

# Safety Considerations of DNA in Food

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## Key Words

DNA · Food safety · Genetically modified organisms

## Abstract

Recombinant DNA techniques are capable of introducing genetic changes into food organisms that are more predictable than those introduced through conventional breeding techniques. This review discusses whether the consumption of DNA in approved novel foods and novel food ingredients derived from genetically modified organisms (GMOs) can be regarded as being as safe as the consumption of DNA in existing foods. It concludes that DNA from GMOs is equivalent to DNA from existing food organisms that has always been consumed with human diets. Any risks associated with the consumption of DNA will remain, irrespective of its origin, because the body handles all DNA in the same way. The breakdown of DNA during food processing and passage through the gastrointestinal tract reduces the likelihood that intact genes capable of encoding foreign proteins will be transferred to gut microflora. The review does not specifically address food safety issues arising from the consumption of viable genetically modified microorganisms but it shows that the likelihood of transfer and functional integration of DNA from ingested food by gut microflora and/or human cells is minimal. Information reviewed

does not indicate any safety concerns associated with the ingestion of DNA per se from GMOs resulting from the use of currently available recombinant DNA techniques in the food chain.

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## Executive Summary

This paper reviews the available data and discusses whether the consumption of DNA in approved novel foods and novel food ingredients derived from genetically modified organisms (GMOs) can be regarded as being as safe as the consumption of DNA in existing foods.

All DNA, including DNA from GMOs, is composed of the same four nucleotides. Genetic modification results in the re-assortment of sequences of nucleotides leaving their chemical structures unchanged. Therefore, DNA from GMOs is chemically equivalent to any other DNA, hence any uniqueness is restricted to differences in the DNA sequence. Taking into account the natural variations of DNA sequences, the present use of recombinant techniques in the food chain does not introduce changes in the chemical characteristics of the DNA.

With our traditional diet we normally ingest gram quantities of DNA and RNA daily. Relatively high concentrations are usually present in edible offals and animal muscle tissues whereas plant-derived foods contain lower concentrations. Consumption of excessive quantities of RNA and, to a lesser extent, DNA, is considered to be a risk factor for gout. However, genetic modification will not increase the overall dietary intake of DNA.

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Ingested nucleic acids are typically contained within cells and become available after cell lysis. Extracellular nucleic acids are extensively broken down in the gastrointestinal tract. Their building blocks (e.g., nucleosides and nucleic bases) are absorbed. Some DNA fragments, though very unlikely complete genes, escape breakdown. There is evidence that such fragments may be absorbed into enterocytes and macrophages. The remainder is excreted with the faeces along with residual cell-bound nucleic acids. There are no indications that genetic modification per se, as used for food applications, has an impact on the digestibility or stability of the nucleic acids.

Due to their chemical identity, toxicological concerns of the building blocks can be excluded. Based on their chemical equivalence and metabolic considerations, the toxicological profiles of DNA/RNA from GMOs and from existing food organisms are equivalent. There is no indication that DNA has allergenic or other immunological properties that would be of relevance for dietary consumption of foods derived from GMOs. Safety considerations, therefore, could only arise in relation to the small proportion of extracellular DNA that remains undegraded. This DNA may interact with either the mammalian cells of the gastrointestinal tract or the gut microflora and may result in genetic transformation.

In the case of gut bacteria, the cells would need to have the capacity to take up DNA. Then the DNA would need to be integrated into the genome either as a linear fragment, which would require extensive sequence homology, or by formation of an independent replicon. In addition, for a gene on the integrated DNA to be expressed, it would need to be associated with appropriate regulatory sequences. A new trait may be maintained without selection, but for the transformed bacterium to become a large part of the population there would need to be selection for the trait. Each of these events is rare and they would need to happen sequentially.

In mammals, DNA fragments may be taken up by cells of the intestinal wall, including cells of the immune system. DNA fragments, after passing through the intestinal wall, may be actively removed by cells of the gut immune system or they may enter the circulation. Here they would also be subjected to the activity of cells of the immune system in which endosomal digestion takes place. In mice, fragments of orally administered plasmid DNA were shown to be taken up by immune cells. Also, there is some evidence that DNA can be taken up into the nuclei of various types of cells.

Mammals possess effective mechanisms to avoid incorporation of foreign DNA into the genome. There is no evidence to suggest that ingested foreign DNA has been incorporated into the genome, although humans and other mammals have always been exposed to foreign DNA in their food.

DNA from GMOs is equivalent to DNA from existing food organisms that has always been consumed with human diets. Any risks associated with the consumption of DNA will remain, irrespective of its origin, because the body handles all DNA in the same way. The breakdown of DNA during food processing and passage through the gastrointestinal tract reduces the likelihood that intact genes capable of encoding foreign proteins will be transferred to gut microflora. The work presented here shows that the likelihood of transfer and functional integration of DNA from ingested food by gut microflora and/or human cells is minimal.

Information reviewed in this paper did not indicate any safety concerns associated with the ingestion of DNA per se from GMOs resulting from the use of currently available recombinant DNA techniques in the food chain. Therefore, such DNA from GMOs is considered to be as safe as any other DNA in food.

## Introduction

Strategy papers by international bodies on the safety assessment of foods derived from genetically modified organisms (GMOs) contain statements that the presence of DNA from GMOs per se does not raise health concerns [WHO, 1991; FDA, 1992]. This paper discusses the chemical structure of DNA and its occurrence in food as well as possible consequences of the presence in food of naked DNA related to its structure (e.g., toxicity) and function (e.g., uptake, integration and expression by other organisms). Distinction is made between novel DNA sequences per se introduced by recombinant DNA techniques (referred to as recDNA) and the complete DNA (i.e., the genome), from GMOs, only a very small proportion of which will be recDNA. Food safety of proteins expressed by GMOs is not discussed here since it is well covered in other guidelines, nor is the food safety of viable genetically modified micro-organisms in food discussed as this is the subject of a separate report [ILSI Europe, 1999]. This paper was developed by an expert group of the Novel Foods Task Force of ILSI Europe and discussed with a wider audience at a workshop held on 26–28 June 2000 in Limelette, Belgium. Workshop participants (see appendix) agreed on an executive summary and a list of conclusions, which have been incorporated into the current text and are summarised in figure 1. The paper provides the food safety specialist with a framework to facilitate discussion, on a case by case basis, of the food safety of GMO and derivatives containing DNA from GMOs.

## Structure and Function of Nucleic Acids

This report discusses both:

- **DNA** (deoxyribonucleic acid): In its double-stranded form DNA is the genetic material of most organisms, the two strands form a double helix with the strands running in opposite directions as determined by the sugar-phosphate backbone of the molecule; and
- **RNA** (ribonucleic acid): RNA is a polynucleotide that can hold genetic information, as in viruses, but is also the primary agent for transferring information from the genome to the cells' protein synthesis machinery.

Recombinant DNA is DNA in which the nucleotide sequence has been altered in a way that does not occur naturally by mating and/or natural recombination, as defined in Directive 90/220/EEC [European Commission, 1990].

Nucleotides are the building blocks from which DNA and RNA are made. Nucleic acids are single- or double-stranded, thread-like polymers, made up of a linear array of monomers, the nucleotides. All nucleotides are constructed from three components: a nitrogen-containing heterocyclic base and a pentose sugar (which together make up a nucleoside) and a phosphate residue attached to the 5' position on the sugar. The bases are derivatives of monocyclic pyrimidines or bicyclic purines. The major purines are adenine (A) and guanine (G), the major pyrimidines are cytosine (C) and thymine (T) in DNA, the latter replaced by uracil (U) in RNA. The sugars are D-ribose in RNA and 2-deoxy-D-ribose in DNA. The bases are covalently attached to the C-1' of the pentoses via N-glycosidic bonds, the hydroxyl group of the pentoses in C-5' is esterified as phosphate esters resulting in the corresponding nucleotides.

The primary structure of nucleic acids results from the covalent linkage of nucleotides via phosphodiester bonds. The 5'-hydroxy group of one pentose is joined to the 3'-hydroxy group of the next nucleotide by a phosphodiester bond. Thus, the backbone of nucleic acids consists of alternating phosphate and pentose moieties; the uniqueness of any primary DNA structure is determined by the sequence of the characteristic bases attached as side chains to this backbone.

The three-dimensional secondary structure of DNA is due to the formation of a right-handed double helix of two polynucleotide strands. They are arranged in an anti-parallel way, i.e. the directions of the 5',3'-phosphodiester bonds are opposite, and are attached via hydrogen bonds between the bases A-T and C-G, respectively;  $\pi$ -interactions (pi-bonds) between the bases (base stacking) result in additional stabilisation of the conformation.

The structure of DNA (diameter of the right-handed helix 1.9 nm; complete turn every 3.4 nm; 10 base pairs per turn) as elucidated by Watson and Crick, also called B-DNA, is the most stable arrangement of a DNA molecule with random sequence under physiological conditions. Under specific circumstances two alternative conformations have been observed: In dehydrated solutions so-called A-DNA (complete turn of helix every 2.6 nm; 11 base pairs per turn), may occur. Z-DNA (left-handed helix, zigzag folding of the backbone, 12 base pairs per winding) shows the strongest deviation from the B-structure. Alternating purine-pyrimidine sequences have the strongest potential to adapt this type of conformation.

Other structural features are also favoured by specific sequences. Bends of the DNA helix occur if there are 4 or more adenines in a row in a strand; 6 consecutive ade-

#### Statements

ILSI Europe Workshop on Safety Considerations of DNA in Food, 26–28 June 2000

These statements reflect the opinion of the expert panel based on the evidence of currently available data

- All DNA including recDNA is composed of the same four nucleotides.
- In view of the variability of dietary intake of DNA, consumption of foods derived from GMOs does not measurably change the overall amount of DNA ingested through the diet.
- Taking into account the natural variations of DNA sequences, the present use of recombinant techniques in the food chain does not introduce changes in the chemical characteristics of the DNA.
- There is no difference in the susceptibility of recDNA and other DNA to degradation by chemical or enzymatic hydrolysis.
- The metabolic fate of DNA digestion products is not influenced by the origin of the DNA.
- DNA is not toxic at levels usually ingested. Where there is potential for adverse effects, e.g., gout, this is due to excessive intake, not the origin of the DNA.
- There is no indication that ingested DNA has allergenic or other immunogenic properties that would be of relevance for consumption of foods derived from GMOs.
- Uptake, integration and expression of any residual extracellular DNA fragments from foods by microorganisms of the gastrointestinal tract cannot be excluded. However, each of these circumstances is a rare event and would have to happen sequentially.
- In vivo uptake of DNA fragments by mammalian cells after oral administration has been observed. However, there are effective mechanisms to avoid genomic insertion of foreign DNA. There is no evidence that DNA from dietary sources has ever been incorporated into the mammalian genome.

Fig. 1.

nines result in an angle of about 18°. The presence of complementary sequences, such as palindromes or reverse repetitions, can result in so-called hairpins or cross-structures.

Variations of the described basic structure may arise from modifications of nucleotide bases by reactions such as methylation, hydroxylation and glycosylation. In bacteria, enzyme-catalyzed methylation is involved in the protection of DNA from the action of endogenous nucleases or in DNA repair mechanisms. In eucaryotes, 5-methylcytosine and N<sup>6</sup>-methyladenosine are the most common modified bases. 5-Methylcytosine mainly results from

methylation of the dinucleotide CpG. In vertebrate DNA more than 50% of CpG sequences may be methylated. The presence of 5-methylcytosine in an alternating CpG sequence increases the tendency of this sequence to be present in the Z-conformation.

These general principles of the structure of DNA also apply to recDNA. The application of recombinant techniques does not lead to any additional structural elements. RecDNA consists of the same building blocks as any other DNA present in nature; the compounds are attached via the same type of covalent (N-glycosides, phosphodiester) and non-covalent (hydrogen and  $\pi$ -interactions) bonds. This is in contrast to synthetic oligonucleotides being developed for anti-sense therapies, in which the type of linkage between the nucleotides (phosphorothioates rather than phosphodiester) and the structure of the sugar moiety (2'-O-methylribose rather than ribose) are intentionally modified in order to increase their stability [Agrawal, 1996].

