afssa
AGENCE FRANÇAISE
DE SECURITE SANITAIRE
DES ALIMENTS

GMOs and food: is it possible to identify and assess health benefits?

A study based on 4 examples:

Insect-resistant crops
Glyphosate-tolerant sugar beet
Vitamin A fortification: the case of golden rice
Genetically-modified microorganisms

- June 2004 -

WORKING METHOD AND LIST OF AUTHORS

Subsequent to the symposium organised by Afssa (French Food Safety Agency) in 2001 on the subject of "GMOs and food: can benefits for health be evaluated?", members of the specialist expert committee on "Biotechnology" undertook to draft a report making a detailed analysis of four GMO cases that could provide health benefits in comparison with conventional products:

Mr. Louis-Aimé AUMAITRE	Department of animal nutrition, INRA – Saint-Gilles
Mr. Pierre BESANÇON	Biological engineering and food sciences laboratory, University of Montpellier II - Montpellier
Mr. Michel BRANCHARD	Institut de sciences agroalimentaires (Institute of Agrifood Sciences) - Plouzané
Mr. Gérard BRANLARD	Wheat quality and genetics, INRA - Auzeville
Mr. Gérard CORTHIER	Jouy research centre, INRA – Jouy en Josas
Mr. Joël GUILLEMAIN	Sesame – Chambray-les-Tours
Mr. Louis-Marie HOUDEBINE	Cellular differentiation unit, INRA - Jouy en Josas
Mr. Philippe JOUDRIER	Cereal biochemistry and molecular biology unit - INRA - Montpellier
Mr. Nicolas LINDLEY	Department of biochemical and food engineering, INSA - Toulouse
Mr. Guy MOULIN	Industrial microbiology and microorganism genetics laboratory, ENSAM - Montpellier
Mr. Alain PARIS	Xenobiotics laboratory, INRA - Toulouse
Mr. Thierry PINEAU	Toxicological pharmacology laboratory, INRA - Toulouse
Mr. Alain RAYNAL	University of Paris-Sud - Orsay
Mr. Jean Pierre ZALTA	Institut de biologie cellulaire et de génétique (Institute of cellular biology and genetics), CNRS - Toulouse

In October 2003, the specialist expert committee on “Biotechnology” was reviewed in part. A few of the new members in this committee conducted an in-depth re-reading of all four documents and proposed several modifications:

Mrs. Chantal BARTHOMEUF	Department of pharmacognostics and biotechnology, UMR - INSERM 484 – Clermont-Ferrand
Mrs. Francine CASSE	University of Montpellier II - Montpellier
Mr. Didier MONTET	CIRAD – AMIS Food processing programme - Montpellier
Mr. Henry-Eric SPINNLER	INA-PG, GER de Technologie et Procédés Alimentaires (Study group on Food Technology and Processes) - THIVERVAL GRIGNON
Mrs. Maria URDACI	Microbiology and applied biochemistry laboratory, ENITA - Bordeaux

The whole committee examined and amended this report several times, with the new committee undertaking the drafting of the introduction and the conclusion. During its meeting of 13 May 2004, the specialist expert committee on “Biotechnology” adopted this report.

Mr. Maxime SCHWARTZ, Chairman of the specialist expert committee, and Mrs. Sophie GALLOTTI, scientific coordinator, were responsible for the scientific coordination and formatting of this document.

Contents

INTRODUCTION

Any technological advance involves risks and plant transgenesis is no exception to this rule. Irrespective of whether these risks are low or high, they are only acceptable to the population if the technological advance brings benefits. It is therefore important to weigh up the benefits against the risks. However, in the case of transgenic plants, which are the most widely known examples of genetically-modified organisms or GMOs, only the risks are highlighted, without enough questions being asked about the benefits. This is largely due to the fact that the transgenic plants currently on the market were developed primarily for economic reasons, to make production easier and cheaper for farmers and to increase profits for companies marketing these crop varieties. The consumer, who perceives a potential risk to health, or the environment, related to GMOs, does not see anything in them for him. He really does not see what GM foods contribute at the present time. He does not see GMOs in terms of a benefit/risk ratio but only in terms of risks.

Aware of this difficulty GM plant producers have therefore emphasised probable benefits of these GM plants for health. Furthermore, they have highlighted the fact that other transgenic plants, known as "second-generation" GM plants, could be developed not only for economic purposes but also with the aim of providing health benefits to consumers, in both industrialised and developing countries.

For the specialist expert committee on "Biotechnology" at Afssa, which is responsible for assessing risks related to the consumption of GM plants and derived products by both humans and animals, it was therefore necessary to try to assess the potential health benefits.

In December 2001, Afssa therefore organised a symposium[1] "GMOs and food: can benefits for health be evaluated?" intended to trigger debate on this theme. In order to further this reflection, the "Biotechnology" Committee then undertook a detailed analysis of four GMO cases that could provide health benefits in comparison with conventional products. It opted to consider the case of two GMOs already on the market or close to being so, initially developed for primarily economic reasons but for which health benefits have since been alleged, and the case of two GMOs currently undergoing assessment and developed to provide health benefits.

The first example is that of crops which are specifically resistant to certain insect pests (but with no effect on others). At the present time, several varieties of corn and cotton resistant to insects and obtained by the introduction of a gene originating from a bacterium present in the environment, *Bacillus thuringiensis*, are cultivated around the world. A first advantage claimed for these varieties is that they limit the use of insecticides and therefore exposure of the human population to these. Another advantage related to this resistance is said to be that they limit the development on the plants of fungi producing mycotoxins, some of which are carcinogenic. A critical examination of these allegations is presented.

The second example is that of sugar beet tolerant to a herbicide, glyphosate, which, although not yet authorised in Europe, has been the subject of numerous studies and research, notably with respect to the environmental impact in terms of gene transfer to self-propagating plants, but also in terms of the quantities of herbicides used and their qualities in comparison with conventional varieties. The present document analyses the possible consequences on human health of these modifications in the use of herbicides.

The third example is that of "golden rice", a rice potentially enriched in a vitamin A precursor but for which we do not yet have any set variety. This is a transgenic plant developed for nutritional purposes and intended to combat a serious vitamin deficiency widespread in developing countries. This is, therefore, clearly a GM plant intended to provide benefits in terms of health. Insofar as this GM plant has been the subject of debates relative to the reality of its value at the current state of research, the "Biotechnology" committee wanted to conduct as objective an analysis as possible of the benefits it is meant to provide.

The last chapter concerns genetically-modified microorganisms, intended for nutritional or medicinal purposes. Indeed, although no GMOs of this type are currently on the market, a number could be within a relatively short period of time. A brief inventory of the benefits that these may provide thus seemed to be appropriate.

[1] The minutes of the symposium "GMOs and food: can benefits for health be evaluated?" held by Afssa on 17 and 18 December 2001 can be consulted in the form of a pdf file on the Afssa website: www.afssa.fr

INSECT-RESISTANT PLANTS

1 INTRODUCTION

A large number of strains of *Bacillus thuringiensis* have been identified for almost 100 years. Each of these produces proteins, delta-endotoxins, with insecticide properties, with the specificity with respect to the target varying with the strain [1,2]. *Bt* toxins in bacterial form are almost totally biodegradable and have been widely used for several decades in biological pest control, and are still commonly used [3].

The emergence since the start of the 1980s of resistance to synthetic insecticides, the increase in environmental protection through a necessary reduction in the quantities of insecticides used and, finally, the use of new methods for genetic improvement of plants integrating biotechnologies have encouraged the creation of new agronomic crop varieties expressing a gene encoding a *Bt* protein resistant to insects. At the present time, the main crops concerned are corn and cotton, with potatoes, soja and rice still representing only small surface areas.

Between 1996 and 2002, their insect-resistant character was the second most dominant trait in all the transgenic crops grown around the world (58.7×10^6 ha[2]) with, in 2002, 17% made up of Bt crops (10.1×10^6 ha) and 8% (4.4×10^6 ha) made up of crops combining insect-resistant characters with herbicide tolerance [4]. This character is in 2nd place, behind tolerance to herbicides alone, which, in 2002, accounted for 75% of the total surface area of genetically-modified crops grown, i.e. 44.2×10^6 ha [4]. Of the various Bt crops grown, it is corn that predominates, with 7.6×10^6 ha cultivated in 7 principal countries; it represents 13% of land cultivated with transgenic plants. Transgenic cotton bearing herbicide tolerance and insect resistance, either alone or in combination, represents 20% of the world's total GM crop surface area in 2002, with no increase in comparison with 2001 [4]. China accounted for 4% of the world's total GM crop surface area in 2002, corresponding to the strongest increase in cultivated surface areas. In fact, Bt cotton crop surface areas increased there by 40% over 2001, thus representing more than half of the total cotton crop area in the country and concerning more than 5 million small farmers [4]. In Europe, Spain is beginning to grow insect-resistant cotton crops.

In 2003, the areas dedicated to GM crops went up by 15%, reaching a total of 67.7 millions ha. This increase is primarily due to the USA (+ 9.7%), Canada (+ 25.7%) China (+ 33%) and, above all, Brazil, which was not previously counted and for which the surface areas represent 3 million ha (http://www.isaaa.org/).

These world statistics relative to cultivated transgenic plants and, in particular corn and Bt cotton crops, have become very significant in the last few years in comparison with conventional crops, which are still dominant, with the annual growth rate for GMPs since 1996 being more than 10% [4]. They now concern a growing number of farmers.

Given this recent evolution in farming practices and, over and above the already well documented positive agronomic and economic consequences, those which exist in terms of quality of the foodstuffs produced from the genetically-modified plant varieties grown ought now to become clear:

- either as a direct consequence of the reduction in the amount of insecticides used on a reduction in the risks to farmers inherent in their handling and a decrease in the amount of insecticide residues found on harvesting,

- or as an indirect consequence, related, in the case of corn, to better protection of the crop against insect damage, leading to the better sanitary quality of the kernels linked to a reduction in mycotoxin contamination.

Similarly, the safety of by-products of cotton harvests, such as the seeds or cake intended for animal feed may be improved due to a reduction in the insecticide residues present.

[2] ha : hectare

2 PEST CONTROL METHODS (INSECTS)

2.1 USE OF INSECTICIDES

2.1.1 Corn

Although in practice it concerns relatively limited surface areas, chemical pest control is the most frequently used method to curb insect damage in corn crops. The insecticides used are mainly organophosphates (chlorpyrifos), carbamates or pyrethrinoids. Their effectiveness makes it possible to limit yield losses to 5-10% versus 30-50% in the absence of any treatment. The precautions for use of chemical products must be complied with in order to avoid any contact with the skin, the respiratory tract and the eyes and any risk of poisoning of those handling them or any person accidentally coming into contact with these products.

The methods of use are dependent on the plot and growth stage of the corn and must take into account the hatching seasons of insects, which are difficult to predict due to a spread and multiplication over time. Insecticide treatments must be applied before European corn borer larvae become established on the stems or rootworm larvae become established in the roots, therefore becoming invulnerable. The methods use may range from spray treatments, to aspersion or air treatments. All these difficulties therefore limit the effectiveness of treatments on corn crops.

The use of chemical insecticides also eradicates another useful entomological fauna involved in the control of other insects which are harmful to the crop, such as plant-feeding mites or aphids. The latter can also be carriers in the transmission of barley yellow dwarf virus or corn dwarf mosaic virus, leading to reductions in yields in the region of 10% [5], and requiring, in certain cases, an additional insecticide treatment (imidacloprid).

Chemical pest control can be implemented preventively during sowing, using coated seed containing products such as imidacloprid or fipronil[3] or on uncoated seed by adding granules containing a carbamate-type insecticide (carbofuran) to the seed beds. Treatments on the growing plant are applied according to the extent of insect attacks. At the seedling stage or post-flowering, ground or air spray treatments use pyrethrinoids (deltamethrin, cypermethrin).

In the United States, chemical control of these insects only concerns 5% of corn crop surfaces. This is mainly due to the difficulty in determining the right time to eradicate European corn borers or corn rootworms. Consequently, the introduction of Bt corn varieties has not led to a significant reduction in the amounts of insecticides sprayed [6].

2.1.2 Cotton

Insect pest control in cotton crops requires the use of frequent insecticide treatments. Generally, 2 to 10 treatments are required. But Huang *et al* [7] report that in conventional farming conditions (manual application of pesticides) in China, as many as around 20 insecticide treatments may be applied due to an acceleration in insect generations in tropical climates, with the quantity of products spread sometimes being as much as 55 kg/ha, all packagings combined. Following the introduction of new genetically modified insect-resistant plants, the quantity of insecticide products spread and the number of treatments have been divided by three. The use of organochloride or organophosphate substances is tending to die out. This has direct implications for the safety of those handling pesticide products. Among farmers who do not use cotton varieties resistant to insects, 30% show signs of health problems during spraying, versus only 9% of farmers using Bt cotton.

In India, the use of 50% Bt cotton varieties would lead to a reduction in the amounts of insecticide products of up to 9200 tonnes per year [6].

In the United States, the reduction in the amount of insecticides used between 1995, the year prior to the introduction of Bt varieties, and 1999 is estimated to be 1200 tonnes, i.e. 14% of the total quantity used, with the number of treatments having fallen by 22% [8]. This marked reduction in the use of insecticides is confirmed by other authors [9].

[3] without prejudice to the measures taken recently to control these substances

2.1.3 Toxicological assessment of the main insecticides

- ***Deltamethrin, cypermethrin***

  These compounds belong to the type-II Pyrethrinoids class [10] and act on the central nervous system of the insect, disrupting the action of the GABA neurotransmitter. They act by contact or after ingestion.

  - *Deltamethrin* [11]

    As a single oral dose (acute toxicity), the LD_{50} ranges from 31 to 139 mg/kg bw[4] in rats, and from 21 to 34 mg/kg bw in mice. The signs of intoxication are similar to those caused by other pyrethrinoids: mastication, salivation, agitation of the limbs, convulsions, paralysis and death. In humans, the signs of poisoning are: ataxia, convulsions leading to muscular fibrillation and paralysis, dermatitis, oedema and diarrhoea, vomiting, breathing difficulties which can cause death by asphyxia. Fatal doses in humans after ingestion range from 2 to 250 mg/kg. Cases of allergic reactions have been described in humans after contact with the skin.

    In chronic administration, the no observed effect level (NOEL) is obtained with a concentration of 12 mg/kg feed over a duration of two years in mice and of 2.1 mg/kg in rats. The no observed effect level in rats treated for 90 days is 10 mg/kg/d. Cases of skin reactions or irritation of the mucous membranes have been reported in workers involved in the production of the substance over a period of 7-8 years, with these signs being prevented by the wearing of masks and gloves.

    The reproductive effects in rats and mice are significant. In contrast, deltamethrin does not present any teratogenic or mutagenic effects. No information is available relative to the carcinogenic effects.

    On an environmental level, deltamethrin disappears in the soil within 1-2 weeks and in vegetation within 10 days. Deltamethrin is highly toxic to fish. However, under normal conditions of use, the aquatic fauna (fish, crustaceans) is not exposed.

  - *Cypermethrin* [12]

    This is a mixture of 8 isomers, each of them with their own chemical and biological characteristics. It is moderately toxic following ingestion or skin contact. At high levels of cutaneous exposure, the symptoms range from numbness, to formication, itching and burning sensations, to disappearance of motor coordination, paralysis or even death. After poisoning by ingestion, the symptoms range from nausea to vomiting, stomach pains, diarrhoea, convulsions and coma. Cypermethrin is mildly irritant for the skin and eyes and could cause allergic reactions.

    The LD_{50} in rats is 250 mg/kg bw when it is distributed in corn oil, but 4123 mg/kg bw when it is administered in water. It is between 82 and 779 mg/kg bw in mice and depends on the ratio between *cis* isomers and *trans* isomers.

    No data relative to chronic toxicity appear to be available. Cypermethrin does not appear to have any effects on reproduction, nor any teratogenic or mutagenic effects. This product may be a potential carcinogen although tests are not fully conclusive.

    Elimination of the product in rats in rapid (a few hours) and its metabolism is very active (hydroxylation, hydrolysis). Elimination of residues stored in adipose tissue is slower.

    Cypermethrin is highly toxic to fish and aquatic invertebrates, but the risks remain limited under normal conditions of use on crops.

    The persistence of cypermethrin in soils ranges from a few days to a few weeks, depending on the soil type. On plants, it is limited to a few days due to its significant photodegradation.

- ***Phenylpyrazoles* (*fipronil*** [13]***, imidacloprid*** [14]**)**

  These two substances act on the central nervous system of insects. Fipronil and imidacloprid have LD_{50} values of 97 and 450 mg/kg bw (oral administration), respectively, and are less toxic for mammals than for certain birds and fish and most insects. Following chronic administration, fipronil is carcinogenic in rats and mice, causing cancer of the thyroid [15,16]. Imidacloprid does not appear to have any mutagenic or teratogenic properties.

[4] bw : body weight

- ***Carbofuran***
  This is a carbamate with a cholinesterase inhibiting activity. This product is considered to be a probable endocrine disrupter. It leads to changes in spermatozoids and the male reproductive system in rats [17,18] and rabbits [19]. This product is also known to disrupt the thyroid system in ewes, leading to an increase in thyroxine concentrations in the blood [20].

- ***Chlorpyrifos*** [21,22]
  This compound belongs to the organophosphate family, the insecticide properties of which were reported as early as 1965. Cases of acute intoxication in humans have been reported: Chlorpyrifos induces inhibition of plasma cholinesterases, which act as biomarkers of exposure. No studies have reported any effect on an increase in cancer cases after exposure, which is corroborated by studies conducted in animals or those conducted to assess the genotoxicity. Reproductive toxicology studies show that this product may be toxic for the foetus. Finally, this compound does not appear to have endocrine disrupting properties, which would rank it in the xeno-oestrogen category but, however, it does disrupt the thyroid system in ewes, leading to a reduction in plasma thyroxine concentration [20].

2.2 BIOLOGICAL PEST CONTROL

2.2.1 Bacteria: *Bacillus thuringiensis*

Bacterial preparations are used in North America, whereas these products are not approved for use on corn crops in France. This bacterium found in soil produces a protoxin (protein crystals) which, when it is ingested by European corn borer or pink stem borer larvae, is transformed by the gastric juices into a toxin disturbing the absorptive capacity of the digestive tract.

Bacillus thuringiensis (Bt) toxins are not toxic for humans or animals [23]. A large number of toxins have been identified amongst the various strains of *B. thuringiensis*. Currently, 40 toxin families from the Cry family, totalling 251 toxins, and 2 Cyt families, representing 22 toxins, are known.

No toxicity has been found in rats, mice, birds, dogs or guinea pigs having received Bt protein crystals extracted from *Bacillus thuringiensis* var. *israelensis* by the oral route [66] due to the absence of receptors to these toxins in the epithelial cells of the intestines of mammals, birds and fish. Bt is irritant for the eyes and can cause mild irritation of the respiratory tract following inhalation. Following chronic administration in rats by the oral route (8.4 g/kg bw /d) for 13 weeks, no toxic effect was detected. Reproductive toxicology, teratogenicity, mutagenicity and carcinogenicity tests are all negative.

2.2.2 Insect: *Trichogramma maidis*

Trichogramma releases lead to parasitisation of the eggs of European corn borers present on corn leaves. This technique is not very effective in the case of significant infestation.

2.2.3 Entomopathogenic fungus: *Beauveria bassania*

This control method involves disseminating the spores of a soil fungus: *Beauveria bassania*, a parasite of the European corn borer larvae.

2.2.4 Conclusion

These three biocontrol methods are known to be environmentally friendly. However, their effectiveness is not total and depends on climatic conditions, the intensity of the infestation, the intervention stage and the number of insect generations during growing of the crop. To remain below the threshold of being harmful, the effectiveness of the intervention is strongly dependent on good knowledge of the parasite's biology.

2.3 BT GENES

Insertion of the Bt gene encoding a specific protein for a particular family of insect pests confers on the modified plant more effective resistance properties than those provided by biocontrol or chemical control conducted in the event of a significant attack. Thus, various Bt corns are resistant to damage by European corn borers (*Ostrinia nubilalis*) and pink stem borers (*Sesamia nonagrioides*) thanks to Cry1Ab, Cry1F or Cry9C genes, and corn rootworms (*Diabrotica virgifera*) thanks to Cry3Bb1, Cry34Ab1 or Cry35Ab1 genes. Bt cotton is resistant to cotton worm and bollworm attacks (Cry1Ac gene) and potato to Colorado beetle attacks (Cry1Ab gene).

3 BT CORN CROPS AND AGRONOMIC CONSEQUENCES

3.1 EVOLUTIONS IN CULTIVATED SURFACE AREAS

There was a marked increase worldwide in the surface areas sown with Bt corn between 2001 and 2002, rising from 5.9×10^6 to 7.6×10^6 ha [4,24]. In the United States, the areas sown with Bt corn have been stagnating since 1998 at close to 22-25% of total sown surface areas [25]. This limit corresponds to an economic compromise between the additional cost of the seed and the extent of harvest losses caused by European corn borer attacks but also depends on the importance of corn exports from the United States to other countries (2.5 Mt/year in Europe). The apparent ceiling observed in recent years may also be explained by a reduction in corn borer populations, an important argument in the decision of farmers to buy Bt corn seed or otherwise.

