not been any systematic review of the *n*-3 fatty acid concentrations in organic versus conventional foods of animal origin. An aim of this chapter is to remedy this situation.

A review of all available literature was undertaken but since changes in *n*-3 fatty acids may be accompanied by changes in other fatty acids, this work also included a review of all key fatty acids reported. Initially, only studies which described a direct comparison between foods produced under conventional conditions and certified organic conditions were included, but few studies meeting this criterion were available, and, out of these, many reported experimentally set up 'simulated' organic systems. To compensate for the lack of data from direct comparisons, the outcome of studies on retail products and those reporting the composition of organic products alone were also included in the analysis. In the tables summarizing all the data (Tables 2.6–2.9) any significant differences between organic and conventionally produced food within studies as reported by the authors are shown. In addition, where adequate numbers of comparative studies were available, the significance of any difference between the means of fatty acids in organic and conventionally produced food across all studies was estimated using a two-sample t-test.

Fatty acids in organic versus conventional milk and dairy products

A total of eight milk, six cheese, two butter and one cream truly organic versus conventional study comparisons were identified plus two more organic-only milks. The SFA composition of these is summarized in Table 2.6 and unsaturated fatty acids are in Table 2.7. Only one study (Jahreis *et al*., 1996) reported EPA concentrations and none reported DHA. Accordingly no valid comparison could be made between organic and conventional systems for these two important LC *n*-3 fatty acids. More data were available for αLNA and, overall, organic milk had a significantly ($P < 0.01$) higher αLNA content than conventional milk, with cheese also having a tendency in that direction ($P = 0.063$). The data for αLNA in milk and dairy products are summarized in Fig. 2.3. Because of the low efficiency of *in vivo* desaturation and elongation (see above), the increased concentration of αLNA will have only limited impact on EPA and DHA supply in the consumer. In any event, at present milk and dairy products only provide about 2% of αLNA intake by UK adults (Henderson *et al*., 2003), and based on these data, if all milk and dairy products consumed were organically produced, this would only rise to 5%.

It is worth noting that the concentrations of αLNA recorded for organic milk are of identical magnitude to those typically seen in milk produced from cows fed diets rich in fresh grass and legume-based forages. This subject has been reviewed by Dewhurst *et al*. (2003) but essentially, although grasses and legumes contain only low concentrations of lipids, these lipids contain high concentrations of αLNA which, when the plants are consumed in large quantities, can have a substantial effect on the αLNA content of milk fat. The effects of this are much reduced if the fresh forage is conserved by ensiling. It is likely therefore that the effects on αLNA seen in milk from organic systems

Table 2.6. Saturated fatty acid content of conventional and organic milk, dairy products and eggs.

System	Details	Fat content (g/kg)	Fatty acid concentration (g/100 g total fatty acids)											Reference
			C8:0	C10:0	C12:0	C14:0	C15:0	C16:0	C17:0	C18:0	C20:0	C22:0	C24:0	Total SFA	
Milk[a]															
Conventional	Jersey breed	65.0	–	–	–	–	–	–	–	–	–	–	–	69.1	Lund (1991)
Organic		61.1	–	–	–	–	–	–	–	–	–	–	–	70.7	
Conventional	Heavy breed	41.5	–	–	–	–	–	–	–	–	–	–	–	64.8	
Organic		44.4	–	–	–	–	–	–	–	–	–	–	–	65.7	
Conventional	Indoor	45.2	1.59[c]	4.86[b]	5.01[b]	13.6[b]	1.51[b]	33.1[b]	0.77	8.84[d]	0.15[d]	0.07[b,c]	0.04	69.6[b]	Jahreis *et al.* (1996)
Conventional	Pasture	43.3	1.55[c]	4.01[d]	4.55[c]	12.6[c]	1.26[c]	30.2[c]	0.77	11.6[b]	0.19[b]	0.09[b]	0.03	66.8[c]	
Organic		36.4	2.06[b]	4.59[c]	5.10[b]	13.5[b]	1.51[b]	30.5[c]	0.82	9.40[c]	0.18[c]	0.06[c]	0.04	67.7[c]	
Conventional	Small farms	42.8	–	–	–	–	–	–	–	–	–	–	–	–	Toledo *et al.* (2002)
Organic		42.5	–	–	–	–	–	–	–	–	–	–	–	75.4	
Conventional	Large farms	43.2	–	–	–	–	–	–	–	–	–	–	–	–	
Organic		43.7	–	–	–	–	–	–	–	–	–	–	–	70.5	
Conventional		–	1.27	2.68[f]	3.09[e]	10.1[e]	0.99[c]	27.4	0.56[f]	9.58[f]	–	–	–	–	Fievez and Vlaeminck (2006)
Organic		–	1.20	2.52[e]	2.59[f]	9.56[f]	1.07[b]	27.0	0.63[e]	10.7[e]	–	–	–	–	
Conventional		–	–	–	–	–	–	–	–	–	–	–	–	67.25	Ellis *et al.* (2006)
Organic		–	–	–	–	–	–	–	–	–	–	–	–	68.13	
Conventional	Buffalo	79.0	–	–	–	1.61	–	4.78[b]	–	1.74	–	–	–	–	Bergamo *et al.* (2003)
Organic	Milk	80.0	–	–	–	1.55	–	4.55[c]	–	1.67	–	–	–	–	
Cheese															
Organic	Buffalo	253	–	–	–	0.51	–	1.42	–	0.57	–	–	–	–	Bergamo *et al.* (2003)
Conventional	Mozzarella	246	–	–	–	0.47	–	1.40	–	0.56	–	–	–	–	
Eggs															
Conventional		251	–	–	–	–	–	26.1	–	8.9	–	–	–	35.2	Cherian *et al.* (2002)
Organic		246	–	–	–	–	–	25.6	–	9.0	–	–	–	34.6	

[a]Note all milk and milk products derived from cows unless otherwise stated.
[b,c,d]Within studies fatty acids with different superscripts differ significantly ($P < 0.05$).
[e,f,g]Within studies fatty acids with different superscripts differ significantly ($P < 0.01$).

Table 2.7. Unsaturated fatty acid content of conventional and organic milk, dairy products and eggs.

			Fatty acid composition (g/100 g total fatty acids)												
System	Details	Fat content (g/kg)	C16:1	C18:1 *cis*-9	C18:1 *trans*-11	Total MUFA	C18:2 *cis*-9, 12	C18:2 *cis*-9, *trans*-11 (CLA)	C18:3 *n*-3 (αLNA)	C20:5 *n*-3 (EPA)	C22:6 *n*-3 (DHA)	Total *n*-3	Total *n*-6	Total PUFA	Reference
Milk[a]															
Conventional	Jersey breed	65.0	–	–	–	25.4[e]	2.1	–	0.5[c]	–	–	–	–	–	Lund (1991)
Organic		61.1	–	–	–	22.3[f]	1.9	–	1.0[b]	–	–	–	–	–	Lund (1991)
Conventional	Heavy breed	41.5	–	–	–	28.8[e]	2.1	–	0.5[c]	–	–	–	–	–	Lund (1991)
Organic		44.4	–	–	–	26.2[f]	2.0	–	1.0[b]	–	–	–	–	–	Lund (1991)
Conventional	Indoor	45.2	2.31[e]	19.2[f]	1.2[g]	24.6[f]	1.8[e,f]	0.34[f]	0.27[g]	0.03[f]	–	–	–	2.5[f]	Jahreis *et al.*(1996)
Conventional	Pasture	43.3	2.21[e,f]	20.6[e]	2.2[f]	25.7[e]	0.1[e]	0.61[e]	0.43[f]	0.05[e]	–	–	–	2.7[e,g]	Jahreis *et al.*(1996)
Organic		36.4	2.17[f]	18.5[g]	2.7[e]	23.6[g]	0.3[f]	0.80[g]	0.89[e]	0.05[e]	–	–	–	2.9[g]	Jahreis *et al.*(1996)
Conventional	Small farms	42.8	–	–	–	–	–	–	–	–	–	–	–	–	Toledo *et al.*(2002)
Organic		42.5	–	–	–	–	–	–	–	–	–	–	–	–	Toledo *et al.*(2002)
Conventional	Large farms	43.2	–	–	–	–	–	–	–	–	–	–	–	–	Toledo *et al.*(2002)
Organic		43.7	–	–	–	–	–	–	–	–	–	–	–	–	Toledo *et al.*(2002)
Conventional		–	–	21.1	1.44[c]	–	1.34	0.69[c]	0.48[c]	–	–	–	–	–	Fievez and Vlaeminck (2006)
Organic		–	–	20.6	2.05[b]	–	1.46	0.93[b]	0.72[b]	–	–	–	–	–	
Conventional		–	–	–	1.75	27.6[e]	–	0.58	–	–	–	0.66[f]	1.68	3.33[f]	Ellis *et al.* (2006)
Organic		–	–	–	2.06	26.2[f]	–	0.65	–	–	–	1.11[e]	1.68	3.89[e]	
Conventional	Fresh milk	–	–	–	1.55	–	3.02	0.51	0.52	–	–	–	–	–	Bergamo *et al.* (2003)
Organic		–	–	–	2.33	–	1.45	0.63	0.60	–	–	–	–	–	
Conventional	UHT milk	–	–	–	1.58	–	2.89	0.62	0.56	–	–	–	–	–	Bergamo *et al.* (2003)
Organic		–	–	–	1.62	–	2.04	1.12	1.10	–	–	–	–	–	
Conventional	Buffalo milk	79.0	0.26	3.12	0.18	–	0.32	0.07	0.05	–	–	–	–	–	Bergamo *et al.* (2003)
Organic		80.0	0.29	2.87	0.35	–	0.24	0.10	0.06	–	–	–	–	–	
Cheese															
Conventional	Parmagiano	–	–	–	–	1.70	–	2.53	0.61	0.69	–	–	–	–	Bergamo *et al.* (2003)
Organic		–	–	–	–	2.01	–	1.98	0.97	1.16	–	–	–	–	

continued

Table 2.7. Continued.

System	Details	Fat content (g/kg)	Fatty acid composition (g/100 g total fatty acids)												Reference
			C16:1	C18:1 *cis*-9	C18:1 *trans*-11	Total MUFA	C18:2 *cis*-9, 12	C18:2 *cis*-9, *trans*-11 (CLA)	C18:3 *n*-3 (αLNA)	C20:5 *n*-3 (EPA)	C22:6 *n*-3 (DHA)	Total *n*-3	Total *n*-6	Total PUFA	
Conventional	Mozzarella	–	–	–	1.29	–	2.46	0.50	0.55	–	–	–	–	–	Bergamo *et al.* (2003)
Organic		–	–	–	1.75	–	1.72	0.58	0.69	–	–	–	–	–	
Conventional	Ricotta	–	–	–	1.45	–	2.45	0.50	0.45	–	–	–	–	–	Bergamo *et al.* (2003)
Organic		–	–	–	2.39	–	1.85	0.70	0.61	–	–	–	–	–	
Conventional	Crescenza	–	–	–	1.89	–	2.71	0.54	0.47	–	–	–	–	–	Bergamo *et al.* (2003)
Organic		–	–	–	2.34	–	2.07	1.18	0.81	–	–	–	–	–	
Conventional	Fontina	–	–	–	1.39	–	2.96	0.62	0.64	–	–	–	–	–	Bergamo *et al.* (2003)
Organic		–	–	–	2.39	–	1.74	1.03	0.96	–	–	–	–	–	
Conventional	Buffalo	253	0.09	1.10	0.06	–	0.10	0.03	0.01	–	–	–	–	–	Bergamo *et al.* (2003)
Organic	Mozzarella	246	0.09	0.96	0.13	–	0.07	0.04	0.02	–	–	–	–	–	
Butter															
Conventional		–	–	–	1.66	–	2.20	0.57	0.55	–	–	–	–	–	Bergamo *et al.* (2003)
Organic		–	–	–	2.35	–	1.61	0.98	1.05	–	–	–	–	–	
Conventional		–	–	–	–	–	–	1.31[c]	–	–	–	–	–	–	Bisig *et al.* (2007)
Organic		–	–	–	–	–	–	1.48[b]	–	–	–	–	–	–	
Cream															Bisig *et al.* (2007)
Conventional		–	–	–	–	–	–	1.35[c]	–	–	–	–	–	–	
Organic		–	–	–	–	–	–	1.54[b]	–	–	–	–	–	–	
Eggs															
Conventional		251	0.32	4.26	–	4.58	1.62	–	–	–	0.07	0.07	1.82	1.89	Cherian *et al.* (2002)
Organic		246	0.38	4.35	–	4.73	1.52	–	–	–	0.06	0.06	1.75	1.72	

[a]Note all milk and milk products derived from cows unless otherwise stated.
[b,c,d] Within studies fatty acids with different superscripts differ significantly ($P < 0.01$).
[e,f,g]Within studies fatty acids with different superscripts differ significantly ($P < 0.05$).

Table 2.8. Fatty acid composition of conventional and organic meat.

System	Details	Fat content (g/kg)	Fatty acid concentration (g/100 g total fatty acids)																Reference
			C 14:0	C 16:0	C 18:0	Total SFA	C 16:1	C 18:1 *cis*-9	C 20:1	Total MUFA	C 18:2	C 18:3 *n*-6	C 18:3 *n*-3 (αLNA)	C20:5 (EPA)	C22:6 (DHA)	Total *n*-6	Total *n*-3	Total PUFA	
Chicken meat																			
Conventional	56 day breast	14.6	–	–	–	34.7[h]	–	–	–	33.9[i]	–	–	–	0.45[k]	0.95[l]	–	4.52[k]	31.4[k]	Castellini *et al.* (2002)
Organic		7.2	–	–	–	37.1[i]	–	–	–	30.2[h]	–	–	–	0.61[l]	1.85[l]	–	5.46[l]	32.7[l]	
Conventional	81 day breast	23.7	–	–	–	35.9[h]	–	–	–	33.0[i]	–	–	–	0.42[k]	0.79[k]	–	4.01[k]	31.2[k]	
Organic		7.4	–	–	–	37.9[i]	–	–	–	29.7[h]	–	–	–	0.56[l]	1.91[l]	–	5.12[l]	32.4[l]	
Conventional	56 day leg	44.6	–	–	–	33.9[h]	–	–	–	38.1[i]	–	–	–	0.21[k]	0.38[k]	–	3.34[k]	28.0[h]	
Organic		24.7	–	–	–	35.9[i]	–	–	–	31.9[h]	–	–	–	0.28[l]	1.14[l]	–	4.85[l]	32.2[i]	
Conventional	81 day leg	50.0	–	–	–	34.6[h]	–	–	–	37.9[i]	–	–	–	0.29[l]	0.45[k]	–	3.12[k]	27.6[h]	
Organic		28.3	–	–	–	36.2[i]	–	–	–	31.7[h]	–	–	–	0.32[l]	1.27[l]	–	4.73[l]	32.1[i]	
Conventional	Fresh breast	9.5	0.55	23.0	17.2	–	3.61	29.9	0.79	–	17.0	0.21	2.03	0.75	2.14	–	–	–	Jahan *et al.* (2004)
Conventional		15.8	0.63	22.7	15.5	–	4.31	30.5	1.05	–	18.5	0.13	2.51	0.69	1.73	–	–	–	
Organic	Fresh breast	7.8	0.40	24.1	17.8	–	3.29	20.4	0.43	–	23.6	0.14	1.59	0.34	1.21	–	–	–	
Organic		9.2	0.54	24.4	15.0	–	3.84	23.7	0.49	–	23.7	0.10	1.81	0.34	0.90	–	–	–	
Conventional	Frozen breast	8.9	0.73	23.8	17.4	–	3.12	26.5	1.07	–	18.1	0.12	1.96	0.96	3.35	–	–	–	
Conventional		9.7	1.02	21.3	17.3	–	2.60	22.1	0.69	–	22.6	0.18	1.96	0.35	0.80	–	–	–	
Conventional	Maize-fed	12.3	0.59	23.3	15.7	–	3.94	28.6	0.69	–	19.9	0.14	1.53	0.45	2.29	–	–	–	
Conventional FR	Farmers-market	9.7	0.60	24.5	15.2	–	3.75	31.6	0.84	–	16.1	0.13	1.28	0.38	1.47	–	–	–	
Conventional FR	Rare-breed	11.4	0.52	25.7	16.0	–	4.65	26.8	0.69	–	18.4	0.13	1.15	0.24	0.67	–	–	–	

continued

Table 2.8. Continued

System	Details	Fat content (g/kg)	Fatty acid concentration (g/100 g total fatty acids)															Reference
			C 14:0	C 16:0	C 18:0	Total SFA	C 16:1	C 18:1 *cis*-9	C 20:1	Total MUFA	C 18:2	C 18:3 *n*-6	C 18:3 *n*-3 (αLNA)	C20:5 (EPA)	C22:6 (DHA)	Total *n*-6	Total *n*-3	Total PUFA	
Organic	Ross breast	11.5	0.57	28.8[k]	9.06[k]	40.0[k]	1.9[k]	25.6[i]	0.17	28.0[l]	21.8[k]	–	0.55[i]	0.19[l]	0.99[i]	30.2[h]	2.82[i]	30.5	Castellini *et al.* (2006)
Organic	Kabir breast	8.5	0.85	27.5[l]	8.19[l]	36.9[l]	2.7[l]	27.0[h]	0.14	30.3[k]	19.2[l]	–	1.52[h]	0.49[k]	1.65[h]	27.2[i]	5.59[h]	32.8	
Conventional	Fresh breast[e]	–	2.14	20.9[h]	12.3[h]	35.4	5.77	23.9	3.41	33.1[l]	8.85[l]	8.24	1.47[l]	3.11	1.26	25.7	5.48	31.6	Jahan and Paterson (2007)
Organic		–	1.03	20.2[h]	9.62[h]	30.8	6.82	30.0[k]	3.71	40.5[k]	9.48	7.41	1.23	6.09	0.88	20.4	8.20	28.6	
Conventional	Maize-fed[e]	–	1.22	22.3[h]	17.0[h]	40.5	1.60	16.0[l]	8.32	25.9	7.11[l]	0.82	3.64[k]	7.60	4.02	18.3	15.3	33.6	
Conventional	FR[e]	–	1.28	8.6[i]	4.19[i]	14.1	27.5	21.4[l]	8.62	57.6	14.4[k]	2.63	0.97	5.06	1.38	21.0	7.42	28.4	
Conventional	Fresh breast[f]	–	0.56[l]	12.8[i]	3.82[k,i]	17.2[i]	13.2[l]	31.2[h,k]	1.95	46.4[h]	17.9	9.70	4.13	0.61	1.28	30.4	6.04	36.5[l]	Jahan and Paterson (2007)
Organic		–	0.75[k]	13.1[i]	5.14[l]	18.9[h,j]	15.8[h,k]	21.6[i]	0.58	38.1[i]	17.5[k]	12.3	5.42	0.50	1.59	35.5	7.51	43.0[k]	
Conventional	Maize-fed[f]	–	0.63	15.3[h]	5.28	21.2[h]	11.7[i]	26.5	2.60	40.8	12.8[l]	13.8	4.36	0.63	1.75	31.3	6.74	38.1	
Conventional	FR[f]	–	0.63	15.6[h]	5.74[h]	22.0[h,d]	13.0[l]	24.5[l]	1.76	39.2[i]	14.7	13.2	4.55	0.74	1.66	31.8	6.95	38.8	
Pig meat																			
Conventional	Muscle[e]	–	trace	17.0[k]	9.0	26.3	0.31	8.89[h]	trace	13.9[h]	28.2[l]	0.27	0.96[h]	1.12[h]	0.90[h]	42.1[h]	5.55[h]	47.6[i]	Hogberg *et al.* (2003)
Organic		–	trace	15.8[l]	9.32	25.5	0.29	7.02[i]	trace	11.0[i]	29.8[k]	0.27	0.67[i]	0.74[i]	0.73[i]	45.0[i]	4.51[i]	49.5[h]	
Conventional	Muscle[f]	–	1.37	23.2	12.1	37.2	2.86	43.0	0.74	51.1	8.62	trace	1.02[h]	trace	trace	9.58	1.39[h]	11.0	
Organic		–	1.35	23.7	12.3	37.8	3.1	42.6	0.75	51.2	8.25	trace	0.70[i]	trace	trace	9.24	0.98[i]	10.2	
Conventional[a]	Pork back fat	–	–	25.0[k]	13.5[k,l]	40.7[k]	2.5[k]	41.9[k]	–	45.3[k]	–	–	–	–	–	–	–	13.7[k]	Hansen *et al.* (2006)
Organic[b]		–	–	24.8[k]	13.7[k]	40.4[k]	2.3[l]	40.3[l]	–	43.3[l]	–	–	–	–	–	–	–	15.4[l]	
Organic[c]		–	–	23.9[l]	12.9[l]	38.6[l]	2.6[k]	39.5	–	42.5[m]	–	–	–	–	–	–	–	17.5[m]	
Organic[d]		–	–	23.4[l]	13.1[k,l]	38.5[l]	2.4[k,l]	39.1	–	41.9[m]	–	–	–	–	–	–	–	18.0[m]	

Lamb																			
Organic[g]	Adipose	731	–	–	–	51.5	–	–	–	–	2.29	–	1.10	0.13	0.05	2.60	1.28	–	Fearon and Strawbridge-Klapkova, personal communication
	Muscle	45.2	–	–	–	47.3	–	–	–	–	2.46	–	1.59	0.31	0.07	2.75	1.98	–	
Organic	Silage-based diet	15.8	1.90	21.4[k]	14.7	40.2	–	34.0	–	–	7.87	–	0.54	0.47	0.30	2.00	11.9	–	Nürnberg *et al*. (2006)
	Soybean meal-based diet	15.4	1.57	18.4[l]	17.4	37.6	–	32.8	–	–	8.54	–	0.60	0.57	0.38	1.83	10.19	–	
Beef																			
Conventional		19.8	0.24	2.62	3.93	–	0.27	3.28	–	–	0.22	–	0.11	–	–	–	–	0.12	Walshe *et al*. (2006)
Organic		33.7	0.14	1.60	1.40	–	0.16	2.06	–	–	0.16	–	0.08	–	–	–	–	0.06	

FR = Free range.
[a]100% conventional concentrate feed.
[b]100% organic feed.
[c]70% organic concentrate and barley/pea silage.
[d]70% organic concentrate + clover/grass silage.
[e]Phospholipid fatty acid composition.
[f]Neutral lipid fatty acid composition.
[g]Produced under simulated organic system.
[h,i,j]Within studies fatty acids with different superscripts differ significantly ($P < 0.01$).
[k,l,m]Within studies fatty acids with different superscripts differ significantly ($P < 0.05$).

Table 2.9. Fatty acid composition of conventional wild, conventional farmed and organic farmed Atlantic salmon.

		Fatty acid composition (g/100 g total fatty acids)											
System	Details	C15:0	C16:0	C18:0	C16:1	C18:1	C18:1 cis-9	C18:2	C18:3 *n*-3 (αLNA)	C20:4 *n*-6	C20:5 (EPA)	C20:6 (DHA)	Reference
Conventional	Wild	0.40	14.8	3.61	8.83	16.0	6.50	2.36	0.85	0.98	8.02	15.9	Molkentin *et al.* (2007)
Conventional	Farmed	0.55	13.9	2.22	9.90	14.9	6.27	6.40	2.18	0.78	8.25	14.4	
Organic	Farmed	0.68	14.3	2.87	8.47	14.1	6.10	6.53	2.45	0.91	7.75	16.3	

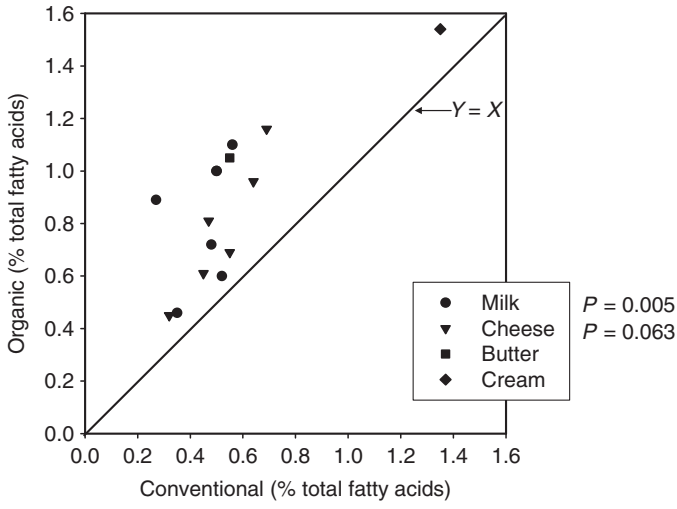

Fig. 2.3. Comparison of the alpha-linolenic acid content of organic and conventionally produced milk and milk products.

are due to the diets given to the dairy cows rather than the whole organic methodology since the same effects could be obtained in non-organic systems. To date there appear to be no studies reported which have tried to differentiate diet from whole organic system effects. Such studies, while difficult to conduct, are needed.

Another outcome was that overall, organic milk had significantly higher *cis*-9, *trans*-11 conjugated linoleic acid (CLA; $P = 0.02$) contents than conventional milk with the higher values in organic milk for *trans*-11 C18:1 (*trans* vaccenic acid, TVA) approaching significance ($P = 0.08$). Organic cheese had higher TVA concentrations ($P = 0.007$) than conventional products and values for CLA tended to be higher ($P = 0.08$) although in all cases the differences were small (Fig. 2.4). Plant oils rich in linoleic acid and αLNA are known to increase TVA synthesis in the rumen of the cow, leading not only to increased concentrations of TVA in milk but increased synthesis of CLA from TVA in the mammary gland. This leads to increased CLA concentration in milk.

Concentrations of CLA are also known to be higher in milk from pasture compared with dried grass, maize, grass or legume silages (Kelly *et al*., 1998; Stanton *et al*., 2003) and under UK conditions, milk fat CLA content is higher during the spring and summer months as a result of higher intakes of fresh grass (Lock and Garnsworthy, 2003). Thus, as for αLNA, it is likely that the effects on CLA and TVA seen in milk from organic systems are due to the diets given to the dairy cows rather than the whole organic methodology.

Few studies showed comparative values for a range of SFA or MUFA. One study which did (Jahreis *et al*., 1996) reported significantly ($P < 0.05$)

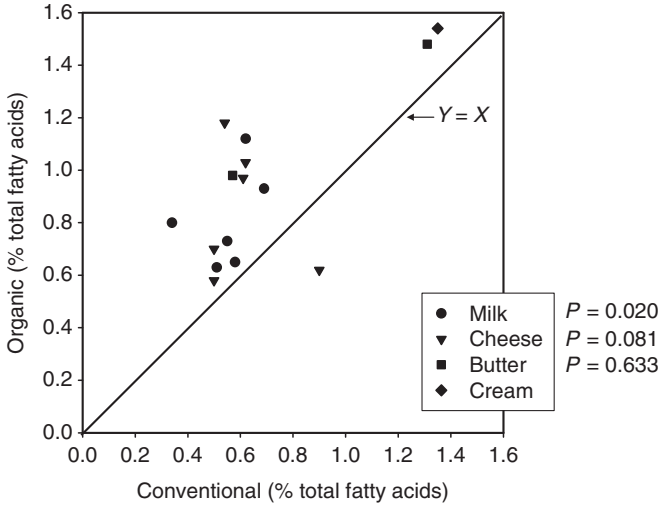

Fig. 2.4. Comparison of the *cis*-9, *trans*-11 conjugated linoleic acid content of organic and conventionally produced milk and milk products.

higher concentrations of C8:0 to C14:0 saturates and lower values for C18:0 in the organic milk. This might be expected with diets richer in forages leading to more SFA being synthesized *de novo* in the mammary gland from acetate and β-hydroxybutyrate units (Givens and Shingfield, 2006). However, the study of Fievez and Vlaeminck (2006) showed the opposite response. In the studies which reported total MUFA (Lund, 1991; Jahreis *et al*., 1996; Ellis *et al*., 2006), contents were shown to be significantly ($P < 0.05$) lower in organic than conventional milk, presumably as a result of less oleic acid. The differences between conventional and organic were rather small but, in general, reducing intakes of monounsaturates is not desirable in relation to risk factors for CHD, especially if accompanied by an increase in saturates (Mensink *et al*., 2003).

Overall, much more sound comparative data are needed before firm conclusions can be reached on the nutrient composition of organic compared with traditionally produced milk and dairy products.

Fatty acids in organic versus conventional eggs

Only one comparative study was found (Cherian *et al*., 2002) and the available data on the saturated (Table 2.6) and unsaturated fatty acid (Table 2.7) composition are summarized. The DHA content of the fat in the eggs was reported with no significant difference between the two production systems. Curiously, no data for αLNA or EPA were reported. There were no significant differences between the two systems for any of the fatty acids reported.

Fatty acids in organic versus conventional meat

Most of the data available for meat related to chicken. A total of eight truly organic versus conventional study comparisons were identified for chicken meat plus a range of studies including free-range birds and one organic-only study. However, some of the chicken meat data relate to fatty acids in phospholipid and neutral lipids fractions (Jahan and Paterson, 2007) and not in whole edible meat, making comparison with other data impossible. Three comparative studies were found for pork but none for beef or lamb although two and one organic-only studies were available for lamb and beef, respectively. The fatty acid composition of all meat types is summarized in Table 2.8.

Six comparative sets of data for EPA and DHA concentrations in chicken meat were provided by two studies (Castellini *et al*., 2002; Jahan *et al*., 2004), although that of Castellini *et al*. (2002) involved comparing breast and leg meat from birds slaughtered at different ages, and Jahan *et al*. (2004) made a study of retail chicken meat. Three out of the four data sets of Castellini *et al*. (2002) showed significantly ($P < 0.05$) higher EPA and all four showed significantly ($P < 0.05$) higher DHA values for the organic meat. The two sets of data from Jahan *et al*. (2004) showed no significant difference between the two systems. Combining the data from these two studies showed no significant effect of organic versus conventional production on EPA and DHA concentrations. Only one study (Hogberg *et al*., 2003) reported EPA and DHA concentrations in organic versus conventional pork. Here, the phospholipid fraction of the organically produced meat had significantly lower concentrations of EPA and DHA than the conventional one. Although based on few data, there was no evidence of differences in αLNA concentrations in chicken, pork or beef.

The higher concentrations of αLNA noted above for organic milk can be explained by the increased use of forages in diets for cows on organic systems and this effect may theoretically also occur in other ruminant production systems. This effect is unlikely to be seen in non-ruminant animal production systems where forage is not normally part of the diet. Indeed, since fishmeal (containing residual EPA and DHA) is not likely to be used in diets for organic production systems, it is likely that organic pig and poultry meat may often have lower EPA and DHA contents than that from conventional systems. This requires further investigation, possibly by means of an extensive study with meats at retail.

Fatty acids in organic versus conventional fish

Only one study providing a comparison between organic and conventionally reared fish was found (Molkentin *et al*., 2007). For farmed Atlantic salmon there were no differences between the two production methods although it is of interest that both types had higher αLNA concentrations than their wild counterpart, which the authors attributed to the use of vegetable oils in aquaculture.

Conclusions

The evidence clearly points to the fact that EPA and DHA are the key bioactive *n*-3 fatty acids and synthesis of these *in vivo* from αLNA is very limited indeed. While increasing intake of αLNA as replacement for *n*-6 linoleic acid is a positive change and will increase EPA supply marginally, the real benefits in terms of reducing chronic disease risk factors only come from EPA/DHA. It is also clear that for the majority of the UK population, intake of EPA + DHA is very much less than the target of 450 mg/day and efforts are needed to increase intake. The literature reviewed above provided no evidence that organic foods have higher concentrations of EPA and DHA than conventional foods. Thus, encouraging consumption of organic foods does not seem to offer a way to increase EPA and DHA intake. Organically produced milk and milk products were seen to be richer in αLNA than conventional comparisons although other evidence confirms that this is a cow diet effect which could be achieved without conversion to an organic system. In any event, the impact at population level on αLNA intake would be small even if all UK milk was organically produced.

A key finding of this exercise was the paucity of robust comparative data to allow a meaningful comparison between the two systems to be made. This clearly needs to be addressed.

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3 Environmental Impacts on the *n*-3 Content of Foods from Ruminant Animals

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Introduction

Inadequate dietary intake of *n*-3 polyunsaturated fatty acids (PUFA) increases the risk of cardiovascular disease and these PUFA are critical for proper brain and visual development in the fetus and maintenance of neural and visual tissues throughout life. Most attention has been focused on the long-chain *n*-3 PUFA, eicosapentaenoic acid (EPA; 20:5*n*-3) and docosahexaenoic acid (DHA; 22:6*n*-3). Plants are the primary source of *n*-3 PUFA, both in the terrestrial and marine ecosystems. Plants have the unique ability to synthesize *de novo* alpha-linolenic acid (αLNA; C18:3*n*-3), which is the building block of the *n*-3 series of essential fatty acids, and elongation and desaturation of this fatty acid results in the synthesis of EPA and DHA. The formation of these long-chain *n*-3 PUFA by marine algae and their transfer through the food chain to fish accounts for the high amounts of these important fatty acids in fish oils. Hence, fish oils are the predominant dietary source of long-chain *n*-3 PUFA for man but since consumption of fish is low in western diets much attention is given to enriching these fatty acids in other foods. It is acknowledged that the natural reserves of marine fish stocks are declining creating an unsustainable situation (Naylor *et al*., 2000) and this has provided further impetus for more sustainable and environmental benign sources of these important fatty acids for man (Napier, 2006).

Despite the low concentration of *n*-3 PUFA in feeds for ruminant animals, considerable effort has been given to increasing these fatty acids in meat and milk. A number of reviews examining the impact of nutrition, including the significant role the rumen plays in the extensive metabolism of dietary lipids, on milk and meat fatty acid composition have recently been published (Chilliard and Ferlay, 2004; Bauman and Lock, 2005; Scollan *et al*.,

2006a; Shingfield and Griinari, 2007; Sinclair, 2007; Wood *et al*., 2008). This chapter is directed towards the role of forages in the diet on lipid metabolism in the rumen and opportunities to beneficially enhance the composition of ruminant lipids, particularly in relation to *n*-3 PUFA. Forages rich in αLNA such as grass are an important feed for ruminants in both conventional and organic farming systems and hence may offer opportunities for increasing the delivery of *n*-3 PUFA to man.

Composition of Ruminant Lipids

Intramuscular fat in muscle of beef and lamb consists proportionally an average of 0.45–0.48 saturated fatty acids (SFA), 0.35–0.45 monounsaturated fatty acids (MUFA) and up to 0.05 PUFA, respectively. The polyunsaturated/saturated fatty acid (P:S) ratio for beef is typically low at around 0.1 except for very lean animals (<1% intramuscular fat) where P:S ratios are much higher ~0.5–0.7 (Scollan *et al*., 2006a). The *n*-6:*n*-3 ratio for beef is beneficially low (usually <3), reflecting the significant amounts of desirable *n*-3 PUFA, particularly αLNA but also EPA, docosapentaenoic acid (DPA; 22:5*n*-3) and DHA. Recent research on dietary intakes indicated that red meat can be an important source of these fatty acids for man (Howe *et al*., 2006). Beef lipids also contain conjugated linoleic acid (CLA) with the *cis*-9, *trans*-11 isomer representing approximately 0.7 of the total CLA. Milk fat generally contains 0.6–0.7 SFA, 0.25–0.35 MUFA and up to 0.05 PUFA (Chilliard and Ferlay, 2004). Linoleic acid (18:2*n*-6) and αLNA are the main PUFA – typically 0.02 and 0.005 of milk fatty acids, respectively. Oleic acid (18:1*n*-9) is on average 0.65 of the MUFA (0.2 of total fatty acids), with the remainder of the MUFA mainly as either *cis* or *trans* isomers of 18:1.

Plant Sources of *n*-3 Fatty Acids

αLNA is found widely distributed at high concentrations in commonly consumed oils and foods including green leafy plants, certain seeds such as linseed and canola and to a lesser extent in beans and nuts. As the precursor of *n*-3 long-chain PUFA, considerable attention has been given to the significance of food sources rich in αLNA and provision of EPA and DHA by conversion through the *n*-3 PUFA elongation-desaturation pathway in both man and animals. Recent studies in man have shown that bioconversion is low, but nevertheless when demands for EPA and DHA are low, primarily supporting membrane turnover and renewal in adults, the synthesis of EPA and DHA from αLNA may be important (Williams and Burdge, 2006). Current studies are also interested in the use of plants (hemp, blackcurrant, echium) containing stearidonic acid (18:4*n*-3). It has the advantage over αLNA in that it does not compete for a desaturase enzymatic step with 18:2*n*-6 and so is more readily converted to EPA and DHA in tissues (William and Burdge, 2006). Other research is exploiting genetically modified (GM) technology to

insert the genes from algae responsible for the capacity to synthesize long-chain *n*-3 fatty acids into linseed and oilseed rape (Napier, 2006). These may offer alternative sources of long-chain PUFA in the future if public concerns about GM crops can be adequately addressed.

Vegetative tissues in plants (leaves and stems), unlike in most animals, have very little storage fat in terms of triacylglycerol and typically make up proportionally only 0.05 of total fat, with diacylglycerol and monoacylglycerol 0.1, free fatty acids (FFA) 0.05 and the remaining 0.8 in the form of cell and organelle membrane lipid (Lee *et al*., 2007a). Galactolipids, sulfolipids and phospholipids make up the cell and organelle lipid membranes of higher plants (Thompson *et al*., 1998). Galactolipids are the most abundant in chloroplasts and are related to organelles involved in photosynthesis, and unlike phospholipids, which in plants can contain high proportions of SFA and MUFA (18:1*n*-9 and 16:0; Murata *et al*., 1982; Yoshida *et al*., 2007), galactolipids contain a high proportion of PUFA. This is a result of a preferential incorporation of highly unsaturated fatty acids to the *sn*-1 and *sn*-2 positions of the glycerol backbone. In pea, this results in up to 0.95 of chloroplastic lipid as αLNA, but in spinach and the 'model' species rockcress ('*Arabidopsis thaliana*'), this is lower at ~0.65 due to the incorporation of 16:3*n*-3 which competes with αLNA at the *sn*-2 position (Wolf *et al*., 1962; Browse *et al*., 1986). This trait results in non-green plant organs such as white leaves, stems and roots having much less total fat than the green leaves and a much lower concentration of αLNA, which as well as being the greatest depot of *n*-3 fatty acids are, along with leucoplasts, the sites of fatty acid anabolism in the cell (Crombie, 1957). Future plant breeding criteria to increase the concentration of αLNA, whether for animals or man, should consider these important organelles.

Effects of Environment on *n*-3 Content of Forage

The amount of αLNA in the forage and hence consumed by the ruminant animal has a large effect on maximizing transfer of αLNA through to meat and milk. The amount of αLNA varies with species, cutting date, cutting interval, growth stage, fertilization and conservation.

Genetic/species effects

Evidence exists for genetic variation in the levels of fatty acids in forages commonly consumed by ruminants. Fatty acid profiles in herbages are distinctive to species under the same management (i.e. at the same cut), which confirms a strong genetic basis. Dewhurst *et al*. (2001) have shown that the content of αLNA varied in ten different herbages and across species of ryegrass (*Lolium perenne*, *L. boucheanum* and *L. multiflorum*). However, with perennial ryegrass (*L. perenne)* varieties that were studied, there was no evidence of an effect of ploidy in relation to levels and patterns of fatty

acids. Similarly, significant variation for all fatty acids including αLNA was observed among 12 species in the study by Boufaïed *et al*. (2003), where Italian ryegrass (*L. multiforum*) had the highest αLNA concentration (20.6 mg/dry matter (DM)) while timothy (*Phelum pratense*) cultivars had the lowest (7.3 mg/kg DM). Legumes had lower αLNA content, ranging 6.0–16.5 g/kg DM from lucerne (*Medicago sativa* L.) to white clover (*Trifolium repens* L.). Clapham *et al*. (2005) reported the fatty acid composition of traditional (i.e. grasses and legumes) and novel forages such as forb, and found that the fractional contribution of αLNA to total fatty acids was lower and more variable in forbs than in grasses, and concluded that intake of fatty acids by grazing animals would be affected by the forage species consumed.

Conservation effects (fresh versus hay versus silage)

Unsaturated fatty acid loss, in particular αLNA, during conservation processes of fresh herbage such as wilting, ensiling and/or silage-making can be substantial (Dewhurst and King, 1998). These losses are associated with the lipoxygenase system, a plant defence mechanism most commonly initiated in damaged plant tissues, which converts PUFA into a range of volatile compounds such as leaf aldehydes and alcohols which act as plant hormones and signalling compounds (Hatanaka, 1993). Some 0.30–0.37 of αLNA were lost solely during wilting (24–68 h either in a glasshouse or laboratory) with ryegrass (Dewhurst and King, 1998; Dewhurst *et al*., 2002; Boufaïed *et al*., 2003) while timothy lost only 0.13 when wilted a few hours in the field (Elgersma *et al*., 2003a).

Hay-making reduced total fatty acids (0.01) with loss of αLNA as high as 0.17 with lucerne herbage, although the amounts and composition of fatty acids for fresh and ensiled lucerne were similar (39.9% versus 40.5% αLNA in total fatty acids for fresh lucerne and silage, respectively; Doreau and Poncet, 2000). Leaf tissues contain more fatty acids than stem, so losses due to leaf shatter during hay-making also contribute to loss of fatty acids (Dewhurst *et al*., 2006). Irrespective of species and stage of maturity, ensiling resulted in a marked increase in the proportions of forage fatty acids as FFA, indicating extensive lipolysis of plant lipids *in silo* (Vanhatalo *et al*., 2007). Other studies have also indicated that ensiling increases FFA concentrations as a proportion of total fatty acids in perennial ryegrass from 0.02 to between 0.27 and 0.49 in silage (Elgersma *et al*., 2003a). A number of studies, at a range of scales (Dewhurst and King, 1998; Boufaïed *et al*., 2003; Shingfield *et al*., 2005), have shown significantly higher levels of total fatty acids and αLNA in grass silages prepared with formic acid additive. The basis of this effect is not clear though it may relate to differential loss of other DM during silage fermentation and perhaps a rapid decline in pH *in silo* will denature plant lipases and lipoxygenases. One possible mechanism for effects of formic acid is an effect on effluent losses. However, effluent losses did not occur in the study of Dewhurst and King (1998).

Stage of maturity and cutting frequency (regrowth) effects

Although numerous studies have provided profound evidence of genetic effects, they have often identified large effects of environment such as light, cutting frequency, stage of maturity, seasonal variation and fertilizer regime (Dewhurst *et al*., 2006). Hence, apparent differences with genetic/species effects must be interpreted with some caution, since most of these studies were conducted under different regimes, which could mask genetic effects. For example, Dewhurst *et al*. (2001) demonstrated that Italian ryegrass had the highest levels of αLNA in vegetative material, but the lowest level during the summer flowering period among tested herbages. Fatty acid concentrations were in general highest in early and late season and lowest during the summer growth. Several other workers agree that αLNA decreases with an increase of 'stemmy' material (Bauchart *et al*., 1984; Mir *et al*., 2006).

Dewhurst *et al*. (2001) also showed that within species, αLNA declined when the regrowth interval was extended from 20 to 38 days. Elgersma *et al*. (2005) supported the effect of cutting frequency on αLNA level in herbage where regrowth period affected the concentration of fatty acids, and especially lowered concentration of αLNA after a longer period of regrowth with proportional increases in 16:0 and 18:2*n*-6.

Fertilizer effect

Some research has been carried out to investigate the effect of nitrogen (N) fertilization on lipids in grass. Boufaïed *et al*. (2003) applied 120 versus 0 kg N/ha on timothy monoculture swards. Increasing the N status of the soil resulted in increases in concentrations of 16:0 (0.18), 18:2*n*-6 (0.12) and αLNA (0.40) acids in the herbage and an overall increase of 0.26 in total lipid concentration. Similarly, the application of N fertilizer from 0 to 100 kg/ha resulted in higher lipid content but did not affect the fatty acid profile in perennial ryegrass (Elgersma *et al*., 2005). It is understood that the effects of regrowth, plant maturity and fertilizer relate to leaf/stem ratio with lower concentrations of fatty acids in 'stemmy' regrowth (Dewhurst *et al*., 2006), although this relationship did not hold for herbage cut in autumn (Elgersma *et al*., 2003b).

Rumen Lipolysis and Biohydrogenation

The metabolism of dietary lipids in the rumen has a large effect on the fatty acid composition of ruminant tissue and milk lipids and is one of the primary explanations for the highly saturated nature of ruminant lipids. Forage lipid, mainly esterified, enters the rumen and the glycerol fatty acid ester bond is cleaved by both plant and microbial lipases releasing FFA (Lee *et al*., 2007a). While bacteria utilize a proportion of released fatty acids for

incorporation into their own lipid membranes and as energy sources, the PUFA are toxic to certain bacterial groups and are detoxified either through hydration forming hydroxy fatty acids or more commonly through a systematic hydrogenation of the double bonds (see reviews by Bauman and Lock, 2005; Palmquist *et al*., 2005; Jenkins *et al*., 2008). This process also results in the formation of CLA and *trans* monoene intermediates, including *cis*-9, *trans*-11 CLA and *trans*-vaccenic acid (TVA; 18:1*n*-7). Since the majority of *cis*-9, *trans*-11 CLA in animal tissues is synthesized by delta-9 desaturase from ruminally derived TVA, factors influencing the production of TVA in the rumen are of interest (Bauman and Lock, 2005). Much research has focused on developing nutritional strategies which influence biohydrogenation and identification of the major microorganisms involved with a view to either increasing delivery of PUFA through to meat and milk, reducing stearic acid (18:0) production and/or increasing the production of *cis*-9, *trans*-11 CLA and TVA.

Dietary strategies to alter biohydrogenation have included the use of: (i) low N diets (Gerson *et al*., 1983); (ii) copper supplementation (Engle *et al*., 2001); (iii) increasing concentrate relative to forage (Kucuk *et al*., 2001); (iv) feeding small particles to alter the kinetics of fermentation and passage in the rumen (Gerson *et al*., 1988); and (v) fish oil (Shingfield *et al*., 2003). The use of fish oil has been very successful at inhibiting the final biohydrogenation step from 18:1 *trans* to 18:0 by its toxic effect on certain bacterial species (Kim *et al*., 2008). Other approaches have adopted protection technologies to bypass the action of the rumen microorganisms, including the use of calcium salts (Schauff and Clark, 1989), fatty acid acyl amides (Fotouhi and Jenkins, 1992) and encapsulation of lipid in a formaldehyde-treated protein matrix (Scollan *et al*., 2003). These vary in the extent of success and expense and as discussed previously, fish oil strategies are questionable in relation to sustainability.

The extent of biohydrogenation of dietary PUFA from a range of different diets is high, ranging between 0.70 and 0.85–1.00 for 18:2*n*-6 and αLNA, respectively. However, biohydrogenation of 18:2*n*-6 and αLNA is lower in red clover (*Trifolium pratense*) relative to grass silage thus delivering more PUFA through to milk and meat (see subsequent section). The red clover effect is related to the enzyme polyphenol oxidase (PPO). This enzyme is widely distributed among higher plants and in the presence of oxygen catalyses the oxidation of ortho-diphenols to ortho-quinones which covalently modify and cross link a variety of nucleophilic cellular constituents, such as proteins, amines and amides, leading to the formation of melanin pigments (Brown, 1983). The browning reaction has been associated with a reduction in the biohydrogenation of PUFA through a combined reduction in plant lipase activity, as a consequence of lipase-phenol complexing and protection of plant membrane lipid from rumen microbial lipases (Lee *et al*., 2007a). Although the exact mechanism of this protection of membrane lipid is yet to be fully determined it has been proposed that lipid micelles and cellular organelles become encapsulated in protein-phenol complexes reducing microbial lipase access to the membrane lipid (Lee *et al*., 2008).

PPO has been found in certain grass species (Marita *et al*., 2005; Lee *et al*., 2006). This may offer plant breeders the opportunity to increase PPO activity in other grass species as a potential mechanism to reduce biohydrogenation of PUFA.

The higher PUFA content in meat and milk of animals grazing 'species rich' grassland relative to improved lowland grass swards (see subsequent section) is considered to relate to changes in rumen lipid metabolism. The changes may relate to alterations in ruminal microbial population due to energy shortages reducing the extent of PUFA biohydrogenation or the action of secondary plant metabolites produced in numerous 'weed' species common in 'species rich' grassland. Such compounds include, PPO (as discussed) but also compounds such as essential oils (Wallace, 2004), saponins (Shi *et al*., 2004; Wallace, 2004) and catecholamines (Lafontan *et al*., 2002), all of which inhibit lipases and possess antimicrobial properties. Condensed tannins found in certain leguminous species inhibited several strains of *Butyrivibrio fibrisolvens* (Min *et al*., 2003), one of the most important bacteria involved in biohydrogenation.

Grazed swards had different effects on milk-saturated FA and *n*-3 when compared with silages made of the same sward type (Ellis *et al*., 2006). As discussed previously such effects may relate to PUFA losses during conservation but they may also relate to differences in rumen lipid metabolism. Lee *et al*. (2007b) suggested that the 'volatile' components (green odour effect), which are released when grass is cut, possess antimicrobial properties which may alter biohydrogenation.

n-3 Fatty Acids in Meat and Dairy Products

Forage effects on *n*-3 fatty acids

Feeding grass or preserved grass (hay, silage) relative to traditional cereal-rich concentrate diets rich in αLNA and 18:2*n*-6, respectively, result in contrasting fatty acid profiles in meat and milk. Grass results in higher concentrations of *n*-3 fatty acids in ruminant milk (reviewed by Dewhurst *et al*., 2006), beef meat (French *et al*., 2000; Nuernberg *et al*., 2005; Warren *et al*., 2008) or lamb (recently summarized by Sinclair, 2007). In beef and lamb, grass-feeding increases αLNA and also EPA and DHA in phospholipid (Tables 3.1 and 3.2; Fisher *et al*., 2000; Dannenberger *et al*., 2004; Warren *et al*., 2008) via elongation and desaturation. The degree of response to grass-feeding depends on the level of inclusion and length of time on diet (French *et al*., 2000 and Noci *et al*., 2005, respectively). In contrast, concentrates rich in 18:2*n*-6 result in higher concentrations of 18:2*n*-6 and associated longer-chain derivatives. A higher proportion of concentrates often increases energy intake resulting in increased fat deposition which increases the proportions of SFA and MUFA at the expense of PUFA and increases the P:S ratio (Scollan *et al*., 2006a; Warren *et al*., 2008).

Results from a selection of the experiments outlined in this section are summarized in Tables 3.1, 3.2 and 3.3.

Table 3.1. Fatty acid proportions (g/100 g total fatty acid), nutritional indices and total fat (g/100 g) in beef longissimus muscle on various diets.

Reference	Animals	Diet	18:2*n*-6	18:3*n*-3 (αLNA)	EPA	DHA	*n*-6:*n*-3	P:S	Fat
Nuernberg *et al*. (2005)	Bulls	Concentrates	5.2	0.46	0.08	0.05	8.34	0.20	2.6
		Grass silage	6.1	2.22	0.94	0.17	2.04	0.33	1.5
Realini *et al*. (2004)	Steers	Concentrates	2.84	0.35	0.30	0.09	3.0	0.12	3.2
		Grass-grazed	3.29	1.34	0.69	0.09	1.44	0.20	1.7
French *et al*. (2000)	Steers	Concentrates	2.96	0.72	0.12	–	4.15	0.09	3.4
		Grazed grass	2.1	1.13	0.23	–	2.33	0.13	4.4
		Grass silage	2.6	0.71	0.2	–	3.6	0.09	4.1
Warren *et al*. (2008)	Aberdeen Angus steers	Concentrates	7.0	0.26	0.09	0.02	15.1	0.18	3.1
		Silage	1.43	0.82	0.35	0.09	1.2	0.05	6.2
		Grazed grass	4.29	1.98	1.0	0.16	3.0	0.28	1.9
Scollan *et al*. (2006b)	Steers	Grass silage	1.4	0.83	0.42	0.04	1.15	1.13	2.1
		Red clover silage	1.9	1.20	0.50	0.11	1.13	0.15	2.0
Noci *et al*. (2005)	Heifers	Grazing 0 day	2.64	1.03	0.22	0.13	2.21	0.12	2.5
		40 days	2.52	1.14	0.28	0.16	1.99	0.14	2.3
		99 days	2.35	1.02	0.25	0.17	1.63	0.12	2.8
		158 days	2.49	1.29	0.30	0.21	1.46	0.15	2.5
Scollan *et al*. (2004)	Steers	0.7 silage and 0.3 concentrates	2.56	0.62	0.28	0.04	2.27	0.07	4.7
		+1000 g/day PLS[a]	6.2	2.83	0.32	0.03	1.88	0.22	1.9
Richardson *et al*. (2004)	Steers	0.7 silage and 0.3 concentrates	12.5	0.84	0.49	0.13	1.56	0.09	4.05
		+200 g/day PFO	1.9	0.77	0.73	0.75	1.04	0.11	4.9
Walshe *et al*. (2006)	Steers	Conventional	4.15	1.97	–	–	2.31	0.06	1.98
		Organic	5.05	2.60	–	–	1.96	0.07	3.37
Razminowicz *et al*. (2006)	Steers	Suckler grass-fed	4.33	1.61	1.0	0.15	1.9	0.45	1.22
		Steers/heifers organic grass	3.43	1.46	0.61	0.11	1.7	0.28	1.57
		Conventional heifers	3.85	0.93	0.41	0.09	3.5	0.26	1.73

[a]PLS, protected lipid supplement; PFO, protected fish oil.

Table 3.2. Fatty acid proportions (g/100 g total fatty acid), nutritional indices and total fat (g/100 g) in lamb muscle on various diets.

Reference	Animals	Diet	18:2*n*-6	18:3*n*-3 (αLNA)	EPA	DHA	*n*-6:n-3	P:S	Fat
Fisher *et al*. (2000)	Suffolk lamb	Concentrates	9.7	0.7	0.4	0.3	5.91	0.44	2.0
		Grass	6.8	2.3	1.3	0.6	1.65	0.41	1.9
Cooper *et al*. (2004)	Suffolk lamb	Linseed oil	4.8	2.70	0.71	0.22	1.37	0.26	3.4
		Fish oil	3.3	1.54	1.29	0.61	1.1	0.19	3.7
		Protected soya/linseed	14.5	3.68	0.56	0.14	3.15	0.57	3.8
		Fish oil/algae	4.1	0.79	2.33	2.55	0.68	0.30	3.7
		Protected soya/linseed and fish oil	10.1	2.5	1.24	2.20	1.70	0.46	3.9
Whittington *et al*. (2006)	Lamb	Control ryegrass pasture	2.63	1.42	0.66	0.17	1.2	0.16	3.2
		Salt marsh	3.11	1.57	0.75	0.22	1.3	0.17	3.1
		Heather	3.85	1.72	0.79	0.33	1.5	0.22	2.8
		Moorland	3.14	1.69	0.80	0.32	1.3	0.21	2.8
Lourenço *et al*. (2007b)	Lamb grazing	Biodiverse pasture	7.06	2.64	2.76	0.43	1.4	0.29	1.6
		Leguminosa-rich	5.28	3.99	1.09	0.29	1.1	0.23	2.4
		Ryegrass	3.37	2.59	1.33	0.34	0.9	0.15	2.0
Lourenço *et al*. (2007a)	Lamb Silages	Biodiverse	4.88	1.53	1.08	0.27	1.98	0.17	1.8
		White clover	4.15	1.62	0.87	0.25	1.79	0.14	1.8
		Red clover	4.29	1.82	0.84	0.25	1.70	0.15	1.9
		Ryegrass	3.60	1.62	0.83	0.27	1.63	0.12	2.4
		Crushed linseed and maize silage	4.95	1.57	0.66	0.23	2.16	0.15	2.5
Vasta *et al*. (2007)	Lamb	Control	14.88	6.84	3.61	2.19	1.46	–	1.43
		+carob pulp	11.20	4.78	3.38	2.07	1.32	–	1.03
		+carob pulp and polyethylene glycol	11.63	5.51	3.18	3.24	1.39	–	1.57

Table 3.3. Fatty acid proportions (g/100 g total fatty acids) in milk or cheese from cows fed on different diets.

Reference	Diet	18:2*n*-6	18:3*n*-3 (αLNA)
Timmen and Patton (1988)	Pasture	–	0.84
	Grass/wheat silage	–	0.36
Aii *et al*. (1988)	Grass	–	1.97
	Hay	–	1.46
Kelly *et al*. (1998)	Grass-white clover	–	0.95
	Maize + legume silage	–	0.25
Dhiman *et al*. (1999)	Grass-white clover	–	2.02
	Lucerne hay-grass-white clover	–	0.81
Ferlay *et al*. (2006)	Cocksfoot hay + concentrates	1.77	0.46
	Maize silage	1.46	0.24
	Ryegrass silage	1.09	0.94
	Ryegrass hay	1.00	1.02
	Mountain grass hay	1.08	1.25
van Dorland *et al*. (2008)	White clover	1.43	1.14
	Red clover	1.43	1.04
	Grass	1.52	0.90
Collomb *et al*. (2002b)	Lowlands 600–650 m, 8 species	–	0.79
	Mountains 900–1210 m, 52 species	–	0.82
	Highlands 1210–2120 m, 53 species	–	1.15
Bugaud *et al*. (2000)	Valley 850–1020 m (mean of three farms)	4.3	0.97
	Mountain 1575 m (I farm)	6.5	1.5
	Mountain 1700–1775 m (three farms)	6.2	1.5 mg/100 g cheese
Hauswirth *et al*. (2004)	Alpine pasture	–	495
	Alpine silage	–	303
	Linseed supplemented	–	245
	Commercial cheddar	–	114

Effect of preserving forage and supplementing with concentrates

Conserving forage through drying or ensiling reduces the content of PUFA with concomitant reductions of CLA and αLNA in milk (Dewhurst *et al*., 2006). Interestingly, hay relative to grass silage or maize silage and concentrate resulted in higher αLNA in milk (Ferlay *et al*., 2006). Indeed Shingfield *et al*. (2005) found that milk from cows fed hay or silage produced from a mixture of timothy and meadow fescue (*Festuca pratensis*) had similar SFA and MUFA, but higher αLNA than silage despite it having about half the intake of αLNA. When wilted and unwilted silages were compared in the rations of finishing steers, the wilted silage had reduced proportions of αLNA. However, the steers fed the wilted silage had a greater DM intake but the fatty acid composition of the meat from both groups was similar (Noci *et al*., 2007).

Effect of forage species

Feeding mixtures of grass and red or white clover (*T. repens*) increased the deposition of both *n*-6 and *n*-3 PUFA, thus increasing the P:S ratio in the muscle of beef steers over and above that of feeding grass alone (Scollan *et al*., 2002). When red clover silage was fed to finishing beef steers at 0.0, 0.5 or 1.0 of the silage component of the diet, it significantly increased the total PUFA content and in particular the 18:2*n*-6 and αLNA, resulting in a beneficially higher P:S ratio and lower *n*-6:*n*-3 ratio (Scollan *et al*., 2006b). Similar results had been found for the effect of red clover on milk fatty acid composition (Al-Mabruk *et al*., 2004). A difference was also found by van Dorland *et al*. (2008) who fed fresh or ensiled red or white clovers to milking cows. They found that white clover produced greater increases in αLNA in milk than red clover when compared to a ryegrass silage control, more so when zero-grazed fresh than when ensiled. However, the composition of milk from fresh and conserved forages was similar despite more than 0.4 (on a DM basis) of the PUFA being lost on ensiling.

Effect of feeding plant and fish oils

Many studies have examined the opportunities to increase the *n*-3 PUFA content of meat or milk by inclusion of *n*-3-rich seeds or oils in the diet, such as linseed or fish oil. Linseed increased αLNA in meat and milk lipids (Choi *et al*., 2000; Scollan *et al*., 2001; Petit *et al*., 2002). In contrast to grass-feeding, the addition of linseed in a concentrate feed increased αLNA and EPA in meat but not DHA (Choi *et al*., 2000; Scollan *et al*., 2001). The difference between grass and linseed-feeding in these studies may relate to length of time on diet. The studies by Warren *et al*. (2008) related to long-term feeding (>200 days) relative to feeding linseed for 80–100 days (Scollan *et al*., 2001). Since the half-life of phospholipid fatty acid turnover is around 180 days, it is thought that feeding αLNA for longer periods will increase EPA and DHA. The potential of fish oil, rich in both the long-chain *n*-3 PUFA, to increase their concentration in beef has been shown (Richardson *et al*., 2004) and the increase is dependent on the level of dietary inclusion (Noci *et al*., 2007). While these strategies can cause sizeable changes in the *n*-6:*n*-3 PUFA ratio they generally do not increase the P:S ratio in the meat above the 0.1–0.15 normally observed. Studies bypassing the rumen either by infusing oil in the small intestine or protecting oils from rumen biohydrogenation have demonstrated the potential for sizeable shifts in the PUFA content of meat and milk. Compared with feeding linseed as part of a concentrate diet, infusing linseed oil into the small intestine of cattle (and thus bypassing rumen biohydrogenation) resulted in αLNA in the total lipid of 26 and 177 mg/100 g muscle, respectively and a P:S ratio of 0.495 (Durand *et al*., 2005). Similar large responses have been noted in milk (Petit *et al*., 2002). Feeding ruminally protected plant oils rich in *n*-6 and *n*-3 PUFA (ratio 2.4:1) increased the P:S ratio (from 0.08 to 0.27) and also increased the *n*-6:*n*-3 PUFA (from 2.75 to 3.59) in muscle (Scollan *et al*., 2003). In a subsequent study, a protected plant oil supplement with *n*-6:*n*-3 PUFA ratio of 1:1 decreased the *n*-6:*n*-3 PUFA ratio in

muscle (from 3.59 to 1.88) while maintaining the high P:S ratio (Scollan *et al*., 2004). No effect was observed on the concentration of EPA or DHA in either study. Protection of fish oil, from degradation in the rumen, increased the concentration of EPA and DHA in tissue but had little effect on the P:S ratio and improved the *n*-6:*n*-3 PUFA ratio only at the highest level fed (Richardson *et al*., 2004). Similar studies with comparable results have been carried out with sheep (see Sinclair, 2007). These studies have demonstrated that n-6:*n*-3 ratio in the diet is an important determinant of the tissue response and that *n*-6 PUFA are more effectively deposited than *n*-3 PUFA in phospholipids.

Concentrate supplementation is frequently practised to enhance growth and to finish animals more quickly during or at the end of the grass-growing season. The negative effects of feeding *n*-6-rich traditional concentrates on fatty acid composition of the resultant product may be ameliorated by including linseed in the diet, helping to maintain the favourable *n*-3 content. This has been demonstrated for both lamb (Marriott *et al*., 2008) and beef (Razminowicz *et al*., 2008). The results of Duckett *et al*. (1993) demonstrate the necessity for including a source of αLNA in a concentrate feed given to animals previously grazing grass. Replacing pasture with concentrate-feeding in steers showed that αLNA in *longissimus* muscle fell from 0.9 g/100 g to 0.4 g/100 g of total fatty acids within 30 days and to 0.05 g/100 g after 5 months.

Effect of biodiversity, Alpine pastures and organic systems

A number of studies have noted positive associations between animals grazing certain biodiverse species relative to improved lowland grass swards and the higher PUFA content in milk (Collomb *et al*., 2002a,b) and cheese made from Alpine regions (Hauswirth *et al*., 2004; Leiber *et al*., 2005). Collomb *et al*. (2002b) studied records of botanical assessment of each pasture and grouped individual plants according to their species. The lowland pastures were characterized by low species diversity and the presence of only two plant families, true grasses (*Poaceae*) and legumes (*Fabaceae*). In contrast, although grasses were the largest family of plants found in mountain and highland pasture, there were much lower numbers of legumes and greater diversity including asters (*Asteraceae*) and members of the buttercup family (*Ranunculaceae*). Pearson correlation coefficients were calculated between the main groups of fatty acids in milk, and the plant families growing on the mountain and highland sites. Grasses were found to be negatively correlated with total PUFA in milk fatty acids (−0.73), whereas asters (0.74), members of the carrot family (*Apiaceae*; 0.63), rose family (*Rosaceae*; 0.41) and sedge family (*Cyperaceae*; 0.41) were positively correlated. It must be noted that most plants within the buttercup family can have a toxic effect and are not normally eaten by cows. Although the higher levels of PUFA may be partly attributable to the lower temperatures and more exercise required at the higher altitudes, the authors suggested that changes in biohydrogenation patterns and differences in PUFA levels of certain fodder plants in the pastures may be responsible. Leiber *et al*. (2005) compared Brown Swiss

cows fed on either silage concentrate indoors, freshly harvested pasture indoors or grazing pasture outdoors. The control group remained on lowland level throughout the study while the two pasture-fed groups spent two periods at low and then high altitude. They showed that when cows were at the low altitude, levels of αLNA in milk from grass-fed cows were 0.33 higher than found in milk from control cows fed silage and concentrate, and 0.96 higher when at high altitude, suggesting that levels in milk were somewhat independent to those in the pasture. The authors suggested that these increases in αLNA in Alpine milk were due to changes in ruminal metabolism as a result of energy shortage or to specific secondary plant metabolites, but may also have been due to selective grazing of the cows on these pastures. Hauswirth *et al*. (2004) compared αLNA levels in cheeses produced by different systems against two types of commercially available cheese. The Alpine cheese contained fourfold higher levels of αLNA, more *n*-3 PUFA and a lower *n*-6:*n*-3 ratio compared with cheddar. Cheese made from cows fed with a linseed supplement contained half the levels of αLNA than the Alpine cheese.

The effect of biodiverse or organic systems on the fatty acid composition of meat is less well documented than that for milk and cheese. As these systems are often forage-rich (including higher proportions of legumes such as red clover), the *n*-3 PUFA intake is likely to be higher than on conventional diets. Adnøy *et al*. (2005) reported higher PUFA in muscle from lambs grazing two biodiverse mountain pastures compared to lowland pasture in Norway. In another study with lambs, involving three unimproved grazing types, the plant species and dominance of cover were evaluated and classed as semi-improved natural grassland (0.80 ryegrass, <10 species), salt marsh (31 species), heather (51 species) and moorland (60 species; Whittington *et al*., 2006). Muscle from lambs grazing on heather and moorland were significantly higher in all *n*-6 PUFA and in 22:6*n*-3, and thus in total PUFA. Moorland lamb was high in *cis*-9, *trans*-11 CLA. Similarly, Lourenço *et al*. (2007b) noted an accumulation of biohydrogenation intermediates in the rumen and an increase of PUFA in intramuscular fat of lambs grazing a botanically diverse pasture compared with an intensive ryegrass pasture.

Tannins have been implicated in affecting the transfer of *n*-3 PUFA into cows' milk. The 18:3*n*-3 concentration in milk was greater, and that of *cis*-9, *trans*-11 CLA lower, for animals grazing bird's-foot trefoil (*Lotus corniculatus*) than those grazing perennial ryegrass (Turner *et al*., 2005). Infusing polyethylene glycol, which blocks the action of tannins, reduced αLNA in the milk implicating the inhibitory effects of tannins on biohydrogenation. Vasta *et al*. (2007) working with carob (*Ceratonia siligua*) high in condensed tannin provided further evidence linking tannins and biohydrogenation. Sulla (*Hedysarium coronarium*), another tannin-containing legume had similar effects on lactating sheep (Addis *et al*., 2005). In these studies, they also identified a herb chrysanthemum (*Chrysanthemum coronarium*) which led to higher concentrations of *cis*-9, *trans*-11 CLA in sheep's milk. The fact that biohydrogenation intermediates such as *cis*-9, *trans*-11 CLA and vaccenic acid (VA) can vary independently in meat and milk, despite common precursors (18:2*n*-6

and αLNA) supports the view that some of this variation is due to the effects of forages on lipolysis, rumen outflow rate and differentially on steps in ruminal biohydrogenation.