The use of recombinant DNA techniques may result in a recombinant DNA which differs in its methylation pattern from the starting DNA, due to the inherent difference(s) in the sequence. However, taking into account that the type of building blocks remain the same and considering the natural variation of DNA sequences, the modified DNA is to be seen as equivalent to other DNA, in that respect.

A gene is a section of DNA, located on a chromosome or plasmid, that in its entirety codes for a protein. It consists of the DNA sequence that codes for the protein together with sequences that promote and regulate gene expression. The size of a gene is dependent on the protein that it codes for but typically a functional gene will be from 150 to 6,000 bases long whilst the control elements will be much shorter, typically from 100 to 500 bases long. DNA is not present as a 'naked' molecule in cells. The negatively charged phosphate moieties are associated with metal ions, polyamines or proteins (in eucaryotes usually histones). Histones are proteins with high proportions of basic amino acids.

The structure and function of DNA in food depend on its origin. Prokaryotes contain higher concentrations of DNA than eukaryotes and a much higher proportion of this DNA forms structural (protein-coding) sequences. In the DNA of eukaryotic organisms, protein-coding sequences (exons) are often interspersed with non-coding sequences (introns). Generally, genes having a similar function (e. g., the synthesis of a particular protein) will be structurally similar (i. e. they are homologous) in a wide range of, often unrelated, organisms.

The nature of the DNA in the diet will also be influenced by the agronomic conditions under which the food is grown and by its further processing. Thus, for example: consumption of crop plants infected with a virus will lead to the consumption of viral DNA and/or plant DNA incorporating viral DNA sequences. Consumption of fermented foods will lead to the consumption of DNA of microbial origin.

## Occurrence of Nucleic Acids in Food

### *RNA and DNA in Food*

Plant and animal foods contain RNA, DNA, nucleotides, and free nucleic bases. Their total amount and pattern in foods varies according to the source depending on the density of the nucleic acids in the cells. Animal muscle tissues comprise a high content of DNA and RNA, though it is lower than in edible offals, which also contain a high quantity of cell nuclei. Plant storage tissues, as in grains or potatoes, with a lower content of cell nuclei, contain less DNA and RNA. The highest DNA and RNA contents are found in bacteria, yeasts and mushrooms and are associated with fast growth. Table 1 shows, for several different foods, the content of material which analyses as RNA and DNA, however, the analytical methods used do not discriminate between intact DNA and nucleic acid fragments resulting from partial degradation.

Thus, dietary intakes of RNA and DNA depend on the origin of the diet and they also depend on the effects of processing. Dietary intakes of RNA and DNA will vary widely between individuals but are typically in the range 0.1–1 g/person/day [Doerfler and Schubert, 1997].

### *RecDNA Content of Genetically Modified Food Crops*

Following conventional breeding (sexual crossing), progeny contain half of their genetic information from each parent. However, the process of genetic modification results in a considerably smaller change to the organisms' genetic information with, typically, only one or two new genes being incorporated. Table 2 shows, for a number of genetically modified crops, the percentage contribution made by recDNA to the total DNA in the genome or in the chromosome that is modified. As will be seen later in this paper, processing can significantly reduce the levels of DNA and recDNA. Highly refined products such as sugar (from *Beta vulgaris*) or soya oil (from *Glycine max*) will contain little or no DNA [Klein et al., 1998].

By combining consumption and intake data with the figures from table 2 it is possible to obtain an estimate of

**Table 1.** Nucleic acid content of selected foods [Herbel & Montag, 1987; Lassek and Montag, 1990]

Food		RNA (g/kg dry matter)	DNA (g/kg dry matter)
Calf	Liver	22.9 (21.2–23)	17.3 (17.1–20.2)
Beef	Liver	22.1 (21.4–22.8)	19.5 (18.9–20)
	Heart	6.1	5.3
	Spleen	17.9 (16.3–19.1)	32.6 (32.2–50.1)
	Lungs	15.5	32.2
	Pancreas	87.9 (73.6–102.1)	16.2 (14.4–18)
	Lymph node	33 (28.5–37.5)	100.9 (86.7–115.2)
Pig	Liver	32.1 (31.2–35.5)	14.8 (14.4–18.1)
	Kidney	15.5 (15.1–15.9)	17.6 (17–18.2)
	Heart	9.4	6.9
	Lymph node	26.5 (25.2–27.8)	68.5 (66.7–70.3)
	Pancreas	71.4 (64.9–77.9)	21.2(18.8–23.6)
Horse	Muscle	10.8	9.2
Saithe	Filet	2.5 (2.1–2.9)	0.6 (0.4–0.7)
Tuna		1.7	0.8
Cod		4.7	0.3
Trout	Smoked	4.7	1.0
Herring	Roe	15.3	0.6
Rye		1.3 (1.1–1.4)	0.7 (0.6–0.8)
Wheat		1.1 (1.0–1.1)	0.6
Oats		3	–
Barley		3.2	–
White millet		1.5	0.7
Senegal millet		2.3	0.6
Lentil		3.9 (3.8–3.9)	0.8 (0.7–0.8)
Broccoli	Fresh	20.6	5.1
Chinese cabbage	Frozen	14.6	2
Cauliflower	Fresh	14.5	2.8
Spinach	Frozen	14	2.6
Parsley leaves	Fresh	8.1	2.7
Potatoes	Fresh	1.4	1
Onion	Fresh	2.6	0.7
Avocado	Fresh	1.5	0.6
Yeast	Baking	66.2	6.0
Oyster fungi		24.1	1.4
Boletus (yellow)		23.1	1
Boletus (yellow)	Dried	16	1
Champignon		20.5	0.9
Chestnut mushrooms		21.1	1.4
Boletus (witch)		18.8	0.6

the potential dietary intake of recDNA for a particular crop. Table 3 gives an estimate of the total per-capita intake of recDNA ( $\mu\text{g}/\text{d}$ ) from genetically modified maize, soya and potatoes as  $0.38 \mu\text{g}/\text{d}$  assuming that only GM crops are consumed. This is around 0.0005% of the total RNA and DNA from these sources or around 0.00006% of a typical daily DNA intake of 0.6 g. Austrian intake data are used but the results will be similar in other EU countries.

## Stability of Nucleic Acids

### Chemical Stability

As befits its role as a carrier of genetic information, DNA is a macromolecule exhibiting relatively high chemical stability. Nevertheless, hydrolysis (non-enzymatic and enzymatic), oxidation and non-enzymatic methylation of DNA are examples of reactions occurring at significant rates [Lindhahl, 1993]. In vivo, these reactions are involved in the generation of spontaneous DNA lesions.

**Table 2.** recDNA content of some genetically modified crops

Crop	Average genome size (Mbp) <sup>1</sup>	Average insert size (kbp) <sup>2</sup>	Trait	% recDNA of genome <sup>3</sup>
<i>Zea mais</i>	2,292	5	Insect protected	0.00022
<i>Solanum tuberosum</i>	1,597	12	Insect protected	0.00075
<i>Glycine max</i>	1,115	2	Herbicide tolerant	0.00018
<i>Beta vulgaris</i> ssp. <i>saccharifera</i>	758	8	Herbicide tolerant	0.0011
<i>Brassica napus</i>	1,129	6	Herbicide tolerant	0.00053

<sup>1</sup> Data from Foster and Twell (1996).

<sup>2</sup> Typical figure for the size of the T-DNA inserted on average in examples of current commercial genetically modified crops (A. Koenig, Personal communication).

<sup>3</sup> Assumes that the crops are homozygous for the trait – this represents a worst case scenario. In some instances the crops marketed may be heterozygous.

**Table 3.** Intake of recDNA from genetically modified maize, soya and potatoes

	Maize	Soya <sup>1</sup>	Potatoes
Per-capita consumption in Austria, kg/year <sup>2</sup>	6.3	0.6	55.8
Per-capita intake in Austria, g/d <sup>3</sup>	14.7	1.4	130
DNA + RNA, g/kg dry matter <sup>4</sup>	5.54	6.46	2.38
DNA + RNA, µg/g food	1,496	6,266	319
Estimated per-capita intake DNA + RNA, µg/d	21,991.2	8,772.4	41,470.0
recDNA, % <sup>5</sup>	0.00022	0.00018	0.00075
recDNA, µg/g food	0.0033	0.0113	0.0024
Estimated per-capita intake recDNA, µg/d	0.049	0.016	0.312
Total recDNA intake (maize, soya and potatoes), µg/d			0.377

<sup>1</sup> Excluding soya oil.

<sup>2</sup> Austrian Food Balance Sheets 1996/97.

<sup>3</sup> Taking into account a correction coefficient of 15% for the conversion from consumption data to intake data (Elmadfa et al., 1999).

<sup>4</sup> Lassek and Montag (1990).

<sup>5</sup> Data from table 2.

In vitro, these reactions set limits for the recovery of DNA from natural sources. As recDNA consists of the same building blocks as any other DNA, the type of reactions leading to changes of recDNA will be the same as for other DNA.

### Hydrolysis

*Nonenzymatic Hydrolysis.* Nucleic acids undergo spontaneous, but not very rapid, decomposition in solution, RNA being more vulnerable than DNA. Because of the presence of the C2-hydroxyl group of ribose, the phosphodiester bonds of RNA molecules are very susceptible to hydrolysis, particularly in the presence of divalent cations.

The rates of hydrolysis of the base-sugar bonds of ribonucleosides are much lower than those of deoxyribonucleosides. However, the chemical price paid for the greatly increased resistance of the nucleic acid phosphodiester bond (gained by removal of the sugar 2'-OH group) is a labile N-glycosyl bond. Guanine and adenine are generated from DNA at similar rates, whereas cytosine and thymine are lost at 5% of the rate of the purines. In consequence, under low pH conditions the depurination of the nucleic acid backbone is the first step in the degradation of DNA followed by hydrolysis of adjacent 3',5-phosphodiester linkages resulting in measurable shortening of DNA strands [Maniatis et al., 1982]. This acid-catalysed hydrolytic degradation is the major type of reaction of

DNA under the physiological low pH conditions in the stomach. This reaction is accelerated by simultaneous heat treatment, as discussed later in the context of DNA susceptibility to food processing.

In addition to the intrinsic lability of glycosyl bonds, DNA base residues are susceptible to hydrolytic deamination. Cytosine and its homologue 5-methylcytosine are the main targets for this reaction. In vivo, there are effective repair mechanisms to restore DNA [Doetsch and Cunningham, 1990; Dianov et al., 1992]. It can be estimated that in each human cell 2,000–10,000 DNA purine bases turn over every day owing to hydrolytic depurination and subsequent repair [Lindahl and Nyberg, 1972]. However, this type of hydrolytic reaction, including repair mechanisms, is of no consequence for dietary DNA intake and its gastrointestinal digestion.

*Enzymatic Hydrolysis.* DNA is susceptible to degradation by both exo- and endonuclease enzymes. There is a discussion of in vivo hydrolysis later in this paper.

#### Nonhydrolytic Reactions

Aerobically growing cells are exposed to active oxygen during normal metabolism and this can cause endogenous DNA damage. The major mutagenic base lesion generated by hydroxyl radicals is 8-hydroxyguanine, which base-pairs preferentially with adenine rather than cytosine and thus generates transversion mutations after replication. This lesion is excised by a specific DNA glycosylase, present in *Escherichia coli* and in mammalian cells [Tchou et al., 1991].

In addition to oxygen, living cells contain several other small reactive molecules that might cause DNA damage and act as endogenous genotoxic agents, such as S-adenosylmethionine. 7-Methylguanine and 3-methyladenine are the major DNA lesions. Both eucaryotic cells and bacteria contain a highly efficient 3-methyladenine-DNA glycosylase that rapidly excises the altered base, generating an apurinic site. 7-Methylguanine, by contrast, is poorly repaired; however, the chemical lability of the 7-methylguanine-deoxyribose bond ensures that a steady state of base modification and loss would be achieved within a few days.