3.2 CONSIDERATION OF RESISTANCE PROBLEMS

In order to reduce the emergence of insects resistant to certain modified and biosynthetised endotoxins in Bt corn as far as possible, refuge area strategies have been introduced. These consist in growing conventional varieties in order to encourage the crossing of insects with a resistant allele, appearing in recessive and heterozygous form, with sensitive homozygotes maintained in the refuge areas. In regions free of cotton crops, such as the Corn Belt, refuge areas represent 20% of surface areas cultivated with corn. This percentage rises to 50% where cotton and corn crops coexist [25].

In these refuge areas, the use of insecticides, with the exception of *Bacillus thuringiensis*, is possible. In practice, both for corn and, even more so, for cotton, the elimination of chemical treatments will not be total.

In the USA, monitoring of the resistance of Bt corn populations has developed with the introduction of these refuge areas. The EPA[5] uses two methods: the application of discriminating levels of Cry1Ab and Cry1F toxins and the comparison of LD_{50} values from one year to another, aimed at detecting the appearance of a resistance allele.

To date, no resistance problems have been identified, including in the regions of the USA in which Bt corn crops have been grown since 1996. Furthermore, no resistance allele has been selected in the various American and European laboratories working on this question. Finally, calculations made in these laboratories [26,27] assess the frequencies of resistance alleles in natural corn borer populations to be less than 10^{-3}.

3.3 PEST CONTROL IN CORN CROPS

New Bt varieties resistant to corn rootworm attacks have been successfully tested in the United States (effectiveness of protection against rootworms and safety in humans and animals -swine, poultry and ruminants-). They have been authorised by the FDA[6] and should come into force in 2003, which may extend the areas sown with Bt corn in North America. In fact, in single-crop corn farming, corn rootworms cause more damage than corn borers. In addition, the presence of rootworms in Europe was detected back in 2000.

3.4 CONSEQUENCES ON THE ENTOMOLOGICAL FAUNA OF BT CORN VARIETIES

In May 1999, a preliminary report was published in *Nature* suggesting that Bt corn pollen may be dangerous for the Monarch butterfly [28].

This result was to very quickly mobilise a number of scientists concerned about this potential problem and tests were to be conducted by a network of different teams over two years. This led to the publication of 6 articles in PNAS (USA) refuting the hasty hypothesis of the article published in Nature.

It emerges clearly from the studies conducted that the risk of Monarch butterfly larvae being affected by the consumption of pollen produced by Bt corn present either on "milkweeds" (their favoured habitat) or even on corn leaves is negligible (cf. Annex).

It is possible to conclude that the use of Bt corn represents a significantly safer environment for Monarch butterflies than the use of chemical insecticides.

---

5 EPA: Environmental Protection Agency (USA)

6 FDA: Food and Drug Administration (USA)

4 BT GENE AND MYCOTOXIN CONTAMINATION LEVELS IN CORN GRAIN

The presence, to variable extents, of various mycotoxins in corn grain has long been documented. The observation of significantly lower mycotoxin levels in genetically modified corn bearing the Bt gene represents an argument opportunely used by professionals in the GMP[7] sector in a strategy to develop their activities, first of all, and also as part of a generic approach intended to restore the image of GMP-derived products and to promote the acceptability to society of the such products. However, this is a benefit which is alleged in a specific context which had not been strategically anticipated in the principle of GMP development and which, in fact, remains an unintentional additional effect which should be measured using rigorous evaluation methods to assess the real impact and the benefits to animal and human health.

4.1 CORN MYCOTOXINS: SOURCE ORGANISMS, CONTAMINATION AND DIVERSITY OF TOXINS.

Corn kernels, primarily, and, to a lesser extent, the whole plant, are usually contaminated by microscopic fungi (*Fusarium, Aspergillus, Penicillium*, etc.), moulds which belong to the field flora and to the storage flora. They are sometimes introduced through the ear and then develop in the cob during growth (plant pathogens) and even after harvesting if the conditions are favourable (saprophyte species).

Mycotoxins are secondary metabolites of these various species of micromycetes. As such, they are not essential to the growth of the fungus and are produced in response to external stimuli related to climatic and environmental conditions, as well as the response to the plant to aggression [29]. In addition, several parameters are linked to the degree of contamination of plants and cereals: the harvesting and storage methods and conditions (pH, aerobiosis, temperature, moisture), any sorting and dust-removing operations on the corn kernel before use [30], damage inflicted on the plant or the integument of the kernel by various species of pests, representing a port of entry for the fungus [31,32].

Aspergillus, Penicillium and *Fusarium* are involved in the production of six families of mycotoxins which, through agricultural foodstuffs, represent a danger to public health. The *Aspergillus* genus produces aflatoxins, and, like the *Penicillium* genus, it also synthesises ochratoxin and patulin. Species from the *Fusarium* genus produce fumonisins, trichothecenes and zearalenone [33,34]. Depending on their exact nature, mycotoxins exert biological, carcinogenic, mutagenic, teratogenic, oestrogenomimetic, neurotoxic and immunotoxic effects in humans, farm animals and laboratory animals. The mycotoxins described in corn are aflatoxins, ochratoxin A, deoxynivalenol, zearalenone and other trichothecenes, fumonisins and moniliformin [35]. Since corn is a relatively specific host of *Aspergillus flavus*, it receives group B aflatoxins, but not group G ones, which are produced by other species of *Aspergillus*.

4.2 PATHOLOGICAL EFFECTS, DANGEROUSNESS, SYNERGY OF EFFECTS AND DETOXIFICATION

The consequences of exposure to each of these mycotoxins are primarily known through initial descriptions of pathognomonic manifestations reported in cases of recognised acute poisoning.

- **Aflatoxins**, originally identified during an episode of fatal hepatotoxicity in turkey farms in the United Kingdom nearly forty years ago, have also given rise to fatal food poisoning in humans, such as the outbreak causing the death of thirteen children in Malaysia in 1988 [36]. Chronic exposure to aflatoxins represents a considerable risk since it has been established that they are carcinogenic and they are classified by the International Agency for Research on Cancer[8] (IARC) in group 1 (carcinogenic in humans), apart from aflatoxin M1, a metabolite of animal origin found in milk and which belongs to group 2B (potentially carcinogenic in humans).
- Although it can be produced before harvesting, **ochratoxin A** is more commonly generated during grain storage stages. Producing strains of *Penicillium* have a more specific tropism for temperate or cold regions whereas producing strains of *Aspergillus* are observed in warm, humid climates. Classified in 1993 as a possible human carcinogen by IARC, ochratoxin A is, due to its nephrotoxicity, a causal factor in the high prevalence of "endemic Balkan nephritis" and urinary tract cancers in Central Europe (classified group 2B).
- **Trichothecenes** represent a vast group of substances with a strong structural homogeneity (more than 150 substances described). Produced by numerous species of *Fusarium* which develop on the plant in relatively cold periods, three toxins are more specifically monitored:

[7] GMP : Genetically modified plant
[8] http://monographs.iarc.fr

nivalenol (group 3 [not classifiable with respect to its carcinogenic potential in humans]), **desoxynivalenol** or "vomitoxin" (classified group 3) and **toxin T-2** (group 3). An exhaustive study in 1988 clearly indicates that Northern hemisphere countries are more particularly affected [37], as is confirmed by a review of the literature [34]. During episodes of acute intoxication, mainly gastrointestinal symptoms are observed (vomiting).

- **Zearalenone** (classified group 3), which results from infestation of the plant with various species of the *Fusarium* genus, exerts a chronic toxicity, which is expressed by oestrogenomimetic effects in female animals observed in pig and sheep farms and leading to episodes of infertility [38].
- **Fumonisins** have only been characterised in the last fifteen years or so and merit particular attention for different reasons. The three substances representative of this family [B_1, B_2, B_3] exert a variety of pathological effects in a range of different animal species: leukoencephalitis in horses, pulmonary oedema in swine and hepatic carcinogenesis in rats [39]. Classified by IARC as a possible carcinogen in humans, fumonisin B_1 (group 2B in 2002) is the predominant form in the event of contamination. A statistical link between its presence in foodstuffs at substantial levels and the observation of an increase in oesophageal cancer cases has been established in South Africa [40] and is very strongly suspected in Italy [41,42]. In the latter case, corn flour used in the preparation of polenta is thought to be the medium for human intoxication [41]. At a molecular level, fumonisins interfere with control of biosynthesis of sphingolipids. As far as *Fusarium* genus toxins are concerned, the risk of mycotoxin contamination exists more specifically at the cultivation site.

These substances present the specificity of being frequently observed in combination, at variable concentrations, in corn grain and derived products. Synergetic toxic effects result from this co-exposure/food intoxication, if they are compared with the intrinsic effects of the various substances considered individually. In addition, the synergetic effect also extends to biological risks. Thus, in China, the superimposition of geographic zones with a high prevalence of exposure to aflatoxins and the endemic disease of hepatitis B reveals an increased incidence of primary liver cancer. Moderate exposure to aflatoxin B1 triples the risks of developing liver cancer in patients carrying the hepatitis B virus [43].

The metabolic routes for detoxification concerning these substances involve the usual hepatic and enterocytic functionalisation (cytochromes P450) and conjugation enzymes. However, cases of inappropriate molecular activation by these same enzymes have been reported in connection with the secondary metabolites of moulds liable to contaminate corn.

4.3 DATA RELATIVE TO STANDARDS AND REGULATORY DATA, INFLUENCE OF CLIMATE AND CULTURAL PRACTICES

With respect to **aflatoxins**, from 12 December 2003, the provisions of regulation 2174/2003, which modifies regulations 194/97 and 466/2001, apply. The maximum level accepted for corn destined directly for human consumption or as an ingredient in foodstuffs is the same as that for cereals in general: 2 µg/kg for aflatoxin B_1 and 4 µg/kg for the sum of aflatoxins B_1, B_2, G_1 and G_2.

With respect to animal nutrition, directive 2002/32/EC of 7 May 2002 relative to undesirable substances in animal feed sets a maximum aflatoxin B1 content of 20 µg/kg in corn (12% moisture content) in the raw material, with contents of 5 µg/kg and 50 µg/kg in complete or complementary feedingstuffs and depending on the animal species for which this feedingstuff is intended.

With respect to **ochratoxin A**, from 5 April 2002, the provisions of regulation 472/2002, which modifies regulation 466/2001, apply. The maximum ochratoxin A limits are, respectively, 5 µg/kg for raw cereal grain and 3 µg/kg for products derived from cereals, along with grain intended for direct human consumption.

Concerning **fusariotoxins**, in the absence of any regulations governing their levels in food products, the “Conseil Supérieur de l’Hygiène Publique de France” (CSHPF – French Public Health Council) recommendations represent references in terms of risk management options (opinion of 8/12/1998). The CSHPF recommendation proposes reducing the maximum **zearalenone** content in cereals and derived products from 200 to 50 µg/kg and that of **fumonisin B_1** to 3000 µg/kg (target value: 1000 µg/kg).

Discussions are currently ongoing at the European Commission to set maximum levels for **zearalenone, deoxynivalenol and fumonisins.**

All the CSHPF recommendations outlined take into account the feasibility of the project in view of the mean mycotoxin concentrations measured in cereals produced in France. A specific comment must be made with respect to the inherent risk of aflatoxins. The climatic conditions in France are unfavourable to the organisms involved in their synthesis. Since this involves production of toxins closely correlated with poor storage conditions, the professional practices of the French cereal industries are unfavourable to the development of *Aspergillus*. Giving up of the practice of drying whole cobs in corn cribs is an illustration of this. It has been established that contamination with aflatoxin can be effectively prevented by artificial drying immediately after harvesting [44]. Similarly, the data available indicate that cases of exceeding the aflatoxin limits are very rare in French corn production (mean for distribution of uncontaminated batches of French cereals: 0.6 µg/kg [45]). All these reliable facts suggest that in the specific context of France, the public health risk associated with exposure to aflatoxins via the consumption of corn grown in France, presents obvious signs of being satisfactorily controlled with respect to the standards in force.

Conversely, fusariotoxins are produced by organisms which are better adapted to the temperate climate in France. They are produced in the context of cultivation of the plant and pre-exist the stored product. Their incidence in corn and its derivatives for animal or human nutrition is high and the maximum values measured in certain contaminated batches can be considerable. In the context of France, the difficulty of controlling corn contamination with fusariotoxins is recognised. Their concentrations are therefore particularly relevant parameters in the assessment of any alleged benefit provided by Bt corn crops in comparison with conventional crops.

4.4 OVER-EXPRESSION OF THE BT TOXIN AND INCIDENCE ON MYCOTOXIN CONTAMINATION OF CORN

The frequency and severity of fungal contamination of corn are dependent on multiple factors. In addition to the influence of local meteorological conditions, it has been established by a substantial number of publications that the degree of impregnation of the kernel with mycotoxins is correlated with the severity of damage caused to the plant by insect pests [46,47,48]. Consequently, there is a legitimate expectation that a measurable impact on the intensity of fungal contamination and/or that of mycotoxin impregnation may be observed in a plant that has become less sensitive to insect predation. However, the rationality of this cause-effect relationship, along with the hoped-for benefits, were not claimed in the initial stages of development of GMPs by the various industrial seed groups. Today, it can be observed that the results of studies authenticating the lower level of impregnation of GMPs by mycotoxins are being widely used when dealing with the industry to promote GMP products and their image. A study by Brookes (2002) evaluated the impact of growing Bt corn on farms in Spain [49]; this study identifies that Spanish farmers perceive Bt corn to be of higher quality on the basis of the lower mycotoxin quantities (fumonisins) that it contains. The reference document cited is the study by Bakan *et al.* published in 2002 [32] implementing field trials in France and Spain. This fact illustrates the rapid use of scientific data for the purposes of communication or even commercial promotion. In this context, it is useful to provide a distanced, objective assessment of the results of a still limited number of studies dealing specifically with the subject in corn.

Table 1 presents a summary of the factors increasing (more) or reducing (less) comparative levels of various mycotoxins measured in **Bt corn** relative to those measured in **conventional corn,** known as isogenic corn.

Table 1. Variations in mycotoxin contamination levels in Bt corn grain compared to conventional near isogenic kernels established by various authors

Studies	Mycotoxins			
	Deoxynivalenol	**Aflatoxins**	**Zearalenone**	**Fumonisin B1**
Brake and Vlachos, 1998 [50]	30 times less	0.5 times less		<1 ppm in both samples
Munkvold and Hellmich, 1999 [51]				0.25 to 9 times less in 1995, 96 and 97
Dowd 2000 [52] MON 810 BT 11 GC 592				30 to 40 times less in 1997 and 98 NS in 1995/98
Pietri and Piva, 2001 [53]	NS	NS	NS	6 to 10 times less in 1997, 98 and 99
Aulrich *et al* 2001 [54]	0.7 to 10 times less			0 to 20 times less
Masoero *et al* 1999 [65]	0.5 times less	2 times less		10 times less
Dowd 2001 [55]				fumonisins 1.8 to 15 times less
Bakan *et al* 2002 [32]	1.5 times more to 13 times less		8 times less (on 1 trial out of 5)	5 to 28 times less

NS: no significant variation.

The context of certain studies must be described in order to assess their scope with more accuracy. Thus, the study by Brake and Vlachos [50] is a comparative nutritional study in chickens. Assessment of deoxynivalenol and aflatoxins was a one-off analysis on two batches of Bt corn (event Bt 176) and conventional corn.

The study by Munkvold and Hellmich [51] presents the remarkable features of having being conducted over three consecutive years, 1995, 1996 and 1997 (different weather conditions), and having compared field trials with infestations of *Ostrinia nubialis* (European corn borer), either natural or controlled using juvenile larvae and reproducing degrees of infestation comparable with natural infestations. The transgenic events affecting the hybrids considered in the study were multiple (e.g.: MON 810, Bt 11, Bt 176, etc.) and all consisted of controlled expression of *cry* genes of *B. thuringiensis*. For each of the three years of the study, the conventional corn crops exceeded at least one of the limits recommended by the American Association of Veterinary Laboratory Diagnosticians for horse feed (5 mg/kg fumonisins) or pig feed (10 mg/kg fumonisins). In 1996 and 1997 the total fumonisin contents (B_1 + B_2 + B_3) of the corresponding transgenic corn crops were, respectively, 4.1 and 7.9 times less. Reproducing previous results, a correlation between the severity of predation of the pest, the presence of *Fusarium* in the plant and the fumonisin content was established. An extensive statistical interpretation of the results attempted to assess, in their own right, the influence of the origin of the various hybrids and the nature of the transgenic event considered.

The studies by Pietri and Piva [53] on event MON 810 were conducted over three years, 1997, 1998, 1999, on a total of 37 sites representing a total of 93 samples. The authors report that the most marked difference relative to the lower fumonisin B_1 content of transgenic corn (10 times less), corresponds to a rainy year during the plant's growth period, offering the best conditions for growth of moulds.

The study by Bakan *et al.* [32] was conducted with transgenic event MON 810 (*cry*1A(b)). Five field trials (1999) were analysed (three in France, two in Spain), in zones in which *Ostrinia nubialis* and *Sesamia nonagrioides* (pink stem borer) are prevalent. Indirect evaluation of the degree of fungal contamination by assessment of the plant's ergosterol plant confirms the correlation between contamination with micromycetes and the mycotoxin content. The presence of micromycetes in transgenic corn was, depending on the study sites, 4 to 18 times less than that of the conventional isogenic corn (fumonisin B_1: 5 to 28 times less). It can be seen that the fumonisin B_1 contents of The conventional corn kernels harvested in France and considered in this study contained higher levels compared to the means usually measured. These contrasting values highlighted the beneficial potential of transgenic corn in conditions which are especially favourable to fungal development. This study also considered the levels of other fusariotoxins, trichothecenes (deoxynivalenol, nivalenol) and zearalenone. However, the results must be considered with caution because of contradictory values between growing sites for deoxynivalenol levels and values close to the detection limit for other mycotoxins. As a consequence, only a small number of statistically significant differences in the level of nivalenol and zearalenol were observed between conventional and transgenic kernels. Discrepancies between a significant favourable effect on fumonisin in transgenic corn only in the absence of the same effect on other mycotoxins synthetised by the same mould are not discussed in the present article. These results must consequently be considered as provisional.

4.5 QUALITY OF ANIMAL PRODUCTS

4.5.1 Incidence of the Bt gene on mycotoxin contamination of corn .

Moist conditions during harvesting or storage can, in certain cases, be favourable to the development of microscopic fungi (*Aspergillus, Fusarium*, etc.). This situation has been particularly well described in the case of long natural drying times of whole cobs in corn cribs. Contamination with aflatoxin is a threat, but can be very effectively prevented by artificial drying immediately after harvesting [44].

More recently, kernel contamination with fumonisins, consecutive to the development of *Fusarium* is promoted by the ear damage caused by European corn borer larvae [50,51]. This type of contamination, which is very significantly higher in conventional corn but highly dependent on climatic conditions and the intensity of corn borer damage, has been reported both in North America [51,56] and in all the corn grain-producing countries of Europe, such as France, Italy and Spain [57] (table 1).

The always moderate contamination of the whole Bt corn plant is also lower than that of conventional corn, especially since the development of fungi during silo storage is also a threat for the latter [56].

Although the level of toxicity of fumonisin in domestic animals has not been formally determined, it is known that it causes leukoencephalitis in horses, pulmonary oedema in pigs, that it is carcinogenic in rodents and that it causes oesophageal cancers in humans (SCAN[9], 2003, personal paper). It may be associated with a relative reduction in the growth performance of chickens fed with control corn contaminated with fumonisin [58].

4.5.2 Nutritional quality and food safety of Bt corn, cotton and soybean

Data relative to the substantial equivalence of genetically modified plants currently growing in certain countries no longer needs to be reported: this is always one of the conditions for acceptance of new plant varieties.

The study references are numerous in the case of Bt corn and the results are statistically confirmed [59]. A number of studies prior to authorisation for dissemination of GMOs have been conducted more specifically on farm animals rather than laboratory animals. Thus, we recalled the results of 19 publications in the scientific literature dedicated to food safety testing of plants carrying a Bt gene, including sixteen in corn, two in cotton and one in soya:

- 3 on the whole corn plant in fattening cattle for a maximum duration of 246 days;
- 3 on the whole corn plant in dairy cows for a maximum duration of 91 days;
- 5 on corn grain in fattening pigs for a maximum duration of 90 days;
- 5 on corn grain in chickens for a maximum duration of 42 days;
- 2 on cotton (cotton seed meal) distributed to dairy cows;
- 1 on soya (soybean oil meal) distributed to chickens.

The most numerous studies on Bt corn concern both the grain, used in all the animals, and the whole plant, reserved for ruminants.

Performance expressed by zootechnical parameters is completed by measurement of product quality: composition of the milk, composition and carcass quality of the cattle, pigs and chickens, along with the weight of the meat cuts. The organoleptic properties of the meat are also sometimes indicated. None of these parameters were modified by consumption of Bt corn, cotton or soybean, authorised for cultivation and animal consumption by the official authorities (FDA[10] and EPA[11] for the USA, SCP[12]/SCAN for the European Union).