Ellis *et al*. (2006) compared the fatty acid composition of organic and conventional milk across 26 farms over a 12-month period. They found that organically produced milk was consistently higher in proportions of PUFA and *n*-3 FA than conventionally produced milk, as well as a lower *n*-6:*n*-3 ratio. Razminowicz *et al*. (2006) examined the fatty acid composition of beef at retail, 'pasture-suckler beef', and organic beef relative to meat from more 'conventional' systems. The *n*-3 fatty acid proportion was highest in the pasture-based systems and numerically highest in the organic. Angood *et al*. (2008) noted similar but larger responses for lamb. The organic lamb chops had more total fatty acids in the *longissimus* muscle (3.43 versus 2.96 g/100 g) and hence higher concentrations of SFA and MUFA and similar amounts of PUFA. They had less 18:2*n*-6 and more αLNA, both in concentration (48.4 versus 65.0 mg/100 g muscle) and as a proportion of total fatty acids (1.67 versus 1.95 g/100 g total fatty acids).

Conclusions

Increased consumption of long-chain *n*-3 PUFA would be beneficial in reducing the incidence of chronic disease. Even though ruminant products, meat and milk are low in long-chain *n*-3 PUFA, for many individuals they represent an important dietary source since consumption of fish and fish-based products rich in these fatty acids is low. Opportunities exist to further increase the concentration of both αLNA and the long-chain *n*-3 PUFA in ruminant meat and milk. Feeding forages rich in αLNA, the building block of the *n*-3 PUFA series, offers considerable sustainability and environmental benefits. In beef and lamb, feeding grass rich in αLNA increases this fatty acid in the tissue lipids and also EPA and DHA, reflecting the *n*-3 elongation-desaturation pathway. Similar responses are noted in milk in relation to grass-feeding for αLNA but not EPA and DHA. Feeding mixtures of grass and red clover relative to grass alone increases the deposition of both 18:2*n*-6 and αLNA PUFA in beef, lamb and milk. Hence, production systems, including organic, containing a higher proportion of forage compared to conventional concentrates generally result in a higher content of *n*-3 PUFA in the product. More botanically diverse forages also impact positively on fatty acids in meat and milk and merit further research. The transfer of αLNA from forage through to meat and milk is dependent on two important processes: (i) increasing the level of αLNA in the forage (and hence into the animal); and (ii) reducing the extent of ruminal biohydrogenation. Research should focus on increasing our understanding of these two major critical control points to increase delivery of αLNA from forage through to meat and milk. Greater integration of research across the various levels of the food chain and increased interaction with industry will help in the delivery of foods with higher nutritional and health benefits for consumers.

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4 Health Benefits and Selenium Content of Organic Versus Conventional Foods

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Summary

The 'trace element' selenium (Se) is essential for the maintenance of health in humans and animals. However, there is no known function for selenium in plants, which often provide a significant proportion of total dietary selenium for animals. Plants accumulate the trace element frequently as selenomethionine, the selenium analogue of methionine. The involvement of selenium in the normal functioning of humans and higher animals revolves around the products of the 24 or 25 selenoprotein genes that have been identified. All of these selenoproteins contain selenocysteine (SeC) at their active site or as an essential factor of their biological function. Selenoproteins are involved in almost every aspect of cell metabolism and include glutathione peroxidases, thioredoxin reductases, iodothyronine deiodinases and proteins involved in selenium transport, selenoprotein synthesis and sperm structure. In Europe, human dietary selenium intake is often below recommended levels and expression of these selenoproteins is lower than maximum levels that are achievable. Many studies associate low selenium status with diseases or potentially detrimental physiological/biochemical changes in humans, leading some to suggest increasing selenium intake as part of a strategy to improve general health in the population. In particular, the elderly appear to be more at risk from selenium-related problems since the micronutrient content in blood decreases with age. Properly controlled intervention trials to assess usefulness and safety of increasing dietary selenium intake need to be carried out. If organic foods contain more selenium than their non-organically produced counterparts, they could contribute towards general health improvements within the population. However, it is clear there are very little or no published data with suitable control groups that would allow us to make such assertions. Data that are available do not provide absolute concentrations of selenium

in organically produced foods. This further highlights the need for carefully designed and conducted research to establish relative amounts of selenium in organically produced food compared with non-organically produced food. Although there is some evidence on potential beneficial effects of increasing selenium intake in the human population, whether organically produced food can contribute to such a process remains an area requiring more research.

Introduction

Background to chapter

There is no doubt that an 'adequate' supply of selenium in the human diet is necessary for the maintenance of optimal health (Levander and Burk, 2007; Maiorino *et al*., 2007). Crucially, dietary intake of the micronutrient can vary throughout the world dependent on differing, available selenium levels in soils being reflected in selenium concentrations in crops and animals produced for food. This chapter will consider why selenium is important to human health, through the activity of selenium-containing proteins. The activity and expression of such proteins are modulated by selenium supply and thus dietary selenium may influence the health of humans. It is important to compare the potential intake of selenium from organic and conventional foods to determine whether this amount is adequate or potentially beneficial.

Selenoproteins and their functions

The involvement of selenium in normal functioning of the human body revolves around the 25 selenoproteins (Table 4.1) that have been identified (Kryukov *et al*., 2003; Gladyshev, 2007), all of which contain SeC at the active site. This SeC, due to its redox activity at physiological pH, is the chemical basis of the biological activity of selenoproteins. When molecular techniques are used to replace SeC with cysteine in recombinant proteins, the modified enzyme has between 100 and 1000 times lower activity with normal substrate concentrations (Gladyshev, 2007). Families of selenoproteins are involved in many aspects of cell metabolism. Glutathione peroxidases are the selenoproteins that have been studied most widely, as this was the first-identified selenoprotein, with the cytosolic form of the enzyme (GPx1) identified as a selenoprotein in 1973. The major function of GPx1 is likely to be as an antioxidant removing potentially toxic lipid hydroperoxides from the cell. In addition, GPx1 may be involved in the production of metabolites of eicosanoids that may have both anti- and pro-inflammatory effects within the cell (Arthur, 2000). Such functions are also associated with glutathione peroxidase 4 (GPx4), a membrane-associated enzyme that also has antioxidant functions. Unlike GPx1, GPx4 is able to metabolize lipid hydroperoxides

Table 4.1. Mammalian selenoproteins. (Updated from Beckett and Arthur, 2005; see Curran *et al*., 2005; Kim and Gladyshev, 2005; Moskovitz, 2005; Rederstorff *et al*., 2006; Burk *et al*., 2007; Hyrenbach *et al*., 2007; Olson *et al*., 2007.)

Selenoprotein	Proposed function
Glutathione peroxidases (GPx)	
GPx-1	Antioxidant in cell cytosol; selenium store?
GPx-2	Antioxidant in gastrointestinal (GI) tract
GPx-3	Antioxidant in extracelluar space and plasma
GPx-4	Membrane antioxidant; Structural protein in sperm; Apoptosis?
GPx-6	Specific antioxidant in nasal cavity?
Thioredoxin reductases (TR)	Multiple roles including: dithiol-disulfide oxoreductase. Detoxifies peroxides, reduces thioredoxin – control of cell growth, Maintains redox state of transcription factors.
TR 1	Mainly cytosolic – ubiquitous
TR 2	Mitochondrial – ubiquitous
TR 3	Expressed by testes
Iodothyronine deiodinases	
Type ID1 and ID2	Converts thyroxine (T4) to bioactive 3,5,3′ triiodothyronine (T3)
Type ID1 and ID3	Converts thyroxine (T4) to bioinactive 3′,3′,5′ reverse T3
Selenoprotein P	Selenium transport protein; receptors in some tissues
Selenoprotein W	Mainly expressed in cardiac and skeletal muscle
Selenophosphate Synthetase (SPS2)	Synthesis of selenophosphate for selenoprotein synthesis
Selenoprotein S	Stress/inflammation response in endoplasmic reticulum
Selenoprotein R (X)	Methionine sulfoxide reductase (stereo-specific)
H, I, K, M, N, O, T, V	Role largely unknown – mutations of N in some myopathies

attached to phospholipids in membranes (reviewed in (Arthur, 2000)). Thus, loss of this activity may be one of the main areas of interaction between selenium deficiency and vitamin E deficiency that produce a wide range of diseases in farm animals (Arthur and Beckett, 1994).

Knockout of GPx4 in mice is lethal at the embryonic stage attesting to the importance of the enzyme for maintaining normal function (Schneider *et al*., 2006). In addition, a polymerized form of GPx4 acts as a structural component of the mid-piece of sperm and is probably thus the basis of selenium function in maintaining male fertility (Ursini *et al*., 1999). Proteins similar to GPx4 have also been found in sperm nuclei and may have functions complimentary to cytosolic and mitochondrial GPx4 (Pfeifer *et al*., 2001).

Glutathione peroxidase (GPx2) which is similar to GPx1 is found predominantly in intestinal cells. Knockout of the protein indicates that it has a function in preventing inflammation caused by bacteria in the intestine. In support of this, the enzyme is induced when bacteria colonize the intestine of mice (Chu *et al*., 2004; Esworthy *et al*., 2005). Glutathione peroxidase 3 (GPx3) has a similar tetrameric structure to GPx1 and GPx2. However, it is a glycoprotein that has proposed extracellular rather than intracellular functions (Kingsley *et al*., 1998; Arthur, 2000). The glycosylation will improve the stability of the enzyme in environments such as the colloid of the thyroid where it may be involved in regulation of hydrogen peroxide levels for synthesis of thyroid hormones (Howie *et al*., 1995).

The 3-iodothyronine deiodinases ID1, ID2 and ID3, which maintain and regulate the levels of thyroid hormones in the circulation and tissues of the body, are all selenoproteins. These enzymes convert thyroxine (T4) to the active triiodothyronine (T3) or inactive, reverse triiodothyronine (rT3). They also carry out further metabolism of T3 and rT3 by specific deiodinations of the tyrosyl ring of the hormone. Thus, selenium can play an important role in whole body metabolism through regulation of thyroid hormone levels in the circulation (Beckett and Arthur, 2005; Schomburg *et al*., 2006).

A third major class of selenoproteins is the thioredoxin reductases. These enzymes use flavin adenine dinucleotide (FAD) as a cofactor in regulating the levels of oxidized and reduced thioredoxin in cells. The enzymes use nicotinamide adenine dinucleotide phosphate (NADPH) as a reductant and through thioredoxin they regulate many metabolic functions including DNA synthesis (Arner and Holmgren, 2000; Conrad *et al*., 2007). Thioredoxin reductase 1 is the cytosolic form of the enzyme and mitochondrial and testis forms have also been identified. Knockout of the cytosolic and mitochondrial forms of the enzyme in mice is lethal at the embryo stage with results pointing towards a role of thioredoxin reductase 1 in cell proliferation and mitochondrial thioredoxin reductase in apoptosis regulation (Conrad *et al*., 2004, 2007). *In vitro* studies also support a role for thioredoxin reductase 1 in protection of circulatory cells from oxidative stress including that caused by oxidized low-density lipoprotein (LDL; Lewin *et al*., 2002; Campbell *et al*., 2007).

Selenoprotein P can account for up to 60% of selenium in plasma and is probably the only selenoprotein containing more than one SeC residue. The protein is thought to have a transport function and is glycosylated presumably to protect against proteolytic degradation in the circulation which allows the protein to reach its target organs (Burk and Hill, 2005). Knockout studies indicate that as well as playing an important role in whole body selenium balance, it is the major source of selenium supply to organs of the body (Hill *et al*., 2003; Schomburg *et al*., 2003; Burk and Hill, 2005; Olson *et al*., 2005; Hoffmann *et al*., 2007). The phenotypes of selenoprotein P deletion include neurological symptoms and defects in sperm structure and motility. The functions of many of the other selenoproteins identified by bioinformatic and radiolabelling techniques are not known, although from amino acid sequences many are predicted to have antioxidant functions. Selenophosphate synthetase 2 (SPS2) is a selenium-containing protein that is required for the

production of selenophosphate, which provides the selenium for the synthesis of many of the other selenoproteins (Tamura *et al*., 2004). No diseases or deleterious effects have yet been associated with changes in SPS2 activity either induced by diet or by genetic means. However, another protein, selenium-binding protein 2 (SBP2), which is involved in the synthesis of selenoproteins but does not contain selenium, has been identified as the source of problems in some patients suffering from thyroid disorders and impaired selenoprotein synthesis. These patients have a mutation in SBP2, which presumably renders it less efficient in selenoprotein synthesis mechanisms (Dumitrescu *et al*., 2005).

Synthesis of selenoproteins

The synthesis of the selenoproteins can be influenced and controlled in many ways (Table 4.2). Thus, the defect in SBP2 described above is just one of many areas where the process can be altered. However, the total amount of selenium in the diet is probably the most influential factor in the synthesis of selenoproteins. The quantity of selenium absorbed will have a 'mass action' effect on the synthesis of selenoproteins. Thus, up to certain maximum levels increasing dietary selenium supply will enhance synthesis of selenoproteins in organs of the body. With further increases in selenium in the diet, although the element will then accumulate in tissues, the expression of selenoproteins will plateau or even decrease by a small amount (Bermano *et al*., 1995; Xia *et al*., 2005; Levander and Burk, 2007). The selenium intake at which the 'break point' of increasing selenoenzyme expression occurs has been used to try and estimate selenium requirements for both humans and animals (Levander and Burk, 2007).

As well as total selenium supply, changes in proteins and nucleic acids involved in synthesis of selenoproteins will influence the eventual expression of the proteins, and in addition to mutations in proteins such as SBP2, other polymorphisms will influence expression of selenoproteins and response to changing selenium levels in the diet (Meplan *et al*., 2007).

Less commonly, dietary antagonists such as high intakes of copper, silver and other metals will decrease synthesis of selenoproteins. Although this has been widely studied in animal models, there are few studies that have revealed such antagonisms occurring in humans (Table 4.2).

Table 4.2. Stages at which synthesis of selenoproteins may be regulated both mechanistically and by selenium supply.

1. UGA stop codon recognized by tRNASeC with transacting factors SBP2 and EFSeC
2. Protein(s) involved in selenophosphate synthesis
3. Overall supply of selenium from the diet
4. Interfering metals, e.g. Hg, Cu, Ag
5. Regulation by other dietary components, e.g. isothiocyanates

tRNA – transfer ribonucleic acid, SeC – selenocysteine, SBP2 – Selenophosphate synthetase 2, EF – elongation factor, Hg – mercury, Cu – copper, Ag – silver.

Human Selenium Deficiency

Selenium in animal diseases

The importance of dietary selenium in maintaining health was first demonstrated in animals in 1957 when it was shown to prevent liver necrosis in rats that were also vitamin E-deficient. Since then selenium deficiency, usually in association with vitamin E deficiency, has been linked to many diseases in farm animals (reviewed by Suttle, 1999). These diseases are predominantly myopathies of skeletal or cardiac muscle, but reproductive and immune processes can also be adversely affected. In addition, improved growth has often been demonstrated when severe selenium deficiency is corrected in farm animals.

Selenium deficiency in human disease

Although the essentiality of selenium for farm animals was recognized in the late 1950s, it was not until the 1970s that low selenium status was associated with Keshan disease in rural human populations in China (reviewed by Foster and Sumar, 1997). Keshan disease is a cardiomyopathy that may be exacerbated by viral infections (Levander and Beck, 1999). Widespread selenium supplementation of the population in affected areas has caused a dramatic decrease in the incidence of Keshan disease. Thus, even if there are exacerbatory factors that predispose towards Keshan disease, increasing selenium status is enough to prevent the onset of the problem (Anon, 2001). Another endemic disease associated with selenium deficiency is Kashin-Beck disease, which has features of an osteoarthropathy affecting joints in the arms and legs. The involvement of selenium deficiency in Kashin-Beck disease is less clear-cut than its role in Keshan disease. Iodine deficiency may be an exacerbating factor in Kashin-Beck disease (Moreno-Reyes *et al*., 1998, 2003). However, in parts of Africa where there are severe combined selenium and iodine deficiencies there is no Kashin-Beck disease. Here selenium deficiency may be involved in the aetiology of cretinism that occurs due to the iodine deficiency (Vanderpas *et al*., 1992). The areas where Keshan disease and Kashin-Beck disease are endemic are among the lowest daily dietary selenium intakes (probably up to 20 μg/day) in the world (Vanderpas *et al*., 1990).

Association of selenium status with chronic diseases

Within other populations that do not have endemic selenium deficiency diseases, relatively lower selenium status has been associated with a wide range of chronic diseases. The most widely studied association is selenium status and the incidence of cancer. Many intervention trials have been carried out, or are currently being carried out, to determine whether increasing selenium intake may be protective against cancer. The Nutritional Prevention of Cancer

(NPC) trial carried out in the USA showed that 200 μg Se/day given in a yeast tablet, when compared with placebo, caused significant decreases in lung, colorectal and prostate cancers (Clark *et al*., 1996). Longer-term follow-ups of the participants in this trial have shown potential small increases in skin cancers and type-2 diabetes in the selenium-supplemented group. Debate continues as to whether the beneficial effects of the selenium supplementation on cancer incidence outweigh smaller, long-term adverse effects (Duffield-Lillico *et al*., 2003; Stranges *et al*., 2007).

The association of low selenium status with impaired immune function has also received attention. Supplementation of UK residents with 50 or 100 μg Se as sodium selenite/day improved response to polio virus vaccination (Broome *et al*., 2004). This was also associated with increased ability of blood cells to produce interleukin-10 and TNFα *in vitro* in response to a viral challenge at 1 and 2 weeks after immunization. 'Pro-inflammatory status' may also be associated with low selenium status in elderly populations (Lauretani *et al*., 2007; McKenzie *et al*., 2007; Murr *et al*., 2007). However, whether selenium status can be shown to control susceptibility to viral and other infections and diseases in free-living populations remains to be determined.

Some epidemiological studies show an association between low selenium status and increased incidence of cardiovascular disease (reviewed by Flores-Mateo *et al*., 2006). The role of selenoproteins in antioxidant systems is hypothesized to be the link between the micronutrient and vascular disease since oxidation of fats is probably a required step in the development of damaged tissue. However, the link between selenium and cardiovascular disease is largely based on epidemiology and the influence of increased dietary selenium intake on the risk of cardiovascular disease is an area that requires further research (Flores-Mateo *et al*., 2006). Blood pressure, a risk factor for vascular disease, is inversely correlated with selenium status in human populations providing a further link between the micronutrient and the disease that may be independent of its antioxidant functions (Nawrot *et al*., 2007). Peroxide intermediates are produced in the synthesis of both prostacyclins and thromboxanes that regulate vascular tone and influence platelet aggregation in the circulation. Both GPx1 and GPx4 may regulate the levels of such peroxides and ensure that appropriate levels of eicosanoid derivatives are produced which should decrease susceptibility to vascular disease (Imai and Nakagawa, 2003).

Potential relationships between selenium status and other abnormal 'physiological functions'

Many other diseases have also been associated with low selenium status including aspects of fertility, reproduction, brain function and visual function (see review of Rayman, 2000). Thus, any increase in dietary selenium intake in European population has the potential to decrease the incidence of many chronic diseases. However, much research still needs to be carried out to show that epidemiological associations of diseases with low selenium

status are not just a consequence of the disease process. Proof that selenium can prevent disease will only come with the completion of long-term, properly conducted, double-blind trials with placebo controls that monitor long-term effects on disease incidence or robust biomarkers of disease.

Selenium Intakes and Requirements (Including 'At-risk' Populations)

Selenium requirements

Since the recognition that selenium was an essential nutrient for animals, many dose–response experiments both with rodents and domestic livestock have been used to set dietary selenium requirements at between 0.1 and 0.2 mg Se/kg diet. These figures have been based on the ability of selenium to prevent diseases, maximize levels of selenoprotein gene or protein expression and on dietary 'balance studies' that involve determination of organ selenium content (Suttle, 1999). Many such experiments cannot be carried out in humans for ethical reasons and thus, as indicated above, expression of selenoproteins in blood fractions have been used as 'functional' indicators of requirement (Brown *et al*., 2000; Meplan *et al*., 2007). In many studies with human volunteers, concentration of ~70–90 μg Se/l plasma (0.9–1.0 μM) is sufficient to maximize levels of GPx3 protein and activity. Calculations have then been made as to the dietary level of selenium that is required to support such plasma selenium concentrations and GPx3 activity. From this dietary selenium requirements for adults are estimated to be between 40 and 80 μg Se/day for male and female adults (Combs, 2001, 2005; Levander and Burk, 2007) These requirements often include a 'safety margin' added to the amount of selenium that maximizes the plasma GPx3 activity. In the UK, the reference nutrient intake (RNI) levels for selenium are calculated at 60 μg/day for adult females and 75 μg/day for adult males. In this context actual selenium intakes in the UK may range from approximately 30 to 50 μg/day and thus may not be considered adequate.

Furthermore, in much of Europe, selenium intakes are less than 50 μg/day. These dietary selenium intakes have been calculated for whole populations and are not available for subgroups such as the young or the elderly. Nevertheless, lower plasma/serum selenium concentrations in elderly populations over the age of 65 indicate they may have lower dietary selenium intakes (Bates *et al*., 2002; Combs, 2005). Many of the estimates, as indicated above, are based on saturation of plasma glutathione peroxidase levels at a particular daily selenium intake. However, a study using plasma selenoprotein P as a marker of selenium status in the population of China indicated that this protein needed a higher selenium intake – provided as selenomethionine – than glutathione peroxidase to reach maximum levels (Xia *et al*., 2005). An additional 100 μg of Se/day as sodium selenite given to volunteers in a UK trial increased their plasma selenoprotein P concentrations to those levels seen in the US population that is considered to have maximum levels of the

protein (Meplan *et al*., 2007). Use of selenoprotein P as a marker of selenium intake is therefore likely to result in the adoption of higher dietary selenium intake recommendations. Thus, if systematic studies are to be carried out comparing selenium intake from organic foods and conventionally produced foods, determination of plasma selenoprotein P may be the preferred method of assessing responses of volunteers to the different diets.

Selenium status in different countries

The selenium content of blood or blood fractions has been determined in many countries and vary greatly. For example, plasma selenium is as low as 20 μg/l in some areas of China and may be as high as 500 μg/l in seleniferous areas of the same country. Values derived from several studies throughout Europe (review of Combs, 2001) show that in general, selenium levels in blood and plasma are in the lower end of this range. Furthermore, in many countries they are lower than the concentrations that would be needed to maximize the expression of GPx3 or selenoprotein P (Combs, 2001; Xia *et al*., 2005). Consistent with this are calculated daily dietary selenium intakes from these countries, the majority of which fall below current dietary recommendations published not only in the UK but by World Health Organization (WHO) and the Food and Drug Administration (FDA) in the USA (Levander and Burk, 2007). These relatively low selenium concentrations in blood fractions and dietary selenium intakes have probably not changed greatly since the year 2000 when they were reviewed (Combs, 2001). In 2007, van Cauwenbergh summarized plasma or serum selenium levels from studies in Europe conducted since the year 2000 (van Cauwenbergh *et al*., 2007). Many of the concentrations are less than 1 μM (~80 μg/l) and again indicate that if selenium intake in the subjects was improved, expression of selenoproteins would be higher. Increasing selenium status in this way would be a desirable course of action if further research confirmed studies that associate lower selenium status with increased risk of chronic disease. A more urgent requirement is, however, for research to determine directly the potential beneficial effects of nutritionally relevant increases in selenium intake in decreasing the risk of chronic diseases. This would be a much more robust reason for increasing selenium intakes than association of lower micronutrient status with disease.

Selenium in the elderly

Many studies of selenium status show that this tends to be lower in elderly populations. Thus, this group may be more 'at risk' from adverse effects of lower selenium status than the general population. It is therefore desirable that improving selenium status, either by supplementation or by changes in the diet, be tested to see if many of the selenium-related problems can be ameliorated in the elderly as is the case with impaired thyroid hormone metabolism (Olivieri *et al*., 1995). In Britain, plasma selenium tended to be

lower in frail and poorly nourished elderly people with concurrent illness compared with a healthier elderly population (Bates *et al*., 2002). These changes were not reflected in whole blood glutathione peroxidase. The blood enzyme activity, which is mainly in red cells, is less influenced by short-term effects of illness that may lower plasma selenoprotein levels. 'Low' selenium status also occurs in older women in Germany (60–70 years; Wolters *et al*., 2006). In France, a 9-year study of plasma selenium in older people (initial age 59–71 years) showed that age of subjects, obesity and occurrence of cardiovascular diseases during follow-up were associated with increased declines in plasma selenium (Arnaud *et al*., 2007). Other factors that did not influence the decline were gender, education, smoking, alcohol intake, dyslipidaemia, diabetes and hypertension. The general decline in selenium status in the elderly is of particular interest since three separate studies have shown that in patients with pre-existing vascular disease or elderly American women or an elderly French population all subjects with the highest quartiles or quintiles of selenium status had better disease prognosis and/or survival (Blankenberg *et al*., 2003; Akbaraly *et al*., 2005; Ray *et al*., 2006). Additionally in an elderly rural Chinese population (2000 subjects aged 65 or older), lower selenium status was associated with lower scores in a series of cognitive tests (Gao *et al*., 2007). A dose response could be seen in these results when selenium levels were split into quintiles. The authors hypothesized that lifelong low selenium level was associated with lower cognitive function. Although selenium deficiency in selenoprotein P knockout mice has been associated with impaired neurological function, studies on the effects of selenium supplementation on neurological functions in humans are contradictory (Schomburg *et al*., 2003; Hill *et al*., 2004). In a randomized double-blind, placebo-controlled trial, 400 subjects (aged 60–74) received selenium supplements or a placebo. Selenium supplementation had no effect on mood or quality of life in the volunteers in contrast to other smaller less well-controlled trials. In this study however initial plasma selenium levels were approximately 1.2–1.3μM and were significantly increased by selenium supplementation. However, the initial selenium level was such that selenoprotein activities were likely to be maximized at baseline. In contrast, the survey carried out in China had at least 50% of participants with selenium intakes of less than 20μg/day, which is likely to be less than half that in the UK population.

Furthermore, in the elderly, low selenium status has also been associated with impaired thyroid hormone metabolism, poor muscle strength and anaemia (Olivieri *et al*., 1995; Lauretani *et al*., 2007; Semba *et al*., 2007).

Selenium in Organic Foods

There are little data in peer-reviewed literature that either give the absolute selenium levels in organic foods or provide direct comparisons with non-organic foods. In addition, such data that do exist are missing descriptions of aspects of methodology or support information that would assist greatly with interpretation. A 'commentary' published by Smith in the *Journal of*

Applied Nutrition in 1993 (Smith, 1993b) provides a comparison of organic foods versus 'supermarket foods' (conventionally grown products) for a range of trace elements including selenium. The foods were purchased over a period of 2 years in 'several stores in the Western suburbs of Chicago'. Unfortunately, the areas where the different types of food were grown are not specified, thus any influence of soil type, fertilization and methods of horticulture cannot be determined. The foodstuffs chosen for analysis included apples, pears, potatoes, wheat and sweet corn. For all the trace elements analysed, an organic versus commercial percentage difference in content is all that is quoted. There is no mention of absolute levels of any of the elements, including selenium, in any of the products. An abstract of the same data also published in 1993 again contains no absolute trace element concentrations (Smith, 1993a). While it is helpful to know that there may be up to two to three times as much selenium in the organic products compared with 'commercial' products, it is not possible to say whether this is caused by method of production or merely reflects the farms on which the food is produced. As mentioned above, available soil selenium will determine the concentration of the element incorporated into crops. In a comprehensive review of selenium (expressed as micrograms per gram), ranges of content are quoted for cereal products, vegetables, fruits, red meats, poultry, fish, milk products and eggs from the USA, the UK, Germany, Finland, New Zealand, China and Venezuela (Combs, 2001). Within countries there is generally a five- to tenfold difference in selenium content of foodstuffs dependent on sampling sites. In the case of animal products this may reflect whether or not selenium supplements are used in diets. Between countries there is an even wider range of selenium contents of foods. Excluding very high selenium areas of China and Venezuela, there can be 1000-fold differences of selenium content between the foods produced, for example, in Finland and the USA (Combs, 2001). Thus, the nutritional significance of the differences between organic foods and supermarket foods quoted by Smith is difficult to predict given that these variations tend to be much smaller than those that can occur naturally both within and between different countries.

In the comparison of the relative selenium contents of organic and conventional foods the research could address two general areas of importance. First, similar food types should be obtained, preferably from the same outlet or producer, and total selenium levels analysed. This would give data as to whether the relative total selenium intake of the consumer could be altered by consuming foodstuffs produced by one particular production method. Present knowledge of selenium nutrition indicates that an important aspect of selenium metabolism in humans is the total amount of the micronutrient in diets rather than its chemical form or apparent bioavailability. Thus, worldwide total selenium content of blood, plasma or serum tends to reflect the calculated total daily intake of selenium in the diet (Combs, 2001). Second, following on from whether the consumers could increase their selenium intake by purchasing organic or conventionally produced foods, research is also required to determine whether the different methods of production will cause changes in selenium content of foodstuffs from closely matched areas. This

may determine whether any differences in the selenium content of the two food types are due to where they are grown (different soils) or the method of production. Thus, crops grown using the different methods of production, on adjacent plots of similar soil type and experiencing similar climatic challenges, will give an accurate picture of how selenium levels are affected. Similarly, animals given feedstuffs grown on otherwise identical, adjacent organic or conventional plots would provide material for a selenium analysis to clarify the effect of management of production on micronutrient content. Clearly, therefore, it will be challenging to make precise comparisons of the effects of organic and conventional production methods on eventual selenium concentrations in plant material or animal-based foodstuffs. Consequently, given the problems in conducting a rigorous comparison of the selenium content of comparable organic and conventional foods, it may probably only be practical to try and assess the effects on the consumer of eating diets either composed mainly of organic foodstuffs or conventionally produced foodstuffs. Any differences that occur in selenium status would then give an insight into any potential health effects from increasing or decreasing intake of organically produced foods.

One study using diets based on foods from conventional versus organic production has given indirect information on the potential impact of organic versus conventional production methods on selenium status in humans (Grinder-Pedersen *et al*., 2003). The objective of the study was to compare the intake and excretion of 'antioxidant' flavonoids on markers of 'antioxidative' defence in humans. The diets were compared in 16 volunteers using a cross-over intervention study. The interventions were carried out over a 22-day period with an intermediate washout of 3 weeks with 'habitual diet'. Where possible, organic and conventional foods were of the same variety and in some cases vegetables came from fields within a similar geographic location, where the organically and conventionally grown vegetables used in each intervention were sowed and harvested in the same week. Pork was used as the only meat in the study and pigs originated from the same litter and were divided into two groups raised either by conventional or organic methods. Among the antioxidants measured was glutathione peroxidase activity (GPx1) expressed as units per gram of haemoglobin in washed erythrocytes. Erythrocyte GPx1 activity reflects selenium intake integrated over a time period since the population of erythrocytes is replaced with a half-life of approximately 140 days. The corrected GPx1 values were not significantly different between the subjects consuming conventionally produced diet and organically produced diet in the experiment. In both cases the activity was less than 5% lower than baseline values. However, the short intervention periods of 3 weeks may not have been sufficient to allow sufficient turnover of glutathione peroxidase within the erythrocytes to reveal any differences. It would have been more useful to have determined selenium concentration or GPx3 activity in serum or plasma that reflect more immediate changes in selenium intake. The experiment was carried out in Denmark where selenium intake has been estimated at 40μg/day (Combs, 2001). Increasing intake from such levels would have the potential to increase expression of glutathione peroxidase in blood and plasma.

Thus, it is possible that both the organic foods and the conventionally produced foods supplied similar amounts of selenium to the volunteers in the study. Although, if selenium intakes were already higher at around 60–70 μg/day any increase in intake caused by the experimental diets would not have been able to increase glutathione peroxidase activities and the only way to determine change in selenium intake would then have been to measure total amounts of the element in blood or plasma.

Two studies report selenium levels in milk produced in organic systems in Sweden. One study showed seasonal variations in milk selenium content with higher levels from October to April and the lowest levels within the grazing period of May to September (Toledo *et al*., 2002). When compared with selenium levels in conventionally produced milk from Swedish farms there was no significant difference. An abstract of a more recent study of bulk tank milk from 20 organic and 20 conventional dairy farms sampled on three occasions during the indoor season confirmed there are no differences in selenium content between milk from the two production methods. This study had the advantage of samples being taken within the same year (Emanuelson and Fall, 2007).

Thus, there is great scope for research to determine the impact of production methods on the selenium content of foods. The validity of the data would be based on the comparison of organic versus conventional foods produced at the same time and preferably of the same variety/strain.

Conclusions

It is likely that a modest increase in selenium intake in many European populations would have beneficial biochemical effects that would be associated with improvements in health. Nevertheless, there are few dietary or supplementation trials that have associated an increased selenium intake with improvements in health or biological function in humans. Most of the evidence for a role of selenium in disease is derived from the observation of a lower selenium status in diseased groups compared with healthy controls. If, however, increased selenium intake can be associated with lower incidence of certain diseases, role-improved diets providing extra selenium may become important. There is a dearth of experimental data comparing selenium content of organic foods with that of conventionally produced products. Thus, the first area for future work on selenium in organic foods should be to determine the levels of the micronutrient. This would then provide a sound basis for dietary trials in which manipulation of selenium intake with organic and conventional food is assessed as a method of disease prevention.

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5 Environmental Impacts Concerning the Selenium Content of Foods

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Introduction

As one of at least 15 micronutrient mineral elements that are essential for higher forms of animal life (Underwood and Suttle, 1999), selenium (Se) can hardly be regarded as unique. Yet Oldfield (2002), in considering the low crustal abundance of Se and appreciating its importance to humans, writes that it 'is a truly remarkable element'. Identified by the Swedish chemist Jöns Jakob Berzelius in 1817, interest in Se initially focused on its toxicity to animals and humans. Marco Polo was probably describing what is now called alkali disease, a form of chronic Se poisoning, when he wrote of his travels on the Silk Road to China in the late 13th century, observing that beasts of burden were affected by the consumption of poisonous plants that produced a necrotic hoof disease causing them to swell and drop off (Komroff, 2002). In the Americas, signs of chronic selenosis (i.e. toxicosis), resembling alkali disease in livestock, and manifest as malformations in chicks and children, and the loss of hair and nails of people, were described and recorded by the missionary Father Pedro Simon in 1560 (Simon, cited by Rosenfeld and Beath, 1964). Selenosis was probably first documented in the USA in 1856 by Madison, a physician with the US Cavalry who noted the loss of hair and sloughing of hooves in horses (*Equus caballus*) in what is now South Dakota (Fig. 5.1). Called alkali disease because early pioneers of the USA associated the malady with alkali (high salt) waters and soils, it was only in the early 1930s that research showed the condition was caused by the grazing of seleniferous plants (Moxon, 1937).

The understanding of the biological importance of Se was dramatically revised when Schwarz and Foltz (1957) inferred the nutritional essentiality of the element from their results that showed how Se is critical in preventing liver necrosis in rats (*Rattus* sp.). This work was quickly followed by research undertaken on domesticated animals which demonstrated that Se deficiency

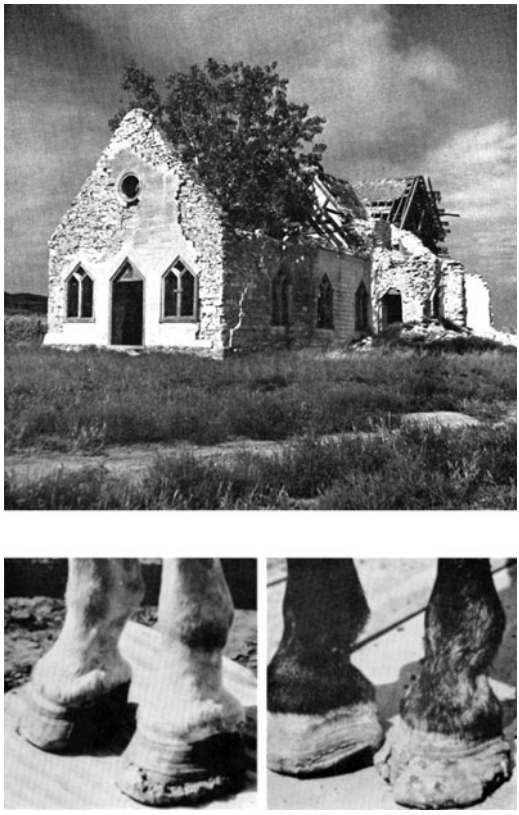

Fig. 5.1. Ruins of the chapel – built of Niobrara limestone – at Fort Randall, South Dakota, location of the Se toxicity described by Madison in the 1850s. Outcrops of Pierre Shale and Niobrara formations supply Se to clay-rich soils which support vegetation that intoxicate animals with the element following grazing. This is clinically evident in the hoof damage of affected animals (Photograph courtesy of O.E. Olsen), a visible symptom of alkali disease. (Reprinted from Rosenfeld and Beath, 1964. Copyright Elsevier. With permission.)

was an underlying cause of previously untreatable diseases such as exudative diathesis (ED) in chicks (*Gallus gallus domesticus*; Patterson *et al*., 1957), white muscle disease (WMD) in lambs (*Ovis aries*) and calves (*Bos taurus*; Schubert *et al*., 1961) and ill-thrift in lambs (Drake *et al*., 1960). Such potentially fatal diseases can result in sick animals of low nutritive quality for human consumption, and with Se also being observed to improve rates of growth (e.g. Blaxter, 1962) and reproduction (Andrews *et al*., 1968), a variety of procedures such as the use of subcutaneous injections, the importation of grains and forages from high Se areas and the use of ruminal pellets are now employed globally in attempts to overcome the problems associated with deprivation of this element (Underwood and Suttle, 1999).

Se deficiency syndromes had been either discovered or induced in 40 animal species before an equivalent condition in humans was reported

(Crounse *et al*., 1983). The essentiality of Se for human health was recognized when the element was shown to prevent Keshan disease (KD), an endemic cardiomyopathy (heart disease) that was first recorded at the beginning of the 20th century in north-east China (Tan, 1989). Se deficiency was suspected as a cause of KD in the 1960s, leading to the undertaking of large-scale studies in China, the results of which were first published in English by Chen *et al*. (1980). These authors suggested that KD is a 'biogeochemical' disease, coincident with areas where soils and staple crops are low in Se. Another overt Se deficiency syndrome, Kashin-Beck disease (KBD; an endemic osteoarthropathy that manifests as deformed joints, shortened fingers and toes and, in severe cases, dwarfism), has a similar north-east–south-west distribution in China, and indeed both KD and KBD can occur in the same areas of the country (Fig. 5.2). The diseases, however, have a complex aetiology, and both syndromes are not just attributable to Se deficiency (WHO, 1996).

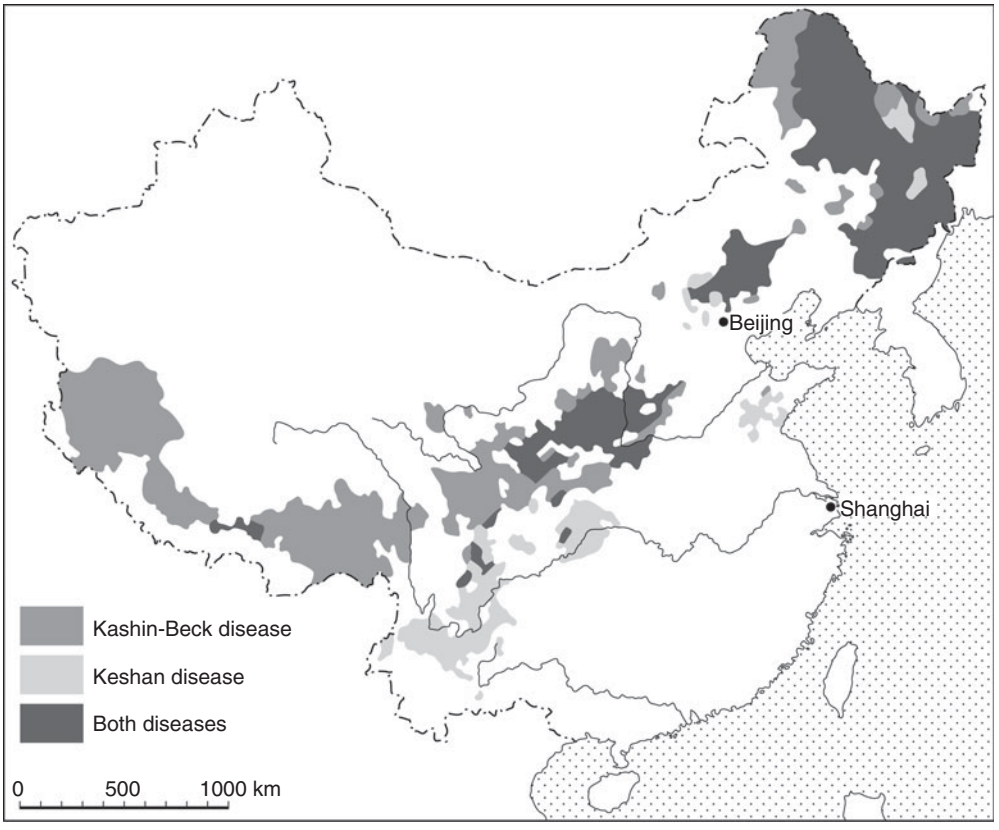

Fig. 5.2. Distribution of KD and KBD in mainland China. The north-east to south-west belt is coincident with very low water-soluble (i.e. plant-available) Se concentrations that are reflected in the composition of staple crops and the dietary intake of this element. However, other variables are likely to be additional factors in the pathogenesis of both diseases. (Reprinted from Tan *et al*., 2002. Copyright Elsevier. With permission.)

Both KD and KBD have also been reported in Korea, and an osteoarthropathy similar to KBD, but known as Urov disease, is associated with the Transbaikalia Province of eastern Siberia (Tan, 1989). Aside from these over diseases, information on Se deficiency in humans remains limited. Nevertheless, evidence is mounting that less-overt deficiency can cause adverse health effects that influence the immune system, the susceptibility to viral infections, reproduction, thyroid function, mood state and the development of chronic conditions such as cancer and cardiovascular disease (Brown and Arthur, 2001; Rayman, 2002). A deprivation of Se in the human diet may therefore not cause deficiency symptoms in the classical sense, but have a significant impact on health in a large number of ways. Combs (2001) considers that the number of Se-deficient people in the world is very likely to be in the range of 500 to 1000 million, with the vast majority having subclinical conditions. Accordingly, he indicates that food systems need to produce enough Se to provide a regular adult intake of at least 40 μg/day (a so-called lower nutritional level), with perhaps as much as 300 μg/day (a supranutritional intake) required to reduce the risks of cancer. The food systems of a number of countries in different parts of the world do not meet the lower nutritional requirement, and few populations have an Se intake approaching the supranutritional level. This generalized deficiency of Se in foodstuffs combined with a recognition that a number of factors (e.g. the use of high-sulfur (S) fertilizers that supply an element that can compete with Se for plant uptake; more intensive crop production; political and trade policies that determine the source of foodstuffs) have contributed to a reduction of the element in human food chains over recent decades (Frost, 1987; Gissel-Nielsen, 1998; Adams *et al*., 2002) has stimulated research in a variety of disciplines related to this subject area. Ultimately all Se can be traced back to geological sources, with the weathering of rocks releasing the element to soils from where Se can be transferred into plants and animals that comprise the human food chain. This chapter discusses the various anthropogenic and natural inputs and outputs of Se that determine the content of this element in soils, and evaluates the numerous factors that influence the transfer of Se from soils to human and animal foodstuffs. Special consideration is given towards the end of this chapter to how the soil–plant–animal transfer of Se in agricultural systems, including organic farming, can be manipulated to enhance dietary intakes of this element.

Selenium in Rocks and Soils

Taylor (1964) records the Se content of the continental crust as being very low, averaging 0.05 mg/kg. Some igneous rocks are associated with higher concentrations (Table 5.1), but relative to sedimentary materials, the amounts generally are not as great. Se concentrations in most limestones and sandstones rarely exceed 0.1 mg/kg, whereas shales have a typical content of 0.6 mg/kg (McNeal and Balistrieri, 1989). However, because of the presence of free iron oxides and other strong adsorbents, some argillaceous sediments

Table 5.1. The Se content of selected rocks. (Adapted from Plant *et al.*, 2004. Copyright Elsevier. With permission.)

Material	Selenium content (mg/kg)
Continental crust	0.05
Igneous rocks	
Granite	0.01–0.05
Mafic rocks	0.05
Ultramafic rocks	0.05
Volcanic rocks	0.35
Volcanic rocks, USA	<0.01
Volcanic rocks, Hawaii	<2.00
Volcanic tuffs	9.15
Sedimentary rocks	
Australian coal	0.21–2.50
Chinese stone-coal	<6500
USA coal	0.46–10.70
Limestone	0.03–0.08
Sandstone	<0.05
Mudstone	0.10–1500
Black shale, China	206–280
Shale, South Korea	0.10–41
Shale, western USA	1–675
Shale, Wyoming, USA	2.30–52
Phosphate rocks	1–300

can be regarded as geochemically anomalous. For example, organic-rich black shales may contain >600 mg Se/kg because of adsorption or complexation of the element (Plant *et al.*, 2004). Coal is typically enriched for the same reasons, while some phosphatic rocks can have concentrations that exceed 300 mg Se/kg.

Generally the Se status of soils can be described as low, with total concentrations ranging from 0.01 to 2 mg/kg, and the global mean equalling 0.4 mg/kg (Fordyce, 2005; Table 5.2). Recent work undertaken in Tibet, one of the least polluted areas on earth and ideal in studying the background concentrations of trace elements, found a mean total soil content of 0.15 mg Se/kg determined from samples collected at 205 locations (Zhang *et al.*, 2002). The total amount of Se in soils is typically influenced by the geochemistry of the parent material, and there is often a strong positive correlation between the soil and rock Se content in any given area. With shales often containing higher concentrations of the element than many other rocks (see above), it is of no surprise that Zhang *et al.* (2002) found greater amounts of Se in soils derived from the weathering of shales ($\overline{X}$ = 0.215 mg/kg, n = 28) than those associated with igneous, limestone or sandstone parent materials (0.133–0.155 mg/kg, n = 119). Working in England and Wales, Thornton *et al.* (1983) likewise found that calcareous and coarse sandy rocks supported soils with lower Se concentrations relative to those derived from fine-textured (i.e. clay,

Table 5.2. The Se content of selected soils. (Plant *et al*., 2004. Copyright Elsevier. With permission.)

Soil	Total Se (mg/kg)[a]	Water-soluble Se (mg/kg)
World, general	0.4	
World, seleniferous	1–5000	
China, general	0.02–3.80	
China, Se-deficient	0.004–0.48	0.00003–0.005
China, Se-adequate	0.73–5.70	
China, seleniferous	1.49–59	0.001–0.25
England and Wales	<0.01–4.70	0.05–0.39
Finland	0.005–1.25	
Greece, Se-deficient	0.05–0.10	
Greece, Se-adequate	>0.20	
India, Se-deficient	0.025–0.71	0.019–0.066
India, Se seleniferous	1–20	0.05–0.62
Ireland, seleniferous	1–1200	
New Zealand	0.1–4	
Norway	3–6	
Sri Lanka, Se-deficient	0.11–5.20	0.005–0.043
USA, general	<0.1–4.3	
USA, seleniferous	1–10	

[a]For interpretation of the total concentrations, the following guidelines can be applied: very low = <0.3; low = 0.3–0.5; average = 0.5–0.9; high = 0.9–1.5; very high = >1.5 (Wells, 1967).

mudstone, shale) parent materials. Because Se can replace S in metal sulfides such as chalcopyrite ($CuFeS_2$), pyrite (FeS_2) and sphalerite ((Zn,Fe)S), the greatest concentrations, up to 4.7 mg Se/kg, were found in soils influenced by the weathering (and mining) of metalliferous minerals. The breakdown of seleniferous rocks can lead to very high amounts of Se in the associated soils (Table 5.2). Thus, Yang *et al*. (1983), working in an area of endemic Se intoxication in humans living in Enshi County (China), found a mean total soil content of 787 mg Se/kg attributable to the weathering of stone-coals (typical content >300 mg Se/kg, though one sample exceeded 80,000 mg/kg), while Moxon (1937) found that the incidence of alkali disease in South Dakota is associated with clay soils derived from seleniferous Cretaceous Pierre Shale and Niobrara formations.

Since young or undeveloped soils retain many of the features of the parent material from which they formed, it can be expected that they will have similar Se concentrations. This was found by Wells (1967) when investigating soils with a minimum of profile development in New Zealand, although Zhang *et al*. (2002) found in their study of young soils in Tibet that limited pedogenesis could still result in a redistribution of Se in the soil profile. Certainly the relationship between the Se status of rocks and soils can be expected to be modified as time progresses and processes operative in the soil profile become more effective. In New Zealand, the Se content of

topsoils ($\bar{X}$ = 0.60 mg/kg) is typically greater than the concentrations found in the parent rocks ($\bar{X}$ = 0.42 mg/kg), indicating an accumulation of the element during soil formation (Wells, 1967). The assimilation arises from the retention of Se by organic material and clay-sized particles, especially kaolinite, gibbsite and ferric iron oxide. Elevated concentrations are consequently associated with clay-rich B horizons, iron-gibbsite concretion layers in brown loam soils, the illuvial iron-enriched horizons of podzols and peat (organic) soils. Similar findings have been found in the UK, where the distribution of Se in soil profiles is governed principally by its associations with iron and organic matter. This is exemplified by the accumulation of Se in the organic surface and sesquioxide-enriched subsurface horizons of stagnopodzols sampled in North Wales (Smith, 1983; Fig. 5.3), and by the enrichment of the element in peat soils ($\bar{X}$ = 1.20 mg Se/kg, n = 30; Thornton *et al*., 1983) in England and Wales. The latter authors also considered that the enrichment of Se that is often found in topsoil relative to subsoil horizons is attributable to the biogeochemical cycling of the element between the soil and plants.

While parent materials are recognized as the primary control influencing the total Se content of soils, there are other natural inputs of the element into soils. For example, there is a significant release of Se into the atmosphere of volcanic regions (with the element escaping as high-temperature volatile gases, so explaining why magmatic rocks are typically associated with low concentrations of the element) following which deposition into soils can occur. Humans also contribute to the Se signature of soils through a variety of deliberate (e.g. the use of Se-bearing fertilizers, an issue that will be discussed in more detail later in the chapter) and inadvertent (e.g. the applications of other fertilizers that contain Se as a trace constituent) additions. Nriagu and Pacyna (1988) calculated that globally 41,000 t Se/year was discharged into soils from industrial/municipal sources. The combustion of fossil fuels are an important source of the element to the environment, releasing volatile Se compounds into the atmosphere from where both gaseous and particle-bound forms of the element can be returned to soils by dry and (especially) wet deposition (Haygarth, 1994). Fly ash from coal-fired power stations is identified by Nriagu and Pacyna (1988) as the major anthropogenic source of Se to soils, and a feature of this material is that it can contain elevated concentrations of water-soluble Se that is plant-available and toxic to sensitive crops. However, using fly ash containing 18 mg Se/l as a soil conditioner applied at rates ranging from 0 to 50 Mg/ha (dry weight basis), Cline *et al*. (2000) found that concentrations of the element did not accumulate in maize (*Zea mays* L.), soybean (*Glycine max* (L.) Merr.) or wheat (*Triticum aestivum* L.) in amounts that would be of concern for plant, animal or human health. Refuse incinerator fly ash can also be enriched in Se, with Wadge and Hutton (1986) collecting samples from a UK refuse incinerator that had a mean content of 4.3 mg/kg. With large quantities of such ash being disposed to landfill that may subsequently be used for agricultural purposes, these authors investigated the transfer of Se from soil amended with fly ash to barley (*Hordeum vulgare*, var. Proctor) and cabbage

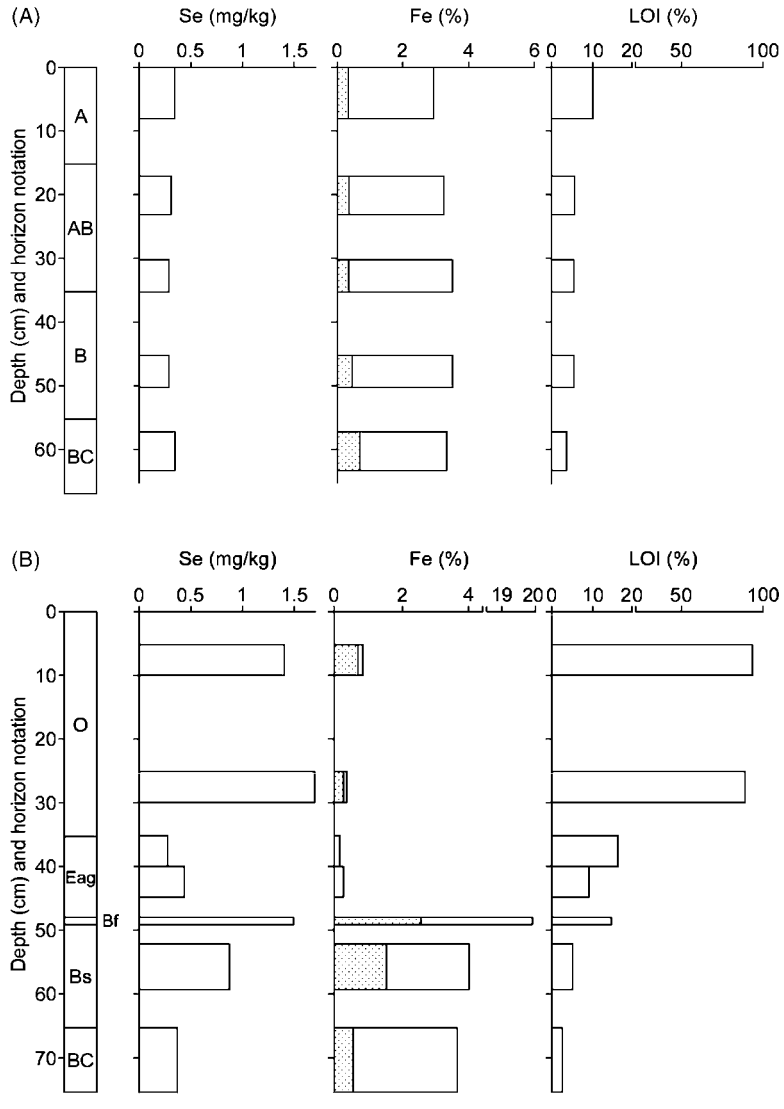

Fig. 5.3. Distribution of total Se, total and pyrophosphate-extractable (stippled) Fe, and organic matter (estimated by loss on ignition, LOI) in soil profiles of (A) the Denbigh series, a freely drained brown earth, and (B) the Hiraethog series, a ferric stagnopodzol, both developed on Silurian shale in North Wales. (From Thornton, 1983.)

(*Brassica oleracea*, var. Golden Acre) plants grown under greenhouse conditions. Elevated Se concentrations were recorded in the plants grown on the amended soils (e.g. contents in cabbage were up to 51-fold greater than the control values), with the authors suggesting that the alkaline nature of the fly ash (pH = 9.5) promoted the plant availability of the element.

Fertilizers and irrigation waters also supply Se to soils. Thus, ammonium sulfate fertilizers contain up to 36 mg Se/kg, while the relatively high concentration of the element in some phosphatic rocks is reflected in the composition of fertilizers manufactured from such source materials (Swaine, 1962 and Bisbjerg, 1972, cited by White *et al*., 2004). Single superphosphate can contain up to 25 mg Se/kg, but its general replacement with triple superphosphate (that typically contains 4 mg Se/kg) has led to a decline in the inadvertent fertilizer input of the element in many of the world's soils in recent years. Lime and manure is reported by Fordyce (2005) to have a typical Se content of 0.08 and 2.4 mg/kg, respectively, while sewage sludge contains 1–17 mg Se/kg. With restrictions in the European Union meaning that the dumping of sewage sludge at sea is no longer permissible, applications to land have increased in recent years. Where sludge is used in agriculture, it is necessary to monitor and control the concentrations in the soil of a number of potentially toxic elements, including Se. In the UK, for arable soils with a pH ≥5.0, the maximum permissible concentration (MAC) of Se in soil following the application of sewage sludge is 3 mg/kg, and the maximum permissible average annual rate of Se addition over a 10-year period is 0.15 kg/ha (DoE, 1996). For soils under grass, the MAC following sludge application is 5 mg Se/kg.

Countering the various natural and anthropogenic inputs of Se to soils is a number of mechanisms that can lead to loss of the element. This was recognized by Haygarth (1994) who summarized the total Se content of a soil by the mass balance equation:

$$\text{Se total} = (\text{Se}_p + \text{Se}_a + \text{Se}_f + \text{Se}_s) - (\text{Se}_{cr} + \text{Se}_l + \text{Se}_v)$$

where the subscripts are *p*, parent material; *a*, atmospheric deposition; *f*, fertilizers; *s*, sewage sludge; *cr*, crop removal following harvesting; *l*, leaching; and *v*, volatilization. Microbial activity leads to the evolution and release into the atmosphere of volatile Se compounds, primarily dimethylselenide (DMSe), from soils (Zieve and Peterson, 1987). The importance of the volatilization process is indicated by the total amount of Se evolved from soil to the atmosphere being estimated to be about almost the same as that released by anthropogenic activity (Tan *et al*., 1994). A variety of microorganisms are known to methylate Se, with factors influencing volatilization being essentially those that: (i) affect microbial activity, such as carbon supply, temperature and soil moisture content; and (ii) affect the availability of Se to the microorganism, such as irreversible adsorption, precipitation and complexation of the element. The latter factors also, in part, determine the vulnerability of Se to leaching. The fact that Se can be removed from soils by this process is dramatically illustrated by making reference to the Kesterson Reservoir site, California, in the USA. The reservoir comprises 12 ponds that in the 1980s collected shallow subsurface agricultural drainage water, essentially soil leachate (Presser, 1994), containing on average 300 µg Se/l as a result of contact with seleniferous soils in the catchment area (Ohlendorf, 1989). The resulting

consequences following the bioaccumulation of Se in the food chain at Kesterson were serious to local wildlife with overt effects on aquatic birds being very evident (although Oldfield (1996) notes that other toxicants may also have been involved).

The Soil–Plant–Animal Pathway of Se in Agricultural Systems

The Se content of plants does not always reflect the total concentrations found in the soils. This can be illustrated by making reference to a seleniferous area in County Limerick, Ireland (Webb and Atkinson, 1965). The Clare Shales found in this locality provide an example of a geochemically anomalous parent material, containing 5–30 mg Se/kg. Soils enriched in Se (i.e. containing >15 mg/kg) overlay the shales, but are also found downstream of the outcrop (Fig. 5.4). Pasture herbage containing excessive amounts of the element, however, is associated almost exclusively with the latter (alluvial) soils, and it is these plants that are toxic to horses and cattle, the animals showing symptoms of chronic Se poisoning in the form of unthriftiness, loss of hair and abnormal growth of the hooves. The authors concluded that the alkaline, organic-rich and poorly drained soil conditions of the alluvial areas promote the availability of Se to the pasture herbage.

Plants primarily obtain Se from the soil solution, but typically only a small proportion (0.3–7%) of the total soil content is in a dissolved form, and water-soluble concentrations are generally <0.1 mg/kg (Byers *et al*. (1938) and Adriano (1986), cited by Elrashidi *et al*., 1989; Table 5.2). Nye and Peterson (1975) found that the water-extractable Se concentrations determined from British soils were very low and *c*.1% of the total content, while Wang and Sippola (1990) analysed 128 samples collected from 13 European countries, finding that the water-extractable concentrations ($\bar{X}$ = 0.006 mg/kg, minimum and maximum = 0.001 and 0.024 mg/kg, respectively) were *c*.2% of soil total Se concentrations. Wang and Chen (2003) used a sequential extraction procedure to assess the various fractions of Se in three important agricultural soils sampled in Taiwan. The low percentage of soluble Se concentrations determined from these soils is illustrated in Fig. 5.5.

A significant association between the soil total and plant-available Se concentrations can be observed. For example, Yang *et al*. (1983) working in the seleniferous region of Enshi County, China, found a very strong positive correlation between total and water-soluble soil Se concentrations (r = 0.99, P < 0.01), while Wang and Sippola (1990) established that there was a moderately strong positive correlation (r = 0.63, P < 0.001) between these two variables determined from 128 European soils. A strong correlation can also be observed when examining the relationship between soil and plant Se concentrations. Yang *et al*. (1983) found such an association (r = 0.83, P < 0.01) between the Se content of cereals and soybeans and the water-soluble soil concentrations in Enshi County, China. However, in contrast Wang and Sippola (1990) calculated a moderately weak (though still highly significant) correlation coefficient (r = 0.33, P = < 0.001) in their work investigating the association between

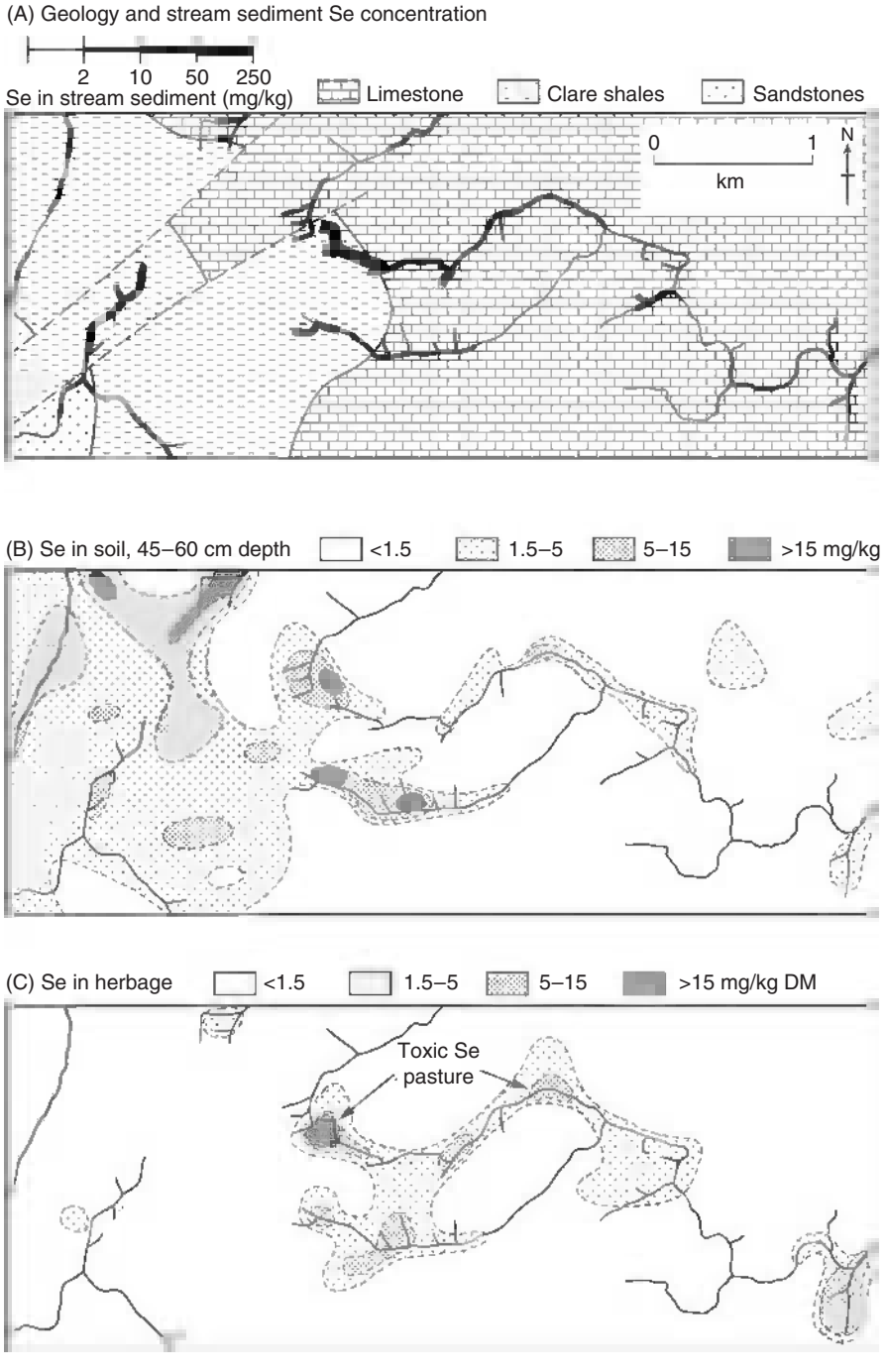

Fig. 5.4. Geology and stream sediment (A), soil (B) and pasture herbage (C) Se concentrations (mg/kg) of a study area investigated in County Limerick, Ireland. Toxic pastures are associated with alluvial areas where soil conditions promote the availability of Se to plants. (Reprinted from Webb and Atkinson, 1965. Copyright Macmillan Publishers. With permission.)

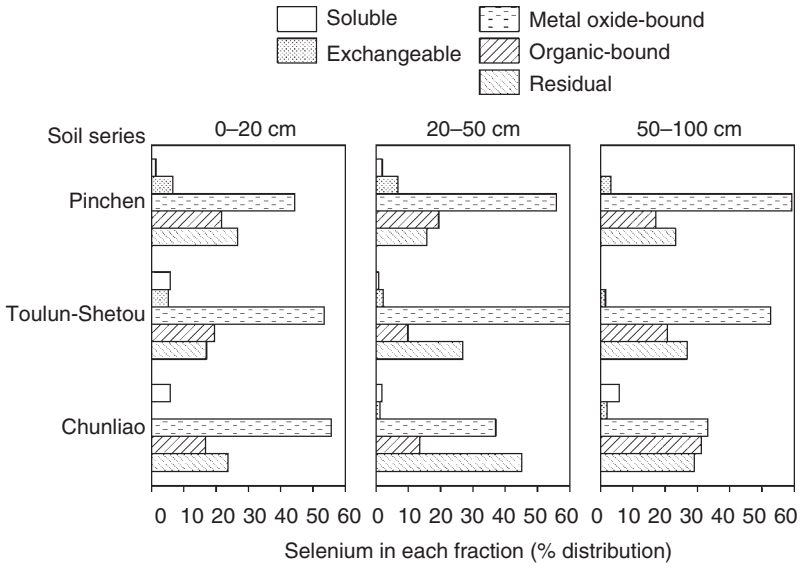

Fig. 5.5. The fractions of Se associated with three important agricultural soils in Taiwan. The different fractions were determined following a sequential extraction procedure: essentially, the metal oxide-bound, organic-bound and residual components are not readily plant-available. Soluble Se is the most immediate fraction that is available to plants, but the exchangeable component (i.e. that held by the adsorption complex of the soil) is easily released into solution by ion exchange and is also important in this respect. (Reprinted from Wang and Chen, 2003. Copyright Elsevier. With permission.)

soil water-extractable and winter wheat Se concentrations. Such a modest correlation coefficient reflects the fact that plant Se uptake is affected by many soil and plant factors (Mikkelsen *et al*., 1989). Thus, a number of variables, including the prevailing pH and redox conditions, organic matter content, texture and mineralogy, the chemical form or speciation of Se, and the presence of competitive ions, are important soil factors that influence the soil solution Se content and the phytoavailability of this element.