These nonhydrolytic reactions may lead to mutagenic lesions and are of relevance for the functional integrity of DNA. In foods, however, neither DNA nor recDNA have a functional purpose.

#### *Susceptibility of DNA to Processing*

In nucleic acids the double helix is unstable and at a temperature between 60 and 90°C or at an alkaline pH

(>12) it melts to produce single strands. After lowering temperature and pH, hydrogen bonds can be reformed. Initial melting of the double-stranded DNA structure may be followed by fragmentation characterised by the removal of side valencies and the loss of the native conformation. Basic conditions lower RNA content because the RNA is unstable and hydrolysis takes place. Acidic pH depurinates DNA and leads to subsequent strand cleavage. UV light can induce the formation of pyrimidine dimers. They are produced through the formation of a cyclobutane ring between two neighbouring pyrimidine bases. Fragmentation can also occur by mechanical treatment (shear forces), enzymatic treatment (nucleases), chemical hydrolysis and ionising radiation.

The stability of DNA is influenced by the matrix within which it is contained. Thus, DNA adsorbed onto clay is more stable and less susceptible to degradation than DNA in solution [England et al., 1998]. Similar phenomena have been observed in foods. Monitoring the fate of recombinant DNA from genetically modified starter organisms in thermally treated fermented sausages revealed strong protection of the DNA against the activity of DNase I in the meat matrix [Straub et al., 1999].

Food processing may lead to partial or complete degradation or removal of the DNA. Physical and chemical parameters, such as shear forces, heat or pH, may cause random cleavage of DNA strands in the course of the processing of foods, thus reducing the average DNA fragment size but not the total DNA content, as determined using techniques such as spectroscopy or dye binding. Some processes, such as the purification of sugar or the production of refined oils will remove most, if not all of the DNA [Klein et al., 1998].

Many foods such as fruits or vegetables are characterized by acidic pH conditions; thus, an acceleration of acid-catalysed reactions in the course of thermal treatment must be expected. The influence of pH on the detectability of the transgene upon heat treatment was demonstrated using polenta made from insect-resistant Bt maize as a model. Random cleavage of maize DNA was considerably accelerated in acidic milieu. At pH 2–3 the cryIA(b) gene region (1,914 bp) was no longer intact after boiling for 5 min, so that PCR failed to amplify the respective fragment. Detection of the transgene (up to 10 min boiling of maize flour at pH 2–3) was only possible via the significantly shorter 211-bp fragment [Hupfer et al., 1998].

In heat-treated pork, for example, a shift in average nucleic acid chain length from 1,100 base pairs (bp) to approximately 300 bp has been observed [Ebbehoj and

Thomsen, 1991]. Similar effects have been described for processed tomato products [Ford et al., 1996]. Shearing in the presence of water at elevated temperatures reduces DNA to fragments of less than 200 bp. DNA fragments of less than 200 bp are unlikely to encode functional proteins, since this would require not only the gene to be present but also the appropriate control sequences.

The crucial importance of the length of the genomic target sequence chosen for amplification by PCR to the detection of recDNA was also confirmed in silage obtained from insect-resistant Bt maize. The ensiling process creates a harsh environment for plant DNA. Chopping of the plant tissue results in disruption of cell walls and membranes, release of DNA and eventually its degradation by endogenous nucleases of the plant and /or the microflora. In addition, the lowering of the pH as a result of the lactic acid fermentation accelerates the degradation process. No significant differences in terms of DNA degradation could be observed between the transgenic crop and the conventional crop. Detection of transgenic Bt maize via the 1,914-bp amplicon was only possible for up to 5 days of ensilage. In contrast, detection of the maize-specific *ivr1* gene and of the Bt gene via amplicons of 226 bp and 211 bp, respectively, could be achieved after 100 days of ensilage [Hupfer et al., 1999]. Looking at these data from a safety assessment angle, they confirm that the presence of intact, functional genes (in the range of 2,000 bp and more) in maize after an extended time of ensilage is highly unlikely. The gene product, the CryIA(b) protein was also no longer detectable after 4 months of ensilage [Fearing et al., 1997].

The fact that the degradation of DNA in the course of heat treatment affects the transgenic sequence to the same degree as any other region of the maize genome of comparable length could be demonstrated in an experiment employing dual-competitive quantitative polymerase chain reaction (PCR) techniques [Hupfer et al., 2000]. Upon heat treatment of Bt maize flour, there was an almost parallel decrease of recovery of a sequence (226 bp) of the maize-specific invertase gene *ivr1* and the target sequence (211 bp) specific for the transgenic Bt maize. On the other hand, the total DNA concentrations as determined by UV spectrophotometry remained constant.

Degradation of DNA was also followed in a thermally treated fermented sausage. The meat matrix strongly protects DNA from the action of DNase. However, upon heat treatment, the detection of recDNA is only possible via PCR amplification of short DNA fragments [Straub et al., 1999].

For reasons discussed below, it is desirable to limit the amount of nucleic acids consumed in the diet [PAG, 1975]. In view of the high level of nucleic acids in yeasts and bacteria, it is recommended that there should not be more than 2 g of nucleic acids introduced into the diet of adults by single cell proteins [Edozien, 1969; Calloway, 1969]. Levels of RNA/DNA in bacteria intended for use as food or feed can be reduced through processing, for example by applying a heat shock [Larsen and Jørgensen, 1996].

In summary, DNA and RNA in food are exposed to various processing conditions which reduce their content in foods and their fragment size; nucleotides and nucleosides are also obtained during passage through the gastrointestinal tract. RecDNA will be subjected to the same conditions yielding comparable products due to its equivalent chemical structure.

### **Safety of DNA – Structural Effects**

This section discusses food safety considerations from the presence in food of DNA per se. Safety considerations arising from the functional properties of genes are discussed later. Safety considerations arising from the presence in food of gene products (proteins) are not discussed in this paper as they are adequately covered in existing guidelines.

#### *Stability of DNA in the GI Tract*

In living organisms, the fate of dietary nucleic acids can be correlated with the known fate of the various components and with the known degradative enzymes. The GI tract is constantly exposed to foreign DNA by the flow of partly or completely digested nutrients. The digestion process applies equally to recDNA as to DNA.

Action by pancreatic nucleases in the intestine cleaves nucleic acids into nucleotides and the latter are cleaved into nucleosides and phosphoric acid by enzymes found on the luminal surfaces of the mucosal cells. Nucleosides are subsequently cleaved to produce sugars and purine and pyrimidine bases.

#### Degradation

Nucleoproteins are converted to nucleic acids in the intestinal tract by the action of proteolytic enzymes [Carver and Walker, 1995]. The nucleic acids are degraded by pancreatic and intestinal epithelial cell nucleases to a mixture of mono-, di-, tri-, oligo- and polynucleotides. Ribonuclease and deoxyribonuclease are specific for RNA and DNA, respectively. Intestinal poly-

nucleotidases or phosphoesterases supplement the action of pancreatic nucleases in producing mononucleotides from fragmented nucleic acids. The liberated nucleotides are then hydrolysed to nucleosides by alkaline phosphatase and nucleotidases, and may be further broken down by nucleosidases. The mammalian nucleosidases are chiefly phosphorylases which cleave the glycosyl linkage to yield purine and to a lesser extent pyrimidine and (desoxy)ribose-1-phosphate. The sugar phosphates are reused for nucleotide synthesis.

Pyrimidine nucleosides are not extensively cleaved by enzymes of the intestine, and the ribosyl-pyrimidines are absorbed intact and utilised for the synthesis of tissue nucleic acids. The known mammalian nucleosidases readily cleave inosine (ribosyl-hypoxanthine) and guanosine. Adenosine is converted by adenosine deaminase to inosine, and the net result leads to purines which are extensively catabolised to uric acid. Nucleic phosphorylases with high activity are responsible for the decomposition to free bases. A high xanthinoxidase activity in the mucosa of the small intestine oxidises most of the purine bases to uric acid [Montag et al., 1989]. Chinsky et al. [1990] found that adenosine deaminase was one of the most abundant proteins of the epithelial lining of the alimentary mucosa in mice. Levels were low at birth but achieved very high levels within the first few weeks of life. Thus dietary DNA, nucleotides, nucleosides, and bases are readily degraded in both the gastric and small intestine compartments.

Investigations in animals suggest that after duodenum passage, over 95% of DNA was hydrolysed and bases are absorbed into the enterocyte. A few parts of the nucleic acids remain as non-diffusible oligonucleotides. There is one observation that, in mice, about 1–2% of orally ingested phage M13mp18 DNA persists transiently. Fragments between 100 and 400 bp in size (and only rarely fragments up to 1,700 bp) were found in the gut and faeces 1 and 7 h after feeding [Schubbert et al., 1997]. DNA present in genetically modified tomatoes was recovered at approximately 60% at the end of the ileal compartment by using an in vitro GI tract model TIM-1 [Van der Vossen et al., 1998]. In the colon, DNA is less rapidly digested and could therefore be available for transformation of competent cells in the micro-flora. However, the introduction of a broad host-range plasmid into the micro-flora of the colon model TIM-2 did not yield transformants at a detectable level. Transformation experiments with DNA from modified tomato and competent *E. coli* cells showed no transformants at the limits of the PCR detection system.

## Absorption

Transport of nucleosides into the enterocyte occurs via both facilitated diffusion and specific Na<sup>+</sup>-dependent carrier-mediated mechanisms [Bronk and Hastewell, 1987; Jarvis, 1989]. The upper region of the small intestine has the greatest absorptive capacity. Once absorbed, most of the nucleosides and bases are rapidly degraded within the enterocyte, and catabolic products such as uric acid are excreted in the urine and intestine [Salati et al., 1984].

Despite extensive catabolism, tracer studies in animals indicate that only 2–5% of dietary nucleotides are incorporated into tissue pools, primarily within the small intestine, liver, and skeletal muscle [Burridge et al., 1976]. Incorporation into tissues is reportedly increased at younger ages. Extensive salvage of purines and pyrimidine nucleotides has been demonstrated in intestinal tissues. Adenine is the most extensively reutilised purine, particularly during the fasted state. In contrast, other purines are extensively degraded to uric acid in the gut. Further, up to 20% of orally administered adenine may be recovered unmetabolised in the portal vasculature [Salati et al., 1984]. Schubbert et al. [1994] reported that a portion of <0.1% of ingested phage M13 DNA was found as fragments (between <200 and 976 bp) in the blood stream, in liver and in different spleen cells in mice (i.e. tissues of the immune system). However, the apparently high rate of uptake and persistence of DNA in leukocytes and even in foetal tissues may be related to the un-methylated CpG sequences in the *E. coli* DNA used [Beever and Kemp, 2000].

There is no reason to suppose that the in vivo hydrolysis and absorption of DNA and recDNA will be different.

## Toxicological Considerations

There is a need for dietary nucleotides in neonatal humans for the first few weeks of life and this need is usually met by the higher nucleotide content of human milk compared to that in cow's milk. However, about 20% of the adult population of the western world suffer from hyperuricaemia, 5–10% of them being taken ill with gout [Colling and Wolfram, 1987]. Apart from genetic disposition, the nucleic acid content of the diet has an important influence on the serum uric acid level. Meat and offal are the key sources of high nucleic acid intakes in Europe and the US.

It has been shown using formula diets that not only the content of orally taken nucleotides, but also their qualitative composition, can influence the serum uric acid level. Increase of serum uric acid level after RNA intake is twice as high as after intake of the equivalent amount of DNA [Zöllner et al., 1972].