Although, for almost all of these studies, the absence of any difference observed for the performance and general condition of farm animals consuming plants carrying the Bt gene leads to the conclusion that neither the genetic construction (DNA) nor the protein(s) expressed are toxic for animals, and, consequently, for humans, a better nutritional quality related to the probable lower consumption of mycotoxins leading to improved growth performance in animals eating Bt corn still remains to be demonstrated. Two studies conducted in growing chickens and pigs by Piva *et al* [58,60] reveal lower contamination with fumonisin B_1 with improved growth performance. More in-depth studies on the nature and quantity of mycotoxin residues in products of animal origin must be conducted in order to assess the possible benefits to consumer health related to lower contamination of products from animals (ruminants, pigs or poultry)[13] with a diet based mainly on feed containing Bt corn.

5 CONCLUSIONS

The introduction of new insect-resistant varieties makes it possible to considerably reduce the amount of insecticide treatments and, in the same proportions, that of active substances, particularly in cotton growing. However, there are geographic variations. Indeed, with respect to all the pesticide products used in the main crops in the USA, estimates for 2001 relative to the domestic American market advance a figure of almost 2000 tonnes for the reduction in active substances used consecutive to the use of Bt varieties for corn and cotton, thought to represent only 10% of the reduction in pesticide products, with most of this "saving" being related to the introduction of herbicide-tolerant varieties of soybean, corn and cotton [61].

---

9 SCAN: Scientific Committee on Animal Nutrition (European)

10 FDA: Food and Drug Administration (USA)

11 EPA: Environmental Protection Agency (USA)

12 SCP: Scientific Committee on Plants (European)

13 A study is currently under way at Afssa to assess exposure of animals to mycotoxins and their impact on the zootechnical performance of these animals.

In developing countries, the introduction of Bt cotton has consequences on the “health” of the environment, with less contamination by insecticide products, on the health of the farmers, who are not always well advised on the chemical risks they are exposed to when using these active substances and do not necessarily take all the precautions required, on the farming economy, by reducing labour and on the quality of the by-products used as home-grown feeds in village farms, such as cotton seed, which will probably be less contaminated by insecticide residues.

However, the introduction of Bt corn is largely dependent on economic reasons. Since few insecticide treatments are currently used due to their high cost during the advanced growth stage and their partial effectiveness, these varieties are only used if the damage caused by insects such as European corn borers, pink stem borers and corn rootworms risks being serious and, consequently, economically penalising. In certain cases, better farming systems can already very significantly limit damage to crops, without requiring the use of Bt varieties.

The introduction of Bt varieties, either for cotton or corn, will not be able to cover all surface areas in order to control the potential development of resistance in the insects concerned. Therefore, for cotton farming in particular, two methods for limiting the population of insect pests will exist alongside one another: one genetic, with the value emphasised previously in terms of health, the other chemical with the toxic risks already mentioned.

In a strategically unintentional way, the introduction of Bt corn varieties makes it possible to markedly reduce mycotoxin contamination following insect attacks on the corn plant and kernels. The results of the studies available are indisputable and highly significant with respect to the fumonisins produced by moulds which are common in the climates of European countries. The results relative to trichothecenes and zearalenone are still provisional pending a definitive conclusion However, the observations, with only one exception, really do highlight a reduction in mycotoxin contents in Bt corn, which is consistent with the results relative to fumonisins. This ought to have a potential incidence, difficult to measure for the time being, on the health of farm animals, for which the production of corn grain is primarily intended, and hence on the quality of the animal products consumed by humans. In their study, Piva *et al* [58,60] already observed better growth in pigs and chickens fed with Bt corn, which was less contaminated with fumonisin B_1 than conventional isogenic corn, a diet based on this better quality probably having an effect on numerous general metabolism parameters. This fact could encourage the creation of new varieties resistant to the development of fungi by conventional plant breeding [62,46] or by the introduction of genes encoding proteins with an antifungal activity [63] or involved in the biosynthesis of antifungal compounds [64].

Although it was detected and confirmed *a posteriori*, the lower level of mycotoxins in Bt corn, in temperate European climates, is to be credited to a genetic intervention strategy on corn which did not intend or even anticipate this. This result, supported by a substantial number of studies on the quality of animal products, must now be evaluated with respect to the actual benefits that the consumer may hope to gain in terms of health. These are of two types: one, direct, through the human consumption of products derived from corn and the second, indirect, through the quality of products of animal origin produced using corn-based feedingstuffs. Indisputably, this fact represents a positive point, from which benefit may be drawn to increase the safety of human and animal foodstuffs.

References

1 Neppl C.C. Managing resistance to *Bacillus thuringiensis* toxins. BA Thesis. Environmental studies. University of Chicago. May 26, 2000.

2 Crickmore N, Zeigler DR, Feitelson J, Schnepf E, Van Rie J, Lereclus D, Baum J & Dean DH. (1998) Revision of the nomenclature for the *Bacillus thuringiensis* pesticidal crystal proteins. *Microbiol. Mol. Biol. Rev.* 62, 807-813.

3 Liu Y.B., Tabashnik B.E. (1997) Experimental evidence that refuges delay insect adaptation to *Bacillus thuringiensis*. *Proc. R. Soc. Lond. B* 264, 605-610.

4 Clive J. (2003) Global status of commercialized transgenic crops: 2002. *ISAAA Briefs* 27.

5 Beuve M., Naïbo B., Foulgocq L., Courbon R., Lapierre H. (2000) La jaunisse nanisante de l'orge chez le maïs-grain. *Phytoma*, 525, 35-37.

6 Phipps R.H., Park J.R. (2002) Environmental benefits of genetically modified crops: global and European perspectives on their ability to reduce pesticide use. *J. Anim. Sci. Feed Sci.* 11, 1-18.

7 Huang J., Hu R., Pray C., Quiao F. (2001) Rozelle S. Biotechnology as an alternative to chemical pesticides: a case study of Bt cotton in China. *5th Internat. Consortium Ag. Biotech. Res.*, Ravello, Italy, 15-18 June 2001, pp 109-110 (abstract).

8 Carpenter J.E. (2001) Case studies in benefits and risks of Agricultural Biotechnology: Roundup Ready soybeans and Bt field corn. National Center for Food and Agricultural Policy, Washington DC.

9 Edge J.M., Benedict J.H., Carroll J.P., Reding H.K. (2001) Bollgard cotton: an assessment of global, economic, environmental and social benefits. *J. Cotton Sci.* 5, 1-8.

10 Pyrethrins and pyrethroids. National Pesticide Telecommunications Network (NPTN), 1998. http://ace.orst.edu/info/nptn or http://ace.orst.edu/info/extoxnet/.

11 Deltamethrin. EXTOXNET PIP (Extension Toxicological Network – Pesticide Information Profiles). Oregon State University (1995). http://ace.orst.edu/cgi-bin/mfs/01/pips/deltamet.htm.

12 Cypermethrin. EXTOXNET PIP (Extension Toxicological Network – Pesticide Information Profiles). Oregon State University (1995). http://ace.orst.edu/cgi-bin/mfs/01/pips/cypermet.htm.

13 http://www.pan-uk.org:80/pestnews/actives/fipronil.htm

14 http://www.bcpcbookshop.co.uk/acatalog/downloads/samplepest.rtf

15 New Pesticide Fact Sheet, 1996, US EPA, Office of Prevention, Pesticides and Toxic Substances, Washington DC, 20460, EPA-737-F-96-005. http://www.epa.gov/fedrgstr/EPA-PEST/199...ay-12/pr-736DIR/Facts/Factsheet.txt.html

16 Hurley PM, Hill RN & Whiting RJ. Mode of carcinogenic action of pesticides inducing thyroid follicular cell tumors in rodents. *Environmental Health Persp.* 106 (1998) 437-445.

17 Pant N., Prasad A.K., Srivatava S.C., Shankar R., Srivastava S.P. (1995) Effect of oral administration of carbofuran on male reproductive system of rat. *Human & Experiment. Toxicol.* 14, 889-894.

18 Pant N., Shankar R., Srivastava S.P. (1997) *In utero* and lactational exposure of carbofuran to rats: effect on testes and sperm. *Human & Experiment. Toxicol.* 16, 267-272.

19 Yousef M.I., Salem M.H., Ibrahim H.Z., Helmi S., Seehy M.A., Bertheussen K. (1995) Toxic effects of carbofuran and glyphosate on semen characteristics in rabbits. *J. Environmental Sci. Health* B30, 513-534.

20 Rawlings N.C., Cook S.J., Waldbillig D. (1998) Effects of the pesticides carbofuran, chlorpyrifos, dimethoate, lindane, triallate, trifluralin, 2,4-D, and pentachlorophenol on the metabolic endocrine and reproductive endocrine system in ewes. *J. Environmental Sci. Health* 54, 21-36.

21 Gandhi R., Snedeker S.M. (1999) Critical evaluation of chlorpyrifos breast cancer risk. Program on breast cancer and environmental risk factors in New York state (BERCF). Critical evaluation n°9, 1999, Cornell University. http://www.cfe.cornell.edu/bcerf/.

22 ATSDR. Toxicological profile for chlorpyrifos. NTIS, 1997, PB98-103088 (Atlanta: Agency of Toxic Substances and Disease Registry, US Public Health Service).

23 *Bacillus thuringiensis*. EXTOXNET PIP (Extension Toxicological Network – Pesticide Information Profiles). Oregon State University (1996). http://ace.ace.orst.edu/info/extoxnet/pips/bacillus.htm.

24 Clive J. (2001).Global review of commercialized transgenic crops: 2001. *ISAAA Briefs* 24.

25 Bourguet D., Desquilbet M., Lemarié S. (2002) Gestion des maïs *Bt* aux Etats-Unis. Mise en place des zones refuges. Inra, Compte rendu de mission.

26 US Environmental Protection Agency (1998). The Environmental Protection Agency's white paper on Bt Plant-pesticide resistance management, may. www.epa.gov/oppbppd1/biopesticides/white_bt.pdf.

27 US Environmental Protection Agency (2001). Biopesticides registration action document (BRAD), october..www.epa.gov/pesticides/biopesticides/reds/brad_bt_pip2.htm.

28 Losey J.E., Rayor L.S., Carter M.E. (1999) Transgenic pollen harms monarch larvae. *Nature* 399, 214.

29 Yiannikouris A., Jouany J-P. (2002) Mycotoxins in feeds and their fate in animals. *Anim. Res.* 51, 81-89.

30 Scudamore K.A., Patel S. (1999) Survey for aflatoxins, ochratoxin A, zearalenone and fumonisins in maize into the United Kingdom. *Anim. Feed Sc. Technol.* 78, 21-37.

31 Le Bars J. Contamination fongique et mycotoxique d'aliments composés fabriqués à la Guadeloupe. *Sci. Alim.* 2 (1982) 61-66.

32 Bakan B., Melcion D., Richard-Molard D., Cahagnier B. (2002) Fungal growth and *fusarium* mycotoxin content in isogenic traditional maize and genetically modified maize grown in France and Spain. *J. Agric. Food Chem.* 50, 728-731.

33 Placinta C.M., D'Mello J.P.F., Macdonald A.M.C. (1999) A review of worldwide contamination of cereal grains and animal feed with *Fusarium* mycotoxins. *Anim. Feed Sc. Technol.* 78, 21-37.

34 Pittet A. (1998) Natural occurrence of mycotoxins in foods and feeds – an updated review. *Rev. Med. Vet.* 149, 479-492.

35 Scudamore K.A., Patel S. (2000) Survey for aflatoxins, ochratoxin A, zearalenone and fumonisins in maize into the United Kingdom. *Food Addit. Contam.* 17, 407-416.

36 Chao T.C., Maxwell S.M., Wong S.Y. (1991) An outbreak of aflatoxicosis and boric acid poisoning in Malaysia: a clinicopathological study. *J Pathol.* 164, 225-33.

37 Tanaka T., Hasegawa A., Yamamoto S., Lee U.S., Sugiura Y. Ueno Y. (1988) Worldwide contamination of cereals by the *Fusarium* mycotoxins nivalenol, deoxynivalenol, and zearalenone. 1. Survey of 19 countries. *J. Agric. Food Chem.* 36, 979-983.

38 Kuiper-Goodman T. Scott P.M. (1989) Risk assessment of the mycotoxin ochratoxin A. *Biomed. Environ. Sci.* 3; 179-248.

39 Dutton M.F. (1996) Fumonisins, mycotoxins of increasing importance: their nature and their effects. *Pharmacol Ther.* 70, 137-61.

40 Rheeder J.P., Marasas W.F.O., Thiel .PG., Sydenham E.W., Shephard G.S., van Schalkwyk D.J. (1992) *Fusarium monoliforme* and fumonisins in corn in relation to human esophageal cancer in Transkei. *Phytopathology* 82, 353-357.

41 Doko M.B., Visconti A. (1994) Occurrence of fumonisins B1 and B2 in corn and corn-based human foodstuffs in Italy. *Food Addit. Contam.*11, 433-439.

42 Franceschi S., Bidoli E., Baron A.E., La Vecchia C. (1990) Maize and risk of cancers in the oral cavity, pharynx and esophagus in northeastern Italy. *J Natl. Cancer Inst.* 17, 1407-1411.

43 Ming L., Thorgeirsson S.S., Gail M.H., Lu P., Harris C.C., Wang N., Shao Y., Wu Z., Liu G., Wang X., Sun Z. (2002). Dominant role of hepatitis B virus and cofactor role of aflatoxin in hepatocarcinogenesis in Qidong, China. *Hepatology* 36, 1046-1049.

44 Shotwell O.L., Hesseltine C.W., Goulden M.L., Vandegraft E.E. (1973) *Cereal Sci. Today* 16 266-273.

45 Les mycotoxines dans l'alimentation: évaluation et gestion du risque. CSHPF, TEC & DOC Eds, Paris 1999.

46 Munkvold G.P., Desjardins A.E. (1997) Fumonisins in maize: can we reduce their occurrence? *Plant Dis.* 81, 556-565.

47 Dowd P.F. (1995) Sap beetles and mycotoxins in maize. *Food Addit. Contam.* 12, 497-508.

48 Sobek E.A., Munkvold G.P. (1999) European corn borer larvae as vectors of *Fusarium moniliforme*, causing kernel rot and symptomless infection of maize kernels. *J. Econ. Entomol.* 92, 503-509.

49 Brookes G. (2002) The farm level impact of using Bt maize in Spain. 1-23. http://www.europabio.org/pages/ne_gbgmcrops.asp.

50 Brake J., Vlachos P. (1998) Evaluation of transgenic Event 176 "*Bt*" corn in broiler chickens. *Poultry Science* 77, 648-653.

51 Munkvold P., Hellmich R.L. (1999) Comparison of fumonisin concentration in kernels of transgenic *Bt* maize hybrids and non transgenic hybrids. *Plant Dis.* 83, 130-138.

52 Dowd P.F. (2000)Indirect reduction of ear molds and associated mycotoxins in *Bacillus thuringiensis* corn under controlled and open field conditions: utility and limitations. *J. Econ. Entomol.* 93, 1669-79.

53 Pietri A., Piva G. (2000) Occurrence and control of mycotoxins in maize grown in Italy. In Proceedings 6th International Feed Production Conference, Piacenza - Italy, 17-28 November 2000, pp226-236.

54 Aulrich K., Bohme H., Daenicke R., Halle I., Flachowsky G. (2001) Genetically modified feeds in animal nutrition 1st communication: *Bacillus thuringiensis* (Bt) corn in poultry, pig and ruminant nutrition. *Arch. Anim. Nutr.* 3, 183-195.

55 Dowd PF. Biotic and abiotic factors limiting efficacy of Bt corn in indirectly reducing mycotoxin levels in commercial fields. *J. Econ. Entomol.*94 (2001) 1067-74.

56 Munkvold P., Faust M., Schnitzler J.A. (2002) Influence of genotype and infestation with European cornborer for nutritive value and quality of fresh and ensiled material from *Bt* and non *Bt* corn. *J. Anim. Sci.* 80 Suppl. 1 301 (Abst. 1119).

57 Cahagnier B., Melcion D. (2000) Mycotoxines de *Fusarium* dans les grains de maïs à la récolte; relation entre la présence d'insectes (pyrales, sésamie) et la teneur en mycotoxines. In Proceedings 6th International Feed Production Conference, Piacenza – Italy, 27-28 November 2000, pp237-249.

58 Piva G., Morlacchini M., Pietri A., Rossi F., Prandini A. (2001) Growth performance of broilers fed insect protected (MON810) or near isogenic control corn. *J. Anim. Sci.* 79 Suppl. 1 320 (Abst. 1324).

59 Aumaître A.L. (2002) Les aliments issus de plantes génétiquement modifiées: équivalence, efficacité et sécurité chez les animaux de ferme. *INRA Prod. Anim.* 15, 97-108.

60 Piva G., Morlacchini M., Pietri A., Piva A., Casadei G. (2001) Performance of weaned piglets fed insect-protected (MON810) or near isogenic corn. *J. Anim. Sci.* 79 Suppl. 1 106 (Abst. 441).

61 Gianessi L.P., Silvers C.S., Sankula S. Carpenter J.E. Plant Biotechnology: Current and Potential Impact For Improving Pest Management In U.S. Agriculture: An Analysis of 40 Case Studies. Juin 2002. http://www.ncfap.org/40CaseStudies.htm.

62 Moreno O.J., Kang M.S. (1999) Aflatoxins in maize: the problem and genetic solutions. *Plant Breed.* 118, 1-16.

63 Jach G., Gornhardt B., Mundy J., Logemann J., Pinsdorf E., Leah R., Schell J., Maas C. (1995) Enhanced quantitative resistance against fungal disease by combinatorial expression of different barley antifungal proteins in transgenic tobacco. *Plant J.* 8, 97-109.

64 Hain R., Reif H.J., Krause E., Langebartels R., Kindl H., Vornam B., Wiese W., Schmelzer E., Schreier P.H. Stocker R.H. (1993) Disease resistance results from foreign phytoalexin expression in a novel plant. *Nature* 361, 153-156.

65 Masoero F., Moschini M. Rossi, Prandini A., Pietri A. (1999) Nutritive value, mycotoxin contamination and *in vitro* rumen fermentation of normal and genetically modified corn (CRYI A(B) grown in northern Italy. *Maydica* 44, 205-209.

66 Roe, R. M. Vertebrate toxicology of the solubilized parasporal crystalline proteins of *Bacillus thuringiensis* israelensis. In Reviews in Pesticide Toxicology 1: Toxicological Studies of Risks and Benefits. Hodgson, E., Roe, R. M. and Motoyama, N., Eds. North Carolina State University, Raleigh, NC, 1991.10-148

Annex

MONARCH BUTTERFLY AND BT CORN

Reminder of a few "historical" facts
In August 1995, the Environmental Protection Agency (EPA) records the first corn genetically modified to be resistant to the European corn borer (Bt corn) [1].

In May 1999, a report is published in *Nature* stating the Bt corn pollen is dangerous for the Monarch butterfly [2]. Transgenic pollen harms monarch larvae, *Nature*, 399, 214).

In November 1999: the first workshop on "monarch/Bt corn" is organised.

In December 1999: the EPA issues a call to obtain data on Bt corn.

In July 2000: an article with a scientific reading committee is published, indicating the absence of Bt corn pollen toxicity on Monarch larvae under field conditions [3].

In October 2000, the EPA sponsors a scientific advisory committee in Washington D.C. [4].

On 9 October 2001: an issue of *Proceedings of the National Academy of Science* publishes a series of 6 articles on the cooperative research carried out on the subject [5-10].

On 16 October 2001, the EPA authorises a new Bt corn variety [11].

Context
Back in 1995, before authorising Bt corn, the EPA had concluded that it did not see any adverse effects of Bt corn crops, including any for butterflies [1].

The EPA's conclusion was based on the fact that the Monarch butterfly does not land on the corn, but, rather, on the weeds surrounding this crop. The fact that pollen is spread for only a small distance around corn therefore limits exposure, as do the low concentrations of this pollen on the neighbouring weeds.

But in 1999, an article in *Nature* [2] indicates that if larvae feed on the Bt pollen spread on the weeds (milkweeds), they grow more slowly and die much earlier than those on milkweeds devoid of Bt corn pollen.

Despite precautions (the authors state that the results cannot be extrapolated to field conditions), the media take the results out of their context and do not indicate the reservations. Europe reacts by blocking new marketing authorisation dossiers and then various "anti-GMO" associations claim a moratorium in the USA.

The scientists respond
First of all, at a symposium held in Chicago in November 1999. At this point, despite the fact that only preliminary results were available, they provided arguments demonstrating that is very unlikely that the Monarch butterfly can be affected by Bt corn pollen.

Another 9 symposia followed. One of these was held in Kansas City and was attended by 40 scientists belonging to various communities (universities, government, industry and environment). The defined research priorities and projects were selected, funded by grants (representing a total amount of more than 200 000 US$) and supervised by a committee representing the various protagonists. The studies focused on exploration of the (potential) effects of Bt corn pollen on Monarch butterflies under typical growing conditions in corn fields.

An important point is that the scientists decided to use methods generating data which were directly comparable from one study to another. This made it possible to collate all the information obtained for the entire corn-growing zone in the USA and Canada.