The chemical species of Se found in soils are:
Selenide (i.e. 2^- oxidation state): Se^{2-}, HSe^-, H_2Se^0
Elemental Se (i.e. 0 oxidation state): Se^0
Selenite (i.e. 4^+ oxidation state): SeO_3^{2-}, $HSeO_3^-$, $H_2SeO_3^0$
Selenate (i.e. 6^+ oxidation state): SeO_4^{2-}, $HSeO_4^-$, $H_2SeO_4^0$
Organic Se

Redox potential (Eh) and pH are the most important parameters controlling the solubility and speciation of inorganic soil Se. Elrashidi *et al*. (1989) recognize three main categories of soil with respect to the inorganic Se species they contain. In gley soils of low redox, inorganic Se predominates as the relatively insoluble selenide and elemental forms; however, in soils containing soluble

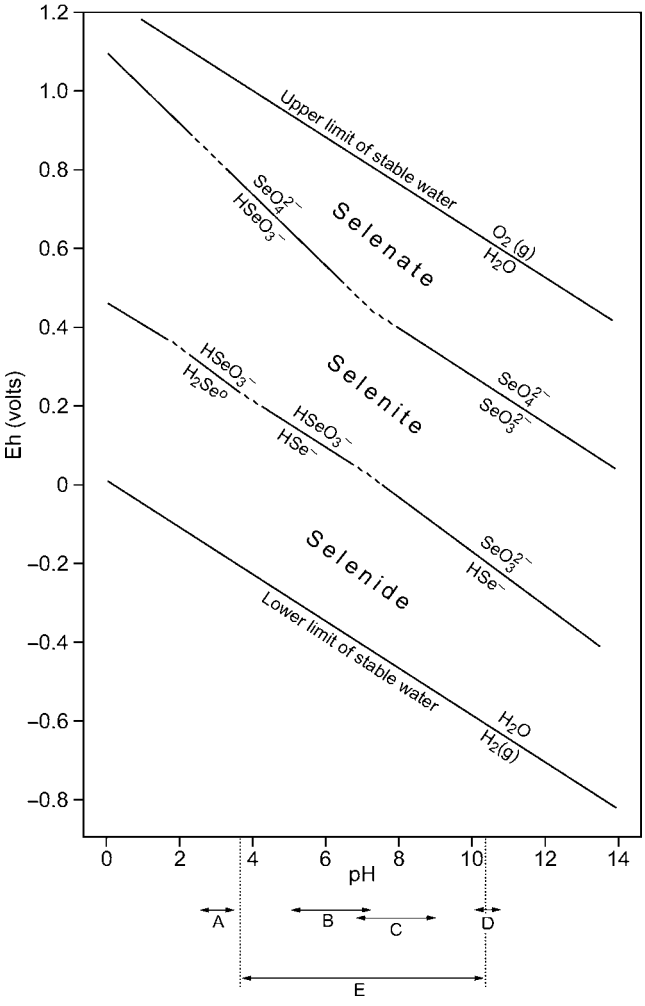

Fig. 5.6. The relation of redox potentials of Se solution species to pH. Key (Brady and Weil, 1999): A = extreme pH for acid peat and acid-sulfate soils; B = range in common for humid region mineral soils; C = range in pH common for arid region mineral soils; D = attained only by alkali mineral soils; E = extreme range in pH for most mineral soils. (Modified from Elrashidi *et al.*, 1989. Copyright Soil Science Society of America. Reproduced with permission.)

Cd^{2+}, Cu^{+}, Cu^{2+}, Pb^{2+} and Sn^{2+} metallic selenides form at a higher redox than that required for the formation of elemental Se. Consequently, at low redox, Se in solution is mainly in the selenide (HSe^-) form (Fig. 5.6). For soils of moderate redox (i.e. a mildly oxidizing soil environment) in humid regions, selenite species prevail: $HSeO_3^-$ at low pH, whereas SeO_3^{2-} is the major species in soils of higher pH. In the well-aerated (i.e. high redox) soils of arid regions, selenate (SeO_4^{2-}) is the major species of Se found in the soil solution. Overall, selenate

and selenite can be recognized as the major species of inorganic Se to be found in cultivated soils. Since selenate is more soluble than selenite, phytoavailability is enhanced in soils where the former species dominate (Gissel-Nielsen and Bisbjerg, 1970). In an investigation of seleniferous soils from South Dakota and County Meath, Ireland, Nye and Peterson (1975) found that selenate predominates in soils of the former region, while selenite is the most important form of soil Se in the latter area. On a quantitative basis soluble selenate, with concentrations of 2 mg/kg, accounted for up to one-half the total Se content of the South Dakota soils. In contrast the Irish seleniferous soils contained <0.2 mg/kg soluble Se, and often only 0.02 mg/kg.

Relative to the inorganic species of Se found in soils, there is less information available regarding the identity and behaviour of organic compounds that contain the element. Neal (1995) states that many studies investigating Se speciation in soils have generally identified soluble organic Se as a single entity because of the analytical problems encountered in determining the various organic forms. Nevertheless, organic Se can be an important component of some soils, accounting for up to 50% of the soil total content of this element (Plant *et al*., 2004). Selenomethionine (SeMet) has been extracted from soils and is two to four times more bioavailable to plants than inorganic selenite. Another organic form, selenocysteine (SeCys), has a lower bioavailability than SeMet. The bioavailability of the different Se species in soils can thus be summarized as:

selenate > SeMet > SeCys > selenite > elemental Se > selenide

In addition to influencing the speciation of Se, soil pH also affects the capacity of organic matter, clay minerals and iron oxides to adsorb the element, with the binding capacity increasing as the pH decreases. Thus, in pots initially containing sandy soils to which 0.5 mg Se/l had been applied, a subsequent increase in the proportion of added clay or peat led to a decrease in the accumulation of Se by spring wheat grain (*T. aestivum* L., var. Drabant) and winter rape plants (*Brassica napus* L., var. Emil). The concentrations of Se in both crops grown in soils of pH 5 were lower than the contents of plants grown in soils of pH 7 (Johnsson, 1991). Hamdy and Gissel-Nielsen (1977) investigated the fixation of selenite by three silicate clays (kaolinite, montmorillonite and vermiculite) and ferric oxide (Fe_2O_3) at different pH values (Fig. 5.7). Maximum fixation of selenite by the silicate clay minerals was observed between pH 3 and 5. The fixation decreased with increasing pH, an observation that the authors attributed to an exchange reaction, with hydroxyl ions displacing selenite ions into solution. However, with 80% of the selenite fixation being observed during the first 24 h of the experimental procedure, and 20% of the fixation occurring during the remaining 10-day study period, there appears to be two mechanisms through which silicate clay minerals can remove selenite out of solution. The first is a surface exchange reaction that proceeds rapidly; the second slower process is attributable to selenite-forming complexes or precipitates with iron originating from the decomposition of the clay minerals.

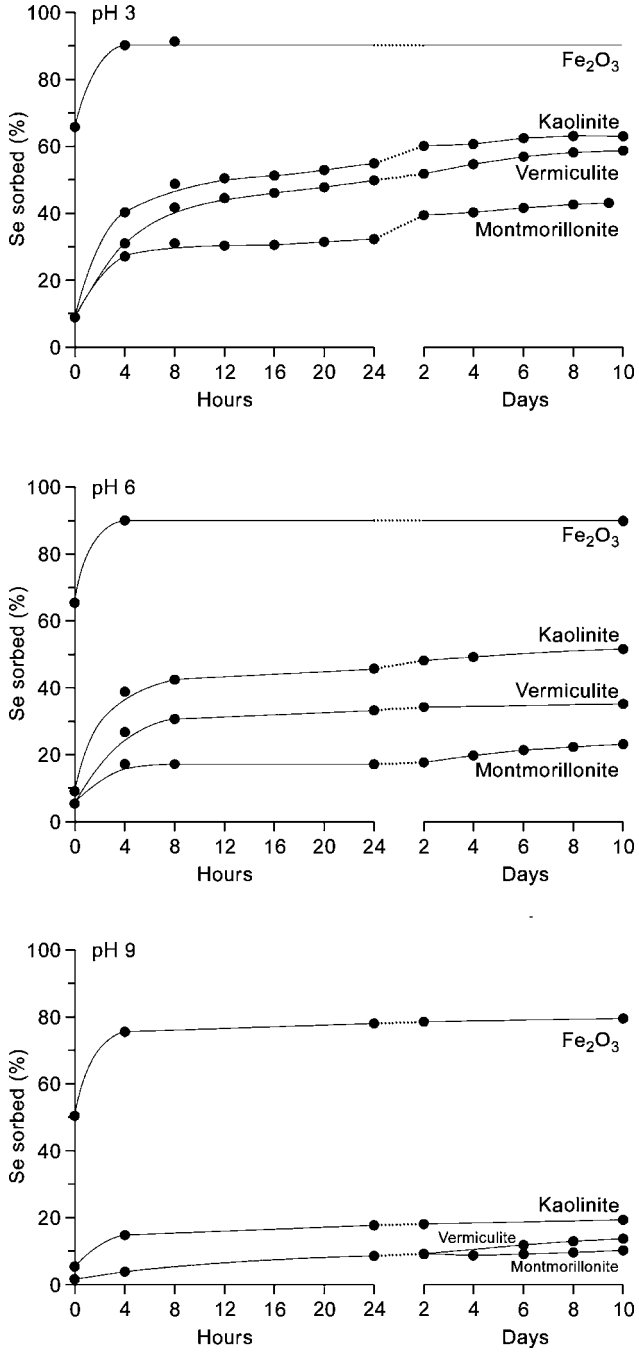

Fig. 5.7. The removal of selenite from solution as influenced by soil mineralogy, pH and time. (Hamdy and Gissel-Nielsen, 1977. Copyright Wiley. Reproduced with permission.)

Figure 5.7 also illustrates how the mineralogy of soil constituents influences the fixation of Se. Kaolinite, a 1:1-type silicate clay mineral, is more effective at fixing selenite than the 2:1-type clays, but Fe_2O_3 has a higher fixation capacity for selenite than any of the silicate clays investigated in this study. Furthermore, the fixation of selenite by Fe_2O_3 occurs very rapidly, with Hamdy and Gissel-Nielsen (1977) suggesting that exchange reactions and the formation of ferric selenite precipitates are important in removing this form of Se out of solution. Use of a fractionation procedure demonstrated that once fixed by Fe_2O_3, *c*.30% of the selenite was 'available with difficulty' to plants; in contrast, the silicate clays, especially the 2:1-type, were more readily capable of releasing this species of Se back into (plant-available) solution. In making these observations, it needs to be appreciated that soils in the field present a considerably more complex system than the silicate clays and Fe_2O_3 used in the laboratory work described here. Relative to the simple silicate clay mineral and Fe_2O_3 systems discussed above, Hamdy and Gissel-Nielsen (1976) found that mineral and muck soils were more effective in retaining selenite following fixation. Forty-six per cent to 66% of the selenite fixed by these soils was available with difficulty.

A variety of chemical amendments such as lime and gypsum are supplied to soils to improve their physical and chemical properties, but such additives can also have measurable effects on the solubility of Se in soil systems with consequent implications to plants (Elrashidi *et al*., 1989). The liming of acid soils to raise pH increases the Se content of the soil solution by ion exchange and through increasing the solubility of ferric hydroxide–ferric selenite complexes (Geering *et al*., 1968). With soil pH influencing the oxidation state of Se, it is also possible that a long-term response to liming may involve the slow oxidation of selenite to the more plant-available selenate form. Liming can thus be expected to increase the soil solution Se concentrations for a variety of reasons, which can be reflected in a greater accumulation of the element in plants. For example, Yang *et al*. (1983) noted that the traditional use of lime was one of the factors implicated in the selenosis observed in Enshi County, China, the fertilizer rendering the soil Se more available to staple food crops.

The presence of 'competitive' ions in the soil – which may occur naturally or via anthropogenic additions such as fertilizer amendments – can also influence the amount of Se that is accumulated by plants, and in this respect sulfate (SO_4^{2-}) and orthophosphate (PO_4^{3-}) can be important. Such ions influence the Se content of plants by either competing for fixation sites in the soil, by acting as an antagonist and so reducing the uptake of the element by plants, or by diluting the Se content of vegetation through causing increased plant growth (Mikkelsen *et al*., 1989). Phosphate fertilizers have previously been noted in this chapter to be a potential source of soil Se, but in terms of PO_4^{3-} being a competitive ion influencing the content of this element in plants, conflicting results have been reported. Most investigations have found that soil additives of phosphorus (P) increase Se accumulation in plants, with PO_4^{3-} having a greater affinity for adsorption than selenite, and therefore promoting the displacement of this species of Se into solution.

P fertilization may also encourage greater root growth, thereby providing the plant with a greater volume of soil from which Se can be extracted. However, Mikkelsen *et al*. (1989) are of the opinion that except for plants growing in conditions with inadequate soil Se to meet animal nutritional needs, Se–PO_4^{3-} interactions are generally not of great consequence. In contrast, the presence of SO_4^{2-} in the soil generally reduces the concentration of plant Se, either by inducing increased plant growth that leads to a consequent dilution of the element in the plant biomass, or by acting as an antagonist that interferes with the absorption of Se (the latter mechanism having more influence on selenate uptake than selenite absorption). With SO_4^{2-} competing with selenate for plant uptake, Terry *et al*. (2000) note that SO_4^{2-} salinity drastically inhibits the absorption of this species of Se. In contrast, chloride salinity has significantly less effect on selenate uptake, though generally there is a small decrease in plant shoots accumulating Se with increasing soil salt content.

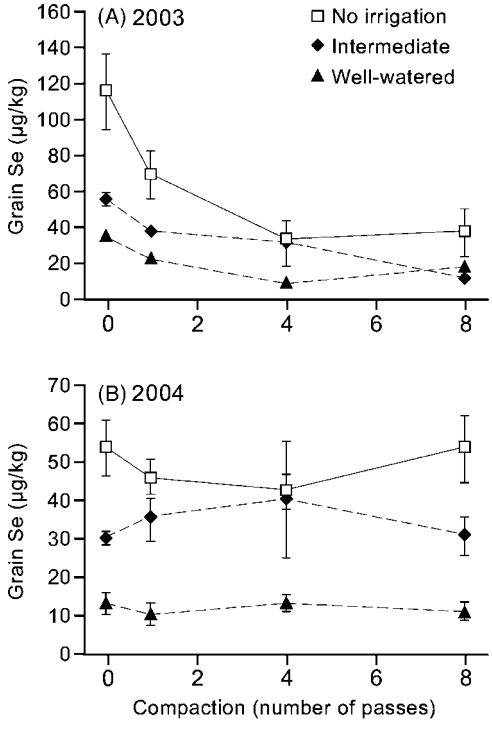

Fig. 5.8. Effects of irrigation and soil compaction on the Se content of wheat grain in (A) 2003 and (B) 2004. The figure illustrates how in both years of study increasing irrigation treatment leads to a reduction in the grain Se concentration. Furthermore, in 2003, increasing soil compaction is also associated with a lowering of the Se content of wheat grain. Vertical bars represent ± the standard error. (Reprinted from Zhao *et al*., 2007. Copyright Elsevier. With permission.)

When investigating how the soil controls the phytoavailability of Se, most studies have concentrated on the various chemical factors involved (pH, Eh, etc.), and there have been few reports on the potential effect of soil physical conditions on uptake of the element by plants. This issue was recently addressed by Zhao *et al*. (2007) who undertook field experiments in the UK to investigate the effect of soil compaction and irrigation on the Se concentrations found in wheat grain (*T. aestivum* L., cv. Clare). Irrigation resulted in a large decrease of grain Se by 30–75% (Fig. 5.8), a finding that the authors considered was attributable to dilution of plant Se content following increased grain yield, a competition of SO_4 added in the irrigation water, and greater leaching losses. Increasing soil compaction also lowered grain concentrations in the 2003 field trial. Overall, the effects observed have implications for human nutrition because the wheat grain concentrations found in this study vary from very low to sufficient according to the soil physical conditions in the field.

The preceding paragraphs indicate that there are a large number of soil variables that influence the phytoavailability – and plant concentrations – of Se. But while soils are recognized as the main source of plant Se, additionally the concentrations of this element found in vegetation are influenced by other inputs and outputs. Thus, vegetation can acquire the element via foliar absorption, as found inadvertently by Davies and Watkinson (1966) who, while investigating Se fertilization rates, sprayed a solution containing selenite on to pasture foliage, subsequently finding high herbage concentrations. Plants are also able to absorb gaseous Se compounds from the atmosphere, while enzymatic activity in vegetation leads to the volatilization of the element and its release (mainly from the roots) from the plant, although soils are recognized as the main source of volatile Se (Zieve and Peterson, 1987; Terry and Zayed, 1994).

Regarding the nutrition of higher plants, the essentiality of Se remains a controversial and unresolved issue (Terry *et al*., 2000), but all plants are capable of absorbing the element from soils. The uptake of selenate and selenite (i.e. inorganic species) has been investigated extensively relative to the absorption of organic forms of the element although it is known that the latter must be considered as sources of plant-available Se (Abrams *et al*., 1990; Ajwa *et al*., 1998). Selenate and selenite uptake into the roots of plants does not appear to follow the same pathway and, following absorption, a differential preference for the translocation of these inorganic species from the root to the plant shoot is evident (Neal, 1995). Selenite is rapidly converted to selenate and organic Se compounds, while selenate moves unchanged through the plant, and may attain concentrations far exceeding those of the soil solution, reaching the leaves where conversion to selenite and then to organic forms can occur.

Like all mineral elements found in the tissues of vegetation, the Se content of plants is influenced by their maturity and the season (Bisbjerg and Gissel-Nielsen, 1969; Gissel-Nielsen, 1975). Concentrations also vary according to plant part (i.e. root, leaves, seeds, etc.), plant species and differences between varieties/cultivars within a species (Thornton, 1983; Alloway,

2005). For example, Wan *et al*. (1988) looked at the Se uptake and partitioning by crops grown in soils to which varying rates of selenate had been applied. For barley (*H. vulgare* L.), beet (*Beta vulgaris* L.) and tomato (*Lycopersicon esculentum* Mill.), the edible portion of the plant contained much less total Se than the generally inedible parts. Apart from the part within a given plant, differences in Se content were also observed between the plant species. Regarding variations between plants, members of the *Cruciferae* family are recognized as accumulating more Se than other crop species (e.g. Bisbjerg and Gissel-Nielsen, 1969). This was first reported by Hurd-Karrer (1935) who proposed that plants with a high-sulfur requirement – such as those of the *Cruciferae* and *Leguminosae* families – also have a tendency to absorb elevated amounts of Se. Fleming (1962) examined the concentrations associated with plants growing on Irish seleniferous soils, and found higher amounts of Se in *Cruciferae* (e.g. cabbage (*B. oleracea capitata* L., var. Wyatts Early)), *Leguminosae* (e.g. white clover (*Trifolium repens* L.)) and *Liliaceae* (onion (*Allium cepa* L., var. James Keeping)), than in *Compositae* (e.g. Daisy (*Chrysanthemum maximum* L.)), *Graminiae* (e.g. wheat (*Triticum vulgare* L., var. Atle)) and *Umbelliferae* (e.g. carrot (*Daucus carota* L., var. St. Valery)).

Rosenfeld and Beath (1964), looking at vegetation established on seleniferous soils, recognized three general groups of plants according to their ability to accumulate Se. Most plants contain only low concentrations of the element (i.e. <25 mg/kg dry matter (DM), and more often <5 mg/kg DM) and can accordingly be termed non-accumulators, although rarely vegetation in this category can contain in excess of 100 mg/kg DM if grown on seleniferous soils. Most cultivated crops, grains and native grasses fall into this group of plants, and much of the Se in these non-accumulating species is found in the form of protein-bound SeMeT (Mayland *et al*., 1989) indicating the capability of plants in converting inorganic soil Se to organic forms. A more limited number of specialized plants associated with Se-rich soils can assimilate higher concentrations in their foliage. Such plants can be divided into two groups: primary accumulators (hyperaccumulators) that store Se mainly in organic compounds, and secondary accumulators (indicator species) where the Se is generally present as selenate and organic forms (Neal, 1995; Ellis and Salt, 2003). The former group can assimilate from hundreds to several thousand mg Se/kg DM in their tissues, while secondary accumulators are typified by concentrations in the hundreds of mg/kg DM. The Se in accumulator plants is mostly water-soluble and found in non-protein forms like methylselenocysteine (MSeC; Mayland *et al*., 1989). This exclusion of Se from protein incorporation is a detoxification mechanism that allows accumulator plants to avoid Se-induced phytotoxicity. Although seleniferous soils can be found on all of the continents permanently settled by humans, clinical signs of Se toxicity affecting plants have only been observed in nature in the region of Enshi County, China. Here, Yang *et al*. (1983) report a number of symptoms – including the mottling of injured soybean leaves, and the discoloration of maize leaf edges – associated with crops growing on seleniferous soils.

Extreme accumulation of Se has been reported in the fruit of some species within the *Lecythidaceae* neotropical family of trees (Broadley *et al*., 2006). The well-known edible Brazil nut (*Bertholletia excelsa* Humb. & Bonpl.) is associated with this family and contains high concentrations of Se up to 150 mg/kg (Tinggi and Reilly, cited in Lyons *et al*. 2003), while the ingestion of other nuts from the same family, such as the coco de mono tree (*Lecythis ollaria* Loefl.), has been known to induce selenosis in human subjects. Kerdel-Vegas (1966) reported that in Venezuela coco de mono nuts can contain 2.23% Se DM, with their ingestion causing hair loss, nausea, vomiting and, for some consumers, death. Just as bioavailability is an important factor in terms of plant uptake of Se, it is also a factor in the diets of humans (and animals). Broadley *et al*. (2006) report that SeMeT is the dominant species of Se in the Brazil nut, a form that is readily bioavailable and absorbed in the human digestive system (Fordyce, 2005). However, exceptions such as the above aside, foodstuffs associated with the two accumulator groups of plants do not usually contribute significantly to the Se intake of humans and animals (WHO, 1987). Instead most plant foodstuffs utilized by humans are associated with the non-accumulator group of vegetation, and while these can contribute large dietary amounts of Se if grown in seleniferous areas, when grown on 'normal' soils the plant tissues generally contain <1 mg/kg DM. This is illustrated by Mikkelsen *et al*. (1989) who compiled information relating to the major food chain crops grown in the USA. The mean Se concentration of any one crop varies by a factor ranging from *c*. two to 20, probably reflecting differing soil properties, but when grouped by crop class differences were apparent. Root and bulb crops were associated with the highest mean Se concentration ($\bar{X}$ = 0.407 mg/kg DM), followed by field crops ($\bar{X}$ = 0.279 mg/kg DM), leafy vegetables ($\bar{X}$ = 0.110 mg/kg DM), seed vegetables ($\bar{X}$ = 0.066 mg/kg), vegetable fruits ($\bar{X}$ = 0.054 mg/kg DM) and tree fruits ($\bar{X}$ = 0.015 mg/kg DM).

The concentrations of Se in forage plants have obvious implications to domesticated animals. Rosenfeld and Beath (1964) defined three different types of Se poisoning in livestock as being attributable to the ingestion of plants enriched in the element:

- Acute poisoning – results following the ingestion, usually of a single feeding, of toxic quantities of Se in the form of highly seleniferous accumulator plants. Death often follows within a few hours following ingestion, but this type of poisoning is rather rare under field conditions since grazing animals generally avoid the relatively unpalatable Se accumulator plants except in times of pasture shortage.
- Chronic poisoning of the blind staggers type – blind staggers, a condition where affected individuals wander, stumble, have impaired vision and eventually succumb to respiratory failure, has been reported in animals that consume moderate amounts of Se-enriched indicator plants over a period of weeks or months. The syndrome has not been replicated by the administration of pure Se compounds, and it is possible that

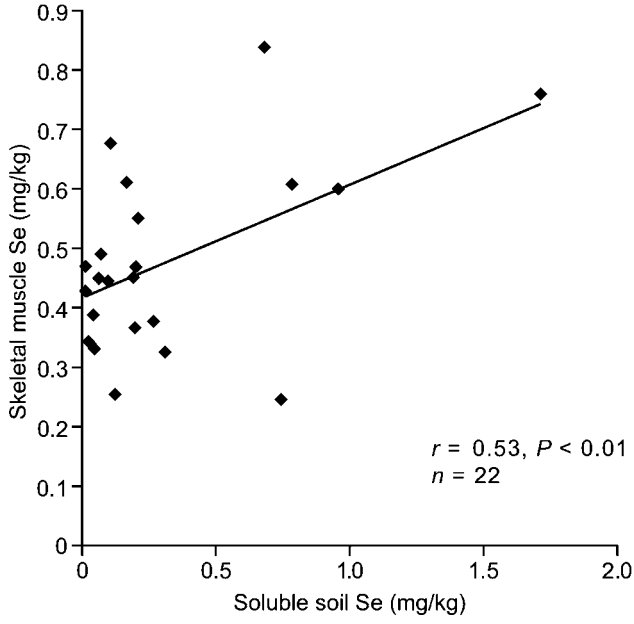

Fig. 5.9. The association between soluble soil Se and skeletal muscle concentrations of the element determined from samples collected from different geographic areas of North Dakota, USA. (Reprinted from Hintze *et al.*, 2001. Copyright American Chemical Society. With permission.)

alkaloids or other toxic substances found in the seleniferous vegetation are contributing to the condition (James *et al.*, 1989).

- Chronic poisoning of the alkali disease type – associated with the consumption of foodstuffs containing >5 mg Se/kg over weeks or months (in seleniferous areas, this threshold is generally accepted as the dividing line between toxic and non-toxic feeds).

Toxic conditions aside, livestock consuming forage of elevated Se content can accumulate higher concentrations of the element in tissues and organs compared to animals grazing pastures with lower Se content. This was found by Hintze *et al.* (2001) who investigated 21 ranches associated with low or high forage Se content in 5 distinct geographic regions throughout North Dakota, USA. Both soluble soil and grass Se concentrations were positively correlated with the content of the element in skeletal muscle (soluble soil: $r = 0.53$, $P < 0.01$ (Fig. 5.9); grass: $r = 0.63$, $P < 0.01$), and the authors concluded that the total Se concentration of beef may be increased by raising the animal in a high Se area.

The association between soil and forage and the Se status of livestock as demonstrated by Hintze *et al.* (2001) is in spite of the complexities regarding the soil–plant–animal pathway of the element in agricultural systems. In this respect, a number of factors influence the utilization and implications of dietary Se to animals, including:

- The form of Se in the diet – generally SeMeT is more readily absorbed following ingestion by animals than selenite, selenate or SeCys (Mayland *et al*., 1989).
- Nutritional factors – for example, vitamin E deficiency can increase the susceptibility of animals to Se toxicosis (WHO, 1987). Vitamin E also decreases the level of dietary Se needed to prevent deficiency disease in animals.
- The effects of other mineral elements in the diet – for example, arsenic may protect against Se toxicity (WHO, 1987).
- The plant species composition of the diet and the amount of ingested soil – livestock can deliberately and accidentally consume soil (Abrahams, 2005). The implications of this direct soil–animal pathway of Se are not necessarily straightforward, but both *in vitro* and *in vivo* experimental procedures have indicated the potential of ingested soils in enhancing the bioavailability of the element to animals. In New Zealand, rumen and duodenum liquors from sheep were used for an *in vitro* study that showed up to tenfold increases in Se content of the digestive fluids following contact with soil (Healy, 1970). Subsequent *in vivo* feeding trials indicated that ingested soil can increase lamb blood Se by up to 60% over a 6-week period (Healy, 1973). Relatively little work has been undertaken on the ability of soils to supply essential mineral nutrients to animals, but the findings detailed above can have practical implications: under farming conditions in England and Wales, in the autumn when sheep were Se-deficient, the intake of soil appeared to increase blood Se concentrations (Russell, 1988).

Dietary intakes of Se by livestock are also influenced by the plant species consumed. In moderate to low Se environments, lucerne (*Medicago* sp.) takes up more Se than other forage crops (Plant *et al*., 2004), and pasture herbage species grown in low Se soils can show some variability in their concentration of the element. For example, Davies and Watkinson (1966) determined the concentrations of Se in four pasture herbage species growing on Atiamuri sand, a New Zealand soil associated with vegetation supporting lambs that experience ill-thrift and WMD. Browntop (*Agrostis tenuis* Sibth.) consistently showed the highest concentrations of Se, and white clover the lowest. Depending on the season, the ratio of Se in browntop to that in white clover ranged from 1.4 to 2.9.

Modifying the Soil–Plant–Animal Pathway of Se to Enhance Human Dietary Intakes

The main pathway of Se exposure in the general population is through food, and despite the complexities of the soil–plant–animal pathway, the amount of the element in the human diet is largely determined by the Se content of the soil and its transfer up the food chain into plants and animals. Analysis of human blood samples from persons living in different areas of the world indicates an association between blood Se and soil concentrations (WHO, 1987).

Low blood Se is thus found in countries with soils depleted in the element (such as New Zealand, Scandinavia and the KD and KBD areas of China), while high blood Se content is coincident with regions of elevated soil concentrations (e.g. the seleniferous areas of Venezuela and China). The geographical distribution of Se in soils is very uneven and large areas of the world can be characterized as Se-deficient or Se-toxic (Oldfield, 2002). In particular, Se-deficient regions are more widespread than adequate or toxic areas, and with the realization that on a worldwide scale vast land areas do not supply enough of this element for the optimal nutrition of humans, procedures to enhance Se intakes are being urgently investigated and implemented.

Lyons *et al*. (2003) outline a number of strategies that could result to increasing human Se intakes, including improved education that can lead to greater consumption of Se-enriched foods or supplements, and food fortification procedures such as the production of selenized salt as occurs in China (Tan, 1989). While such initiatives have proved to be successful in countering micronutrient deficiencies generally, they tend to be expensive and often fail to reach all the individuals at risk, and they require appropriate legislation. There are also problems regarding the supplementation of livestock with Se, since this procedure is considered unlikely to increase levels of the element in the human population (Lyons *et al*., 2003). Breeding for improved Se uptake and concentration in the edible parts of crops may be an effective and sustainable strategy but for wheat, one of the most important sources of the element for humans, such a genetic biofortification strategy does not seem to be as practical or productive as fertilization (i.e. agronomic biofortification; Lyons *et al*., 2005a). The latter strategy has been shown to be an effective way to increase the amount of Se in crops, with the element being applied directly to soils, foliage or seeds (Mikkelsen *et al*., 1989). Regardless of the method of application, compared to selenite forms of the element, selenate additives generally result in much higher plant Se concentrations. Organic additives have also been investigated as a source of plant Se, as studied by Carvalho *et al*. (2003) who supplemented soils used for growing four agricultural crops with either an inorganic (selenite) or organic (SeMeT) form of the element. The results showed that application with the inorganic form of Se led to higher accumulation in the plants, with a maximum of 97.5% retained in the edible portion of lettuce (*Latuca sativa* var. Black Seeded Simpson). The amount of Se left in the soil following application, plant uptake and harvesting, was not high enough to inhibit the germination of any new seeds, and the authors concluded that several crops could be grown and supplemented in the same soil.

The concept of adding fertilizers to improve the Se status of deficient crops and soils is not new, and following the discovery of the essentiality of the element for animals in the late 1950s, it was not long before trials were being followed to investigate the feasibility of correcting deficiencies by amendments. For example, Grant (1965) in New Zealand undertook preliminary trials that indicated the possibility of raising the Se content of pastures in deficient areas by top-dressing with soluble sodium selenite sprayed at rates not exceeding 1 oz Se/acre (i.e. ≈71 g/ha). Higher rates of application were associated with pastures that proved toxic to sheep.

Se fertilization was pioneered in New Zealand, a country with extensive areas of Se deficiency attributable to the volcanic rocks of the North Island, and the excessive leaching of the element from soils in the South Island (Oldfield, 2002). Reilly (1996) notes that Se fertilization is not legally enforced by the New Zealand government, but is strongly encouraged in areas deficient in the element. Sodium selenate is the most common additive – having the advantage over fertilizers that contain selenite by being the more readily available form for plant uptake – with residues in the soil being rapidly converted into less plant-available forms that prevents any build up to toxic concentrations. Oldfield (1996) notes that slow-release fertilizers containing barium selenate have been subsequently used with success, the slow-release principle being environmentally desirable since it reduces the total amount of Se needed.

Se fertilizer amendments in New Zealand are made solely for the benefit of livestock production, but in Finland – the only country where such additives are required by law – the government decision in sanctioning their use was made additionally because of human health concerns. Typically, the Se concentrations of agricultural soils in Finland can be categorized as very low to low (i.e. 0.2–0.3 mg/kg; $\bar{X}$ = 0.21 mg/kg), and the acidic properties and high content of adsorptive oxides favours a low plant bioavailability of this element (Yläranta, 1985; Aro *et al*., 1996; Hartikainen, 2005). The dietary intake of Se was found to be extremely low in Finland during the 1970s, reflecting the content of domestic agricultural products (Eurola *et al*., 2003). Consequently, in 1984 sodium selenate started to be added to fertilizer slurries in order to obtain a uniform concentration of the element in granules that were then added to soils with the primary objective of raising cereal grain concentrations of the element (Hartikainen, 2005). From 1984 to 1990, two supplementation levels were used: 6 mg Se/kg for compound fertilizers intended for fodder and hay production, and 16 mg Se/kg for fertilizers used for grain production. In 1990, the system was simplified by applying the 6 mg Se/kg level to all compound fertilizers, but in 1998, the supplementation level was raised to 10 mg Se/kg because of the observed falling trends in the Se content of foods, feeds and human serum, and the decreasing dietary intake (Eurola *et al*., 2003). Since 1998, the mean Se content of wheat and rye has doubled (Fig. 5.10), concentrations in milk have increased by 30%, and (since 1999) the content in meat has been raised by *c*.30–50%. The average daily dietary intake has increased by about 20% since 1999, being currently higher than in most other European countries. However, despite now meeting recommended dietary intakes of Se, the health outcomes for the Finnish human population have proved impossible to evaluate to date because of the multifactorial causes of disease. No adverse environmental effects – such as detrimental concentrations of Se found in groundwater or freshwater – of the fertilization strategy have yet been observed: Eurola *et al*. (2003) state that utilization of fertilizer Se by crops is usually <10%, and it is assumed that most of the Se not used by plants is immobilized in the soil. A rough calculation suggests that the total Se content of the 20 cm thick plough layer has increased on average by *c*.20% since 1984.

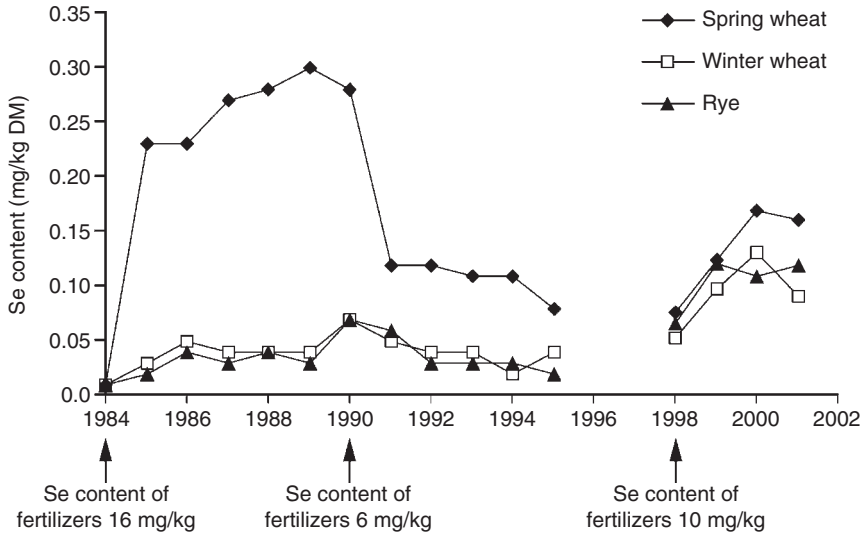

Fig. 5.10. Trends in the Se content of Finnish wheat and rye grains following the fertilization of arable soils, 1984–2001. (Eurola *et al.*, 2003. Copyright MTT Agrifood Research Finland and authors. Republished with permission.)

Concluding Remarks

Of the 88 naturally occurring elements in the earth's crust, Se is ranked as only the 70th most abundant (Plant *et al.*, 2004). Yet it is clear that this element has an impact on the environment and human society that is greater than this statistic suggests. Dietary intakes of Se by people and animals relate to both deficiency and toxicity problems, and of all the essential mineral micronutrients, this element is recognized as having one of the narrowest ranges between human dietary deficiency (<40 µg Se/day for adults) and toxic concentrations (400 µg Se/day (WHO, 1996), although Combs (2001) regards this 'upper safe limit' as being very likely to be too conservative). Grazing animals usually avoid Se-accumulating plants (many of which are unpalatable and give off an offensive odour), and with 'wild' plants being recognized as accumulators of the element rather than crop species, the intake by humans of plant foodstuffs containing excessive amounts of Se is reduced (Reilly, 1996). Nevertheless, humans and especially domesticated animals can suffer the consequences of a diet that contains too much Se, and the toxicity of this element must not be forgotten in a situation where there is an ever-increasing awareness and focus on the widespread deficiency problems that afflict both animal production and people.

It is now apparent that the human population of most countries would probably benefit from an increased Se dietary intake, and while this can be achieved in a number of ways (supplements, etc.), agriculture needs to be recognized as being the major intervention tool to prevent Se – and other

micronutrients – malnutrition (Welch and Graham, 2005). Agricultural food systems can be developed in various ways to satisfy this aim, but agronomic biofortification using selenate fertilizer applied directly to the soil has been found in a variety of countries (Australia, Finland, New Zealand) to be an effective, inexpensive and easy-to-implement strategy that can increase the Se intake of whole populations and has the advantage of not requiring a change in current consumer behaviour (Arthur, 2003; Lyons *et al*., 2005b). Such a biofortification strategy works because of the evidence that the soil geochemical environment influences dietary intakes and human Se status. The association between soil and human blood Se content is not perfect – especially if the correlation is examined over a limited range of soil concentrations – because of the complexities of the soil–plant–animal pathway of the element and other factors such as lifestyle (e.g. smoking), general nutritional status and the effects of certain diseases on the individual. But, it is demonstrably clear that even in the most developed nations such as the USA, where human mobility and greater dietary diversity can be expected to lessen the links between environment and health, the impact of the soil content of the element and its implications on the Se status of humans is still broadly evident.

Se is one of the most intensely studied mineral nutrients, with research on the element covering many disciplines such as agriculture, public and environmental health, soil science and toxicology. Such multidisciplinary investigations will continue, and from an earth science perspective there is an urgent need to provide global up-to-date spatial information on this element in the form of regional geochemical maps that delineate potential problem areas. Geochemical surveys to date have been shown to provide a useful catalogue of baseline information relevant to those with interests in agriculture and human health. However, many surveys have been undertaken only on an ad hoc basis in particular areas, and Se has often not been included because of the analytical difficulties involved in detecting the typical low concentrations found in many regions. Recent improvements in analytical instrumentation has resulted in more robust information on Se concentrations being determined from environmental samples (e.g. BGS, 2000), and future systematic geochemical surveys are increasingly likely to include the element.

Despite the overwhelming literature dedicated to Se – Reilly (1996) states that there are possibly 100,000 publications that consider this element following its recognition in 1817 – very little research to date has been undertaken on investigating the element in organic farming systems. Govasmark *et al*. (2005) have recently evaluated if the current Se feeding practices meet the dietary needs of dairy cattle and sheep on organic farms in Norway. These authors found that the herbage Se concentrations were insufficient to meet the dietary requirements of ruminants, and they recommend supplementation of the element to livestock until alternative means to raise the content of feed constituents are found. From an organic farming perspective the use of rock phosphate, enriched in Se, can be considered an acceptable way to achieve this, although the possibility of Se–PO_4^{3-} interactions, and the presence of potentially harmful elements such as cadmium, justify a continuous level of monitoring in any future use of this resource.

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6 Contaminants in Organic and Conventional Food: the Missing Link Between Contaminant Levels and Health Effects

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Introduction

The production and consumption of organically grown food show an increasing trend, both in Europe (Rohner Thielen, 2005) and in the USA (USDA, 2007). The total area of land farmed organically in the EU-15 increased from 2.3 million hectares in 1998 (representing 1.8% of the total agricultural land area) to 4.9 million hectares in 2002 (representing 3.8% of the total utilized agricultural area). In the USA, organic farming has been one of the fastest-growing sectors for over a decade. In 2005, US producers dedicated over 1.6 million hectares of farmland, comprising 0.7 million hectares of cropland and 0.9 million hectares of rangeland and pasture, to organic production systems. However, the overall adoption level is still low; only about 0.5% of all US cropland and 0.5% of all US pasture was certified organic in 2005.

The growing demand for organic food is associated with an increased consumer concern about animal welfare, environmental quality and personal health. This trend is stimulated by media reports of incidents in conventional food production systems, such as: the outbreak of bovine spongiform encephalopathy (BSE) in Europe; avian influenza in Asia; foot-and-mouth disease in the Netherlands and the UK; and the dioxin crises in Belgium. Although strict official definitions and standards vary slightly (e g. EC, 2008; Soil Association, 2008), organic farming is generally seen by the public as a form of agriculture which avoids or largely excludes the use of

 Health Benefits of Organic Food: Effects of the Environment (Givens *et al.*)

synthetic fertilizers and pesticides, plant growth regulators and livestock feed additives, and, as far as possible, relies on green manures and compost to maintain soil fertility, and crop rotation, mechanical cultivation and natural predators and antagonists to control pests and diseases (Wikipedia contributors, 2007). Many consumers believe that organically grown food is healthier and safer than conventional food (Jolly *et al*., 1989; Williams and Hammitt, 2001; Lea and Worsley, 2005). Organic food is argued to have a higher nutritional value, to stimulate the immune system of its consumers and to contain less residues and contaminants, e.g. pesticides, metals and veterinary drug residues. However, the claim that organic food is healthier than conventional food has not yet been the subject of thorough scientific investigation.

The aim of the present chapter is to critically review the scientific evidence for the claim that organic food is healthier than conventional food. The focus is on potential adverse health effects caused by food contaminants. Other health claims of organic food, for example, those related to nutritional value, are beyond the scope of the current chapter but are covered elsewhere in this volume. First, the scientific evidence comparing levels of food and environmental contaminants in organic food and conventional food is reviewed, with particular emphasis on pesticides, metals, nitrate and other environmental pollutants (dioxins, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs)). Subsequently, the regulatory approaches in place to derive safe concentration levels for food contaminants are reviewed, along with the scientific evidence as to whether violation of these levels may cause adverse health effects. Special focus is then given to the issues of protection of sensitive subgroups in the human population, effects at low exposure levels and potential mixture effects. The chapter concludes with the scientific uncertainties in relation to food contaminants and adverse health effects.

Does Organic Food Contain Less Contaminants than Conventional Food?

Contaminant levels in food are an important indicator for potential health effects. The general assumption is that less contamination implies less health risk. Within this context, it is plausible that organic food will be healthier than conventional food. After all, conventional agriculture uses a wide array of synthetic agrochemicals to fight pests and improve yields. Residues of these agrochemicals have the potential to remain in conventional food products, whereas avoiding synthetic agrochemicals, alternative growth and pest control techniques in organic farming will result in lower contamination levels. This hypothesis is confirmed by results from most monitoring programmes for contaminants in foods. However, it does not apply to all types of contaminants. We reviewed the scientific data on pesticides, nitrate, metals and other environmental pollutants, e.g. dioxin-like compounds, PCBs and PAHs.

Table 6.1. Detection frequency of pesticides in organically and conventionally grown vegetables and fruit in the USA and the detection frequency of multiple pesticide residues. (From Baker *et al*., 2002.)

	Conventional	Organic
Detection frequency (%)	43.0	9.1
Multiple pesticide residues (%)	26.7	2.6

Pesticides

Baker *et al*. (2002) performed an extensive analysis of the USA's pesticide residue data in conventionally and organically grown vegetables and fruits. The detection frequency of pesticide residues in organic products was about one-fourth of that found in conventional products (Table 6.1). The levels of pesticide residues found in organic produce samples were also consistently lower than those found in conventional samples. The geometric mean ratio between the levels found in conventional and organic foods was 1.57. Baker *et al*. (2002) also found that the number of samples containing multiple pesticide residues was significantly higher in conventional than in organic products (Table 6.1). Similar findings have been reported for other countries (Bitaud, 2000; McGowan, 2003; Hajslova *et al*., 2005; Hoogenboom *et al*., 2006; Pussemier *et al*., 2006; Harcz *et al*., 2007a; Tasiopoulou *et al*., 2007) and product groups (Kumpulainen, 2001; Bourn and Prescott, 2002; Magkos *et al*., 2006). These findings confirm that organic foods generally contain fewer pesticide residues and at lower levels than conventional foods.

Fewer and less does not mean that organic foods are free of pesticides; residues in organic foods are occasionally detected. The presence of these pesticides can have several causes, for example: (i) historical pollution; (ii) the dispersion of pesticides from conventional applications, e.g. by spray drift; and (iii) the fraudulent use of pesticides by organic farmers. Baker *et al*. (2002) showed that the detection frequency of pesticide residues in organic food decreases considerably if the category of banned and persistent organic organochlorine pesticides (OCPs) is excluded from the data set. Apparently, pesticides belonging to the 'persistent' category make a major contribution to pesticide detection frequencies in organic crops. This historical pollution originates from conventional agriculture, and, as the name suggests, the environmental degradation rate of these persistent pesticides is very slow, meaning that they can be detected for years after the farming system has switched from conventional to organic. A recent study by Fontcuberta *et al*. (2007) found OCPs in only seven of 1484 samples analysed in the 2001–2006 period (0.5%). Compared with the previous 1989–2000 period, this is a distinct decrease in both the proportion of samples with detectable residues and in the variety of OCPs found, suggesting that concentrations are gradually disappearing.

The general finding that organic food contains fewer different pesticides and at lower concentrations is confirmed by regulatory data on the violation of

pesticide standards in food. These standards are referred to as maximum residue limits (MRLs; see section on violation of food standards). The incidence of MRL violations in conventional foods varies between approximately 1% and 5% (Magkos *et al*., 2006). In Europe, the incidence of MRL violations shows an increasing trend from 3.0% in 1996 to 5.0% in 2005. This upward trend can be explained by the increasing number of pesticides measured over this period as well as the increasing number of MRLs. It is therefore difficult to tell whether the general trend of pesticide residues in food is increasing or decreasing. Specific data on MRL violations in organic food are rare. The UK Pesticide Residues Committee (2007) measured 220 organic samples in the year 2006 and found no MRL violations, whereas 1.8% of the 3342 conventional food samples violated the MRL. A 2002–2006 monitoring survey of organic food products by the Federal State of Baden-Württemberg in Germany (CVBW, 2007) showed that the MRL was violated in 1.2% of the organic samples, whereas the German average percentage of MRL violations in conventional food samples was reported to be 6.7% in 2005 (CEC, 2007). The relationship between health implications and MRL violations is discussed further in the section on violation of food standards.

Nitrate

Whether or not dietary nitrate has toxic or beneficial properties on human health is controversial (see Chapter 12, this volume). Nevertheless, some studies have attempted to compare nitrate levels between conventional and organic products.

Woese *et al*. (1997) reviewed 41 comparative studies that addressed the problem of nitrate content in vegetables and vegetable products from different cultivation forms. Despite shortcomings in some of the studies, they found strong indications for lower nitrate contents in vegetables from organic cultivation or vegetables grown with organic fertilizers. Higher nitrate levels in conventionally cultivated crops or those treated with mineral fertilizers were found in nitrophilic leaf, root and tuber vegetables. These findings are confirmed by most other studies (Worthington, 1998, 2001; Bourn and Prescott, 2002; Williams, 2002; Hoogenboom *et al*., 2006; Pussemier *et al*., 2006). Pussemier *et al*. (2006) hypothesize that the lower nitrate levels found in some organic vegetables during winter in Europe may be related to the fact that these vegetables are often imported from Mediterranean countries where sunshine is abundant. A lack of light is a well-established factor explaining nitrate accumulation in vegetables. The effect of environmental controls on nitrate content of plant parts is discussed in detail in Chapter 13 (this volume).

Overall, organically grown nitrophilic and leafy vegetables are estimated to contain 15–50% less nitrate than their conventionally grown counterparts (Magkos *et al*., 2006). For non-nitrophilic crops, e.g. cereals, potatoes, fruit, seed and bulb vegetables, the data show no consistent differences in nitrate content between organic and conventional products. Some studies even found higher nitrate levels in certain organic products, e.g. tomatoes, spinach, chicory, green salad and rocket lettuce (Malmauret *et al*., 2002; De Martin

and Restani, 2003). This can be explained by the fact that other factors, besides the farming system, can also influence nitrate levels in vegetables, e.g. cultivar, soil type, planting and harvesting times, irrigation water quality, climate, storage conditions and postharvest processing (Magkos *et al*., 2006; see Chapter 13, this volume). The general finding that organic vegetables contain 'less nitrate' may not be applicable to specific individual exposure situations, e.g. organic vegetables purchased from a particular farm could be higher than the average conventional produce.

Metals

Metals do not show a consistent pattern when organic and conventional food products are compared. Malmauret *et al*. (2002) found slightly higher cadmium (Cd) and lead (Pb) levels in several organically produced vegetables, especially carrots and spinach. Contrastingly, Karavoltsos *et al*. (2008) found that organic foodstuffs contained less Cd than conventional foodstuffs for 64% of the wide range of product categories included in their study. For Pb, this percentage was 61%. Olsson *et al*. (2001) found that Cd levels in kidneys, livers and mammary tissue were significantly lower (by *c*.25%) in dairy cows from organic farming than those from conventional farming.

The relatively high Cd concentration in some artificial fertilizers in combination with the limited use of this type of fertilizer in organic farming gives rise to the expectation that Cd concentrations in organic crops will be lower than in conventional crops. This effect could be enhanced by the occasional use of sewage sludge in conventional agriculture. The lower concentrations of Pb and Cd found in organically reared dairy cows (Olsson *et al*., 2001) seem to confirm this hypothesis, but in the scientific literature no convincing evidence was found to suggest that organic crops have lower Cd levels than conventional crops (Woese *et al*., 1997). This contradiction to expectation may have various explanations. Variability in local environmental conditions such as location, soil composition, climatic factors, pH and moisture content can influence the bioavailability of contaminants which is an important determinant of bioaccumulation in crops. Within this context, the local conditions may be much more important than the difference between organic and conventional cultivation techniques and fertilizers. The higher Cd and Pb levels found in conventional dairy cows (Olsson *et al*., 2001) may be caused by differences in feed composition between both production systems. For example, conventional cows received a 55% higher amount of concentrates in their feed than organic cows. Some of the constituents of these concentrates, i.e. soybeans and sugarbeet, are known to have a relatively high uptake of Cd via the root system.

Other environmental pollutants

Other environmental pollutants occasionally found in food products include polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans

(PCDFs), PCBs and PAHs. The dispersion of these substances in the environment typically takes place via particulate aerosols in the atmosphere, which may travel long distances from their source. The presence of these substances in crops and livestock is typically related to pollution incidents (Drotman *et al*., 1983; Bernard *et al*., 1999) and emissions from combustion sources via atmospheric depositions. The persistent substances such as dioxins and PCBs can accumulate in fats of animals and are passed on to the humans who consume them (Fries and Paustenbach, 1990).

In line with the origin and distribution patterns of dioxin-like compounds, PCBs and PAHs, the probability of food products getting contaminated will depend on: (i) the measures taken to prevent pollution incidents; and (ii) the proximity of major pollution sources. Relatively high concentrations are generally found in products of farms located in heavily industrialized and populated areas. There is no reason to assume that there is a systematic difference between levels of these pollutants in organic and conventional products, with maybe one exception; there are indications that organic livestock may accumulate more persistent dioxins and PCBs due to their more frequent contact with the soil. An example is the elevated concentrations of dioxins and dioxin-like PCBs generally found in eggs of free-range chickens (Fürst *et al*., 1993; Schuler *et al*., 1997; Pussemier *et al*., 2004; Schoeters and Hoogenboom, 2006).

Conclusion

Organic food generally contains fewer types of pesticide and at lower concentrations and some products, i.e. leafy and nitrophilic vegetables, would overall contain less nitrate. There are indications that persistent substances such as dioxins may accumulate to higher levels in organic animal products due to the more intensive soil contact of free-ranging animals. The level of pollution of other product categories by other substances (e.g. PAHs, metals) is probably dominated by local conditions, the distance between the area of production and the polluted area, and less so by the difference between organic and conventional cultivation systems.

Are Consumers of Organic Products Less Exposed than Consumers of Conventional Products?

Despite advertising statements such as, 'eating some organic food reduces your exposure to pesticide residues', there are actually very few scientific studies that attempt to estimate differences in measured exposure. For organophosphate (OP) pesticides, Curl *et al*. (2003) and Lu *et al*. (2006a) both show that levels of OP metabolites in preschool children on an organic diet are lower than in those consuming conventional products. The study of Curl *et al*. (2003) classified the children's normal diet as either 'predominantly conventional' or 'organic based' and estimated exposure based on urinary

excretion of OP metabolites. Depending on which OP parent compound was attributed as the source of the metabolites measured, children eating 'conventional' had estimated exposure levels close to, or above, the US Environmental Protection Agency's current guidelines, whereas children on a predominantly organic-based diet were below the exposure guideline. Thus, consuming predominantly organic produce in this case appears to be a simple way of shifting exposures from within the range of uncertain risk to within the range of negligible risk. Instead of classifying existing diets, Lu *et al*. (2006a) replaced 23 children's conventional diets with organic diets for a 5-day period before returning them to normal diets. This showed that the measured OP metabolites decreased substantially during the 5 days on the organic diet and returned to original levels within 2–3 days of returning to the conventional diet. This may not be surprising as OPs are mainly used in agriculture and there are therefore few other exposure routes outside dietary. In contrast, when measuring common pyrethroid metabolites in the same 23 children as above, Lu *et al*. (2006b) found that the main explanatory factor was domestic use of pyrethroids, and that diet had little effect. To our knowledge, no data on dietary exposure differences of other man-made pollutants or metals are available.

There is, however, another issue of exposure, namely that of mycotoxins. Mycotoxins can appear in food as a result of fungal infection of crops (see Chapter 7, this volume). Patulin is associated with a range of fungal species and is found in mouldy fruits, vegetables and cereals. It is a potential carcinogen and can damage both the immune and nervous system. As it is destroyed by alcoholic fermentation, it is not found in alcoholic drinks, but can be a problem in fruit juice. In a survey of over 130 Italian apple juices, Spadaro *et al*. (2007) found a similar incidence of positive samples in conventional and organic-based samples, and, although the mean contamination level was higher in organic (10.92 µg/kg) than in conventional juices (4.77 µg/kg), this was not statistically significant. Based on the available data on Italian intakes of fruit juices, Piemontese *et al*. (2005) estimated that adult daily intakes of patulin were 0.38 and 1.57 ng/kg body weight (bw) from conventional and organic products, respectively. For children, the estimated intakes were higher for both conventional and organic products (3.41 and 14.17 ng/kg bw, respectively) although below the provisional maximum tolerable daily intake (TDI) of 400 ng/kg bw. When evaluating patulin exposure of children in Flanders consuming organic or conventional apple juice through a probabilistic approach, Baert *et al*. (2007) showed that 0.9% of children would exceed the TDI if consuming only organic apple juice as opposed to 0.1% for conventional.

While Harcz *et al*. (2007a) were not able to detect significant differences in chemical contamination, excluding mycotoxins, between organic and conventional winter wheat, they found differences in the mycotoxin levels of the harvested wheat. For deoxynivalenol (DON), conservative estimates showed that consumers could reach 56% and 99% of the TDI through organic and conventional cereal products, respectively, whereas for Cd these figures were 19% and 17%, respectively. All other estimated intakes of contaminants

(including pesticides) were lower than 10% of the TDI. A separate study based on Monte Carlo simulations of exposure showed that 10% of those consuming organic wheat containing DON may be exposed to this natural toxin at levels above the TDI (Leblanc *et al*., 2002). Thus, in some cases the 'natural' toxins made by fungi may be of greater concern than more 'traditional' contaminants.

Another mycotoxin, ochratoxin A (OTA), is also a potential carcinogen and further known to cause kidney damage in humans. OTA production occurs postharvest during storage and increases in wet years or under wet conditions. Unlike patulin, it is not destroyed by alcoholic fermentation and can thus be a problem in beer. A study in Belgium concluded that for the 97.5th percentile beer consumer, organic beer can provide 50–100% of the TDI; this is in contrast to 8–20% of the TDI for a conventional beer consumer (Harcz *et al*., 2007b). This last finding may be especially significant as OTA is a contaminant of all cereals and so exposure can occur through numerous other routes additional to beer consumption.

Conclusion

Although the use and levels of pesticides in conventional crops are strictly regulated and only rarely exceed the set standards, the available evidence shows that consumers of organic products are less exposed to pesticides. Additional research is necessary to determine consumer exposure to other substances such as nitrate and metals. Although fungi are present in both conventional and organic harvests, one could speculate that the risk of mycotoxin exposure is higher from organic products because no pesticides are used during production and storage, or in preservation of the final product. While pesticides are very much an issue in the public eye, mycotoxins are less so. The drive for lower pesticide use could, if not monitored carefully, lead to use of ineffective concentrations and result in a scenario where pesticide residues in food would be present while having no safeguarding against mycotoxins (Harcz *et al*., 2007b).

Does Violation of Food Standards Imply that Adverse Health Effects Will Occur?

In order to tackle the question of whether violation of food standards may or may not result in adverse effects in humans, it is worth first to overview the nature of food standards and how they are set.

Historically, the precautionary principle and the risk assessment paradigm have aimed to protect individuals from adverse health effects that may occur during exposure to environmental toxins, pesticides and contaminants in food. There are different standards to protect consumers in relation to acute and chronic exposure and these depend on the compound's mechanism of toxicity, namely, non-genotoxic 'threshold of toxicity' or genotoxic 'cancer

end point'. The approaches for derivation of standards for both mechanisms, although developed separately, can be divided into four sequential steps: hazard identification, hazard characterization, exposure assessment and risk characterization (Truhaut, 1991; Dorne *et al*., 2007).

For compounds with threshold toxicity, health agencies throughout the world have relied upon the use of a surrogate for the threshold such as the no-observed-adverse-effect-level (NOAEL) or the benchmark dose and a standard uncertainty factor (UF) of a 100-fold to determine safe levels of chronic human exposure. The nomenclature for these safe levels 'without appreciable health risk' when consumed every day for a lifetime differs; in Europe, the term 'acceptable daily intake (ADI)' is used, whereas in the USA the term 'reference dose' is applied instead (Dorne *et al*., 2005). For acute effects, the acute reference dose (ARfD) reflects the amount of a chemical that can be taken over a short period of time (usually during one meal or 1 day) without an appreciable health risk. All these health standards are expressed in milligrams of the chemical per kilogram body weight of the consumer. MRLs have also been set for food products to provide a standard for pesticides, therapeutic drugs and feed additives and to ensure that good agricultural practices are applied. For pesticides, the MRL is derived from field trials; after the crop has been treated with the pesticide following the product licence and guidelines, samples of the crop are analysed to determine residue levels. MRLs are expressed in milligrams of chemical per kilogram of food product.

For genotoxic carcinogens, the human health risk is associated with an estimated exposure or vice versa, and these are usually quantified using dose–response relationships often based on experimental animal data combined with low dose extrapolation (Dorne, 2004). Recently, the margin-of-exposure (MOE) approach has been used for the risk assessment of a number of carcinogens by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the European Food Safety Authority (EFSA; e.g. for aflatoxins, ethylcarbamate, polyaromatic hydrocarbons and acrylamide; EFSA, 2005). MOEs are derived by using reference points from animal carcinogenicity data (i.e. benchmark dose and its lower confidence limit) and human dietary exposure data and it is considered that an MOE of 10,000 or more, 'would be of low concern from a public health point of view and might reasonably be considered as a low priority for risk management actions' (EFSA, 2005; O'Brien *et al*., 2006). A full discussion of genotoxic carcinogens is beyond the scope of this chapter.

Overall, human exposure data for pesticides and food contaminants are often compared with food standards for non-genotoxic carcinogens so that food quality and the potential associated health risk can be assessed. However, these may differ in interpretation. For example, for pesticides, compliance of food products within the EU or national pesticide regulations is checked against the MRLs meaning that regulatory data about the violation frequency of such standards provides no information about potential health risks. Health risks from pesticide and veterinary drug residue exposure are controlled through a system of approval and residue monitoring. Approval for

putting pesticide products on the market is given only if the product can be shown to be effective (i.e. control the pest, weed or disease without adverse effects on the crop) and safe to humans (the consumer, users and bystanders) as well as the environment (including soil, water and non-target organisms). Safety and risk with respect to the consumer is assessed through the following steps:

1. The acceptable chronic or acute intake of a pesticide (ADI, ARfD or acceptable operator exposure level (AOEL)) is determined based on toxicity data from sub-chronic to chronic animal studies and the use of a UF of a 100-fold.
2. The highest level of pesticide residue in crops is determined after application of the pesticide in line with the instructions on the product label.
3. The maximum amount of pesticide intake is determined based on a realistic exposure scenario for consumers and is compared to the acceptable intake level (ADI, ARfD or AOEL).

MRLs are thus only derived for pesticide and veterinary drugs that have been approved, meaning that residue levels at, or below, the MRL are unlikely to result in intake levels that exceed the ADI/ARfD. This disconnection of residue standards (MRLs; which indicate good agricultural use) and health standards (ADI/ARfD; which indicate safe exposure) means that it could be argued that risk characterization of residues is less transparent than for other chemicals present in human food (Renwick, 2002). Messages that are difficult to communicate to the general public, e.g. 'Pesticide standards are being exceeded but there is no health risk', can result and by so doing may raise credibility issues. Moreover, health risks of MRL violations are rarely communicated to the general public. For example, Germany and the Netherlands do publish yearly reports on pesticide measurements and MRL violations in food, but these reports do not contain data about the likelihood of violating health standards such as the ADI or ARfD (VWA, 2006; BVL, 2007). An exception is the UK Pesticide Residues Committee (2007), which reports ARfD violations and provides an indication of the potential health implications. For 2006, the UK Pesticide Residues Committee performed 158 risk assessments and reported a small number of cases in which the intake could be above the ARfD.

Assessing the likelihood of ADI and reference dose (RfD) violations involves complicated exposure calculations including assumptions about the amount of product intake and loss of residue during processing or preparation of the food prior to consumption. The UK Pesticide Safety Directorate has developed guidelines to perform acute and chronic exposure calculations in a consistent manner (PSD, 2001). These calculation procedures could also be applied in a backwards fashion to derive health standards based on predefined exposure scenarios. Recently, the panel on contaminants in the food chain of the EFSA has published a number of opinions related to the health risk associated with the consumption of meat products from non-target animal species fed cross-contaminated feed with coccidiostatic drugs. The risk characterization was based on an exposure assessment taking into

account the toxicokinetics (TK) and the residue levels in the animals, a food basket approach of human consumption and a final step comparing the actual exposure to the ADI of the compound (EFSA, 2008). Overall, the derivation and maintenance of health-based residue standards, next to standards based on good agricultural practice, can significantly improve the communication of health risks associated with pesticide and veterinary residues.

Hence, a violation of acute and chronic human health standards (ADIs, ARfD and AOEL) due to pesticide and veterinary residues in food products is highly unlikely. But even if a health standard is violated, it does not mean that human health will be impaired because human health standards are derived for daily exposure and are based on chronic toxicity data in test species. The maximum dose at which no adverse effects are observed in test species, referred to as the NOEL, is used as the basis for deriving human health standards. The test species NOEL is divided by a UF (usually 100) to generate the human health standard.

The UF is applied to account for the following:

- Humans may be more sensitive to the toxic substance than laboratory animals due to physiological and biochemical differences in species characteristics. This is generally referred to as interspecies variability. Interspecies variability is a typical source of uncertainty when test data derived from laboratory animals are extrapolated to humans and the UF is applied to guarantee a conservative outcome.
- The variation in sensitivity between laboratory animals, generally referred to as intraspecies variability, will typically be small when compared to the human population. After all, limited numbers of laboratory animals are tested which are genetically similar (same breed) and kept under constant laboratory conditions without confounding factors such as environmental exposure to chemicals, use of therapeutic drugs, smoking and alcohol. The NOEL will be higher in populations with small intraspecies variability because the NOEL is derived from the most sensitive individuals in the population. Intraspecies variability is discussed in more detail in the following section.
- Potential experimental errors make the outcome of a laboratory toxicity test uncertain. The statistical power of the toxicity tests is limited and the identification of adverse health effects requires extensive experience and skills. The experimental NOEL may therefore deviate from the true NOEL.

The standard UF of 100 is rationalized as the product of a factor of 10 to account for interspecies variability and a factor of 10 for intraspecies variability (WHO, 1987; Dourson *et al*., 1996; Renwick and Lazarus, 1998). However, the empirical basis for this assertion is weak (Vermeire *et al*., 1999). The factor of 100 has evolved from practical experience and is generally considered to result in a conservative estimate of the true ADI and ARfD (Lehman and Fitzhugh, 1954). The value can be adjusted down if sufficient scientific information is available about the true interspecies or intraspecies variability for

the substance and species involved. Ideally, specific UFs can be derived if sufficient scientific information is available about the true interspecies or intraspecies variability for the substance and species involved, e.g. from studies on the metabolism and/or the mechanism of toxicity/mode of action of the substance.

The procedure outlined above applies to substances that elicit no adverse effect below a certain threshold; the NOEL. The derivation procedure of safe exposure levels such as the ADI, ARfD and MOE is surrounded by further uncertainty. This is caused by the fact that experimental validation of safe exposure levels is extremely difficult and controversial. Validation research is hampered by several obstacles: (i) intoxication experiments or experiments using trace doses of contaminants in humans are unethical; (ii) it is more difficult to show the absence of adverse health effects than the presence of an adverse health effect; and (iii) experimental detection of low effect or risk levels (e.g. a one-in-a-million tumour risk) requires an unrealistic measurement effort. On the rare occasion that epidemiological data on humans are available and suitable for deriving safe exposure levels, uncertainty about extrapolation from high to low dose levels often remains. The ADI, ARfD and virtually safe dose (VSD) thus represent conservative estimates of safe exposure levels, given the fact that uncertainty is unavoidable. However, it should be noted that, although it is highly unlikely that adverse effects will occur at the level of the ADI, ARfD or VSD, uncertainty remains (Ragas, 2000). There are scientific arguments for stricter as well as for less-strict health standards. Three of these issues are discussed in the following sections: (i) the protection of sensitive subgroups; (ii) the potential beneficial effects of toxic substances; and (iii) the effects of mixtures.

Conclusion

The protection of human health against adverse health effects from pesticide and veterinary residues in food is regulated in a two-step procedure. The first step is that only pesticides and veterinary drugs that can be proven to cause no substantial health risks when applied according to the rules of good agricultural practice are allowed on the market for use. For these substances, MRLs are derived indicating pesticide use in line with good agricultural practice. If MRLs are exceeded, the second step consists of an assessment of the likelihood that health standards such as the ADI, ARfD or VSD are being exceeded. These health standards reflect conservative estimates of exposure levels that result in no or acceptable health risks. So even if health standards are being exceeded, the probability that adverse health effects will actually occur is relatively small because of the use of UFs applied in their derivation. However, uncertainty always remains about such UFs and the fact that they may not provide a sufficient degree of protection to exclude all adverse health effects under all potential exposure conditions, for example, when applied to susceptible subgroups of the population, as will be discussed in a later section.

Do Less Contaminants Always Imply Less Adverse Effects?

Toxicology builds upon the viewpoint that xenobiotic substances can trigger adverse health effects. The foundation of this viewpoint was laid down by the Swiss alchemist and physician Theophrastus Paracelsus (1493–1541) who introduced the dose–response concept by stating: 'All things are poison and nothing is without poison, only the dose permits something not to be poisonous'. This focus on adverse health effects in chemical risk assessment is also evident in the traditional and monotonic dose–response models, i.e. those for substances with and without a threshold. Over recent years, this traditional viewpoint is being increasingly challenged by observations that xenobiotics which cause adverse effects at high dose levels can have a stimulatory effect at low dose levels. This dose–response phenomenon is not new and is called hormesis (Stebbing, 1981). Calabrese and Baldwin (2002) define hormesis as an adaptive response characterized by biphasic dose responses of generally similar quantitative features with respect to amplitude and range of the stimulatory response, which are either directly induced or the result of compensatory biological processes following an initial disruption in homeostasis.

The hormesis concept is the subject of an intense scientific debate (Calabrese and Baldwin, 2003a; Kaiser, 2003; Axelrod *et al*., 2004; Thayer *et al*., 2005). It is generally agreed that some chemicals can trigger stimulatory effects at low doses, while triggering adverse health effects at high doses. The controversy focuses on questions concerning: (i) whether hormesis can be generalized and thereby adopted as a default model for dose–response modelling; (ii) the biological mechanisms and interpretations underlying hormesis; and (iii) the regulatory implications. As explained in the preceding section, the default models for threshold and non-threshold substances are based on the assumption that the adverse effects elicited at relatively high exposure levels can be extrapolated (often linearly) to low exposure levels. Several toxicological studies have shown that a substantial number of toxic substances can trigger a stimulatory effect at low doses (Calabrese and Baldwin, 2001, 2003b; Cedergreen *et al*., 2005; Calabrese *et al*., 2006). Cases of hormesis reported in literature include exposure to radiation and substances like arsenic, dioxin and Cd. Calabrese and Baldwin (2001) evaluated 20,000 articles from the mid-1960s to the late 1990s. Out of the 1.5–2.0% of these articles that passed the entry criteria (i.e. having an appropriate study design), approximately 40% reported a hormetic response. A similar study utilizing the US National Cancer Institute yeast screening database showed that hormetic response patterns were observed approximately four times more often than would be expected by chance alone, outperforming the conventional threshold model (Calabrese *et al*., 2006). Based on these types of findings, proponents of the hormesis concept argue that a biphasic dose–response relationship should be adopted as the default model in risk assessment. It should be noted that some of the cases originally identified as showing a hormetic response have been criticized by a number of scientists. For example, low doses of dioxin have been shown to suppress breast tumours in animals but have been implicated in having a role in promotion of liver tumours

(Kaiser, 2003). Only when all tumours are combined do the dioxins exhibit a hormetic response.