Toxicology studies demonstrated that nucleotides fed to animals at 8% of the diet for several months did not produce adverse effects seen as significant by the authors [Kojima, 1974]. Oral intake of nucleotides, nucleosides, and nucleic acids increases serum and urinary degradation products in animals and adult humans. Dietary nucleic acids have the greatest influence upon serum uric acid levels and a maximum safe limit of RNA in the diet of 2 g/day has been suggested [Griebsch and Zöllner, 1974]. Foods high in nucleic acids, such as glandular meats, increase the uric acid output. Adenosine and its derivatives normally reach hypoxanthine and uric acid through inosine, but free adenine, if present in large amounts, is oxidised in part directly to 2,8-dihydroxyadenine, and this may cause kidney damage through deposition of crystals in the tubules. Alterations in tissue levels of very long chain fatty acids and serum levels of lipoproteins have been reported in term and pre-term infants fed nucleotide-supplemented formula [Sanchez-Poza et al., 1986].

Yeast extracts, rich in 5'-ribonucleotides, in particular 5'-GMP and 5'-IMP, are used in the food industry as taste enhancers, in soups and snacks. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated these food additives in 1974 and concluded that an acceptable daily intake (ADI) had not to be specified [JECFA, 1993]. In addition, nucleotides have been evaluated by the EU Scientific Committee for Food as food additives and for use in infant formulae and follow-on formulae [SCF, 1991] and are permitted for both purposes in the EU, in the latter case to a maximum level of 1.2 mg/100 kJ [European Commission, 1996].

It has been recommended that a limit be placed on the amount of nucleic acids that should be introduced into human diets from single cell proteins [PAG, 1975]. At the time, it was foreseen that the single cell protein intake by some individuals could be considerable and, if unprocessed, single cell proteins may contain around 10% of RNA and/or DNA. However, gout is essentially the only health problem associated with the consumption of non-viral DNA.

In conclusion, DNA in amounts found in typical human diets is not toxic. Since recDNA and DNA have the same structure and are composed of the same building blocks, there is no indication that toxicological properties of recDNA might differ from conventional DNA. Thus, there are unlikely to be any differences in the risk of gout in people fed GM or non-GM crops or from the consumption of products of animals fed GM or non-GM crops.

### *Human Defence Systems*

Investigators have demonstrated the role of dietary nucleotides in the maintenance of cellular immune response. While the mechanism is unclear, data suggest that exogenous nucleotides supplied by the diet contribute to the pool of nucleotides available to stimulated leukocytes, which rapidly turnover and thus have increased nucleotide requirements. Some studies suggest that proliferating lymphocytes require an exogenous supply of nucleotides for optimum function [Marijnen et al., 1989]. Orally administered RNA had no effect against methicillin-resistant *Staphylococcus aureus* infection in mice [Adjel et al., 1993]. Van Buren et al. [1985] proposed that dietary nucleotides exert effects upon immune responsiveness by acting upon the T helper/inducer population with the predominant effect upon the initial phase of antigen processing and lymphocyte proliferation. The presumed mechanism is suppression of uncommitted T lymphocyte responses, as demonstrated by higher levels of a specific intracellular marker for undifferentiated lymphocytes in primary lymphoid organs in mice fed a nucleotide-free diet [Rudolph et al., 1986].

Dietary nucleotides may produce in vivo effects upon humoral immunity. Specific antibody responses to T cell-dependent antigens were significantly decreased in mice fed a nucleotide-free diet [Zhang et al., 1993]. Responses to T cell-independent antigens and to a non-specific polyclonal B cell activator, however, were not affected. Dietary nucleotide enhancement of immunity may be particularly important for individuals at increased risk of acquiring infections.

The mechanisms of dietary nucleotide effects upon immunity are unknown. Most dietary nucleotides are readily metabolised and excreted, however, a significant proportion of retained nucleotides are found in gastrointestinal tissues. Gut-associated lymphoid tissue can initiate and regulate T-cell development and may act as a thymus analogue [Mosley and Klein, 1992]. Dietary nucleotide effects upon peripheral immunity may be mediated in part via effects upon this important, but poorly understood, immune tissue. Dietary nucleotides are also reported to have significant effects upon lymphoid, intestinal and hepatic tissues, and lipid metabolism.

There is no indication that DNA per se, or specific sequences such as might be found in DNA/recDNA have allergenic or other immunological properties that would be of relevance for dietary aspects of these materials. Protein-related allergies are typically associated with larger molecular units than nucleotide sequences of DNA [Busch and Helge, 1996]. Moreover, the possibility of

recDNA to bind to protein to form a hapten is the same as for DNA. It has been demonstrated that unmethylated microbial DNA is immuno-stimulatory [Yi and Kreig, 1998; Klinman et al., 1997; Sato et al., 1996].

### **Safety of DNA – Functional Effects**

Gene transfer from organisms used as food poses a number of potential food safety concerns from the possible transformation of gut microorganisms, gut epithelial cells and human stem cells. In particular, (i) transformation, although not resulting in expression, might interfere with existing cell metabolism and (ii) transformation, if accompanied by expression, might lead to the production of new products or the suppression of existing products, with adverse consequences. The latter may be of particular concern for GMO containing genes that encode proteins not normally found in food organisms (e.g., genes encoding antibiotic resistance). However, there is no evidence that gut epithelial cells or human stem cells are transformed by food DNA with or without expression of any foreign genetic material. Indeed, if this were the case, the epithelium would routinely express many foreign genes and human stem cells would have many genes showing significant homology with all types of plant genes and there is no evidence for either.

In addressing potential concerns from possible transformation, it is first necessary to understand the mechanisms by which DNA is transferred between cells, and then integrated and expressed.

#### *Gene Transfer*

##### *Horizontal Gene Transfer in Bacteria*

##### Conjugation, Transduction, and Transformation

Promiscuous gene transfer provides a mechanism for the availability of a huge pool of genes for bacterial evolution [Guiney, 1993]. Indirect – but nevertheless impressive – evidence for gene transfer can be drawn from published genome sequences, where highly conserved DNA regions and sequences encoding prophages indicate recent DNA transfer events. The *Bacillus subtilis* genome may serve as an example for this kind of evidence [Kunst et al., 1997].

An example of gene transfer based on the selective value of acquired genes is the development of resistance to antibiotics in microorganisms in humans as well as in animals [DeFlaun and Levy, 1989; Perreten et al., 1997;

European Commission, 1999]. However, such transfers require either a replicative mechanism or the insertion into the genome of the complete resistance determinant gene. In addition, expression elements are required and selection pressure is needed to allow proliferation of the transformed organism. Most genetically modified plants made to date do not contain ORIs or phage sequences and thus gene transfer from these plants to bacteria requires proper insertion in the genome. Even under conditions that strongly favour transfer, deVries and Wackernagel [1998] and Gebhard and Smalla [1998] were only able to demonstrate rare transfer in the situation where there would be homologous recombination, with a short deletion in *nptII*, and under strong selection pressure. These experiments and others [Schlüter et al., 1995; Nielsen et al., 1997 and reviewed in Dröge et al., 1998 and Nielsen et al., 1998] indicate that the transfer of an antibiotic resistance marker gene from a genetically modified plant to bacteria that do not already contain the same resistance gene is extremely unlikely.

Gene transfer may occur by one of three mechanisms: conjugation, transduction, or transformation. Several books and reviews on gene transfer have been published [Clewel, 1993; Levy and Miller, 1989; Lorenz and Wackernagel, 1994; Gauthier, 1992].

Bacterial conjugation mediates genetic exchange not only between cells of the same species but also between members of distantly related or even unrelated genera [Guiney, 1993, Bertram et al., 1991, Heinemann and Sprague, 1989]. It requires a complex set of trans- and cis-acting factors within the donor cell [Ippen-Ihler, 1989; Ippen-Ihler and Skurray, 1993], which are usually located on large plasmids, called conjugative plasmids. Transfer of chromosomal DNA can be mediated after integration of a conjugative plasmid into the chromosome [Low, 1987].

Bacteriophage-mediated transfer of non-phage DNA between bacteria is called transduction. Two types of transduction, generalised and specialised, are distinguished [Margolin, 1987; Weisberg, 1987]. Generalised transduction results from infrequent, accidental packaging of chromosomal as well as plasmid DNA of the host cell into phage heads [Margolin, 1987; Lefringhausen, 1996]. Specialised transduction, which is restricted to temperate phages only, results from an imprecise excision of the prophage genome upon induction [Weisberg, 1987].

Natural genetic transformation is characterised by the active uptake by a cell of free, extracellular DNA (chromosomal and plasmid) and the heritable incorporation of

its genetic information [Lorenz and Wackernagel, 1994]. Cells capable of actively taking up DNA from the environment are called competent. During competence only a single DNA strand enters the cytoplasm while the other strand is degraded. The list of naturally competent species is steadily growing. However, amongst microbial species, the proportion of species known to exhibit natural competence is limited to about 1 or 2%, based on the number of ca. 4,000 validly described bacterial species (<http://www.dsmz.de>) and the number of species known to be naturally competent [Lorenz and Wackernagel, 1994]. In some cases only DNA with a very specific sequence, e.g., *Haemophilus influenzae* [Sisco and Smith, 1979], or specific codons can be taken up [Berche, 1998; Nielsen et al., 1998].

There exist some peculiar natural plasmid transformation systems for which cell-to-cell contact appears to be necessary [Paul, 1992]. Transfer occurs with heat-killed donor cells, but is inhibited by nalidixic acid and rifampicin. However, although cell-cell contact is necessary, transfer occurs by transformation and not by conjugation, since the process is DNase-sensitive. In the presence of appropriate salts (e.g. CaCl<sub>2</sub>) duplex DNA, and also plasmids, can penetrate the cell wall of *E. coli* K12 [Mandel and Higa, 1970], a situation that may occur under natural conditions [Baur et al., 1996].

#### Integration of DNA into the Genome of Bacteria

*Physical Integration of Chromosomal DNA.* Heritable incorporation of transferred chromosomal DNA requires the physical integration into the chromosome of the recipient cell. In closely related donor and recipient cells the degree of sequence homology is very high. Thus, integration by homologous recombination can occur at any site of the chromosome. With increasing phylogenetic distance the sites for homologous recombination become rarer. Insertion sequences or transposons, as well as flanking bacterial linker sequences, may provide sites for recombination. Homologous recombination has been observed between unrelated microorganisms within rRNA genes [Strätz et al., 1996] which decreases in frequency with increasing sequence divergence. Recently it was shown that the absence of a homologous sequence in the recipient bacteria decreased the integration of a gene (*nptII*) by more than 10<sup>9</sup>-fold to below the detection limit [deVries et al., 2001]. However, it should be noted that *Campylobacter coli* is able to take up and integrate non-homologous plasmid DNA, albeit with very low efficiency of about 3 × 10<sup>-13</sup> transformants per microgram of DNA [Richardson and Park, 1997].

The genetic information of plasmids that cannot replicate in the recipient cell may be rescued by physical integration into the chromosome. All mechanisms that facilitate rearrangements in the genome of an organism may facilitate integration of foreign DNA into the chromosome. The systems, e.g. insertion elements, transposons [Berg et al., 1989], site-specific recombination systems like DNA inversion or phage integration systems, may either be located on the chromosome of the recipient cell or on the plasmid. Homologous as well as illegitimate recombination [Ehrlich, 1989] may be involved. However, while DNA involved in rearrangements within an organism is stable, transferred foreign DNA may be less stable. Thus, the probability of such DNA being integrated into the genome is much lower than the probability of rearrangements occurring by the same recombination mechanism.

*Establishment of Plasmids.* Because of their self-replicating nature, plasmids do not have to be integrated into the chromosome in order to become part of the genome of the recipient cell. Depending on their replication functions and their selective markers, plasmids show either a broad or a narrow host range [Guiney, 1993]. Such plasmids may either be natural isolates or constructs obtained through genetic engineering. In the latter case, different origins of replication and antibiotic resistance genes are often combined to allow replication in distantly related bacterial species.