The EPA acts
It puts several questions to the Biotech industry, giving it a deadline in which to respond. The public and private sectors collate all the available information concerning: the habitat zones of the Monarch butterfly and its behaviour, the spread of corn pollen, the distribution of milkweed, the toxicity of the Bt protein and Bt corn pollen on the Monarch butterfly.

It creates a "scientific advisory panel" (SAP) made up of experts charged with re-examining BT corn authorisations and examining the new data relative to the Monarch butterfly between 1999 and 2000.

The scientists return their conclusions

* **Swallowtails study**
  During the summer of 2000, scientists from the University of Illinois conducted a study demonstrating the absence of any Bt pollen toxicity on the swallowtail under normal field conditions. With the exception of Bt 176 corn, the scientists failed to find a higher Monarch mortality, even at pollen doses 5 times higher than those typically found in fields.

* **Studies on the Monarch butterfly**
  39 American and Canadian scientists conducted field studies in 1999 and 2000 to make a detailed evaluation of any impact of Bt corn pollen on the Monarch butterfly. Specialists in weeds, corn, entomology and other fields pooled their expertise to analyse the data, which made it possible to quickly build up a complete picture of the problem. This led to the publication of 6 articles in the *Proceedings of the National Academy of Sciences* in October 2001 [5-10].

Various points examined:

- The sensitivity of Monarch larvae to purified toxins and the pollen of various Bt corn varieties containing the *Cry1Ab, Cry1Ac, Cry9C & Cry1F* genes. They were tested either:
  - as purified toxins, incorporated in a specific diet;
  - from the pollen of hybrid Bt corn applied directly to the leaf discs of milkweeds;
  - from Bt pollen collected from corn cobs and applied to the leaf discs of milkweeds.

  The main conclusions of this study indicate that only pollen from corn event Bt 176 may affect Monarch larvae.

- The overlap periods between the presence of butterfly larvae and dissemination of corn pollen.

- Pollen deposits on milkweeds and neighbouring corn fields.

- Identification of the risks of a Bt pollen impact on Monarch populations, the conclusion being that the risk is negligible.

- Assessment of the impact of the Cry1AB toxin expressed in Bt corn pollen on Monarch larvae under normal field conditions.

Conclusions
It clearly emerges from all these studies that the risk of Monarch larvae being affected by the consumption of pollen produced by Bt corn, either on milkweeds (which is their favourite habitat), or even on the leaves of the corn, is negligible.

Although the article by Losey *et al*. [2] made both the biotech industry and the general public aware of a potential non-targeted negative effect of a Bt transgenic plant, it also had the effect of first of all triggering a set of negative reactions on the part of organisations opposed to the development of biotechnologies, but, at the same time, a very rapid reaction on the part of the scientific community since, within a range of two years [5-17], it has been able to provide answers to the questions raised and, ultimately, to conclude that the use of Bt corn represented a safer environment for the Monarch butterfly than the use of chemical pesticides.

References

1 U.S. Environmental Protection Agency. *Pesticide fact sheet for Bacillus thuringiensis ssp. kurstaki Cry1(A)b delta-endotoxin and the genetic material necessary for the production (plasmid vector pCIB4431) in corn*. EPA Publication No. EPA731-F-95-004 (1995). Washington, D.C.

2 Losey J.E., Rayor L.S., Carter M.E. (1999) Transgenic pollen harms monarch larvae. *Nature* 399, 214.

3 Wraight C.L., Zangerl A.R., Carroll M.J., Berenbaum M.R. (2000) Absence of toxicity of *Bacillus thuringiensis* pollen to black swallowtails under field conditions. *Proc. Natl. Acad. Sci. USA* 97, 7700-7703.

4 U.S. Environmental Protection Agency. Bt plant-pesticides biopesticides registration action document. 2000. www.epa.gov/scipoly/sap/2000/#october.

5 Hellmich R.L., Siegfried B.D., Sears M.K., Stanley-Horn D.E., Daniels M.J., Mattila H.R., Spencer T., Bidne K.G., Lewis L.C. (2001) Monarch larvae sensitivity to *Bacillus thuringiensis*-purified proteins and pollen. *Proc. Natl. Acad. Sci. USA* 98 11925-11930.

6 Oberhauser K.S., Prysby M.D., Mattila H.R., Stanley-Horn D.E., Sears M.K., Dively G.P., Olson E., Pleasants J.M., Lam W-K.F., Hellmich R.L. (2001) Temporal and spatial overlap between monarch larvae and corn pollen. *Proc. Natl. Acad. Sci. USA* 98, 11913-11918.

7 Pleasants J.M., Hellmich R.L., Dively G.P., Sears M.K., Stanley-Horn D.E., Mattila H.R., Foster J.E., Clark P.L., . Jones G.D. (2001) Corn pollen deposition on milkweeds in and near cornfields. *Proc. Natl. Acad. Sci. USA* 98, 11919-11924.

8 Sears M.K., Hellmich R.L., Stanley-Horn D.E., Oberhauser K.S., Pleasants J.M., Mattila H.R., Siegfried B.D., . Dively G.P. (2001) Impact of *Bt* corn pollen on monarch butterfly populations: A risk assessment. *Proc. Natl. Acad. Sci. USA,* 98, 11937-11942.

9 Stanley-Horn D.E., Dively G.P., Hellmich R.L., Mattila H.R., Sears M.K., Rose R., Jesse L.C.H., Losey J.E., Obrycki J.J., Lewis L.C. (2001) Assessing the impact of Cry1Ab-expressing corn pollen on monarch butterfly larvae in field studies. *Proc. Natl. Acad. Sci. USA* 98, 11931-11936.

10 Zangerl A.R., McKenna D., Wraight C.L., Carroll M., Ficarello P., Warner R., Berenbaum M.R. (2001) Effects of exposure to event 176 *Bacillus thuringiensis* corn pollen on monarch and black swallowtail caterpillars under field conditions. *Proc. Natl. Acad. Sci. USA* 98, 11908-11912.

11 U.S. Environmental Protection Agency. Biotechnology corn approved for continued use. [News release (16 October 2001)].

12 Jesse L.C.H., Obrycki J.J. (2000) Field deposition of *Bt* transgenic corn pollen: lethal effects on the monarch butterfly. *Oecologia* 125, 241-248.

13 Jones G.D. (2001) Corn pollen deposition on milkweeds in and near cornfields. *Proc. Natl. Acad. Sci. USA* 98, 11913-11918.

14 Minorsky P.V. (2001) The monarch butterfly controversy. *Plant Physiol.* 127, 709-710.

15 Shelton A.M., Sears M.K. (2001) The monarch butterfly controversy: Scientific interpretations of a phenomenon. *Plant J.* 27, 483-488.

16 Irwin R., Krishna P.J. Nontarget impacts of *Bt* corn: A risk assessment. *Information Systems for Biotechnology (ISB) News Report* (2002), pp. 6-8.

17 Gianessi L.P., Silvers C.S., Sankula S., Carpenter J. *Plant biotechnology: Current and potential impact for improving pest management in U.S. agriculture, An analysis of 40 case studies*. Washington, D.C.: National Center for Food and Agricultural Policy. 2002.

18 Gatehouse A.M.R., Ferry N., Raemaekers R.J.M. (2002) The case of the monarch butterfly: A verdict is returned. *Trends in Genetica* 18, 249-251.

1 PRELIMINARY COMMENTS RELATIVE TO THE CHOICE OF THIS CROP

The choice of glyphosate-tolerant sugar beet as an example of a GM plant which can be used to document the expected benefits in terms of human health is based on the following points.

- First of all, sugar beet farming is part of an industrial production process. The technical elements relative to production, processing and marketing are part of an industry sector.
- Production (generally under contract) is carried out using monogerm seed purchased every year; traceability is possible. Sugar beet is a biennial plant; the seeds are obtained in the second year. Sugar beet does not lead to any risk of pollination of neighbouring plant species and hence transmission of genes (GMO or otherwise) since the root is harvested in the first year.
- It is industrially processed using a crystallisation process to obtain sugar, with this process enabling control or even elimination of unwanted ingredients (fibres, polyphenols, contaminants, etc.).
- This crop is conventionally the subject of frequent herbicide applications, not devoid of risks to both the farmer and the environment.
- Finally, it should be noted that fodder beet, which is less widely grown and intended as an animal feed, is produced using a technical itinerary similar to that of sugar beet.

Finally, in comparison with other crops, such as corn or oilseed rape, in which there may be risks of pollen contamination (gene flows), GM sugar beet crops offer, outside seed production zones, apparently controlled, or controllable, conditions within a well integrated industry. It is therefore necessary to examine in what ways GM glyphosate-tolerant sugar beet crops could provide one or more health benefits.

2 REVIEW OF SUGAR BEET PRODUCTION

Sugar beet production in France covers almost 400,000 ha. Production is concentrated mainly in the North, Alsace and the Puy de Dôme region. This crop, which is highly localised with respect to sugar refineries, is in decline; from 438,000 ha in 2002, it fell to 395,000 ha in 2003, which can be broken down as follows: 356,000 ha for sugar production, 28,500 for industrial alcohol, 11,000 ha for ethanol.

Fodder beet crops are cultivated on less than 20,000 ha in France, primarily, in decreasing order of surface area, in the regions of Seine Maritime, Pas de Calais, Eure, Côtes d'Armor, the North, Ile-et-Vilaine, Somme and Morbihan. Although the surface areas are tending to decrease, this production (15 to 20 t of dry matter/ha) is one of the only fodders which can secure "grass pasture plus hay" systems, at the same time increasing the energy value of the basic diet of dairy cows. Consequently, the surface area is tending to increase in AOC cheese zones, where the use of silage crops is not advised.

The genetic improvement of sugar and fodder beet is carried out by a dozen plant breeding establishments in France and Europe. Every year, new varieties are listed in the French catalogue, which in 2003 had 93 and 37 varieties of sugar and fodder beet, respectively. Over the last 25 years, average sugar beet yields have risen sharply, notably in terms of white sugar per hectare, which went up from 7 t/ha in 1977 to 11.8 t/ha in 2002, which was a record yield [1].

This result is a consequence of both genetic progress and farming techniques, including weed control, which plays a major role in the success of this crop.

3 WHY IS WEED CONTROL NECESSARY IN SUGAR BEET CROPS?

Sugar beet growth and yields are largely dependent on climatic factors. Light plays a key role during the period between ground cover and harvest, particularly for sugar production. CO_2 fixing and its conversion into sugar closely match the evolution of the sun's rays over the course of the day; a figure of 1.72 g of dry matter (i.e. 1 gram of sugar) per megajoule of light intercepted per m² has been reported [1]. For this reason, it is essential to obtain rapid ground cover and to maintain this under optimum plant health conditions until harvesting, in order to draw maximum benefit from sunlight.

Prior to the 1970s, weed control was primarily manual and accounted for 120 hours of labour per hectare. Mechanical weed control (scarification, hoeing) is incomplete. The development of chemical methods of weed control represents a solution now widely used by sugar beet producers.

4 CONVENTIONALLY USED HERBICIDES

Several substances are used as herbicides on sugar beet, mainly to control dicotyledons but also graminaceous weeds. In France, the following substances are primarily used: Chloridazone, Cycloxydim, Ethofumesate, Lenacil, Metamitron, Phenmedipham and Triflusulfuron-methyl. These substances are the active ingredients of the herbicide, which also contains additives (solvent, surfactant, etc.). These active substances are in the public domain and companies producing pesticide products propose several different formulations, which they own, including these active substances and additives which are chemical agents, such as xylene, ethylene oxide, paraffin mineral oils, terpenic alcohols, complex ethylene and propylene polymers, etc. In the context of European harmonisation of pesticide products, these formulations are to be phased out by 2006 and replaced by those including vegetable oils not requiring any toxicological classification as additives.

Some of the toxicological characteristics of these substances are summarised in table 1 (MRL: maximum residue limit, ADI: acceptable daily intake).

Table 1: Characteristics of active substances applied to sugar beet crops

Name of the active substance	Toxicity (rat)		ADI	MRL on sugar beet	Toxicological classification*
	by the oral route LD50 (mg/kg bw.)	by inhalation LC50 (mg/L air)	mg/kg bw./d	mg/kg	
Chloridazone	2200	>5.4	ND	0.500	Xi N R43 R50 553
Cycloxydim	>2000	>5.28	0.06	0.100	exempt
Ethofumesate	>5000	>0.3	0.07	0.100	N R51 R53
Lenacil	>5000	ND	ND	ND	exempt
Metamitron	1830-3343	>0.33	0.025	0.050	Xn N R22 R50 R53
Phenmedipham	>8000	ND	0.03	0.100	N R50 R53
Triflusulfuron-methyl	>5000	>5.1	0.04	0.020	exempt
Glyphosate	>2000	>5.0	0.3	on vegetable 0.100	Xi N R41 R51 R53

* Key:

source: AGRITOX [2]

ND: not determined; Xi: Irritant; Xn: Harmful; N : Dangerous for environment
R22: Harmful if swallowed
R41: Risk of serious damage to eyes
R50: Very toxic to aquatic organisms
R51/ R53: Toxic to aquatic organisms, may cause long term adverse effects in the aquatic environment

The treatments are applied pre-emergence on around 65% of surface areas, generally between 20 March and 1 April, then post-emergence. Two or even four applications are then conducted after emergence of the sugar beet (between the cotyledon stage and 1 June) on the entire crop, by spraying on a mixture of three or four of these substances whenever weeds appear. For example, for each of these applications, the following doses are conventionally used [3] (table 2).

Table 2: Examples of herbicide doses (active substances) applied per treatment

Name	Dose applied	
	kg/ha	Active substance g/ha
Ethofumesate	0.6	120
Metamitron	2	700
Phenmedipham	1.2	192

The total mass of herbicide applied to the crop can therefore be as much as 12 kg/ha with three treatments, i.e. around **2.7 kg active substances per ha** according to surveys conducted over several years in France by the “Institut technique de la betterave” (Technical institute for sugar beet) [1].

These doses can be compared with those applied to a genetically modified (GM) sugar beet which is tolerant to glyphosate. In this case, there would be no pre-emergence application, but only two or three post-emergence applications, 10 to 15 days apart. The recommended doses per application

would be between 2 and 3 kg of products, i.e. 720 to 1000 g active substance per ha. The total herbicide mass for this glyphosate-tolerant sugar beet crop would be 4 to 6 kg/ha i.e. **1.4 to 2.1 kg active substance per ha** [1,3]. This dose is actually quite close, although nonetheless lower, to that used for production with conventional herbicides.

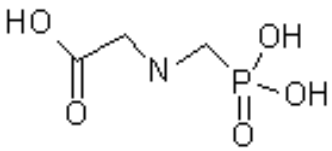
In order to attempt to identify health advantages of GM glyphosate-tolerant sugar beet crops, it is necessary to examine the genetic modification made to the sugar beet and the action of this herbicide in more detail.

5 GM SUGAR BEET

In order to be genetically tolerant to glyphosate, the GM plant must contain several genes: the 5-enolpyruvylshikimate-3-phosphate synthase gene (EPSPS), the 3- phosphoshikimate 1-carboxyvinyltransferase gene and a glyphosate oxidoreductase gene (GOX) [4,5]. These three genes, which are often of bacterial origin (*Agrobacterium tumefaciens* for EPSPS and *Achromobacter* for GOX) make it possible to avoid the effect of glyphosate on EPSPS and to degrade (either partially or totally) glyphosate into amino-methyl-phosphonic acid (AMPA) due to the action of GOX. These genes are introduced into *Agrobacterium tumefaciens* which is used as a processing agent for sugar beet cotyledons. Seedlings are regenerated from these cotyledons by *in vitro* culturing. The GM plants are selected by the joint use of molecular markers for the genes introduced and by application of the herbicide. A negative correlation has been observed between the number of copies of transgenes and the level of tolerance to glyphosate. GM sugar beet plants with only one copy of the transgenes have demonstrated the highest level of tolerance to glyphosate [4]. The success of the transformation depends on the genotype of the sugar beet.

6 MODE OF ACTION OF GLYPHOSATE

The glyphosate or N–phosphonomethyl glycine molecule is a non-selective herbicide with a very wide scope. Its mode of action is systemic and not due to a straightforward local contact effect.



Glyphosate moves in the sap, is found in the various tissues of the plant and is concentrated in meristematic zones. This substance inhibits the synthesis of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which is a key enzyme in the biosynthesis of three aromatic amino acids in microorganisms and plants. Animals are devoid of shikimic acid and the EPSPS enzyme and must therefore find these aromatic amino acids in their diet. Glyphosate is irreversibly bound to EPSPS which catalyses the reaction of shikimate-3-phosphate and phosphoenolpyruvate to produce 5-enolpyruvateshikimate-3-phosphate. This reaction occurs in the chloroplast.

Glyphosate is never applied in its natural state. Depending on the formulation, it is often found in the form of isopropylamine glyphosate salt and of course, like many herbicides, combined with “inert” surfactant agents. The formulation containing glyphosate applied to GM sugar beet would most likely be that marketed under the name of "Roundup Bioforce"; i.e. in the form of isopropylamine salt without ethoxylated amines (surfactants which are often phytotoxic). It should be noted, however, that the compounds generally present in herbicide formulations containing glyphosate are far from devoid of any effects on health (table 3), with this comment generally applying to all pesticides.

Table 3: List of the main compounds present, either alone or in combination, in commercial formulations containing glyphosate or glyphosate salt [6]

Name	Effects attributed
Benzisothiazolone	eczema, skin irritation [7]
Isobutane	nausea, breathing difficulties [8]
Isopropylamine	attacks the mucous membranes, nausea, headaches [9]
Methyl pyrrolidinone	eye irritation, spontaneous abortion in lab animals [10]
Ammonium sulphate	eye irritation, respiratory allergy [8]
Sodium sulphate	eye irritation, skin irritation, vomiting [11]
Polyethoxylated tallow amine (POEA)	burning of the eyes, nausea, diarrhoea [12]
3-iodo-2-propynyl butylcarbamate (IPBC)	eye irritation, spontaneous abortion in lab animals [13]

Statistical assessment of the health risks related to application of glyphosate cannot, for the time being, be conducted independently of the effect of one or more of these compounds. However, based on several of the physicochemical and toxicological characteristics of these substances, comparisons are possible with respect to the potential risks which may be linked to their use.

7 COMPARISON OF THE EFFECTS OF CONVENTIONAL HERBICIDES AND GLYPHOSATE

7.1 RELATIVE TO THE MODE OF ACTION OF THESE HERBICIDES

Briefly, a herbicide acts by contact and penetration into the plant: physiopathologists make a distinction between three modes of action:

(1) by contact, the plant dies without there being any penetration of the herbicide into the phloem,
(2) by shallow penetration, leading to death of the plant, with access to the phloem and transport of the molecule in the leaves,
(3) by transport in both types of sap and accumulation in both the leaves and the roots; the product is then said to be systemic.

The mode of action (1) is that used by ethofumesate and phenmedipham. Chloridazone and triflusulfuron-methyl are said to be relatively immobile and are found on the leaves. But it should be remembered that it is the roots, from which the sugars are extracted, that are ingested by animals.

Systemic herbicides, such as cycloxydim, lenacil, metamitron and glyphosate may be found in the storage organ, the root. In fact, although their maximum residue limits (MRL) are equivalent, GM sugar ought to contain less since glyphosate is actually metabolised, if not avoided, by enzymes produced by transgenes. But, it appears that (i) depending on the species (in particular, oilseed rape, corn), (ii) depending on the transformation element integrated in the GM plant, and (iii) depending on the growing conditions, residues can take various forms and range from 100% glyphosate and 0% amino-methyl-phosphoric acid (AMPA) to several percent glyphosate and almost 100% AMPA (Cujier, 2003, personal communication). Toxicologists also consider AMPA to be a residue, which must be measured at the same time as glyphosate residues in GM plants. New transformation events have been developed which could degrade this AMPA [14]. It is therefore necessary to check whether these products are effectively catabolised in the storage organ of GM sugar beet.

Finally, it should be remembered that MRLs are obtained from determination of the no observed effect level (NOEL) allocated a safety coefficient and that they apply to the whole sugar beet (root + leaves). Consequently, herbicide levels in the root are generally lower than the MRL and it must also be remembered that, irrespective of its mode of action, the product may have been partially or totally degraded (applications are carried out 3 months before harvesting).

We can conclude that, although GM sugar beet may contain less herbicide, the advantage of using glyphosate is not established here since the other herbicides are also in very low quantities or even non-existent at the harvesting stage. Measurement or residues in the organs used for consumption is obviously conducted by the relevant laboratories before any herbicide is approved for use.

7.2 RELATIVE TO THE CONSEQUENCES OF LEACHING

Rain can carry herbicides and the concentration in run-off water, water courses and ground water can be very variable. For example, it was observed that rainwater in 2000 was less contaminated than that in 1996 [15]. Ground water is contaminated with atrazine, a herbicide which is now prohibited in France.

Independently of the intensity and frequency of rain, relief and proximity or otherwise to water courses or ground water, at least two physicochemical characteristics of herbicides must be taken into account to be able to compare them in this respect: their solubility and their stability in water. Table 4 presents these two characteristics recorded in the Agritox base [2], which must also be established prior to approval of each of the active substances.