Adoption of hormesis as the default dose–response model in risk assessment would require plausible biological explanations which can be compound and mechanism-specific. Without such explanations, it is difficult to specify under what conditions hormesis occurs, and whether it is a stimulatory effect that can be labelled as beneficial. Finally, the beneficial effect itself should be defined and quantified on a case-by-case basis. Several biological response mechanisms such as activation of the DNA repair system and the immune system could perceivably result in hormetic responses. However, Stebbing (2003) states that before hormesis can begin to gain wider recognition, there is a need to involve researchers from other disciplines than toxicology to identify physiological mechanisms and a satisfactory theoretical basis to account for the diversity of instances of hormesis; without this the concept has little meaning beyond what is obvious from the defining biphasic curve. Calabrese and Baldwin (2003a) argue that there is no single hormetic mechanism, but that hormetic processes represent a common strategy for resource allocation when systems need to respond to low-level metabolic perturbations. Others argue that the concept of hormesis is largely based on empirical observations without understanding the underlying mechanisms, and that it is therefore inappropriate to conclude that hormesis is a uniformly adaptive phenomenon (Thayer *et al.*, 2005). It is an important controversy because detection of hormesis is experimentally costly and a case-by-case approach for the acceptance of hormesis for regulatory purposes would essentially derail the hormesis for widespread practical use in risk assessment (Calabrese and Baldwin, 2003a; Calabrese *et al.*, 2007). Another argument put forward against the general adoption of hormesis is that it does not account for variations in susceptibility in the human population, i.e. the adaptive response may not apply to sensitive subgroups in a population (Calabrese and Baldwin, 2002; Axelrod *et al.*, 2004). But even if hormesis would be accepted as a general adaptive response, the question remains whether this effect should be labelled as 'beneficial'. Stimulatory responses such as faster growth or larger offspring are not necessarily a beneficial effect, but can be a strategy of an individual or a population to adapt to environmental stressors. However, the energy allocated by the organism to develop an adaptive response cannot be used for other processes (Kooijman and Troost, 2007). This may not be problematic if food is abundant, e.g. in laboratory studies with rats, but this may be a very different story under field conditions. Indeed, it would be interesting to explore whether hormetic responses also occur under field conditions where organisms are exposed to multiple stressors and food availability is limited.

Conclusion

The hormesis concept has the potential to challenge some of the basic paradigms of toxicology and risk assessment, but its applicability is still a subject of complex debate and its biological basis *in vivo* is still not clear. However, if

hormesis can be proven to be generally applicable and to result in responses that are beneficial to health, it may have far-reaching consequences in the way we assess and perceive risks from xenobiotic exposure. Thus, it is currently too soon to conclude whether or not the benefits of low-level exposure outweigh the risks. Additional research is needed to determine under what conditions hormesis is likely to occur, and to define and quantify what can be considered as beneficial. Finally, assessment of susceptible subgroups of the population (e.g. neonates) would be required since toxicity may occur at lower doses compared to healthy adults.

Are Sensitive Subgroups in the Population Sufficiently Protected by Current Standards?

A critical aspect of risk assessment is the protection of susceptible subgroups within the human population, such as neonates and the elderly. Broadly speaking, two types of susceptible subgroups can be identified in relation to chemical exposure: (i) individuals for whom there is an increase in the external exposure due to food consumption and behavioural patterns; and (ii) individuals for whom there is an increase in internal exposure due to physiological and metabolic processes.

An increase in external exposure often leads to an increase in internal exposure (TK), which, ultimately may lead to increased toxicity at the site of action (toxicodynamics (TD)). Before one can answer the question as to whether current risk assessment frameworks ultimately provide a sufficient degree of protection for susceptible individuals, it is worth reminding the reader how these frameworks are applied to cancer and non-cancer risk assessment. Cancer risk assessment has been performed for genotoxic and carcinogenic substances and differs from assessment for compounds assumed to show a threshold (non-genotoxic compounds) below which no toxicity occurs (see section on violation of food standards). For five decades, health agencies throughout the world have relied upon the use of a surrogate for the threshold such as the NOAEL or the benchmark dose and a standard UF of a 100-fold to determine those levels 'without appreciable health risk' in humans. As stated earlier, this 100-fold UF has been used to account for interspecies differences (tenfold) and human variability (tenfold; Crump, 1984; Dorne and Renwick, 2005; Dorne *et al*., 2005; WHO, 2006). Further refinement in the early 1990s has suggested the subdivision of these two tenfold UFs to quantify differences in elimination (TK, 3.2) and the expression of target organ toxicity/mechanisms of toxicity (TD, 3.2; Renwick, 1993; Dorne and Renwick, 2005; Dorne *et al*., 2005; Dorne, 2007). Such refinements allow for a chemical-specific adjustment factor (CSAF) and physiologically based pharmacokinetic models to replace these default UFs (WHO, 2006). A critical aspect to refine these UFs is to incorporate differences between potentially susceptible individuals of the human population at both the levels of TK and TD (Renwick, 1993; Dorne *et al*., 2001a; Dorne, 2004). Recently (1998–2006), the analysis of human variability in TK for phase I and phase II metabolism (including polymorphic

pathways (CYP2C9, CYP2C19, CYP2D6 and N-acetyltransferase, NAT 2)) and renal excretion has identified a number of cases for which the current UF would not allow for this variability (e.g. Dorne *et al*., 2001a,b). Potential susceptible subgroups of the population have been identified and these are associated with a decrease in xenobiotic elimination, i.e. an increase in internal dose and susceptibility to toxicity. In these cases, the UF allowing for differences in TK (3.2) would not cover subgroups of the human population.

Human variability in phase I (CYP2C9, CYP2C19 and CYP2D6) and phase II (NAT-2) polymorphic metabolism constitutes the first category of high-risk conditions. For CYP2D6 metabolism, such high variability has been associated with a range of copies of the gene in the human population (zero copies in poor metabolizers, one to four copies in intermediate individuals and 13 copies in super-fast extensive metabolizers). The same concept applies to CYP2C19 and NAT-2 genes. This analysis assumed that the proximate toxicant is the parent compound and fast metabolism gives you an advantage. However, the reverse situation can be true; bioactivation could frequently lead to a toxic metabolite and here the extensive metabolizer subgroup would be most susceptible to toxicity. An example of such bioactivation to toxic metabolites is the organophosphorothioate chlorpyrifos, which is activated by CYP2D6 and CYP2C19. The effect of the quantitative involvement of CYP2D6 and CYP2C19 in extensive metabolizers and the differences in clearance rate between extensive metabolizers and poor metabolizers have also been investigated for major and minor substrates of each CYP isoform (10–100% metabolism) and exponential relationships have been shown to relate the two variables. In terms of UFs, the kinetic UF would only cover inter-phenotypic differences for compounds metabolized to a minor extent (30% of an oral dose; Dorne *et al*., 2002, 2003).

Interethnic differences constitute the second high-risk condition. Lower enzyme activities have been demonstrated for CYP2D6 in Africans, CYP2C19 and NAT-2 in Asians, and CYP3A4 in both South Asian and African healthy adults. These lower activities are associated with lower hepatic and gut metabolism and P-glycoprotein activity. CYP2D6 activity in Asian poor metabolizers is equivalent to Caucasian poor metabolizers, but it is higher in the overall Asian population and these differences are known to be due to differences in CYP2D6 poor-metabolizer frequency (2% in Asian and 8% in Caucasians). The reverse situation applies to CYP2C19 with 18% poor metabolizers in Asian subgroups compared with 3% in Caucasian subgroups (Dorne *et al*., 2006).

Age differences in metabolism and TK particularly for the elderly and neonates constitute another source of variability. This variability applies to most metabolic routes as a result of lower hepatic metabolism and renal excretion due to slower and immature metabolism in the elderly and the neonate, respectively. In contrast, for most elimination routes, hepatic metabolism and renal excretion is faster in children than adults due to growth and a general polymorphic CYP2D6 and CYP2C19 metabolism. However, no reliable data were available for poor-metabolizer subjects in any of these subgroups.

Chemical mixtures can also be a potential source of high-risk scenarios; however, these aspects are difficult to assess since the molecular interactions

between the components of the mixture need to be understood at the TK and TD level. Mixtures are discussed in the following section.

Conclusion

Polymorphic metabolism has the potential to modify exposure to chemicals considerably and create high-risk conditions for subgroups of the population exposed to single compounds and mixtures. The quantitative aspect of CYP2D6 and CYP2C19 metabolism in extensive metabolizers has been shown to drive the differences in elimination rate between extensive and poor metabolizers so that the kinetic UF currently in use (3.2) would only be protective for compounds metabolized to a minor extent by each isoform (30% of an oral dose; Dorne *et al*., 2002, 2003). Other high-risk subgroups of the population are the elderly and neonates due to their lower and immature hepatic and renal metabolism. Additionally, the mixture issue generates yet another high-risk scenario due to the potential TK interaction between chemicals metabolized via a particular route and inhibitors/inducers of that route. Any of these high-risk scenarios may result in decreased elimination of toxic species and potential TD consequences. Such findings have implications for human risk assessment of environmental contaminants since a number of pesticides are metabolized by polymorphic routes such as CYP2D6, CYP2C19 and CYP2C9. Hence, it is essential that the metabolism of such compounds is well characterized before they reach the market. Cell lines (expressing specific CYP isoforms) and liver microsomes can be used consistently to depict metabolic routes *in vitro* as well as their potency to inhibit or induce such routes (Dorne *et al*., 2006). Use of *in vitro* cell culture can possibly identify the specific CYP involved, but can yield no information regarding effects at the TK and TD level. Experimental studies should be conducted to investigate such effects.

Once in the possession of such metabolism, TK, TD data from either *in vitro* or *in vivo* sources, regulators can then make informed decisions regarding the use of default factors, pathway-related UFs or, ideally, CSAFs. Until then there are indications that some subgroups can be particularly sensitive to certain pollutants, and that this variability may in special cases and under special conditions be higher than the factor 10 generally accounted for in human effect assessment procedures. However, most data and insights on these issues have been obtained at relatively high (internal) exposure levels and it is not certain whether the results and insights are also applicable to low exposure levels.

Do Standards for Single Substances also Protect Against Potential Effects of Exposures to Whom It May Concern: Mixtures?

It is important to realize that the standards covered above (see section on violation of food standards) are derived specifically for single substances.

Obviously in reality more than one pollutant can be present in food, for example, multiple pesticides or a mixture of pesticides and other contaminants. While it is sometimes argued that the UFs applied in the derivation of single substance standards are sufficiently high to protect against mixture effects, but the empirical basis for this assertion is lacking. The need for more data on mixture exposures was highlighted in the 1996 US Food Quality and Protection Act (FQPA), which directed the US Environmental Protection Agency (US EPA) to consider cumulative (multiple chemicals) and aggregate (multiple routes) exposures in the risk assessment process.

Conventional mixtures such as PCBs and PAHs are regulated as mixtures, often through the use of what is called the relative potency factor (RPF) method, but this is the exception rather than the rule. The US EPA RPF approach basically expresses the potency of each chemical in a cumulative assessment group in relation to the potency of another member in the group which has been selected as the index chemical. An RPF is calculated for each chemical for each route of exposure (e.g. oral, dermal, inhalation). For example, if compound A is determined to be one-tenth as toxic as the index compound, the RPF for compound A is 0.1. Using this approach for each route of exposure for each chemical, exposure is expressed as exposure equivalents of the index chemical. The exposure equivalents are calculated by multiplying the residues and the RPF for each route. These exposure equivalents are then summed to obtain an estimate of total exposure by route in terms of the index chemical (US EPA, 2003).

There are two generally recognized reference concepts for estimating the joint effect of chemicals, namely: concentration addition (CA; also known as Loewe Additivity); and, independent action (IA; also known as Bliss Independence, response addition or effect multiplication; Loewe and Muischnek, 1926; Bliss, 1939). Both these reference models rely on the assumption of non-interaction between mixture components. CA is aimed at chemicals with similar mode of action, describes the effect of their combination by considering them dilutions of each other and estimates overall toxicity through summation of the concentration of the individual chemicals scaled by their potency (e.g. $EC_{50;}$ US EPA, 2000). While CA may seem similar to the RPF approach, it relates the amount of each chemical to its own effect concentration rather than a reference chemical. IA, in contrast to CA, is based on the assumption that the distribution of the sensitivities of individuals to the toxicants is statistically independent (Backhaus *et al*., 2004). The IA model is, therefore, a measure of the joint probability of individual sensitivity to the compounds in the mixture assuming independence. When comparing the IA and CA models for similar acting compounds, the CA model in general yields the best predictions (Altenburger *et al*., 2000). Equally, it has been shown that the IA model predicted the effects of dissimilarly (independently) acting chemicals more closely than the CA model (Backhaus *et al*., 2000). Although useful as default models, interaction between chemicals can potentially limit the predictive capability of these models to describe joint effects when looking at mixtures with few compounds (Jonker *et al*., 2005).

In a recent review, McCarty and Borgert (2006) identified that while most tested mixtures are near or below simple dose/concentration additivity,

exceptions (both positive and negative) tend to occur when tested mixtures have only a few components or where sensitive whole organism or sub-organismal changes are used as the response metric (McCarty and Borgert, 2006). Similarly, in a review of the 1985–1998 literature on low dose mixtures, Carpy *et al*. (2000) showed that while a combination of compounds with the same target organ and the same or very similar mechanisms of action may cause additive or synergistic effects, the chance of such effects will most likely diminish with decreasing exposure levels to such combinations. The same is said to hold when the number of chemicals increase, as synergism and antagonism may both occur at the same time at different organs or targets in the same organism. While this does not mean the interactions do not occur, it does mean that they mask each others potential effects. Carpy *et al*. (2000) concluded that despite some exceptions, it has been demonstrated that interaction between components is not a common event at low levels of human exposure such as those that may occur through pesticide residues in food and that exposure to mixtures of pesticides at low doses of the individual constituents does not represent a potential source of concern to human health.

Wade *et al*. (2002) undertook experiments with a complex mixture of ubiquitous persistent environmental contaminants, including PCBs, organochlorines, other pesticides, solvents and metals, all present in the mixture at the minimum risk level (MRL) or TDI. Sexually mature male rats were exposed to this complex mixture at 1, 10, 100 and 1000 times the estimated safe levels daily for 70 days. The conclusion was that additive or synergistic effects of exposure at levels representative of those in contemporary human tissue are unlikely to result in adverse effects on immune function or reproductive physiology. However, the exceptions are real, as demonstrated by Moser *et al*. (2006) who found significant greater-than-additive responses for all but two end points (motor activity and tail-pinch response) when exposing preweanling rats to a mixture of four OPs. The magnitude of the greater-than-additive response increased and was seen in all end points if malathion was added to the mixture. The mechanism responsible here is reasonably well known; malathion toxicity is potentiated by OP pesticides which inhibit the carboxylesterase (CaE)-mediated hydrolysis of malaoxon (i.e. degradation of the toxic metabolite).

In recognizing that interactions between contaminants occur, the mixture issue generates yet another high-risk scenario for the sensitive subgroups discussed in the previous section. The risk scenario relates to the potential TK interaction between chemicals metabolized via a particular route and chemicals also present in the mixture that are inhibitors/inducers of that route.

Conclusion

Although mixture effects cannot be excluded, adverse effects seem to be the exception rather than the rule. Exceptions are most likely to occur where mixtures are dominated by, or contain, significant amounts of specifically acting chemicals (e.g. pesticides). Additional mechanisms of action-based studies are required in order to rule out effects on sensitive subgroups.

Are There Empirical Data About the Relation Between Pesticide/Contaminant Exposure at Dietary Levels and the Occurrence of Adverse Health Effects?

If it can be demonstrated that pesticide intake at current dietary exposure levels from conventional crops can cause adverse health effects, organic products can be claimed to be healthier since they contain less pesticides. However, epidemiological studies do not provide evidence for an association between health effects and dietary exposure levels. There are some indications that pesticide exposure can result in increased health risks, for example, a relationship between pesticide exposure and Parkinson's disease has been suggested (Elbaz and Tranchant, 2007). However, this type of study refers to relatively high exposure levels relating to the professional use of pesticides rather than intake through diet. Pesticide use has also been put forward as a possible explanation for a downward trend in male fertility in Western societies (Carlsen *et al*., 1992; Colborn, *et al*., 1996), but others refute such a trend (Brake and Krause, 1992; Sherins, 1995). Certain types of occupational exposure to pesticides may contribute to reduced male fertility (Oliva *et al*., 2001), but there is no evidence that this occurs at dietary exposure levels. Other studies have suggested a relationship between breast cancer and exposure to OCPs (Wolff *et al*., 1993; Mathur *et al*., 2002), but a recent review and meta-analysis of epidemiological data did not reveal any statistically significant association, except for the insecticide, heptachlor (Khanjani *et al*., 2007). Biomonitoring studies show that pesticides are metabolized in individuals exposed to dietary levels (Curl *et al*., 2003; Lu *et al*., 2006a,b) and show responses in related biochemical parameters, but effects that can be considered adverse have not been established.

Conclusion

The scientific evidence to support a cause-and-effect relationship between pesticide exposure at current dietary exposure levels and the occurrence of adverse health effects is scarce. On theoretical grounds, dietary exposure could have adverse effects, but potential beneficial effects at low doses (hormesis) cannot be ruled out even though *in vivo* evidence is lacking and beneficial effects still remain to be defined and quantified on a case-by-case basis. This implies that claims that organic food is healthier because it contains less pesticides cannot be substantiated based on current scientific evidence.

Overall Conclusions

Organic foods generally contain less pesticide residues but may contain as many environmental and food contaminants as conventional food. However, conclusive scientific evidence to support the claim that lower

pesticide contamination levels will result in a better health status is lacking. While there is some evidence that consuming organic produce will lead to lower exposure to pesticides compared to the consumption of conventional produce, there is no evidence of effect at contemporary concentrations. In fact, pesticide and veterinary drug use is well regulated and the potentially higher risk of being exposed to genotoxic mycotoxins from unpreserved organic products may be of actual higher concern; however, quantifying such effects is difficult because of scarce data and the lack of quantitative tools. Susceptible subgroups (neonates, poor metabolizers) exposed to single compounds or mixtures could constitute a potential scientific argument for choosing organic food over conventional since these groups may not be completely covered by the UFs employed in risk assessment. However, this risk may be very small and further research is needed to investigate biological effects of single chemicals and mixtures at low dose to set the foundations of a truly quantitative risk assessment and minimize the uncertainties related to exposure assessment and extrapolation steps.

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7 Mycotoxins in Organic and Conventional Foods and Effects of the Environment

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Introduction

The most important mycotoxin-producing moulds belong to the genera *Aspergillus*, *Fusarium* and *Penicillium*. Today, several hundreds of mycotoxins have been identified, but risk assessments have only been performed for a handful of them by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the European Food Safety Authority (EFSA). For these mycotoxins (aflatoxins, ochratoxin A (OTA), patulin, deoxynivalenol (DON), zearalenone and fumonisins), maximum limits have been established (European Commission, 2006). A few more mycotoxins (e.g. T-2 and HT-2) are expected to be regulated in the near future.

Mycotoxins differ considerably in both their chemical structure and in their toxicological effects. Aflatoxins, OTA and fumonisins are all carcinogens, whereas DON and T-2/HT-2 are immunosuppressants and zearalenone is oestrogenic. The commodities most commonly exposed to fungal invasion and consequent mycotoxin formation are those that are preserved by drying, i.e. cereal grain, dried fruit, nuts, pulses, coffee, spices. Fungi may invade the growing crop and produce mycotoxins in the field (e.g. *Fusarium* toxins such as DON, T-2 and HT-2 and zearalenone) and there may later be a further build-up as a consequence of improper drying and storage. Other fungi, such as the OTA-producing species *Penicillium verrucosum*, primarily invade the crop and produce toxin during drying and storage.

Comparisons of amounts of mycotoxins in organic versus conventional foods have increased considerably during the last few years. However, the investigations are not consistent and it is still very difficult to assess the cause of any differences; namely, whether toxin formation is due to the lack of adequate equipment and handling procedures or the farming system. Some results indicate that DON levels are lower in organic cereals and cereal products and that patulin levels are higher in organic fruit products, but, in most

cases there is no difference between organic and conventionally produced food products. In those cases where significant differences have been observed, the estimated intakes do not constitute any matter of concern for consumers, i.e. the intakes are still below the tolerable daily intakes (TDIs) that have been established by JECFA or EFSA. In order to resolve whether differences seen are due to the farming system or other factors, there is a strong need for investigations under strictly controlled conditions.

The influence of climate change on mycotoxin-producing fungi and mycotoxin contamination may change the occurrence of mycotoxins in the future. Heavy rain fall during unfavourable time periods, increased temperature and introduction of new crop species may favour the propagation of toxigenic moulds into new geographical areas and make it more difficult to uphold good agricultural practices in both organic and conventional farming systems.

Background on Moulds and Mycotoxins

Mycotoxins are fungal secondary metabolites and consist of a diverse group of chemical substances which are toxic to man and animals. The most important mycotoxins known today are produced by mould species belonging to *Aspergillus*, *Fusarium* and *Penicillium*. Frisvad *et al*. (2006a) have listed all the important mycotoxin-producing fungi of these genera together with information regarding the types of commodities with which they are associated. Unfortunately, the literature is full of examples where mycotoxin production has been incorrectly attributed to a particular species (Frisvad *et al*., 2006b). The chapters by Frisvad *et al*. (2006a,b) are accurate and useful references for professionals working on food safety issues concerning moulds and mycotoxins. The possible presence of mycotoxigenic fungi in foods is an ever-present cause for concern in the food industry. Rational decisions regarding the safety of foods suspected of containing mycotoxins are required and these should be supported by accurate tools for identification of mycotoxin-producing fungal species.

Aspergillus, *Fusarium* and *Penicillium* species have different demands on environmental conditions, such as water availability and temperature, for optimum growth and mycotoxin production. These different demands determine which species dominate and produce toxins, and, whether mycotoxin production occurs pre-harvest or postharvest. For example, *Fusarium* species grow and produce mycotoxins in grain in the field, whereas *Aspergillus* and *Penicillium* are more related to postharvest conditions, but, there are no strict barriers. Furthermore, all commodities, due to their environment and composition, are associated with specific fungal species and consequently mycotoxins are also associated with specific commodities.

Today, several hundreds of mycotoxins have been identified, but risk assessments have only been performed for a handful of them by the JECFA and the EFSA. Table 7.1 lists the major mycotoxins, the fungal species responsible for their production, the most common commodities affected, the main

Table 7.1. Producing fungal species, most commonly affected commodities, the main toxic effects and provisional tolerable daily intakes (TDIs) for selected mycotoxins.

Mycotoxin	Major fungal sources[a]	Most common commodities	Main toxic effects	TDI (ng/kg body weight per day)
Aflatoxins	*Aspergillus flavus* *A. parasiticus*	Nuts, maize, figs	Carcinogenic, genotoxic (human carcinogen)[c]	NA[b]
Deoxynivalenol	*Fusarium graminearum* *F. culmorum*	Cereals, especially wheat and maize	Immunotoxicity Gastrointestinal disorders	1000[d,e]
Fumonisins	*Fusarium verticillioides* *F. proliferatum*	Maize	Nephrotoxic, Carcinogenic, lack of evidence of genotoxicity	2000[d,e]
Ochratoxin A	*Aspergillus ochraceus* *A. westerdijkiae* *A. carbonarius* *A. steynii* *Petromyces alliaceus* *Penicillium verrucosum* *P. nordicum*	Cereals, coffee, wine, dried vine fruits, grape juice	Nephrotoxic Carcinogenic, lack of evidence of genotoxicity	14[d] 17[e]
Patulin	*Penicillium expansum* *P. griseofulvum* *P. carneum* *Byssochlamys nivea*	Apple and pear products including juices and pasteurized fruit juices	Reduced growth rate Gastrointestinal disorders Cytotoxic Genotoxic	400[d,e]
T-2 and HT-2	*Fusarium sporotrichioides* *F. langsethiae*	Cereals, especially oats	Immunotoxicity Haematotoxicity Gastrointestinal disorders	60[d,e]
Zearalenone	*Fusarium graminearum* *F. culmorum*	Cereals, especially wheat and maize	Hormonal effects	500[d] 200[e]

[a]Frisvad *et al*. (2006a).
[b]Not applicable for genotoxic carcinogens, i.e. no threshold dose below which no tumour formation would occur.
[c]According to IARC (International Agency for Research on Cancer, 1993).
[d]According to FAO/WHO Joint Expert Committee on Food Additives (2007).
[e]According to EFSA (2007).

toxic effects and provisional TDIs established by recognized international expert committees. For these mycotoxins (aflatoxins, OTA, patulin, DON, zearalenone and fumonisins) maximum limits have been established (European Commission, 2006). A few more mycotoxins (e.g. T-2 and HT-2) are expected to be assessed in the near future.

Comparison of Current Published Data on Amounts of Mycotoxins in Organic and Conventional Foods

Overview articles published on the issue of consumer safety in connection with organic versus conventional foods (Woese *et al*., 1997; Magkos *et al*., 2003; Burke, 2004; Magkos *et al*., 2006) have, so far, not been able to show any clear difference between the two systems when it comes to mycotoxins. Magkos *et al*. (2006) made an extensive review of mycotoxins in general, but, with a special focus on *Fusarium* toxins in cereals and patulin in apples. Facing limited and conflicting data, the authors were not able to distinguish any clear difference between organic and conventional produce. They concluded, in accordance with Kumpulainen (2001), that in order to carry out a valid comparison between organic and conventional food products, it is required that plants be cultivated in similar soils, under similar climatic conditions, be sampled at the same time, pretreated similarly, and analysed by accredited laboratories employing validated methods.

Olsen and Möller (1995) investigated the occurrence of moulds and trichothecenes in wheat and rye from organic and conventional farming systems (n = 83) from 1992 to 1994. Most of the samples came from control field trials and a few paired samples came from neighbouring farms using conventional or organic systems. In this study, samples were taken at the same time and pretreated similarly. The only trichothecene which could be detected was DON at very low levels (all below 100 ppb) and there was no significant difference between the organic and conventional system. This study was only published in Swedish and consequently not included in any of the international reviews.

One of the few large studies performed under well-controlled conditions focused on the effects of agronomic practices on the concentration of *Fusarium* toxins in cereals. This study (Edwards, 2007a,b) has only recently been finished and therefore is not included in previous reviews; its main conclusions are included below.

Despite the serious toxicity of the aflatoxins, very few papers have been published dealing with their occurrence in conventional versus organic farming. Woese *et al*. (1997) include an early study on aflatoxin in peanut butter from alternative and conventional shops in their review. Alternative production had higher contents of total aflatoxins than conventional. However, alternative production is not equivalent to organic production. On the other hand, two other studies presented in the same review showed that aflatoxin M_1 was lower in organic than in conventional milk.

A substantial number of new studies comparing the occurrence of other mycotoxins in organic and conventional products have been published during the last years (Table 7.2). The findings of studies for which it is possible for the reader to distinguish a statistical difference or no difference between the systems are summarized below. It is striking that some authors have concluded in their abstracts that there is a difference between the two systems, yet, reading carefully the whole article, it is clear from the statistical analysis that the difference is not significant. It is also astonishing that conclusions on differences between the two systems sometimes are made on the basis of 10–12 samples.

Fusarium toxins

There is today a substantial amount of investigation on grains and *Fusarium* toxins in organic and conventional farming giving us a good opportunity to compare the two farming systems. Since *Fusarium* may invade cereal grain and produce mycotoxins in the field, most of the occurrence data are obtained on raw cereals and not from retail.

Deoxynivalenol (DON)

Hietaniemi *et al*. (2004) investigated trichothecenes in oats during official variety, organic cultivation and nitrogen fertilization trials between 1997 and 1999 at six locations in Finland. More mycotoxins were produced during the warm, dry summers of 1997 and 1999 than in 1998. In all, 55% of the oat samples (n = 162) in the official variety trials contained DON within the range 50–896 µg/kg (i.e. all below the European Union (EU) maximum limits). The differences in DON concentrations between organic and conventional cultivation were small. The results showed also that various nitrogen fertilization levels only slightly affected trichothecene concentration.

DON was examined in wheat flour produced from cereals (n = 60) of conventional or organic origin in south-west Germany in 1999. The DON content was significantly higher in flour originating from wheat of conventional (median 295 µg/kg) than of organic production (median 120 µg/kg; Schollenberger *et al.*, 2002). However, the incidence of DON in conventional and organic flour was nearly the same (100% and 96%, respectively).

Schollenberger *et al*. (2005) examined 101 commercially different groups of bread widely consumed by the German population. In bread samples produced with cereals that were organically grown, the median values of DON were lower compared to those of conventional bread samples, with a significant effect in mixed wheat bread (52 and 232 µg/kg DON, respectively).

Wheat quality in organic and conventional farming systems was investigated in a 21-year field experiment in Switzerland introduced in 1978 (Mäder *et al.*, 2007). The conventional farming system was converted to an integrated farming system in 1985 and DON concentrations were analysed during the years 1998 and 2000 (n = 8). No significant system differences were found in DON concentrations in cereals from organic and conventional farming systems.

Table 7.2. Recent investigations of mycotoxins in organic and conventional crops/food.

Food/crop sampled (product origin)	Mycotoxin	Organic < conventional[a]	No difference[a] organic versus conventional	Organic > conventional[a]	Reference
Oats (Finland)	Deoxynivalenol		×		Hietaniemi *et al*. (2004)
Flour of wheat (Germany)	Deoxynivalenol	×			Schollenberger *et al*. (2002)
Bread of rye/wheat (Germany)	Deoxynivalenol	×[c]	×		Schollenberger *et al*. (2005)
Wheat (Switzerland)[b]	Deoxynivalenol		×		Mäder *et al*. (2007)
Wheat and rye (Germany)[b]	Deoxynivalenol	×			Meister and Springer (2004)
Wheat (Belgium)	Deoxynivalenol		×		Harcz *et al*. (2007a)
Wheat (UK)	Deoxynivalenol		×		Edwards (2007a)
Wheat (Italy)	Deoxynivalenol	×			Rossi *et al*. (2006)
Beer (Belgium)	Deoxynivalenol		×		Harcz *et al*. (2007b)
Cereal grain (France)	Deoxynivalenol		×		Malmauret *et al*. (2002)
Oat and wheat (UK)	T-2 and HT-2	×			Edwards (2007a,b)
Wheat (Belgium)	Zearalenone		×		Harcz *et al*. (2007a)
Maize (Spain)	Fumonisins		×		Ariño *et al*. (2007)
Beer (Belgium)	Ochratoxin A			×	Harcz *et al*. (2007b)
Wheat Italy	Ochratoxin A		×		Rossi *et al*. (2006)
Bread (several)	Ochratoxin A		×		González-Osnaya *et al*. (2007)
Wine (several)	Ochratoxin A		×		Chiodini *et al*. (2006)
Apple products (Italy)	Patulin		×		Ritieni (2003)
Apple products (The Netherlands)	Patulin		×		Boonzaaijer *et al*. (2005)
Apples (France)	Patulin		×		Malmauret *et al*. (2002)
Apple products (Italy)	Patulin		×	×	Beretta *et al*. (2000)
Fruit products (Italy)	Patulin			×	Piemontese *et al*. (2005)
Fruit products (Italy)	Patulin		×		Spadaro *et al*. (2007)

[a]Significantly different ($P < 0.05$).
[b]Conventional system was indicated as an integrated farming system.
[c]Depending on product there were different results.

In Germany, wheat (n = 60) and rye (n = 60) samples from integrated and organic cultivation were analysed for DON over a period of 4 years for DON (2000–2003). DON incidence was significantly lower and concentrations, where detected, were also lower in cereals from organic systems (Meister and Springer, 2004).

Harcz *et al*. (2007a) examined DON (n = 117) in organically and conventionally produced winter wheat in Belgium between 2002 and 2005. No significant differences in concentrations were detected between the conventional and organic systems. However, the average and the calculated 95 percentile were higher for the conventional cereal. The authors also estimated the daily intakes from cereals, assuming no further change in contamination levels during processing and preparation of foodstuffs. Expressing those intakes as a percentage of the TDI indicated that the DON intakes from organic and conventional cereals were 56% and 99% of TDI, respectively. In addition, Harcz *et al*. (2007b) examined the occurrence and levels of DON in beer (n = 84) and found no difference between organic and conventional production.

Rossi *et al*. (2006) examined 35 samples of organic (n = 20) and conventional (n = 15) wheat and found that DON levels were significantly lower in the organic system. In addition, the frequency of DON-positive samples was much lower in organically (25%) as compared to conventionally (100%) grown samples.

In a limited French study, organic wheat and barley (n = 11) were more highly contaminated with DON than corresponding conventional grains (n = 11), but, the difference was not statistically significant (Malmauret *et al*., 2002).

In the UK, an extensive study has been performed during 2001–2005 to ascertain the effect of agronomic practices on concentration of *Fusarium* mycotoxins in wheat (Edwards, 2007a). It involved the collection of 300 samples of wheat per year from fields of known agronomy over a number of seasons. The samples were analysed for ten mycotoxins, including DON. There was no significant difference between the DON concentration in organic and conventional samples. Within conventional samples, those which received an azole fungicide ear spray had significantly lower DON than those which received no ear spray.

Other Fusarium *toxins*

The extensive UK study described above also included a 4-year project to ascertain the effects of agronomic practices on the concentration of *Fusarium* mycotoxins in UK barley and oats over a number of seasons (Edwards, 2007b). One hundred samples both of barley and oats were collected each year at harvest, together with agronomic details and analysed for ten trichothecenes, including DON and T-2 and HT-2. The incidence of DON was low in both barley and oats compared to wheat. However, the incidence and concentration of T-2 and HT-2 in oats was high with quantifiable concentrations in 92% of the samples and a combined mean concentration of T-2 and HT-2 of 570 ppb for all samples analysed from 2002 to 2005. The concentration of T-2 and HT-2

was modelled against agronomic practices applied to each field. Year, region, practice (organic or conventional), previous crop, cultivation and variety all had statistically significant effects on the T-2 and HT-2 concentration.

In the UK study on wheat (Edwards, 2007a), T-2 and HT-2 occurred in much lower concentrations than in oats (Edwards, 2007b). However, T-2 and HT-2 occurred less frequently in organic samples and when they did occur they were present in lower concentrations.

Harcz *et al*. (2007a) found no significant differences between the zearalenone concentrations in conventionally and organically produced wheat (n = 115), despite the observation that average levels and the calculated percentiles were systematically higher for conventional cereals. The estimated daily intake for zearalenone was 16% for organic and 32% for conventional cereals when expressed as percentage of the TDI.

The occurrence of fumonisins B_1 and B_2 in conventional (n = 30) and organic maize was studied in Spain by Ariño *et al*. (2007). For the conventional maize, 13.3% of the samples contained fumonisins B_1 and B_2 at mean levels of 43 and 22 µg/kg, respectively, while 10% of the organic maize samples contained fumonisins at a somewhat lower level of 35 µg/kg (FB_1) and 19 µg/kg (FB_2), but the difference between production systems was not significant. Overall the fumonisins levels in the maize samples were much lower than the maximum permitted level of 2000 µg/kg. The intake estimated from the values from conventional and organic maize gained in this study represented a very low percentage (0.21% and 0.17%, respectively) of the established TDI for fumonisins of 2 µg/kg body weight.

Ochratoxin A (OTA)

As described above, OTA occurs in a wide range of products. Comparisons between organic and conventional food products are mainly performed for the more important products such as cereals; the main contributors representing approximately 50% of the total intake, and, products of grapes, such as wine and dried vine fruit. OTA in grain is mainly produced during storage (Olsen *et al*., 2006), but, in grapes, OTA production may start in the vineyard (Battiliani and Pietri, 2004).

Harcz *et al*. (2007b) found that OTA presence in organically produced beer is significantly higher than in conventional beers analysed during the period 2003–2005. The authors stress that during a year with a high content of OTA in beer (2004), a high consumer (97.5th percentile) of organic beers would have an intake of OTA corresponding to 109% of the TDI. The equivalent figure for a high consumer of conventional beer would be 43%. However, the authors used a TDI of 5 ng/kg body weight which does not agree with values for provisional tolerable weekly intake (PTWI) established by either the JECFA (the FAO/WHO expert group) or the EFSA (see section on 'Background on Moulds and Mycotoxins'). The intake figures for high consumers of organic beers in the study of Harcz *et al*. (2007b) actually correspond to 38% and 32% of the PTWI established by JECFA and EFSA (see Table 7.1).

In an Italian study by Rossi *et al*. (2006), samples were taken from lots of organic (20) and conventional (15) wheat using the European rules for official control. All data were below the maximum limit set by the EU (5 µg/kg) and there were no differences between organic and conventional grain.

A Spanish study estimated the dietary intake of OTA from conventional (n = 74) and organic bread (n = 26; González-Osnaya *et al*., 2007). The incidence of OTA was 20.3% and 23% for non-organic and organic bread, respectively. The highest levels of OTA were found in non-organic bread, of which five samples exceeded the European maximum limit of 3 µg/kg. The authors do not indicate whether there was a statistical difference between the organic and non-organic bread.

In a Dutch study (Chiodini *et al*., 2006) the OTA content was determined in 19 organic and 25 conventional wines originating from different geographical regions. The concentrations of OTA in organically produced wines (ranging from 'not detected' to 0.72 µg/l) were not significantly different from those in conventional products (ranging from 'not detected' to 0.75 µg/l).

Patulin

Patulin is predominantly produced in fruits after harvest. Patulin formation in apples is very sensitive and responsive to several harvesting techniques, storage conditions and other processing practices that are irrelevant to the farming system per se. It may in some cases also be produced in foodstuffs after processing, for example, in jams, due to secondary infection of moulds.

The occurrence of patulin in four different apple products (n = 40) was studied in the Italian market (Ritieni, 2003). The products were apple juice, apple-containing baby food, apple vinegar and apple purée. No significant differences were found when organically and conventionally produced products were compared. Similarly, no significant difference could be detected between organic and conventional systems when six apple samples (2 kg from each system) from different producers in France were compared (Malmauret *et al*., 2002).

Sixty-three commercial apple products available on the Dutch market were used to examine patulin levels (Boonzaaijer *et al*., 2005). The samples consisted of baby food (14 conventional, 12 organic), apple purée (5 conventional, 5 organic), apple juice (5 conventional, 5 organic), fruit juice (5 conventional), apple cider (5 conventional) and apples (5 conventional). Patulin was detected (detection and quantification limit = 25 µg/l) in only one sample (organic apple juice), but no significant difference could be found between conventional and organic samples.

Beretta *et al*. (2000) surveyed patulin in apple-based products such as baby foods (n = 23) and apple juices (n = 33) available on the Italian market. In the case of baby foods, all samples had a patulin level below the maximum permitted for baby food (10 µg/kg) and there were no significant differences between conventionally and organically produced products. In contrast, apple juices from organic agriculture contained significantly higher mean

levels of patulin (7.7 µg/kg) than conventional products (1.0 µg/kg). However, the maximum levels found in organic products (28.2 µg/kg) were well below the maximum permitted levels for patulin in apple juices (50 µg/kg) and would not likely constitute a significant risk for the consumer.

Piemontese *et al*. (2005) investigated the occurrence of patulin in 100 conventional and 69 organic fruit-based foodstuffs which were commercially available in Italy. The incidence of positive samples was greater in organic products (45%) than in conventional fruit products (26%). Mean patulin concentrations in apple juices, pear juices, other fruit juices and fruit purées were also higher in organic products as compared to conventional products. Statistical analyses of all commercial samples revealed that mean concentrations in organic products were significantly higher. Moreover, the maximum levels found in this study were always found in organic products. The authors also estimated the daily intake of patulin in the Italian population using the levels found in their study. For adults, these were 0.4 and 1.6 ng/kg body weight for conventional and organic products, respectively. The corresponding intake figures for children were 3.4 ng/kg body weight for conventional products and 14 ng/kg body weight for organic products. However, it is very unlikely that these differences between intakes from conventional and organic products will have consequences for the health of the population since the estimates for both groups of products are far below the established TDI for patulin (400 ng/kg body weight; Table 7.1).

Another Italian survey on the occurrence of patulin was conducted during 2005 on commercial pure apple juices (n = 53) and mixed apple juices (n = 82; Spadaro *et al*., 2007). With the exception of one sample, the levels of patulin were lower than 50 µg/kg, which is the maximum permitted level in Europe for fruit juices. There was a similar incidence of positive samples found in conventional and organic apple-based juices, and the differences between the mean contamination levels, although higher in organic (10.92 µg/kg) than in conventional juices (4.77 µg/kg), were not statistically significant.

Other studies

In an unpublished investigation performed by the Swedish National Food Administration (Möller, T. and Brostedt, S., 2007, National Food Administration, Sweden, personal communication) the amounts of mycotoxins in organic and conventional foods were surveyed at retail level in two geographic regions during 2001–2003 (Table 7.3). The samples included cereal products and fruit jams; no significant difference between conventional and organic origin could be detected.

The Effects of the Environment on Mycotoxins in Organic and Conventional Foods

This review of mycotoxins in food and food raw material such as cereal grain is in accordance with the findings of Magkos *et al*. (2006), i.e. organ-

Table 7.3. Occurrence of mycotoxins in organic and conventional foods at retail. (Unpublished data (2001–2003) from the Swedish National Food Administration, Uppsala, Sweden; Möller, T. and Brostedt, S., 2007, personal communication.)

Mycotoxin	Commodities/ farming system	No. of samples	No. of positive samples	Range (μg/kg)
Ochratoxin A[a]	Cereals/conventional	137	15	0.1–0.8
	Cereals/organic	134	30	0.1–1.5
Trichothecenes				
Deoxynivalenol	Cereals/conventional	137	127	10–289
	Cereals/organic	134	122	10–1522
Nivalenol	Cereals/conventional	137	78	10–324
	Cereals/organic	134	79	10–291
Acetyl-Deoxy-nivalnol	Cereals/conventional	137	40	10–115
	Cereals/organic	134	38	10–103
HT-2	Cereals/conventional	137	14	25–224
	Cereals/organic	134	18	25–246
Patulin[a]	Fruit jams/conventional	75	5	3.3–40.6
	Fruit jams/organic	75	2	4.9–6.8

[a]Performed with accredited analytical methods.

ically grown foods are reported to be either more, less, or in most cases equally contaminated with mycotoxins as conventional foods. As stressed above, data are in most cases not directly comparable due to different varieties, geographic location, time of harvest and postharvest handling. In other words, it is not clear to what extent the differences observed can be attributed to the cultivation system per se. The best selection of studies comparing the two farming systems deals with mycotoxins in cereals and cereal-based food products in connection with contamination by *Fusarium* mycotoxins. It is well known that contamination with *Fusarium* mycotoxins increases in practices where cereal is followed by another cereal (especially where maize is grown before wheat). Leaving residues of a previous crop on the soil surface, as a consequence of reduced or zero tillage practices, and, using *Fusarium* non-resistant varieties can also enhance mycotoxin contamination. Edwards (2007b) noted that many conventional farms grow the oat variety 'Gerald' after another cereal, usually wheat, whereas organic farmers were more likely to grow other oat varieties after a non-cereal. That the organic samples had significantly lower T-2 and HT-2 contents compared to conventional samples, therefore, could be partly due to organic growers not growing 'Gerald' and not following a cereal as frequently as conventional growers. As alluded to earlier, Edward's analysis revealed that previous crop and variety all had statistically significant effects on T-2 and HT-2 concentration, in addition to year, region and practice (organic or conventional). Another possible factor, not accounted for in the statistical analysis, is rotation; organic growers tend to use longer and less cereal-intense rotation. Edwards (2007a) also noted that conventional wheat samples receiving

azole fungicide ear spray had significantly lower DON than those which received no ear spray. The efficacy of fungicide application to reduce *Fusarium* Ear Blight (FEB) and subsequent DON formation depends on the fungicide used and the rate and the timing of application. In particular, azoles, tebuconazole, metconazole and prothioconazole have been shown to significantly reduce FEB symptoms and *Fusarium* mycotoxin concentration (Nicholson *et al*., 2003). Another fungicide, azoxystrobin had little impact on mycotoxin concentration in harvested grain when *Fusarium* species dominated the site but could result in an increase in mycotoxin concentration in grain when *Microdochium nivale* was the predominant species present. A number of trials in Germany (Ellner, 2006) have indicated that some strobilurin fungicides applied before flowering can also result in increased DON compared to unsprayed plots.

The above description of the complexity in *Fusarium* mycotoxin production in cereals indicates that both organic and conventional systems may include important practices to avoid *Fusarium* infection and subsequent toxin formation. Furthermore, global climate change may very likely lead to increased levels of mycotoxins in raw food material. There is already an apparent shift in prevalence of *Fusarium* species on infected heads in Northern Europe towards *Fusarium graminearum*. It has been suggested that *F. graminearum* is displacing other species such as *F. culmorum*, presumably due, in part, to short-term climate variations and ecological differences among fungi, but also perhaps due to differences in aggressiveness and pathogenicity of the various species (Osborne and Stein, 2007). Relative to other species, *F. graminearum* is favoured in warmer, wetter conditions in terms of conidial production and infection rates.

Furthermore, climate change may lead to the introduction of new plants to regions where climate used to be an obstacle for successful production. One example is the increased production of maize in Northern Europe. Increased production of maize and the dramatic increase in maize and other residues that remain on the soil surface may provide an increased reservoir of fungal spores, especially of *F. graminearum* (Osborne and Stein, 2007).

Cotty and Jaime-Garcia (2007) have summarized the influences of climate on aflatoxin-producing fungi and aflatoxin contamination. They concluded that as climate shifts, so do the complex communities of aflatoxin-producing fungi. This includes changes in the quantity of aflatoxin producers in the environment, and, alterations to fungal community structure. Fluctuations in climate also influence predisposition of hosts to contamination by altering crop development and by affecting insects that create wounds in which aflatoxin producers proliferate. Aflatoxin contamination is prevalent both in warm humid climates and in irrigated hot desserts. In temperate regions, contamination may be severe during drought. Maize has become a staple for many millions in warm regions throughout Africa, Asia and the Americas. This crop is particularly vulnerable to influences of climate as exemplified by recent outbreaks of lethal aflatoxicoses in Kenya (Azziz-Baumgartner *et al*., 2005). These outbreaks of aflatoxin contamination of home-grown maize have been a recurrent problem and reliable methods to prevent future outbreaks are necessary.

Whether a conventional or organic farming system is the best suited to meet future challenges due to climate change is a complex question. As indicated above, both systems may have advantages. However, it will be important to consider preventive measures from both systems in order to meet future challenges caused by climate change.

Conclusions

This review has not been able to identify significant or emerging differences concerning mycotoxin levels in organic or conventional food products. Some results indicate that DON levels are occasionally lower in organic cereals/cereal products and that patulin levels are occasionally higher in organic fruit products but in most cases there is no difference between organic and conventionally produced food products (Table 7.2). In those cases where significant differences have been observed, the estimated intakes do not promote any cause for concern for consumers, i.e. the intakes are still below the TDIs that have been established by JECFA or EFSA.

Finally, there is a strong need for investigations under strictly controlled conditions which could give an unbiased answer to the question whether the differences seen are due to the farming system or other factors. Such new knowledge may also provide useful information for future decisions to understand and predict climate change impacts on the development of mycotoxin-producing fungi.

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8 Human Pathogens in Organic and Conventional Foods and Effects of the Environment

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Introduction

Food-borne illnesses have become increasingly problematic, with a sixfold increase in gastro-enteritis and food poisoning in industrialized countries in the last 20 years (Nicholson *et al*., 2000). The most frequently reported food-borne diseases in the EU in 2005 were those caused by *Campylobacter* and *Salmonella* (European Food Safety Authority, 2006). In the USA and Canada, salmonellosis is economically the most important disease followed by Shiga toxin-producing *Escherichia coli* (STEC; primarily *E. coli* O157:H7; Mead *et al*., 1999). STEC infections occur worldwide but are most common in the USA and Canada (Nataro and Kaper, 1998). However, STEC infections are also on the increase in Europe (Fisher and Meakens, 2006).

Because animals are reservoirs and carriers of these human pathogens, most attention has been paid to contamination of animal products. A large proportion of animal products can be contaminated with particular pathogens. For example, 36% of poultry samples were contaminated with *Salmonella Enteritidis* in a Belgian retail market (Uyttendaele *et al*., 1999). Around 12% of minced beef samples in Spain, sampled between 1995 and 2003, contained STEC (Mora *et al*., 2007).

Increasingly, outbreaks of intestinal infections have been associated with bacterial pathogens ingested with vegetables and fruits (Tauxe *et al*., 1997; Sivapalasingam *et al*., 2004). Contrary to animal products, which are generally cooked, various fruits and vegetables are consumed raw. Most outbreaks related to consumption of fresh vegetables have been associated with *Salmonella* spp. and to a lesser extent with *E. coli* O157:H7 (Sivapalasingam *et al*., 2004). The best-known examples are outbreaks of *E. coli* O157:H7 from unpasteurized apple cider, lettuce or spinach, followed

by salmonellosis associated with tomatoes, lettuce, melons and sprouts (Guo *et al*., 2001; Sivapalasingam *et al*., 2004). In addition, *Listeria* can be found on cut vegetables and salads (Jemmi and Stephan, 2006). Recently, a large multi-state outbreak of *E. coli* O157:H7 associated with spinach occurred in the USA, with at least 187 cases, including 97 hospitalizations and three deaths (Anon, 2006).

Consumers perceive organic products as safer and healthier than conventional products, and the demand for organic products has increased in recent years (Magkos *et al*., 2003). However, is organic food indeed safer than conventional food? Some people attribute the increase in outbreaks of enteric diseases, at least in part, to the increase in organic food production with its use of animal manure and/or compost, and the absence of antibiotics allowing growth of human pathogenic bacteria in agricultural animals and their products (Magkos *et al*., 2003, 2006; Avery, 2004). Yet, only one outbreak, namely the *E. coli* O157:H7 outbreak in 2006, has been associated with organic produce (spinach) so far (FDA, 2006). In this chapter, we compare the risks associated with organic and conventional foodstuffs based on scientific evidence.

First, an overview is given of the most important pathogens associated with enteric diseases, their biological characteristics, the epidemiology of the diseases they cause and effects of the environment. Next, the prevalence of bacterial pathogens on poultry, pork, dairy and cattle products and fresh produce from organic and conventional production systems is compared. This is followed by a discussion of risk management strategies. We end with a general discussion and conclusions.

Pathogens Associated with Enteric Diseases

A wide variety of organisms can cause disease in humans via the food production chain. This chapter is restricted to the most important zoonotic bacterial pathogens, namely *E. coli* (including *E. coli* O157:H7), *Campylobacter jejuni*, *Campylobacter coli*, *Listeria monocytogenes and Salmonella enterica* (Woolhouse and Gowtage-Sequeria, 2005). These bacterial species can cause infections in the human intestinal tract, or induce food poisoning through the production of toxins either inside or outside of the body. The effects vary from mild diarrhoea to extreme kidney damage and death, especially in people with a weak immune system such as children, the elderly and the immunocompromised.

Descriptions of enteric pathogens

Escherichia

E. coli is a facultative anaerobic bacterium, metabolically active between 15°C and 45°C, with an optimal growth temperature of 37°C and pH of

7.0–7.4. *E. coli* is part of the normal microbial community in warm-blooded animals, including humans, and is considered an indicator organism of faecal contamination of food and the environment. Some strains are pathogenic, producing enterotoxins and haemolysins. *E. coli* is the closest 'relative' of *Shigella*, and its toxins are similar to those of *Shigella*, also called shiga toxins. Among the enteropathogenic *E. coli*, the following types are distinguished: Enteropathogenic (EPEC), enterotoxigenic (ETEC), enterohaemorrhagic (EHEC) and Shiga toxin-producing (STEC). EHEC constitutes a subset of STEC, inducing severe clinical symptoms in humans. The infectious dose for STEC/EHEC types is low (<100 cells); for ETEC it is 10^6 cells and for EPEC 10^8–10^9 cells (Altwegg and Bockemuhl, 1998). EHEC pathovars tolerate very acid conditions (pH ~2.5) for short periods. *E. coli* O157:H7 seems to be more virulent than other STEC (Vanselow *et al*., 2005). This high virulence is partially determined by its ability to survive environmental stresses, such as resistance to low pH levels encountered in the human stomach. *E. coli* O157:H7 can induce bloody diarrhoea and kidney damage, especially in young children.

Salmonella

S. enterica is a facultative anaerobe with an optimal growth temperature of 37°C and range of 7–48°C. Under special conditions, *S. enterica* may proliferate at temperatures of <4°C. Growth occurs between pH 4 and 9. *Salmonella* spp. are closely related to *E. coli* and *Shigella*. Strains of *S. enterica* are subdivided serologically in to *Typhimurium*, *Enteritidis*, *Paratyphi* and *Typhi* (Old and Threlfall, 1998). *Salmonella* spp. are general parasites, pathogenic for humans and many animal species. *Salmonella* is a cosmopolite with many serovars. The infectious dose depends on the serovar, immune system and age of people and can range from 100 cells for children to 10^5 and even 10^9 cells for adults. *S. Typhi* causes systemic infections in humans, and does not have an animal reservoir. We will only consider non-typhoidal salmonellosis caused by *S. Typhimurium* or *S. Enteritidis*. These serovars induce diarrhoea, nausea, abdominal pain and mild fever in humans (Forshell and Wierup, 2006).

Campylobacter

C. jejuni and *C. coli* are microaerophilic to anaerobic; they require oxygen (O_2; 3–15%) and carbon dioxide (CO_2; 3–10%) for growth. They consume amino acids and products of the tricarboxylic acid cycle but not carbohydrates. Their optimal growth temperature is 37°C (some species 42°C), and growth occurs in the range 15–43°C. They are resistant to 4°C and even −20°C. The optimal pH for growth is neutral. *Campylobacter* spp. are commensals in many animal species and rarely cause disease. *Campylobacter* can survive in manure contaminated water for 3 months. Vegetables can become contaminated through manure or irrigation water. The pathogen survives well in soil and in the rhizosphere of vegetables (Brandl *et al*., 2004). *C. jejuni* and *C. coli* are pathogenic for humans (Nachamkin and Skirrow, 1998). The

infectious dose is between 10^3 and 10^5 cells/g, respectively. Diarrhoea, abdominal cramps and nausea are commonly associated with campylobacteriosis (Wagenaar *et al*., 2006).

Listeria

L. monocytogenes is aerobic or facultatively anaerobic. It can grow between 0°C and 45°C and at 4°C with a doubling time of 1–2 days. Optimal growth occurs at temperatures between 30°C and 37°C and at pHs between 5.5 and 9.6. It is ubiquitous, being found in decaying plant material, healthy vegetation, healthy animals, faeces, soil, water, and fresh and frozen meat. It is stress-resistant and can survive food processing. *L. monocytogenes* is pathogenic to animals as well as humans. The infectious dose can be as high as 10^9 colony-forming units (cfus). In inoculated chick embryos the LD_{50} is around 10^2 cfus. In intramammary-injected cows bacteraemia does not occur, but 10^3–10^4 *L. monocytogenes* cfus/ml is shed into milk for 9–12 months (McLauchlin and Jones, 1998). In humans, this pathogen causes not only gastro-enteritis but can also induce listeriosis, which may result in meningitis, sepsis, abortion and death. Out of the food-borne pathogens, it is responsible for the highest hospitalization rate (91%; Jemmi and Stephan, 2006).

Epidemiology and effects of the environment

The pathogens mentioned above have an animal reservoir. Farm animals normally carry the pathogens asymptomatically, but certain strains of *Salmonella*, *E. coli* and *Listeria* can cause clinical disease in animals, leading to signs of fever and diarrhoea (especially in calves and piglets). Some of the bacterial pathogens, in particular *E. coli*, *Campylobacter* and *Salmonella* spp., are also carried by wildlife, including mammals and birds (Waldenstrom *et al*., 2002; Daniels *et al*., 2003). The various human pathogens are associated with different animals. Poultry and pigs are the predominant reservoirs of *Salmonella* (Nicholson *et al*., 2000), although cows can also carry substantial numbers (Calvert *et al*., 1998). *E. coli* O157:H7 is mostly associated with cattle, whereas the main reservoir for *Campylobacter* sp. is poultry (Table 8.1).

Besides particular associations between animals and pathogens at the species level, the prevalence of serovars of a single species, for example

Table 8.1. Incidence of four bacterial species in animal manures in the UK. (From Nicholson *et al*., 2000.)

Pathogen	Cattle (%)	Pigs (%)	Poultry (%)
Salmonella spp.	0–11	0–38	1–100
Escherichia coli O157:H7	16	0.4	0.5
Campylobacter jejuni	89	95	>75
Listeria monocytogenes	>75	<5	8

S. enterica, can also be more prevalent in some animal species (including humans) than in others (van Duijkeren *et al*., 2002).

The prevalence of *E. coli* O157:H7 in dairy herds can vary from 10% to 50% or higher depending on the time of year (Bouwknegt *et al*., 2004; Franz *et al*., 2007a) with frequency of Shiga toxin genes reported as being as high as 80% (Franz *et al*., 2007a). Higher cattle densities and increased cattle movements may have contributed to the emergence and spread of *E. coli* O157:H7 (Vanselow *et al*., 2005). The increased use of cattle slurry on pastures may also have contributed to a rise in STEC (Armstrong *et al*., 1996). *E. coli* O157:H7 has been found in deer (Renter *et al*., 2001) and flies (Alam and Zurek, 2004). STEC is relatively rare in pigs and poultry.

The incidence of *Campylobacter* is moderately high in cows and pigs, but can be up to 100% in poultry (Heuer *et al*., 2001). Avian wildlife is considered a significant reservoir for *Campylobacter* and *Salmonella* (Refsum *et al*., 2002). The level of contamination of farm animals can be influenced by various factors, such as season, animal breed, age, housing, nutrition, antibiotic use, pathogen exposure, stress and on-farm hygiene (Daniels *et al*., 2003; Brabban *et al*., 2004). Young animals are frequently more susceptible to pathogens, including human pathogens, especially when they are exposed to outdoor conditions. For example, young chicks may carry more *Salmonella* than older chickens due to their undeveloped immune system.

The effect of feed composition on the prevalence of *E. coli* O157:H7 in cows has been well documented (Russell and Rychlik, 2001). Feeding high-carbohydrate diets like maize silage or maize generally results in a higher prevalence of *E. coli* O157:H7 than feeding a high-fibre diet with older grass silage, hay or straw (Couzin, 1998; Franz *et al*., 2005; Vanselow *et al*., 2005). Sugars from high-starch feeds, like maize silage and maize, pass on to the lower intestinal tract where fermentation results in an increase in fatty acid concentrations and a decrease in pH (Diez-Gonzalez *et al*., 1998). Cattle fed large amounts of raw maize had approximately 1000-fold more generic *E. coli* than cattle fed hay, and these *E. coli* strains were much less sensitive to an acid-shock mimicking the human stomach (Diez-Gonzalez *et al*., 1998). In addition, ionophores are sometimes added to conventional cattle feed to increase production efficiency by increasing nitrogen and carbon retention in the digestive system (Callaway *et al*., 2003). Ionophores, which are fermentation products of several actinomycetes, inhibit gram-positive bacteria and may thereby promote gram-negative bacteria like *E. coli* (Ipharraguerre and Clark, 2003).

Manure and irrigation water are the main avenues for spread of human pathogens to fields and the crops growing there (Solomon *et al*., 2002; Islam *et al*., 2004). Manure can harbour high numbers of human pathogenic bacteria like *Salmonella* spp. and *E. coli* O157:H7, which can remain viable for extensive periods of time, even up to 1 year (Kudva *et al*., 1998). The survival period in soil is dependent on soil management (e.g. organic versus conventional), available substrate for survival of the pathogens in relation to microbial competition, bacterial diversity, temperature, moisture and availability of O_2 (Franz *et al*., 2008). Soil survival of *E. coli* O157:H7 was longer when

injected with slurry than when surface-applied or incorporated with manure (A.V. Semenov, 2008, Moscow State University, Moscow, unpublished data).

Subsequent to manuring or irrigation of land, crops can become contaminated via the roots, spread by wildlife, or by splashing rain or irrigation water on to the leaf surface (Natvig *et al*., 2002). Superficial disinfection of contaminated plants generally does not eliminate the pathogens (Solomon *et al*., 2002; Johannessen *et al*., 2005; Franz *et al*., 2007b; Klerks *et al*., 2007b) suggesting that contamination not only occurs on plant surfaces, but also inside plant tissues. Endophytic occurrence of *E. coli* and *S. enterica* has been demonstrated microscopically (Solomon *et al*., 2002; Klerks *et al*., 2007b), but endophytic pathogen growth during crop growth has not yet been demonstrated. When plants or plant products are cut, bacterial contaminants can spread throughout the food processing and packing operations, leading to their widespread distribution, for example on shredded lettuce, diced tomatoes or cut cabbage (Kapperud *et al*., 1995; Guo *et al*., 2001).

Prevalence in Organic and Conventional Foods and Farms

Poultry products

A lot of research has been done to determine the presence and spread of *Campylobacter* and *Salmonella* in conventional poultry products. Less attention has been paid to the occurrence of pathogens in alternative farming systems, such as organic and free-range (Heuer *et al*., 2001). Information relating to organic and free-range egg production is particularly lacking. With the change from conventional cage to various free-range systems the *Salmonella* incidence in laying flocks could be expected to increase as a result of the risk of contamination outdoors. However, one German study has shown that the incidence of *Salmonella* (mostly *S. Enteritidis*) was higher in conventional than in organic and other free-range systems (Methner *et al*., 2006). In contrast, penetration of eggshells by *S. Enteritidis* was enhanced for organic compared to conventional free-range and battery cage eggs. This difference was attributed to the feed rather than the chicken breed (Messens *et al*., 2007). The eggs were obtained from a commercial source, and batches were not replicated.

More information is available about organic versus conventional broilers and meat. Most studies show a significantly higher prevalence of *Campylobacter* in organic broilers, which is likely associated with the mandatory free-range period outdoors for organic chickens (Heuer *et al*., 2001; Rodenburg *et al*., 2004; Van Overbeke *et al*., 2006). The incidence was generally high, ranging from 26% to 100% (Table 8.2). When *Campylobacter* prevalence in broilers from organic, intensive and extensive indoor rearing systems in Denmark was compared, the prevalence of *Campylobacter* in organic flocks was significantly higher than in the flocks of the other systems, while there was no significant difference between the intensive and extensive indoor flocks (Heuer *et al*., 2001). The prevalence of *Campylobacter* on broiler and turkey farms in

Table 8.2. Prevalence[a] of *Campylobacter* in chickens, reared in different systems.

Reference	Location	Organic (O) (%)	Conventional (C) (%)	Extensive indoor or outdoor (E) (%)	Significant difference
Heuer *et al.* (2001)	Farm				
	Flocks	100	37	49	Yes: O–C ($P < 0.001$) No: C–E
	Individuals	65	68	60	No
Kramer *et al.* (2003)	Retail	49	43		No
Van der Zee *et al.* (2003)	Retail	36	26		Yes ($P < 0.01$)
Van der Zee *et al.* (2004)	Retail	44	29		Yes ($P < 0.01$)
Cui *et al.* (2005)	Retail	76	74		–
Van Overbeke *et al.* (2006)	Farm	67	27		Yes ($P = 0.024$)
El-Shibiny *et al.* (2005)	Farm				
	Individuals	69		90	–
Luangtongkum *et al.* (2006)	Farm	89	66		Yes ($P < 0.05$)

[a]Percentage of flocks with at least one positive broiler or percentage of positive individuals per flock.

Ohio was also higher in organic than in conventional birds, but the difference was significant for broilers only (Luangtongkum *et al.*, 2006). No significant differences in *Campylobacter* incidence were found between four organic and four conventional chicken farms in Belgium (Tuyttens *et al.*, 2005). In a Belgian study (Van Overbeke *et al.*, 2006) with nine organic and 11 conventional broiler farms, *Campylobacter* incidence was similar in organic and conventional flocks until the organic chickens gained access to the outside area. At slaughter (12 and 6 weeks for organic and conventional broilers, respectively), the *Campylobacter* prevalence in caecal samples was significantly higher in the organic broiler chickens. *Campylobacter* is widespread in nature, for example in birds, rats, mice and insects, and survives very well in soil. Thus, the slaughter age of the organic chickens and the requirement to have outdoor access seem to be associated with the higher infection level (Luangtongkum *et al.*, 2006; Van Overbeke *et al.*, 2006). When organic and free-range chickens, both with outdoor access, were monitored at single farms, *Campylobacter* infection started at a younger age and the incidence was higher for the free-range farm but the diversity of *Campylobacter* strains and the number of *Campylobacter* phages were higher for the organic farm (El-Shibiny *et al.*, 2005). The dominant *Campylobacter* spp. changed from *C. jejuni* in young chickens to *C. coli* in older chickens in both farms. Isolates from free-range chickens were resistant to a wider range of antibiotics than those from organic chickens, although antibiotics were not used for rearing any of the chickens sampled.

At retail, incidence of *Campylobacter* in organic and conventional chicken meat has often been similar, levels in both systems ranging from 43–49% in The Netherlands (Kramer *et al*., 2003) to 74–76% in Maryland, USA (Cui *et al*., 2005). However, in another study, the prevalence of *Campylobacter* in organic chicken meat was 36.3%, which was significantly higher than that in conventional chicken meat (25.9%; van der Zee *et al*., 2003). This observation was confirmed in 2004, when the percentages of *Campylobacter*-contaminated poultry meat were 43.9% and 29.3% for organic and conventional, respectively (van der Zee *et al*., 2004). However, the incidence on meat is likely dependent on slaughtering procedures.

For *Salmonella*, results relating to pathogen contamination of organic and conventional poultry are mostly reverse to those for *Campylobacter* (Table 8.3). However, exceptions can be noted. In one American study, organic chickens were more frequently contaminated with *Salmonella* than various non-organic chickens, but only one organic rearing system was included and statistical analyses were not carried out (Bailey and Cosby, 2005). The authors attributed the higher incidence in chickens from the organic farm to exposure to wild animals and their droppings (Bailey and Cosby, 2005). Once outdoor chicken runs are contaminated, they can remain a source of infection for a long time as *Salmonella* can survive in contaminated chicken faeces for 64–77 days (Siemon *et al*., 2007). In another American study, *Salmonella* contamination rates in organic and conventional poultry meat from retail stores were 61% and 44%, respectively, but again statistical analyses were not included (Cui *et al*., 2005). The authors attributed the difference to the regular use of antimicrobials by conventional producers, a practice that is not allowed in organic production. The broiler feed antimicrobial additive flavophospholipol indeed reduced the degree and incidence of *Salmonella* shedding in faecal samples of 6-week-old chickens (Bolder *et al*., 1999). However, when the effect of feed with and without flavophospholipol was tested on pathogen

Table 8.3. Prevalence of *Salmonella* in chickens, reared in different systems.

Reference	Location	Organic (%)	Conventional (%)	Free-range, pasture, natural (%)	Significant difference
Kramer *et al*. (2003)	Retail[a]	4	8		No
Van der Zee *et al*. (2003)	Retail	3.4	11.2		Yes ($P < 0.01$)
Van der Zee *et al*. (2004)	Retail	2.1	7.4		Yes ($P < 0.01$)
Van Overbeke *et al*. (2006)	Farm[b]	11	9		No
Tuyttens *et al*. (2005)	Farm	0	2.5		No
Bailey and Cosby (2005)	Farm	60	11.3	25	–
Cui *et al*. (2005)	Retail	61	44		–
Siemon *et al*. (2007)	Farm		47	33	No

[a]Percentage of *Salmonella*-positive birds per sample.
[b]Percentage of total farms tested which were contaminated with *Salmonella*.

incidence in broilers reared in free-range and pastured pen systems, no significant effect on the occurrence of *Campylobacter* and *Salmonella* was found (Lund *et al*., 2003).