If plasmids are transferred as linear molecules, either co-integrated in chromosomal DNA or as concatemers, they have to undergo recombination in order to become re-established as a self-replicating unit. Such recombinations often result in alterations of the physical structure of the plasmid, e.g. creation of deletions [van der Lelie and Venema, 1987; Lefringhausen, 1996].

*Expression of DNA.* The expression of DNA depends on its topology and requires transcription, translation, and proper folding of the polypeptides synthesised. Whether transferred DNA is expressed in the recipient cell, therefore, depends on the physiology of the recipient cell and on the compatibility of transcription and translation signals of the incoming DNA with the corresponding transcription/translation machinery of the recipient cell.

Cells from extreme environments have different demands for the supercoiling of DNA. The supercoiled structure of DNA molecules may significantly influence the availability of transcription signals.

The basic structure of promoters – or more specifically, the structure of housekeeping promoters – is more or less universal among prokaryotic microorganisms. However,

in order to be recognised by RNA polymerase many promoters need different sigma factors, host factors which are involved in DNA bending, or regulatory proteins which are needed for induction or repression of transcription. Thus, expression of many promoters will only proceed in the proper way if the correct factors are present.

Translation initiation signals are basically the same among all microorganisms. The genetic code is universal. Therefore, any gene should be recognised as such in any organism and should be translated. However, the frequency of codon usage which is the consequence of the availability of tRNA species within the cell, may vary considerably. In some cases this may prevent translation, because not all tRNA species needed for translation are present in the cell.

Proper folding of the polypeptide chain is essential for proteins to function correctly. Folding and/or stability of proteins have to be adapted to the environment the organism is living in. This is immediately obvious for the so-called extremophiles. Many proteins from organisms living in moderate environments will not be able to function correctly at, e.g., the extreme temperatures some other organisms are living in.

Finally, whether DNA will be expressed and to what extent, depends – in addition to the above – on its integration into the genome. Transferred DNA that is not integrated, will – at best – only be expressed transiently. The dependence for expression on physical integration is immediately obvious for linear DNA, since such DNA is usually very unstable. However, physical integration into the chromosome requires two cross-over events to occur at the same time. The minimal length of homologous DNA required for a cross-over to occur is species specific; it ranges from 20–40 bp in *E. coli* [Watt et al., 1985] to about 200 bp in *Bacillus subtilis* [Michel et al., 1983] and 280 bp in *Campylobacter coli* [Richardson and Park, 1997]. When depending on such short sequences, a cross-over becomes a very rare event [Richardson and Park, 1997; Lloyd and Low, 1996; Weinrauch and Dubnau, 1983], the frequency of recombination being as low as about  $10^{-6}$  per cell and generation-time [Lloyd and Low, 1996]. For two recombinations to occur at the same time, the frequency is the product of the frequency of the single event, i.e. about  $10^{-12}$  in the latter case. However, the final value and thus the probability by which integration into the genome will occur is influenced by negative interference, degree of homology, physical nature of the DNA construct etc.

**Barriers to the Transfer of DNA.** One important barrier to DNA transfer is restriction [Lorenz and Wackernagel, 1994]. Transduction and transformation seem to be the

transfer mechanisms susceptible to restriction. However, when taken up by transformation, chromosomal DNA appears to be less sensitive to restriction than plasmid DNA [Lorenz and Wackernagel, 1994]. Sensitivity or insensitivity to restriction depends on the physical nature of the DNA taken up into the cytoplasm. Restriction enzymes are only able to cleave double-stranded DNA. Thus, all mechanisms based on the transfer of single-stranded DNA may be less sensitive to restriction.

The development of competence governs DNA uptake by transformation. If environmental factors favour induction of competence DNA can be transferred at a considerable rate.

Besides the often advantageous outcome of a gene transfer event, deleterious effects may also occur. One general aspect is the induction of a prophage following uptake and integration of foreign DNA [Lorenz and Wackernagel, 1994]. Other aspects may be rather specific for the genes transferred. The integration of DNA fragments may, for example, result in gene disruption, gene silencing, alteration of gene expression, and expression of altered (fusion) proteins. However, every event resulting in reduced fitness of the recipient will reduce the rate of formation of transformed cells.

An important aspect of plasmid transfer is the phenomenon of incompatibility. Plasmids are incompatible when they share the same replication system. Thus, a resident plasmid may interfere with the establishment of a foreign plasmid. Incompatibility is best known for plasmids of the ColEI- and IncFII-group [Helinski et al., 1996]. Besides, it is an important tool for the differentiation of the different plasmid groups.

A barrier observed in conjugation only is the phenomenon of surface exclusion. It inhibits transfer of F-factor or similar conjugative plasmids to cells harbouring the same factor [Ippen-Ihler and Skurray, 1993].

A barrier to transduction is the absence of the proper phage receptor on the cell surface. However, it has to be noted that DNA transfer is not strictly restricted to host bacteria of the phage. It is often observed that the host range for DNA transfer is broader than the host range for phage propagation [Lefringhausen, 1996]. However, transducing DNA delivered by phages can be subject to DNA restriction.

Finally, and most importantly, stable and heritable incorporation of heterologous DNA into the genome of a recipient cell will only occur if there is homology between the heterologous DNA and that of the recipient and is most likely if the transformation offers a selective advantage to the recipient cell. Such a selective advantage could

theoretically arise through the formation of a new gene product expressed by the transferred gene or through the suppression of an existing gene product, e.g. through disruption of an existing gene. Experimentally it has been shown that under optimised conditions during transformation, the integration chance of kanamycin resistance gene into a bacterial genome was less than  $10^{-13}$  per *nptII* gene [Wackernagel and deVries, 2000].

*DNA Transfer from Food.* Gene transfer with respect to food systems has only been studied in few instances [Bräutigam et al., 1997; Zenz et al., 1998; Hertel et al., 1995; Kleinschmidt et al., 1993]. The data show that transfer by all of the three mechanisms outlined earlier is potentially possible in foodstuffs, at least from microorganisms consumed as food. However, as can be expected, the food matrix plays a key role in gene transfer, and while one kind of transfer may be possible in a given food matrix the other kind(s) may be excluded. Recently it has been shown that *Bacillus subtilis* is able to develop natural competence within certain milk products [Bräutigam et al., 1997; Zenz et al., 1998]. Indirect evidence for gene transfer to occur in lactic acid bacterial co-cultures comes from studies analysing the distribution of specific IS elements [Guédon et al., 1998]. The observed sequence similarities are very indicative for recent gene transfer events to have occurred between *Lactococcus*, *Streptococcus*, *Lactobacillus*, *Leuconostoc*, and *Enterococcus*. The results of Perreten et al. [1997] on the transfer of antibiotic resistance genes are very much along this line.

The gut, and the colon in particular, is likely to be one of the most important environments for gene transfer, because of its high density of microorganisms. Direct cell-cell contact favours conjugation, and natural transformation is favoured because of the relatively high DNA concentration at the recipient cell's surface [Paul, 1992]. Despite its importance for gene transfer, studies on gene transfer in the human or animal gut are rather limited. However, at least for naked DNA, there is only a very low probability per gene and per passage through the GI tract, of uptake and stable integration into the genome of a bacterial cell. This is mostly due to the degradation of the DNA during the gastric and ileal passage, which makes it highly unlikely that linear DNA molecules of sufficient size enter the colon as well as the enterocytes, which food molecules must traverse to be absorbed systemically. For transformation by linear DNA there must also be homology between the heterologous DNA and that of the bacterial cell. To ensure the spread of the trait in the bacterial population it must confer a competitive advantage to any transformed cells.

Mercer et al. [1999] have demonstrated that DNA released from bacteria or food sources in the mouth has the potential to transform naturally competent oral bacteria with whole or linear plasmids, although the frequency of transfer in vivo has yet to be established.

#### *Horizontal Gene Transfer from Plants to Bacteria*

There is no well-documented evidence for gene transfer from plants to bacteria. For transfer and functional integration, a large number of discrete steps would be required, each of which would be expected to occur only extremely infrequently. One reason is the lack of significant homologies between bacterial and plant nuclear DNA. However, for bacterial DNA and plant chloroplast and mitochondrial DNA, higher homologies are observed [Markmann-Mulisch and Subramanian, 1988; Andersson et al., 1998]. In agreement with the endosymbiont theory, homologies are highest between chloroplast and cyanobacterial DNA [Andersson et al., 1998] and between mitochondrial and obligate intracellular parasite (*Rickettsia*) DNA. However, due to the evolutionary very long separation, DNA homologies are less significant between the organelles and the presently living cyanobacteria and *Rickettsia* than between related species of bacteria [Andersson et al., 1998]. Furthermore, many bacteria have restriction enzymes that reduce the probability of viral DNA infection and which might be expected to cleave certain foreign DNA. Even if integration into the bacterial genome occurred, expression of the newly introduced gene would not be expected due to the lack of proper microbial expression signals on the plant DNA.

DeVries and Wackernagel [1998] studied the ability of *Acinetobacter* sp. strain BD413, which carried an incomplete *nptII* gene, to take up and integrate transgenic plant DNA under optimised laboratory conditions. Restoration of *nptII* was observed with plasmid DNA and plant DNA carrying the *nptII* gene from seven transgenic crop plants including potato, tomato, sugarbeet, oilseed rape and tobacco. Similar results were obtained by Gebhard and Smalla [1998] using sugarbeet DNA and sugarbeet homogenates. In some transformants not only was the gene restored but additional DNA obtained. However, this relatively rare event occurred only by homologous recombination [Salayers, <http://www.healthsci.tufts.edu/apua/salayersreport.htm>]. Similar observations were also made using *Pseudomonas stutzeri* as a recipient for transgenic plant DNA [deVries et al., 2001].

Schlüter et al. [1995] used a model system to study the frequency of horizontal gene transfer from a plant (transgenic potato, containing a  $\beta$ -lactamase gene linked to a pBR322 origin of replication) and a tightly associated bacterial pathogen (*Erwinia chrysanthemi*). From the in vitro experiments, the frequency of transformation was estimated to be extremely low or non-existent ( $<2 \times 10^{-17}$  per cell) under natural conditions.

Selective screening of soil bacteria from field trials of genetically modified plants containing antibiotic resistance marker genes have shown no bacterial transformants [Becker et al., 1994; Smalla, 1995; Smalla et al., 1994]. These studies are particularly relevant to gene transfer in the gut since many soil organisms are carried into the gut on food or colonise the gut. Since soil can act as a gene reservoir, thus enhancing transfer, it can be predicted that transfer in soil is likely to occur, if at all, at a higher rate than in the gut.

#### *Uptake of DNA by Mammalian Cells*

Functional gene transfer from bacteria to mammalian cells is possible in cell culture using genetically optimised constructs [Grillot-Courvalin et al., 1998]. However, gene transfer from plants to mammalian cells has not been reported. Most mammalian cells that might be expected to take up naked or bacterial DNA produce at least one DNase with exonuclease activity and these would be expected to degrade most exogenous DNA. However, if the exogenous DNA is not degraded, the consequences are likely to be transitory due to the intervention of cell repair mechanisms as discussed earlier.

A study, tracing by fluorescent in situ hybridisation has indicated that when orally administered to mice, M13mp18 DNA fragments were taken up by intestinal wall epithelia and reached the nuclei of leukocytes and spleen and liver cells. The isolation of recombinant clones can be interpreted to suggest that phage DNA fragments were covalently linked to mouse DNA [Schubbert et al., 1997]. Later studies [Doerfler and Schubbert, 1998; Schubbert et al., 1998] showed that foreign DNA can be found in the foetuses and new-born mice of pregnant mice consuming the foreign DNA fragments. This DNA is invariably found in the nuclei and never in all of the cells of the foetus suggesting a transplacental pathway rather than germline transmission. Schubbert et al. [1998] concluded that naked DNA from the dam had crossed the placenta and had been taken into foetal somatic cells. However, Beever and Kemp [2000] have postulated that

leukocytes of the dam phagocytosed the DNA before crossing the placenta to enter the circulatory system and ultimately foetal tissue. These are rare events based on the amount of DNA used in the gavage studies and the numbers of cells shown to contain M13 or plasmid DNA and there was no evidence of long pieces of DNA equivalent to cDNA, or of gene expression.