Table 4: Some physicochemical characteristics of herbicide active substances.

Active substance	Solubility in water mg/L	Stability in water	Henry's constant Pa*m^3/mole	Liposolubility Log P
Chloridazone	340	> 24 h	3.7	2.7
Cycloxydim	40	234 days (at pH 7)	ND*	2.31
Ethofumesate	40	stable (>2000 days at pH 5)	6.8 10^{-4}	2.7
Lenacil	6	stable	ND	2.31
Metamitron	1820	410 days (pH 4), 30 days (p H7)	1 10^{-7}	0.83
Phenmedipham	3.1	20 h (pH 7)	8.3 10^{-8}	3.59
Triflusulfuron-methyl	110	32 days	2.4 10^{-3}	1.95
Glyphosate	10500	> 30 days	2.1 10^{-7}	-3.2

* ND not determined *Source: AGRITOX[2]*

It can be seen that the various herbicides used on sugar beet do not have the same solubility in water. It is observed that glyphosate is 5 times to 3300 times more soluble in water than conventional herbicides.

The stability is evaluated by the degradation time of 50% of the active substance (DT50) in water, expressed in days or hours at a given pH. Phenmedipham, which is highly toxic for aquatic organisms, very fortunately has a low solubility and stability. However, it can be seen that metamitron and, particularly, glyphosate, are substances which can act on other organisms (bacterial or telluric fauna) or can be found in well water due precisely to their solubility and stability in water. The most widely used glyphosate formulation, Roundup, is known to have a very harmful effect on aquatic fauna [16].

The action of these substances on microorganisms, on aquatic fauna and, more generally, on the environment, will not be dealt with in this report. Let us simply remember that the pluriannual studies conducted in England on GM herbicide-tolerant sugar beet have revealed that using this crop has a real effect on floral and also on the biodiversity of insects [17]. However, it is necessary here to examine the health risks linked to the use of these herbicides, particularly for farmers and people living in rural areas.

7.3 RELATIVE TO AIR CONTAMINATION

Analyses conducted on air contamination during spraying show that the first 50 to 100 metres around the field are highly contaminated and that concentrations in the region of 5 to 10 micrograms per cubic metre can be detected [15]. And yet, whilst we consume two litres of water per day, we also breathe in between fifteen and eighteen cubic metres of air per day. It is therefore very important to examine the capacity of these herbicide substances to become volatile. Henry's constant characterises the capacity of an active substance in solution to become volatile. It is expressed in Pascal x m^3/mole and it can be seen in table 4 that there are marked differences between these substances. Chloridazone is a highly volatile substance, whereas phenmedipham and glyphosate are transformed less readily into vapour state when they are in solution in water. The risks associated with the use of these substances are first of all dependent on how much will be volatilised in the air. In terms of herbicide effectiveness, it is considered that the percentage of losses due to air contamination represents a few tens of percent of the active substance applied whereas the amount carried away by leaching is not considered to exceed an average of 1% [15].

From these observations, it appears that the risks to the health of people living in rural areas should first of all be investigated in relation to the volatility of the substances. On this basis, glyphosate, like metamitron and phenmedipham, presents a lower risk of damage to health than that run by the use of chloridazone.

7.4 RELATIVE TO THE BIOACCUMULATION OF SUBSTANCES

Study of the impact on the environment of the repeated use of herbicides and, in particular, glyphosate has been the subject of numerous publications. In health terms, it is important that herbicides are not present (or only at doses of less than the ADI) in the air, water or foods. It should be remembered that

the ADI is set either by the committee for study of the toxicity of pesticide products for agricultural use or by the European Union Commission, or by international bodies (FAO/WHO).

In the event of repeated ingestion, it is necessary to examine whether the product is susceptible to bioaccumulation. To do this, toxicologists notably use an octanol/water partition coefficient P. This dimension-free value defined at a given temperature and pH is often expressed as a decimal logarithm. Log P is an indicator of the liposolubility of a substance. If log P $\geq$ 3 the active substance is susceptible to bioaccumulation. Table 4 shows us that phenmedipham presents serious risks of bioaccumulation whereas glyphosate is the most interesting substance in this respect. The other herbicides reported in this table have a coefficient of less than 3.

8 ADVANTAGES OF GLYPHOSATE-TOLERANT SUGAR BEET

We have seen that the active substance dose applied to GM glyphosate-tolerant sugar beet crops would be 22 to 48% less per hectare than that applied to a crop using conventional herbicides. This involves a saving, particularly in the number of applications, and thus a lower risk to the farmer and his environment. This risk of air contamination is made even lower precisely because glyphosate is one of the least volatile substances. This herbicide also has the advantage of having very low liposolubility, thus meaning that there is less chance of finding it in milk and cheese produced in areas where fodder beet is grown. The low liposolubility of glyphosate, which can be translated into a lower risk of bioaccumulation, is therefore an element in favour of this herbicide over most of the others used on sugar beet.

9 GLYPHOSATE AND THE ENVIRONMENT

The advantages outlined above should not, however, conceal the very high hydrosolubility of glyphosate (the highest of all the substances used on sugar beet) along with a relatively high stability in water. The consequences of these two characteristics are known by manufacturers since toxicological analysis of this substance ranks it in the category of products that are toxic to aquatic organisms and which may cause long term damage to this medium and the environment more generally. It is therefore necessary to highlight the consequences, often reported in the literature, of repeated applications of glyphosate herbicides on microbiological life and soil development (reduction in organic matter and bacterial flora, acidification of soil, in particular). And yet it is known that this microbiological life is actually important in the water detoxification and purification process. We also raised the question of additives, important supplements which improve the penetration, and even the mode of action, of the active substance. These formulation agents are often prepared for a crop type. It is necessary to examine their harmfulness to the environment and also to human health, since conditions have been reported for Roundup, for example (eye and skin irritation, nausea, abdominal pains, etc.) [18-21].

10 CONCLUSION

Sugar beet crops concern around 2.4% of useful farmland in France, i.e. 400,000 hectares. Weed control, which was primarily manual until 1970, has since been carried out using herbicides, represented by 7 main substances. Some authors agree that the use of herbicides in general, due to the application of low Maximum residue limits (MRLs), does not present any particular health risks. In the specific case of sugar beet, the physical purification and crystallisation processes lead to the absence of any detectable herbicide residues in white sugar. Under these conditions, there is no direct benefit to be expected for consumers through the consumption of a finished product produced from GM glyphosate-tolerant sugar beet.

The problem is rather different for the farmer and, indirectly, for public health, via the environment. The physicochemical characteristics of the substances may, in this case, suggest a potential environmental risk. The solubility and stability in aqueous medium are high for glyphosate in comparison with other herbicides and represent negative elements for its use. However, its low liposolubility and, consequently, the limited risk of bioaccumulation, represent a definite advantage compared to the other herbicides used in non-GM sugar beet crops (non-glyphosate tolerant). The limited risk of dispersion in air during spraying is also an advantage for glyphosate, with this mainly being a benefit for the farmer. These data appear to be in line with the results of studies reported on the basis of the environmental indicators of Yardstick [22] and I-Phy [19], favourable to glyphosate compared with other conventional treatments.

In conclusion, GM sugar beet (glyphosate tolerant) does not modify the quality of the resulting finished product. Furthermore, the absence of contaminants such as pesticides, related particularly to the production method of the finished product resulting from non-GM sugar beet, did not lead one to expect any benefits to consumers in this respect. However, glyphosate-tolerant sugar beet could represent an interest to the farmer and, to a certain extent, the environment. The potential advantages, other than economic [23], nonetheless required arguments better supported by studies targeting the population at risk. Thus, the potential beneficial effects expected for the environment in the context of less use of herbicides, the lower volatility and lower liposolubility of glyphosate must be compared, for example, with the conclusions of an environmental impact study conducted by the British authorities [17] reporting that that the farming of herbicide-tolerant plants could have an impact on wild dicotyledon plant populations, with repercussions on insects and, consequently, the birds that eat these insects.

References

1 ITB Institut technique de la betterave, 45 r. de Naples 75008 Paris. site: www.institut-betterave.asso.fr

2 AGRITOX 2003 Base de données sur les substances actives phytopharmaceutiques. Site: www.inra.fr/agritox/

3 Phipps R.H., Park J.R. (2002) Environmental benefits of genetically modified crops: global and European perspectives on their ability to reduce pesticide use. *J. Animal Feed Sci*. 11, 1-18

4 Mannerlof M., Tuvesson S., Stenn PO., Tenning P. (1997) Transgenic suger beet tolerant to glyphosate. Euphytica, 94, 83-91.

5 Issa A.A., (1999) Interference of glyphosate with the shikimate pathway by Cyanobacteria in chemostat culture. *Microbios.* 395, 47-55.

6 Cox C. (1998) Glyphosat (Roundup). *J. Pesticide Reform*. 18, 3-17.

7 Damsira R.J.,Van Vicien W.A., Van Ginkel C.J.W. Allergic contact dermatitis from the preservative 1,2-benzisothiazolin-3-one (1,2-BIT: Proxel®): a case report, its prevalence in those occupationally at risk and in the general dermatological population and its relationship to allergy to its analogue Kathon® CG. *Cont. Dermatit*. 27,105-109.

8 Monsanto Co, 1996. Material safety data sheet: Roundup Sure Shot Foam. www.ortho.com/content/products/Solaris_mads/SOLMSDS, HTML, Aug.

9 Temple W.A., Smith N.A. (1992) Glyphosate herbicide poisoning experience in New Zealand. *N.Z. Med. J.* 105, 173-174.

10 Hass U., Jacobsen G.M., Lund S.P. (1995) Developmental toxicity of inhaled n-methylpyrrolidinone in the rat. *Pharm. Toxicol.* 76, 406-409.

11 Lodi A. Chiarelli G, Mancini LL, Crosti C. (1993), Contact allergy to sodium sulfite contained in antifungal preparation. *Cont. Dermatit* 29; 97.

12 Monsanto Co. (1997) Material safety data sheet: surfactant. www.monsanto.com. Aug.

13 Bryld L.E, Agner T, Rastogi SC, Menne T. (1997). Iodopropynyl butylcarbamate: a new contact allergen. *Cont Dermatit*. 36, 156-158.

14 Dale M.F.B., Dewar A.M., Fisherr S.J., Haydock P.P.J. Jaggard K.W. (1998) Transgenic herbicide tolerant sugar beet –present status and future developments. *Aspects Appl. Biology* 52, 273-278.

15 Seux R. (2003) Quelles sont les différentes sources de pollution des eaux ? Quelle est la part imputable aux produits phytosanitaires ? Quels sont les risques potentiels pour l'environnement et la santé ? In: "Pesticides : les enjeux du débat." UIPP ed. ISBN: 2-84541-061-1, 27-30.

16 Sopinska A., Grochola A., Niezgoda J. (2000) Influence of water pollution with Roundup herbicide on fish health. *Medycyna Weterynaryjna*. 56(9), 593-597.

17 ACRE Advice on the implications of the farm-scale evaluations of genetically modified herbicide-tolerant crops. 13 January 2004, http://www.defra.gov.uk/environment/gm/fse.

18 Joost A.W., Reus A., PeterC. (2000) The Environmental Yardstick for Pesticides: a Practical Indicator Used in the Netherlands, Leendertse in Crop Protection 19,

http://www.clm.nl/en/crop/articles/yardstick.phtml

19 Van der Werf H. M. G., Zimmer C. (1997) Un indicateur d'impact environnemental de pesticides basé sur un système expert à logique floue., *Le Courrier de l'Environnement*, 34.

http://indicateurs.agrienv..free.fr/fra/liens.html

20 Adam A., Marzuki A., Rahman H.A., Aziz M.A. (1997) The oral and intracheal toxicities of Roundup and its components to rats. *Veterinary Hum. Toxicology*. 39(3), 147-151.

21 Eisenbrand G., Aulepp H., Dayan A.D., Elias P.S., Grinow W., Ring J., Schlatter J. (1996) Assessment of the allergenic potential of foods derived from genetically engineered plants: glyphosate tolerant soybean as a case study. Food allergies and intolerances: symposium. VCH Verlagsgesellschaft mbH, Weinheim, Germany, 212-221

22 Richard-Molard D. (2002) Première approche de l'analyse des bénéfices de l'utilisation des betteraves tolérantes au glyphosate. In "Rapport de la commission du génie biomoléculaire et du comité provisoire de biovigilance sur l'analyse des bénéfices des OGM." Paris, 29 January 2002.

23 May M.J. (2003). Economic consequences for UK farmers of growing GM herbicide tolerant sugar beet. *Ann. Appl. Biol*. 142, 41-48.

VITAMIN A FORTIFICATION: THE CASE OF "GOLDEN RICE"

1 ROLE OF RICE IN NUTRITION

Rice is eaten by half the world's population. It is the staple food in at least 33 developing countries (15 in Asia and the Pacific, 10 in Latin America and the Caribbean, 8 in Africa) where it accounts for 27% of calories, 20% of protein and 3% of fat provided by diet. It contains thiamine, riboflavin, niacin (group B vitamins) and zinc in nutritionally valuable quantities, but no vitamins C and D and beta-carotene (FAO – International Rice Committee – July 2002).

The nutritional content of rice depends on a number of factors: genotype (cultivar), cultural practices, environmental conditions, storage time and conditions, method of preparation (hulling, processing, sterilising, cooking). The energy quantity provided by rice has increased considerably in 40 years (411 kcal/person/day in 1960, 577 today).

According to studies conducted in China and India (FAO, 1998), average daily consumption is 300 grams of uncooked rice per adult male and 250 grams per adult female.

The nutritional value of rice can be improved by conventional plant breeding methods, but a few specific programmes are now employing transgenesis (increase in beta-carotene and ferritin contents; obtaining a thermo-resistant phytase). In addition, direct fortification of rice composition has also been attempted, particularly by adding iron, but this method is awkward to implement and the difficulty depends on the element to be added.

Another approach is to modify eating habits, encouraging populations to grow and eat fruits and vegetable with a high carotene content, in particular, but also to eat brown rice (whole) rather than white rice. The latter, apparently simple, approach runs into the problem of deeply entrenched sociocultural habits in the population, extolling the use of white rice.

2 VITAMIN A REQUIREMENTS AND DEFICIENCIES

2.1 GENERAL CONTEXT

The major worldwide nutritional problems, particularly in developing countries, are considered to be malnutrition due to a lack of protein and calories along with iron, iodine, zinc and vitamin A deficiencies. With respect to the latter, millions of people show clinical signs of vitamin A deficiency [1]. Of the areas concerned, Southern Asia presents the highest prevalence of anaemia and vitamin A deficiency [2]. The countries in this area of the world are big rice consumers.

Vitamin A deficiency (VAD) is one of the most important deficiencies in terms of its effects on health and is generally manifested by severe clinical symptoms. UNICEF estimates that 124 million children worldwide were suffering from this deficiency in 1992 [3]. Thus, every year, 5 million children in Southern Asia develop xerophthalmia and, on a worldwide scale, 500,000 children go irreversibly blind.

2.2 VITAMIN A SOURCES

Products of animal origin are sources of vitamin A (retinol, retinal, retinol esters) and those of plant origin contain beta-carotene or provitamin A. In fact, the body only needs carotenoids to potentially transform these into active retinol. The availability of the latter (release of the substance in the intestinal lumen) is related to the nature of the matrix in which they are found (fibres, leaves, etc.) and the fat composition of meals. Indeed, the use of carotenoids is strongly associated with that of fats. Carotenoids are dissolved and integrated in lipid micelles thanks to the action of biliary salts and phospholipids: the dissolved form is the only one enabling their passage into the enterocytes, the site of their bioconversion into retinol or their transfer into the chylomicrons (lipoprotein complex ensuring transportation of fats in the body following their intestinal absorption).

2.3 METABOLISM, BIOCONVERSION OF PROVITAMIN A INTO VITAMIN A

The bioconversion of carotenoids into retinol, the active form of vitamin A, occurs partially in the intestinal cells during absorption or in the liver, thanks to a cleavage enzyme, beta-carotene dioxygenase, which cuts the beta-carotene, releasing retinal, which is itself then reduced into retinol by a reductase, acting with zinc as a cofactor. The retinol can also be oxidised into retinoic acid. Vitamin A circulates in the blood in the form of retinol bound to a specific transport protein, RBP (Retinol Binding Protein).

The bioconversion of provitamin A into vitamin A varies greatly from one species to another; for example, conversion rates of 100% are reported in chickens and of 10% in horses. In humans, it is generally accepted that the average conversion factor is 6 – i.e. 6 micrograms of beta-carotene per 1 microgram of retinol -.There is a relatively high level of variability between individuals and also between men and women [4]. Two other carotenoids are also a source of retinol, alpha-carotene and beta-cryptoxanthin but the conversion factor is thought to be at least 2 times less than that of beta-carotene. For beta-carotene, various bodies propose the following mean conversion factors .

a) x 12 (US Natl. Acad. Sci. Inst. Med. 2001) [5]
b) x 6 (FAO/WHO,1988) [6]
c) x 4 (Indian Council of Medical Research).

Although this conversion factor was recently revised upwards by the US Institute of Medicine, Food and Nutrition [5], some authors consider that this is still lower than the real figure. They believe that 21 µg of beta-carotene is required to obtain 1 µg of retinol from a complex food [28]. This key point relative to the bioavailability of provitamin A from foods and its conversion factors was the subject of a recent review by Li and Tso [29].

2.4 BIOAVAILABILITY

The overall bioavailability of carotenoids is therefore a result of the effectiveness of digestion and of their absorption and their subsequent conversion into active metabolites.

The availability of carotenoids therefore depends on their origin, their nature and the dietary context of their ingestion. Thus, carrots produce a provitamin A in crystal form, difficult to digest in its natural state. Cooking and the simultaneous consumption of an oil aid its absorption [7]. Absorption from oranges is easier. The intestinal absorption of provitamin A depends on a number of factors, notably the concomitant presence of fats at the time of ingestion. According to de Benoist (WHO), populations suffering from a deficiency often have diets with an insufficient fat content, which is not favourable to adequate vitamin A intake. Furthermore, infectious diseases or diarrhoea can singularly reduce the absorption of the vitamin A precursor. Although the bioavailability of vitamin A incorporated in rice in the form of palmitate at a rate of 800 µg/g has been the subject of a study concluding that there was a positive effect in 48 of the 83 subjects treated [8], we were unable to identify any study of this type with golden rice.

2.5 STABILITY

Provitamin A, like vitamin A, is sensitive to heat, light and oxidation. What is considered to be a high quantity of provitamin A is lost during drying and storage of plant products. Thus, the quantity of provitamin A could be close to zero 6 months after harvesting certain crops. According to Flores *et al* [8], a premix containing 800 µg/g vitamin A palmitate, stored out of direct light, would lose 25% of its content in 3 months. Cooking (5 min in boiling water and 25 min in warm water) leads to a loss of 25.9% $\pm$ 9.1% (n = 70). Depending on the storage conditions, traditional Thai foods lose up to 40% of their provitamin A content after 3 months. Conversely, fermentation of certain preparations may lead to increases in beta-carotene levels of about 68% after 2 months and up to 1265% after 12 months [9].

2.6 REQUIREMENTS

According to Azaïs-Braesco *et al* [10], the recommended daily intakes (RDIs) range from 350 RE[14] (Retinol Equivalents), i.e. 1155 IU per day, in babies to 950 RE, i.e. 3125 IU per day, in breast-feeding women. In a child aged between 1 and 3 years, the recommended intakes are 450 RE, i.e. 1320 IU or 0.3 mg per day. The RDIs for adult men and women are 800 and 600 RE/day, respectively.

2.7 VITAMIN A DEFICIENCY

Vitamin A deficiency affects the eyes, first of all by slowing down the rate of regeneration of pigments involved in vision, following exposure to bright light, then by gradually breaking up the integrity of the epithelium, ultimately resulting in blindness.

Vitamin A also appears to have an activity on the immune system and on the integrity of the cell system. Retinoic acid, obtained by oxidation of retinol, plays an important role in expression of the

[14] 1 mg retinol = 1000 RE (Retinol Equivalent) = 3330 IU (International Units)

genome and cell differentiation. In the event of a deficiency, these combined effects could result in the annual deaths of around 1 to 3 million children under the age of 5 years [11].

2.8 HYPERVITAMINOSIS A

Hypervitaminosis A is a pathological process related to excessive retinol or free retinoic acid concentrations [12]. It results in two different types of toxicity:

- **acute toxicity** at massive doses 1000 times higher than the minimum requirements. The doses causing disturbances are 1 to 2 million vitamin A units in adults and 75 to 100,000 IU in children [13,14] .The symptoms observed in humans, which may be isolated or occur simultaneously, are severe headaches, nausea, loss of appetite, accompanied by skin and scalp conditions, asthenia and mild haemorrhages;
- **chronic toxicity** following the long-term ingestion of quantities in the region of 100 to 1000 higher than the minimum recommended intake. In adults, chronic intoxication is very rare for consumption of levels of less than 50,000 IU vitamin A per day and up to 100,000 IU/d. In children, and especially in babies, disturbances develop with lower intakes (in the region of 2500 IU vitamin A A/kg body weight). The symptoms observed are the same as those for acute intoxication but the intensity may be variable. To these are added skin disorders (damage to the mucous membranes, gingivitis, etc.), insomnia and personality disturbances, along with bone pain and bone metabolism disturbances. Liver damage (hepatomegaly, fibrosis) and teratogenic effects occur in the most severe cases.