Contrary to the studies cited above, four other studies comparing broilers and poultry meat from organic and conventional systems have revealed a lower *Salmonella* prevalence in the organic systems. In three Dutch studies, *Salmonella* contamination ranged from 2% to 4% in organic meat and from 7% to 11% in conventional meat. The differences were significant in two of the three studies (Kramer *et al*., 2003; van der Zee *et al*., 2003, 2004). Other studies have found no difference in the prevalence of *Salmonella* between organic and conventional (Tuyttens *et al*., 2005) or conventional and free-range pasture (Siemon *et al*., 2007) at the farm level. However, in the latter study, the conventional farms had significantly higher percentages of infected chickens than pasture farms. The effects of different production systems and feeding regimes on the occurrence of *Salmonella* in broiler chickens were determined in a controlled experiment with pastured pen and free-range systems combined with organic or conventional feed in the USA. No effects of feed were detected. Although more cases of *Salmonella* were found in the free-range system, the difference was not significant (Lund *et al*., 2003). In the survey of organic and conventional broiler chickens of Van Overbeke *et al*. (2006), attention was paid to the age of broilers when infection with pathogens occurred (see also section under '*Campylobacter*'). No significant differences were found in *Salmonella* infection between the two rearing systems at any time. However, the slaughter age of the chickens might explain the higher *Salmonella* prevalence in conventional than organic poultry meat in other studies. Heyndrickx *et al*. (2002) have shown that the highest shedding of *Salmonella* by conventional broilers was in the first 2 weeks of rearing. It seems that young animals can recover from a *Salmonella* infection (Kramer *et al*., 2003). This may be the main reason why *Salmonella* is often less prevalent in organic broilers, as these are slaughtered at an older age. Another reason may be a better developed immune system in organic chickens associated with differences in bacterial composition in the feed (Huber, 2007). Furthermore, *Campylobacter* may be more prevalent in the outdoor environment than *Salmonella*, outcompeting *Salmonella* in the chickens. Finally, *Salmonella* presence in organic chickens might be lower than in conventional chickens thanks to lower stress levels (Van Overbeke *et al*., 2006). Using the tonic immobility test, Tuyttens *et al*. (2005) showed that stress levels among conventional chickens were higher than among organic chickens.

Pig meat

Similar to poultry, pigs can harbour high numbers of *Campylobacter* and *Salmonella* spp. To what extent pork products are responsible for human campylobacteriosis and salmonellosis has not yet been fully elucidated.

Only few studies compare *Campylobacter* or *Salmonella* prevalence in organic versus conventional pigs or pork. However, because preventive use of antibiotics is not allowed in organic pig production, of possible relevance are comparative studies of conventional production systems which use antibiotics with those which do not (antibiotic-free, ABF). In a study in North Carolina (Thakur and Gebreyes, 2005), faecal samples were taken at conventional and ABF nursery and finishing farms, in addition to carcass samples in slaughterhouses. For the nursery farms, the prevalence of *C. coli* was significantly higher in manure of pigs reared at the ABF farms (77%) than at the conventional farms (28%). In contrast, there were no significant differences in *C. coli* prevalence in manure at the finishing farms and in carcass samples at the slaughterhouses. Thus, the absence of preventive antibiotic use at organic farms may not affect the final contamination of pork meat with *Campylobacter* spp. However, the mandatory outdoor husbandry in organic systems may enhance the risk of infection by various pathogens that survive in the outdoor environment, such as *Campylobacter* and *Salmonella* spp. (Jensen *et al*., 2006a,b; Kijlstra and Eijck, 2006). The dominant *Campylobacter* sp. in pigs has been shown to shift from *C. coli* to *C. jejuni* (more pathogenic to humans) after exposure to a paddock contaminated with *C. jejuni* by birds and rats (Jensen *et al*., 2006a). In experimental organic paddocks, *S. Typhimurium* was transmitted from inoculated pigs to tracer pigs, and this strain survived in soil, water and shelter huts for several weeks (Jensen *et al*., 2006b). More than one study reports *Salmonella* sero-prevalences to be significantly higher in meat juice from organic and free-range pig herds than from conventional herds (Wingstrand *et al*., 1999; van der Wolf *et al*., 2001). However, Bonde and Sørensen (2007) report no difference in *Salmonella* antibodies between outdoor (organic and conventional) and indoor (conventional) pigs, but *Salmonella* shedding on-farm and at slaughter was higher in pigs from the conventional indoor farms. Thus, sero-prevalence in meat need not be correlated to on-farm incidence. Sero-prevalence indicates immune activation during the lifetime of an animal, while shedding of live *Salmonella* cells may decrease over time. Altogether, we cannot conclude from these studies that the prevalence of *Salmonella* cells is higher in organic versus conventional pigs or pork. Besides the outdoor access and absence of antibiotics, other potentially important differences between conventional and organic practice include suckling period (longer for organic piglets); age at slaughter; and feed (potentially affecting the microbial composition of the gut and the development of the immune system; Jensen *et al*., 2006b).

Dairy products

Most dairy products are pasteurized, which explains the sporadic isolation of enteric pathogens from these products (European Food Safety Authority, 2006). We have not found any comparative studies of human pathogens in

organic versus conventional dairy products. However, soft cheese from unpasteurized milk can be a source of various pathogens, in particular *L. monocytogenes* (Jemmi and Stephan, 2006), and organic cheese is more often made from unpasteurized milk than conventional cheese (Zangerl *et al*., 2000). Yet, this does not necessarily mean that organic cheese may be more risky, as contamination of milk is primarily determined by cleanliness of the cows. Results from Ellis *et al*. (2007) indicate that, at least during the winter period, organically farmed cows may be cleaner than those farmed conventionally. In a survey of 175 cheese samples form 58 organic farms in Austria, *E. coli* abundance was higher than 10^5/g for 17% of the samples, a statistic similar to that found for conventional cheeses (Zangerl *et al*., 2000). Despite the lack of directly comparable studies for diary products, the findings above indicate that hygiene standards need to be improved for both conventional and organic practices.

Because cows are considered to be an important reservoir of pathogens (Rasmussen and Casey, 2001), studies which compare pathogen prevalence in manure and the farm environment between organic and conventional dairy farms have been summarized in Table 8.4. None of the studies summarized in the table report significant differences between farming systems. However, there was a lot of variability in prevalence between farms.

Table 8.4. Prevalence of pathogens in organic and conventional dairy farms.

Reference	Pathogen	Sampling details	Organic (%)	Conventional (%)	Significant difference
Sato *et al*. (2003)	*Campylobacter*	Manure samples pathogen-positive[a]	26.7	29.1	No[b]
Fossler *et al*. (2004)	*Salmonella*	Farms having >1 pathogen-positive faecal or environmental sample	92.3	92.8	No
		Faecal samples pathogen-positive	4.7	4.9	No
Kuhnert *et al*. (2005)	STEC[c]	Faecal samples pathogen-positive	58.3	57.0	No
	Escherichia coli O157:H7	Faecal samples pathogen-positive	5.7	3.5	No
Cho *et al*. (2005)	*E. coli* O157:H7	Faecal samples pathogen-positive	7.4–13.3	2.0–3.6	–[d]
Cho *et al*. (2006)	Shiga toxin-encoding bacteria	Faecal samples positive	6.6	2.3	No

[a]Thirty pairs of neighbouring organic and conventional farms compared.
[b]Significant differences were associated with season, animal age and herd size.
[c]Sixty organic and 60 conventional farms compared; STEC was detected on all 120 farms.
[d]Not tested.

In a large study of *Salmonella* incidence in the USA, 92–93% of both organic and conventional farms had at least one *Salmonella*-positive faecal or environmental sample (Fossler *et al*., 2004). About 5% of the individual faecal samples were *Salmonella*-positive. There were no significant differences between management systems.

In another American study, 30 pairs of neighbouring organic and conventional dairy farms were compared with respect to *Campylobacter* prevalence in manure (Sato *et al*., 2003). *Campylobacter* was detected in 27% of the organic samples and in 29% of the conventional samples. Again type of farm was not the decisive factor for observed differences. However, significant differences were associated with differences in season, age and herd size.

Similarly, no significant differences between farming systems were found for the prevalence of STEC or EHEC (Kuhnert *et al*., 2005; Cho *et al*., 2006). Shiga toxin-encoding bacteria (STB) occurred in 2% of conventional faecal samples and in 7% of similar organic samples, but this difference was not significant (Cho *et al*., 2006). Of all STB isolates 2% was STEC; there were no differences between farming systems. *E. coli* O157:H7 was detected in more faecal samples from organic than conventional farms, but the number of *E. coli* O157:H7-positive farms was not significantly different between organic and conventional farms (Cho *et al*., 2005). Similarly, no significant differences in STEC prevalence between 60 organic and 60 conventional dairy farms were found in Switzerland (Kuhnert *et al*., 2005). STEC was detected on all 120 farms, on average in 58% of the faecal samples. STEC O157:H7 was detected in 6% and 4% of the samples from organic and conventional farms, respectively (not significantly different).

The shedding rate of *E. coli* in dairy cattle appears very variable, depending on season, region, infection time, composition and storage of feed, age of the animals, herd size, hygiene, housing and detection methods (Cho *et al*., 2005; Franz, 2007). Pasture access was suggested as an important risk factor for STEC in cattle (Kuhnert *et al*., 2005). Organic cows usually spend more days outside than conventional cows (Hoogenboom *et al*., 2006) and therefore are at higher risk of contamination with *E. coli* from sources such as water and grass (Kuhnert *et al*., 2005). The stable floor is another factor; the presence of a rubber mat instead of other flooring could decrease the risk of *E. coli* O157:H7 contamination (Kuhnert *et al*., 2005). The use of straw, as often practiced in organic farms, can also reduce contamination, as cows may be cleaner, although the greater cleanliness of organic cows can mainly be attributed to higher fibre content in feed and manure (Ellis *et al*., 2007). Cows from conventional farms often receive a diet with a higher proportion of protein- and energy-rich concentrates and bulk feed to increase milk production. In many countries limits are set for the percentage of concentrates in organic cattle feed. The impact of feed composition on the presence of STEC is somewhat controversial; while populations of *E. coli* sometimes seem to be higher in (energy-rich) grain-fed than in forage-fed cows (Callaway *et al*., 2003; Brabban *et al*., 2004); Hovde *et al*. (1999)

have shown that *E. coli* O157:H7 is shed for a longer period in cows fed with hay than in cows fed with grains. High-protein feed results in high urea concentrations in milk which may favour the presence of STEC (Kuhnert *et al*., 2005). The age of cows can be another risk factor for STEC; calves between 2 and 6 months old are the highest risk group (Nielsen *et al*., 2002); however, older dairy cows also have an increased risk of being STEC carriers (Kuhnert *et al*., 2005), and organic cows are usually maintained for more years than conventional cows. Finally, the use of antibiotics may also affect the prevalence of enteric pathogens in cattle. Preventive antibiotic use is forbidden for organic cattle anywhere and for conventional cattle in many EU countries but not in the USA. Antibiotic use on conventional dairy farms may reduce the prevalence rates of STEC in the cows. On the other hand, antibiotic use may increase antibiotic resistance in bacterial subpopulations. For example, ampicillin and multi-drug resistance has been shown to be more common for *E. coli* strains isolated from conventional farms compared to organic farms (Walk *et al*., 2007).

Vegetables

Although food-borne outbreaks are often associated with animal products, increasing numbers of outbreaks have been linked to the consumption of contaminated fresh vegetables and fruits. Moreover, consumption of convenience foods has increased, and these may enhance the risk of microbiological contamination due to cross-contamination during the process of washing and cutting (Phillips and Harrison, 2005). Literature comparing organic and conventional produce in terms of contamination with coliform bacteria or *E. coli* is scarce. Problematic is the fact that the proportion of pathogen-positive vegetables is often very low, so that meaningful information can only be obtained when sample sizes are very large (typically 1000 or more). For example in a Dutch study, the numbers of samples of organic and conventional vegetables were too low to detect any differences; *E. coli* O157:H7 and *Salmonella* were not found (Hoogenboom *et al*., 2006). Results of the few investigations that do report quantitative comparisons are summarized in Table 8.5.

In extensive multiyear comparative studies with 40–63 participating farms in the USA (Minnesota and Wisconsin), the density of coliform bacteria was similar or higher in organic compared to conventional produce (Table 8.5), including leafy greens, cabbages, peppers and tomatoes (Mukherjee *et al*., 2004, 2006). Coliforms were present in 92% of all the samples. When coliform density was higher in organic produce, the difference was mostly due to the contribution from non-certified semi-organic farms. In 2002, the *E. coli* incidence in organic produce (including non-certified) was roughly sixfold greater than in conventional produce (Mukherjee *et al*., 2004). However, this difference did not hold when only certified organic farms were considered. In 2003 and 2004, the percentages

Table 8.5. Coliform counts and *Escherichia coli* prevalence in vegetables, grown in different systems.

Reference	Pathogen	Unit	Organic (O)	Semi-organic (S)	Conventional (C)	Significant difference
Mukherjee *et al.* (2004)	Coliforms	log MPN/g	2.9		2.9	No
	Escherichia coli	% +	4.3	11.4	1.6	Yes: O + S– C
		+/total	5/117	41/359	21/29	Yes: O–S
Mukherjee *et al.* (2006)	Coliforms					
	2003	log MPN/g	2.1	2.3	2.0	Yes: C–S
	2004	log MPN/g	2.3	2.3	1.5	Yes: C–S, O
	E. coli					
	2003	log MPN/g	2.0	2.3	2.4	No
		% +[a]	8.8	9.4	1.7	–
		+/total[a]	14/160	29/307	4/230	–
	2004	log MPN/g	2.3	2.4	2.0	No
		% +[a]	7.1	6.8	2.8	–
		+/total[a]	17/238	31/455	8/282	–
Phillips and Harrison (2005)	Coliforms	log CFU/g	2.9		2.7	No
	E. coli	% +	3.7		8.3	No
		+/total	4/108		9/108	No

[a]Derived from Table 5 in Mukherjee *et al.* (2006).

of *E. coli*-positive samples were higher in organic and semi-organic than in conventional produce (Mukherjee *et al.*, 2006). The average *E. coli* densities were about 2.2 log most probably number (MPN)/g and were not significantly different between farming systems (Mukherjee *et al.*, 2006). *E. coli* O157:H7 was not detected, and *Salmonella* was found on one organic lettuce sample and one organic green pepper sample (Mukherjee *et al.*, 2004). It was not reported whether these samples originated from certified organic or non-certified semi-organic farms. Organically and conventionally produced spring mix (108 samples each) from the same processor in California did not differ in total bacterial counts, coliform counts or *E. coli* incidence (Phillips and Harrison, 2005). *Salmonella* and *L. monocytogenes* were not detected.

As organic farms commonly use manure to maintain soil fertility, several researchers suggested that this entails an increased risk of pathogen contamination of fruits and vegetables (Bourn and Prescott, 2002; Magkos *et al.*, 2003, 2006). However, enteric bacteria, including pathogens, can occur naturally in soil (Mukherjee *et al.*, 2006) and water (Solomon *et al.*, 2002; Muniesa *et al.*, 2006), and these routes of infection are potentially as common in conventional as in organic fields. So far, out of all outbreaks of *E. coli* O157:H7 infection associated with fresh vegetables, only one (baby spinach in the USA in

2006) originated from an organic farm, and there is no indication that the occurrence of human pathogens would be greater for organic than for conventional produce.

Risk Management Strategies

Preventive control

At the farming systems and production chain level

Governments and the food industry have become concerned about the repeated outbreaks of food-borne diseases, as the economic and social consequences can be very large. Working groups are developing integrated approaches to risk management with the goal to prevent or minimize the risk of outbreaks. Hazard analysis and critical control point (HACCP) protocols were first developed for food processing industries, and more recently also for large, industrialized farms and retail markets. Critical points of potential contamination are identified and checked for the occurrence of pathogens, and introduction and transmission of pathogens are as much as possible controlled (Noordhuizen *et al*., 2008). Testing for sero-positivity in meat instead of the presence of live pathogens is not recommended, because animals may have been exposed but may have combated the infection before slaughter (Bonde and Sørensen, 2007). This holds especially for organic animals (Huber, 2007). Similarly, polymerase chain reaction (PCR) techniques used on DNA extracted directly from a substrate will give false positives in terms of risk, as DNA can remain intact long after an organism has died. Moreover, these tests could lead to unnecessary bans on animals, plants and their products and to trade barriers (Mathews *et al*., 2003). Nevertheless, it is prudent to restrict the movement of animals and animal feed, especially from sources with unknown risk conditions (OIE Animal Production Food Safety Working Group, 2006).

Microorganisms move through cycles in agroecosystems (Fig. 8.1), with a reservoir in soil or water, and a secondary reservoir in warm-blooded animal species in the case of human pathogens. In a simple cycle, the plant rhizosphere selects for a subset of microbes from the total soil community with a further selection and reduction in diversity in the plant endosphere. The plant-associated microbial community will directly or indirectly enter the animal gut, and a selected community ends up in the faeces to be returned to soil. Contaminated water can also play an important role in the cycle. This cycle needs to be broken by specific management practices.

First and foremost the animals need to be kept in a healthy condition to minimize shedding; they need to have proper feed, free from pathogens, enough space and ventilation to minimize cross-contamination, clean drinking water and minimal stress (OIE Animal Production Food Safety Working Group, 2006). Infected animals need to be kept separate from healthy animals; rotational pasturing is recommended. Introduction of infected animals

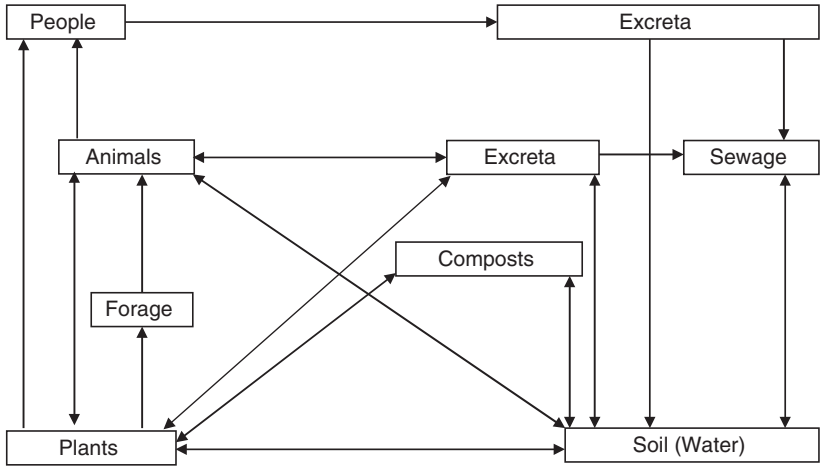

Fig. 8.1. General diagram of a common cycle of microorganisms in (agro)ecosystems.

needs to be avoided. Proper records need to be kept of all animal movements, feed stocks, health status, etc. Second, transmission from animals via soil or water to plants needs to be minimized by proper storage or composting of manure. Transmission by rodents and insects from manure to feed needs to be minimized as well. These general safety precautions would be the same for organic and conventional farms, although the approaches to rodent and insect control would be different.

Organic farms also differ from conventional farms in animal welfare aspects (more space per animal and the use of bedding material), the avoidance of synthetic pesticides, minimal use of antibiotics and absence of mineral fertilizers. Moreover, traditional organic farms attempt to close the nutrient cycle as much as possible, minimizing introduction of animals and general inputs from other farms. They may also use high-fibre feed and manure, preferably composted, and few concentrates with a low nitrogen content. Concentrations of soluble nitrogen and phosphorous and easily utilizable organic carbon and nitrogen are relatively low; these conditions are typical for an oligotrophic system with a high biodiversity and resilience after a disturbance (van Bruggen and Semenov, 2000; Semenov *et al*., 2008). The animals and plants grow more slowly than in conventional farms, but are frequently more resistant to invasion by various pathogens (van Bruggen and Termorshuizen, 2003). Oligotrophication (as opposed to eutrophication) of the farming system may be the best strategy for the preventive control of human pathogens in the food chain (Franz, 2007).

At the animal production level

Specific animal management practices can also be used to complement the farm-level practices. Organically raised animals need to have access to the outdoors, at least during part of their lives. Exposure to soil, water and wildlife

that may be contaminated with pathogens is a realistic risk. Chickens and pigs can become infected with *Campylobacter* and *Salmonella* from soil and faeces, and the higher *Campylobacter* incidence in organic chickens is associated with outdoor access (Van Overbeke *et al*., 2006). Infection of broilers by *Campylobacter* spp. can possibly be reduced by using slow-growing breeds on organic farms, because these are often less susceptible to *Campylobacter* colonization (Heuer *et al*., 2001). Immunization of young chicks against *Salmonella* is advisable (Methner *et al*., 2006), and is practised in both organic and conventional farms in The Netherlands (Kijlstra and Eijck, 2006). This may also become feasible for the control of *Campylobacter* in the future. In addition, short- and medium-chain fatty acids can be used in chicken feed to control *Salmonella*, decreasing its ability to invade human cells (van Immerseel *et al*., 2006). In the future it might be possible to combine probiotic bacteria and prebiotic components in feed, so that anti-*Salmonella* fatty acids are produced in the gut (van Immerseel *et al*., 2006). Finally, it may be possible to cover outdoor areas with organic materials like saw dust that can be removed and composted on a regular basis.

The differences in raising conditions between organic and conventional cattle and dairy cows are less pronounced than those for pigs and chickens (Kijlstra and Eijck, 2006). However, the feed can differ substantially: organic cows often receive more fibrous feed (mostly organically produced) and less concentrates. Fibrous feed can lead to significantly lower shedding of STEC than high-energy and high-protein feed (Diez-Gonzalez *et al*., 1998; Russell and Rychlik, 2001). A high-fibre diet also leads to more solid manure and greater cow cleanliness in organic farms (Ellis *et al*., 2007). Moreover, increasingly, organic farmers produce dual-purpose cows by mixing Friesian Holstein with traditional disease-resistant breeds. This may also affect their susceptibility to human enteric pathogens.

A large difference between organic and conventional animal rearing is in the use of antibiotics. Preventive use of antibiotics in animal feed is not allowed in organic production. Curative use is allowed, when an animal is sick, but the withdrawal time of the products after antibiotic administering is about twice as long for organic as for conventional farms. Thus, organic farmers rarely use antibiotics. As a consequence, the incidence of antibiotic resistance in bacteria is commonly lower in organic or ABF than in conventional farms as is the incidence of bacteria resistant to more than one antibiotic (Gebreyes *et al*., 2006). Alternative treatments are being investigated, in particular the use of probiotic bacteria or their antibacterial products, such as non-pathogenic *E. coli*-producing colicin that is effective against STEC (Cutler *et al*., 2007). Organic farmers also use mixtures of herbs with antibacterial properties, but their effectiveness has not been proven unequivocally (Bullita *et al*., 2007).

At the plant production level

The main sources of enteric pathogens on plants are contaminated animal manure and water. The type, quality and handling of manure may determine the actual risk of particular pathogens. *E. coli*, including STEC, is mostly

associated with cattle manure (Table 8.1), and significantly higher numbers of *E. coli*-positive vegetable samples were detected, when cattle manure, especially that of young cows, was used rather than other kinds of manures in the USA (Mukherjee *et al*., 2004). However, in Norway, *E. coli* contamination of organic iceberg lettuce was not affected by the type of manure (Johannessen *et al*., 2004).

Organic farmers prefer cattle manure over other types of manures, as the nitrogen/phosphorus ratio of the former is more suitable for plant production. Nevertheless, pig and poultry manures are also used. Organic vegetable producers are not unique in their use of manure; conventional growers also use substantial amounts of manure in areas with a high farm animal density like the Netherlands (van Diepeningen *et al*., 2006).

In a Dutch study on pathogen risks in organic and conventional lettuce production chains, the cattle diet, the resulting manure quality and soil management significantly influenced the decline rates of *E. coli* O157:H7 and *S. Typhimurium* in manure and soil (Franz *et al*., 2005). Maize-based silage resulted in manure with a lower pH and fibre content than grass silage or straw, which allowed longer survival of both pathogens (Franz *et al*., 2005, 2007a) than higher-fibre manure did. Both pathogens declined faster in some organic than conventional soils (Franz *et al*., 2005), but not in others (Franz *et al*., 2008). Survival in soil was enhanced at higher available substrate concentrations and decreased at higher microbial diversity in organic soils (Franz *et al*., 2008). When lettuce was planted in soil amended with either pathogen, lettuce seedlings became contaminated internally as well as externally (Franz *et al*., 2007b). A probabilistic risk model indicated that the risk of obtaining a lettuce head contaminated with *E. coli* O157:H7 was about tenfold lower in an extensive organic farming system than in an intensive conventional farming system, primarily as a result of differences in cattle feed and manure handling (Franz, 2007). Manure is used in different ways, as slurry or dry manure, as pure manure or mixed with litter or straw, after a shorter or longer period of storage, and finally applied on the soil surface or injected into soil (slurry only). Surface application and exposure to sunlight may reduce survival more than injection into soil (Nicholson *et al*., 2000; A.V. Semenov, 2008, unpublished data).

Pathogen content can be reduced by long-term storage (Nicholson *et al*., 2000) and eliminated by composting at high temperatures but not at low temperatures (Termorshuizen *et al*., 2001). In the USA and the UK, there are strict regulations for the use of manure. To minimize the risk of contamination of food crops, manure must be composted or stored for extensive periods before being added to fields where food crops are grown. Although most pathogenic bacteria are susceptible to the heat produced during storage or composting of manure, composting is not always 100% effective (Tauxe *et al*., 1997). In Europe there is no obligation to use composted manure but there are advisories with respect to the number of days between fertilizer application and planting/sowing of the crops (approximately 120 days), in order to allow a maximum die off of pathogens. Thus, the origin, form, storage, composting treatment of manure and the way it is applied all influence the risk of contamination of the crops grown in manured soil.

Besides manure, contaminated water can be a major source of contamination for vegetables and fruits. Surface and shallow ground water should not be used for irrigation if there are farm animals in the area. Potentially contaminated water should not be used for washing of produce either.

Curative control

In the conventional food industry, the emphasis is often on technological solutions for control of the risk of pathogens in food products at the end of the production and processing chain. Hygiene is emphasized, especially in slaughter houses and processing industries. Contamination of meat takes place during the slaughtering process, but this is not different for organic and conventional animals. Differences have been demonstrated, however, in the extent of stress endured by the animals before slaughtering, stress levels being lower in the organic animals, and stress promotes shedding of pathogens (Tuyttens *et al*., 2005).

Various disinfectants are used for decontamination of food products and surface areas, for example chlorinated or ozonated water and detergents. Vegetables are sometimes washed in chlorinated or ozonated water or solutions of organic acids (Akbas and Ölmez, 2007). Chlorine is also used to sanitize chicken meat in the USA, but its use is forbidden in many European countries. Organic acids can be used for meat preservation as well, but acid-tolerant strains may develop over time (Theron and Lues, 2007). Ionizing radiation has been in use in many countries (Kamat *et al*., 2005). However, none of these methods are allowed in organic food production, except for treatment with naturally produced organic acids. These appeared to be more effective than chlorine or ozone (Akbas and Ölmez, 2007); thus, the risk of contamination need not be higher in organic than in conventional products. Indeed, a large retail company in the USA that implemented HACCP and checked 55,000 produce samples for *E. coli* contamination did not find significant differences between organic and conventional produce (Parker, 2006).

Heating has been traditionally used to control human pathogens on food, for example pasteurization or sterilization of milk. Exceptions are cheeses produced from unpasteurized milk, which may contain various pathogens. Most bacteria are killed at 70°C, with the exception of spore-forming species. However, it is difficult to eliminate pathogens inside solid materials; therefore, radiofrequency heating has been proposed as an alternative, for example to sterilize animal feed ingredients (Lagunas-Solar *et al*., 2005).

Cooling is another common method to control multiplication of pathogens in food during transportation and in storage. Most of the pathogens discussed here, with the exception of *L. monocytogenes* (McLauchlin and Jones, 1998), do not grow at temperatures typical of refrigeration units (4°C).

Most methods employed to decrease the pathogen burden on food (except cooling) also eliminate non-pathogenic microorganisms. Such treat-

ments would not be preferred by organic consumers who consider balanced microbial communities as a beneficial by-product of organic food that may stimulate a healthy immune system (Huber, 2007).

A final point relates to the fact that the retail of packaged meat and vegetables has increased over the last decades. The atmosphere in the packages is often enriched with CO_2 to delay food spoilage. This may, however, present a competitive advantage to enteric bacteria which are all facultative anaerobes; *Campylobacter* even needs elevated CO_2 concentrations for growth (Nachamkin and Skirrow, 1998). Consumers of organic produce frequently prefer unprocessed, unpackaged fresh products and may in this respect run a lower exposure risk than consumers of conventional products.

Discussion and Conclusions

Organic production systems were established worldwide as alternative to conventional approaches to food production to solve various environmental problems. Organic food is considered more tasty and healthy by increasing numbers of consumers, and there is some scientific evidence for this contention (Finamore *et al*., 2004; Huber, 2007; Rist *et al*., 2007). The organic way of farming is also considered friendlier for farm animals and the natural environment (Kijlstra and Eijck, 2006). Organic farms are less dependent on industrial inputs, keeping the cycles of elements and associated microorganisms more closed. However, there is a wide range of approaches to organic farming as well as to conventional farming; the extremes on the spectrum comprise the biodynamic farms on one end and the intensive, specialized conventional farms on the other (Franz, 2007).

At present, no significant differences were found in pathogen prevalence in most of the studies comparing organic and conventional production systems. A clear exception is organic poultry, which frequently has a higher incidence of *Campylobacter* than conventional poultry. On the other hand, the *Salmonella* incidence in organic broilers is usually similar or lower than in conventional broilers. The factors that have a considerable influence on these general trends are outdoor access, slaughter age and antibiotic use. The ability of agricultural animals to be outdoors is considered as an important risk factor with respect to the microbiological safety because of the increased probability of contact with wild animals such as mice, rats, birds and insects. However, this would also hold for conventional production systems which are animal-friendly, like the free-range farms. Although very few studies have been conducted on organic pig production, the risks associated with outdoor access are likely to be similar to those for poultry. Similar to conventional pig and poultry production, organic production has also been intensified. Although the animal densities are generally lower in organic rearing systems, they are still relatively high compared to traditional rearing systems. High densities of animals indoors lead to high concentrations of CO_2 and volatile organic compounds, unless excellent ventilation systems are installed. These are exactly conditions where *Campylobacter* can thrive, and it

is therefore understandable why this pathogen is so common in poultry and pig farms when animals are kept indoors, even when there is partial access to the outdoors.

No significant differences were found in the occurrence of *E. coli*, *Salmonella* and *Campylobacter* between organic and conventional cows. The main reason may be that both conventional and organic dairy cows and cattle have pasture access. Large differences have been found between individual farms, probably due to individual management practices. Risk factors for the prevalence of STEC in cows are duration of pasture access, way of housing, kind of feed, age and herd size. Many of these factors stack up in favour of the microbial safety of traditional organic production methods, in particular the use of high-fibre feed, solid manure (often composted), absence of pesticides and antibiotics and absence of synthetic fertilizers. These practices ultimately lead to oligotrophication of the system, with a higher resilience and resistance to disturbances as a result of a higher biodiversity throughout the agroecosystem (van Bruggen and Semenov, 2000; Bengtsson *et al*., 2005). The increased use of slurry injected into soil rather than solid manure (to reduce ammonia emissions to the atmosphere) has led to increased eutrophication of soil and a reduction in diversity of the soil food web (Domene *et al*., 2007; Franz *et al*., 2008) and possibly to increased risks of enteric pathogens in the human food chain. The use of solid manure, if possible composted, is therefore preferred.

In most studies on the occurrence of pathogens in organic and conventional vegetables, *Salmonella* and *E. coli* O157:H7 were not detected, except for two *Salmonella*-positive samples (Mukherjee *et al*., 2004). The prevalence of generic *E. coli* was not higher in vegetables from certified organic growers than in those from conventional farmers. Coliform counts were seldom higher in certified organic than in conventional produce (Mukherjee *et al*., 2006). Produce of farmers who used organic practices but were not certified as organic generally had higher coliform or *E. coli* counts than produce from certified farmers (Mukherjee *et al*., 2004, 2006). The USDA requirements for certified organic agriculture, namely composting or long-term storage of manure, likely contributed to the reduced prevalence of *E. coli* in certified organic produce. Generic *E. coli* may actually be a poor predictor of the risk of pathogenic *E. coli*, because non-pathogenic strains could be effective competitors of pathogenic strains (Franz *et al*., 2007a, 2008). Furthermore, prevalence of *E. coli* in manure and vegetables generally differs so much among farms that individual farm management is a more important determining factor than type of farming system. Finally, the composition of pathogenic strains and their virulence may differ between farming systems, with potentially less-virulent strains in organic than in conventional systems (Kijlstra and Eijck, 2006; Franz *et al*., 2007a), so that prevalence data at the bacterial genus level may not be very meaningful.

In conclusion, there is no scientific evidence that the occurrence of human pathogens would be categorically higher in organic than in conventional production systems. Besides arguments that certain organic management

practices lead to more risk of microbiological contamination, there are several scientifically well-founded facts that argue for the proposition that traditional organic systems are safer with respect to human pathogens. As mentioned earlier, a higher microbial diversity (in soil, manure and on/in plants) might function as a buffer against the invasion and spread of human pathogenic bacteria analogous to the often found negative relation between biodiversity and plant root diseases (van Bruggen and Termorshuizen, 2003; van Diepeningen *et al*., 2006). In addition, a higher fibre content and higher pH in manure of cows fed a high-fibre diet, as is more commonly done at organic dairy farms, reduces the survival capabilities of human pathogens compared to manure resulting from a more conventional dairy diet (Franz *et al*., 2005).

Modern intensive farming has led to eutrophication of soil and waterways and an intrinsic increase in the risk of pathogen outbreaks. Globalization of trade has allowed intensive farming, but has increased the risk of international movement of contaminated animals, plants and food products. Intensive farming contributes to CO_2, N_2O and methane emissions and thereby to global climate change. In turn, higher CO_2 concentrations will contribute to eutrophication due to enhanced exudation and carbon losses in the rhizosphere of plants. Eutrophication of the rhizosphere is in turn very conducive to the survival of human pathogens, in particular *Campylobacter* and *Salmonella* (Klerks *et al*., 2007a). Intensive animal farming with the preventive use of antibiotics has also led to increased antibiotic resistance in a wide array of bacterial pathogens, and even to multiple drug resistance, for example in *Salmonella* (Gebreyes *et al*., 2006). The preventive use of antibiotics may temporarily reduce the prevalence of enteric pathogens in food, but is likely in the long run to be more harmful than beneficial, and is increasingly banned (Pugh, 2002). Intensification and globalization of the food chain and the interest of consumers in convenience food may also have contributed to enhanced risks due to cross-contamination during cutting and packaging and the widespread industry practice of increased CO_2 concentrations inside the packages, which may promote the survival and growth of enteric pathogens. The answer of the food industry to these enhanced risks is focused on increasing control at the end of the chain by instituting HACCP protocols with emphasis on hygienic practices and the application of sanitizing agents. In the long run, this may not be the best answer to the problem of food-borne pathogens. Extensification, diversification and oligotrophication of farming systems may be a better answer to the many global problems of our current food production system. Increased yields at the farm level come at a tremendous cost to society. Organic production systems may become forerunners of more sustainable farming systems. However, the organic farming, processing and marketing industry needs to analyse its practices to decrease the survival and spread of pathogens like *Campylobacter*. Although the intentions of the organic community are to promote biodiversity and become more self-reliant in terms of nutrients, organic farms are also becoming more intensive and specialized, enhancing

the risks of contamination of organic food with enteric pathogens. A scientific risk analysis of the whole production system combined with experiential knowledge of stakeholders may lead to the design, development and implementation of a system with minimized microbiological risks. This may result in a different approach to HACCP certification, with more emphasis on agroecosystem sustainability rather than sanitation of the industrialized food chain.

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9 What Does Consumer Science Tell Us About Organic Foods?

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Introduction

This chapter is concerned with consumer perceptions of environmental and nutritional aspects of organically produced foods and their impact upon the market for organic products. We use market and survey data for the UK as a case study to illustrate the answer to the question 'What Does Consumer Science Tell Us?' By 'consumer science' we mean the application of various social science disciplines to understanding the behaviour of consumers, at both an individual and aggregate (market) level. This can be broken down into three main areas.

First, there is the application of what are sometimes called 'the behavioural sciences' – essentially sociology, psychology and anthropology – to the behaviour of consumers of food, usually now referred to as 'food choice'. Prominent within this are psychology-based models of food choice, such as the 'theory of reasoned action' and 'the theory of planned behaviour' (Köster and Mojet, 2007).

Second, economists have also developed models of consumer behaviour (which sometimes sit rather uncomfortably alongside those derived from the behavioural sciences). They have the advantage of the capacity for aggregation at the level of market demand (Ritson and Petrovici, 2001).

Third, there is the contribution of consumer research within the subject of marketing. This in turn is often divided into 'qualitative' and 'quantitative' consumer research. In the former category, for organic products, much of this has been focus groups or individual depth interviews, with the information collected and often analysed by a procedure known as 'means-end chain', in which attributes of organic food products which attract consumers are related to an individual's fundamental personal values and beliefs (Zanoli, 2004).

'Quantitative research' is based on large sample questionnaire survey data, in which relationships between organic purchase and casual factors can be

established using tests of statistical significance. Models of consumer behaviour can inform the structure of consumer questionnaires, the kinds of questions asked and the analytical procedures followed. For example, the questionnaire used in the Newcastle University survey reported on later in this chapter was structured on the basis of an adapted model of planned behaviour.

Another research technique which has been popular for attempting to explain organic purchase decisions is 'choice experiments', underpinned by economic theory. These attempts to establish how consumers trade off different product attributes when coming to a purchase decision. Almost always, one of these attributes is different price levels, but it is only possible to include two or three additional factors. One such factor could be 'produced organically', together with other production systems.

This chapter reflects all the aspects of 'consumer science' outlined above; in particular, though, we draw on the results of a large survey of UK consumers conducted under the EU Framework 6 Project 'Improving quality and safety and reduction of cost in the European organic and low input supply chains' (known as QLIF).

First, we outline recent developments in the UK market for organic food and follow this by showing how analysis of market data can help to explain what has caused the recent (rapid) growth in sales of organic food products. Next, there is a brief review of the reasons why consumers choose to purchase organic products, drawing mainly on various pieces of qualitative research. The remainder of the chapter is based on the Newcastle survey of UK consumer attitudes to organic and low-input food products.

Recent Developments in the UK Market for Organic Food Products

The total value of organic products sold in the UK has shown spectacular growth over the past 10 years, reaching, in 2007, more than four times 1998 sales (Fig. 9.1). In year-to-year percentage terms, growth was very rapid at first, then levelling off at, a still respectable, 11–13% from 2002.

The immediate reaction to Fig. 9.1 typically is that the cause of this growth must be a fundamental favourable shift in UK consumer attitudes to organic food products. But organic markets (like many others) are more complex than this. In particular an increase in the value of retail sales may reflect a growth in quantity (volume) or a rise in average prices; and an increase in volume will not be matched by a similar rise in value if it is accompanied by declining prices.

Table 9.1 summarizes the various forces which can cause a market to grow. First, a growing market can be 'supply led'. The usual reason for this in agriculture is the introduction of a new cost-reducing technology (as has happened, for example in the case of poultry production and farmed fish). Government subsidies can also stimulate supply. Consumption reacts to the market signal of falling prices and grows along with supply, without any fundamental change in consumer attitudes.

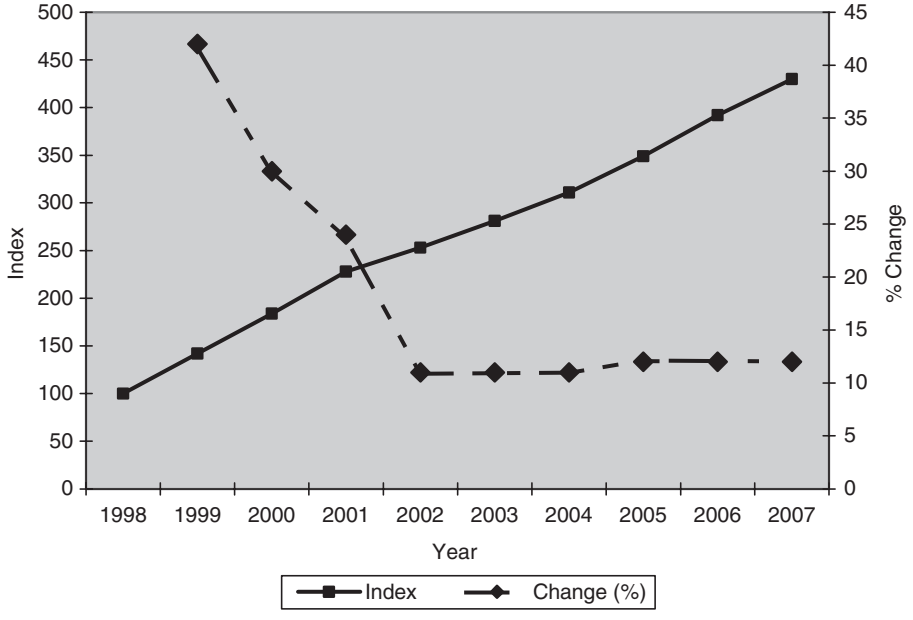


Fig. 9.1. Indexed retail value of organic food and percentage of change year on year (index 1998 = 100, percentage of change from previous year). (Constructed from data published in Mintel Organic Reports, 2003, 2007.)

Table 9.1. Theoretical background to the expanding organic market.

- Supply-led – (cost-reducing technology, government subsidies, producer values); consumption responds to market signals
- Demand-led – (income growth, tastes, lifestyle); production responds to market signals
- Coincidental, independent, market growth
- Supply growth generates demand growth
- (Awareness, availability, product range and quality)

In the case of organic agriculture, there are two additional, unusual, features of supply which complicate market analysis. First is what might be described as 'producer values'. Many of the early suppliers of organic products were motivated by a belief in the environmental benefits of the production system, rather than being market aware, and their production 'had' to find a market, one way or another. Second is the delay of several years between a decision to convert land to organic production and supplies becoming available to market. This means that increased supplies of organic products are reacting to market signals from several years previously.

A growing market will, of course, often be demand-led. Changing tastes and lifestyles make the product more attractive, and supply reacts to market

signals. However, the delay mentioned above means that, in these circumstances, at first much of the growth in value will be price-related, rather than volume-related.

Both supply and demand can grow together independently; in which case the rise is market value will be mainly volume-related. Supply growth can also generate increased consumption, without either a major shift in consumer attitudes, or a fall in prices. More widespread availability of organic products stimulates demand simply by increasing consumer awareness. An extension in the range of organic products can also be important, so that the introduction of a new organic product might stimulate demand for an existing one. Even something as simple as the recent change in supermarket policy to one of integrating organic products into normal shelf areas (rather than having an 'organic section') can attract new consumers who would have avoided them otherwise.

Figure 9.2 shows that, although in value terms, fruit and vegetables still comprise the largest sector; it is milk and dairy products which demonstrate the most rapid growth. This has taken the organic share of the dairy market, at nearly 5%, to first place, ahead of fruit and vegetables (4.2%) and meat and poultry (3%; Soil Association, 2007). The pattern of the development of the UK organic milk market (rapid growth levelling off, than sudden acceleration)

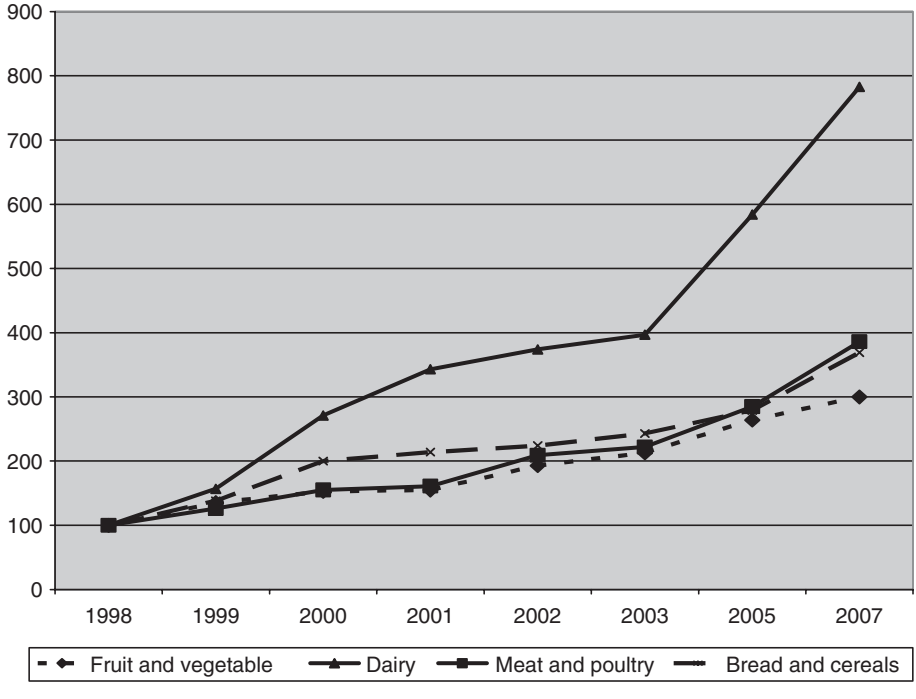
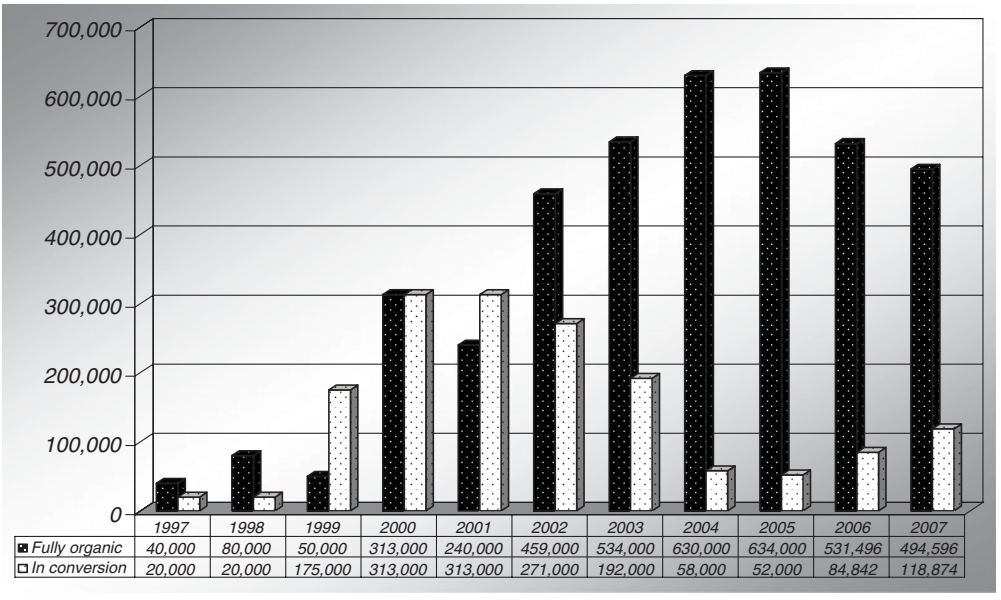


Fig. 9.2. Index of growth in retail sales by product category. Market value – 2007: fruit and vegetable – £502 m.; dairy – £310 m.; meat and poultry – £207 m.; bread and cereals – £99 m. (Constructed from data published in Mintel Organic Reports, 2003, 2007.)

also provides the best illustration of the interplay of supply and demand within the UK, as virtually all the market is domestically supplied (97%, compared with 70% for vegetables, 40% for salad and only 12% for fruit). Most of the land converted for organic production in the UK is grassland for meat and milk production.

Figure 9.3 illustrates the evolution of organically managed land in the UK over the past 10 years. The area peaks in 2004 as the land under conversion between 1999 and 2003 becomes available to supply organic products to the market. This is matched by a big drop in the amount of land in conversion between 2003 and 2005, though in 2006 there are signs of new land entering the conversion process.

Combining the information in Figs 9.2 and 9.3 allows us to identify a pattern in the development of the UK organic market, which is summarized in Table 9.2. After an extended period in which organic agriculture was the preserve of a small number of committed producers and consumers, the late 1990s sees a surge in demand. Organic product prices rise, organic agriculture becomes commercially attractive, and a substantial area of land goes into conversion for organic production. By 2001/2002 there has been a large increase in domestically produced supplies of grassland products. Prices fall, holding back the growth in the value (though not the volume) of sales. Some organically produced products are sold through normal marketing channels. In 2000, all organically produced milk is sold as organic, whereas in 2001, 35% of it is simply absorbed into normal milk retail sales (Hamm and Gronefeld, 2004).



	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007
Fully organic	40,000	80,000	50,000	313,000	240,000	459,000	534,000	630,000	634,000	531,496	494,596
In conversion	20,000	20,000	175,000	313,000	313,000	271,000	192,000	58,000	52,000	84,842	118,874

Fig. 9.3. Organically managed land in the UK – 1997–2007 (hectares). (Constructed from data published in Soil Association Annual Organic Reports, 2005, 2007.)

Table 9.2. Phases in the evolution of the UK market.

Phase	Evolution progress
Pre-1997	Slow growth: supply-driven
1997–2002	Rapid growth: demand-led ('The expansion in organic production is racing to keep up with the growth in customer demand' – House of Commons, 2001)
2003–2005	Consumption growth stabilizes: supply response overshoots demand growth; organic area declines
2006 →	Renewed surge in demand aided by better availability and more mainstream marketing

Agriculture reacts to falling organic profitability by a virtual end to new conversion, and some land is 'de-converted'. Demand continues to grow, aided by better marketing, prices recover, the value of sales increases more rapidly and conversion recommences.

The Price Premium

This analysis of the development of the UK organic market, in which one factor that can stimulate (or impede) consumption is change in organic prices, is dependent on evidence that consumption *is* sensitive to price. Table 9.3 provides some estimates of price elasticities of demand for organic and conventionally produced food products in the UK. It implies, for example, that an increase of 10% in the price of organic milk (with no change in other food prices, including conventional milk) would lead to an approximately 15% decline in organic milk sales.

Table 9.3. Price sensitivity of organic consumption: own price elasticities of demand for organic and conventional products in the UK.[a] (Based on data in ADAS, 2004.)

Product	Conventional	Organic
Milk	–0.76	–1.54
Eggs	–0.26	–0.52
Cheese	–0.34	–0.67
Beef	–1.64	–3.28
Pork	–1.87	–3.74
Chicken	–1.37	–2.75
Vegetables	–0.31	–0.62
Potatoes	–0.21	–0.43
Fruit	–0.21	–0.43

[a]Percentage change in quantity purchased in response to a 1% price increase.

The price elasticities for organic products are typically about double those for the conventional equivalent and so organic consumption appears to be the more price-sensitive. This is because when, for example, organic milk consumption increases as a result of a price fall, part of the increase will reflect consumers moving from conventional to organic purchase. Similarly a fall in conventional milk prices will lead some organic consumers to switch (back). This switching of consumption between organic and conventional has, proportionately, a much bigger impact on the organic market than on the much larger conventional market.

The switching of consumption between conventional and organic has led to organic prices usually being couched in terms of the 'price premium' – the percentage excess of the organic price over the conventionally produced equivalent.

It is of course difficult to collect reliable data to calculate price premiums and, for some products, to know which 'conventional' comparative to use, but Hamm and Gronefeld (2004) have attempted to collect estimates of organic price premiums for various European countries. Those for the UK are shown in Table 9.4.

During this period, therefore, price premiums were typically between 40% and 70% above the conventional equivalent. Price is often cited as the main 'barrier' to increasing consumption of organic products, but we have virtually no market information to tell us how consumers might react if price premiums were much lower or eliminated.

Table 9.5 collects information from a variety of studies in different European countries of consumer's 'willingness to pay' for organic food. The studies are either choice experiments, or based on 'willingness to pay' questionnaires. The data in the table imply that there is a substantial proportion of consumers who would choose to purchase organic products at low price premiums – much lower than those prevalent in the UK (and other European countries). In addition, there appears to be a core of 'committed' consumers who are insensitive to the size of price premiums.

Table 9.4. Consumer price premiums for organic products in the UK 2000/2001. (Based on estimates in Hamm and Gronefeld, 2004.)

Product	Price premium (%)
Milk	48
Yoghurt	30
Cheese	49
Chicken	115
Steak	72
Carrots	58
Potatoes	75
Bread	42

Table 9.5. The price premium: European consumer's willingness to pay for organic food. (Based on data in Weir and Calverley, 2002 and Soler and Gil, 2002.)

Price premium (%)	Proportion of consumers willing to buy (%)
5–10	45–80
10–20	20–50
20–30	10–25
30–40	5–20
40–50	3–18
50–60	3–15

Reasons Why Consumers Purchase Organic Foods

Table 9.6 provides a brief synthesis of the results of a number of qualitative consumer studies exploring the motivation behind consumer decisions to choose to buy organic. Within this, an important distinction can first be made between those factors which are directly associated with the act of consumption, sometimes known as 'private' or 'use values', and those which are not, known as 'non-use' or 'public interest' values.

Thus, 'Own Health and Well-being' (use values) encompasses beliefs that organic products may have superior organoleptic properties, the positive association with superior nutrient content and the negative association attached to modern food production of the perceived harmful effects of chemical fertilizers, the use of pesticides and additives in food processing. For organic products we have described the latter as the 'without list'.

The second category ('non-use' or public interest values) reflects the view that organic agriculture is more environment- and animal-friendly. Research

Table 9.6. Qualitative research: reasons why consumers purchase organic foods.

1. Own health and well-being
   a) The 'without' list
   b) Superior nutrient content
   c) Consumption satisfaction
2. Environment
   a) Animal welfare
   b) Sustainable production
3. Associated values
   a) Local production
   b) Fair trade
   c) Small-scale farming
4. Reasons for not buying
   Price – appearance – availability – variety – taste – trust

using panel data from Denmark (Weir *et al*., 2005) implies that organic consumers would like to see organic farming supported by Government out of taxation for environmental, public good reasons (and may 'say' that this motivates them to buy organic). However, analysis of the purchase data suggests that it is the private good (use) values of 'Own Health and Well-being' which are the main causes of consumers choosing to purchase organic products.

We find that organic consumers are often also consumers who value other aspects of agricultural production regarded as 'socially responsible' – such as local and small producers, and fair trade. They may even believe, sometimes incorrectly, that buying organic will also allow them to respect these 'associated' values. Finally, qualitative consumer research has also explored what are often described as 'barriers' to (or 'reasons for not buying') organic products. This leads on to another interesting distinction, first noted by Ritson and Oughton (2006), as described below.

In food marketing it has been found useful (Ritson and Mai, 1998) to distinguish between the following attributes:

- Search attributes – which consumers are aware of before they purchase (e.g. colour, size);
- Experience attributes – which consumers become aware of after consumption (e.g. taste); and
- Credence attributes – which consumers can never be sure about on the basis of personal experience and most therefore 'believe'.

Most of the attributes which consumers value in organic products are credence attributes (e.g. 'without chemicals', 'animal friendly'), whereas most of the reasons given for not buying are search or experience attributes such as price, appearance and availability.

A brief résumé of the content of this chapter so far is that we first reported on the rapid recent growth in the UK market for organic products but drew attention to evidence which suggests that not all of this is due to a fundamental change in consumer attitudes. We then highlighted the fact that organic consumption appeared to be sensitive to price, and in particular to the size of the gap between organically and conventionally produced products. We noted that motivation for purchasing organic products can be divided into private (use) and public (non-use) values, and that most of the reasons given for *not* purchasing organic products represent search or experience attitudes, whereas features which attract consumers to organic products are usually credence attributes.

In the remainder of this chapter, we explore in more detail consumer attitudes and perceptions of these quality and safety attributes of food products, concentrating on how the views of organic consumers differ from those of consumers in general.

The Newcastle Survey

In the autumn of 2006, a survey of 1012 UK consumers' attitudes to safety and quality aspects of organic and low-input foods was undertaken as

part of an EU Framework VI research project 'Quality Low Input Food'. The survey was also administered in five other European countries, but here we draw on only the results from the UK survey. A full analysis of the survey results for all countries can be found in the project report (Ness *et al*., 2008).

Many of the questions related to a specific product normally bought by the respondent, either bread, tomatoes, eggs or yoghurt, with approximately 250 questionnaires for each of the four products. This was because earlier focus group research (Francois, 2006) indicated that many food quality and safety attributes were very product-specific.

No attempt was made in the survey to 'capture' organic consumers, but towards the end of the questionnaire respondents were asked whether they 'almost always', 'frequently', 'sometimes', 'rarely' or 'never' bought 'organic versions' of eight products. The percentages for the four survey products, and a basket of eight products, are reported in Table 9.7.

Even allowing for some respondents having a liberal interpretation of 'frequent purchase', it is clear that reported purchase exceeds the level of known sales of certified organic products. Respondents were asked how often they had purchased 'organic versions' of eight specific food products. No attempt was made to define 'organic' and it was not the intention of this question to estimate market share of certified organic produce. Rather we wished to identify respondents who believed they frequently purchased products which had been produced in a way that they understood to be consistent with organic principles. Clearly many respondents will have interpreted 'organic' to cover a range of purchases which do not subscribe entirely to those produced under EU organic regulation (and marketed as such). This is particularly the case with eggs (the product with the highest level of reported organic purchase in the survey) where many consumers will have interpreted free-range/outdoor production as organic.

Nevertheless, we will now label those who reported frequent consumption as 'organic consumers' and contrast them with the rest of the sample. Membership of this 'organic group' was found to be significantly related to level of education for all four products; to gender in the case of eggs and yoghurt; to income for eggs and tomatoes; and to area of residence for bread and eggs. Thus, we can say that the British organic consumer is a little more

Table 9.7. Reported incidence of organic consumption, proportion of sample which reported buying 'organic versions' of specific food products.

	'Frequently' or 'Almost always' (%)	'Rarely' or 'Never' (%)
Bread	18	57
Eggs	38	38
Tomatoes	27	43
Yoghurt	23	52
All products	12	63

likely to be educated to higher level, be female, have a high income and live in rural locations.

Food Quality

One of the most informative questions asked respondents how important they regarded a series of attributes as indicators of quality of the product they were being questioned about, on a 5-point scale (very unimportant (1) to very important (5)). Mean scores were then calculated. Fifteen attributes were common across the four products and in addition each product included a series of product-specific attributes.

Irrespective of the product, most importance was attached to 'freshness', 'taste', 'free from chemical residues', 'without artificial ingredients', 'naturalness' and 'nutritional content'; and least importance to 'brand name' and some product-specific attributes (e.g. 'colour of shell'). This was the case for organic and non-organic consumers alike. However, a statistically significant difference in the mean scores between organic consumers and the rest of the sample was identified for many of the attributes. A complete record of this is provided in Tables 9.8–9.11, and this allows us to paint an intriguing picture of how the UK organic consumer differs from consumers in general in the attributes which they regard as important in determining product quality.

The first thing to note is that organic consumers tend to express a greater degree of importance for many of the attributes – they appear to be more 'involved' and careful in their choice of food product. Second, the attributes to which organic consumers attach significantly more importance are ranked in the tables according to size of the gap between mean scores and 'organic

Table 9.8. Product attributes for which there was a significant difference between the mean scores for organic and non-organic consumers: bread.

- Organic consumers attached significantly more importance to:
  1. Organic label
  2. Not mass-produced
  3. No genetically modified ingredients
  4. Made from wholegrain
  5. Local or regional identity
  6. Free from chemical residues
  7. Processed without artificial ingredients
- They attached less importance to:
  1. Texture
  2. Appearance
  3. Reputation of seller
  4. Brand name
  5. Freshness

Table 9.9. Product attributes for which there was a significant difference between the mean scores for organic and non-organic consumers: eggs.

- Organic consumers attached significantly more importance to:
  1. Organic label
  2. Local or regional identity
  3. Not mass-produced
  4. Outdoor production
  5. Produced with freedom to move
  6. Quality of poultry feed
  7. From your country
  8. Without artificial ingredients
  9. Naturalness
  10. Nutritional content
  11. Free from chemical residues
  12. Taste
- They attached less importance to:
  1. Brand name
  2. Colour of shell
  3. Size of egg
  4. Appearance
  5. High price
  6. Colour of yolk
  7. Reputation of seller

Table 9.10. Product attributes for which there was a significant difference between the mean scores for organic and non-organic consumers: tomatoes.

- Organic consumers attached significantly more importance to:
  1. Organic label
  2. Not artificially ripened
  3. Not mass-produced
  4. Not genetically modified
  5. Quality assurance label
  6. In season
  7. The variety
  8. Without artificial ingredients
  9. The country of origin
  10. Naturalness
- They attached less importance to:
  1. Brand name
  2. Pre-packed

label' comes first in the case of all four products. This may seem an unsurprising result, but it is worth noting that this was the first point in the questionnaire that the word 'organic' appeared; and to remember that each table reflects the views of a different group of respondents. Thus, this result adds support to the reliability of the reported incidence of organic purchase.

Table 9.11. Product attributes for which there was a significant difference between the mean scores for organic and non-organic consumers: yoghurt.

- Organic consumers attached significantly more importance to:
  1. Organic label
  2. Local or regional identity
  3. Not mass-produced
  4. Naturalness
  5. Quality assurance label
  6. Free from artificial ingredients
  7. Nutritional content
- They attached less importance to:
  1. High price
  2. Fat content
  3. Fruit content
  4. Texture

Next, the six attributes listed above to which all consumers attach greatest importance are typically *not* the attributes for which the greatest gap between mean scores is recorded; and in many cases there is no significant difference between the importance attached by organic and non-organic consumers for those attributes. This implies that we need to look beyond what organic consumers say are most important to them as indicators of product quality in order to explain organic food choice.

The views or organic consumers are noticeably stronger with respect to genetic modification, local and small-scale production, and product-specific attributes associated with avoiding aspects of modern production technology (made from wholegrain, outdoor/freedom production, artificial ingredients). Finally, virtually all of the characteristics to which organic consumers attach less importance are search or experience attributes. This finding is consistent with our previous comment that it is usually search or experience attributes which are cited as reasons for not buying organic.

Are Organic Products 'Better'?

Respondents were asked, on a scale of 1 (much worse) to 5 (much better), what they thought of organic foods compared to non-organic. Table 9.12 summarizes the results for non-organic consumers. This emphasizes the general favourable image of organic agriculture, even among consumers who rarely, if ever, purchase the products; with many of the attributes which consumers regard as important as quality indicators being ranked as 'better' or 'much better'. This again implies that it is the small number of negative features (barriers to consumption) rather than a lack of positive attributes which explains non-organic purchase. The mean scores for organic consumers were significantly higher than those for the non-organic group for all 24 product attributes listed in Table 9.12. But the opinions of the organic group exceed those of non-

Table 9.12. What non-organic consumers think of organic products.

Much better	Chemical residues
	Artificial ingredients
	Impact on environment
Better	Naturalness
	Not mass-produced
	Quality of animal feed
	Unnecessary use of veterinary medicines
	Animal welfare
	Nutritional content
	Taste
	Food safety
	Made in your country
	Local or regional identity
A little better	Hygiene standards in production and processing
	Freshness
	Reputation of seller
	Distance transported
	Quality assurance label
About the same	Providing a fair price to producers
	Appearance
Worse	Shelf life/keeping quality
	Value for money
	Range of types available
	Price

organic consumers by the greatest amount in the three price-related features – 'Value for Money', 'Provide and Fair Price to Producers' and 'Price'. This suggests that organic consumers have overcome the price-related barrier to organic purchase often reported by non-organic consumers. Other attributes for which the opinions of organic consumers were substantially more favourable than non-organic consumers were 'Taste', 'Animal Welfare', 'Nutritional Content', 'Impact on the Environment' and 'Freshness'.

Are Organic Consumers More Health-Conscious?

Respondents were asked how concerned they were that a series of aspects of production could be a risk to their health. Some of these were very product-specific (e.g. 'Diseased Grain', 'Use of Hormones in Milk Production', 'Artificial Ripening Techniques', 'Cholesterol Content'). Others were more generic across three or all four of these products. Five potential sources of risk were listed for bread and tomatoes, seven for eggs and nine for yoghurt (reflecting additional diet-related hazards). Here a very clear picture emerged of the health-related concerns of organic consumers compared to non-organic consumers. Organic consumers expressed significantly more concern about pesticides (either in the product or in animal feed) and genetic modification

Table 9.13. Public interest value in agriculture production: ranked according to the degree to which the importance attached by organic consumers exceeds that of non-organic consumers.

1. Support small producers
2. Non-transported a great distance
3. Produced and supplied in an energy-efficient way
4. No impact on environment
5. Support local producers
6. Producers receive a fair price
7. Traced to original supplier
8. Support local producers in your country
9. Animal welfare-friendly

(of the product or in animal feed) for all four products; and the use of additives in the case of three (not included as a health risk for tomatoes). Organic consumers also expressed significantly more concern about battery cage production for eggs and artificially ripening techniques for tomatoes. There was no significant difference between the views of organic and non-organic consumers for the other health-related aspects of food.

Again it is necessary to emphasize that it is the views of different groups of respondents being reported here – *not* the same people expressing concern about genetic modification in the case of all four products.

Public Interest Values in Food Consumption

Respondents were asked how important it was to them that the foods they buy should conform to a series of public interest ('non-use') values. Respondents attached most importance to animal welfare, food standards regulations, fair prices and impact on the environment. They regarded support of small and local producers, energy efficiency and distance transported as less important. Organic consumers rated nine of the eleven public interest production values as significantly more important than non-organic consumers. These are listed in Table 9.13, ranked according to the degree to which the importance attached by organic consumers exceeded that of non-organic consumer. Thus, the main contrast between organic and non-organic consumers concerns support for small, local producers and the contemporary issues of energy use and air miles.

Conclusions

The rapid growth in the UK market for organic products reflects more than just a fundamental shift in consumer attitudes. The growth in demand for organic products has been underpinned by growing health and safety-related

concerns in food consumption. Organic consumers attach more importance than others to environmental aspects of food production (pollution, animal welfare, energy use) but they are motivated to purchase mainly by own-consumption values. Organic consumers value the credence attributes of food products. They are less concerned about some search and experience attributes, some of which may act as a deterrent to organic purchase. Perceived health benefits clearly constitute strong motivation for organic purchase, but concern about 'chemical residues', 'additives' and 'GM' appears to exceed 'nutritional content'.

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10 The Beneficial Effects of Dietary Flavonoids: Sources, Bioavailability and Biological Functions

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Introduction

Various epidemiological investigations, as well as dietary supplementation studies in humans and animals, have established a positive correlation between the intake of flavonoid-rich foods or beverages and the attenuation or delayed onset of cardiovascular disease (CVD), cancer and neurodegeneration. Flavonoids are found ubiquitously in the human diet, although some foods and beverages, such as tea, wine, cocoa, berries and citrus fruits, are particularly rich sources of these plant phytochemicals. They are extensively metabolized at various sites within the human body, most notably the small intestine, large intestine and liver, where they are converted into a variety of metabolic forms. Due to this metabolism and the low extent of their overall absorption, it is likely that their beneficial actions are mediated by their abilities to interact with both protein and lipid kinase signalling cascades, rather than via their potential to act as classical antioxidants. The concentrations of flavonoids encountered *in vivo* are sufficiently high to exert pharmacological activity at receptors and on kinases and transcription factors. Presently the precise sites of action are unknown, although it is likely that their activity depends on their ability to: (i) bind to ATP sites on enzymes and receptors; (ii) modulate the activity of kinases directly, i.e. mitogen-activated protein kinase kinase kinase (MAPKKK), mitogen-activated protein kinase kinase (MAPKK) or mitogen-activated protein kinase (MAPK); (iii) affect the function of important phosphatases, which act in opposition to kinases; (iv) preserve Ca^{2+} homeostasis, thereby preventing Ca^{2+}-dependent activation of kinases in neurons; and (v) modulate signalling cascades lying downstream of kinases, i.e. transcription factor activation and binding to promoter sequences. Such interactions will be discussed in relation to the precise site(s)

 Health Benefits of Organic Food: Effects of the Environment (Givens *et al.*)

of action of flavonoids within signalling pathways and the sequence of events that allow them to regulate cellular function at various sites within the body.