It has been documented in several biological systems that plant [Ledoux and Huart, 1971] and mammalian cells [Groneberg et al., 1975; Bhargava and Shaninugam, 1971] can take up foreign DNA and incorporate it into their genomes. Considering the exposure to foreign DNA, entire genomes or fragments of them in the course of the evolution, it is suggested that mammalian cells have developed several mechanisms of defence against the uptake, integration, and continued expression of foreign DNA. The defence mechanisms are thought to be: (i) degradation and/or excretion of foreign DNA, (ii) excision and loss of previously integrated DNA from the host genome, and (iii) targeted inactivation of foreign genes by sequence-specific methylation [Doerfler, 1991]. Furthermore, the nuclear membrane is a very strong barrier against the penetration of proteins and nucleic acids. There is evidence that entry is tightly regulated by nuclear pores and that nuclear localisation signals are required for penetration, especially in the case of cells that have terminated their division and in which the nuclear envelope is not disrupted [Zeimienowicz et al., 1999; Guralnick et al., 1996; Saphire et al., 2000; Popov et al., 1998; Palacios et al., 1997; Collas and Alestrom, 1997; Gorlick and Mattaj, 1996].

A number of studies have examined the take up by mammalian cells of DNA from the diet. Thus, Klotz and Einspanier [1998] examined the transfer of foreign DNA from the diet into the meat and milk of domestic animals via Peyer's patches in the small intestine and lymphatic system. They were able to detect the presence of soya DNA in the blood of cattle fed with genetically modified soya and also in the white corpuscles isolated from it although the novel gene was not detected. Soya DNA was not found in the milk produced by the cattle. In a more recent study of cattle and chickens fed genetically modified modified maize under normal feeding conditions, it was found [Einspanier et al., 2001] that the presence of short DNA fragments ( $<200$  bp) derived from plant chloroplasts could be detected in the blood lymphocytes of cows. In all other cattle organs investigated, plant DNAs were not found although there were faint traces in milk. Maize chloroplast gene fragments were found in all chicken tissues examined, but not in eggs. Fragments originat-

ing from the novel gene were not detected in any of the cattle or poultry samples.

Taking into account the tremendous flow of DNA via food into the GI tract of mammals, the detection of traces of fragments of degraded DNA after penetration through the epithelial lining in mice described by Schubbert et al. [1994, 1997] is not as surprising as it seems at first glance. Actually, these results could be considered as reassuring arguments in the discussions on the presence of recDNA if they turned out to be generally valid. The only transient detection of DNA fragments and the time course of the degradation of M13 DNA after incubation with faecal suspensions or in mouse whole blood [Schubbert et al., 1994] indicate the availability of the enzymatic repertoire of mammals to degrade foreign DNA (fragments). There is no evidence to suggest that the movement of DNA in general from food into the blood is anything other than a random event and the probability of recDNA being present in the blood of a human consumer is correspondingly very small. Furthermore, many genes used for the genetic modification of food organisms come from organisms for which there is a history of human exposure.

In conclusion, DNA in food, including recDNA, is likely to be broken down during food processing and during passage through the human gastrointestinal tract. If fragments of DNA or recDNA from food enter the blood stream, existing defence mechanisms of the body help to dispose of it.

### **Food Safety Consequences of Gene Transfer**

As discussed earlier, recDNA and DNA are composed of the same building blocks linked together in the same way. There is no reason to suppose that, were it to occur, transformation per se by DNA from a food organism to gut microorganisms, gut epithelial cells or human stem cells is anything other than a random event determined in part by sequence homology between the exogenous DNA and that of the recipient cell. Thus, since recDNA comprises only a minute proportion of the DNA in a food organism the proportion of transformed cells containing recDNA will be correspondingly small.

As indicated previously, mammalian cells have developed sophisticated methods to excise and destroy exogenous DNA, furthermore, the cells most immediately at risk, the gut epithelial cells, are rapidly lost from the human gut. Thus, food safety concerns from transformation focus on the transformation of gut microorganisms through the incorporation of whole genes or of DNA frag-

ments that combine with other sequences. Without stable and heritable incorporation of the heterologous DNA any safety concerns arising from transformation, if it occurred, will be transient. Stable and heritable incorporation of heterologous DNA into the genome of a recipient cell will only occur, if the transformation offers a selective advantage to the recipient cell. Such a selective advantage could theoretically arise: (i) through the formation of a new gene product expressed by the transferred gene or (ii) through the suppression of an existing gene product through disruption of an existing gene.

The risk from the integration of fragments of recDNA is no different from that of other DNA. Selective advantage through the formation of a new gene product, if any, will only hold true for a very limited number of possible genetic modifications, since the modifications have been constructed for entirely different reasons. Thus, in food systems, selection markers (e.g. antibiotic resistance markers) effective under laboratory conditions lose their selective advantage.

Although bacteria have developed sophisticated systems for DNA uptake and horizontal DNA exchange, horizontal transfer into and expression of naked recDNA present in food in a recipient cell can only be an extremely rare event. The presentation of general quantitative data is not possible, because transfer and incorporation rates for DNA vary greatly with different organisms and under different environmental conditions. However, based on the potential selective advantage, the physical structure, the most likely mechanism of transfer, and the microorganisms involved in the transfer of the recDNA molecules, a relatively reliable prediction of the magnitudes of transfer and incorporation rates is possible. Thus, Redenbaugh et al. [1994] concluded that consumption of the Flavr Savr™ tomato containing a gene for resistance to the antibiotic kanamycin would lead to a maximum projected increase in the number of kanamycin resistant bacteria in the gut of  $2.6 \times 10^{-13}\%$ .

RecDNA is equivalent to DNA which has always been consumed with human diets. Any risks from the consumption of recDNA are, per gene and per passage through the GI tract, the same as those from the consumption of DNA since DNA and recDNA are handled by the body in the same way. The work presented here shows that the likelihood of transfer and functional integration by gut microflora and/or human cells, of DNA from ingested food is minimal; the breakdown of DNA during food processing and passage through the GI tract reduces the likelihood that intact genes capable of encoding foreign proteins will be transferred to gut microflora. The

source of the DNA (i.e., whether it is from food or non-food organisms, synthetic or recombinant) is immaterial. However, it is prudent that the food safety consequences of the transfer of recDNA from food to human gut microflora should be assessed on a case by case basis when the recDNA is not known to be present in organisms safely consumed as/with food. The assessment should take into account the human consequences of foreign protein expression, in the unlikely event that it might occur, based on its properties and evidence from previous human exposure. If food safety consequences cannot be ruled out, the probability of gene transfer, integration and expression will require further investigation.

It is important that genes used for the genetic modification of food source organisms are selected with due regard to their possible food safety consequences. New technologies make it possible to use genes (e.g., as marker genes) that are less likely to present food safety concerns than hitherto.

## Conclusion

DNA from GMOs is equivalent to DNA from existing food organisms that has always been consumed with human diets. Any risks associated with the consumption of DNA will remain irrespective of its origin because the body handles all DNA in the same way. The breakdown of DNA during food processing and passage through the gastrointestinal tract reduces the likelihood that intact genes capable of encoding foreign proteins will be transferred to gut microflora. The work presented here shows that the likelihood of transfer and functional integration of DNA from ingested food by gut microflora and/or human cells is minimal.

Recombinant DNA techniques are capable of introducing into food organisms genetic changes that are more predictable than those introduced through conventional breeding techniques. Information reviewed in this paper did not indicate any safety concerns associated with the ingestion of DNA per se from GMOs resulting from the use of currently available recombinant DNA techniques in the food chain. Therefore, such DNA from GMOs is considered to be as safe as any other DNA in food.