Furthermore, the first trimester of pregnancy is a critical period in the event of excessive vitamin A intake, with multiple risks of foetal malformation.

These toxicity data have led various bodies to formulate recommendations in terms of maximum intakes. Consequently, in 1996 the Conseil Supérieur d'Hygiène Publique de France (CSHPF - French Public Health Council) set maximum vitamin A values, above which there are risks of toxicity:

- 50,000 IU as a chronic intake,
- 1 to 2 million IU as an acute intake for the general population,
- 25000 IU as an acute or chronic intake in pregnant women (lowest observed adverse effect level).

The safety limit[15] of 5500 IU set by the CSHPF in 1996 was recently revised on a European level in the light of new studies and set at 10,000 IU for the general population (including women of childbearing age[16]).

3 STRATEGIES TO PREVENT VITAMIN A DEFICIENCY

Reducing the prevalence of the various forms of vitamin A deficiency has been the subject of numerous interventional programmes and nutritional trials around the world. Their effectiveness varies greatly depending on the experimental conditions and the strategies adopted. Furthermore, the causes of vitamin A deficiency are very numerous and can be placed into four categories:

- inadequate dietary intake of vitamin A sources: in general, this is the case for retinol contained in animal products;
- low bioavailability: this more specifically concerns carotenoids of plant origin;
- inadequate maternal breast-feeding practices
- microbial and parasitic infections.

Finally, it is necessary to take into account the numerous nutritional interactions which are liable to affect the bioavailability and bioefficiency of vitamin A [15]. Hence, the coexistence of vitamin A, iron and zinc deficiencies is observed. These nutrients are involved in the metabolism of vitamin A, such as zinc, which is a retinal-reductase cofactor. Fat and protein intakes are also key factors, either for intestinal absorption mechanisms (fats) or for control of transport proteins (RBP-TTR-retinol complex), storage proteins, cellular receptors and enzymes such as beta-carotene dioxygenase.

---

[15] The safety limit[13] corresponds to a dose for which it is reasonable to think that the probability of observing an adverse effect is the lowest possible (without being nil), taking into account differences in sensitivity between individuals.

[16] Assessment of nutritional requirements of animals in vitamins A, D and E and of the risks to animal and consumer health related to high intakes in food-producing animals. Afssa report, March 2004. www.afssa.fr.

The numerous factors causing vitamin A deficiency involve and warrant different and complementary strategies to combat the effects of the deficiencies. Three broad approaches are possible:

A the approach of medicinal supplementation using vitamin A capsules;

B nutritional approaches, grouping together all the measures and interventions relative to nutrition. This involves acting on three levels:
- the production of vitamin A-rich foods and the adoption of appropriate processing and storage habits, remembering that vitamin A is sensitive to oxidation and light;
- diversification of diet;
- the fortification of vitamin-A carrying foods.

C public health measures: promotion of breast-feeding and control of infections and parasite infestations.

3.1 MEDICINAL SUPPLEMENTATION

These programmes involve the distribution of vitamin A capsules to children aged between 6 months and 6 years at doses liable to enable them to build up vitamin A stores in the liver. Generally, supplementation campaigns are coupled with vaccination campaigns, for example against poliomyelitis. In this way, one can expect to cover 90% of children, but distribution is only possible once a year, whereas the capsules need to be distributed two to three times per year. The question of the effectiveness of a supplementation programme in an unfavourable context is raised. Finally, when vaccination programmes have achieved their objective (eradication of poliomyelitis), in what form can supplementation be continued?

Beaton *et al* [16] estimate that supplementation programmes may reduce cases of infant morbidity and mortality by around 25% in developing countries. However, Zagré [15] notes that despite a supplementation programme conducted in Burkina-Faso, the prevalence of night blindness in children under the age of 10 years remains high.

3.2 NUTRITIONAL APPROACHES

Nutritional approaches – fortification and diversification – are generally considered to be the only methods that are effective in the long term and in different contexts: diversity of age groups, of geographic zones. The successes already recorded plead in favour of such strategies [15,17,18]. However, these fortified foods have generally been distributed free of charge in experimental contexts, which limits the impact in terms of feasibility. Only Zagré [15] successfully conducted a marketing trial of red palm oil, in real purchasing and free consumption conditions.

3.2.1 Fortification of foods with vitamin A

Several protocols and approaches are possible.

Vitamin A fortification
This usually involves adding vitamin A to a carrier food, in a specific form (retinol esters, beta-carotene) at a fortification dose which depends on the estimated consumption of this food. Carrier foods must be socio-economically acceptable and regularly consumed by the majority of the population or more specifically by the target groups. This technique has two advantages: non-dependence on dietary habits, since the carrier food is part of the usual dietary model and absence of risk of overdose relative to medicinal supplementation, if the dose is properly calculated. One constraint is linked to the technological aspect: it must be possible to master and rigorously control the addition process, which implies that the operation can only be carried out in a limited number of industrial production units.

Trials with vitamin-A fortified foods have mainly been carried out in Latin American and Asian countries: they have concerned sugar, oil, wheat, rice, tea, glutamate [19]. A recent trial in the Philippines demonstrates an improvement in the vitamin A status of children given vitamin A-fortified wheat [20].

Genetic improvement
Here we can cite genetic improvement programmes using conventional methods (carrots with a high beta-carotene content in India, fortified sweet potatoes in Mozambique and Peru) or genetic engineering methods (golden rice).

Food-to-food fortification
A last approach consists in combining a conventional carrier food with an ingredient with a particularly high vitamin A content. This was the case in Tanzania where cassava flour was fortified with red palm oil [21]. Another trial conducted in Vietnam [22] shows that combination of rice with a fruit particularly rich in beta-carotene (gac) improves retinol concentrations in children, whereas rice directly fortified with beta-carotene does not achieve the desired results. Finally Garcia-Casal *et al* [23] show that fortification of cereals (rice, wheat, corn) with vitamin A improves non-heme iron absorption.

3.2.2 Dietary diversification

In this case, it is the diversity of vitamin A sources that is acted upon, making a distinction between:

- **animal products**, rich in retinol, with a good bioavailability: butter, cheese, eggs, whole milk, oily fish. It should be noted that some of these products are not very economically accessible, particularly in developing countries.
- **plant products**: vegetables (carrots, spinach, broccoli, leeks) and yellow or orange-fleshed fruits melon, mango, apricot, papaya). Particular mention must be made of non-refined red palm oil. A recent inventory of plant sources of carotenoids was compiled by a team at IRD-Montpellier: it demonstrates very high potentials for cultivated plants, particularly in Sahelian regions, which are worth analysing and putting to use [24].

In plants, vitamin A is found in the form of carotenoids, the bioavailability of which is mediocre: it is lower in green leaves (carotene bound to chloroplasts) than in yellow fruits (carotene bound to chromoplasts). However, the relatively low bioavailability of plant carotenoids is compensated for by the wide diversity of sources.

Dietary diversification involves acting on the following levels:
- promoting
  * the production of vitamin A-rich foods,
  * access to these foods,

  their consumption, through educational actions,
- improving the processing and storage of products, reducing losses (due to oxidation or UV light),
- improving the bioavailability, for example by increasing the consumption of associated fats.

The few programmes implemented combine the promotion of diversified and vitamin-A rich foods with educational nutritional actions or the introduction of family or village gardens. In order for them to be evaluated, these programmes require the existence of nutritional monitoring systems. It is possible to cite a few trials conducted in recent years in Kenya, Thailand and India. Finally, we must mention the work of Zagré in Burkina-Faso, in which he carried out a longitudinal study over two years, consisting of the introduction of red palm oil in a region not traditionally consuming this product. The originality of the approach lay in the fact that the red palm oil was introduced according to a commercial basis under free and voluntary purchasing and consumption conditions. The results obtained – an increase in vitamin A consumption, improvement in retinol concentrations in children and mothers – demonstrate the feasibility and effectiveness of this approach [25,15].

These various nutritional approaches by means of fortification or diversification also show that, in addition to the context linked to the nutritional condition of the groups targeted and the health status, the role of economic and sociocultural factors is crucial.

4 OBTAINING A PROVITAMIN A-RICH RICE USING BIOTECHNOLOGICAL METHODS: GOLDEN RICE [26]

The endosperm of immature rice grains is capable of synthesising geranylgeranyl diphosphate which can then be converted into phytoene ("colourless carotene") in the presence of phytoene synthetase, not initially present. But obtaining beta-carotene requires the additional action of another 3 enzymes: phytoene desaturase, beta-carotene desaturase (each one involved in the formation of two double bonds) and lycopene beta-cyclase. The difficulty of introducing the 4 genes governing the synthesis of these 4 enzymes can be reduced by using a desaturase of bacterial origin which, on its own, can create the 4 double bonds required.

The protocol followed by the authors will be presented briefly here. It is based on the use of immature rice embryos and *Agrobacterium* to introduce the genes concerned in a single transgenesis operation. The pB19hpc includes the sequences of phytoene synthetase (produced by dahlia, *Narcissus*

pseudonarcissus) and those of phytoene desaturase (produced by the bacterium *Erwinia uredovora);* they lead to the formation of lycopene in the plasts of the endosperm, where geranylgeranyl diphosphate is synthesised. Another two carriers are used: pZPsC, which has the same genes as the plasmid pB19hpc, and pZLcyH, which includes the gene encoding lycopene β cyclase (sequence produced by dahlia).

Following new trials, Beyer et *al* [27] show that transgenesis can produce results which are just as good as those obtained with the protocol described above, without co-transformation, i.e. using only one construction including only phytoene synthetase and phytoene desaturase. Other protocol modifications are currently being studied.

The quantity of beta-carotene produced per grain is currently in the region of 1.6 to 2.0 μg/g. If we refer to the lowest conversion rate cited previously (12 μg beta-carotene to obtain 1 μg retinol) and to the recommended daily intakes (400 to 500 μg retinol for children), this leads to a daily consumption of around 2.5 kg of golden rice in the form in which it is currently produced. Furthermore, it is clear that rice produced in the field may no longer present the same production capacity (but trials are currently under way to introduce the same construction in local rice varieties). However, one must be aware that golden rice does not claim to cover all provitamin A requirements on its own, and that, even if it only partially compensates for current deficiencies, it will still have achieved a very useful objective.

5 EFFECTIVENESS OF GOLDEN RICE

Like most cereals, whole “brown” rice only contains low quantities of provitamin A (0.1 μg beta-carotene equivalent per gram) whereas hulled "white" rice does not contain any.

It is the very extensive consumption of this food worldwide, particularly by the poorest populations, which led the “inventors” of golden rice to propose it as a method for supplementing the diet. Given the practice of hulling rice, the expression of provitamin A was targeted in the endocarp.

On the basis of an estimated provitamin A content of between 0.16 and 0.20 mg/100 g golden rice, and a bioavailability of the precursor of 100% (high hypothesis) or 50% (low hypothesis) and considering that the recommended intake in children is 0.3 mg/day, the factors for conversion of provitamin A into vitamin A depending on the organisms (cf. point 2.3): x12, x6 or x4, led Beyer and Potrykus to the following conclusions relative to the “golden rice” consumption necessary to cover all vitamin A requirements (1st estimate):

1st estimate	“Golden rice” consumption in g/day	
Conversion factor depending on the body	Availability of the precursor: 100%	Availability of the precursor: 50%
X12	1800 to 2250	3600 to 4500
X6	900 to 1125	1800 to 2250
X4	600 to 750	1200 to 1500

If one considers that the recommended intake is an idealistic objective, but that 30 to 40% would be sufficient to reduce mortality, morbidity and sight problems, the daily consumptions are estimated to be (2nd estimate):

2nd estimate	“Golden rice” consumption in g/day	
Conversion factor depending on the body	Availability of the precursor: 100%	Availability of the precursor: 50%
X12	540 to 675	1080 to 1350
X6	270 to 337	or 540 to 674
X4	180 to 225	360 to 450

And if one considers that the provision of 100% of these requirements **by "golden rice" alone** is a maximalist objective but that 50% would be a good objective, with the other 50% being provided by other sources, the daily “golden rice” consumptions would thus be reduced to (3rd estimate):

3rd estimate	“Golden rice” consumption in g/day	
Conversion factor depending on the body	Availability of the precursor: 100%	Availability of the precursor: 50%
X12	270 to 337	540 to 674
X6	135 to 168	270 to 336
X4	90 to 112	180 to 224

But the authors consider that the project is still in the "proof of concept" stage and that improvements in the current variety may lead to the production of provitamin A being increased by a factor of 3 to 5, leading to the following estimates (4th estimate):

4th estimate	“Golden rice” consumption in g/day	
Conversion factor depending on the body	Availability of the precursor: 100%	Availability of the precursor: 50%
X12	90 to 112	180 to 224
X6	45 to 56	90 to 112
X4	30 to 38	60 to 76

Thus, the authors consider that a daily consumption of golden rice (new variety) of 30 to 224 g could significantly reduce deficiencies and their consequences, an estimate that is not shared by certain NGOs, which work on the basis of several kilograms being required to provide a sufficient intake.

In conclusion, everyone agrees that there is a genuine problem of vitamin deficiencies in certain countries and, in particular, vitamin A deficiency. It also appears that many people believe that the response must be multi-directional, with golden rice being a possible element in this strategy.

We have already seen that various problems related to the bioavailability and stability of provitamin A exist, without, to our knowledge, any studies having explained these various aspects for golden rice. It is true that Potrykus considers that beta-carotene is stored in the lipid membranes and that, consequently, it is highly bioavailable with, furthermore, a conversion factor of 2. Studies are required to support these statements, with it also being necessary to take into consideration the nutritional and health situation of the populations targeted. The capacity of this variety to be grown and combined with the other rice varieties essential to the traditions of the country concerned is also a factor that must be taken into account, and for which there does not appear to be any information. In the daily consumption approach reported above, Potrykus is already considering a new variety of golden rice, which confirms the current situation for this proposition, a "proof of concept", but which primarily incorporates research elements and rather forgets the field aspects …. The questions raised at the time of Potrykus's presentation during the Afssa symposium still appear to be largely unanswered:

- digestibility of the storage protein?
- trials on transfer to the Philippines, Malaysia, Vietnam, China, etc.?
- clinical trials conducted?

Some important pieces of the jigsaw are still missing, preventing us from being able to consider the proposition as totally convincing at this stage, and tailored to the multiple aspects of the problem.

6 CONCLUSIONS AND PERSPECTIVES

The golden rice story is only just beginning and already a great deal of words have been written on the subject. It is true that the project is ambitious in more than one way. In fact, it is the first ever case of a project with the sole aim of improving the health of human groups suffering badly from nutritional deficiencies. Obtaining a rice containing useful quantities of vitamin A is not simple. Establishing varieties of rice containing vitamin A is not easy from either a technical, agronomic, medical or social point of view, nor in terms of industrial patent rights.

The first indisputable success is that three genes are sufficient for substantial quantities of vitamin A to be found in a rice grain which does not naturally contain any. However, we are forced to observe that the existing golden rice seeds are only really in the prototype stages and that we have still not gone beyond the proof of concept stage which, it is true, is an essential point. One still has to assess the behaviour of the rice in real agronomic conditions. The 3 to 5-fold increase in vitamin A concentrations in golden rice, which is highly desirable, is not yet guaranteed. The effectiveness of conversion of the provitamin A in golden rice into active vitamin A still seems to be little known and experiments based on the use of animal models have not been published if they have been conducted. The rapid success of the first part of the project is therefore now coming up against the problems of human nutrition, which are far more complex.

The rapid transposition of the process to local varieties was hoped for by the authors of the project and is now under way. However, it should be noted that two varieties of rice widely used in Asia have recently been genetically modified to produce substantial quantities of vitamin A [30]. Furthermore, the Philippines have also launched a project to develop the growing of golden rice [31]. Hundreds of different varieties are used due to their suitability to local agronomic conditions and the tastes of consumers. The number of varieties that could be fortified with vitamin A can only be very limited in practice. In the event of success, the consumption of golden rice could gradually accelerate the abandonment of local varieties representing a precious biodiversity.

The acceptability of golden rice by consumers is not a given. The colour of golden rice and the element of the unknown represented by this novelty may lead to its rejection by certain communities which, in contrast, would derive the most benefit from adopting these new varieties.

Industrial patent rights represent one of the key points in the story. The project's authors have been able to obtain licenses free of charge for the 72 patents held by 30 companies. However, we still have to ensure that freedom to operate is genuinely guaranteed and that farmers really will have the right to grow golden rice. As for the various authors, scientists and bodies supporting the project, they have planned to give seed to farmers free of charge. The Monsanto company, which developed vitamin A-fortified varieties of mustard and oilseed rape proposes to give up its rights for needy farmers, on condition that the latter do no sell their products at a level exceeding 10,000 dollars per year and per farmer.

In addition to all these considerations, it is important to assess the validity of the approach which led to the existence of golden rice.

It cannot really be disputed that the project is designed to improve the health of needy human populations and that it is conducted as independently as possible from private companies. The successes of the initial stages have probably given rise to excessive enthusiasm amongst some people, particularly the main players involved in the project. However, these are not false promises but, rather, real hopes.

It seems that golden rice is not the solution to eliminate vitamin A deficiencies since no solution could resolve the problem on its own. One of the strong points of the project is, in theory at least, that substantial levels of vitamin A would be provided without the populations having to modify their farming and eating habits.

It also appears that the other solutions (use of local plants naturally rich in vitamin A, impregnation of rice with synthetic vitamin A before marketing) have not been the subject of any studies or funding commensurate with the hopes that they raise. However, we must avoid making any simplistic judgments on this matter. It is probable that rapid and massive urbanisation has been accompanied by abandonment of cultivation and consumption of micronutrient-rich plants. However, nutritional deficiencies are not new. It would in any case be prejudicial to oppose the development of traditional plant crops or the addition synthetic vitamin A to the use of golden rice. There are too many uncertainties in these various approaches for one to reasonably be able to take the risk of favouring one over another. The importance of the problem and the urgent requirement to find satisfactory solutions do not leave us much choice.

Nothing indicates that the approach that led to the creation of the first varieties of golden rice is heading towards failure. It is therefore important that it be allowed to continue without interference, with the critical encouragement of public opinion.

Bibliographic references

1 United Nations ACC/SCN. Fourth report on World Nutrition Situation. (2000) UN ACC/SCN in collaboration with IFPRI, Geneva, 2000, 132pp. www.ifri.org

2 Mason J., Hunt J., Parker D. Jonsson U. (1999) Investing in child nutrition in Asia. Asian Development Review 17(1,2).

3 Humphrey J.H., West K.P., Sommer A. (1992) Vitamin A deficiency and attributable mortality among under-5-year-olds. *Bull World Health Organ* 70.: 225-232.

4 Hickenbottom, S. J, Follett, J. R, Lin, Y., Dueker, S. R, Burri, B. J, Neidlinger, T. R, Clifford, A. J (2002). Variability in conversion of {beta}-carotene to vitamin A in men as measured by using a double-tracer study design. *Am. J. Clin. Nutr.* 75: 900-907

5 US Natl. Acad. Sci. Inst. of Medicine (IOM), January 9, 2001. In Beyer P. Tables on provitamin A amount, Forum US AgBioView, 2001.

6 FAO/WHO Expert Consultation (1988) Requirements of Vitamin A, Iron, Folate, and Vitamin B12. Food and Nutrition Series no. 23, FAO, Rome The latest international report to consider requirements for vitamin A.

7 Koechlin, F. (2000) The "golden rice" - a big illusion?' Third World Resurgence #114/115, 33-35.

8 Flores H., Guerra N.B., Cavalcanti A.C.A., Campos F.A.C.S., Azevedo M.C.N.A., Silva M.B.M. (1994). Bioavailability of Vitamin A in a synthetic Rice Premix. *J. Food Sci*., 59, 2, 371-372 and 377.

9 Chavasit V., Pisaphab R., Sungpuag P., Jittinandana S., Watsantwisut E. (2002) Changes in Beta-carotene and vitamin A contents of vitamin A-rich food in Thailand during preservation and storage. *J.Food Sci*., 67, 1, 375-379.

10 Azaïs-Braesco V. et Grolier P. (2001) Vitamines liposolubles, vitamine A et caroténoïdes provitaminiques. In " Apports nutritionnels conseillés pour la population française ". 3e éd Tec & Doc. Martin A. coord. 221-228.

11 Underwood B.A. (1998) Vitamin A deficiency. *Bull World Health Organ.* 76 Suppl 2:124-5.

12 Smith F.R. and Goodman D.W. (1976). Vitamin A transport in human vitamin A toxicity. *N. Engl. J. Med*. 294, 805-808.

13 Omaye F. (1984) Safety of megavitamin therapy. *Adv. Exp. Med. Biol*. 117: 169 -203.

14 Azaïs-Braesco V., (1993) Hypervitaminose A et tératogenèse, incidence et mécanismes. *Cah. Nutr. Diet*., XXVIII, 28: 143-150.