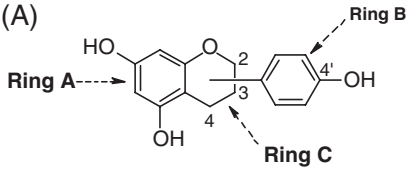
Flavonoids are secondary metabolites found widely in the plant kingdom. They display a wide array of structures, which are partly responsible for the vibrant colours of flowers and fruits. These phytochemicals are found ubiquitously in plants and are therefore abundant in the human diet. Increased flavonoid consumption has been associated with a reduced risk of development of a range of chronic diseases, including cancer, CVD and neurodegenerative disorders. Initially the antioxidant properties of flavonoids were believed to underlie their beneficial effects *in vivo*. However, as they are poorly absorbed and are subject to extensive metabolism in the small intestine, liver and colon, other potential mechanisms of action have emerged which include their interaction with cell signalling pathways and modulation of gene expression.

Flavonoid Structure

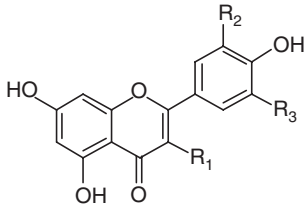
Polyphenols represent a wide variety of compounds that possess multiple hydroxyl groups on aromatic rings. Thousands of molecules possessing polyphenol structure have been identified in plants. These compounds are classified into different groups based on the number of phenol rings and the way in which these rings interact. Flavonoids are polyphenolic compounds that share a common structure consisting of two aromatic rings (A and B), which are bound together by three carbon atoms, forming an oxygenated heterocycle (ring C; Fig. 10.1A). Based on the variation in the type of heterocycle, flavonoids may be divided into seven subclasses: flavonols, flavones, flavanones, flavanonols, flavanols, anthocyanidins and isoflavones. Individual differences within each group arise from the variation in number and arrangement of the hydroxyl groups and their alkylation and/or glycosylation.

Flavonols and flavones share a similar structure based on the 2-phenylchromen-4-one skeleton. Hydroxylation on position 3 of this structure gives rise to the 3-hydroxyflavones also called flavonols. The diversity of these compounds stems in the different positions of the phenolic-OH groups (Fig. 10.1B). Flavanols, also referred to as flavan-3-ols, have a structure based on the 2-phenyl-3,4-dihydro-2H-chromen-3-ol skeleton. Variations in structures lie in the hydroxylation pattern of the B ring and the creation of ester bonds with gallic acid in position 3 (Fig. 10.1C). The lack of a double bond at the 2–3 position and the presence of a 3-hydroxyl group on the C ring create two centres of asymmetry. This latter gives rise to four different structures with the (−)-epicatechin (2*R*,3*R*-3,5,7,3′,4′-pentahydroxyflavan) and the (+)-catechin (2*R*,3*S*-3,5,7,3′,4′-pentahydroxyflavan) being the most common optical isomers found in nature. Moreover, flavanols are also encountered as oligomers or polymers, referred to as condensed tannins or proanthocyanidins (because they release anthocyanidins when heated under acidic conditions; Bate-Smith, 1953, 1954). These compounds differ in nature based on their

(A)

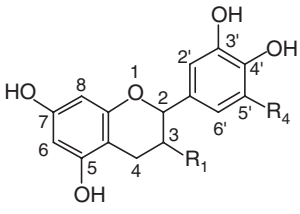


(B)



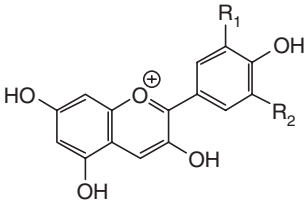
Flavonols			
	R1	***R2***	***R3***
Kaempferol	OH	OH	H
Quercetin	OH	OH	H
Myricetin	OH	OH	OH
Isorhamnetin	OH	OCH_3	H
Flavones			
Luteolin	H	OH	H
Apigenin	H	OH	H

(C)



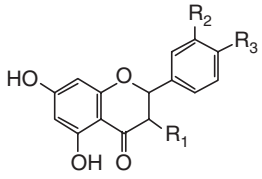
Flavanols		
	R1	***R4***
Catechin	OH	H
Epicatechin	OH	H
EGC	OH	OH
ECG	gallate	H
EGCG	gallate	OH

(D)



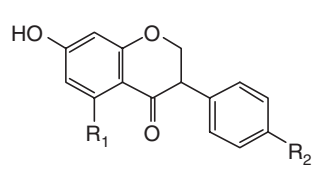
Anthocyanidins		
	R1	**R2**
Pelargonidin	H	H
Cyanidin	OH	H
Delphinidin	OH	OH
Paeonidin	OCH_3	H
Petunidin	OCH_3	OH
Malvidin	OCH_3	OCH_3

(E)



Flavanones			
	R1	**R2**	**R3**
Naringenin	H	H	OH
Hesperetin	H	OCH_3	H
Flavanonols			
Taxifolin	OH	OH	OH
Astilbin	O-rhamnosyl	OH	OH
Engeletin	O-rhamnosyl	H	OH

(F)



Isoflavones		
	R1	**R2**
Genistein	OH	OH
Daidzein	OH	H

Fig. 10.1. The structures of the main classes of flavonoids. The major differences between the individual groups reside in the hydroxylation pattern of the ring-structure, the degree of saturation of the C ring and the substitution of the 3-position: (A) general structure of flavonoids, (B) structure of flavonols and flavones, (C) structure of flavanols, also referred as flavan-3-ols, (D) structure of anthocyanidins, (E) structure of flavanones and flavanonols and (F) structure of isoflavones.

constitutive units (e.g. catechins and epicatechin), their sequence and the positions of interflavanic linkages (C4–C6 or C4–C8 in the B-type series, with additional C2–O–C7 or C2–O–C5 bonds in A-type structures; Cheynier, 2005). Anthocyanidins are the aglycone forms of the anthocyanins and have a structure based on the flavylium (2-phenylchromenylium) ion skeleton. These compounds are encountered as glycoside forms and are water-soluble flavonoid pigments that appear red to blue according to pH. Individual structures arise from the variation in number and arrangement of the hydroxyl and methoxy groups (Fig. 10.1D). Flavanones and flavanonols share a similar structure based on the 2,3-dihydro-2-phenylchromen-4-one skeleton. Hydroxylation in position 3 of the C ring allows the differentiation of flavanonols from flavanones (Fig. 10.1E). Finally, isoflavones are a subclass of the isoflavonoid that have a structure based on the 3-phenylchromen-4-one skeleton. Isoflavonoids have a greater structural variability and higher presence as aglycones in plants than other classes of flavonoids (Head, 1998). More than 600 isoflavones have been identified to date and are classified according to oxidation level of the central pyran ring (Fig. 10.1F).

Biosynthetic Routes within the Plant

Flavonoids are secondary metabolites synthesized by plants. All flavonoids derive from a chalcone precursor, the product of the condensation of 4-coumaroyl CoA (a product of the centralphenyl-propanoid pathway) and 3 molecules of malonyl-CoA (formed from acetate via a cytoplasmatic form of acetyl CoA carboxylase) by the action of the enzyme chalcone synthase (CHS; Dixon and Steele, 1999; Winkel-Shirley, 2001a). Chalcone is isomerized to a flavanone by the enzyme chalcone flavanone isomerase (CHI). From these central intermediates, the pathway diverges into several side branches, each resulting in a different class of flavonoids. Flavones are synthesized by the flavone synthase (FSI/FS2) while isoflavones are formed by the isoflavone synthase (IFS). Flavanone 3-hydroxylase (F3H) catalyses the 3β-hydroxylation of (2*S*)-flavanones to dihydroflavonols. The latter are transformed into flavan-3,4-diols (leucoanthocyanidins) by the dihydroflavonol reductase (DFR), which is then converted to anthocyanidins by anthocyanidin synthase (ANS). The enzyme leucoanthocyanidin reductase (LAR) removes the hydroxyl in position 4 to produce the corresponding flavan-3-ol (e.g. catechin from leucocyanidin) and the anthocyanidin reductase (ANR) converts anthocyanidin to the corresponding 2,3-flavan-3-ol (e.g. epicatechin from cyanidin). The formation of glucosides is catalysed by UDP glucose-flavonoid 3-*o*-glucosyl transferase (UFGT), which stabilizes the anthocyanidins and flavonols by 3-*o*-glucosylation (Bohm, 1998). The basic flavonoid biosynthesis pathway is conserved, although further modifications are possible in particular plant species. The flavonoid biosynthetic pathway responds to environmental and developmental factors by altering the quantities and proportions of compound generated (Bais *et al*., 2003). After synthesis in the cytosol, flavonoids accumulate in vacuoles and cell walls (Winkel-Shirley, 2001b). A multidrug

and toxin extrusion (MATE) transporter and a glutathione transporter have been identified as mechanisms of transport into the vacuole, whereas into the cell wall the transport is via endoplasmic reticulum-derived vesicles. It is thought that different species utilize different mechanisms to distribute flavonoids within cells, or that more than one mechanism is used in some species (Fig. 10.2).

Major Sources within the Diet

Flavonols are the most ubiquitous flavonoids in food, as they are present in almost all fruit and vegetables. Quercetin, the main flavonol in our diet, is particularly abundant in onions (0.3 mg/g fresh weight; Hertog *et al*., 1992) and tea (10–25 mg/l; Hertog *et al*., 1993b) but also in reasonable concentrations in red wine, apples and berries. Other flavonols in the diet include kaempferol (broccoli), myricetin (berries) and isorhamnetin (onion). These compounds are present in plants in glycosylated forms, where the associated sugar moiety is predominantly glucose or rhamnose, although other sugars may also be attached (Table 10.1). The daily intake of quercetin was estimated to range between 3 and 38 mg in the seven countries studied (Hertog *et al*., 1995), whereas in the United States, the estimated intake of flavonols, of which quercetin constituted 73%, was 20–22 mg/day (Sampson *et al*., 2002).

Flavanones are the main type of flavonoids present in citrus fruit and juices. The highest concentrations are found in the solid parts of the fruit, particularly the albedo and the membranes, but concentrations up to 200–600 mg/l of flavanones have also been found in the juice (Tomas-Barberan and Clifford, 2000). Flavanones are generally glycosylated but the main aglycones are hesperetin and naringenin in oranges. The main flavonoid of grapefruit is naringenin. Other sources include tomatoes and aromatic plants such as mint (Table 10.1). The average intake of naringenin in Finland has been estimated to be 8.3 and 28.3 mg/day for hesperetin (Kumpulainen *et al*., 1999).

Flavanols are a unique class of flavonoids because they exist both in the monomeric form (catechins) and in the polymeric form (proanthocyanidins). In contrast to other classes of flavonoids, flavanols are not glycosylated in food and they usually occur as aglycones or gallate esters on the 3-position of the flavonoid ring. Catechin and epicatechin are the main flavanols and are found in various fruit such as apples and apricots and are also present at high concentrations in red wine and cocoa. Gallocatechin, epigallocatechin and epigallocatechin gallate (EGCG) are found in certain seeds of leguminous plants, in grapes and at a high concentration in tea (Table 10.1). The daily intake of catechin in monomeric form as well as dimers and trimers has been estimated to be 18–50 mg/day, with the main sources being tea, chocolate, apples, pears and grapes (Arts *et al*., 2000a,b). The related group, the anthocyanins, is a class of water-soluble flavonoids which are abundant in some vegetables and fruit, particularly in berries to which they impart pink,

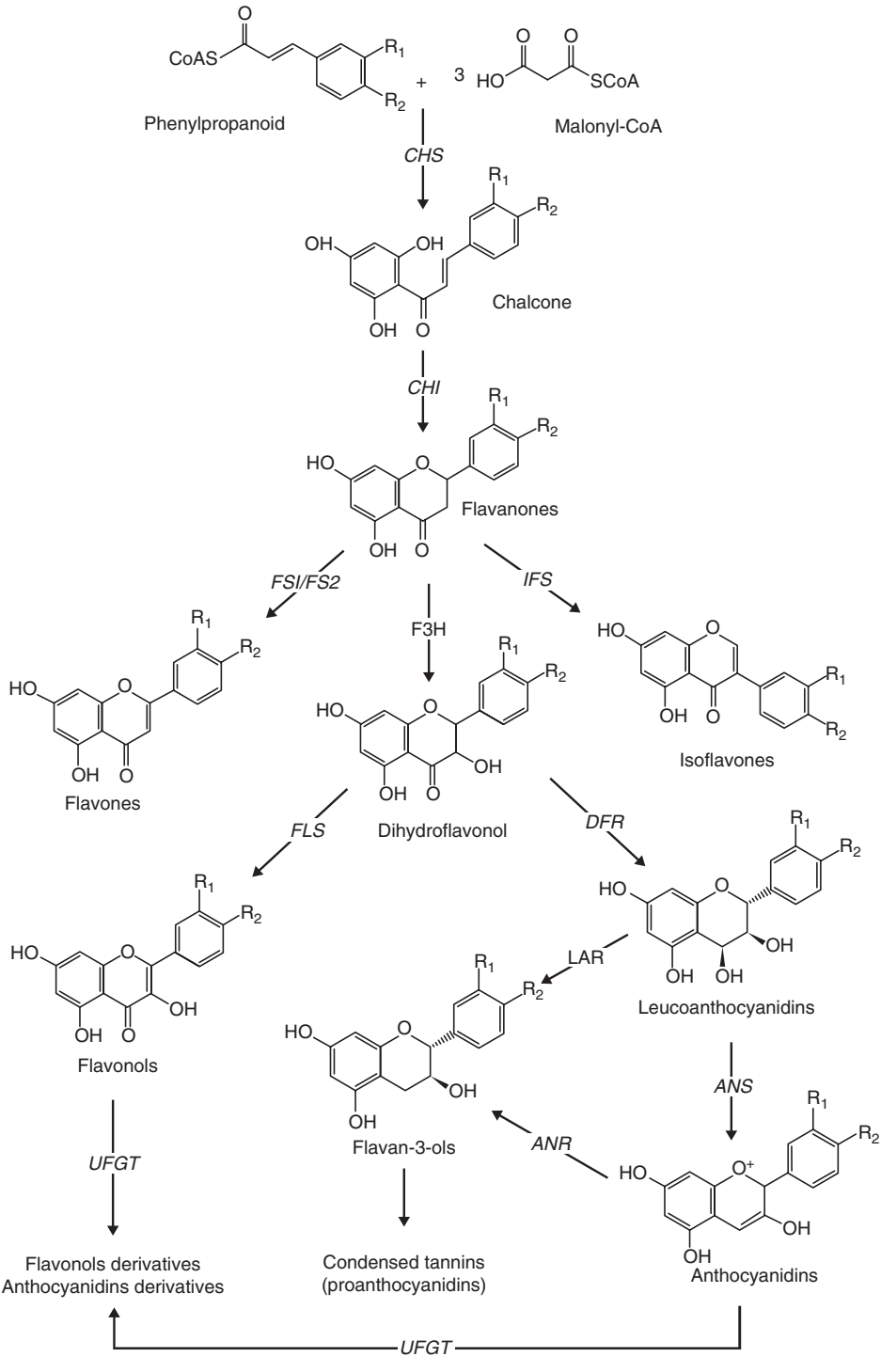


Fig. 10.2. The biosynthesis of flavonoids in plants.

Table 10.1. Polyphenol containing foods.

Flavonoid subclass	Dietary source	Polyphenol content (mg/kg or mg/l fresh weight)
Flavonols	Onion, kale	300–1200
	Leek, cherry tomato, broccoli,	50–300
	blueberry, blackcurrant, apricot, apple,	0–50
	green bean, black grape, tomato,	
	black tea, green tea	
Flavones	Parsley	200–2000
	Celery	20–200
	Capsicum pepper	1–20
Flavanones	Orange, orange juice	200–1000
	Grapefruit, grapefruit juice	100–700
	Lemon juice	50–300
Flavanols	Chocolate, green tea,	500–1000
	Beans, black tea	300–500
	Apricot, cherry, grape, peach,	10–300
	blackberry, apple, red wine, cider	
Anthocyanins	Aubergine, blackberry, blackcurrant,	1000–5000
	blueberry, black grape, cherry	
	Rhubarb	1000–2000
	Strawberry, red wine, plum, red cabbage	10–1000
Isoflavones	Soya flour	1000–2000
	Soybeans, miso, tofu, tempeh, soya milk	10–1000

red, blue or purple colour, and exist mainly as glycosides. The high intake of anthocyanidin relates to the high concentrations found in vegetables and fruit; for example, a serving of 200 g aubergine or black grapes can provide up to 1500 mg of anthocyanins and a 300 ml glass of red wine can contain 100 mg of anthocyanins (Manach *et al*., 2004). The mean dietary intake in Finland has been estimated to be 82 mg/day, with the main sources being berries, red wine and juices (Manach *et al*., 2005; Table 10.1).

Flavones are much less common than other flavonoids in the human diet. The main sources of dietary intake are herbs such as parsley and celery. They exist mainly as glycosides of luteolin and apigenin. In essential oils derived from citrus fruits, a group of poly-methoxylated flavones exist such as

Fig. 10.2. Continued. All flavonoids are derived from chalcone precursors that are derived from phenylpropanoid and three malonyl-CoA and biosynthesized by chalcone synthase (CHS). Various enzymes act to bring about the formation of the various flavonoid classes: chalcone isomerase (CHI), flavone synthase (FSI/FS2), isoflavone synthase (IFS), flavanone 3-hydroxylase (F3H), dihydroflavonol reductase (DFR), anthocyanidin synthase (ANS), leucoanthocyanidin reductase (LAR), anthocyanidin reductase (ANR), UDP glucose-flavonoid 3-*o*-glucosyl transferase (UFGT), flavonol synthase (FLS).

tangeretin, which are considered the most hydrophobic forms of flavonoids (Table 10.1). Isoflavones are naturally occurring plant components having a 1,2-diarylpropane structure. These compounds belong to the phyto-oestrogen class having a similar chemical structure to mammalian oestrogens. They are present in several legumes but soybeans have been identified as the principal dietary source for humans. The isoflavone content of soybeans varies depending on the variety of soybean, year harvested, geographic location and plant part. Non-soy legumes such as lentils and other types of beans do not contain appreciable amounts of isoflavones (Franke, 1997). Isoflavones are present in plants either as the aglycone (mainly genistein or daidzein) or as different glycosides, including their β-glycosides, namely genistein and daidzein or their methoxylated derivatives, namely biochanin A and formononetin (Ruiz-Larrea *et al*., 1997; Table 10.1).

Metabolic Fate of Dietary Polyphenols

Although flavonoids have been identified as powerful antioxidants *in vitro*, their ability to act as effective antioxidants *in vivo* is dependent on the extent of their biotransformation (Vitaglione *et al*., 2005) and conjugation during absorption from the gastrointestinal (GI) tract, in the liver and finally in cells. Native polyphenols in the diet are subjected to extensive metabolism following oral ingestion. Indeed in the upper GI tract, dietary polyphenols act as substrates for a number of enzymes and are subjected to extensive metabolism by glucosidase enzymes, phase I enzymes (hydrolysing and oxidizing), such as cytochrome P450, and phase II enzymes (conjugating and detoxifying) found both in the small intestine and the liver. Further transformations have been reported in the colon, where the enzymes of the gut microflora act to breakdown flavonoids to simple phenolics acids, which may also be absorbed and further metabolized in the liver (Fig. 10.3).

Gastrointestinal tract metabolism

Modifications of polyphenol structure may occur at many points in the GI tract. In the upper GI tract saliva has been found to cause degalloylation of flavanol gallate esters, such as EGCG (Yang *et al*., 1999), but to have little effect on the stability of green tea catechins (Tsuchiya *et al*., 1997). The quercetin rutinoside, rutin, is hydrolysed by cell-free extracts of human salivary cultures (Laires *et al*., 1989) and by *streptococci* isolated from the mouths of normal individuals (Parisis and Pritchard, 1983), but quercetin-3-rhamnoside (quercitrin) is not susceptible to hydrolysis, suggesting that only rutin-glycosidase-elaborating organisms occur in saliva (Macdonald *et al*., 1983). An interaction of flavanols and procyanidins with salivary proteins has been shown and indicates that (+)-catechin has a higher affinity for proline-rich proteins than (−)-epicatechin, and C(4)–C(8) linked procyanidin dimers bind more strongly than their C(4)–C(6) counterparts (De Freitas and Mateus,

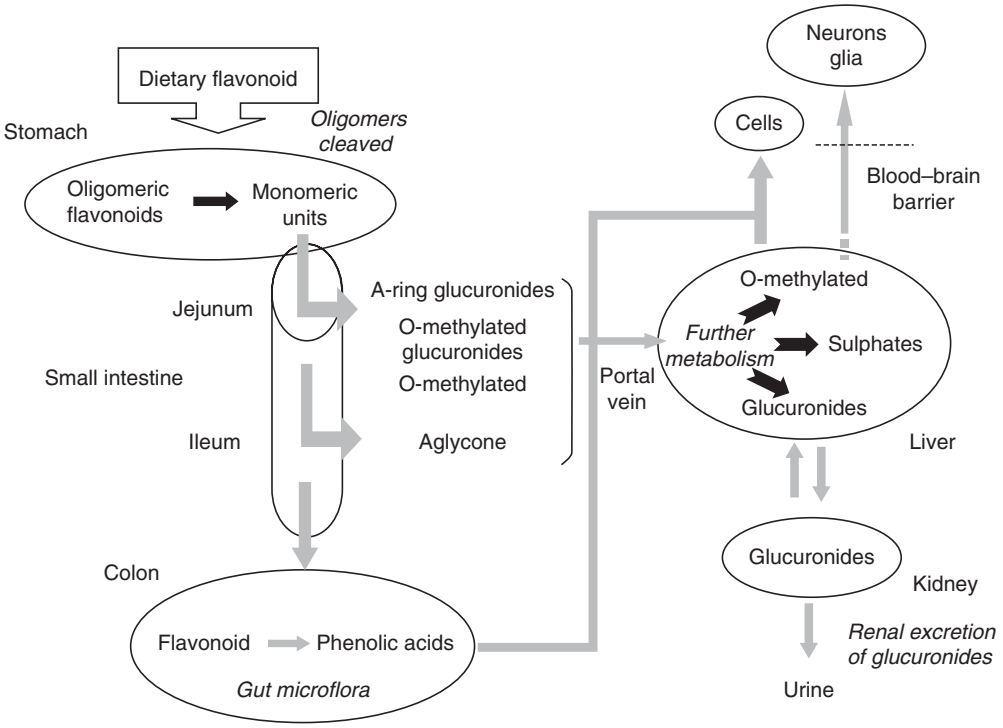


Fig. 10.3. Summary of the formation of gastrointestinal tract and hepatic metabolites and conjugates of polyphenols in humans. Cleavage of oligomeric flavonoids such as procyanidins may occur in the stomach in an environment of low pH. All classes of flavonoids undergo extensive metabolism in the jejunum and ileum of the small intestine and resulting metabolites enter the portal vein and undergo further metabolism in the liver. Colonic microflora degrade flavonoids into smaller phenolic acids that may also be absorbed. The fate of flavonoids is renal excretion, although, some may enter cells and tissues.

2001). This polyphenol-protein binding in the form of adsorption with high molecular weight salivary proteins, bacterial cells and mucous materials may be one explanation for the observed decrease in quercetin mutagenicity after incubation with saliva (Nishioka *et al*., 1981).

There are many factors that influence the extent and rate of absorption of ingested compounds by the small intestine (Lin *et al*., 1999). These include physiochemical factors such as molecular size, lipophilicity, solubility, pKa and biological factors such as gastric and intestinal transit time, lumen pH, membrane permeability and first pass metabolism (Higuchi *et al*., 1981; Ho *et al*., 1983). Procyanidin oligomers ranging from dimer to decamer (isolated from *Theobroma cacao*) have been observed to be unstable under conditions of low pH similar to that present in the gastric juice of the stomach (Spencer *et al*., 2000). During incubation of the procyanidins with simulated gastric juice, oligomers rapidly decompose to epicatechin monomeric and dimeric units but also to other oligomeric units trimer

and tetramer units also formed (Spencer *et al*., 2000). Thus, absorption of flavanols and procyanidins, for example after consumption of chocolate or cocoa, is likely to be influenced by pre-absorption events in the gastric lumen within the residence time. However, consideration needs to be given to the food matrix, which may influence the pH environment of the procyanidins and their subsequent decomposition. Monomeric flavonoid glycosides have been observed to be stable in the acidic environment of the stomach and are not observed to undergo non-enzymatic deglycosylation (Gee *et al*., 1998). Because glycoside derivatives of polyphenols are relatively polar molecules, their passive diffusion across the membranes of small intestinal brush border is unlikely. However, many studies have suggested that flavonoid glycosides are subject to the action of β-glucosidases prior to their absorption in the jejunum and ileum (Spencer *et al*., 1999; Day and Williamson, 2001) and it is generally believed that the removal of the glycosidic moiety is necessary before absorption of the flavonoid can take place. The majority of polyphenol glycosides and in some instances the aglycones, present in plant-derived foods, are extensively conjugated and metabolized during absorption in the small intestine and then again in the liver. In particular there is strong evidence for the extensive phase I de-glycosylation and phase II metabolism (by UDP-glucuronosyltransferases, sulfotransferases and catechol-*o*-methyltransferases (COMT)) to yield glucuronides, sulfates and *o*-methylated derivatives. Indeed, in the jejunum and ileum of the small intestine there is efficient glucuronidation of nearly all polyphenols to differing extents by the action of UDP-glucuronosyltransferase enzymes. In the case of catechol containing B-ring flavonoids, there is also extensive *o*-methylation by the action of COMT. Unabsorbed polyphenols will reach the large intestine where they will be further metabolized by the enzymes of the gut microflora to simple phenolic acids.

Colonic metabolism

Studies have suggested that the extent of absorption of dietary polyphenols in the small intestine is relatively small (10–20%; Kuhnle *et al*., 2000a,b). The implications of this low absorption in the small intestine means that the majority of ingested polyphenols, including those absorbed and conjugated in the enterocytes and/or the liver before transport back out into the lumen either directly or via the bile (Crespy *et al*., 1999), will reach the large intestine where they encounter colonic microflora. The colon contains approximately 10^{12} microorganisms/cm^3, which has an enormous catalytic and hydrolytic potential and this enzymatic degradation of flavonoids by the colonic microflora results in a huge array of new metabolites. For example, bacterial enzymes may catalyse many reactions including hydrolysis, dehydroxylation, demethylation, ring cleavage and decarboxylation as well as rapid de-conjugation (Scheline, 1999). Unlike human enzymes, the bacteria of the large intestine catalyse the breakdown of the flavonoid backbone itself to simpler molecules such as phenolic acids. Specific metabolites have been

observed in urine after consumption of a variety of phenolics. For example, the glycine conjugate of benzoic acid, hippuric acid, is primarily derived from plant phenolics and aromatic amino acids through the action of intestinal bacteria. Consequently, the level of hippuric acid would be expected to increase in the urine of individuals consuming diets rich in flavanols or polyphenols in general. It must be noted, however, that hippuric acid could possibly derive from other sources such as quinic acid or, in quantitative terms, more importantly from the aromatic amino acids tryptophan, tyrosine and phenylalanine, as well as from the use of benzoic acid as a food preservative.

The 5,7,3,3′,4′-hydroxylation pattern of flavan-3-ols is believed to enhance ring opening after hydrolysis (Spencer *et al*., 2001b) and metabolism of flavanols by enzymes of the microflora of the large intestine results in many metabolites: 3,4-dihydrophenylacetic acid, 3-hydroxyphenylacetic acid, homovanillic acid and their conjugates derived from the B ring (Scheline, 1999) and phenolic acids from the C ring. Flavanols, because of their structures (no C-4 carbonyl group), can also degrade to the specific metabolites phenylvalerolactones. Phenylpropionic acids (which may undergo further metabolism to benzoic acids) may also be the products of flavanol metabolism in animal studies, which demonstrates fission of the A ring (Scheline, 1999). The metabolism of flavan-3-ol oligomers may also take place in the colon. Colonic-derived metabolites of flavanols have been detected in human plasma and urine after a single ingestion of green tea (Li *et al*., 2000), which suggest that there may be significant metabolism by gut microflora in the colon. Flavonols such as quercetin-3-rhamnoglucoside and quercetin-3-rhamnoside may also undergo metabolism by the colonic flora with *Bacteroides distasonis*, *Bacteroides uniformis* and *Bacteroides ovatus* capable of cleaving the sugar using α-rhamnosidase and β-glucosidase to liberate quercetin aglycone (Bokkenheuser *et al*., 1987) and other phenolic metabolites (Baba *et al*., 1983). Other bacteria, such as *Enterococcus casseliflavus*, have been observed to degrade quercetin-3-glucoside (Schneider *et al*., 1999), luteolin-7-glucoside, rutin, quercetin, kaempferol, luteolin, eriodictyol, naringenin, taxifolin and phloretin (Schneider and Blaut, 2000) to phenolic acids and *Eubacterium ramulus* is capable of degrading the aromatic ring system of quercetin producing the transient intermediate, phloroglucinol (Schneider *et al*., 1999). Other flavonoid glycosides, hesperidin, naringin and poncirin are also metabolized to phenolic acids, via aglycones, by human intestinal microflora that produce α-rhamnosidase, exo-β-glucosidase, endo-β-glucosidase and/or β-glucuronidase enzymes (Kim *et al*., 1998). In addition, baicalin, puerarin and daidzin were transformed to their aglycones by the bacteria producing β-glucuronidase, C-glycosidase and β-glycosidase, respectively.

Role in Human Health

Epidemiological studies suggest that high dietary intake of polyphenols is associated with decreased risk of a range of diseases including CVD, specific

forms of cancer (Kuriyama *et al*., 2006) and neurodegenerative diseases (Checkoway *et al*., 2002). Flavonoids in particular have been extensively linked with beneficial effects in many human, animal and *in vitro* studies (Schroeter *et al*., 2001). With respect to cardiovascular health, flavonoids may alter lipid metabolism (Zern *et al*., 2005); inhibit low-density lipoprotein (LDL) oxidation (Jeong *et al*., 2005), atherosclerotic lesion formation (Fuhrman *et al*., 2005) and platelet aggregation (Hubbard *et al*., 2006); decrease inflammation and vascular cell adhesion molecule expression (Ludwig *et al*., 2004); and improve endothelial function (Hallund *et al*., 2006). With respect to neurodegeneration, flavonoids may positively influence cognitive function and reverse certain age-related declines (Joseph *et al*., 1999). Neuroprotective mechanisms of flavonoids and other polyphenols include modulation of neuronal and mitochondrial function (Schroeter *et al*., 2001) and inhibition of glial-induced neuroinflammation (Chen *et al*., 2005). The anti-carcinogenic effects of polyphenols include induction of apoptosis (Mantena *et al*., 2006), inhibition of cell proliferation (Wang *et al*., 2000), modulation of detoxification enzyme activity (Bacon *et al*., 2003), inhibition of angiogenesis and prevention of tumour invasion (Piao *et al*., 2006).

Cardiovascular effects of flavonoids

Numerous epidemiological and human intervention studies suggest that regular consumption of a diet rich in fruit, vegetables and beverages, such as tea and wine, may have cardioprotective effects in human populations (Hertog *et al*., 1993a). There has been accumulating evidence to suggest that dietary flavonoids, in particular flavanols, may in large part explain the health benefits of increased intake (Mink *et al*., 2007). However, there are also conflicting results regarding the role that flavonoids play in cardiovascular health (Farouque *et al*., 2006). Cocoa has been shown to be an important contributor to the total dietary intake of flavonoids (Arts *et al*., 2001), particularly flavanols and procyanidins, with higher antioxidant capacity and flavonoid content than tea and red wine (Lee *et al*., 2003b). For these reasons, the potential effects of cocoa and chocolate on CVD have been extensively researched (Keen *et al*., 2005). Suggested reasons for this beneficial action include an ability of flavanols to increase antioxidant status (Rein *et al*., 2000), lower blood pressure (Taubert *et al*., 2007), inhibit LDL oxidation (Mathur *et al*., 2002), improve endothelial function (Heiss *et al*., 2003) and inhibit platelet aggregation (Pearson *et al*., 2002) and the inflammatory response (Mao *et al*., 2002).

The Kuna amerinds of San Blas Island off the coast of Panama are an interesting study population as they have a diet which is extremely rich in flavanols, through the consumption of large amounts of cocoa. They have a very low risk of developing CVD, despite high salt intake. Hypertension is very rare in this community and their blood pressure alters little with age. In this population, the protection against CVD risk appears to be environmental rather than genetic as the Kuna amerinds who migrate to urban Panama

city reduce their daily cocoa intake (Schroeter *et al*., 2006) and as a consequence begin to develop hypertension with age (Hollenberg *et al*., 1997). These observations suggest that flavanol-rich cocoa has the potential to significantly reduce hypertension and therefore CVD risk. Schroeter *et al*. (2006) reported that the levels of urinary nitric oxide metabolites were more than twice as high in traditional Kuna island communities compared to levels of those living on the mainland, supporting the theory that chronic consumption of flavanol-rich cocoa is associated with an increase in nitric oxide production, which is known to be a potent vasodilator. Studies to support these observations have indicated that the ingestion of a high-flavanol cocoa drink is capable of increasing flow-mediated dilation (FMD) of the brachial artery 4 h after consumption and that these correlated well with increases in plasma levels of flavanol metabolites and plasma nitroso species. Ingestion of pure epicatechin produced similar vascular effects to that of the cocoa drink, strengthening the hypothesis that it is the flavanols in cocoa which exert these vascular effects (Schroeter *et al*., 2006). These findings provide compelling evidence that consumption of flavanol-rich cocoa can contribute to vascular benefits and reduced CVD risk.

The mechanism by which these effects are mediated is currently unknown, although there has been much recent interest in defining their tissue, cellular and molecular actions. Several studies have shown a correlation between flavanol-rich cocoa consumption and improvements in endothelial function. *In vitro* experiments have shown that tetramers and higher oligomers of epicatechin induced endothelium-dependent relaxation in aortic ring experiments (Karim *et al*., 2000). The mechanism proposed was the activation of the endothelial nitric oxide synthase. Heiss *et al*. (2003) reported that flavanol-rich cocoa improves endothelium-dependent dilation in humans by increasing the bioavailability of nitric oxide. In this study, endothelium-dependent dilation was measured by FMD and it was found that ingestion of a flavanol-rich cocoa drink (176 mg) increased FMD and plasma nitric oxide species in patients with coronary artery disease, hypertension or diabetes, whereas the ingestion of a similar low flavanol drink (less than 10 mg total flavanol content) had no effect on either parameter. Other vascular parameters such as forearm blood flow, blood pressure, heart rate and diameter of the brachial artery did not change following consumption of the cocoa drink, indicating again that flavanols appear to induce arterial dilation via increases in nitric oxide bioavailability rather than by other mechanisms. Similarly, increases in FMD in hypertensive subjects have been observed after 2 weeks of flavanol-rich chocolate consumption (Grassi *et al*., 2005). It has also been shown that flavanol-rich cocoa can reverse endothelial dysfunction in smokers (Heiss *et al*., 2005) and hypercholesterolemic postmenopausal women (Wang-Polagruto *et al*., 2006). Fisher *et al*. (2003) provided additional evidence that the activation of endothelial nitric oxide synthase (eNOS) is the mechanism responsible for the vasodilator effects of cocoa. Increases in FMD in patients with coronary artery disease have also been observed following consumption of black tea or grape juice (Stein *et al*., 1999; Duffy *et al*., 2001).

As well as effects on vascular tone, the beneficial effects of flavanols have been attributed to their ability to inhibit platelet activation. Platelets play an important role in the early stages of atherosclerosis and coronary thrombosis. An inhibition of platelet activation has been observed 2 and 6 h following high-flavanol cocoa consumption by healthy subjects (Rein *et al*., 2000) and the consumption of flavanol-rich chocolate has also been found to affect platelet function (Holt *et al*., 2002). In agreement with these findings, the inhibitory effects of flavanol-rich cocoa on platelet activation and function have been reported to be similar to that of aspirin (Pearson *et al.*, 2002). Finally, Murphy *et al*. (2003) reported a moderate but significant decrease in platelet function after 28 days of supplementation with cocoa flavanols and procyanidins.

In summary, there is growing evidence that the dietary intake of flavonoids, in particular flavanols, is associated with a reduced risk of CVD, although conclusive evidence is still lacking with regard to the long-term effects of flavonoid consumption. It appears that flavanol-rich foods may improve endothelial function and platelet function in healthy humans as well as those at risk of CVD. The synthesis of bioactive nitric oxide, via the action of eNOS, appears to be an important factor underlying the health effects of flavanol-rich foods, although additional studies aimed at understanding their mechanisms of action are required.

Beneficial effects of flavonoids on brain function

Recently, there has been intense interest in the potential of flavonoids to modulate neuronal function and prevent against age-related neurodegeneration. The use of flavonoid-rich plant or food extracts in humans and animal dietary supplementation studies have shown improvements in cognition function possibly by protecting vulnerable neurons, enhancing existing neuronal function or by stimulating neuronal regeneration (Youdim and Joseph, 2001). Their neuroprotective potential has been shown in both oxidative stress- (Inanami *et al*., 1998) and Aβ-induced neuronal death models (Luo *et al*., 2002). Evidence also exists for the beneficial and neuromodulatory effects of flavonoid-rich ginkgo biloba extracts, particularly in connection with age-related dementias and Alzheimer's disease (Bastianetto *et al*., 2000). Furthermore, individual flavonoids such as the citrus flavanone tangeretin have been observed to maintain nigro-striatal integrity and functionality following lesioning with 6-hydroxydopamine, suggesting that it may serve as a potential neuroprotective agent against the underlying pathology associated with Parkinson's disease (Datla *et al*., 2001).

Flavonoids and their access to the brain

In order to understand whether flavonoids and their metabolic derivatives are able to exert neuroprotective effects, it is crucial to ascertain whether they are capable of entering the central nervous system (CNS). Initial studies by Youdim *et al*. provided evidence that the flavanones hesperetin, narin-

genin and their *in vivo* metabolites, along with some dietary anthocyanins, cyanidin-3-rutinoside and pelargonidin-3-glucoside, were able to traverse the blood–brain barrier (BBB) in relevant *in vitro* and *in situ* models (Youdim *et al*., 2004). In these studies the ability of flavonoids to enter the brain was dependent on compound lipophilicity and on their interactions with specific efflux transporters expressed in the BBB (Youdim *et al*., 2003). Evidence also exists from animal feeding studies which emphasize the fact that flavonoids may access the brain and act as neuroprotective substances. The citrus flavonoids, hesperetin, naringenin and their relevant glucuronidated metabolites, were localized within the brain after intravenous administration of flavonoids in rats (Peng *et al*., 1998), whereas the flavanol EGCG was able to access the brain after oral administration of tea in mice (Suganuma *et al*., 1998). In a study by Abd El Mohsen *et al*., oral ingestion of pure epicatechin resulted in the detection of epicatechin glucuronide and 3′-*o*-methyl-epicatechin glucuronide in rat brain tissue (Abd El Mohsen *et al*., 2002). Anthocyanidins are another flavonoid group which may enter the CNS. Two recent studies have shown that pelargonidin and cyanidin 3-glucoside are able to penetrate rat brains after oral administration of pure pelargonidin (El Mohsen *et al*., 2006) and a blackberry extract (Talavera *et al*., 2005), respectively. Furthermore, several anthocyanidins have been identified in different regions of rat brain of blueberry-fed animals as well as in brains of anesthetized rats following intra-gastric administration (Passamonti *et al*., 2005). These results furthermore support the fact that flavonoids can indeed transverse the BBB and therefore be localized in the brain, making them potential candidates for protection against neurodegenerative diseases.

Protection against neurodegeneration

Neurodegeneration in Parkinson's, Alzheimer's and other neurodegenerative diseases appears to be triggered by multifactorial events including neuroinflammation, glutamatergic excitotoxicity, increases in iron and nitric oxide and depletion of endogenous antioxidants. All these events contribute to neuronal injury and may underlie pathological conditions (Mandel and Youdim, 2004; VafeiAdou *et al*., 2007). There is a growing body of evidence to suggest that flavonoids may be neuroprotective. For example, a Ginkgo biloba extract has been shown to protect hippocampal neurons from nitric oxide- or beta-amyloid-induced neurotoxicity (Bastianetto *et al*., 2000) and studies have demonstrated that the consumption of green tea may have beneficial effect in reducing the risk of Parkinson's disease (Checkoway *et al*., 2002). In agreement with the latter study, tea extracts and (−)-epigallocatechin-3-gallate (EGCG) have also been shown to attenuate 6-hydroxydopamine-induced toxicity (Levites *et al*., 2002) and to protect against hippocampal injury during transient global ischaemia (Lee *et al*., 2000) and to prevent nigral damage in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of Parkinson's disease (Levites *et al*., 2001). Regarding the latter study,

pre-treatment of mice with either a green tea extract (0.5 and 1 mg/kg) or EGCG (2 and 10 mg/kg) prevented the depletion of striatal dopamine and tyrosine hydroxylase protein levels caused by the MPTP neurotoxin. Other flavanols, such as epicatechin, are also effective at preventing neurotoxicity, with low micromolar concentrations completely protecting against oxidized low-density lipoprotein (oxLDL)-induced death in primary striatal neurons (Schroeter *et al*., 2000). Although the precise mechanism of action in unknown, it seems to involve an attenuation of caspase-3 activation and apoptosis (Schroeter *et al.*, 2001).

The death of nigral neurons in Parkinson's disease is also thought to involve the formation of the endogenous neurotoxin, 5-*S*-cysteinyl-dopamine. Recent investigations have shown that 5-*S*-cysteinyl-catecholamine conjugates are strong neurotoxins and initiate a sustained increase in intracellular reactive oxygen species (ROS) in neurons leading to DNA oxidation, caspase-3 activation and delayed neuronal death (Hastings, 1995; Spencer *et al*., 2002). Moreover, the oxidation of dopamine and L-3, 4-dihydroxyphenylalanine (L-DOPA) by superoxide leads to the formation of o-quinone species that react rapidly with cellular thiols to form adducts (Spencer *et al*., 1995). Such adducts have been observed to have been elevated in the human substantia nigra of patients who died of Parkinson's disease (Spencer *et al*., 1998), suggesting that such species may be potential endogenous nigral toxins. In presence of the flavanol (+)-catechin, tyrosinase-induced formation of 5-*S*-cysteinyl-dopamine was inhibited by a mechanism linked to the capacity of catechin to undergo tyrosinase-induced oxidation to yield cysteinyl-catechin adducts. In contrast, the inhibition afforded by flavanones, such as hesperetin, was not accompanied by the formation of cysteinyl-hesperetin adducts, indicating that it may inhibit via direct interaction with tyrosinase (Vauzour *et al*., 2007b). Flavanones, such as hesperetin and its metabolite, 5-nitro-hesperetin, have also been observed to inhibit oxidative stress-induced neuronal apoptosis via a mechanism involving the activation/phosphorylation of signalling proteins important in the pro-survival pathways and thus leading to the inhibition of the pro-apoptotic events (Vauzour *et al*., 2007a).

Inhibition of neuroinflammation

Increasing evidence suggests that neuroinflammatory processes mediated by glial cells (mainly astrocytes and microglia) may contribute to the cascade of events leading to the progressive neuronal damage observed in Parkinson's disease and Alzheimer's disease (Hirsch *et al*., 2005). Central to glial-induced neurotoxicity is the generation of nitric oxide ($NO^{\bullet}$) via increases in the expression of inducible nitric oxide synthase (iNOS), an increased cytokine production, such as interleukin-1β (IL-1β) and tumour necrosis factor-alpha (TNF-α; Kozuka *et al*., 2005) and an activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase which can generate superoxide ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2; Bal-Price *et al*., 2002). The transcriptional and post-transcriptional regulation of iNOS and cytokines in activated glial cells

is dependent on signalling through pathways such as MAPK signalling cascades (Marcus *et al*., 2003). The use of non-steroidal anti-inflammatory drugs, such as ibuprofen may delay or even prevent the onset of neurodegenerative disorders, such as Parkinson disease (Casper *et al*., 2000).

Emerging evidence suggests that flavonoids may exert neuroprotective effects via modulation of neuroinflammatory processes that contribute to the progressive neuronal damage observed in neurodegenerative disorders. Studies on various classes of flavonoids including the flavones wogonin and bacalein (Lee *et al*., 2003a), the flavonol quercetin (Chen *et al*., 2005), the isoflavone genistein (Wang *et al*., 2005) and the flavanols catechin and EGCG (Li *et al*., 2004) on astrocytes and microglia suggest that flavonoids may attenuate the release of cytokines, such as IL-1β and TNF-α2, inhibit the expression of the iNOS and subsequent $NO^{\bullet}$ production as well as inhibit the activation of NADPH oxidase and subsequent ROS generation. More recently, blueberry extracts rich in flavonoids inhibited the inflammatory mediators $NO^{\bullet}$, IL-1β and TNF-α production from activated microglia suggesting that dietary flavonoids could be potentially useful in modulation of inflammatory conditions in the CNS (Lau *et al*., 2007). One of the potential underlying cellular mechanisms of the anti-inflammatory effects seems to involve downregulation of pro-inflammatory transcription factors including nuclear factor-κB (NF-κB) and the signal transducer and activator of transcription-1 (STAT-1) which mediate neuroinflammatory processes in the CNS (Chen *et al*., 2005). Interestingly, NF-κB responds to MAPK p38 signalling leading to iNOS induction (Bhat *et al*., 2002) and MAPK signalling seems to regulate the activation of iNOS and TNF-α expression in activated glial cells (Bhat *et al*., 1998) suggesting that an interplay between signalling pathways, transcription factors and the production of inflammatory molecules is pivotal in determining a neuroinflammatory response in the CNS. Previously, the polyphenolic compound resveratrol attenuated the production of inflammatory molecules via downregulation of p38 in activated microglia (Bi *et al*., 2005). Since there is strong evidence to suggest that flavonoids are able to interfere with signalling pathways within the CNS (Spencer *et al*., 2003), it is highly plausible that they may also mediate their anti-inflammatory effects in the brain via inhibition/modulation of MAPK signalling cascades.

Flavonoid-induced improvements in memory, learning and cognitive performance

There is a growing interest in the potential of phytochemicals to improve memory, learning and general cognitive ability. A recent prospective study aimed at examining flavonoid intake in relation to cognitive function and decline has provided strong evidence that dietary flavonoid intake is associated with better cognitive evolution, i.e. the preservation of cognitive performance with ageing (Letenneur *et al*., 2007). In this PAQUID study (Personnes Agées QUID), a total of 1640 subjects (aged 65 years or older) free from dementia at baseline and with reliable dietary assessment data were

examined for their cognitive performance (Mini-Mental State Examination, Benton's Visual Retention Test, 'Isaacs' Set Test) four times over a 10-year period. After adjustment for age, sex and educational level, flavonoid intake was found to be associated with significantly better cognitive performance at baseline and with a significantly better evolution of the performance over time. In particular, subjects included in the two highest quartiles of flavonoid intake had better cognitive evolution than subjects in the lowest quartile and after 10 years' follow-up; subjects with the lowest flavonoid intake had lost on average 2.1 points on the Mini-Mental State Examination, whereas subjects with the highest quartile had lost 1.2 points. Such data provide a strong indication that regular flavonoid consumption may have a positive effect on neuro-cognitive performance as we age, although it does not provide information regarding the activity of specific flavonoid groups.

There has been much interest in the neuro-cognitive effects of soy isoflavones, primarily in postmenopausal women (Lee *et al*., 2005). The rationale behind the potential of isoflavones to exert positive effects on cognitive function is believed to lie primarily in their potential to mimic the actions and functions of oestrogens in the brain (Birge, 1996). For example, epidemiological investigations have provided evidence that postmenopausal women who undertake oestrogen-replacement therapy have a significantly lower risk for the onset of Alzheimer's disease than women who do not (Henderson, 2006). Furthermore, animal behavioural studies have shown that ovariectomy results in the development of cognitive dysfunction, which may be prevented by oestrogen replacement, suggesting that normal mammalian cognitive function is impaired by oestrogen reduction (Birge, 1996). Isoflavone supplementation has been observed to have a favourable effect on cognitive function (Casini *et al*., 2006), particularly verbal memory, in postmenopausal women (Kritz-Silverstein *et al*., 2003) and a 6- and 12-week supplementation was observed to have a positive effect of frontal lobe function (File *et al*., 2005). However, other large intervention trials have reported that dietary isoflavone supplementation does not improve cognitive function (Fournier *et al*., 2007). If isoflavones do possess the potential to influence human memory and cognitive performance it is likely that their mechanism of action would include their role as weak oestrogens, their ability to inhibit tyrosine kinase-dependent signal transduction and their ability to act as weak antioxidants (Barnes *et al*., 2000).

Other flavonoid-rich foods, in particular those containing flavanols, have been observed to improve peripheral blood flow and surrogate markers of cardiovascular function in humans (Schroeter *et al*., 2006). In the context of the CNS, brain imaging studies in humans have demonstrated that the consumption of flavanol-rich cocoa may enhance cortical blood flow (Dinges, 2006). This is important as increased cerebrovascular function, especially in the hippocampus, a brain region important for memory, may facilitate adult neurogenesis (Gage, 2000). Indeed, new hippocampal cells are clustered near blood vessels, proliferate in response to vascular growth factors and may influence memory (Palmer *et al*., 2000). As well as new neuronal growth, increases in neuronal spine density and morphol-

ogy are considered vital for learning and memory (Harris and Kater, 1994). Changes in spine density, morphology and motility have been shown to occur with paradigms that induce synaptic, as well as altered sensory experience and lead to alterations in synaptic connectivity and strength between neuronal partners, affecting the efficacy of synaptic communication. These events are mediated at the cellular and molecular level and are strongly correlated with memory and learning. The flavanol (−)-epicatechin, especially in combination with exercise, has been observed to enhance the retention of rat spatial memory in a water maze test (van Praag *et al*., 2007). This improvement in spatial memory was associated with increased angiogenesis and neuronal spine density in the dentate gyrus of the hippocampus and with the upregulation of genes associated with learning in the hippocampus.

There is also extensive evidence that berries, in particular blueberries, are effective at reversing age-related deficits in motor function and spatial working memory (Casadesus *et al*., 2004). For example, the latency period to find a platform and the distance swum to a platform in a Morris water maze task are significantly reduced following blueberry supplementation (Joseph *et al*., 1999). Such results may suggest favourable effects of the blueberry diet on locomotor activity in old animals (Andres-Lacueva *et al*., 2005). However, reductions in the time taken to make a choice may also reflect an improved memory component, where rats 'remember' more rapidly and thus respond quicker. Animal studies with tea (Chan *et al*., 2006), grape juice (Shukitt-Hale *et al*., 2006), or flavonols such as quercetin (Patil *et al*., 2003), have provided further evidence that dietary flavonoids are beneficial in reversing the course of neuronal and behavioural ageing. Although such effects have been linked with antioxidant actions it is more likely that these effects are mediated by a modulation of neurotransmitter release (Joseph *et al*., 1999), a stimulation of hippocampal neurogenesis (Casadesus *et al*., 2004) and changes in neuronal signalling (Goyarzu *et al*., 2004).

Mechanisms of Action of Flavonoids

Antioxidant effects of flavonoids

For a long time it was postulated that the beneficial effects of flavonoids are due to their antioxidant capacity. As a consequence, their ability to act as hydrogen-donating molecules was used to explain their protective effects against oxidative stress-associated diseases (Rice-Evans *et al*., 1995). Several *in vitro* reports have demonstrated the ability of flavonoids to act as classical electron- (or hydrogen-)donating antioxidants (Hertog and Hollman, 1996), their capacity to scavenge reactive species (Garcia-Alonso *et al*., 2005) and their potential to quench singlet oxygen (Halliwell *et al*., 2005). Many cell culture studies have shown the ability of flavonoids to protect against the oxidation of cellular biomolecules *in vitro* including oxidation of LDL (Yamamoto *et al*., 1999), proteins or DNA (Duthie and Dobson, 1999). Furthermore,

evidence from *in vivo* studies have shown the capacity of flavonoids to increase plasma total antioxidant capacity (Rietveld and Wiseman, 2003) and to decrease specific markers of oxidative stress such as F_2-isoprostanes concentration (O'Reilly *et al*., 2001) and lymphocyte 8-hydroxy-2'-deoxyguanosine levels (Hodgson *et al*., 2002). Nevertheless, a range of human studies also exist that have produced conflicting results on the potential of flavonoids to attenuate markers of oxidative stress (Halliwell *et al*., 2004), thus suggesting a lack of systemic antioxidant effects of flavonoids *in vivo*. Since oxidative stress has been suggested to play a pivotal role in the development of most diseases, it has been sensible to hypothesize that the beneficial effects of flavonoids against age-related disease are mostly attributed to their antioxidant properties (Spencer *et al*., 2003).

Non-antioxidant effects: interaction of flavonoids with the cell signalling cascades

Recent evidence suggests that the antioxidant capacity of flavonoids per se cannot be the only explanation for their bioactivity and that other mechanisms such as modulation of signalling pathways, may also contribute to their postulated beneficial effects (Williams *et al*., 2004). Flavonoids' extensive metabolism *in vivo* results in structural changes which are accompanied by a significant loss in their antioxidant capacity. It is now well established that the circulating form of flavonoids such as the sulfated and glucuronidated metabolites have lower antioxidant capacity compared to their corresponsive aglycones (Turner *et al*., 2004). Flavonoids are also subject to intracellular metabolism such as conjugation with thiols, especially glutathione and oxidative metabolism, which may also alter their redox capacity (Abd El Mohsen *et al.,* 2002). Importantly, intracellular concentrations of flavonoids required to affect cell signalling pathways are considerably lower than those required to impact cellular antioxidant capacity, and flavonoid metabolites may still retain their ability to interact with cell signalling proteins, even if their antioxidant activity is diminished (Spencer *et al*., 2003). Furthermore, circulating concentrations of flavonoids and their metabolites that are likely to exert biological effects are not more than 1μmol/l (Halliwell *et al*., 2000), which is much lower than the concentrations recorded for well-known small-molecule antioxidants such as ascorbic acid and α-tocopherol (Schroeter *et al*., 2000). Consequently, it is likely that flavonoids, at the concentrations found *in vivo*, cannot outcompete the action of other antioxidants present in circulation. Additionally, flavonoids possess a multitude of biological activities other than antioxidant effects which are suggestive for additional cellular mechanisms of action. For example, flavonoids bind to ATP-binding sites of proteins (Di *et al*., 1975), such as mitochondrial ATPase (Barzilai and Rahamimoff, 1983), calcium membrane ATPase (Gamet-Payrastre *et al*., 1999), protein kinase C (Boege *et al*., 1996), topoisomerase (Medina *et al*., 1997) as well as to benzodiazepine-binding sites of GABA-A and adenosine receptors (Spencer *et al*., 2001a). Flavonoids may also directly

interact with mitochondria (Laughton *et al*., 1991) and alter enzyme activity including cyclooxygenase, lipoxygenase and xanthine oxidase activity (Williams *et al*., 2004).

Recent *in vitro* studies indicate that a number of biological effects of flavonoids are mediated by interactions with signalling proteins central to intracellular signal transduction pathways (Kong *et al*., 2000). Specifically, flavonoids may exert cellular effects through selective actions at different components of a number of protein kinase and lipid kinase signalling cascades, such as the Akt/PKB tyrosine kinase, PKC, PI3-kinase and members of the MAPK family such as the extracellular signal-related kinase (ERK), c-jun amino-terminal kinase (JNK) and p38 kinases (Schroeter *et al*., 2002). Their interaction with MAPK pathway is of particular importance since the MAP signalling cascades are important enzymes involved in gene expression, cell proliferation and cell death (Chang and Karin, 2001). In general, activation of the ERK pathway is regulated by growth factors and is associated with cell survival, whereas that of JNK and p38 is activated by inflammatory stimuli and stress and is involved in cell death (Torii *et al*., 2004). Oxidative stress may alter the signalling pathways by activating pro-apoptotic signalling proteins such as JNK and thus propagate the apoptotic mechanisms within the cells (Kwon *et al*., 2003; Fig. 10.4). Interestingly,

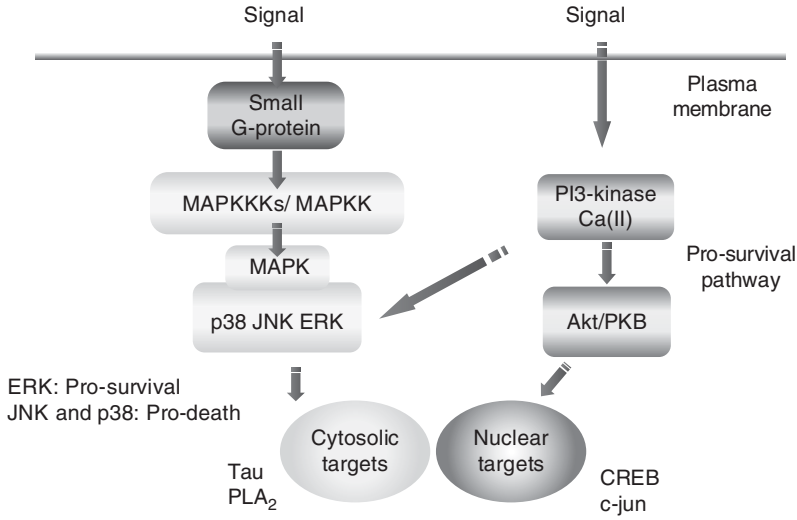


Fig. 10.4. Diagrammatic representation of the MAP kinase and Akt/PKB signalling pathways. Extracellular signal-related kinase (ERK), c-jun amino-terminal kinase (JNK) and p38 are involved in growth, differentiation, development, apoptosis and inflammation. ERK and JNK are generally considered as having opposing actions in cells with signalling through ERK usually regarded as pro-survival and JNK pro-apoptotic. The serine/threonine kinase, Akt/PKB, is one of the main downstream effectors of phosphatidylinositol 3-kinase (PI3-kinase) and a pivotal kinase in cell survival.

flavonoids have close structural homology with pharmacological inhibitors of cell signalling cascades, such as the LY294002, a phosphatidylinositol-3 kinase (PI3) inhibitor which was modelled on the structure of quercetin and PD98059 which is a MAPK inhibitor (Vlahos *et al*., 1994). LY294002 and quercetin fit into the ATP-binding pocket of the enzyme and it appears that the number and substitution of hydroxyl groups on the B ring and the degree of unsaturation of the C2–C3 bond determine this particular bioactivity. Similar to the PI3 inhibitor LY294002, quercetin and other flavonoids have been shown to inhibit PI3-kinase activity (Spencer *et al*., 2003), with inhibition directed at the ATP-binding site of the kinase (Vlahos *et al*., 1994). There are a number of potential sites within signalling pathways where flavonoids or their metabolites may interact. For instance, flavonoids may maintain calcium homeostasis, an important factor in MAPK activation and may also influence one of the many upstream MAPKKK activating proteins that transduce signals to JNK thus inhibit JNK activation and subsequently prevent oxidative stress-induced apoptosis (Zippel *et al*., 2000). Alternatively, flavonoids may directly interact with mitochondria by modulation of the mPT that controls cytochrome *c* release during apoptosis (Green and Reed, 1998), or by modulation of other mitochondrial associated pro-apoptotic factors such as DIABLO/smac (Srinivasula *et al*., 2001).

The capacity of flavonoids to modulate signalling cascades may prove to play significant role in disease prevention such as cancer, CVD or neurodegenerative diseases. Emerging data from *in vitro* studies suggest that flavonoids may induce apoptotic cell death and promote cell cycle arrest in cancer cells by modulation of MAPK signalling pathways (Shen *et al*., 2007). With respect to CVD, flavonoids may exert beneficial effects though modulation of endothelial signalling (Kyaw *et al*., 2004). For example, quercetin may inhibit cell proliferation and migration in smooth muscle cells by downregulation of JNK and ERK pathways (Yoshizumi *et al*., 2002) and may also induce haem oxygenase 1 (HO-1) expression, which is known to inhibit atherosclerosis progression, through p38 activation in aortic smooth muscle cells (Lijima *et al*., 2002).

Conclusion

Flavonoids are found ubiquitously in plants and represent a major part of a human diet. Much data has emerged on the potential health effects of several classes of flavonoids in a number of chronic and age-related diseases. Over the recent years we have gained an understanding of how flavonoids are absorbed and metabolized within the body. However, better designed human studies, that measure appropriate biomarkers, are warranted in order to assess the role of flavonoids in health and disease. In addition to investigation *in vivo*, it is also essential that we better understand their mechanisms of action. An understanding of the precise cellular and molecular actions of flavonoids is crucial in order to evaluate the beneficial effects of these phytochemicals against chronic disease. When considering such actions in cellular models, emphasis should be given to the mode of action of circulating flavo-

noids metabolites, which are the forms most likely to reach the target tissues and penetrate cells. Thus far, numerous *in vitro* studies suggest that flavonoids may affect chronic disease by selectively interacting with signalling pathways involved in growth, differentiation and apoptosis, rather than by acting as antioxidants. However, further studies are required in order to determine precise modes of action underlying clinical responses in humans. The outcomes of these studies may in future be used to make dietary recommendations for flavonoid intake.

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11 Environmental Regulation of Flavonoid Biosynthesis

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Introduction

Flavonoids are a large family of polyphenolic secondary metabolites synthesized by a wide variety of plant species from mosses to angiosperms. They comprise several ubiquitous groups of compounds, including flavonols, anthocyanins and condensed tannins. The structures of the different flavonoids are based on the same 15-carbon chemical skeleton (Fig. 11.1) and numerous modifications are possible, for example by glycosylation and methylation. Consequently, several thousand different flavonoids have been identified in nature (Harborne and Williams, 2000).

Flavonoids have diverse, important functions in plants (Winkel-Shirley, 2001a,b). They provide pigmentation of flowers, leaves and other tissues (Mol *et al*., 1998), affect seed dormancy (Debeaujon *et al*., 2000) and are involved in regulating auxin transport (Brown *et al*., 2001). In some species flavonoids are involved in defence against pathogens (Dixon and Paiva, 1995; Dixon, 2001), fertility (Shirley, 1996) and signalling between plants and microorganisms (Shaw *et al*., 2006). In addition, flavonoids are important in UV protection (Bornman *et al*., 1997; Jenkins and Brown, 2007), protection against photooxidative damage (Havaux and Kloppstech, 2001) and may act as antioxidants to alleviate the effects of reactive oxygen species (ROS) produced in abiotic stresses (Winkel-Shirley, 2002). Moreover, flux from primary to secondary metabolism provides a 'safety valve' for excess carbon under conditions of stress.

Since flavonoids are present in diverse species and are found in a variety of plant tissues and organs they are ubiquitous constituents of plant-derived foods and drinks. Hence, products of the pathway are important components both of the human diet and of animal feeds. Epidemiological studies have shown a positive correlation between the consumption of flavonoids and a reduced incidence of cardiovascular disease (Scalbert *et al*., 2005). Moreover,

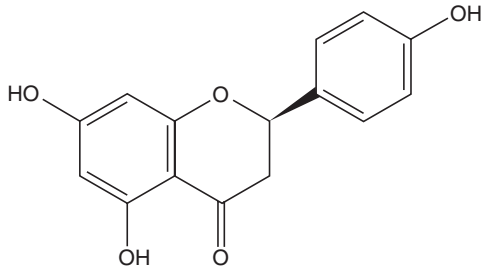

Fig. 11.1. The 15-carbon chemical skeleton, which is the basis of the structure of the different flavonoids.

there is evidence that certain flavonoids may have a positive impact on other conditions, including certain cancers (Birt *et al*., 2001), although questions remain regarding the extent of flavonoid absorption in the gut and the use of *in vitro* models of carcinogenesis (Yang *et al*., 2001). It has been claimed that the beneficial effects of flavonoids may be due to their potent antioxidant activities, which give them the potential to scavenge ROS produced in cells, but recent evidence suggests that their activity may derive from interactions with cellular signal transduction components (Williams *et al*., 2004; see Spencer, Chapter 10, this volume). Other research has shown that flavonoids affect the quality of animal feeds. For instance, condensed tannins in seeds affect processing and meal quality and in forage grasses relieve ruminant bloat (Marles *et al*., 2003). Hence, there is considerable interest in identifying which flavonoids have beneficial nutritional properties and in understanding how plants produce different types and amounts of flavonoids.

The focus of this chapter is on the regulation of flavonoid biosynthesis by environmental factors. It includes an outline of the biosynthesis of the principal flavonoids and identifies differences between species. The article discusses the importance of various environmental factors in regulating flavonoid accumulation and emphasizes the molecular basis of regulation.

Flavonoid Biosynthesis

The flavonoid biosynthesis pathway has been the subject of detailed biochemical characterization and the description below provides an outline of the key steps (see Fig. 11.2). Most of the flavonoid biosynthesis enzymes are located in the cytosol and several are loosely associated with the endoplasmic reticulum. There is evidence that some enzymes form a complex that facilitates channelling of metabolites (Winkel-Shirley, 2001a). Some aspects of flavonoid biosynthesis such as transfer into the vacuole and the biosynthesis of polymeric flavonoids are still not fully characterized and the reader should consult detailed reviews for further information (Dixon and Paiva, 1995; Holton and Cornish, 1995; Weisshaar and Jenkins, 1998; Winkel-Shirley, 2001a,b; Marles *et al*., 2003; Schijlen *et al*., 2004). The principal products of the

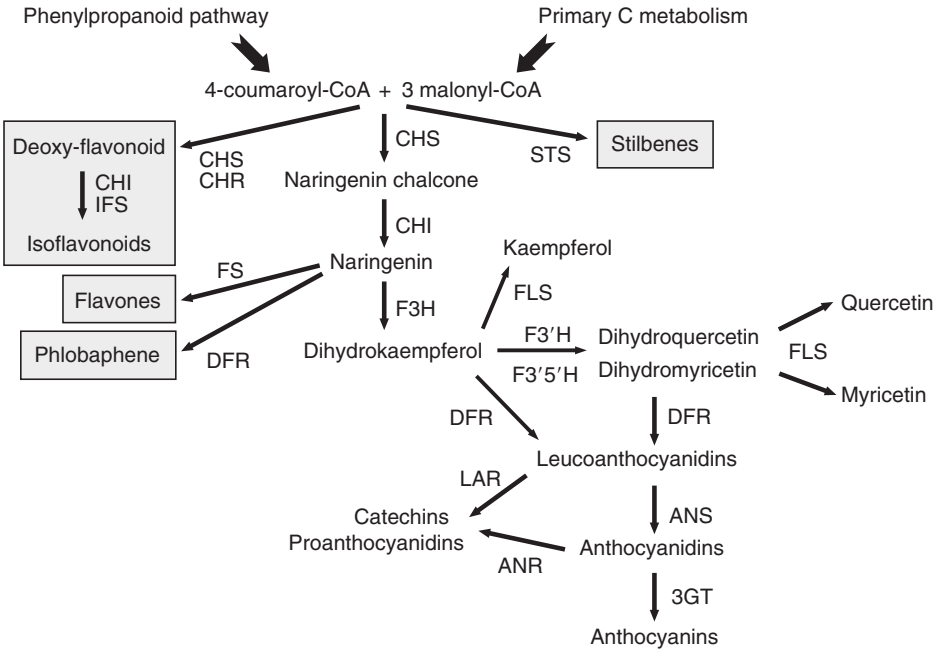

Fig. 11.2. Key steps in the flavonoid biosynthesis pathway.

pathway include chalcones, flavones, flavonols, flavandiols, anthocyanins, catechins and condensed tannins. Species differ in the compounds accumulated and in modifications such as glycosylation. Moreover, as noted below, some products are found only in particular genera.