## Appendix: List of Participants

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## References

- Adjei, A.A., Takamine, F., Yokoyama, H., Shiakowa, K., Matsumoto, Y., Asato, L., Shinjo, S., Imamura, T. and Yamamoto, S. (1993) The effects of oral RNA and intraperitoneal nucleoside-nucleotide administration on methicillin-resistant *Staphylococcus aureus* infection in mice. *J. Parenter. Enter. Nutr.* 17:148–152.
- Agrawal, S. (1996) Antisense oligonucleotides: towards clinical trials. *Trends Biotechnol.* 14: 376–387.
- Andersson, S.G.E. et al. (1998) The genome sequence of *Rickettsia prowazekii* and the origin of mitochondria. *Nature* 396:133–140
- Baur, B., Hanselmann, K., Schlimme, W., and Jemmi, B. (1996) Genetic transformation in freshwater: *Escherichia coli* is able to develop natural competence. *Appl. Environ. Microbiol.* 62: 3673–3678.
- Becker, J., Siegert, K., Logemann, J. and Schell, J. (1994) Begleitende Sicherheitsforschung zur Freisetzung gentechnisch veränderter Petunien Bd. 3. pp 563–578, in Bundesministerium für Forschung und Technologie (Ed.), Biologische Sicherheit. *Forschung Biotechnologie.*
- Beever, D.E. and Kemp, C.F. (2000) Safety issues associated with the DNA in animal feed derived from genetically modified crops. A review of scientific and regulatory procedures. *Nutrition Abstracts and Reviews*, 70(3):197–204.
- Berche, P. (1998) Les Plantes transgéniques et la résistance aux antibiotiques. *Médecine Thérapeutique*, 4, 9, Novembre.
- Berg, C.M., Berg, D.E. and Groisman, E.A. *Transposable Elements and the Genetic Engineering of Bacteria*, pp. 879–925 (1989) In *Transposable Elements*, pp. 799–832 (Eds. D.E. Berg and M.M. Howe) ASM Press, Washington D.C.
- Bertram, J., Strätz, M. and Dürre, P. (1991) Natural transfer of conjugative transposon Tn916 between Gram-positive and Gram-negative bacteria. *J. Bacteriol.* 173:443–448.
- Bhargava, P.M. and Shaninagam, G. (1971) Uptake of Nonviral Nucleic Acids by Mammalian Cells. *Progr. Nucl. Acid Res.* 11:103–192.
- Bräutigam, M., Hertel, C. and Hammes, W.P. (1997) Evidence for natural transformation of *Bacillus subtilis* in foodstuffs. *FEMS Microbiol. Lett.* 155:93–98.
- Bronk, J.R. and Hastewell, J.G. (1987) The transport of pyrimidines into tissue rings cut from rat small intestine. *J. Physiol.* 382:475–488.
- Burrige, P.W., Woods, R.A. and Henderson, J.F. (1976) Utilisation of dietary nucleic acid purines for nucleotide and nucleic acid synthesis in the mouse. *Can. J. Biochem.* 54:179–190.
- Bush, R. and Hefle, S. L. (1996) Food Allergens. *Crit. Rev. Food Sci. Nutr.* 36, Supplement, 119–164.
- Calloway, D.H. (1969) Safety of Single-Cell Protein, FAO/WHO/UNICEF Protein Advisory Group, Document 2.23/2, United Nations, New York.
- Carver, J.D. and Walker, W.A. (1995) The role of nucleotides in human nutrition. *Nutr. Biochem.* 6:58–72.
- Chinsky, J.M., Ramamurthy, V., Fanslow, W.C., Ingolia, D.E., Blackburn, M.R., Shaffer, K.T., Higley, H.R. and Rundolph, F.B. (1990) Developmental expression of adenosine deaminase in the upper alimentary tract of mice. *Differentiation* 42:172–183.
- Clewell, D.B. (1993) *Bacterial Conjugation*, Plenum Publishing Press, New York.
- Collas, P. and Alestrom, P. (1997) Rapid targeting of plasmid DNA to zebrafish embryo nuclei by the nuclear localization signal of SV40 antigen. *Mol. Mar. Biol. Biotechnol.* 6(1):48–58.
- Colling, M. and Wolfram, G. (1987) Einfluss des Garens auf den Puringehalt. *Z. Ernährungswiss.* 26(4):214–218.
- DeFlaun, M.F. and Levy, S.B. (1989) Genes and their varied hosts. In *Gene Transfer in the Environment*, pp. 1–32 (Eds. S.B. Levy and R.V. Miller) McGraw-Hill Publishing Company, New York.
- DeVries, J. and Wackernagel, W. (1998) Detection on nptII (kanamycin resistance) genes in genomes of transgenic plants by marker-rescue-transformation. *Mol. Gen. Genet.* 257:606–613.
- DeVries, J., Meier, P. and Wackernagel, W. (2001) The natural transformation of the soil bacteria *Pseudomonas stutzeri* and *Acinetobacter* sp. by transgenic plant DNA strictly depends on homologous sequences in the recipient cells. *FEMS Microbiol. Lett.* 195:211–215.
- Dianov, G., Price, A. and Lindahl, T. (1992) Generation of single-nucleotide repair patches following excision of uracil residues from DNA. *Mol. Cell. Biol.* 12:1605–1612.
- Doerfler, W. and Schubert, R. (1997) Fremde DNA im Säugersystem. *Deutsches Ärzteblatt* 94, 51–52, 22 Dezember 1997.
- Doerfler, W. and Schubert, R. (1998) Uptake of foreign DNA from the environment: the gastrointestinal tract and the placenta as portals of entry. *Wien. Klin. Wochenschr.* 110(2):40–44.
- Doerfler, W. (1991) Patterns of DNA Methylation – Evolutionary Vestiges of Foreign DNA Inactivation as a Host Defence Mechanism. *A Proposal. Biol. Chem. Hoppe-Seyler* 372:557–564.
- Doetsch, P.W. and Cunningham, R.P. (1990) The enzymology of apurinic/apyrimidinic endonucleases. *Mutat. Res.* 236:173–201.
- Dröge, M., Pühler, A. and Selbitschka, W. (1998) Horizontal gene transfer as a biosafety issue: A natural phenomenon of public concern. *J. Biotechnol.* 64:75–90.
- Ebbelohj, K.F. and Thomsen, P.D. (1991) Species differentiation of heated meat products by DNA hybridisation. *Meat Science* 30:221–234.
- Edozi, J.C. (1969) *Yeast for Human Feeding: New Data on Safety*, FAO/WHO/UNICEF Protein Advisory Group, Document 2.23/1, United Nations, New York.
- Ehrlich, S.D. (1989) Illegitimate Recombination in Bacteria. In *Transposable Elements*, pp. 799–832 (Eds. D.E. Berg and M.M. Howe) ASM Press, Washington D.C.
- Einspanier, R., Klotz, A., Kraft, J., Aulrich, K., Poser, R., Schwägele, F., Jahreis, G. and Flachowsky, G. (2001) The fate of forage plant DNA in farm animals: a collaborative case-study investigating cattle and chicken fed recombinant plant material. *Eur. Food Res. Technol.* 212:129–134.
- Elmadfa, I., Burger, P., Derndorfer, E., Kiefer, I., Kunze, M., König, J., Leimüller, G., Manafi, M., Mecl, M., Papanthasiou, V., Rust, P., Vojir, F., Wagner, K.H. and Zarfl, B. (1999) Österreichischer Ernährungsbericht 1998. Bundesministerium für Gesundheit, Arbeit und Soziales und Bundesministerium für Frauenangelegenheiten und Verbraucherschutz (Hrsg.), Wien.
- England, L.S., Holmes, S.B. and Trevors, J.T. (1998) Review: Persistence of viruses and DNA in soil. *World J. Microbiol. Biotechnol.* 14: 163–169
- European Commission. (1990) Council Directive of 23 April 1990 on the deliberate release into the environment of genetically modified organisms. *Official Journal of the European Communities*, No. L 117/5, May 8, 1990.
- European Commission. (1996) Commission Directive 96/4/EC of 16 February 1996 amending Commission Directive 91/321 of 14 May 1991 on infant and follow-on formula. *Official Journal of the European Communities*, No. L 49, February 28, 1996.
- European Commission. (1999) Opinion of the Scientific Steering Committee on Antimicrobial Resistance. 28 May, 1999.
- Fearing, P.L., Brown, D., Vlachos, D., Meghji, M. and Privalle, L. (1997) Quantitative analysis of CryI(A)(b) expression in Bt maize plants, tissues and silage and stability of expression over successive generations. *Mol. Breed.* 3:169–176.
- Food and Drug Administration. (1992) Statement of Policy: Foods Derived from New Plant Varieties; Notice. *Federal Register* 57(104):22984–23002.
- Ford, M., Du Prat, E., Barallon, R.V., Rogers, H.J. and Parkes, H.C. (1996) Authenticity '96, IFR, Norwich, September 1–3:1996. Poster/Conference Abstract.
- Foster, G.D. and Twell, D. (1996) in *Plant gene isolation: principles and practice*, John Wiley and Sons, Chichester.
- Gauthier, M.J. (1992) *Genetic Transfers and Environment*. Springer Verlag Berlin, Heidelberg, New York.
- Gebhard, F. and Smalla, K. (1998) Transformation of *Acinetobacter* sp. Strain BD413 by Transgenic Sugar Beet DNA. *Appl. Env. Microbiol.* 64(4):1550–1554.
- Gorlick, D. and Mattaj, I.W. (1996) Nucleocytoplasmic transport, *Science*, Mar 15, 271(5255): 1513–1518.
- Griebisch, A. and Zöllner, N. (1974) Effect of ribonucleotides given orally in uric acid production in man. *Adv. Exp. Med. Biol.* 41B, 443–449.

- Grillot-Courvalin, C., Goussard, S., Huetz, F., Ojcius, D.M. and Courvalin, P. (1998) Functional gene transfer from intracellular bacteria to mammalian cells. *Nature Biotechnology* 16: 1–5.
- Groneberg, J., Brown, D.T. and Doerfler, W. (1975) Uptake and Fate of the DNA of Adenovirus Type 2 in KB Cells. *Virology* 64:115–131.
- Guédon, G., Bourgoïn, F. and Decaris, B. (1998) Does horizontal gene transfer occur in lactic acid bacteria co-cultures? *Lait* 78:53–58.
- Guiney, D.G. (1993) Broad host range conjugative and mobilizable plasmids in gram-negative bacteria, pp.75–103 (ed. D.B. Clewell) Plenum Press, New York.
- Guralnick, B., Thomsen, G. and Citovsky, V. (1996) Transport of DNA into the nuclei of *Xenopus* oocytes by a modified VirE2 protein of *Agrobacterium*. *Plant Cell* 8(3):363–373.
- Heinemann, J.A. and Sprague Jr. G.F. (1989) Bacterial conjugative plasmids mobilize DNA transfer between bacteria and yeast. *Nature* 340:205–209.
- Helinski, D.R., Toukdarian, A.E. and Novick, R.P. (1996) Replication control and other stable maintenance mechanisms of plasmids. In: *Escherichia coli and Salmonella*, pp 2295–2324 (Eds. F.C. Neidhardt et al.) ASM, Washington D.C.
- Herbel, W. and Montag, A. (1987) Nucleo-compounds in protein rich food. *Z. Lebensm. Unters. Forsch.* 185:119–122.
- Hertel, C., Probst, A.J., Cavadini, C., Meding, E. and Hammes, W.P. (1995) Safety assessment of genetically modified microorganisms applied in meat fermentation. *System. Appl. Microbiol.* 18:469–476.
- Hupfer, C., Hotzel, H., Sachse, K. and Engel, K.H. (1998) Detection of the genetic modification in heat-treated products of Bt-maize by polymerase chain reaction. *Z. Lebensm. Unters. Forsch.* A 206:203–207.
- Hupfer, C., Hotzel, H., Sachse, K., Moreano, F. and Engel, K.H. (2000) PCR-based quantitation of genetically modified Bt maize: single-competitive versus dual-competitive approach. *Eur. Food Res. Technol.* 212:95–99.
- Hupfer, C., Mayer, J., Hotzel, H., Sachse, K. and Engel, K.H. (1999) The effect of ensiling on PCR-based detection of genetically modified Bt maize. *Eur. Food Res. Technol.* 209:301–304.
- ILSI Europe. (1999) Safety Assessment of Viable Genetically Modified Micro-organisms Used in Food, ILSI Europe Report Series, ILSI Europe, Brussels.
- Ippen-Ihler, K. (1989) Bacterial Conjugation. In *Gene Transfer in the Environment*, pp. 1–32 (Eds. S.B. Levy and R.V. Miller) McGraw-Hill Publishing Company, New York.
- Ippen-Ihler, K. and Skurray, R.A. (1993) Genetic organization of transfer-related determinants on the sex factor F and related plasmids. In: *Bacterial conjugation*, pp.23–51 (ed. D.B. Clewell) Plenum Press, New York.
- Jarvis, S.M. (1989) Characterisation of sodium-dependent nucleoside transport in rabbit intestinal brush-border membrane vesicles. *Biochim. Biophys. Acta* 979:132–138.
- Joint FAO/WHO Expert Committee on Food Additives (1993) Disodium 5'-Guanylate and Disodium 5'-Inosinate, in *Toxicological evaluation of certain food additives and contaminants*, pp 67–84, WHO Food Additive Series 32, World Health Organization, Geneva.
- Klein, J., Altenbucher, J. and Mattes, R. (1998) Nucleic acid and protein elimination during the sugar manufacturing process of conventional and transgenic sugar beets. *J. Biotechnol.* 60:145–153.
- Kleinschmidt, J., Soeding, B., Teuber, M. and Neve, H. (1993) Evaluation of horizontal and vertical gene transfer and stability of heterologous DNA in *Streptococcus thermophilus* from yogurt and yogurt starters. *System. Appl. Microbiol.* 16:287–295.
- Klinman, D.M., Yarruschikov, G. and Ishigatosubo, Y. (1997) Contribution of CpG motifs to the immunogenicity of DNA vaccines. *J. Immunology* 158:3635–3639.
- Klotz, A. and Einspanier, R. (1998) Nachweis von 'Novel Feed' im Tier? Beeinträchtigung des Verbrauchers von Fleisch oder Milch ist nicht zu erwarten. *Mais* 3:109–111.
- Kojima, K. (1974) Safety evaluation of disodium 5'-inosinate, disodium 5'-guanylate and disodium 5'-ribonucleate. *Toxicology* 2:185–206.
- Kunst, F., Ogasawara, N., Moszer, I., Albertini, A.M., Alloni, G., Azevedo, V., Bertero, M.G., Bessieres, P., Bolotin, A., Borichert, S., Borriss, R., Boursier, L., Brans, A., Braun, M., Brignell, S.C., Bron, S., Brouillet, S., Bruschi, C.V., Caldwell, B., Capuano, V., Carter, N.M., Choi, S.K., Codani, J.J., Connerton, I.F., Danchin A., et al. (1997) The complete genome sequence of the gram-positive bacterium *Bacillus subtilis*. *Nature* 390:249–256.
- Larsen, J. and Jørgensen, L. (1996) Reduction of RNA and DNA in *Methylococcus capsulatus* by endogenous nucleases. *Applied Microbiology and Biotechnology*. 45(112):137–140.
- Lassek, E. and Montag, A. (1990) Nucleic-acid components in carbohydrate-rich food. *Z. Lebensm. Unters. Forsch.* 190:17–21.
- Ledoux, L. and Huart, R. (1971) Fate of exogenous DNA in *Arabidopsis thaliana*. Translocation and integration. *Eur. J. Biochem.* 23:96–108.
- Lefringhausen, A. (1996) Transduction with *Streptococcus thermophilus*. Doctoral thesis, Christian-Albrechts-Universität, Kiel.
- Levy and Miller (1989) *Gene Transfer in the Environment*. McGraw-Hill Publishing Company, New York.
- Lindahl and Nyberg (1972) Rate of depurination of native deoxyribonucleic acid. *Biochemistry* 11: 3610–3618.
- Lindahl, T. (1993) Instability and decay of the primary structure of DNA. *Nature* 362:709–715.
- Lloyd, R.G. and Low, K.B. (1996) Homologous recombination. In: *Escherichia coli and Salmonella typhimurium*, pp. 2236–2255 (Eds. F.C. Neidhardt et al.) ASM Washington D.C.
- Lorenz, M.G. and Wackernagel, W. (1994) Bacterial gene transfer by natural genetic transformation in the environment. *Microbiol. Rev.* 58:563–602.
- Low, K.B. (1987) Hfr strains of *Escherichia coli* K-12. In: *Escherichia coli and Salmonella typhimurium*, pp. 1134–1137 (Eds. F.C. Neidhardt et al.) ASM Washington D.C.
- Mandel, M. and Higa, A. (1970) Calcium dependent bacteriophage DNA infection. *J. Mol. Biol.* 53:159–162.
- Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) *Molecular Cloning*. CSH Press.
- Margolin, P. (1987) Generalized transduction. In: *Escherichia coli and Salmonella typhimurium*, pp. 1154–1168 (Eds. F.C. Neidhardt et al.) ASM Washington D.C.
- Marijnen, Y.M.T., deKorte, D., Haverkort, W.A., den Breejen, E.J.S., van Gennip, A.H., Ross, D. (1989) Studies on the incorporation of precursors into purine and pyrimidine nucleotides via 'de novo' and 'salvage' pathways in normal lymphocytes and lymphoblastic cell-line cells. *Biochim Biophys Acta* 1012:148–155.
- Markmann-Mulisch, U. and Subramanian, A.R. (1988) Nucleotide sequence and linkage map position of the genes for ribosomal proteins L14 and S8 in the maize chloroplast genome. *Eur. J. Biochem.* 170:507–514.
- Mercer, D.K., Scott, K.P., Bruce-Johnson, W.A., Glover, I.A. and Flint, H. (1999) Fate of free DNA and transformation of the oral bacterium *Streptococcus gordonii* DL1 by plasmid DNA in human saliva. *Appl. Env. Microbiol.* 65:6–10.
- Michel, B., Niaudet, B. and Ehrlich, D.S. (1983) Intermolecular recombination during transformation of *Bacillus subtilis* competent cells by monomeric and dimeric plasmids. *Plasmid* 10: 1–10.
- Montag, A., Kölling, I., Jänicke, S., Benkmann, S. and Lou, N. (1989) Purine Base Contents in Food. *Akt. Ernähr.* 14:243–247.
- Mosley, R.L. and Klein, J.R. (1992) Peripheral engraftment of fetal intestine into athymic mice sponsors T cell development: Direct evidence for thymopoietic function of murine small intestine. *J. Exp. Med.* 176:1365–1373.
- Nielsen, K.M., Bones, A.M., Smalla, K. and van Elsas, J.D. (1998) FEMS Microbiology Reviews 22:79–103.
- Nielsen, K.M., van Weerelt, M.D.M., Berg, T.N., Bones, A.M., Hagler, A.N. and van Elsas, J.D. (1997) Natural transformation and availability of transforming DNA to *Acinetobacter calcoaceticus* in soil microcosms. *App. Env. Microbiol.* 63(5):1945–1952.
- Palacios, I., Hetzer, M., Adams, S.A. and Mattaj, I.W. (1997) Nuclear import of U snRNPs requires importin beta. *EMBO J.* 16(22):6783–6792.
- Paul, J.H. (1992) Intergeneric Natural Plasmid Transformation between *Escherichia coli* and a marine *Vibrio* Species. In: *Genetic Transfers and Environment*, pp. 61–67, (Ed. M.J. Gauthier) Springer Verlag Berlin, Heidelberg, New York.