15 Zagré N. (2002) Projet-pilote d'introduction de l'huile de palme non raffinée comme source de vitamine A au Burkina-Faso. Evaluation de l'impact. Thèse Université Montpellier 2 et PhD Université de Montréal (September 2002).

16 Beaton G.H. , Martorell R. , Aronson K.J. (1993) Effectiveness of vitamin A supplementation in the control of young child morbidity and mortality in developing countries. ACC/SCN State-of-the-art Series, Nutrition Policies Discussions, paper n°13, Geneva, United Nations,

17 De Pée S., Bloem M.W., Kiess L. (2000) Evaluating food-based programmes for their reduction of VAD and its consequences. *Food Nutr. Bull*. 21

18 Gibson R.S., Hotz C., Temple L. (2000) Dietary strategies to combat deficiencies in iron, zinc and vitamin A in developing countries. Development, implementation, monitoring and evaluation. Food Nutr. Bull. 21, 219-231

19 Darnton-Hill I., Mora J.O., Weinstein H., Wilbur S., Nalubola P.R. (1999) Iron and Folate Fortification in the Americas to Prevent and Control Micronutrient Malnutrition: An Analysis. Nutrition Reviews®, Vol. 57, No. 1.

20 Solon F.S., Klemm R.D., Sanchez L., Darnton-Hill I., Craft N.E., Christian P., West K.P. Jr. (2000) Efficacy of a vitamin A-fortified wheat-floor bun on the vitamin A status of Filipino schoolchildren. *Am. J. Clin. Nutr.* 72, 738-744

21 Mosha T.C., Laswai H.S., Mtebe K. (1999) Control of vitamin A deficiency disorders through fortification of cassava flour with red palm oil: a case study of Kigoma district, Tanzania. *Ecol. Food Nutr.* 569-593

22 Le Vuong T., Dueker S.R., Murphy S.P. (2002) Plasma beta-carotene and retinol concentrations of children increase after a 30-d supplementation with the fruit *Momordica cochinchinensis (gac). Am. J. Clin.Nutr*. 75, 872-879

23 Garcia-Casal M.N. (1998). Vitamin A and beta-carotene can improve non-heme absorption from rice, wheat and corn by humans. *J.Nutr*. 128, 648-650.

24 Mathieu-Daude C., Chevalier P., Barrot L. (2001) Produits végétaux riches en carotènes. CD-Rom IRD-OMS Ed. WHO/NHD/01.6, AFR/NHD 01.01

25 Delisle H., Zagré N., Ouedraogo V. (2001) Marketing of red palm oil as a source of vitamin A in Burkina-Faso: a pilot project involving women's groups. *Food Nutr. Bull*. 22, 4, 388-394

26 Ye X., Al-Babili S., Klöti A., Zhang J., Lucca P., Beyer P., Potrykus I. (2000) Engineering provitamin A (beta-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. *Science* 287: 303-305

27 Beyer P., Al-Babili S., Ye X., Lucca P., Schaub P., Welsch R., Potrykus I. (2002) Golden rice: introducing the beta-carotene biosynthesis pathway into rice endosperm by genetic engineering to defeat vitamin A deficiency. *Am. Soc. Nutr. Sci*., 506S-510S

28 West C.E., Eilander A., Van Liieshout M. (2002) Consequences of revised estimates of carotenoid bioefficacy for dietary control of vitamin A deficiency in developing countries. *J. Nutr*., 132:2920S-2962S.

29 Li E., Tso P. (2003) Vitamin A uptake from foods. *Curr. Opin. Lipidol*., 14: 241-247.

30 Hoa T.T.C., Al-Babili, Schaub P., Potrykus I., Beyer P. (2003) Golden Indica and Japonica rice lines amenable to deregulation. *Plant Physiology*, 133, 161-169.

31 Chong M. (2003) Acceptance of golden rice in the Philippines "rice bowl". *Nature Biotechnology*, 21, 971-972.

PROSPECTIVE ANALYSIS OF THE POTENTIAL BENEFICIAL EFFECTS OF GENETICALLY-MODIFIED MICROORGANISMS (GMMS): BACTERIA AND YEASTS

The scope of this prospective analysis concerns live Genetically-Modified Microorganisms (GMMs) to be included in pharmaceutical preparations or be ingested with food or drink or be used in various bioremediation processes. They may be designed for medical, nutritional or environmental purposes. These strains are not yet available on the market (apart from one exception in Russia), but they could be the subject of marketing authorisation applications if "anti-GMO" concerns are removed, at least partially, and if they represent a real benefit to consumers.

The purpose of this text is to conduct a brief review of what may emerge in the future and the potential benefits resulting from these developments. Like any prospective study, it contains an element of uncertainty but we assert this right of error.

1 REVIEW OF CURRENT CONSUMPTION OF NON-GMM LIVE MICROORGANISMS

Every day, we ingest a large number of live bacteria. Some are part of the saprophytic or accidental flora present in our foods, such as those found in fresh fruit and vegetables or even in certain bottled waters. The number of opportunistic microorganisms is low, however, for quality products. For other foods, bacteria are introduced deliberately by humans and are active in the processing of the product. This is the case for certain salt meats, such as sausage, along with cheeses and fermented milks. The greatest consumption of bacteria comes from yoghurts and fermented milks since the number of bacteria is about 10^8 bacteria/gram and daily intake of these dairy products often exceeds 200 g.

In the last ten years or so, new types of bacteria have been added to these products. These modify the taste or texture but they are mainly chosen to provide beneficial effects to human health. These are known as probiotics, i.e. living microorganisms (usually bacteria) which, when they are ingested in sufficient quantities, have a positive effect on health beyond the traditional nutritional effects. With the exception of one probiotic yeast [1] with a medicinal role (*Saccharomyces boulardii*), probiotics are principally lactic bacteria (LB), i.e. they ferment carbohydrates producing lactic acid, or occasionally *Bacillus*. A number of papers have dealt with the beneficial effects of probiotic LB on human health. Here, we cite the most recent [2-6] and encourage instructive consultation of an issue of the British Journal of Nutrition (Vol 88, Supplement 1, 2002) dedicated to "probiotics and health". The main positive effects primarily concern regulation of transit (reduction of diarrhoea and constipation), stimulation of immunity markers, suggesting a positive effect on the immune system. Recent reports suggest an effect on a type of allergy (infantile atopic eczema) and a reduction in digestive inflammatory responses. Re-balancing of the intestinal flora is often claimed but usually it is simply that the probiotic ingested when eating the product is detected in the stools.

The way is therefore open for genetically-modified microorganisms (GMMs) having acquired new properties and consumed live in products. They could exert a medicinal role but also modify the organoleptic properties of certain foods or change industrial processes.

2 CURRENT USE OF GENETICALLY MODIFIED MICROORGANISMS

From the 1980s onwards, GMMs cultivated under controlled confinement conditions have been used as organisms producing substances of therapeutic interest in medicine, such as hormones (e.g.: insulin, human growth hormone) or vaccines (hepatitis B vaccine produced by *Saccharomyces cerevisiae* yeast) and also in the context of production of enzymes, flavourings and processing aids in human or animal nutrition. GMMs are thus used as metabolic micro-factories, and the substances produced are then purified and marketed. This is the case, for example, of chymosin, an enzyme used in the cheese-making industry, which can be produced by genetically-modified strains of *Escherichia col*i, *Aspergillus Niger* and *Kluyveromices lactis* or amylases of *Bacillus* sp. used in bread-making or the brewing industry.

In the future, GMMs could also be directly present – and no longer indirectly as is already the case with the production of enzymes, for example – in the production of certain foods. Indeed, in the last 10 years, a set of

studies have made it possible to significantly increase the number of tools available to obtain genetic constructions in GMMs. No GMMs are currently authorised for marketing to be used live in human food. However, one genetically-modified bacterium and two viruses are authorised for non-nutritional purposes. These involve a bacterium that is the basis for a kit to detect antibiotic residues in milk and two veterinary vaccines (one against rabies and one against pseudorabies in swine).

Various GMMs are currently considered – by their constructors at least – to be ready for use in food technology. Amongst the most advanced products, of particular note are those concerning the lactic bacteria *Lactococcus lactis*, some strains of which are genetically modified to enable acceleration of ripening of cheeses or better resistance to certain viruses specific to it [7][17]; other projects concern the construction of genetically-modified yeasts to optimise the production of wine or bread.

A new field for the use of GMMs appears to be emerging: live GMMs which could replace an active substance (drug, enzyme, other probiotic effect) and would be consumed by humans via food preparations. This new approach could potentially permit the use of GMMs to deliver a drug to the lower parts of the gastrointestinal tract without recourse to more sophisticated drug delivery forms.

It is important to note that the designers of these new strains of microorganisms make a distinction between transgenic GMMs, i.e. those carrying one or more genes or genetic structures produced from an organism different from the one being modified, and non-transgenic GMMs, constructed by self-cloning (without the addition of an external gene) and hence not subject to the same regulations.

3 TOWARDS "MEDICINAL" BACTERIA

A first field concerns oral, parenteral or nasal vaccination. The expression of viral or tumoural antigens, neutralising antibodies or the concomitant production of various interleukins and antigens appears to lead to an interesting modulating effect on immune reaction. In the case of food allergies, it may be possible to target oral desensitisation using a GMM producing a modified allergen.

A second field concerns secretion by the GMM ingested into the digestive tract of enzymes of mediators that could have a beneficial role (preventive or curative) on human health. The most striking example at the current time, is that of Steidler's team [8] which concerns the production of interleukin 10 by *Lactococcus lactis* in the digestive tract. Using this process, it was possible to reduce experimentally-induced digestive inflammation and this type of approach is currently being tested in patients suffering from Crohn's disease.

It has also been demonstrated in an animal model that the release of a lipase by *Lactococcus lactis* could improve the digestibility of lipids in animals which have had their pancreatic duct ligated to mimic lipase deficiency.

All these approaches require the consumption of live bacteria, some expressing eukaryote proteins. Benefit/risk studies must be implemented.

Tables 1 and 2 present a panorama of possible applications of live GMMs in the medical field.

Table 1: List of heterologous proteins produced by lactic bacteria (except *Lactococcus lactis)* and liable to lead to medical or technological and medical applications

Producing organism	Substance	Objective	References
Streptococcus gordonii	Microbicidal single-chain antibody (H6)	Vaginal candidiasis due to *Candida albicans*	[32]
Streptococcus gordonii	Anti-idiotypic single-chain recombinant antibody simulating the type III capsular polysaccharide of group B streptococci	Passive protection against neonatal infections caused by group B streptococci	[33]
Streptococcus gordonii and Lactococcus casei	E7 protein of the human papilloma virus	Immunisation against papilloma virus	[42,57]
Lactobacillus. plantarum	Cholera toxin B	Protection against cholera	[65]
Bacillus subtilis	Ure B (subunit of *Helicobacter pilori* urease)	Protection against *Helicobacter pilori*	[71]
Bacillus subtilis	Human Interferon alpha-2	Anti-viral protection	[68,31]
Bacillus subtilis	C fragment of tetanus toxin	Protection against *Clostridium tetani*	[43,56]

[17] http://www.inra.fr/Internet/Directions/DIC/ACTUALITES/DOSSIERS/OGM).

Table 2: List of heterologous proteins produced by *Lactococcus lactis* and liable to lead to medical or technological applications

Proteins	Gene	Origin	Production method	References
Bacterial antigens				
L7/L12	*L7/L12*	*Brucella abortus*	Secreted/Anchored/ Cytoplasmic	[62]
TTFC	*ttfc*	*Clostridium tetani*	Secreted	[74]
Viral antigens				
E7	*E7*	Human papilloma virus type16	Secreted	[34]
NSP4	*NSP4*	Bovine coronavirus	Cytoplasmic	[44]
BCV epitope	*BCV*	Bovine coronavirus	Secreted	[49]
Subunit B of urease		*Helicobacter pilori*	Secreted	[53]
Interleukins				
IL-2	*IL-2*	Mouse	Secreted	[67]
IL-6	*IL-6*	Mouse	Secreted	[66]
IL-10	*IL-10*	Mouse	Secreted	
IL-12	*IL-12*	Mouse	Secreted	
Allergens				
BLG	*blg*	Bovine	Secreted / Cytoplasmic	[35,36]
Virulence factor				
Firbonectin-binding protein A	*fnbpA*	*Staphylococcus aureus*		[64]
Clumping factor	*clfA*	*Staphylococcus aureus*		[61]
Protein A	*spA*	*Staphylococcus aureus*	Anchored	[66]
Enzymes				
Listeriolysin	*Ply511*	*Listeria monocytogenes* bacteriophage	Secreted	[13]
Streptodornase		*Streptococcus equisimilis*	Secreted	[75]
Prochymosin	*PC*	Bovine	Secreted	[63]
Lipase	*lip*	*Staphylococcus hyicus*	Secreted	[77] [79]
Plasmin		Bovine	Secreted	[78]

4 AN APPROACH FOCUSING ON HUMAN NUTRITION

Defining the regulatory limit between "medicinal GMM" and "nutritional GMM" claims will be very important. Only the latter would fall into the scope of Afssa. In human nutrition, the GMMs that seem likely to be available soon primarily concern improvements to industrial processes or modification of the flavours of food products.

In the case of bread and beer production, the microorganisms traditionally used (yeasts) are destroyed by heat or eliminated by filtration. However, for dairy products, the lactic bacteria remain present in the food, especially for yoghurts and fermented milks.

4.1 BREAD-MAKING, WINE-MAKING AND BREWING PRODUCTS

A (rapid) review of the patents reveals that the four major sectors using yeast as a fermentation agent – the bread-making, wine-making, brewing and ethanol production industries – all now have GMM yeasts.

Analysis of the main patents shows that, at present, the objectives concern improving processes in the majority of cases and improving organoleptic qualities in a few cases. There is also one patent concerning transgenic yeasts with a higher resistance to cold temperatures [9].

No patent clearly targets the health aspect. Indeed, in most cases, the microbial substances are separated from the final product. The only case in which yeasts remain in the product concerns bread-making. Given the bread-baking temperatures, it is probable that a high proportion, or even all, of the yeasts will be destroyed during this process.

In this sector of activity, the use of live yeasts is not currently envisaged, in contrast with the use of lactic bacteria in the dairy product sector.

Table 3 is simply intended to plot the current situation relative to research in this field without aiming to be exhaustive, given the high number of patents registered since 1985-1986.

Table 3: Applications in wine-making and brewing

Producing organism	Application	Objective	References
Saccharomyces cerevisiae	wine-making	To improve fermentary performance and simplify the process	[40,41]
Saccharomyces cerevisiae	wine-making	To improve the organoleptic and hygienic quality of the wine	[40,41]
Saccharomyces cerevisiae	baking	Fermentation of maltose et melibiose	[40] Patent 87/201670
Saccharomyces cerevisiae	baking	Slow fermentation at low temperatures, cold storage of dough	Patent WO 97/28693
Saccharomyces cerevisiae	baking	Resistance to cold temperatures and high sugar contents	Patent EP 0 921 190 A2
Saccharomyces cerevisiae	baking	Better hydrolysis of starch and hemicellulose	Patent WO 98/03630
Brewer's yeast	brewing	Improvement of flavour	Patent US 6,468,567 B1

Comment

Methylglyoxal is a compound the formation of which was reported back in 1970 following the catabolism of glucose in *Escherichia coli* [10]. During glycolysis, di-hydroxyacetone-phosphate can be converted into methylglyoxal then D-lactic acid. In 1995, Inose and Murata [11] demonstrated that the use of a strain of transformed *Saccharomyces cerevisiae* in alcohol production leads to intracellular accumulation of methylglyoxal at a concentration such that the yeast extract can induce a mutagenic effect. In 1997, Hashimoto *et al* [12] supplemented these studies by showing that the synthesis of methylglyoxal is markedly reduced in the event of a methylglyoxal synthase-deficient mutant, along with the mutagenic effect of the cellular extracts tested. Although these two articles are, to our knowledge, the only ones reporting this problem, they are frequently cited in articles debating the risks relative to the use of genetically-modified microorganisms in foods. This example first of all demonstrates the limits of the supposedly predictive nature of genetic engineering and, secondly, the inadequacy of the principle of substance equivalence to guarantee the safety of GMMs.

4.2 DAIRY PRODUCTS

Although there are relatively few patents in this field, analyses of the literature reveal a significant potential, suggesting that GMM strains have a strong chance of soon being used in dairy products, whenever public opinion becomes favourable to this. A certain number of applications are potentially concerned by these studies, notably for the improvement of production processes (resistance to stresses, resistance of phagic infections, etc.) [13,14], the improvement of food safety thanks to the production of wide-spectrum bacteriocins from other bacteria [15-17], more rapid improvement of the acidification potential of milk or improvements in the organoleptic quality of dairy products (better degradation of proteins [18-20], production of texturing agents, production of flavouring compounds [21-27]. Obviously, in all these cases, since the bacteria genetically modified by self-cloning or by heterologous modification remain in the final product, the sanitary safety of these products must be assessed.

There are recipient lactic bacteria which are termed as "Generally Regarded As Safe" (GRAS status). In theory, these bacteria present fewer risks in human food use. A working group of the Scientific Committee on Food[18] studied the possibility of introducing a system similar to GRAS, applicable to microorganisms and, possibly, their products, at the same time taking into account social specificities and European regulations.

5 LIVESTOCK NUTRITION

There are few publications relative to the ingestion of live GMMs in animal feed. Only one article reports the use of bacteria over-producing a phytase in poultry feed [28]. Therefore we can simply observe the following:

- a certain number of additives for use in animal feeds - threonine, tryptophan and lysine - are already produced by recombinant bacteria;
- enzymes (cellulase, xylanase, beta-glucosidase, phytase, etc.) and live microorganisms are authorised as additives;
- a high number of genes of very diverse origins encoding such enzymes are cloned and characterised, particularly a number of phytases. It should be noted that, with respect to phytases, practically all the "major companies" have a patent for cloning and expression of phytases;

[18] On a generic approach to the safety assessment of microorganism used in feed/food and feed/food production. Working paper, Directorate C – Scientific opinions

- a number of studies concern the use of probiotics (yeasts, lactobacilli, *Bacillus*), their effects on growth performance and their potential interactions with the resident intestinal flora, along with the fate of microorganisms ingested and their dissemination;
- industrial companies market live yeasts and a whole range of enzymes for animal feeds. It is entirely conceivable that these companies have already developed or are studying strains of recombinant yeasts producing these enzymes. However, it is evident today that, from an economical point of view, the enzymes on the market are available at very low cost, whereas the construction and constraints relative to marketing GMMs would certainly be both lengthy and expensive;
- finally, the producing microorganisms currently used in industry are "recycled" in animal feed (brewing yeasts, for example); what about any future GMMs?

Table 4 presents enzymes/peptides/amino acids which are produced in a digestion chamber; no GMMs are consumed live.

Table 4: Applications in livestock feed

	Gene	Origin	Expression host	References
Glucohydrolases				
Cellulase	*EG1/Cel7B*	*Trichoderma reesei*	*Trichoderma reesei*	[37,38]
Cellulase	*Cel7B*	*Trichoderma reesei*	*Trichoderma reesei*	[39]
Cellulase	*engB*	*Clostridium cellulovorans*	*Bacillus subtilis*	[58,59]
Cellulase	*CelA/CelD*	*Neocallimastix patriciarum*	*Clostridium beijerinckii*	[54]
Xylanase Cellulase	*Xys1/Cel1*	*Streptomyces halstedii*	*Brevibacterium lactofermentum*	[29]
Xylanase	*xynA*	*Erwinia chrysanthemi*	*Escherichia coli*	[48]
Xylanase	*xynA*	*Thermotoga neapolitana*	*Escherichia coli*	[76]
Phytases				
thermo^R phytase	*fphy*	*Aspergillus fumigatus*	*Pichia pastoris*	[60]
	phyL	*Bacillus licheniformis*	*Bacillus subtilis*	[70]
	phyA	*Bacillus subtilis*		
Fungal phytases		*Peniophora lycii*	*Aspergillus oryzae*	[50]
		Agrocybe pediades		
		Ceriporia sp.		
		Trametes pubescens		
	synth. gene	*Aspergillus niger*	*Pichia pastoris*	[30]
	appA	*Escherichia coli*	*Pichia pastoris*	US Pat 6,451,572 Lei, Xingen
	phyA, phyB	*Aspergillus niger*		
	phyA	*Aspergillus niger*	*Saccharomyces cerevisiae*	[45]
	appA	*Escherichia coli*	*Saccharomyces sp., Pichia sp., Hansenula sp, Candida sp…...*	US app.0192791 Lei, Xingen
Amino acid, antibacterial activated peptide, antitoxins, etc.				
Buforin II	peptide 21aa	Amphibian	*Escherichia coli*	[51,52]
Plantaricin 423	*plaA, B, C, D*	*Lactobacillus plantarum*	*Saccharomyces. cerevisiae*	[72]
Indirubin and others		Soil DNA library	*Escherichia coli*	[55]
Cecropin B-Thanatin	CB-Tan	Synthetic	*Escherichia coli*	[73]
Magainin-melittin	MA-E	Synthetic	*Escherichia coli*	[46]
Lactonohydrolase	*zhd101*	Fungal	*Escherichia coli, Schizosaccharomyces pombe*	[69]
Epoxide hydrolase	mEH + CYP1A2	Human	*Saccharomyces cerevisiae*	[47]
Elongase		*Thraustochytrium aureum*	*Escherichia coli, Bacillus subtilis*, yeasts	US pat 0138874 Mukerji

6 GREEN CHEMISTRY AND BIOREMEDIATION TECHNOLOGY

The advantage of green chemistry to the consumer resides mainly in the development of more environmentally-friendly processes, particularly with respect to the toxic residues associated with petrochemical production.