The flavonoid biosynthesis pathway is a branch of the general phenylpropanoid pathway (Fig. 11.2). The first committed step is catalysed by the enzyme chalcone synthase (CHS). CHS catalyses the condensation of three acetate residues from malonyl-CoA, derived from carbohydrate metabolism, with 4-coumaroyl-CoA, to produce 4,2′,4′,6′-tetrahydroxy-chalcone or naringenin chalcone. The substrate 4-coumaroyl-CoA is a product of the phenylpropanoid pathway. In most plants the chalcone does not accumulate but is used to produce a variety of products through downstream steps in the pathway. The naringenin chalcone product of CHS activity is a substrate for chalcone isomerase (CHI), which catalyses the formation of the flavanone naringenin. Naringenin is hydroxylated by the enzyme flavanone-3-hydroxylase (F3H) to produce the dihydroflavonol dihydrokaempferol, which can be further hydroxylated at one position on the B ring by flavonoid 3′-hydroxylase (F3′H) to form dihydroquercetin or at two positions by flavonoid 3′5′-hydroxylase (F3′5′H) to form dihydromyricetin. Dihydrokaempferol, dihydroquercetin and dihydromyricetin are substrates for the enzyme flavonol synthase (FLS), which converts them into kaempferol, quercetin and myricetin, respectively. These flavonols are important products of the flavonoid biosynthesis pathway as they have high antioxidant activity and strong UV absorbance.

The dihydroflavonols, dihydrokaempferol, dihydroquercetin and dihydromyricetin are precursors of anthocyanin biosynthesis. Anthocyanins are red-purple pigments that frequently colour flower petals and provide pigmentation of leaf tissues. Dihydrokaempferol, dihydroquercetin and dihydromyricetin are converted into the leucoanthocyanidins leucocyanidin, leucopelargonidin and leucodelphinidin, respectively, by the enzyme dihydroflavonol reductase (DFR) using nicotinamide adenine dinucleotide phosphate oxidase (NADPH). Leucoanthocyanidins are converted into anthocyanidins by the enzyme anthocyanidin synthase (ANS), also called leucoanthocyanidin oxidase (LDOX). Leucocyanidin, leucopelargonidin and leucodelphinidin are converted into cyanidin, pelargonidin and delphinidin, respectively, by ANS. These compounds have different colours because of their degree of hydroxylation; cyanidin is pink/magenta, pelargonidin orange/red and delphinidin purple/blue.

The anthocyanidins are stabilized by the addition of a glucose moiety from UDP-glucose catalysed by the enzyme UDP-glucose:flavonoid 3-*o*-glucosyltransferase (3GT) and are then transferred to the vacuole. Indeed other flavonoids including the flavonols accumulate as sugar conjugates that are deposited in the vacuole. A variety of glycosylated products, involving different sugars and addition at different positions within the molecule, have been reported. The mechanism of vacuolar sequestration is not fully understood. There is evidence from mutant characterization that glutathione *S*-transferase (GST) proteins are required for the uptake of anthocyanins into the vacuole (Marrs *et al*., 1995; Alfenito *et al*., 1998; Kitamura *et al*., 2004) and the GSTs are thought to bind to the glycosylated anthocyanins to mediate their transfer. In maize, a transporter of the multidrug resistance-associated protein (MRP) class mediates the vacuolar transport of anthocyanin glutathione conjugates (Goodman *et al*., 2004). The different colours of the anthocyanins are dependent not only on their degree of hydroxylation, but also the vacuolar pH and any further modifications that may occur prior to vacuolar sequestration, such as additional glycosylation or methylation.

Catechins are flavan-3-ol molecules found in a variety of foodstuffs but are particularly abundant in green tea and cocoa. They are antioxidants and have been implicated in protection against cardiovascular disease and certain cancers. Catechins are produced by reduction of either leucoanthocyanidins or anthocyanidins by the enzymes leucoanthocyanidin reductase (LAR) and anthocyanidin reductase (ANR), respectively. The latter enzyme was identified through characterization of the Arabidopsis *BANYULS* gene product (Devic *et al*., 1999), which has ANR activity (Xie *et al*., 2003). Proanthocyanidins (PAs), otherwise known as condensed tannins, are flavonoid polymers that provide brown pigmentation to seeds and are also found in leaves of some forage crops where they are beneficial to grazing ruminants. PAs are additionally important in limiting seed deterioration during storage by inhibiting attack by pathogens and acting as a physical barrier to prevent water uptake (Debeaujon *et al*., 2000). The steps in PA synthesis are not fully characterized but involve the progressive addition of leucoanthocyanidin-derived units to a catechin initiating unit (Marles *et al*., 2003). Both LAR and

BANYULS provide the units required for condensed tannin formation. PAs are transferred to the vacuole and this is reported to involve both GST (Kitamura *et al*., 2004) and a multidrug and toxic compound extrusion (MATE)-type transport protein in Arabidopsis (Debeaujon *et al*., 2001).

Several variations and additions to the core flavonoid biosynthesis pathway described above are present in some species. For instance, legumes and a few non-leguminous species possess an enzyme chalcone reductase (CHR) that acts with CHS to produce a deoxy-flavonoid 2′,4′,4-trihydroxychalcone, in contrast to the 4,2′,4′,6′-tetrahydroxy chalcone produced by CHS alone. The deoxy-flavonoid is the precursor of isoflavonoids. The production of these compounds involves the action of CHI and the enzyme isoflavone synthase (IFS). Isoflavonoids are involved in pathogen defence and act as signalling molecules in nodulation of roots by nitrogen-fixing bacteria. Some isoflavonoids, such as genistein and daidzein, are reported to have anti-cancer properties and also estrogenic activity (Cos *et al*., 2003).

Some species, such as *Vitis vinifera* (grapevine), possess an enzyme termed stilbene synthase (STS) which acts on malonyl-CoA and 4-coumaroyl-CoA, the substrates of CHS, to produce stilbenes. One of these, resveratrol, is found in the skins of red grapes and is present in red wines. Resveratrol is credited with several beneficial properties, including anti-cancer activity and cardioprotective effects (Baur and Sinclair, 2006).

Variations on the flavonoid biosynthesis pathway produce additional pigments in some species. Maize and some cereals possess a branch within the pathway that converts flavanones into 3-deoxy-flavonoids, including the phlobaphene pigments found in maize floral organs. DFR is involved in this pathway, producing a flavan-4-ol from a flavanone substrate. In some other species the flavanone naringenin can be converted into flavone pigments, which are important in colouring the flowers of dahlia and chrysanthemum. A further class of flavonoid pigments, the yellow aurones, are derived from hydroxy-chalcones in species such as *Antirrhinum*.

Molecular Basis of the Regulation of Flavonoid Biosynthesis

Flavonoid biosynthesis is regulated substantially through transcription of genes encoding enzymes of the pathway (Mol *et al*., 1996, 1998; Koes *et al*., 2005). There are numerous examples of factors, such as environmental treatments, stimulating an increase in the abundance of transcripts of flavonoid biosynthesis genes resulting in accumulation of the corresponding flavonoids. Hence, there is generally a very close correlation between the level of flavonoid accumulation and the level of expression of genes encoding enzymes of the pathway. Therefore, considerable attention has been given to understanding the regulation of expression of flavonoid biosynthesis genes at the transcriptional level.

Transcription of genes is controlled by transcription factor proteins that associate with regulatory DNA sequence elements of the target gene, leading to the initiation of transcription via RNA polymerase activity. The identifica-

tion of transcription factors controlling the production of flavonoids has been achieved by molecular genetic studies of the pigmentation of vegetative tissue, flowers and seeds in principally three species, *Zea mays*, *Petunia hybrida* and *Arabidopsis thaliana*. These species have been used because of the ease of isolation of mutants altered in flavonoid pigment accumulation and the ability to identify genes corresponding to the mutations. Characterization of the altered phenotype of a mutant provides information about the function of the wild-type gene. The results obtained with the above species provide a consistent model of regulation that is applicable to a wide range of other plants.

Anthocyanin biosynthesis has become a paradigm for understanding the molecular regulation of flavonoid production. In maize, Arabidopsis and Petunia three types of proteins act together to regulate anthocyanin biosynthesis genes, basic helix-loop-helix (bHLH) transcription factors, Myb transcription factors and WD40 proteins (Mol *et al*., 1996, 1998; Koes *et al*., 2005). The WD40 proteins facilitate interactions between the transcription factors, although their precise role is unclear (Broun, 2005; Koes *et al*., 2005). The above proteins regulate transcription of the genes encoding DFR and other 'late' biosynthetic enzymes involved in anthocyanin biosynthesis. Thus, mutants in either bHLH, Myb or WD40 regulatory genes fail to express the 'late' biosynthetic enzymes but are unaffected in production of 'early' enzymes such as CHS.

Each of the above species contains more than one transcription factor of each type that can regulate anthocyanin biosynthesis genes. The genes encoding these factors are expressed in particular tissues and are responsible for controlling pigmentation in those tissues (Mol *et al*., 1996). For instance, in maize (Dooner *et al*., 1991), anthocyanin biosynthesis is regulated by different bHLH family members, B, R, Lc and Sn, and the Myb factors, C1 and Pl. R is expressed in the kernel, seedling, anthers and coleoptile, whereas B controls anthocyanin production in mature tissues such as husk leaves. Lc and Sn are expressed in the leaf blade, scutellar node and mesocotyl. The bHLH factors function with Myb factors expressed in the different tissues. For example, R functions with C1, expressed in the kernel, whereas B functions with P1 in husk leaves. In Petunia, the AN1 bHLH factor functions with the Myb factors AN2 and AN4, expressed in the corolla and anthers, respectively, and with the WD40 AN11 (Mol *et al*., 1996; Koes *et al*., 2005).

In Arabidopsis three bHLH factors GL3, EGL3 and TT8 regulate processes in the leaf and root epidermis, including root and leaf hair production as well as anthocyanin biosynthesis. They act redundantly, that is one can take over the function of the other if it is removed by mutation. Thus, a triple *gl3 egl3 tt8* mutant is needed to see a complete reduction in anthocyanin biosynthesis, whereas the single and double mutant combinations retain anthocyanin to varying degrees (Zhang *et al*., 2003). The identity of the Myb factors that function with the bHLH factors is not entirely clear, although two proteins, PAP1 and PAP2, are strongly implicated in regulating anthocyanin production. These proteins were identified because they cause massive

overproduction of anthocyanins when they are over-expressed in transgenic plants. However, they elevate expression of 'early' as well as 'late' flavonoid biosynthesis genes and cause overproduction of a range of phenolic compounds (Borewitz *et al*., 2000; Tohge *et al*., 2005). It is possible that overexpression causes aberrant effects on target gene expression and so the molecular targets of PAP1 and PAP2 *in vivo* are not entirely defined. Nevertheless, there is strong evidence that they regulate aspects of flavonoid biosynthesis *in vivo* because their expression is closely correlated with changes in anthocyanin biosynthesis gene expression (see below) and, moreover, suppression of expression of PAP1 leads to a reduction of both anthocyanin and proanthocyanidin biosynthesis (Matsui *et al*., 2004). It has been shown that PAP1 and PAP2 interact directly with specific bHLH proteins (Zimmerman *et al*., 2004).

Other flavonoid products are regulated by different transcription factors. In Arabidopsis a mutant in the AtMYB12 transcription factor fails to synthesize flavonols (Mehrtens *et al*., 2005). AtMYB12 regulates transcription of the *FLS* gene but has very little effect on DFR transcription. A further protein, AtMYB4, represses flavonoid biosynthesis in seedlings and promotes synthesis of sinapic acid esters that help to protect against damage by UV-B (Jin *et al*., 2000). In maize, phlobaphene synthesis is regulated by another Myb factor, termed P (Grotewold *et al*., 1994).

Detailed information has been obtained regarding the regulation of PA biosynthesis in seeds of Arabidopsis. Mutants were isolated lacking pigmentation of the seed testa: the *transparent testa* (*tt*) mutants (Shirley *et al*., 1995). The bHLH, Myb and WD40 proteins controlling PA biosynthesis in Arabidopsis are TT8, TT2 and TTG1, respectively (Walker *et al*., 1999; Nesi *et al*., 2000, 2001). Mutants in either of these genes result in seeds deficient in PAs. However, additional *tt* mutants have identified further genes involved in the regulation of PA biosynthesis, *TT1*, *TT16* and *TTG2*. Molecular characterization of the *tt1* mutant showed that *TT1* encoded a Zn-finger type transcription factor (Sagasser *et al*., 2002). The mutant is altered in development of the endothelial layer in the seed as well as PA accumulation and thus TT1 appears to regulate several aspects of gene expression in this tissue. Similarly, the MADS-domain transcription factor TT16 regulates both endothelial development and PA biosynthesis (Nesi *et al*., 2002). TTG2 is a WRKY-family transcription factor involved in the regulation of leaf trichome formation as well as seed PA biosynthesis (Johnson *et al*., 2002).

The pivotal importance of transcription factors in the regulation of flavonoid biosynthesis has been demonstrated very effectively in experiments to modify flavonoid production in transgenic plants. As indicated above, when the anthocyanin regulators PAP1 and PAP2 are strongly over-expressed in transgenic Arabidopsis the vegetative tissue becomes highly pigmented with anthocyanin. The same is observed when these factors are expressed in tobacco plants and in this case the flowers also show increased pigmentation (Borewitz *et al*., 2000). Similarly, expression of the maize Lc and C1 transcription factors in tomato causes purple foliage and flavonol accumulation in the flesh of fruits, which normally lacks flavonoids (Bovy *et al*., 2002).

Factors Regulating Flavonoid Biosynthesis

Numerous publications have concerned the regulation of flavonoid biosynthesis by various factors in diverse species. It is beyond the scope of the present article to provide a comprehensive review of the literature. Rather, the aim is to highlight the main ways in which flavonoid accumulation is regulated, in particular by environmental factors, by reference to some of the most informative studies. In many cases these have been undertaken with the model species Arabidopsis, simply because the genetic and molecular biological tools available for this species enable particularly incisive experiments to be undertaken. Nevertheless, many of the conclusions drawn from experiments with model species such as Arabidopsis, maize and tomato appear to be applicable to a range of other species. Even though other species may accumulate different flavonoids and have particular features of interest, the principles of regulation appear to be well conserved among diverse species.

Flavonoid biosynthesis is regulated by a combination of endogenous factors and environmental stimuli. The endogenous factors include developmental signals that determine spatial and temporal regulation of flavonoid biosynthesis. The levels of endogenous plant growth regulators and metabolites are additionally important in regulation. Among the environmental factors, light, soil nutrient status and abiotic stresses have major effects on flavonoid biosynthesis.

As discussed above, flavonoid biosynthesis is regulated principally by controlling the rate of transcription of genes encoding the enzymes of the pathway. Hence, many studies have reported changes in the expression of genes encoding flavonoid biosynthesis enzymes in parallel with changes in flavonoid accumulation. Gene expression is most frequently assayed at the transcript level by either hybridization of gene-specific DNA probes to blots of fractionated RNA samples (northern blots) or reverse transcription-polymerase chain reaction (RT-PCR) with gene-specific primers. Increasingly, there are reports of the effects of environmental factors on whole genome expression – transcriptome analysis using microarrays – that enable conclusions to be drawn about regulation of the entire set of flavonoid biosynthesis genes in a single study.

It has been demonstrated that environmental factors regulate expression of the transcription factors controlling flavonoid biosynthesis genes. Several examples of this will be given below. Thus, to understand the regulation of flavonoid biosynthesis by environmental factors it is important to determine how the transcription factors are regulated.

Regulation by endogenous developmental signals and the role of plant growth regulators

Flavonoids do not accumulate uniformly in plant tissues. First, the different flavonoids accumulate in specific cells and tissues consistent with their functions. Second, in addition to spatial regulation, temporal regulation of

flavonoid biosynthesis is observed. One of the best illustrations of this is the developmental regulation of PAs in the seed. In terms of spatial regulation, PAs accumulate in the seedcoat and specifically in the endothelial layer (Debeaujon *et al*., 2003). Temporally, PAs are synthesized during the course of seed development. The pigments are initially colourless but become oxidized and brown as seed development proceeds (Devic *et al*., 1999). As discussed above, a number of transcription factors that control PA biosynthesis in the seedcoat have been identified. Specific factors, e.g. TT1, are expressed in the endothelial layer (Sagasser *et al*., 2002). Mutants in the *TT1* and *TT16* genes have aberrant development of the endothelial layer (Nesi *et al*., 2002; Sagasser *et al*., 2002). Thus, the spatial expression of the transcription factors specifies the spatial expression of PA biosynthesis genes (Debeaujon *et al*., 2003).

Spatial regulation is also observed in vegetative tissues, in that most flavonoids accumulate in the outer epidermal layers. Studies of parsley showed very clearly that *CHS* gene expression occurred principally in the epidermis of leaves accumulating flavonoids (Schmelzer *et al*., 1988).

Several plant growth regulators affect flavonoid biosynthesis. The wound signalling molecule jasmonic acid promotes anthocyanin accumulation and induces the expression of several flavonoid biosynthesis genes, including *CHS* and *DFR* (Franceschi and Grimes, 1991; Tamari *et al*., 1995). Gibberellic acid (GA) promotes anthocyanin accumulation and *CHS* expression in Petunia flower petals and these effects are antagonized by abscisic acid (Weiss *et al*., 1992; Mol *et al*., 1996). Cytokinins also stimulate anthocyanin accumulation and this has been observed in a variety of species including maize (Piazza *et al*., 2002) and Arabidopsis (Deikman and Hammer, 1995; Wade *et al*., 2003). Deikman and Hammer (1995) reported that cytokinin stimulated the transcript levels of several flavonoid biosynthesis genes in Arabidopsis. It is likely that these and possibly other plant growth regulators are involved in mediating or modulating the effects of environmental factors on flavonoid biosynthesis but little information is available on this point. However, it has been reported that GA diminishes the stimulatory effect of phosphate starvation on anthocyanin accumulation in Arabidopsis (Jiang *et al*., 2007).

Regulation by sugars

Flavonoids are secondary, non-essential metabolites. One of the functions of secondary metabolism appears to be that it provides a 'safety valve' for channelling excess carbon. Thus, treatments that promote the accumulation of carbon under conditions where its use in primary metabolism is impaired are likely to lead to increased secondary metabolism and the accumulation of flavonoids. For instance, when plants are grown on an exogenous carbon source the potential for flux into secondary metabolism is increased. If, for example, under the same conditions nitrogen is deficient the potential for carbon flux into amino acid biosynthesis is decreased and flux into secondary metabolism is promoted. This phenomenon can be demonstrated simply by growing seedlings such as Arabidopsis on sucrose in the presence of

limiting nitrogen – in this case the seedlings become pink/purple in appearance due to the accumulation of anthocyanin (Martin *et al*., 2002; Lea *et al*., 2007). Similarly, the higher the ambient light intensity, the more carbon will be produced through photosynthesis and if plants are concomitantly exposed to environmental stresses that impair primary metabolism, flux into secondary metabolites will be increased. Thus, stressed plants will often appear pink/purple due to anthocyanin accumulation. It is possible that the accumulated secondary metabolites in some way help to ameliorate the effects of stress, perhaps through antioxidant activity, apart from acting as a sink for excess carbon (Winkel-Shirley, 2002).

It can be predicted that an increase in the availability of carbon for secondary metabolite biosynthesis should occur when plants are grown in elevated atmospheric CO_2. This has been verified in experiments with wheat (Estiarte *et al*., 1999) and tobacco (Matros *et al*., 2006) plants, which showed that CO_2 enrichment increased the levels of flavonoids.

The induction of anthocyanin accumulation in response to sucrose has been observed in several studies with Arabidopsis (Tsukaya *et al*., 1991; Martin *et al*., 2002; Wade *et al*., 2003) and other species, such as Petunia (Weiss, 2000), *V. vinifera* (Larronde *et al*., 1998) and radish (Hara *et al*., 2003). Transcriptome analysis in Arabidopsis has revealed that sucrose strongly stimulates expression of a range of flavonoid and anthocyanin biosynthesis genes (Solfanelli *et al*., 2006). Moreover, sucrose enhances expression of regulatory transcription factors, including PAP1 (Lloyd and Zakhleniuk, 2004; Teng *et al*., 2005; Solfanelli *et al*., 2006). Genetic analysis indicates that the extent of anthocyanin induction by sucrose in Arabidopsis genotypes is determined by PAP1 (Teng *et al*., 2005).

It is important to note that the effect of sucrose on anthocyanin accumulation is not simply an effect of carbon supply and that sucrose acts as a regulatory signalling molecule. The stimulation of flavonoid biosynthesis gene expression occurs specifically in response to sucrose and equivalent amounts of glucose or fructose are ineffective, demonstrating that the effect of sucrose is not simply nutritional (Teng *et al*., 2005; Solfanelli *et al*., 2006). It may be that sucrose is important as a regulatory signal in some of the environmental effects on flavonoid biosynthesis described below. If, for example, a stress results in sucrose accumulation, sucrose signalling may lead to the stimulation of flavonoid biosynthesis genes.

Regulation by light

It is well established that light is one of the most important factors regulating flavonoid biosynthesis (Beggs *et al*., 1986; Hahlbrock and Scheel, 1989; Dixon and Paiva, 1995; Jenkins *et al*., 2001). Seedlings grown in darkness and plants grown in low light intensities have very low levels of flavonoids but the amounts increase substantially and rapidly following illumination as a result of the stimulation of expression of the biosynthetic genes (Feinbaum and Ausubel, 1988; Batschauer *et al*., 1991; Kubasek *et al*., 1992). Similar results are

observed when plant cell cultures are illuminated (Bruns *et al.*, 1986; Hahlbrock and Scheel, 1989). 'Early' biosynthesis genes such as *CHS* are expressed more rapidly than 'late' genes such as *DFR* (Hahlbrock and Scheel, 1989).

One of the reasons that flavonoid production is strongly induced by light is that some flavonoids, in particular the flavonols, are very effective in absorbing UV-B radiation that has the potential to damage plant tissues exposed to sunlight (Bornman *et al.*, 1997; Ryan *et al.*, 2001, 2002). It is therefore not surprising that UV-B wavelengths are very effective in stimulating expression of flavonoid biosynthesis genes and flavonoid accumulation (Jenkins *et al.*, 2001; Jenkins and Brown, 2007). Recent transcriptome analyses in Arabidopsis and maize have shown that all the key flavonoid biosynthesis genes are UV-stimulated (Casati and Walbot, 2003; Ulm *et al.*, 2004; Brown *et al.*, 2005; Brown and Jenkins, 2008).

The flavonoids are deposited in vacuoles of epidermal cells where they produce a UV-absorbing protective screen. Studies using microspectrophotometry demonstrated that this decreases penetration (Reuber *et al.*, 1996; Bornman *et al.*, 1997). The UV-inducible flavonoids act in conjunction with other phenolic compounds, such as hydroxycinnamic acid esters that provide a largely constitutive level of UV-absorbance (Burchard *et al.*, 2000). Studies of Arabidopsis and maize genotypes altered in flavonoid content show that both types of compounds contribute to UV protection (Li *et al.*, 1993; Lois and Buchanan, 1994; Stapleton and Walbot, 1994; Landry *et al.*, 1995).

Although UV-B wavelengths are very effective in inducing flavonoid biosynthesis little is known about the mechanisms of UV-B perception. No UV-B photoreceptor has yet been identified and it is possible that no such molecule exists. However, a protein has recently been identified in Arabidopsis that acts specifically in UV-B to mediate increases in expression of flavonoid biosynthesis genes. This protein is termed UVR8 (Jenkins and Brown, 2007). The *uvr8* mutant fails to induce flavonoid biosynthesis genes and other UV-protective genes in response to UV-B and is defective in flavonoid accumulation (Kliebenstein *et al.*, 2002; Brown *et al.*, 2005). In consequence the *uvr8* mutant has greatly reduced viability when exposed to UV-B.

UV-B wavelengths are not the only spectral region that stimulates expression of flavonoid biosynthesis genes and flavonoid accumulation. UV-A and blue light, detected by the cryptochrome 1 photoreceptor, stimulate expression of flavonoid biosynthesis genes such as *CHS* and *DFR*. An Arabidopsis mutant lacking this photoreceptor is defective in expression of the biosynthetic genes and anthocyanin accumulation in response to UV-A and blue light (Ahmad *et al.*, 1995; Jackson and Jenkins, 1995; Wade *et al.*, 2001). Phytochromes, which are photoreceptors principally of red and far-red light, also mediate the expression of flavonoid biosynthesis genes and anthocyanin accumulation (Frohnmeyer *et al.*, 1992; Kubasek *et al.*, 1992). In particular, in young seedlings exposure to red and in some cases far-red light promotes the response. In some species the competence to induce anthocyanin accumulation via phytochrome is lost soon after seedling establishment (Frohnmeyer *et al.*, 1992; Kaiser *et al.*, 1995). Hence, there is a developmental control of the response.

It is evident from the above discussion that the photoregulation of flavonoid biosynthesis is complex as it involves multiple photoreceptors. It is in fact even more complex than it might first appear because there are interactions between the signal transduction pathways mediating the responses to different photoreceptors. For instance, although phytochrome does not induce *CHS* expression in mature Arabidopsis leaf tissue, phytochrome both potentiates the response to UV-A/blue light mediated principally by cryptochrome 1 (cry1) and co-acts with cry1 to induce *CHS* expression (Wade *et al*., 2001). In contrast, the UV-B induction of *CHS* is repressed by phytochrome B. The UV-B induction is synergistically enhanced by distinct interactions with UV-A and blue light mediated by unidentified photoreceptors (Fuglevand *et al*., 1996). Thus, a complex interacting network is involved in the light induction of flavonoid biosynthesis genes in leaf tissue (Wade *et al*., 2001).

Information has been obtained about the roles of several transcription factors in the regulation of flavonoid biosynthesis by light. For instance, in maize, transcripts of some of the bHLH and Myb transcription factors that regulate anthocyanin biosynthesis genes in vegetative tissues increase in abundance following exposure to light (Procissi *et al*., 1997; Piazza *et al*., 2002). However, light induction of specific Mybs is most closely correlated with stimulation of the biosynthetic genes. Similarly, in Arabidopsis light stimulates expression of the *PAP1* and *PAP2* Myb transcription factors involved in controlling anthocyanin accumulation (Cominelli *et al*., 2007). In addition, *MYB12* expression is stimulated by UV-B in leaf tissue (Cloix and Jenkins, 2008), facilitating the UV induction of flavonol biosynthesis. Expression of another *Myb* gene, *MYB4*, is stimulated by UV-B in Arabidopsis seedlings, but in this case the Myb represses *CHS* expression, allowing an increase in sinapate ester biosynthesis from a branch of the general phenylpropanoid pathway (Jin *et al*., 2000). Furthermore, light stimulates expression of a *Myb* gene in apple fruit that is responsible for regulating the production of anthocyanin pigmentation in the skin (Takos *et al*., 2006). Together these studies emphasize that light regulation of expression of key Myb transcription factors is particularly important in controlling flavonoid accumulation.

An additional transcription factor important in regulating flavonoid biosynthesis genes by light is the basic leucine zipper factor HY5. The pivotal importance of HY5 in plant responses to light is well established (Lee *et al*., 2007), but most information about the role of HY5 in regulation of flavonoid biosynthesis has come from studies of UV-B responses. The Arabidopsis *hy5* mutant is impaired in the stimulation of early (e.g. *CHS*) and late (e.g. *DFR*) flavonoid biosynthesis genes by UV-B and is UV-sensitive (Brown *et al*., 2005). Expression of the *HY5* gene is itself stimulated by UV-B and this response is mediated by the UVR8 signalling protein (Brown *et al*., 2005).

Circadian clock

Circadian clocks are present in organisms to ensure that processes occur at the correct time of day. They act to maintain endogenous rhythms of biological

activities. It has been demonstrated that many plant genes are subject to circadian regulation and this includes flavonoid biosynthesis genes. Harmer *et al*. (2000) demonstrated that flavonoid biosynthesis genes increase in expression before dawn of the diurnal cycle. It seems likely that the increase in expression is timed to ensure that maximal protection against UV-B wavelengths is present each day before plants are exposed to sunlight. Interestingly, the circadian regulation and timing of expression of the transcription factors *PAP1* and *PAP2* was consistent with their playing a key role in regulation of the biosynthetic genes.

Soil nutrient availability

Several nutrient deficiencies are characterized by the accumulation of flavonoids and, particularly anthocyanin. For instance, tomato plants starved of nitrogen showed increased levels of anthocyanin and the flavonol quercetin (Bongue-Bartelsman and Phillips, 1995). Stewart *et al*. (2001) examined the effects of nitrogen and phosphorus limitation on flavonol content in Arabidopsis and tomato plants. Growth of Arabidopsis seedlings on media lacking either phosphate or nitrate resulted in three- to fourfold increases in total flavonol content, with increases being detected in the major flavonols quercetin and kaempferol. Similar results were obtained for tomato seedlings, although the increase in flavonols in response to phosphate starvation was only about twofold. In leaves of mature tomato plants grown under commercial conditions nitrate limitation again increased the flavonol content but in this case phosphate limitation did not. Little effect of altered nitrogen and phosphate status was observed on flavonols in the tomato fruits. It should be noted that the mature plants were not totally starved of nitrogen and phosphate as that would have severely prevented growth and fruit production. These experiments therefore demonstrate that reduced nitrogen and phosphate levels stimulate flavonol accumulation in leaf but not fruit tissue.

Further studies with Arabidopsis have shown that phosphate deficiency stimulates anthocyanin accumulation in seedlings (Jiang *et al*., 2007) and that nitrogen deficiency has a stronger stimulatory effect on anthocyanin production than on flavonol levels (Lea *et al*., 2007). Both nitrate and phosphate deficiency promoted the expression of several genes concerned with flavonoid and, particularly anthocyanin biosynthesis (Schieble *et al*., 2004; Jiang *et al*., 2007). Furthermore, nitrate deficiency stimulated expression of the regulatory Myb factors PAP1 and PAP2 (Schieble *et al*., 2004; Lea *et al*., 2007), although the increase in *PAP2* transcripts was much stronger. An increase was also observed in transcripts of the bHLH factor GL3 (Lea *et al*., 2007).

Abiotic stresses

It is well known that plants exposed to abiotic stresses, such as cold temperatures and drought, often acquire red/purple pigmentation in the leaves due

to anthocyanin accumulation. Several studies have shown that specific stresses stimulate expression of flavonoid biosynthesis genes leading to flavonoid accumulation. In maize, anthocyanin accumulation in response to low temperature was correlated with increases in expression of genes encoding the general phenylpropanoid pathway enzyme phenylalanine ammonialyase (PAL) and CHS (Christie *et al*., 1994). Similarly, low temperature stimulates *PAL* and *CHS* transcript levels in Arabidopsis (Leyva *et al*., 1995). In this case the response was shown to be dependent on light, indicating that light, either through photosynthesis or a specific photoreceptor, interacts with the low temperature signalling pathway to regulate expression. A recent transcriptome analysis indicates that a range of flavonoid biosynthesis genes increase in expression in response to low temperature in Arabidopsis and these were among several classes of transcripts whose expression correlated with freezing tolerance (Hannah *et al*., 2006). *PAP2* expression was stimulated during cold acclimation and may be responsible for regulating the expression of at least some flavonoid biosynthesis genes in response to low temperature (Hannah *et al*., 2006).

One of the most important regulatory factors in response to abiotic stress is the production of ROS, such as superoxide radicals and hydrogen peroxide. ROS accumulate in many stress responses and can damage and even cause death to cells. However, ROS also act as signalling molecules regulating gene expression responses that help to protect cells against abiotic stresses (Mittler, 2002; Apel and Hirt, 2004). Vanderauwera *et al*. (2005) examined the transcriptome of Arabidopsis plants deficient in peroxisomal catalase, which is an important enzyme in removal of cellular hydrogen peroxide. The control and catalase-deficient plants were subjected to high light, which can cause photooxidative stress. This treatment caused anthocyanin accumulation in wild-type plants and the rapid, coordinated induction of all the flavonoid biosynthesis genes. It is possible that the antioxidant activity of the flavonoids helps to reduce the level of oxidative stress. In contrast, catalase-deficient plants lacked the strong induction of flavonoid biosynthesis genes, indicating that hydrogen peroxide has a negative effect on the induction of flavonoid biosynthesis genes. Expression of the Mybs PAP1 and PAP2 was stimulated by high light and diminished in the catalase-deficient plants, consistent with the proposed regulatory role of these transcription factors. The mechanism through which ROS regulates expression of these proteins is not understood.

Chemical treatments

Various chemicals, in particular a variety of pesticides and herbicides, are often used in the production of food plants. These have the potential to influence plant metabolism and hence may affect flavonoid content (Daniel *et al*., 1999). Different chemicals have different sites of action and so do not have equivalent effects on metabolism. For instance, triazine and phenylurea herbicides inhibit photosynthetic activity and hence will decrease the amount of

carbon available for flavonoid biosynthesis. Glyphosate inhibits EPSP synthase, a key enzyme in the shikimate pathway, and thereby reduces the biosynthesis of phenylalanine that feeds into the general phenylpropanoid pathway. In consequence, flavonoid biosynthesis is reduced. In contrast, some other herbicides actually stimulate flavonoid production. Compounds that generate ROS production, such as methyl viologen (paraquat) and acifluorfen, increase the synthesis of PAL, the first enzyme in the general phenylpropanoid pathway, leading to flavonoid biosynthesis (Kömives and Casida, 1996; Daniel *et al*., 1999). Similarly, the diphenylether herbicide lactofen causes ROS production in soybean, leading to increased *CHS*, *CHR* and *IFS* expression and isoflavonoid accumulation (Graham, 2005).

Pathogens

The relationship between flavonoid production and plant–pathogen interactions is complex. Several phenolic compounds derived from the general phenylpropanoid pathway and the flavonoid biosynthesis pathway act as phytoalexins, i.e. have antipathogenic activity and it is well established that pathogen infection or treatment of plants with pathogen-derived elicitors induces expression of genes of these pathways (Lamb *et al*., 1989; Dixon and Paiva, 1995; Dixon, 2001). In contrast, in some species pathogen attack actually impairs the production of flavonoids. In parsley treatment with a fungal elicitor represses the induction of *CHS* expression in response to UV-B (Lozoya *et al*., 1991).

Is the Method of Crop Production Likely to Affect Flavonoid Content?

There is increasing interest in the use of organic methods to produce crops. Since flavonoids are important nutritional components of food crops it is appropriate to consider whether an organic as opposed to conventional mode of crop production would be likely to influence flavonoid content. The first point to emphasize is that species differ in many respects, so it is extremely difficult to generalize. Nevertheless, many of the effects of environmental factors on flavonoid biosynthesis discussed above are seen in a range of species and so a degree of commonality can be expected in responses to major factors such as light, nutrient deprivation and response to abiotic stresses. Having said that, there is genetic variation between genotypes within the same species with regard to the regulation of flavonoid biosynthesis, as illustrated by the different effects of low temperature on flavonoid accumulation in Arabidopsis accessions (Hannah *et al*., 2006), so caution must be exercised.

As discussed above, research has demonstrated that deprivation of soil nitrogen and phosphate promotes flavonoid accumulation. Although organic and conventional farming differ with respect to the method of supply of

these nutrients, neither is likely to result in nutrient starvation to the extent that it would substantially elevate flavonoid content. The methods of farming also differ with respect to the use of chemical treatments but, as noted above, these do not have consistent effects on flavonoids. In contrast, the major environmental effects on flavonoid content are differences in light quantity and spectral quality and abiotic stresses, which are not associated with organic or non-organic methods. Hence, the method of crop production would not be expected, in general, to have a consistent, predictable effect on flavonoid content. Moreover, any studies undertaken to compare the effect of organic versus conventional production on flavonoid accumulation would have to very carefully ensure that variations in other environmental factors did not influence the results.

Concluding Remarks

It is evident that flavonoid biosynthesis is regulated by a range of endogenous and environmental factors. The principal point of regulation is the level of expression of the biosynthetic enzymes, and this in turn is controlled by specific transcription factor proteins. These proteins regulate specific sets of genes and often are tissue-specifically expressed. Moreover, several key transcription factors are regulated in response to endogenous and environmental stimuli. Thus, understanding how the transcription factors themselves are regulated and how they target specific biosynthetic genes is key to understanding the control of flavonoid accumulation. Studies with model plants such as Arabidopsis, maize and Petunia have provided extensive insights into the identity of transcription factors controlling flavonoid biosynthesis and their regulation at the transcriptional level. Nevertheless, even in these extensively studied species a comprehensive knowledge of the relevant transcription factors is still lacking. Furthermore, relatively little information is available on the nature and regulation of these proteins in important crop species, apart from maize, and this is an important direction for future research.

It is clear that environmental regulation of genes of the pathway has a major control over flavonoid biosynthesis. Some studies have identified complex interactions between stimuli regulating genes of the pathway (e.g. Wade *et al*., 2001), but more extensive research is needed to reveal the full extent of interactions in the signal transduction networks regulating expression of the genes. In addition, most studies have focused on relatively few genes, often *CHS* and *DFR*. Only recently, largely through the use of transcriptome analyses has the full spectrum of genes of the pathway received attention. This research has been important in revealing correlations in expression of the biosynthetic genes with transcription factors such as PAP1 and PAP2, thereby highlighting the regulatory role of these factors in responses to various stimuli (Harmer *et al*., 2000; Schieble *et al*., 2004; Teng *et al*., 2005; Vanderauwera *et al*., 2005; Hannah *et al*., 2006; Lea *et al*., 2007; Cominelli *et al*., 2008). Hence, the application

of transcriptome analyses to a wider range of species should help to identify further key regulators of the pathway.

Numerous studies have highlighted the beneficial effects of various flavonoids in human and animal nutrition, although the basis of these effects is not fully understood. It is therefore important to try to maximize the flavonoid content of plants harvested for foods and beverages. To this end, it is important to understand the mechanisms regulating flavonoid biosynthesis and to apply the knowledge gained to the methods of crop production and the breeding of new varieties. Methods can be used to promote flavonoid biosynthesis, such as UV-B supplementation of leafy crops grown under glass, but the key determinant of the capacity for flavonoid production is genetic. For instance, some genotypes of crops such as lettuce, grapes and apples express much higher levels of anthocyanins in the leaves or fruits than others, simply because they express higher levels of the biosynthetic enzymes via regulatory transcription factors. Knowledge of the relevant transcription factors will be useful in producing new varieties with high flavonoid content.

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12 Nitrates in the Human Diet

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Introduction

There has been considerable controversy concerning the possible harmful effects of nitrate in our diet and in the environment. Nitrate has, in general, been considered harmful, although the evidence for this has become less convincing with time. Recently, since the discovery of nitric oxide (NO) as an important biological molecule which regulates many bodily functions and provides host defence against numerous microorganisms, the image of nitrate has, to some extent, been rehabilitated. This is because of the discovery of a novel biochemical pathway which can lead to the formation of large, possibly protective, amounts of NO in mammals from the sequential reduction of inorganic nitrate. The purpose of this chapter is to consider the possible harmful and beneficial properties of inorganic nitrates which are an essential component of biological systems and which are encountered in large amounts in our diet every day.

For centuries inorganic nitrates (NO_3^-) have been added as a food preservative, especially to pig meat, to make ham and bacon. As well as its beneficial effect to limit the growth of serious pathogens such as *Clostridium botulinum* (Reddy *et al*., 1983), nitrate, or more specifically its reduction product nitrite (NO_2^-), has also the dubious benefit of rendering muscle tissue a bright pink colour, by the formation of nitrosomyoglobin. It has subsequently become clear that nitrate is generally non-reactive with organic molecules and has to be chemically or enzymatically reduced to nitrite to be effective as an antimicrobial agent (Binkerd and Kolari, 1975). Nitrate is also found in large quantities in green, leafy vegetables such as lettuce and spinach, particularly when grown under low light conditions (see below and Table 12.1) as well as some root vegetables such as beetroot.

Despite its long use, there have been considerable concerns about the use of nitrate and nitrite as a preservative in food and about the content of these

Table 12.1. Contribution (%) of various foodstuffs to dietary intake of nitrate and nitrite. (After Committee on Nitrite and Alternative Curing Agents in Food, 1981.)

Food	Nitrate	Nitrite
Cured meats	1.6	39
Fresh meats	0.8	7.7
Vegetables	87	16
Fruit/juices	6	1.3
Baked foods/cereals	1.6	34
Milk/milk products	0.2	1.3
Water	2.6	1.3

ions in vegetables. In both Europe and the USA, there are strict limits which prohibit in excess of 50 mg/l nitrate in drinking water. There are two reasons for this. First, the theoretical possibility of forming carcinogenic *N*-nitroso compounds in food to which these ions are added. These *N*-nitroso compounds could also theoretically be formed in humans *in vivo*, due to nitrosation of secondary amines also present in the diet or ingested as drug therapy (Tannenbaum *et al*., 1974). Nitrosation of amines and other chemicals will occur rapidly under acidic conditions (such as in the human stomach) when nitrite is present, due to the formation of nitrous acid (Williams, 1988). It will also occur in the stomach under more neutral pH when there are bacteria present. The mechanism of this presumably enzymatic nitrosation is not understood.

Nitrous acid is an effective nitrosating agent due to its ability to donate an NO^+ group. These nitrosation reactions are catalysed by halide ions (such as chloride) and thiocyanate due to the formation of nitrosohalide (such as NOCl) and nitrosothiocyanate (NOSCN), respectively, and these intermediates will more effectively donate NO^+ groups. Both chloride and thiocyanate ions are present in gastric juice in high concentrations; the latter is derived from the diet (particularly brassicas such as cabbage) and is concentrated in saliva. Nitrate in the diet is similarly concentrated in saliva following absorption and reduced to nitrite in the mouth. It therefore seems that the stomach is an ideal reaction chamber for the nitrosation of susceptible swallowed chemicals.

This scheme of human metabolism of nitrate and nitrite seems to be counterproductive for human health and has prompted a reconsideration of exactly how these ions are handled following ingestion. We have formulated a scheme whereby the formation of nitrogen oxides in the stomach and on the skin surface, derived from dietary nitrate and nitrite, may protect against bacterial, and possibly viral, pathogens. More recent studies suggest that this mechanism may also protect against stomach ulcers and lower high blood pressure.

A second potential mechanism for nitrite toxicity is the formation of methaemoglobin due to oxidation of the iron in haemoglobin. This is due to the reaction of nitrite with oxyhaemoglobin, where it is oxidized to form

nitrate. It should also be noted that NO also reacts quickly with oxyhaemoglobin to produce methaemoglobin and nitrate. This may be more important than nitrite in causing this problem in infants fed on well water.

Methaemoglobinaemia is generally only a problem in young infants, but has been the main reason for statutory limitations on the concentrations of nitrate in drinking water. For nitrate to cause methaemoglobinaemia, it has to be reduced to nitrite in food, in water or in the body. The mechanisms by which this may occur will be considered in the next section.

Nitrate Metabolism by Plants and Bacteria

In nature, nitrogen is continually cycled between nitrogen gas, and the fully oxidized nitrogen molecule, nitrate and the fully reduced nitrogen molecule, ammonia. Plants and bacteria have somewhat different uses for nitrogen molecules. In general, plants need nitrogen as ammonia as a precursor to protein synthesis. They have the appropriate enzymes to reduce nitrate through to ammonia, a process requiring energy provided by photosynthesis. This occurs in at least two distinct steps catalysed by separate enzymes, nitrate reduction to nitrite (nitrate reductase) and then nitrite reduction to ammonia (nitrite reductase). Nitrate is present in the soil due to inorganic fertilizer application, organic decomposition or the fixing of atmospheric nitrogen by nitrogen-fixing bacteria which may be associated with roots of certain leguminous plants. Nitrate is commonly stored in the leaves of vegetables such as lettuce and spinach, and accumulates to very high concentrations under low light conditions. This is presumably because of a reduction in the flux of nitrate through to ammonia when insufficient energy is available from photosynthesis. As a result, especially in cool climates, green vegetables contribute to our nitrate intake more than any other single source (Cantliffe, 1972). The high concentrations stored in certain plant roots such as beetroot may serve a different function, where the nitrate ion is acting as an osmotic regulator.

Although some microorganisms will also reduce nitrate to ammonia for protein synthesis, many also use nitrate reduction for the purpose of anaerobic respiration. The enterobacteriacae such as *Escherichia coli* can switch from using oxygen to burn available fuel to using nitrate as an electron acceptor (or oxidant), which is then converted to nitrite. The facultative anaerobes which inhabit the human mouth allow little further reduction of nitrite, presumably because they lack the nitrite reductase enzyme.

Nitrate and Nitrite Metabolism in Man

It was first shown in 1916 that humans excrete more nitrate than they consume (Mitchell *et al*., 1916). This was confirmed in careful studies by Tannenbaum's group in the 1970s and 1980s (Green *et al*., 1981a,b) and is now known to be due, at least in part, to the formation of NO by mammalian cells from the amino acid L-arginine.

Nitric oxide synthesis via NO synthase

Following the demonstration by Furchgott and Zawadzki (1980) that an intact vascular endothelium was necessary for blood vessels to relax when exposed to the neurotransmitter acetylcholine, it was clear that endothelial cells were able to synthesize a short-lived vasodilator substance. It took 7 years to identify this substance which turned out to be the simple molecule NO (Palmer *et al*., 1987). It is now known that there are three distinct NO synthase enzymes. Two of these, the endothelial isoform and the neuronal isoform, continually synthesize NO, whereas the inducible isoform produces this molecule from arginine only when the appropriate cell is exposed to bacterial cell wall products or pro-inflammatory cytokines such as interferon gamma and tumour necrosis factor alpha.

The function of the endothelial isoform is to provide continual vasodilatation. Inhibition of NO synthesis with arginine analogues such as methylarginine causes high blood pressure in animals and man, and intriguingly, it has recently been shown that NO synthesis is impaired in patients with hypertension (Forte *et al*., 1997). Although many central (brain) neurones contain the neuronal form of NO synthase, the precise function of NO in the central nervous function is as yet unclear. It is likely that these two isoforms contribute to the majority of NO synthesis which is rapidly converted to nitrate when this molecule encounters oxidized haemoglobin or superoxide.

Inducible NO synthase is easily demonstrated within a few hours in mouse macrophages exposed to bacterial lipopolysaccharide (LPS). Indeed if rodents are exposed to LPS or made septicaemic there is a large rise in plasma and urinary nitrate concentration. It has been much more difficult to demonstrate synthesis of NO in human cells exposed to LPS, although it is clear that overwhelming infection will increase nitrate synthesis in man (Neilly *et al*., 1995). Merely injecting killed bacteria, as in a vaccine, is not effective in enhancing NO synthesis (Macallan *et al*., 1997). The one infection which will cause a large increase in NO synthesis is gastroenteritis. It is not yet known that this is due to induction of NO synthase. The large rise in plasma nitrate in this condition may, however, be effective in preventing the recirculation of pathogens through the stomach by mechanisms discussed below.

Nitrate and Nitrite in the Diet

Nitrate

Because of the potential for toxicity, there have been many studies in the last 20 years which have studied the effect of nitrate in the human diet and estimated the intake of this ion in different populations. For those people who eat a large amount of vegetables, the main source of nitrate will be the green leaves of plants such as lettuce and spinach. Significant amounts are also found in root vegetables such as beetroot and carrots. For those who eat few vegetables, the nitrate concentration of tap water becomes an important fac-

tor determining nitrate intake. The concentration of nitrate in drinking water has been limited to 50 mg/l in Europe, mainly because of concern about methaemoglobinaemia in infants (although this is now extremely rare). As it is now known that nitrite is the effective antimicrobial product of nitrate, it is less common that nitrate itself is used as a preservative for meat products such as sausage and ham, and these foods generally have little nitrate content, although nitrate is available from pharmacies to use as a meat preservative.

Nitrite

The average intake of nitrite is considerably less than that of nitrate. The main source is again vegetables which will convert nitrate to nitrite on storage and when contaminated by nitrate-reducing bacteria. Occasionally this can result in very high concentrations of nitrite in vegetable juices in particular. Nitrite is also a component of preserved meats such as sausage and ham. However, much of this nitrite is chemically altered following addition to food (Table 12.1). The amount of nitrite ingested from food, however, is only a fraction of that swallowed as a result of nitrate reduction in the mouth. The average concentration of nitrite in saliva is around 200 μM. Given that humans swallow approximately 500 ml saliva each hour, each person must ingest approximately 2.4 mmol of nitrite per day from this mechanism. For this reason, limitations on the nitrite content of food seem inappropriate.

Metabolism of Nitrate in Humans

It was found in the mid-1970s that this anion was handled in a peculiar way in the human body (Spiegelhalder *et al*., 1976; Tannenbaum *et al*., 1976). When swallowed it is rapidly absorbed and at least 25% is concentrated in the salivary glands by an as yet uncharacterized mechanism, so that the nitrate concentration of saliva is at least ten times that found in plasma. The nitrate is then rapidly reduced to nitrite in the mouth by mechanisms which will be discussed below. Saliva containing large amounts of nitrite will be acidified in the normal stomach to produce nitrous acid which could potentially nitrosate amines to form *N*-nitrosamines, which experimentally are powerful carcinogens (Crampton, 1980). From this theoretical understanding of nitrate metabolism a number of studies have been performed which looked at the relationship between nitrate intake and cancer (particularly gastric cancer) in humans. In general it was found that there was either no relationship or an inverse relationship, so that those individuals who had a high nitrate intake had a lower rate of cancer (Forman *et al*., 1985; Al-Dabbagh *et al*., 1986; Knight *et al*., 1990; Vittozzi, 1992). Similarly, in animal studies, it has been generally impossible to demonstrate an increased risk of cancer (or any other adverse effect) when nitrate intake is increased (Committee on Nitrite and Alternative Curing Agents in Food, 1981).

It is now thought that endogenous nitrate synthesis derives from constitutive NO synthetase (NOS) enzymes acting on L-arginine (Hibbs *et al*., 1992). The NO formed is rapidly oxidized to nitrate when it encounters superoxide or oxidized haemoglobin. It is still not clear whether all endogenous nitrate synthesis derives from this route as, following prolonged infusion of ^{15}N-labelled arginine, the enrichment of urinary nitrate with this heavy isotope is only about one-half of the steady state of ^{15}N arginine enrichment (Li *et al*., 1997). This may mean that nitrate also derives from another source, or that the intracellular enrichment of labelled arginine is less than that in the plasma due to transamination reactions. This means that even on a nitrate-free diet, there are considerable concentrations of nitrate in plasma (around 30 μM) and in the urine (around 800 μmol per 24 h). Although it is not protein bound, nitrate has a long half-life of 5–8 h (Wagner *et al*., 1983), which seems to be because it is about 80% reabsorbed from the renal tubule by an active transport mechanism (Kahn *et al*., 1975).

This peculiar metabolism of nitrate, i.e. renal salvage, salivary concentration and conversion to nitrite in the mouth, made us consider that this may be a purposeful mechanism to provide oxides of nitrogen in the mouth and stomach to provide host defence against swallowed pathogens (Benjamin *et al*., 1994). The first studies we performed were to investigate the mechanism of nitrate reduction to nitrite in the mouth.

Oral nitrate reduction

Although Tannenbaum *et al*. (1976) had considered that salivary bacteria may be reducing nitrate to nitrite, Sasaki and Matano (1979) showed that in humans this activity is present almost entirely on the surface of the tongue. They considered that the nitrate reductase enzyme was most likely to be a mammalian nitrate reductase. Using a rat tongue preparation, we also found that the dorsal surface of the tongue in this animal had very high nitrate reductase activity, which was confined to the posterior two-thirds (Duncan *et al*., 1995). Microscopic analysis of the tongue surface revealed a dense population of gram-negative and gram-positive bacteria, 80% of which, *in vitro*, showed marked nitrate-reducing activity.

Our suspicion that the nitrate reduction was being accomplished by bacteria was strengthened by the observation that the tongues of rats bred in a germ-free environment, which had no colonization of bacteria, demonstrated no nitrate-reducing activity on the tongue. Furthermore, treatment of healthy volunteers with the broad spectrum antibiotic amoxycillin results in reduced salivary nitrite concentrations (Dougall *et al*., 1995). Although we have not been able to characterize the organisms in normal human tongues (this would require a deep biopsy as the majority of the bacteria are at the bottom of the papillary clefts of the tongue surface), the most commonly found nitrite-producing organisms in the rat were *Staphylococcus sciuri*, followed by *S intermedius*, *Pasteurella* spp. and finally *Streptococcus* spp. (Li *et al*., 1997). Both morphometric quantification of bacteria on tongue sections and

enumeration of culturable bacteria showed an increase in the density of bacteria towards the posterior tongue.

We now believe that these organisms are true symbionts, and that the mammalian host actively encourages the growth of nitrite-forming organisms on the surface of the tongue. The bacteria are facultative anaerobes which, under hypoxic conditions, use nitrate instead of oxygen as an electron acceptor for oxidation of carbon compounds to derive energy. For the bacteria, nitrite is an undesirable waste product of this process, but is, we believe, used by the mammalian host for its antimicrobial potential elsewhere.

Acidification of nitrite and production of NO in the mouth and stomach

Nitrite formed on the tongue surface can be acidified in two ways. It can be swallowed into the acidic stomach, or it may encounter the acid environment around the teeth provided by organisms such as *Lactobacillus* or *Streptococcus mutans* which are thought to be important in caries production.

Acidification of nitrite produces nitrous acid (HNO_2) which has an acid dissociation constant of 3.2, so that in the normal fasting stomach (pH 1–2) complete conversion will occur. Nitrous acid is unstable and will spontaneously decompose to NO and nitrogen dioxide (NO_2). Under reducing conditions more NO will be formed than NO_2. Lundberg *et al*. (1994) were the first to show that there was a very high concentration of NO in gas expelled from the stomach in healthy volunteers, which increased when nitrate intake was increased and reduced when stomach acidification was impaired with the proton pump inhibitor omeprazole. We have conducted further studies on the amount of NO produced following ingestion of inorganic nitrate, measured more directly using nasogastric intubation of healthy human volunteers. Following ingestion of 1 mmol of inorganic nitrate, the amount of nitrate found in a large helping of lettuce, there follows a pronounced increase in stomach headspace gas NO which peaks at about 1 h and continues to be elevated above the control for at least 6 h (McKnight *et al*., 1997). The concentration of NO measured in the headspace gas of the stomach in these experiments would be lethal after about 20 min if breathed continuously.

The concentration of NO in the stomach is much higher than would be expected from the concentration of nitrite in saliva and the measured pH in the gastric lumen. *In vitro* studies suggest that these concentrations of nitrite and acid would generate about one-tenth of the NO that is actually measured (G.M. McKnight, L.M. Smith and N. Benjamin, 2008, Plymouth, unpublished data). It is likely that a reducing substance such as ascorbic acid (Sobala *et al*., 1989, 1991; Schorah *et al*., 1991), which is actively secreted into the stomach, or reduced thiols (which are in high concentrations in the gastric mucosa) are responsible for the enhanced NO production. We were surprised to find that NO is also generated in the oral cavity from salivary nitrite (Duncan *et al*., 1995) as saliva is generally neutral or slightly alkaline.

The most likely mechanism for this production is acidification at the gingival margins as noted above. It will be important to determine if this is the

case as the NO formed in this way may be able to inhibit the growth of organisms which generate acid. Such a mechanism for local NO synthesis from nitrite may in part explain the importance of saliva in protection from caries. As in the stomach, acidification of saliva results in larger amounts of NO production than would be expected from the concentration of nitrite present.

Nitric oxide synthesis from the skin

Generation of NO from normal human skin can also readily be detected using a simple apparatus such as a glass jar sealed around the hand, with NO-free gas passed through it to a chemiluminescence detector (Weller *et al*., 1996). As NO has the ability to diffuse readily across membranes, we first considered it most likely that we were measuring NO which had escaped from vascular endothelium to the skin surface, manufactured by constitutive NOS. However, when the NOS antagonist monomethyl arginine was infused into the brachial artery of healthy volunteers in amounts sufficient to maximally reduce forearm blood flow, we found that the release of NO from the hand was not affected. Furthermore, application of inorganic nitrite substantially elevated skin NO synthesis. This coupled with the observations that NO release was enhanced by acidity, and reduced by antibiotic therapy makes it likely that again NO is being formed by nitrite reduction. Normal human sweat contains nitrite at a concentration of about 5 μM, and this concentration is precisely in line with that which we would predict would be necessary to generate the amount of NO release we observed from the skin. The source of nitrite is not clear, but is likely to be from bacterial reduction of sweat nitrate by skin commensal organisms which are known to elaborate the nitrate reductase enzyme.

This observation has led us to the hypothesis that skin NO synthesis may also be designed as a host defence mechanism to protect against pathogenic skin infections, especially against fungi. The release of NO is inevitably increased following licking of the skin (due to the large amount of nitrite in saliva), which may explain why animals and humans have an instinctive urge to lick their wounds (Benjamin *et al*., 1997).

Importance of Nitrogen Oxides in Host Defence

Much of the evidence for the importance of the arginine-NO system in host defence comes from the observation that inhibition of NO synthesis using arginine analogues impairs the ability of inflammatory cells (such as macrophages) or whole animals to kill invading pathogens (DeGroote and Fang, 1995). This is particularly the case for intracellular organisms such as *Leishmania* major and *Mycobacterium tuberculosis* (Mannick *et al*., 1994; Stenger *et al*., 1996; MacMicking *et al*., 1997), where there is particular evidence that the ability of the host to synthesize NO may be important in containing latent infections. The ability to synthesize adequate amounts of NO may also be

important in reducing the severity of *Plasmodium falciparum* malaria infections in humans (Anstey *et al*., 1996).

Mechanisms of nitric oxide-mediated microbial killing

This subject has been extensively reviewed recently by Fang (1997). It is clear that many organisms are not killed by NO alone, but require the synthesis of other, more reactive nitrogen oxide species. The reaction of NO with superoxide anion (which is also produced by activated inflammatory cells) to produce peroxynitrite has received most attention (Fang, 1997), i.e.:

$$NO + O_2^- = ONOO^-$$

This reaction is very rapid, indeed more rapid than the reaction of superoxide with the enzyme superoxide dismutase. Peroxynitrite is a very reactive species, which can easily be protonated to form peroxynitrous acid (ONOOH) which may then cleave to produce nitrogen dioxide and hydroxyl.

Antimicrobial activity of acidified nitrite

Following the observation that nitrite is manufactured in the mouth and then acidified in the stomach, we went on to determine the susceptibility of common food pathogens to acidified nitrite solutions. We found that the susceptibility varied as follows: *Yersinia enterocolitica* > *Salmonella enteriditis* > *S. typhymurium* = *Shigella sonnei* ($P < 0.05$). *E. coli* 0157 and *Shig. sonnei* are most resistant to acid; they survive exposure at pH 2.1 for 30 min which kills the other microorganisms. However, *E. coli* 0157 shows inhibition of growth up to pH 4.2 when the other organisms apart from *Y. enterocolitica*, manage to maintain growth unless nitrite is present in the solution. It seems that *E. coli* 0157 manages to survive a relatively acid environment by slowing down growth activity. Its ability to survive this way is undone by the addition of nitrite to the medium.

Helicobacter pylori clearly must be able to tolerate the nitrosative stress found in the stomach, and indeed, this organism is more resistant than some other organisms to the combination of nitrite and acid (MacMicking *et al*., 1997). The reason for this is not evident. It may be that it can withstand the effect of nitrogen oxides as it is protected against acid stress by the generation of ammonia from urea via urease enzyme. Alternatively it may have developed specific biochemical mechanisms for protection. If this is the case, such mechanisms would be an attractive target for eradication of this important pathogen.

Cardiovascular Effects of Nitrate

It has been known for some time that individuals who eat a lot of green, leafy vegetables have lower blood pressure than those who do not (Sacks *et al*.,

1995). It has been widely speculated that a vitamin such as ascorbic acid or vitamin E may be important, but study of the individual vitamins shows no antihypertensive effect. Similarly, antioxidants, which are claimed to confer benefits in humans, generally do not in themselves lower blood pressure. From our knowledge of nitrate metabolism (as described above), we hypothesized that a high nitrate intake could increase circulating nitrite concentrations in humans, which would lower blood pressure and prevent platelet aggregation by acting as a precursor to NO synthesis. Studies recently published by Larsen *et al*. (2006) and from the author's laboratory (Webb *et al*., 2008) convincingly show that a high nitrate intake lowers blood pressure in healthy volunteers, as well as having an 'aspirin-like' effect to inhibit platelets and therefore presumably to protect against coronary artery and brain artery thrombosis (heart attacks and strokes). At present we do not know the optimal dose of nitrate to have this effect, or whether it will be long-lasting, but the magnitude of the effect is impressive, a dose of beetroot juice causing a greater effect on blood pressure in healthy volunteers than conventional antihypertensive agents.

Conclusions

Although nitrate and nitrite have been used for centuries, it has only recently been discovered that nitrate is manufactured in mammals by the oxidation of NO and that the nitrate formed also has the potential for disinfecting the food we eat. Nitrate from this endogenous source as well as nitrate from vegetables is continually recycled in our bodies to help protect against pathogens and keep our blood pressure under control. We do not yet completely understand the mechanisms by which NO and other nitrogen oxides provide selective toxicity towards pathogens, and it is likely that the mechanisms will be different with different organisms. While it is clear that acidified nitrite is produced on mucosal surfaces, and that this combination is effective in killing a variety of human gut and skin pathogens, we have no definite evidence as yet that this mechanism is truly protective in humans exposed to a contaminated environment. The exciting new finding that nitrate in the diet has major beneficial effects on blood pressure will certainly make us re-evaluate what was previously considered a toxic contaminant. This is particularly relevant in the context of organically produced plant-derived foods which are generally considerably lower in nitrate than their conventional counterparts (Woese *et al*., 1997; Worthington, 2001).

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13 Impacts of Environment and Management on Nitrate in Vegetables and Water

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Introduction

Dietary nitrate arises from two main sources, i.e. vegetables and drinking water. The estimated intake of nitrate (NO_3), as well as the relative contribution of these sources, varies according to diet, resulting in regional differences at a global scale (Fig. 13.1). Over the last 40 years, there has been increasingly stringent regulation to reduce the levels of nitrate in both food and water. Legislation is based primarily on the premise that dietary nitrate, when converted by the digestive system to nitrite and *N*-nitrosocompounds, can have detrimental effects on human health, such as an increased risk of gastric cancer and infantile methaemoglobinaemia (Mensinga *et al*., 2003). Direct nitrite intake also occurs mainly arising from the use of nitrite during the curing of meat products; significant intake of nitrite can also occur from potatoes (Walker, 1996).

Recently meta-analysis of epidemiological and nutritional studies has failed to find good evidence of an increased risk of gastric or intestinal cancer in groups with high dietary nitrate intake (L'hirondel and L'hirondel, 2002); the UK government formally accepted that there was no link in 1985 (Acheson, 1985). Gastroenteritis has been shown to be a more important factor than nitrate in the occurrence of infantile methaemoglobinaemia (Hegesh and Shiloah, 1982). Physiological studies have even shown that dietary intake of nitrate may have beneficial impacts (Addiscott and Benjamin, 2004; Chapter 12, this volume). However, increased levels of nitrate in surface waters are a major contributing factor to the degradation of river, lake and estuary habitats as a result of eutrophication (Vitousek *et al*., 1997).

The FAO/WHO (2003) set Acceptable Daily Intake figures for both nitrite and nitrate in its consideration of food additives. However, few studies of nitrite and nitrate intake have been published and no statutory

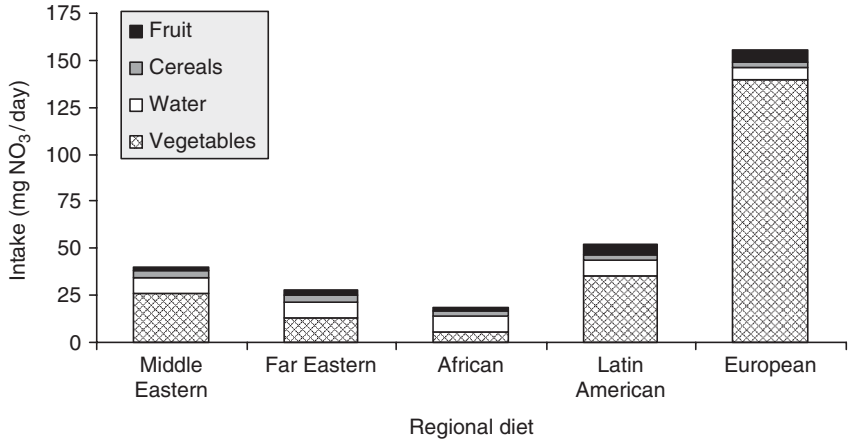

Fig. 13.1. Estimated intakes of nitrate in typical diets showing proportions of nitrate from various food sources (excluding food additives). (Drawn from data given by Santamaria, 2006.)

limits for nitrate contents in vegetables have been set outside the EU (Santamaria, 2006). Maximum permitted levels of contaminants in food, including nitrate, are set across the EU by Commission Regulation (EC) No 466/2001. This regulation set limits for nitrate in fresh lettuce and fresh, frozen or preserved spinach. These limits have been adjusted, or added to, by amendment. For example, EC Regulation 655/2004 added new limits for nitrate at 200 mg NO_3/kg on an as sold basis for baby foods. EC Regulation 563/2002 added a new limit for iceberg lettuce, introduced the optional 'derogation' from the limits and altered the limits for lettuce based on the time of the year the crop was harvested (Table 13.1). Several Member States, including the UK, have applied an optional derogation from the limits for nitrate in lettuce and spinach where it is grown and sold within the individual Member State. A code of Good Agricultural Practice is required to ensure that levels of nitrate in lettuce and spinach are as low as possible. In the UK, the code was formulated by the National Farmers Union in association with the Food Standards Agency and is implemented through a product assurance scheme operated by Assured Produce. EC Regulation 563/2002 also stipulates that all Member States carry out monitoring for nitrate in lettuce and spinach and report the results annually to the European Commission. The European Food Safety Authority is currently undertaking a risk–benefit evaluation of nitrate in food; results are due in early 2008.

No limits have been set for the nitrate content of other vegetables, though a number of countries have guidelines for crops such as celery, beetroot, cabbage, endive, radish and carrot which can show levels greater than 1000 mg NO_3/kg fresh weight (Santamaria, 2006). Guidelines have also been set for potatoes in Germany (<200 mg NO_3/kg fresh weight) and

Table 13.1. Summary of maximum levels of nitrate (mg NO_3/kg) for lettuce and spinach established by European Commission Regulation (EC) No. 563/2002, amending Regulation (EC) No. 466/2001.

Product	Maximum level of nitrate (mg NO_3/kg fresh weight)
Fresh spinach	
Harvested 1 November to 31 March	3000
Harvested 1 April to 31 October	2500
Preserved/frozen spinach	2000
Fresh lettuce (excluding iceberg)	
Harvested 1 October to 31 March	
Grown under cover	4500
Grown in the open air	4000
Harvested 1 April to 30 September	
Grown under cover	3500
Grown in the open air	2500
Iceberg type	
Grown under cover	2500
Grown in the open air	2000

Poland (<183 mg NO_3/kg; Cieslik and Sikora, 1998). Sales contracts for vegetables often contain stringent requirements with regard to nitrate content – for example Santamaria (2006) noted that rocket is not able to be exported from Italy if the nitrate content exceeds 2500–4000 mg NO_3/kg fresh material.

The EC 1980 Drinking Water Directive established a limit of 50 mg NO_3/l in potable waters; the equivalent limit in the USA is 44 mg NO_3/l (USEPA, 1987). In the EU, the Nitrates Directive (91/676/EEC) seeks to reduce the impact of agricultural losses of nitrate on the concentration of nitrate in ground and surface waters. Nitrate Vulnerable Zones have been designated identifying surface waters which are nitrate polluted or could become polluted, groundwaters which are nitrate polluted or could become polluted and waters which are eutrophic or could become eutrophic. Each Member State has implemented Action Programmes in these areas, which require action by farmers to reduce nitrate pollution (Table 13.2). Currently, 55% of England has been designated as a Nitrate Vulnerable Zone (Environment Agency, 2007).

Given this legal framework, which defines maximum levels of nitrate for several vegetable crops and other foodstuffs, I will consider how environment and management variables affect the nitrate levels in plants used as food. I will also briefly review the extensive literature which considers the impacts of agricultural practice on nitrate in surface waters and groundwaters. This review will then consider the extent that changes in farm management practices, particularly the adoption of organic farming systems, might have on dietary intake of nitrate.

Table 13.2. Key requirements of the NVZ Action Programmes in the UK (Environment Agency, 2007).