- Perreten, V., Schwarz, F., Cresta, L., Boeglin, M., Dasen, G. and Teuber, M. (1997) Antibiotic resistance spread in food. *Nature*. 389:801–802.
- Popov, S., Rexach, M., Zybarth, G., Reiling, N., Lee, M.A., Ratner, L., Lane, C.M., Moore, M.S., Blobel, G. and Bukrinsky, M. (1998) Viral protein R regulates nuclear import of the HIV-1 pre-integration complex. *EMBO J.* 17(4):909–917.
- Protein Advisory Group (PAG). (1975) Ad Hoc Working Group Meeting on Clinical Evaluation and Acceptable Nucleic Acid Levels of SCP for Human Consumption, Geneva.
- Redenbaugh, K., Hiatt, W., Martineau, B., Lindemann, J. and Emlay, D. (1994) Aminoglycoside 3'-phosphotransferase II (APH(3')II): Review of its safety and use in the production of genetically engineered plants. *Food Biotechnol.* 8: 137–165.
- Richardson, P.T. and Park, S.F. (1997) Integration of heterologous plasmid DNA into multiple sites on the genome of *Campylobacter coli* following natural transformation. *J. Bacteriol.* 179(5):1809–1812.
- Rudolph, F.B., Fanslow, W.C., Kulkarni, A.D., Kulkarni, S.S. and Van Buren, C.T. (1986) Effect of dietary nucleotides on lymphocyte maturation. *Aqv. Exp. Med. Biol.* 195A:497–501.
- Salati, L.M., Gross, C.J., Henderson, L.M. and Saviano, D.A. (1984) Absorption and metabolism of adenine, adenosine-5'-mono-phosphate, adenosine and hypoxanthine by the isolated vascularly perfused rat small intestine. *J. Nutr.* 114:753–760.
- Sanchez-Poza, A., Pita, M.L., Martinez, A., Molina, J.A., Sanchez-Medina, F. and Gil, A. (1986) Effects of dietary nucleotides upon lipoprotein pattern or newborn infants. *Nutr. Res.* 6, 76) 717:11.
- Saphire, A.C., Guan, T., Schirmer, E.C., Nemerow, G.R. and Gerace, L. (2000) Nuclear import of adenovirus DNA in vitro involves the nuclear protein import pathway and hsc70. *J. Biol. Chem.* 275(6):4298–4304.
- Sato, Y., Roman, M., Tighs, H., Lee, D., Corr, M., Nguyen, M-D., Silverman, G.J., Lotz, M., Carson, D. A. and Raz, E. (1996) Immunostimulatory DNA sequences necessary for effective intradermal gene immunization. *Science* 273: 352–354.
- Schlüter, K., Fütterer, J. and Potrykus, I. (1995) 'Horizontal' Gene Transfer from a Transgenic Potato Line to a Bacterial Pathogen (*Erwinia chrysanthemi*) Occurs – if at all – at an extremely low frequency. *Biotechnology* 13: 1094–1098.
- Schubbert, R., Hohlweg, U., Renz, D. and Doerfler, W. (1998) On the fate of orally ingested foreign DNA in mice: chromosomal association and placental transmission to the fetus. *Mol. Gen. Genet.* 259(6):569–576.
- Schubbert, R., Lettmann, C. and Doerfler, W. (1994) Ingested foreign phage M13 DNA survives transiently in the gastrointestinal tract and enters the bloodstream of mice. *Mol. Gen. Genet.* 241:495–504.
- Schubbert, R., Renz, D., Schmitz, B. and Doerfler, W. (1997) Foreign M13 DNA ingested by mice reaches peripheral leukocytes, spleen, and liver via the intestinal wall mucosa and can be covalently linked to mouse DNA. *Proc. Natl. Acad. Sci. USA* 94:961–966.
- Scientific Committee for Food (1991) Reports of the Scientific Committee for Food (Twenty-fifth series): First series of food additives of various technological functions, Commission of the European Communities, Brussels.
- Sisco, K.L. and Smith, H.O. (1979) Sequence-specific DNA uptake in *Haemophilus influenzae* transformation. *Proc. Natl. Acad. Sci. USA* 76(2):972–976.
- Smalla, K. (1995) Horizontal gene transfer from transgenic plants into plant associated microorganisms and soil microorganisms. pp 29–34 in Safety of transgenic crops. Environmental and Agricultural Considerations. Proc. Basel Forum of Biosafety. BATS, Agency for Biosafety Research and Assessment of Technology Impacts of the Swiss Priority Programme Biotechnology, Basel.
- Smalla, K., Gebhard, R., van Isas, J.D., Matzk, A. and Schiemann, J. (1994) Bacterial communities influenced by transgenic plants. In: Proceedings of the 3rd International Symposium on the Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms. 157–167.
- Strätz, M., Mau, M. and Timmis, K.N. (1996) System to study horizontal gene exchange among microorganisms without cultivation of recipients. *Mol. Microbiol.* 22:207–215.
- Straub, J.A., Hertel, C., Hammes W.P. (1999) The fate of recombinant DNA in thermally treated fermented sausages. *Eur. Food Res. Technol.* 210:62–67.
- Tchou, J., Kasai, H., Shibutani, S., Laval, J., Grollman, A.P. and Nishimura, S. (1991) 8-oxoguanine (8-hydroxyguanine) DNA glycosylase and its substrate specificity. *Proc. Natl. Acad. Sci., USA* 88:4690–4694.
- Van Buren, C.T., Kulkarni, A.D., Fanslow, W.C. and Rudolph, F.B. (1985) Dietary nucleotides, a requirement for helper/inducer T lymphocytes. *Transplantation* 40:694–697.
- Van der Lelie, D. and Venema, G. (1987) *Bacillus subtilis* generates a major specific deletion in pAM#1. *Appl. Environm. Microbiol.* 51:2458–2463.
- Van der Vossen, J.M.B.M., Havakers, W.A.L.M., Koster, D.S., Ten Brink, B., Minekus, M., Havenaar, R., Huis in't Veld, J.H.J., Overseem, J., Hendriks, N. and Hofstra, H. (1998) Development and application of in vitro intestinal tract model for safety evaluation of genetically modified foods. TNO Report.
- Wackernagel, W. and deVries, J. (2000) Transformation von Bakterien durch Antibiotikum-Resistenzgene aus transgenen Pflanzen und Nahrungsmitteln, Ernährungsforschung, in press.
- Watt, V.M., Ingles, C.J., Urdea, M.S. and Rutter, W.J. (1985) Homology requirements for recombination in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 82:4768–4772.
- Weinrauch, Y. and Dubnau, D. (1983) Plasmid marker rescue transformation in *Bacillus subtilis*. *J. Bacteriol.* 154(3):1077–1087.
- Weisberg, R.A. (1987) Specialized transduction. In: *Escherichia coli* and *Salmonella typhimurium*, pp. 1169–1176 (Eds. F.C. Neidhardt et al.) ASM Washington D.C.
- World Health Organization. (1991) Strategies for Assessing the Safety of Foods Produced by Biotechnology. Report of a Joint FAO/WHO Consultation. WHO, Geneva.
- Yi, A-K. and Krieg, A.M. (1998) Rapid induction of mitogen-activated protein kinases by immune stimulatory CpG DNA. *J. Immunology* 161:4493–4497.
- Zeimienowicz, A., Gorlich, D., Lanka, E., Hohn, B. and Rossi, L. (1999) Import of DNA into mammalian nuclei by proteins originating from a plant pathogenic bacterium. *Proc. Natl. Acad. Sci. USA* 96(7):3729–3733.
- Zenz, K., Neve, H., Geis, A. and Heller, K.J. (1998) *Bacillus subtilis* develops competence for uptake of plasmid DNA when growing in milk products. *System. Appl. Microbiol.* 21:28–32.
- Zhang, L., Jynouchi, H., Yokoyama, H. and Tomita, Y. (1993) Immunomodulating actions of nucleotides on Ab/ig production by murine spleen lymphocytes in vivo and in vitro. *FA-SEB J.* 7:A413.
- Zöllner, N., Griebisch, A. and Gröbner, W. (1972) Einfluss verschiedener Purine auf den Harnsäurestoffwechsel. *Ernähr. Umsch.* 19:79–82.