Record keeping

- Records must be kept annually on the use of all organic and chemical nitrogen (N) fertilizers, on a field-by-field basis
- A fertilizer and manure plan must be prepared and implemented each year

Nitrogen application limits

- Farm and field-based limits for N that can be applied in the form of organic manure
- N from organic and inorganic sources must not exceed the crop or grassland requirement

Closed periods

- For chemical fertilizers closed periods cover the months where there is unlikely to be plant uptake and the risk of nitrate leaching is highest
- For slurry, poultry manure and liquid digested sewage sludge closed periods cover sandy and shallow soils during the times when nitrate leaching is most likely

Other restrictions on nitrogen application

- Restrictions on the spreading of N fertilizer under conditions where there is a risk of runoff
- Restrictions on the spreading of organic manure close to water courses and drinking supplies
- Restrictions on the timing of incorporating vegetable crop residues into the soil, due to their high nitrogen content

Storage of slurry/poultry manure and farmyard manure

- The capacity of storage facilities must be sufficient to hold all the slurry/poultry manure that cannot be applied due to closed periods
- Manure produced in excess of the storage requirement must not be used in a manner that will cause harm to the environment
- Field middens/stores must be sited away from inland and coastal waters, wells, boreholes or similar water supplies

Nitrate Levels in Plants Used as Food

How do nitrate concentrations vary between crops; how does this relate to dietary intake?

Genetic factors affect the uptake and accumulation of nitrate so that it is possible to identify crop species which are more likely to have high nitrate concentrations in the crop parts consumed as food (Fig. 13.2). The highest accumulation of nitrate has been observed in the leafy vegetable known as rocket (>9000 mg NO_3/kg fresh weight; Santamaria *et al.*, 1999), which is

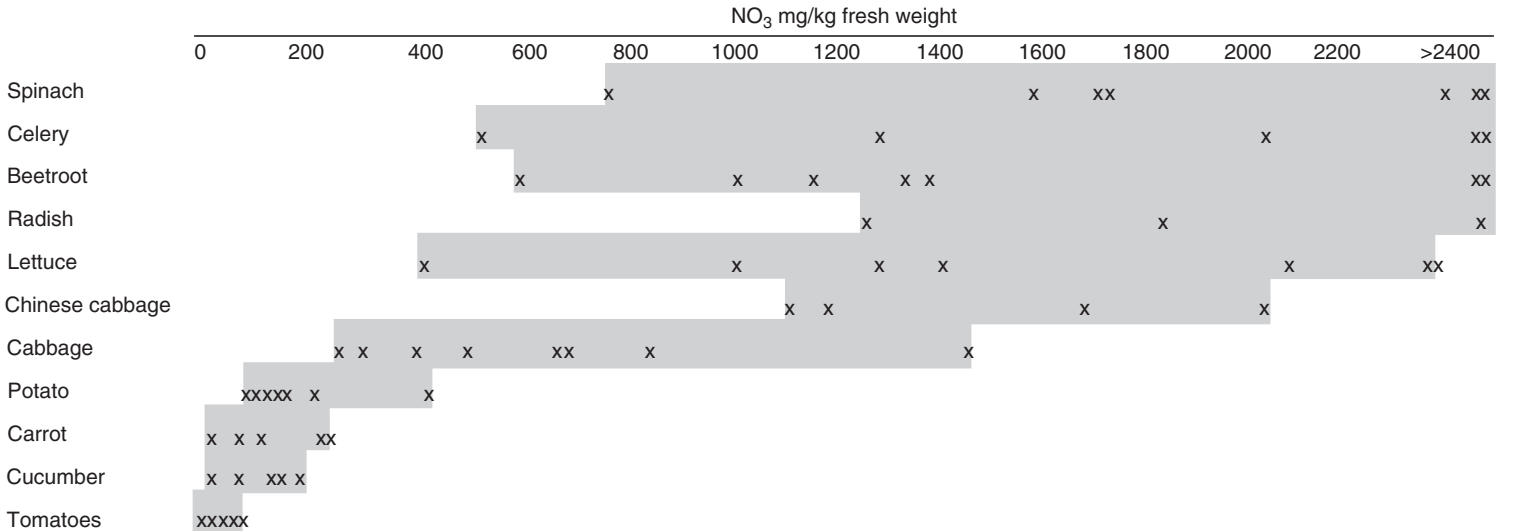

Fig. 13.2. Typical range of nitrate concentration (NO_3 mg/kg) in a number of vegetable crops showing mean values (*x*) obtained in a number of independent studies. (Drawn from data given by Thomson *et al.*, 2007.)

made up of a number of species of the *Brassicaceae* family. The highest concentrations in vegetables are often associated with plants in which the leaf (and stem) is consumed. However, because a higher proportion of the diet is made up by potatoes, this can contribute a significant proportion of the total dietary intake despite medium-low nitrate concentrations (Santamaria, 2006). None the less a wide range of nitrate concentrations is measured even within a species. Roorda van Eysinga (1984) highlighted the potential of using this genetic variation to allow selection of new varieties with low risks of nitrate accumulation. Differences between plants parts in nitrate accumulation can also be seen. In vegetables such as cabbage where the whole head is harvested, it has been shown that the outer leaves (often discarded before consumption) often have nitrate more than twice those of the inner leaves (Greenwood and Hunt, 1986). Parts of crop plants that are above ground, do not transpire and grow by means of phloem-transported material (e.g. seeds, cauliflower curds) often contain no detectable nitrate, even where they are grown with excessively high fertilizer application (Greenwood and Hunt, 1986). In addition to genetic controls, a range of environmental and management factors can affect the level of nitrate accumulating in plant tissues.

Nitrogen uptake and assimilation in plants

Nitrogen (N) is quantitatively the most abundant mineral nutrient in plant tissue and has a range of specific and essential functions in plants. It is a major constituent of proteins and nucleic acids and, in the form of nitrate, can also have an important role in osmoregulation. N is taken up by crop plants from the soil as ammonium (NH_4^+), nitrate or small soluble organic compounds (Fig. 13.3). Within individual cell walls, free space occurs which forms a continuum with the external soil solution and within which ammonium, nitrate and small soluble organic compounds move as a result of diffusion or mass flow (Marschner, 1995). Apparent uptake of N from the soil into the plant can therefore occur without the need for the plasma membrane to be crossed. Within this cell wall continuum, known as the apoplasm, carboxylic groups act as cation exchangers (Marschner, 1995). Consequently, ammonium (and to some extent amino acids) is not able to move freely (Fig. 13.3). Cation binding in the apoplasm can increase the concentration of ammonium in the vicinity of uptake sites in the plasma membrane. In contrast as an anion, nitrate shows little to no adsorption in the apoplasm and can be rapidly transported through the apoplastic pathway (Fig. 13.3). Entry of nitrate into the xylem is likely to be mediated by anion channels (Kohler and Raschke, 2000) and concentrations of nitrate in xylem sap can be high; in many plants much of the nitrate taken up is transported as nitrate to the shoot (Lewis *et al*., 1982).

Small N-containing monomers such as amino acids are usually present at very low concentrations within the soluble organic N pool in soils due to rapid turnover (minutes to hours; Jones *et al*., 2004). Although uptake of amino acids has been measured for wheat (Owen and Jones, 2001), as well as plants more associated with low N environments (e.g. Chapin *et al*., 1993),

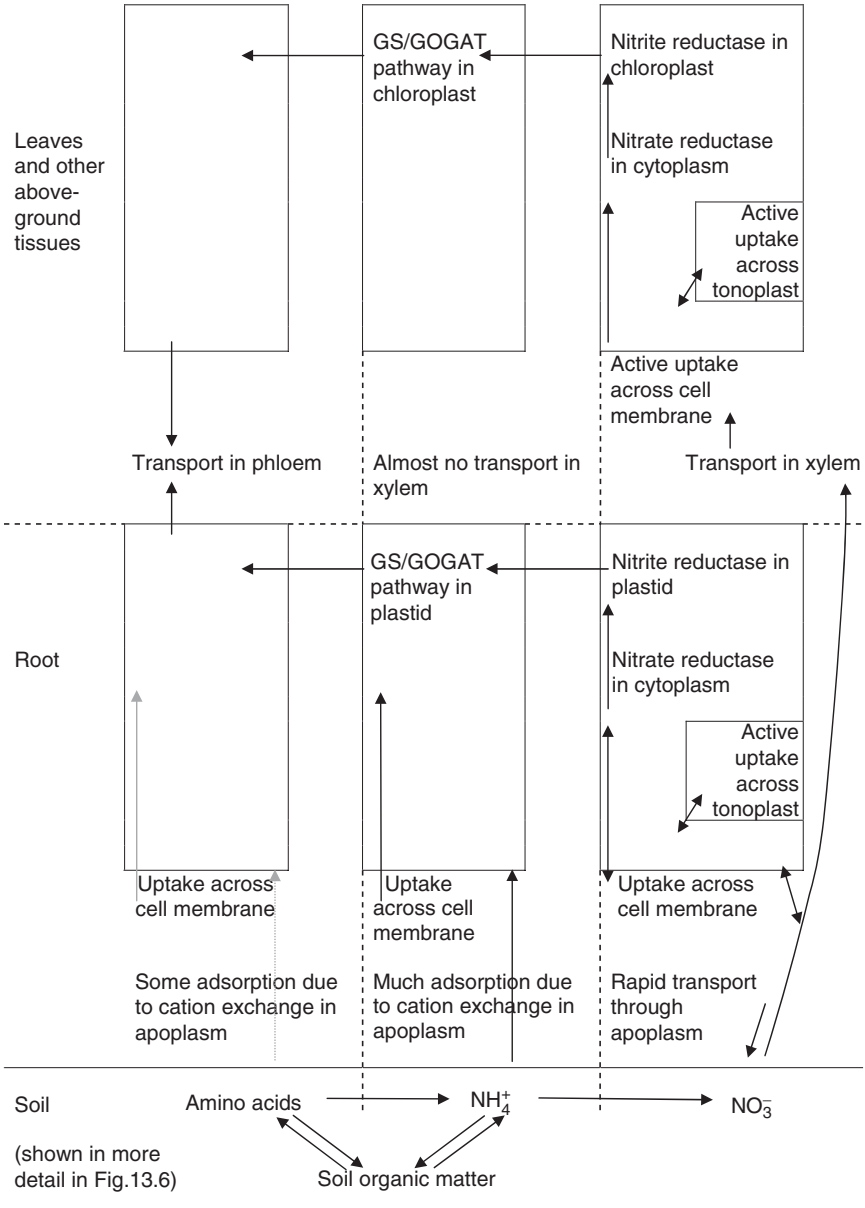

Fig. 13.3. Schematic diagram showing the main pathways of uptake and transformation of ammonium (NH_4^+), nitrate (NO_3^-) and amino acids in crop plants.

the importance of soluble organic N in the soil–crop N cycle is not very well understood (Jones, 1998; Murphy *et al*., 2000). The expression of transporters for amino acids in the root epidermis and cortex (Ortiz-Lopez *et al*., 2000) does not necessarily imply direct uptake from the soil, as amino acids are one of the main buffer, transport and transient storage pools for N within the plant following assimilation (Marschner, 1995). Passive root exudation of

low molecular weight organic molecules (amino acids, sugars, organic acids) also occurs, driven by concentration gradients between the cytosol and soil solution (Farrar *et al*., 2003; Jones *et al*., 2005).

Uptake of ammonium from soil solution across the plasma membrane may occur passively, as a result of electrical potential gradients arising across the membrane when external concentrations of ammonium are around 1 mM (Glass, 2003). However, NH_4^+ uptake requires active transport at lower external concentrations; the source of energy for this flux may result from proton cotransport (Wang *et al*., 1994). The symbiosis of some plants with soil microorganisms (most commonly legumes with rhizobia) allows fixation of dinitrogen gas from the atmosphere. This is reduced by the microbe to NH_4^+ (Marschner, 1995); consequently fixed N is also taken up into plant cells in the form of ammonium. Higher plants possess genes encoding at least 6 high-affinity ammonium transporters, which can be separated into two distinct groups (Miller and Cramer, 2004). The regulation of these transporters is partly controlled by cell glutamine concentrations (Glass *et al*., 2002) and by external ammonium concentrations (von Wirén *et al*., 2000). However, the role of plant ammonium transporters in uptake from the soil and their relative importance compared to passive uptake has not been clearly established (Miller and Cramer, 2004).

Ammonium is toxic to plant cells because of its ability to uncouple respiration at low concentrations (Britto and Kronzucker, 2002); consequently it is rapidly assimilated (Wang *et al*., 1993). Rice is a clear exception to this general rule, and transporters have been identified in rice, which enable NH_4^+ to be stored in the vacuole (Loqué *et al*., 2005). It is debated whether ammonium is transported from roots to above-ground plant parts via xylem, but this has been demonstrated to be possible, if not common (Schjoerring *et al*., 2002). Within plant cells, ammonium is assimilated to glutamine and other amino acids via the glutamine synthetase (GS) glutamate-2-oxoglutarate amino transferase (GOGAT) pathways (Fig. 13.3, Temple *et al*., 1998). GS catalyses the combination of ammonium with carbon molecules to form glutamine; there are a number of other enzyme systems which can also result in ammonium assimilation (Brugière *et al*., 2001), though they are quantitatively much less important. In roots, GS is dominantly found in the cytosol rather than the plastid; the converse is true in leaf and shoot tissue (Miller and Cramer, 2004). GOGAT catalyses the transfer of an amide group from 2-oxoglutarate to glutamine resulting in two molecules of glutamate. There are two types of GOGAT which are distinguished by the use of either NADPH or reduced Fd as the electron donor (Miller and Cramer, 2004). NADH-GOGAT activity is very much lower than that of Fd-GOGAT and is only associated with non-photosynthetic tissues (Ireland and Lea, 1999). In contrast, Fd GOGAT is common in root plastids and in chloroplasts (Miller and Cramer, 2004).

For nitrate transport across the plasma membrane a proton-anion cotransport or symport is needed – the steep electrical potential difference and pH gradient for protons is the driving force (Miller and Smith, 1996). Consequently, active uptake of nitrate through the plasma membrane is balanced by export of protons (two for each nitrate anion taken up; Miller and Smith, 1996). Two

low-affinity nitrate transporters (active at high nitrate concentrations) and two high-affinity nitrate transporters (active at low nitrate concentrations) have been identified in the plasma membrane (Glass, 2003). Some redundancy occurs; nitrate transporters may also transport amino acids and peptides across the membrane (Miller and Zhou, 2000). The plant response is also relatively plastic and plants respond to a change in form of N supplied through expression of over 200 genes likely to result in the up-regulation of alternative transporters very rapidly (Wang *et al*., 2001, 2003). There has been shown to be a close coordination between the expression of nitrate, ammonium and sulfate transporters and the photosynthetic pattern, which may be mediated directly or indirectly as a result of cell sucrose supply (Lejay *et al*., 2003). Glutamine has also been identified as an important regulator (Vidmar *et al*., 2000). Numerous studies have also shown an immediate inhibition of nitrate influx in the presence of high NH_4^+ concentrations; however, the mechanism leading to this effect remains unclear (Glass, 2003). Despite the large energy requirements for nitrate uptake, there is also significant passive efflux of nitrate across the plasma membrane which is proportional to whole tissue nitrate concentrations (Fig. 13.3; Aslam *et al*., 1996).

Within the cell, nitrate may be further taken up across the tonoplast and stored in the vacuole (Fig. 13.3). Different transporters have been found in the tonoplast than the plasma membrane (de Angeli *et al*., 2006). Nitrate stored in the vacuole is by far the most abundant form of nitrate in plants; around 90% of cell nitrate is stored in the vacuole (Miller and Smith, 1996; Chen *et al*., 2004). Nitrate accumulation in the vacuoles can be of considerable importance for maintaining cation–anion balance and for cellular pH stabilization (Marschner, 1995). In some situations the rate of efflux of stored nitrate from the vacuole may become the limiting step for further assimilation (Rufty *et al*., 1982). In some species, nitrate, rather than chloride, is the dominant mineral anion reducing the osmotic potential of vacuolar sap and hence maintaining cell turgor for plant growth (Smirnoff and Stewart, 1985). Nitrate and chloride ions compete during uptake and there are also negative feedback effects on uptake resulting from very high vacuolar concentrations of either ion (Marschner, 1995). When light levels are low, use of nitrate as an osmoticum reduces the energy cost to the plant of maintaining turgor using organic acids and sugars (McCall and Willumsen, 1998). At concentrations greater than 6000 mg NO_3/kg, not uncommon in spinach and rocket, nitrate fully replaces the osmotic functions usually performed by sugars (Marschner, 1995).

Before nitrate can be truly used as an N source by the plant, it must be reduced to ammonia (Fig. 13.3). Reduction of nitrate is a two-stage enzyme-mediated process (Crawford *et al*., 2000). In the cytosol nitrate reductase is active and reduces nitrate to nitrite (Warner and Kleinhofs, 1992); in this form it is taken into the plastid (or chloroplast in leaf cells) where it is further reduced by nitrite reductase to ammonium (Oaks, 1991). The ammonium produced enters the GS/GOGAT pathway as described above; in this case the GS step occurs in the plastid. There is no re-oxidation pathway in plants which can transform organically bound forms of N back to nitrate, though recycling of NH_4^+ released during photorespiration has been shown to occur

(Hirel and Lea, 2001). Both nitrate and nitrite reductase are rapidly induced in the presence of nitrate (Crawford, 1995); induction by the first substrate in the chain reduces the possibility of nitrite, which can be toxic, accumulating and despite the spatial separation of the enzymes, nitrite rarely accumulates in plants (Marschner, 1995).

The nitrate concentration in any individual cell (wherever it lies in the plant) is therefore the result of the balance between rates of uptake, intake into the vacuole and rates of transformation/assimilation as a result of the action of the nitrate reductase enzyme. A range of other factors in addition to nitrate concentrations also affect the activity of nitrate reductase and N assimilation, including the availability of carbon substrates as reductant and to provide skeletons for amino acid synthesis, light/dark conditions, as well as the presence of the end products of assimilation (Miller and Cramer, 2004). Diurnal changes in N assimilation especially in leaves have been studied intensively. Whole leaf tissue nitrate concentrations (presumably in the vacuole) have also been shown to decrease during daylight and then recover overnight (Steingröver *et al*., 1986). This is the result of complex interactions between the expression and activity of a number of nitrate transporters and assimilatory enzymes with nitrate reductase activity found to be at very high levels early in the light period and then decreasing later as the availability of nitrate declines (Stitt *et al*., 2002). The availability of reductants as a result of photosynthesis and phloem transport is also important (Miller and Cramer, 2004).

Nitrate can be reduced in both roots and shoots; Marschner (1995) unhelpfully gives a range of 5–95% for the proportion of nitrate assimilated in the roots. The main site of nitrate reduction in a plant depends on species, developmental stage and environmental factors (Andrews, 1986). In many herbaceous plants nitrate is dominantly transported through the apoplastic pathway and via xylem to the stem before assimilation. Apoplastic transport through root tissues isolates the nitrate from the cell cytoplasm and hence nitrate reductase (Lewis *et al*., 1982). In rice, nitrate is dominantly assimilated in the root; whereas in barley, maize and white lupin the shoot is the dominant site of nitrate assimilation (Miller and Cramer, 2004). Transport of nitrate to shoot/leaves is also highly correlated with transpiration rates; much nitrate is moved through the soil–plant system as a result of mass flow. In general the proportion of nitrate reduced in roots for any given species increases with temperature, plant age and where calcium or sodium is the accompanying cations, rather than potassium (Marschner, 1995). The main site of nitrate reduction has a significant impact on the carbon economy of the plant as reduction and assimilation of nitrate in roots has a high energy demand; Bloom *et al*. (1992) calculated that 15% of the energy from root respiration is directly needed for nitrate reduction. In contrast, for nitrate reduction in leaves, reducing equivalents can be directly provided from the photosynthetic pathway. Developmental stage is also important. Santoro and Magalhaes (1983) have shown a strong relationship between the nitrate reductase activity and rates of leaf expansion (Fig. 13.4). While nitrate might therefore accumulate to some extent due to lower nitrate reductase activity in fully expanded leaves, this nitrate is of limited use in N metabolism for the

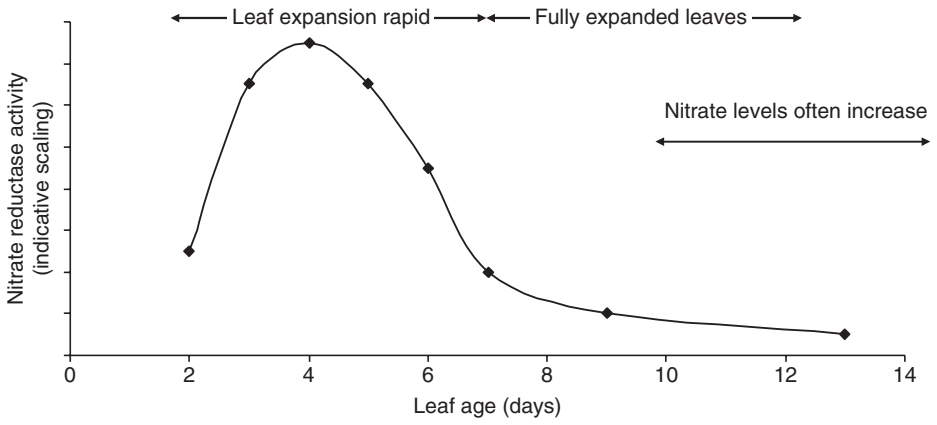

Fig. 13.4. Indicative time course of nitrate reductase activity in relation to leaf expansion. (Adapted from data provided for the first trifoliate leaf of soybean in Santoro and Magalhaes, 1983.)

remainder of the plant due to its low phloem mobility. While nitrate uptake and assimilation may seem to be part of a simple linear chain of dependent reactions, none the less, the nitrate concentration in any part of the plant is the result of complex interactions. This is because nitrate uptake, nitrate assimilation, ammonium assimilation interact with one another and with pH regulation and flows of ions, carbon and other assimilates at both the cell and whole plant level (Stitt *et al*., 2002).

Impacts of environmental and management factors

For some parts of the plant, i.e. storage tissues, fruits, seed, additional internal plant controls operate over their composition (Murray, 1984), hence, there is likely to be less scope for environmentally driven variability than within leaves. Given the physiological understanding of plant N metabolism outlined above, it is clear that both light intensity and N fertilization are likely to have significant effects on the nitrate content of crops. To optimize crop management with regard to yield and other aspects of crop quality, it is also important to understand whether any other factors have a measurable impact on nitrate content. In the field, seasonal variations can be difficult to untangle due to the interactions between the impacts of light and temperature changes on metabolism in leaves and roots and also their impact on the rate of chemical transformations and availability of nutrients in the soil.

Light intensity

Light intensity has been found to be the main factor affecting nitrate concentration (e.g. Blom-Zandra and Lampe, 1985; Steingröver *et al*., 1986). As discussed above, nitrate reductase activity is strongly linked to photosynthetic

rate and hence to light intensity. Irigoyen *et al*. (2006) showed a very strong negative correlation between the accumulated solar radiation in the 10 days preceding harvest and the nitrate content of spinach leaves at harvest across a number of field sites and fertilizer rates. Kage *et al*. (2003) successfully modelled the nitrate fraction of N stored in cauliflower leaves across a range of N application regimes from an empirical relationship based solely on radiation intensity (averaged over the previous 10 days) which had been derived under conditions where N supply was non-limiting (Kage *et al*., 2002). Under controlled conditions, Prioletti *et al*. (2004) showed that under low light conditions, plant have slower growth rates, a smaller leaf area and higher concentrations of oxalate, as well as nitrate in their leaves. Legislation recognizes this interaction between nitrate content and light conditions so that EC Regulation 563/2002 sets a higher target for winter lettuce and spinach in recognition of the fact that where plants are cultivated permanently under low light conditions (e.g. in a heated greenhouse out of season) then nitrate concentrations in leaves are often several times higher than the same cultivars grown under natural light during the summer. Under field conditions or in unheated tunnels, conditions of low light intensity are often accompanied by low temperatures so nitrate availability may be restricted and low nitrate reductase activity counteracted by low rates of nitrate uptake (Gent, 2002) and nitrate accumulation does not occur to the same degree.

As well as allowing cultivation when natural light intensity is low, greenhouse cultivation can also modify the spectrum of light received by the plant, as a result of filtering and the use of supplementary lighting. For example, the radiation received by the plants in greenhouses contains virtually no UV-B (280–320 nm; Gruda, 2005). Kleeman (2004) also showed that when far red wavelengths were filtered from light in the evening excessive elongation was not stimulated and nitrate concentrations in lettuce leaves could be reduced.

Fertilization

AMOUNT OF NITROGEN FERTILIZER Wang and Li (2004) showed significant positive correlations between the amounts of N applied in fertilizer and nitrate content in leaves of spinach and a range of brassica species – indicating that, within the constraints set by species, the rate of N fertilization is a major cause of nitrate accumulation in vegetables grown under the same field conditions. The nitrate concentration of the leaf petiole has been suggested as a rapid indicator of N stress in the field (Barraclough, 1993). Chen *et al*. (2004) showed significant increases in the nitrate reductase activity in leaves when small amounts of N fertilizer were applied to crops; however, at higher rates of N application nitrate reductase activity was not increased further. Fertilization at levels above that required for optimum growth has been shown to lead to significant increases in nitrate content (Greenwood and Hunt, 1986; Irigoyen *et al*., 2006; Fig. 13.5). McCall and Willumsen (1998) highlighted the balance between optimization of yield and nitrate content; achieving low nitrate content in some circumstances might lead to recommending fertilizer rates that

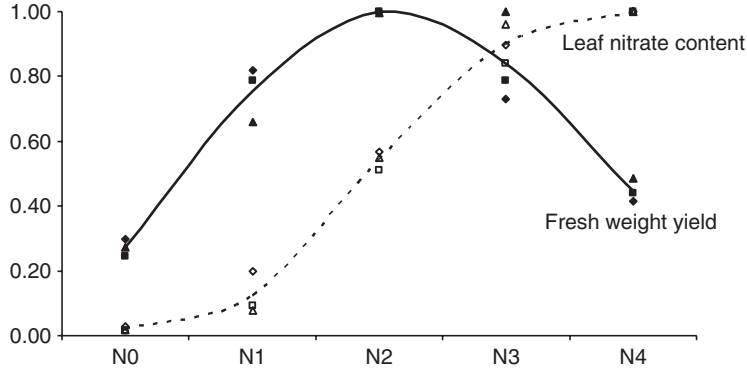

Fig. 13.5. Schematic diagram showing the relative response of yield (solid line and filled symbols) and leaf nitrate content (dotted line and hollow symbols) in leafy vegetables to increasing rates of nitrogen application supplied as potassium nitrate (where N0 = no N fertilizer and N1 to N4 are increasing rates of N supply). (Based on data provided in Chen *et al*. (2004) for crops of rape (▲), Chinese cabbage (♦) and spinach (■).)

will give lower than optimum yield. Greenwood and Hunt (1986) estimated that the use of optimum fertilization roughly doubled nitrate intake in vegetables in the UK compared to no fertilization in low-fertility soils; if over-fertilization was common then nitrate contents in vegetables would further double. If over-fertilization leads to a yield reduction, then the absolute nitrate removal of the crop may be reduced despite increasing nitrate concentrations, this may also lead to an increase in the residual fertilizer left in soil (McCall and Willumsen, 1998; Chen *et al*., 2004).

TYPE OF NITROGEN FERTILIZER Higher plants can use both ammonium and nitrate to meet N demand, as discussed above. When grown under hydroponic conditions, very clear effects of N supply to the crops in the form of NH_4^+ or NO_3 can be seen. Nitrate rarely occurs at detectable levels in the tissues of plants grown solely with ammonium (Lasa *et al*., 2001; Kim *et al*., 2006), but under these growing conditions NH_4^+ can be toxic with different species showing different levels of sensitivity to sole ammonium nutrition (Lasa *et al*., 2001). Kim *et al*. (2006) showed that for rocket a mixture of 1:3 NO_3:NH_4 in the nutrient solution had no significant effect on yield but led to low nitrate contents in leaves compared to high proportions of NO_3 in the nutrient solution; other studies have shown a reduction in yield for cabbage where ammonium comprises more than 50% of the N source (Zhang *et al*., 1997). Similar impacts have been shown in potato tubers grown in a soil-less culture; Serio *et al*. (2004) showed at least a fourfold increase in tuber nitrate contents where NO_3 was supplied in nutrient solutions as well as, or in place of, NH_4^+. Competition between chloride and nitrate was also observed with chloride concentrations in the tubers reduced to negligible amounts where 100% NO_3 was supplied (Serio *et al*., 2004). For hydroponic cultivation the

recommendation is therefore to supply a mixed nitrate:ammonium solution; in practice proportions ranging from 1:1 to 3:1 are common (Wolf, 1998).

In soil, urea and ammonium forms of fertilizer are rapidly transformed to nitrate as a result of bacterially mediated processes (Fig. 13.3); none the less the effects of sole sources of NH_4Cl or urea compared to NH_4NO_3 or $NaNO_3$ fertilizer at the same rate significantly reduced nitrate content in spinach and cabbage (Wang and Li, 2004). Nitrification inhibitors are available which slow the transformation of NH_4^+ to NO_3 in the soil (Amberger, 1989). Use of nitrification inhibitors has been shown to reduce nitrate content without an impact in yield for greenhouse lettuce (McCall and Willumsen, 1998; Montemurro *et al*., 1998), and field-cultivated spinach (Pasda *et al*., 2001). However, with slow growth rates of lettuce at low temperatures, Vaughan (1985) found that the use of nitrification inhibitors could cause problems with NH_4^+ toxicity. Irigoyen *et al*. (2006) showed that nitrification inhibitors could reduce nitrate accumulation in spinach when applied with ammonium sulfate nitrate fertilizer; however, their effectiveness in reducing nitrate content was affected by soil conditions particularly high temperatures and pre-existing nitrate levels.

The use of organic fertilizer is also relatively common in horticultural systems both as a soil conditioner and a source of nutrients. Most organic fertilizers in use in horticultural systems are comprised of composts derived from animal manure, sewage sludge and a range of green wastes. Given the variability and complexity of these materials, it is not surprising that a range of impacts have been observed when comparing organic and mineral fertilization on crop yield and nutrient contents in leaves shown in the controlled trials carried out by, and those reported in, Gent (2002).

TIMING OF NITROGEN FERTILIZATION Under hydroponic conditions Santamaria *et al*. (1998) reported that nitrate concentrations in rocket and chicory were approximately halved when nitrate supply in the nutrient solution was reduced by 75% or completely replaced by NH_4^+ in the week before harvest. In crops where nitrate is a key ion in osmoregulation, such as lettuce and spinach, partial replacement of nitrate application by chloride can also be successful in reducing nitrate concentrations without loss of yield (Blom-Zandra and Lampe, 1985). The most significant reductions in nitrate concentrations have been found where both NH_4^+ and Cl are supplied (Van der Boon *et al*., 1990). Similar interactions have been found in soil-based cultivation in greenhouses (McCall and Willumsen, 1998). However, Roorda van Eysinga (1984) showed little success with complete removal of nitrate from nutrient solutions shortly before harvest; nitrate stored in the vacuole of lettuce was only very slowly released. In the field Feller and Fink (2004) found that nitrate was increased in beetroot at later sowing dates and that higher nitrate contents were measured at lower rates of N application for later sowing dates. Adjustment of type and/or timing of fertilizer close to harvest, and in particular adjustment of fertilizer rates to take account of light conditions, as well as predicted yield, is important for crops at risk of nitrate accumulation (Breimer, 1982), whether crops are grown in the greenhouse or field.

OTHER FACTORS Temperature is a key driver of metabolism and rates of reaction in the plant–soil system. Temperature is therefore a key factor affecting sink strength and consequently photo-assimilate partitioning (Dorais *et al*., 2004). In field studies it can be difficult to separate the influence of solar irradiation from changes in temperature as these are interdependent and hence synchronous (Gent, 2002). None the less, studies have been carried out to study the impact of temperature under controlled conditions. Physiological studies indicate that less nitrate is translocated to the shoot for assimilation as temperatures increase (Marschner, 1995). This is consistent with the observation of Nieuwhof (1994) that the root vegetable, radish, has higher nitrate content at higher temperature. Cantliffe (1972) also measured lower nitrate concentration in leaves of lettuce at lower temperatures when it was grown under a range of controlled conditions.

The observation that outer leaves of cabbage often have nitrate concentrations more than twice those of the inner leaves (Greenwood and Hunt, 1986) may be linked to leaf age. Santoro and Magalhaes (1983; Fig. 13.4) elucidated a mechanism for nitrate accumulation in older leaves as a result of declining nitrate reductase activity and the low phloem mobility of nitrate. Wang and Li (2004) also showed the nitrate concentration in leaves increased with their age – harvesting 50–53 days after sowing crops had much lower concentrations of nitrate than where crops grew on for 65–68 days; in this case this was not linked to the timing of fertilization.

Manipulation of CO_2 levels in greenhouse conditions can have a variety of impacts on the nitrate concentrations in vegetables; CO_2 enrichment commonly stimulates plant growth and affects both the rates of nitrate uptake (by manipulating sink strength and water demand) as well as leading to stimulation of nitrate reductase; the eventual balance is what controls nitrate content (Hucklesby and Blanke, 1990; Cardenas-Navarro *et al*., 1999).

Little impact of other fertilizer nutrients has been recorded on nitrate accumulation. Wang and Li (2004) found no impact of phosphorus fertilizer on nitrate accumulation, though they were working in soils which were not deficient in phosphorus. Marschner (1995) suggests that where potassium is the accompanying cation to nitrate, more nitrate will be translocated to shoot before assimilation. However, no specific impacts of potassium nitrate compared to calcium or sodium nitrate as a fertilizer have been recorded.

Nitrate Levels in Surface Water and Groundwater

How do nitrate concentrations vary in surface waters and groundwaters?

The nitrate concentration in surface water is normally low (0–18 mg NO_3/l) but can reach high levels as a result of agricultural runoff, landfill runoff, or contamination with human or animal wastes (European Commission, 2007). Agriculture is typically responsible for 50–80% of the total nitrate load in the aquatic environment (European Commission, 2007). Nitrate concentrations

in surface waters have gradually increased in many European countries in the last few decades and have sometimes doubled over the past 20 years. In the UK, for example, an average annual increase of 0.7 mg NO_3/l has been observed in some rivers (Young and Morgan-Jones, 1980). In 2000–2003, 4.5% of sampling points in the UK showed nitrate concentration above 50 mg NO_3/l; values above 40 NO_3/l were recorded in 11% of monitoring stations (European Commission, 2007). Nitrate concentrations in rivers fluctuate and show strong seasonal trends, being low in summer and high in winter. Typically nitrate concentrations are greater during times of high runoff after a dry summer. More recently surface waters have been showing some decline or stabilization in nitrate levels (European Commission, 2007); in 2006, 28% of rivers in England had concentrations of nitrate greater than 30 mg NO_3/l (Environment Agency, 2007).

The natural nitrate concentration in groundwater under aerobic conditions is a few milligrams per litre and depends strongly on soil type and on the geological situation; some naturally occurring minerals may release significant amounts of NO_3 into groundwaters. In the USA, naturally occurring levels in groundwater do not exceed 4–9 mg/l for nitrate and 0.3 mg/l for nitrite (USEPA, 1987). Nitrate levels in some groundwaters in the UK have increased since 1980 (Environment Agency, 2007). In the period 2000–2003, 17% of EU monitoring stations (average values) had nitrate concentrations above 50 mg NO_3/l, 7% were in the range 40–50 mg NO_3/l and 15% were in the range 25–40 mg NO_3/l (European Commission, 2007).

Processes regulating nitrate in soil–water system

Nitrate leaching occurs from all agricultural systems both fertilized and legume-based, cropping systems and grazed pastures, across a range of environments (Crews and Peoples, 2005). Nitrate losses from agricultural soils to surface and groundwater are controlled by interacting biological, chemical and physical factors in the soil and the environment. The key pools and processes regulating nitrate transfer can be separated into those which control water movement over, into and through soils and those which control the presence of nitrate in the mobile water (Fig. 13.6). The root of the problem of nitrate leaching from agricultural systems is 'untimely nitrate', i.e. nitrate which is found in the soil solution during periods of no/low crop uptake, which therefore is vulnerable to loss in drainage. Some leaching loss of N seems inevitable, however efficiently N is taken up by the crop, as plant uptake processes have higher threshold temperatures for activity than the processes occurring in the soil which release nitrate (Vos, 1992). In a whole catchment study Kyllman *et al*. (2006) showed that large nitrate loads in the water leaving the catchment were associated with a combination of meteorological and management factors: mild winters, high precipitation, sandy/organic soils, high livestock density and cropping systems with a high proportion of bare ground. The potential losses of nitrate from agricultural soils to ground and surface water are regulated by combinations of environmental

factors and management, which interact with a complex web of soil processes (Fig. 13.6). These interacting controls are complex and still relatively poorly understood.

Snapshot measurements of either soil solution nitrate or nitrate concentrations in mobile water only give a partial prediction of nitrate at risk of loss to water since the bulk of soil N is found in organic forms. In a long-term monitoring scheme the average amount of mineral N measured in topsoil during autumn in the UK was only 76 kg/ha compared to 7000 kg N/ha found in the soil organic matter (Shepherd *et al*., 1996). The balance between the microbial processes of mineralization and immobilization largely controls N released from soil organic matter (Jarvis *et al*., 1996). Mineralization is the process by which ammonium is released by soil microorganisms as they utilize soil organic materials as an energy source, while immobilization of ammonium and nitrate by microorganisms is determined by the demands of protein synthesis. Soil organisms are able to immobilize NO_3, but NH_4^+ immobilization is more energetically favourable (Recous *et al*., 1990). The balance of mineralization and immobilization is strongly related to the properties of the substrate being mineralized and its interaction with the environment (Jarvis *et al*., 1996).

The main rate limiting process controlling the availability and loss of the mobile nitrate ion is often nitrification, the process by which ammonium is converted to nitrate, rather than release of N by mineralization. Nitrification in soils is dominated by the chemoautotrophic oxidation of NH_4^+ to NO_3 via

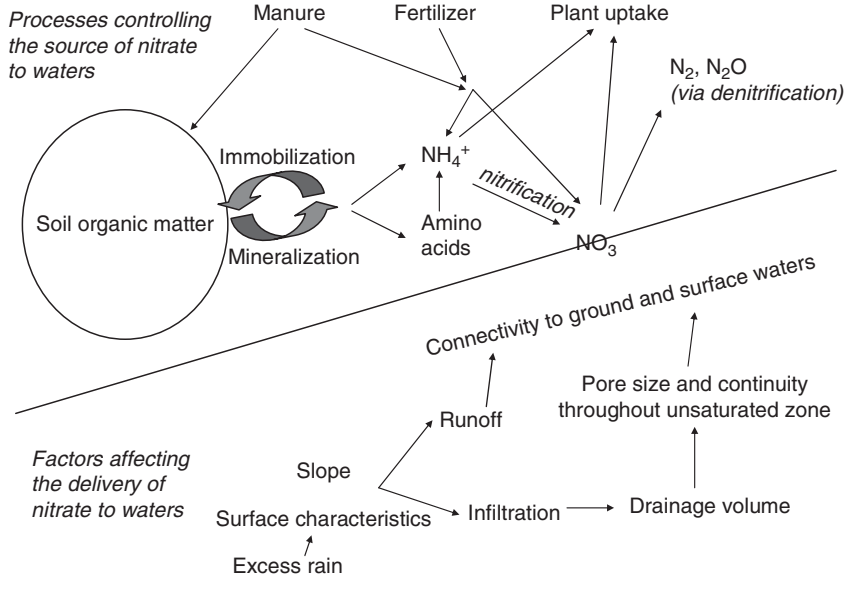

Fig. 13.6. Schematic diagram showing the main pathways of transformation and transport of nitrogen in soils highlighting those controlling nitrate transfer to ground and surface water.

nitrite. This is a two-step process mediated by *Nitrosomonas* and *Nitrobacter*, respectively; rates are usually limited by the availability of NH_4^+ (Booth *et al*., 2005). Heterotrophic nitrification, which releases NO_3 directly from organic N without NH_4^+ as an intermediary, is also known to occur, particularly under acid uncultivated situations (Pennington and Ellis, 1993). In temperate tilled agricultural soils, nitrification rates are usually limited by mineralization (Harmsen and van Schreven, 1955). However, in grassland soils significant quantities of ammonium may accumulate where swards are grazed or farm wastes are applied (Jarvis and Barraclough, 1991). Despite a reasonable knowledge of the ecology of the bacteria involved (Prosser, 1986), nitrification remains a poorly defined process in many soils. While the factors controlling nitrification are known at a microsite scale, more work needs to be done to understand the controlling factors operating at larger scales. The balance between mineralization and nitrification is changed under changing environmental conditions and management (Booth *et al*., 2005). The pool of ammonium released by mineralization is subject to rapid consumption as a result of plant uptake, nitrification (*N*) or the immobilization of N by the microbial biomass (*I*). In the absence of a significant plant sink the relative dominance of the pathways of ammonium consumption, expressed in the ratio *N/I*, which has been linked to the potential to lose N from the system via leaching or denitrification in forest (Tietema and Wessel, 1992), grassland and arable systems (Stockdale *et al*., 2002). The component of available N that could be leached may also depend on combinations of factors: other processes competing for NO_3^- (e.g. denitrification, plant uptake) may, under certain soil conditions, alter the simple relationship of *N/I* with leaching.

Nitrate concentrations in solution may be reduced as a result of denitrification, i.e. the biological reduction of nitrate to nitrous oxide and/or N_2. Denitrification is associated with a number of bacterial genera, which are able to use nitrate or nitrite as the terminal electron acceptor in respiration under anaerobic conditions; there is evidence of high adaptability and ubiquity of bacterial groups with regard to this process (Knowles, 1982). Hotspots of denitrification activity are often associated with pockets of organic matter in soil (Parkin, 1987) as the process dominantly occurs during decomposition where both anaerobic conditions and nitrate occur in soil.

Volumes of drainage control both the total amounts of N leached and the concentrations of nitrate in drainage water (Addiscott *et al*., 1991). Very high rates and amounts of nitrate leaching have been measured in soils with high hydraulic conductivities or artificially drained soils under conditions of high precipitation or with flood irrigation (Crews and Peoples, 2005). A range of soil characteristics is known to control water flow: soil permeability, water storage capacity, texture, depth and slope, to name but a few. These characteristics are variable both spatially and temporally within soils. Water storage capacity may vary annually while soil texture, depth and slope can be considered to be stable properties changing only over decades, unless catastrophic erosion events take place (Halvorson *et al*., 1997). Hydraulic conductivity depends on the size and continuity of the conducting pores and so

varies with soil moisture content reaching a maximum value when the soil is saturated. Measurements under field conditions show that hydraulic conductivity is highly variable both temporally and spatially (Warrick and Nielsen, 1980). The movement of water in soil is reviewed by many textbooks of soil physics (e.g. Hillel, 1980; Hanks, 1992); Elrick and Clothier (1990) give an excellent review of the factors controlling solute transport from the microscopic to field scale.

The factors controlling the transport of nutrients, here nitrate, to the receiving water body are at least as important as the nitrate reservoir in the soil (Heathwaite *et al*., 2005). Both should be managed if nitrate losses are to be minimized. However, the routes, and the factors which control them, for nitrate flow from within the soil profile to either channellized surface water flow or aquifers are complex and much less well understood than the sources of nitrate and mobilization and transport of nitrate within the soil profile discussed so far (Haygarth *et al*., 2005). In part this is because of the increased difficulties of data collection so that those data that exist are at inappropriate scales and lack the resolution necessary to elucidate the mechanisms (Haygarth *et al*., 2005).

Impact of environmental and management factors

RAINFALL AND DRAINAGE Total amounts of rainfall and its intensity together with evapotranspiration are the dominant climatic factors controlling the soil water balance and hence the runoff and leaching risk. The presentation of any rainfall data always carries warnings of the extreme variability of the data both temporally and spatially (e.g. Collins and Cummins, 1996). Evapotranspiration is much less spatially variable, but data are more difficult to obtain. Estimates of winter drainage for England and Wales show that it is independent of annual precipitation and also more variable (Rose, 1991). Differences in soils, slopes and vegetative covers also modify moisture budgets temporally and spatially (Jones, 1976). Because the nitrate limit for waters is expressed in terms of concentrations rather than amounts leached, where excess winter rainfall is low (<100–200 mm) even relatively low mineral N concentrations in soil can lead to drainage nitrate concentrations above 50 mg NO_3/l (Whitmore and Schröder, 2007).

MANAGEMENT OF SOIL WATER: INFILTRATION/RUNOFF On the basis of site characteristics (e.g. slope, vegetation), rainfall (intensity and amount), and the more stable soil properties (depth, texture, stoniness), soil series may be grouped into nitrate leaching risk categories (e.g. Smith and Cassel, 1991) or runoff risk categories (e.g. Collins and Cummins, 1996). However, they give no indication of the timing of nitrate loss. Farmers may be advised to use such classes to assist management decisions such as timing of fertilizer or slurry applications and thus to mitigate the nitrate source. As well as managing the risks of nitrate accumulation in soil, there are also opportunities for farm management practices to disconnect pollution from receiving waters through strategies to control, intercept, buffer and remediate polluting flows

on farm. Cultivation, buffer zones, wetlands, temporary storage ponds and ditch management can all be used to mitigate the flow path for nitrate loss, especially when surface runoff is an important pathway (Hewett *et al*., 2004). Where soils are very permeable or effectively underdrained then measures might be taken to intercept flow, e.g. in managing flows of water in ditches creating a series of shallow cascading ponds or wetlands to reduce water flow rate and increase opportunities for nitrate uptake and/or denitrification (Heathwaite *et al*., 2005). There are some concerns that approaches which focus solely on the management of the diffuse losses of one pollutant, here nitrate, could increase surface runoff and hence phosphorus and/or sediment loss. However, it is possible where both routes of loss occur in the same area for land management options to be selected that reduce nitrate losses without necessarily increasing surface runoff (Hewett *et al*., 2004).

FERTILIZATION The N balance (N input – N export by the crop) is often used to estimate the risk of N leaching from arable land. Sieling and Kage (2006) showed that increasing N balance progressively raised N leaching in the subsequent period with all crops, however, only 13–25% of the N balance surpluses originating from the preceding crop seem to leave the system via leaching. Rosen and Allan (2007) concluded that, where nutrient supply potential was equal, yields of vegetable crops tended to be similar whether mineral or organic fertilizers were used, but lack of synchrony of N supply from organic sources with plant demand tended to limit yield and could increase the risk of nitrate leaching. Residual mineral N measured at or soon after harvest can be used to indicate the likely nitrate available for leaching (Chaney, 1990; Sylvester-Bradley and Chambers, 1992). The breakpoint for optimum environmental fertilizer application, i.e. residual mineral N similar to unfertilized control plots, is usually close to that of the economic optimum in wheat crops (Glendining *et al*., 1996). In some vegetable crops the optimum environmental fertilizer application may be significantly lower than the economic optimum and high concentrations of mineral N may therefore be measured in the soil at harvest (Rahn *et al*., 1992). Residual mineral N levels may also be significant following disastrous yields due to drought, pest or disease (e.g. Macdonald *et al*., 1997).

CROP AND RESIDUE MANAGEMENT Crop residues provide a source of mineralizable N. They return between 20 and 145 kg N/ha in arable crops (Shepherd *et al*., 1996) and residues can be larger in vegetable crops (Rahn *et al*., 1992). Soil organic N may also be mineralized during the autumn and winter period supplying nitrate which may be lost by leaching (Macdonald *et al*., 1997), even where residual mineral concentrations are low. The greatest risk of leaching where legumes occur in a rotation occurs during periods after residue incorporation but prior to establishment/rapid growth of subsequent crops (Fillery, 2001). Similarly where grass swards are cultivated and resown or brought into arable production, large quantities of N are incorporated into the soil (Francis *et al*., 1992) and large leaching losses commonly result (Whitehead, 1995). However, use of legumes in rotation, with consequent

reduced use of N fertilizer, does not necessarily need to result in higher nitrate leaching losses, if management is good (Owens *et al*., 1994; Drinkwater *et al*., 1998).

In a whole catchment study, reductions in the nitrate load in the stream were mainly achieved by smaller manure applications and smaller areas of bare fallow over winter (Kyllman *et al*., 2006). Crews and Peoples (2005) also highlight that the most effective management approach to increase synchrony and reduce nitrate leaching is to minimize the time fields are left exposed without growing vegetation. This can be achieved by integrating arable and perennial crops (agroforestry approaches), using undersowing to establish the following crop or by cultivating as close to sowing as possible. Whitmore and Schröder (2007) showed that in well-designed systems intercropping can reduce nitrate leaching without concomitant loss of yield. In situations where residual nitrate in the soil at harvest is particularly likely then agricultural rotations may be modified to include catch or cover crops, which are able to absorb residual N and reduce losses of N by leaching (Martinez and Guiraud, 1990; Jensen, 1991). Under Mediterranean conditions, rocket has been suggested as a possibly useful cover crop due to its rapid uptake of nitrate and its ability to store high levels of nitrate in leaves (Santamaria *et al*., 2002). Such practices are usually effective in their year of application and have been shown to reduce nitrate leaching 30–40% (Askegaard *et al*., 2005). However, some work has shown that the N is mineralized in subsequent years, and the leaching loss can be delayed, rather than prevented altogether unless managed carefully (Berntsen *et al*., 2006a). Macdonald *et al*. (2005) showed that the inclusion of catch crops into a crop rotation had the greatest positive impact on very free draining soils in wet winters; in poorer draining medium-heavy textured soils natural regeneration of weeds and/or crop volunteers showed a similar benefit.

LIVESTOCK MANAGEMENT Higher levels of nitrate pollution in tile drains (which feed into watercourses) have been shown to arise under grazing compared to fields receiving slurry and cut for silage (McGechan and Topp, 2004); much of this increased nitrate leaching occurs late in the grazing season when grass growth has slowed and consequently deposition of N in dung and urine gives rise to 'untimely nitrate'. In grazed pastures in New Zealand, about twice as much N was added to the grass pastures than was estimated to have been fixed by clover; however, leaching losses were 6–7 times higher on the grass-based pastures (Ruzjerez *et al*., 1995). In the same study leaching from urine patches accounted for 55% of the total N leached from clover-based pastures, but only 25% of the total leached from N-fertilized grass. Increased stocking rates might therefore be expected to increase nitrate leaching risk, but stocking rates are restricted by the grass yield and so this link is somewhat self-regulating. However, supplementary feeding in-field to prolong the grazing period in early spring or into the autumn/winter is likely to significantly increase leaching risk. Observations show that livestock often congregate in certain areas of a field giving very high localized stocking rates. However, the fact that urine and faeces patches are concentrated over a small

proportion of the field area did not give an increase in overall loss when this was considered along with field areas receiving no excretions (McGechan and Topp, 2004).

Are Organic Farming Practices Likely to Have Distinct Impacts on Dietary Nitrate?

In organic farming systems, use of manufactured fertilizers is prohibited (or at least significantly restricted), crops must be grown in rotation or as mixtures and the cropping plan is designed with regard to the fertility building and depleting role of the crops with a focus on nutrient recycling to reduce the need for external input (Watson *et al*., 2002). Nutrients are dominantly added to the soil as organic (manures, compost, crop residues, legumes) or slow-release sources (e.g. rock phosphate). Consequently in organic farming systems a greater reliance is placed on chemical and biological processes within the soil to release N in forms available for plant uptake. Berry *et al*. (2002) clearly showed that yields in organic farming systems were reduced largely due to N limitation, but this does not necessarily equate to good synchronization of N supply with crop demand.

Brand and Molgard (2001) suggest that when considered as a whole organic farming has the potential of reducing nitrate levels in vegetables due to the lower N supply for most crops. Merino *et al*. (2006) showed low nitrate levels in lettuce grown in organic farming systems (826 mg NO_3/kg fresh weight) compared to integrated (1708 mg NO_3/kg) and conventional systems (2484 mg NO_3/kg) in 2000. However, this was not the case for iceberg lettuce, where genetic factors masked any impact of environmental or management variation (Merino *et al*., 2006). Guadagnin *et al*. (2005) also showed mixed responses to farming system when they compared organic, conventional and hydroponic cultivation in Brazil, with organic farming systems giving lower nitrate content for rocket and lettuce but high concentrations for watercress. Variation in the nitrate content for any crop species was high and varied significantly between producers, as well as farming systems (Guadagnin *et al*., 2005). However, they did not attempt to relate the variations directly to management practices used within the systems, e.g. rates of N fertilizer. Other studies have shown low or insignificant differences between systems and high variability of nitrate contents observed within farming system types (Lyons *et al*., 1994; Woese *et al*., 1997; Malmauret *et al*., 2002).

Berntsen *et al*. (2006b) studied the dynamics of nitrate leaching within an organic rotation and showed large differences between rotation phases with moderate leaching under legume-based pasture but high losses in the 2 years after its incorporation on a very sandy soil. Stopes *et al*. (2002) also showed lower leaching losses under organic grass-clover than conventional grass pastures at similar rates of N input across a range of soil types. With similar cropping systems and averaged over the whole rotation, organic farming systems showed similar/slightly smaller nitrate leaching looses than conventional farms under best environmental management

(Stopes *et al*., 2002). Stark *et al*. (2006) also showed a trend of lower nitrate leaching from organic cropping systems though the difference was not statistically significant. Kirchmann and Bergstrom (2001) compared nitrate leaching in organic and conventional arable systems and highlighted differences; these were strongly linked to the different input intensity of N and the variations in the type and sequence of crop grown. Where they considered losses on a per unit product compared to a per unit area basis differences were reduced and where different input intensities were taken into account there was no difference between management systems. Stark *et al*. (2006) showed that the crop rotation and the presence/absence of cover crops was more important in controlling nitrate leaching than the presence/absence of N fertilizer even where organic and conventional systems were compared.

Success of on-farm management in matching N demand with N supply in time and space is more important than system per se in controlling nitrate leaching.

What Scope Is There for Management of Nitrate in Vegetables and Waters?

The factors leading to accumulation of nitrate in vegetable crops are reasonably well understood, as outlined above. In conventional systems careful management of N fertilizer (amount, type and timing) is critical and effective management guidelines have been developed for spinach and lettuce. It is also possible with reasonable confidence to identify the sources and mechanisms which lead to nitrate accumulation in soils and some of the likely pathways of any 'untimely nitrate' from agricultural land to water. Crop selection and careful rotation design are critical to both the minimization of nitrate concentration in crops and also waters derived from all farming systems. However, the direct evidence needed to quantify the linkages between source, land management and impacts is lacking (Haygarth *et al*., 2005).

Management guidelines to reduce nitrate accumulation by vegetables and nitrate transfer to ground or surface waters in both conventional and organic farming systems can be identified. This is not necessarily as easy as it sounds, as best management practices need to provide defined and explicit advice tailored to work with the complex interactions of site and management factors which occur within agricultural systems. In the UK codes of practice have often been too general, not targeted to particular farms or catchments and too narrowly focused on single rather than multiple objectives (D'Arcy and Frost, 2001). However, the scope for reducing nitrate levels is often tempered by farmers' need for robust economic return and willingness to implement additional management for little obvious reward. Even with improved livestock, crop and soil management techniques there will still be periods of the year when excess rainfall will occur when soil is bare and so nitrate leaching cannot be completely prevented. Equally consumer taste will also lead to the cultivation of crops

which preferentially use nitrate in osmoregulation. There is scope therefore to reduce dietary nitrate in European diets but not to eliminate it completely. Evidence suggests that organic farming practices do lead to reductions in nitrate accumulation by vegetables and smaller leaching losses. However, compared to the background variability resulting from climate (radiation, excess rainfall) and other environmental factors (soil type, crop selection), these reductions are modest. Differences in human intake of nitrate resulting from personal preferences among vegetables are likely to be much more significant.

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14 Effects of the Environment on the Nutritional Quality and Safety of Organically Produced Foods: Round-up and Summary

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Introduction

The aim of the workshop was to explore in depth certain key aspects of the effects of the environment on the health benefits of organic foods and to address the three key questions set out in Table 14.1. Overall, the organic food market has witnessed buoyant developments over the last decade, particularly in the retail sector, with significant growth evolving around the basic staples such as fresh vegetables and fruit, milk and dairy products, grass-fed beef and lamb and bread products. Professor Chris Ritson discussed the consumer demand-led and supply-led aspects to explain the market changes. He highlighted the facts that, despite the huge awareness of organic foods, they still have a relatively small share of the market and total agricultural land area in use. Consumer expectation is for organic food to be safe and when it is bought, prepared and processed, to be attractive, taste better and be competitively priced and better for health. Typically, however, organic foods command a price premium of 40–140% over conventional foods. Table 14.2 summarizes the points raised by speakers in discussions on what people think organic means from the consumer perspective.

'Organic' is not a word that can be used legally without strict controls. Table 14.3 summarizes the key aspects of the European Commission regulation on organic production and labelling of organic products that give consumers the assurances and confidence that organic food is what it claims to be. Professor Alan Swinbank highlighted the wider organic ideal, which embraces the social impact of food production, protection of the environment, animal welfare, keeping track of the journey that organic food makes from the field to the shelf and trust in the organic label.

 Health Benefits of Organic Food: Effects of the Environment (Givens *et al.*)

Table 14.1. Three key questions to be addressed over the course of the conference.

Q1	Are there quantifiable effects of organic rather than conventionally produced food on human health?
Q2	How does the environment impact on these possible health benefits?
Q3	How do the public perceive these benefits?

Table 14.2. What do people think organic means?

- Not mass-produced/fresher
- No pesticides, artificial fertilizers
- Food is GM-free
- Local/regional supplies
- Food is free from artificial additives and E numbers, artificial sweeteners, flavourings and colourings
- Better welfare standards for animals and freedom from bovine spongio encephalopathy (BSE)/environmentally friendly
- Food has more vitamins, minerals and phytoprotective substances
- Less likelihood of allergies/intolerances, since food contains no additives and contaminants
- Better for overall health
- Tastes better
- Sustainable agriculture
- Organic label provides reassurance of product quality

Identification of Possible Health Benefits

Nutrition and chronic disease

Dr Anne-Marie Minihane addressed the health benefits of the long-chain *n*-3 polyunsaturated fatty acids (EPA and DHA) and pointed out that, although dietary intakes of these 'omega-3' fatty acids are well below recommended levels of intake and that there is clear evidence of benefit in relation to reduced risk of coronary heart disease, improved cognitive performance and modulation of inflammatory responses, there was no direct comparison of the effects of organic versus conventional foods and that any difference was likely to be small. Professor Ian Givens highlighted that there was no evidence of increased concentrations of EPA/DHA in organic foods and although there was some evidence that organic milk and dairy products may have modestly higher alpha-linolenic acid (αLNA) contents, the effect is likely to be due to the increased use of forage in the diets of these animals, rather than organic production methods per se. Professor Nigel Scollan discussed the exploitation of beneficial components of grass and legumes and grass management techniques that can influence αLNA levels and the *n*-6:*n*-3 ratios of animal products. However,

Table 14.3. New regulation on organic production and labelling of organic products, (EC) No. 834/2007, 28 June 2007.

- Encourages natural plant and animal health, husbandry and feeding practices
- Applies to all stages of production, preparation and distribution
- Controls use of indications referring to organic production in labelling and advertising
- Clarifies GMO rules, notably that GMO products are strictly banned (0.9% threshold for accidental presence)
- Only foods containing at least 95% organic ingredients can be labelled as organic
- Non-organic products to indicate organic ingredients only on ingredient list
- Requires indication of where the products were farmed
- Applies from 1 January 2009 (repeals regulation 2092/91)

because αLNA is poorly converted in the body to the bioactive *n*-3 polyunsaturated fatty acids, EPA and DHA, the contributions of organic foods rather than conventional foods to a healthier diet are likely to be small. Similarly with the trace element selenium, Professor John Arthur identified the need for biomarkers for selenium nutritional status and that dietary intakes of selenium in the UK are low, with accumulating evidence suggesting that greater population intakes would be associated with considerable health benefits. He concluded that there are no controlled experiments which provide information on the selenium content of organic versus conventional foods, and that any specific benefits of organic foods are most likely masked by the huge natural variations in soil selenium with subsequent influence on the selenium concentrations of plant and animal products derived from various regions. These observations on the health aspects of selenium were followed by those of Dr Peter Abrahams, who discussed the factors influencing solubility of selenium in different soil types, plants such as nuts, which are selenium accumulators, and grasses and grains, which are non-accumulators. He concluded that there needs to be a more systematic approach to surveying the selenium content of soils.

Dr Jeremy Spencer set out the health benefits of several flavonoid components that are found in foods such as fruits and vegetables, tea, wine and cocoa, and the effects of their metabolites on gene expression, the immune system, gut microflora and blood pressure control. Professor Gareth Jenkins discussed the factors involved in regulation of flavonoid biosynthesis in plants and how environmental factors such as protection against UV light, soil nutrient limitations, abiotic stresses, chemical treatments and pathogens can modulate flavonoid production significantly. In contrast, methods of production are likely to have only a small impact on flavonoid content of plants.

In conclusion, based on the evidence presented, there is little indication of any meaningful differences in nutrient and phytochemical composition between organic and conventional foods. The composition appears to be highly dependent on agronomic factors, pre-harvest and postharvest

conditions. Furthermore the benefits of organic foods to human health have neither been proven nor disproven.

Food-borne pesticides, herbicides, nitrates and environmental toxicants such as dioxins, PCBs and mycotoxins

Dr Claus Svendsen presented a critical review of the potential adverse health effects caused by food contaminants and risk assessment methodologies to determine safe levels in foods. The main conclusions were that organic foods contain lower levels of pesticides and nitrates than conventional foods but similar amounts of other contaminants. Conclusive evidence to support the claim that organic foods with lower concentrations of contaminants than conventional foods will result in better health is lacking. Similarly, comparisons of the amounts of mycotoxins in organic versus conventional foods give inconsistent results. The overriding conclusion was that agronomic practices, handling procedures, not to mention the vagaries of the weather, were more likely to impact on levels found in foods. Dr Monica Olsen concluded that there was no significant difference in levels of mycotoxins between organic and conventionally grown foods, and that there is a need for studies under strictly controlled conditions to give unbiased answers. In cases where significant differences have been observed, the levels in the foods and estimated intakes are not of concern. Dr Olsen also highlighted the impact of global climate change on the introduction of new crop varieties, crop rotations and pre- and postharvesting conditions, which may result in different toxigenic moulds on plants and levels of mycotoxins in products.

Professor Nigel Benjamin gave a fascinating insight into the role of nitrate and its metabolism in plants and in human physiology. He raised the question of nitrate in the diet – good or bad? Like other potential environmental toxicants, nitrates are treated as contaminants under existing legislation and the amounts in plants are influenced by the use of both artificial fertilizers and manures, the characteristics of the plant and the amount of sunlight. In man, there are both exogenous sources of nitrate from food and endogenous sources. The latter reflect the body's defences against infection and other vital physiological functions. The challenges for science relate to gaining a greater understanding of the beneficial role of nitrate and its metabolism in human health and to reassessing the existing scientific and regulatory perception of nitrate as being only a contaminant with a negative health impact. Dr Elizabeth Stockdale reviewed the metabolic regulation of nitrate uptake and nitrogen assimilation in vegetable plants and the impact of agricultural management factors such as artificial fertilizers and crop rotations to maintain soil fertility. Optimal nitrogen application greatly affects crop yield and must match crop need. Untimely fertilization can lead to rapid leaching from the soil, pollution and eutrophication of waterways.

Overall, dietary exposure to synthetic pesticides, herbicides and nitrates is likely to be significantly lower in organic foods compared with conventional foods, but the levels of most contaminants in both systems of produc-

tion are more highly dependent on agronomic and other environmental factors. Furthermore there is little evidence that the concentrations of these contaminants commonly observed in conventional foods are associated with any adverse health effects.

Food-borne infections

Professor Ariena van Bruggen discussed the prevalence of human pathogens in poultry, meat, dairy products and vegetables and made comparisons between organic and conventional farming systems. Typically, the reasons for greater numbers of outbreaks of gastroenteritis are due to poor handling of foods after purchase and during preparation. Changes in processing and packaging, the evolution of more virulent strains of bacteria, the globalization of trade and movement of food products have all contributed to increased risk of infection. In particular, there are concerns about the microbial contamination of fresh vegetables and fruits including lettuce, salads, spinach and herbs, and the need for more controls on the use of solid and liquid manures, a greater understanding of pathogen survival in manures and storage times of manures to reduce risk of survival of pathogenic organisms. Overall, the risk of microbiological contamination appears to be no greater with organic than conventional food production systems. Nevertheless, good hygiene and agricultural practices are of vital importance for both types of foods throughout the food supply chain.

Research Activities

Although not an exhaustive list of possible areas of future research, the following broad groups of activities were identified to help provide the solid evidence base for the development of organic farming methods and to promote greater consumer understanding of the differences between organic and conventional food products.

Nutritional aspects of organic foods

- Establish using carefully controlled plant-growing and animal-feeding trials, the concentration of the beneficial nutrients and non-nutrients (fibre, flavonoids) in organic versus conventional foods.
- Assess the potential health benefits of any observed differences in composition between organic versus conventional foods.
- Conduct research to further establish animal-feeding conditions designed to maximize the EPA/DHA content of animal products following increase αLNA in the diet.
- Promote greater understanding of the regulation of flavonoid production in plants.
- Improve understanding of the impact of nitrate/nitrite on human health.

Food safety and reduction of risk of microbial pollution

- Expand knowledge of microbiological quality of organic versus conventional food.
- Pay attention to hygienic production and handling of both organic and conventional foods.
- Promote efforts to use fewer synthetic pesticides and nitrogen fertilizers and gain more knowledge of biological control agents, crop rotations and methods for maintaining and enhancing soil fertility.
- Reduce crop and food spoilage and maintain quality with integrated harvesting, processing and packaging systems.
- Improve and monitor management of manure and livestock to control pathogens.
- Support effective fertilizer management techniques and nitrate uptake and assimilation in plants.

Economic/ecological impact of organic farming

- Identify opportunities for diversification as vital component of farm businesses and rural economy.
- Overcome agronomic problems to growing crops without pesticides.
- Balance locally produced foods versus imported foods/assessment of energy use and implications for 'green miles'.
- Address fragmentation of organic producers, small and medium enterprises (SMEs) and supply chain management/shortages.
- Move to explore opportunities to develop more added-value processed foods and branded products.
- Balance costs and benefits.

Consumer demand for organic foods and health

- Stimulate development of high added-value organic foods and organic meal products with good quality, taste and nutritional value.
- Create and validate effective ways to communicate and target information on healthy eating/organic foods to various groups (children, elderly).
- Monitor consumer perceptions and consumer understanding of the benefits of organic foods.

Concluding Remarks

As our lives and the environment in which we live change, consumer demands and tastes are constantly evolving. The advent of genetically modified crops, along with a range of different food issues has caused some people to ask questions about the food they buy and consume, such as where it comes from and how it is grown and processed.

Many people are choosing organic foods because they believe they are safer, free from genetic modification, better tasting and more nutritious. They also believe that organic food production is based on a more sustainable, environmentally friendly and safer way of life. Whatever the motivation, people expect the food to be safe, attractive, competitively priced and possibly better for health.

There are pros and cons for both organic and conventional methods of food production, and as this workshop has demonstrated, there are many questions that remain unanswered. What information does exist about the microbiological quality of organic foods indicates that these products are as safe as conventionally produced products. Similarly, surveys provide little or no objective evidence to support assertions that organic foods have more vitamins, minerals and trace elements and fewer contaminants than conventional foods. It is also claimed that organic foods taste better than their conventional counterparts. However, blind tasting of organic versus conventional produce rarely results in an overwhelming case for organic food production on the basis of taste. On the other hand, the taste of organic foods may reflect the use of older or more unusual varieties of plants.

Organic is not simply a claim: it is a legal system that must be followed before something can be marketed as organic. The choice of organic foods is as much about lifestyle as about choice. For those people with a particular type of lifestyle, organic may seem the only ethically justifiable option. For those who want choice, organic products allow them to choose particular foods that suit their tastes.

This workshop provided the opportunity to listen and debate as well as to identify the need for the generation of new knowledge through scientific discovery. Speakers and delegates from different and traditional scientific disciplines sought to share and provide insights and sustainable solutions to many of the issues concerning the food chain and our food supply. The most exciting advances often arise when multidisciplinary approaches and collaborative scientific research work together. The workshop has aired some of the strategic and scientific priorities and will contribute to our greater understanding of organic farming and products.

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