Bioremediation can be applied to the degradation of a whole range of contaminants, generally in the water and soil, such as aromatic hydrocarbons, polychlorobiphenyls (PCBs), fuel oil, petrol, heavy metals (such as copper, cadmium, chromium and mercury), pesticides, oils, waste from a variety of industries.

A patent concerning a strain of *Pseudomonas aeruginosa* genetically modified to be used in the degradation of hydrocarbons was registered in 1980 by Ananda Chakrabarty. Historically, this was the first patent registered concerning a transgenic organism.

Since then, there have been numerous GMM constructions to treat pollutants in laboratories, but no recombinant strain is currently marketed for bioremediation. However, several academic groups in the USA, such as at the University of Tennessee for example, have obtained government approval to test their recombinant bacteria on the ground.

GMMs could be useful in the case of degradation of recalcitrant products, such as xenobiotics, or for products for which no natural means of degradation exist.

Patents
There are many patents relative to techniques themselves and fewer for products (table 5).

Table 5: Green chemistry technology

Compound	Microorganism	Patent
Oxaloacetate derivatives	*Escherichia coli*	US Pat 6 455 284
L-isoleucine	*Corynebacterium*	US Pat 6 451 564
L-amino acids	*Corynebacterium*	US Pat 6 355 454
Beta-Carotene	*Fusarium*	US Pat 6 372 479
Keto-carotenoids	?	US Pat 5 910 433
Glucosamine	?	US Pat 6 372 457
Polyhydroxyalkanoates (plastic)	Miscellaneous	US Pat 6 329 183
Xylitol	Yeast	US Pat 6 271 007
4-hydroxybenzoate	?	US Pat 6 114 157
1,3-propanediol	*Escherichia coli*	US Pat 6 136 576
Ethanol	Yeast, *Zymomonas*	US Pat 5 424 202
Cis-cis muconate/catechol	*Pseudomonas* sp	US Pat 5 616 496
Hydrocortisone	*Saccharomyces cerevisiae*	CNRS-Aventis

Publications
The very large number of publications in this field reflect the chemicals industry's interest in a whole range of compounds, some of which have chemical applications proper and other which more specifically concern pharmaceutical applications (synthesis intermediates).

Ongoing studies
If we look at the financial resources mobilised, particularly in the United States but also in Europe, it can be seen that a number of wide-ranging programmes are being developed. This is a marker of the growing interest in green chemistry, mainly intended to increase the use of renewable resources.

Examples
Bioethanol (construction of strains for the production of ethanol from lignocellulose)
Degradation of organophosphate substances
Solvents (improvement of tolerance to solvents)
Isoprenoids
Non-natural porphyrins with a strain of recombinant *Escherichia coli*
Carotenoids
Synthesis of food flavourings using a strain of recombinant *Escherichia coli*

7 SANITARY SAFETY OF GMM PROBIOTICS

Current procedures for the development of GMMs for the purpose of the production of miscellaneous substances (confined use) require that the final products be purified (at least partially), that there is no residual DNA and that the residues of fermentation processes no longer contain any live GMMs. The general idea is to prevent any gene transfer from these GMMs to the native digestive microflora in humans or that of the environment. This field of the “genetically correct”, more advanced than that of plants, is mastered both by public sector scientists and scientists working for major private companies.

The probable development of live GMMs used in human and animal nutrition has led the *Codex Alimentarius* to draft directives governing the conduct of food safety assessment of foods produced using recombinant-DNA microorganisms[19]. These directives identify the potential risks related to the consumption of products containing or produced by these GMMs and define the relevant data for assessing these risks.

7.1 POTENTIAL DANGERS AND RISKS

From the above considerations, and restricting ourselves to non-medicinal GMM probiotics, the following potential risks can be determined:

a- Modification of the metabolism of the GMM could lead to an unexpected detrimental effect on the consumer, either directly or indirectly. The construction conditions mean that it should be possible to control this risk. The product of the transgene is not, in theory, dangerous in itself. However, its over-expression may lead to disturbances in the intestinal tract.

b- The GMM may be resident or a transient host, which changes its functional status and its relationship with the flora.

c- The GMM may modify the balance of the intestinal flora due to unexpected reactions with resident bacteria, or promote the establishment of pathogens (whether the GMM is resident or not). Current probiotics do not have this effect but the presence of an additional function provided by the GMM must be considered.

7.2 CONTROL OF DANGERS AND RISKS

Control of the dangers and risks of these GMMs requires prior evaluation, which can be based on a comparison with a microorganism used in a traditional “reference” product. Although, in the majority of cases, these GMMs and the genes introduced will be produced by microorganisms already known for their use in the food industry, a risk analysis for each recipient bacterium/genetic construction pairing remains essential.

The genetic constructions will have to meet certain essential criteria:
- the absence of antibiotic resistance genes used as a marker;
- constructions inserted in the chromosome and not including any transposon or plasmid-type mobile genetic elements;
- the insertion point in the identified chromosome;
- no foreign encoding sequence apart from the gene considered at the origin of the GMO classification.

The tools available in the field of GMMs make these requirements accessible. A conditional promoter governing the concerned gene (for example, nisin in certain lactic bacteria), could potentially be used.

The risks that should be more specifically examined would, due to the introduction of a new microorganism, be the loss of the barrier effect exerted by the native flora in the intestines, thus promoting the proliferation of pathogenic microorganisms and possible impairment of the metabolic functions of the normal intestinal flora. These effects are not observed with the probiotics currently used.

The report of the FAO-WHO expert group of September 2001 entitled "Safety assessment of food derived from genetically modified microorganisms" notably devotes a chapter to safety and nutritional

[19] Guideline for the conduct of food safety assessment of foods produced using recombinant-DNA microorganisms. Alinorm 03/34A. Codex Alimentarius Joint FAO/WHO Food Standards Programme.

assessment along with the interactions between the GMM, the intestinal flora and the mammalian host.

The points to be documented include:
- colonisation and persistence of the microorganism in the intestine and any impact on the digestive ecosystem,
- the nutritional and toxicological aspects of any bacterial or fungal metabolites, and their possible impairment due to genetic modifications,
- the allergenic potential of these modified microorganisms,
- the survival and propagation of these microorganisms in the environment (direct or indirect via humans or animals) and the consequences in terms of public health.

Control of the metabolism of probiotic GMMs (transcriptomic, proteomic, metabolomic approaches?), although it does not necessarily reflect the metabolic effects *in situ*, represents an interesting approach.

Safety studies on the effects on intestinal flora identify methods that could be used, for example by bacteriological analysis of the stools of a cohort of "normal" volunteers. These cumbersome studies should only be considered where there is an assumed risk.

The Afssa document[20] entitled "Recommandations pour la présentation des données permettant l'évaluation de l'innocuité des microorganismes utilisés dans le secteur agroalimentaire – souches nouvelles ou modifiées – application différente de souches déjà utilisées" ("Recommendations for the presentation of data enabling assessment of the safety of microorganisms used in the agrifood sector – new or modified strains – different application of strains already used") devotes a chapter to the "case of a strain intended to be consumed live in a foodstuff". In addition to the necessary prerequisite of good characterisation of the strain (reliable and relevant taxonomic data) and the absence of any pathogenic or toxinogenic risk, it is suggested that tests be conducted in laboratory animals (90 days) aimed at documenting the local tolerance and any systemic toxicity of the GMM administered by the oral route (parenteral route).

The environmental risk is an important point to consider insofar as it can have an impact on microbial ecological balance but also on humans via the contamination of soil and animals. Assessment of the risks to the environment is a matter for the biomolecular engineering committee.

In any case, GMMs must be examined on a case-by-case basis and analysed in terms of benefits and risks.

[20] Document available on the website: www.afssa.fr

References

1 Periti P., Tonelli F. (2001) Preclinical and clinical pharmacology of biotherapeutic agents: *Saccharomyces boulardii*. *J Chemother* 13:473-93.

2 Drouault S., Corthier G. (2001) Health effects of lactic acid bacteria ingested in fermented milk.. *Vet Res* 32:101-17.

3 Isolauri E., Kirjavainen P.V. Salminen S. (2002) Probiotics: a role in the treatment of intestinal infection and inflammation? *Gut* 50:III54-9.

4 Marteau, P.R., de Vrese M., Cellier C.J., Schrezenmeir J. (2001) Protection from gastrointestinal diseases with the use of probiotics. *Am J Clin N*utr 73:430S-436S.

5 Saarela M., Mogensen G., Fonden R., Matto J. Mattila-Sandholm T. (2000) Probiotic bacteria: safety, functional and technological properties. *J Biotechnol* 84:197-215.

6 Mercenier A, Pavan S, Pot B. (2003) Probiotics as biotherapeutic agents: present knowledge and future prospects *Curr Pharm* 9:175-91.

7 Forde A., Fitzgerald G.F. (1999) Bacteriophage defence systems in lactic acid bacteria. Antonie van Leeuwenhoek 76:89-113.

8 Steidler L, Hans W, Schotte L, Neirynck S, Obermeier F, Falk W, Fiers W, Remaut E. (2000) Treatment of murine colitis by *Lactococcus lactis* secreting interleukin-10. *Science* 289(5483): 1352-5.

9 Bourgeois E, Gautier M.F, Joudrier P. (2000) Levures transformées par des gènes augmentant leur tolérance au stress froid. WO 02051978.

10 Cooper R.A. and Anderson Anne (1970). The formation and catabolism of methylglyoxal during glycolysis in *Escherichia coli*. *FEBS Letters*, 11: 273276.

11 Inose T. and Murata K. (1995) Enhanced accumulation of toxic compound in yeast cells having high glycolytic activity: a case study on the safety of genetically engineered yeast. *Int. J. of Food Science and Technology, 30: 141-146.*

12 Hashimoto W., Inose T., Masuda K., Murata K. (1997) Safety assessment of genetically engineered yeast: elimination of mutagenicity of the yeast *Saccharomyces cerevisiae* by decreasing the activity of methyglyoxal synthase. *Int. J. of Food Science and Technology* 32: 521-526.

13 Gaeng S, Scherer S, Neve H, Loessner MJ. (2000) Gene Cloning and Expression and Secretion of *Listeria monocytogenes* Bacteriophage-Lytic Enzymes in *Lactococcus lactis*. *Appl Environ Microbiol.* 66: 2951-2958.

14 Wouters J.A., Mailhes M., Rombouts F.M., de Vos W.M., Kuipers O.P., Abee T. (2000) Physiological and Regulatory Effects of Controlled Overproduction of Five Cold Shock Proteins of *Lactococcus lactis* MG1363. *Appl Environ Microbiol.* 66: 3756-3763.

15 Horn N., Martínez M.I., Martínez J.M., Hernández P.E., Gasson M.J., Rodríguez J.M., Dodd H.M. (1999). Enhanced Production of Pediocin PA-1 and Coproduction of Nisin and Pediocin PA-1 by *Lactococcus lactis*. *Appl Environ Microbiol*. 65: 4443-4450.

16 Martínez J.M., Kok J, Sanders J.W., Hernández P.E. (2000) Heterologous Coproduction of Enterocin A and Pediocin PA-1 by *Lactococcus lactis*: Detection by Specific Peptide-Directed Antibodies. *Appl Environ Microbiol.* Aug; 66: 3543-3549.

17 Li H., O'Sullivan D.J. (2002) Heterologous Expression of the *Lactococcus lactis* Bacteriocin, Nisin, in a Dairy Enterococcus Strain. *Appl Environ Microbiol.* Jul; 68: 3392-3400.

18 Luoma S., Peltoniemi K., Joutsjoki V., Rantanen T., Tamminen M., Heikkinen I., Palva A. (2001) Expression of Six Peptidases from *Lactobacillus helveticus* in *Lactococcus lactis*. *Appl Environ Microbiol*., 67: 1232-1238.

19 Leenhouts K., Bolhuis A., Boot J., Deutz I., Toonen M., Venema G., Kok J., Ledeboer A. (1998).Cloning, Expression and Chromosomal Stabilization of the *Propionibacterium shermanii* Proline Iminopeptidase Gene (pip) for Food-Grade Application in *Lactococcus lactis*. *Appl Environ Microbiol.* 64:4736-4742.

20 Wegmann U, Klein JR, Drumm I, Kuipers OP, Henrich B. (1999) Introduction of Peptidase Genes from *Lactobacillus delbrueckii* subsp. lactis into *Lactococcus lactis* and Controlled Expression. *Appl Environ Microbiol.* 65: 4729-4733.

21 Lapierre L, Germond JE, Ott A, Delley M, Mollet B. (1999) d-Lactate Dehydrogenase Gene (ldhD) Inactivation and Resulting Metabolic Effects in the *Lactobacillus johnsonii* Strains La1 and N312. *Appl Environ Microbiol.* 65: 4002-4007.

22 Lopez de Felipe F., Kleerebezem M., de Vos W.M., Hugenholtz J. (1998) Cofactor Engineering: a Novel Approach to Metabolic Engineering in *Lactococcus lactis* by Controlled Expression of NADH Oxidase. *J Bacteriol.* 180: 3804-3808.

23 Hugenholtz J, Kleerebezem M, Starrenburg M, Delcour J, de Vos W, Hols P. (2000) *Lactococcus lactis* as a Cell Factory for High-Level Diacetyl Production. *Appl Environ Microbiol.* 66: 4112-4114.

24 Van Kranenburg R, de Vos WM. (1998) Characterization of Multiple Regions Involved in Replication and Mobilization of Plasmid pNZ4000 Coding for Exopolysaccharide Production in *Lactococcus lactis*. *J Bacteriol.* 180: 5285-5290.

25 Van Kranenburg R, Vos HR, van Swam II, Kleerebezem M, de Vos WM. (1999) Functional Analysis of Glycosyltransferase Genes from *Lactococcus lactis* and Other Gram-Positive Cocci: Complementation, Expression, and Diversity. *J Bacteriol.* 181: 6347-6353.

26 Rijnen L., Bonneau S., Yvon M. (1999) Genetic Characterization of the Major Lactococcal Aromatic Aminotransferase and Its Involvement in Conversion of Amino Acids to Aroma Compounds. *Appl Environ Microbiol.* Nov; 65: 4873-4880.

27 Andersen H.W., Solem C., Hammer K., Jensen P.R. (2001) Reduction of Phosphofructokinase Activity in *Lactococcus lactis* Results in Strong Decreases in Growth Rate and in Glycolytic Flux. *J Bacteriol.* 183: 3458-3467.

28 Lan G.Q., Abdullah N., Jalaludin S., Ho Y.W. (2002) Efficacy of supplementation of a phytase-producing bacterial culture on the performance and nutrient use of broiler chickens fed corn-soybean meal diets. *Poult. Sci*. 81(10): 1522-32

29 Adham S.A., Campelo A.B., Ramos A., Gil J.A.. (2001) Construction of a xylanase-producing strain of *Brevibacterium lactofermentum* by stable integration of an engineered xysA gene from *Streptomyces halstedii* JM8. *Appl Environ Microbiol*. 67(12):5425-30.

30 Bei J.L., Chen Z., Yang L., Liao L., Wang X.Z., Jiang Z.Y. (2001) Overexpression of artificial synthetic gene of *Aspergillus niger* NRRL3135 phytase in *Pichia pastoris*. Sheng Wu Gong Cheng Xue Bao. 17(3):254-8.

31 Beliavskaia V.A., Kashperova T.A., Bondarenko V.M., Il'chev A.A., Sorokulova I.B., Malik N.I. (2001). Experimental evaluation of the biological safety of gene-engineered bacteria using a model strain *Bacillus subtilis* interferon-producing strain]. Zh Mikrobiol Epidemiol Immunobiol. 2001, 2:16-20.

32 Beninati C, Oggioni M.R., Boccanera M., Spinosa M.R., Maggi T., Conti S., Magliani W., De Bernardis F., Teti G., Cassone A., Pozzi G., Polonelli L (2000). Therapy of mucosal candidiasis by expression of an anti-idiotype in human commensal bacteria. *Nat Biotechnol* 18(10): 1060-4.

33 Beninati C., Oggioni M., Mancuso G., Midiri A., Polonelli L., Pozzi G., Teti G. (2001) Anti-idiotypic vaccination against group *B streptococci*. *Int Rev Immunol* 20(2): 263-73.

34 Bermudez-Humaran L.G., Langella P., Miyoshi A., Gruss A., Guerra R.T., Montes de Oca-Luna R., Le Loir Y. (2002). Production of human papillomavirus type 16 E7 protein in *Lactococcus lactis*. *Appl Environ Microbiol* 68(2): 917-22.

35 Bernasconi E., Germond J.E., Delley M., Fritsche R., Corthesy B. (2002) *Lactobacillus bulgaricus* proteinase expressed in *Lactococcus lactis* is a powerful carrier for cell wall-associated and secreted bovine beta-lactoglobulin fusion proteins. *Appl Environ Microbiol* 68(6): 2917-23.

36 Chatel J.M., Langella P., Adel-Patient K., Commissaire J., Wal J.M., Corthier G. (2001). Induction of mucosal immune response after intranasal or oral inoculation of mice with *Lactococcus lactis* producing bovine beta-lactoglobulin. *Clin Diagn Lab Immunol* 8(3): 545-51.

37 Collen A., Ward M., Tjerneld F., Stalbrand H.. (2001) Genetic engineering of the *Trichoderma reesei* endoglucanase I (Cel7B) for enhanced partitioning in aqueous two-phase systems containing thermoseparating ethylene oxide–propylene oxide copolymers *J Biotechnol.* 4;87(2):179-91.

38 Collen A., Ward M., Tjerneld F., Stalbrand H. (2001) Genetically engineered peptide fusions for improved protein partitioning in aqueous two-phase systems. Effect of fusion localization on endoglucanase I of *Trichoderma reesei*. *J Chromatogr* A. 2;910(2):275-84.

39 Collen A, Selber K, Hyytia T, Persson J, Nakari-Setla T, Bailey M, Fagerstrom R, Kula MR, Penttila M, Stalbrand H, Tjerneld F. (2002) Primary recovery of a genetically engineered *Trichoderma reesei* endoglucanase I (Cel 7B) fusion protein in cloud point extraction systems. *Biotechnol Bioeng.* 20;78(4):385-94.

40 Dequin S. (2001) The potential of genetic engineering for improving wine-making and baking yeasts. *Appl. Microbiol.Biotechnol*, 56: 577-588.

41 Dequin S., Salmon J.M., Huu-vang Nguyen and B. Blondin (2002). Wine Yeast's.

42 Di Fabio S., Medaglini D., Rush C.M., Corrias F., Panzini G.L., Pace M., Verani P., Pozzi G., Titti F. (1998). Vaginal immunization of Cynomolgus monkeys with *Streptococcus gordonii* expressing HIV-1 and HPV 16 antigens. *Vaccine* 16(5): 485-92.

43 Duc le H., Hong H.A., Fairweather N., Ricca E., Cutting S.M. (2003) Bacterial spores as vaccine vehicles. Infect Immun. 71:2810-8.

44 Enouf V, Langella P, Commissaire J, Cohen J, Corthier G. (2001) Bovine rotavirus nonstructural protein 4 produced by *Lactococcus lactis* is antigenic and immunogenic. *Appl Environ Microbiol* 67(4): 1423-8.

45 Han Y., Wilson D.B., Lei X.G. (1999) Expression of an *Aspergillus niger* phytase gene (phyA) in *Saccharomyces cerevisiae. Appl Environ Microbiol*. 65(5):1915-8.

46 Huang Y., Liu F.P., Zhou T.H., Zhu J.M. (2001) Cloning and expression of a synthetic gene encoding magainin-melittin hybrid peptide in *Escherichia coli* and studies on its antibacterial activity. Sheng Wu Gong Cheng Xue Bao. 17(2):207-10

47 Kelly E.J., Erickson K.E., Sengstag C., Eaton D.L. (2002) Expression of human microsomal epoxide hydrolase in *Saccharomyces cerevisiae* reveals a functional role in aflatoxin B1 detoxification. *Toxicol Sci*. 65(1):35-42.

48 Keen N.T., Boyd C., Henrissat B. (1996) Cloning and characterization of a xylanase gene from corn strains of *Erwinia chrysanthemi*. *Mol Plant Microbe Interact.* 9(7):651-7.

49 Langella P., Le Loir Y. (1999) Heterologous protein secretion in *Lactococcus lactis*: a novel antigen delivery system. Braz *J Med Biol Res* 32(2): 191-8.

50 Lassen S.F., Breinholt J., Ostergaard P.R., Brugger R., Bischoff A., Wyss M., Fuglsang C.C.. (2001) Expression, gene cloning, and characterization of five novel phytases from four basidiomycete fungi: *Peniophora lycii, Agrocybe pediades*, a *Ceriporia* sp., and *Trametes pubescens. Appl Environ Microbiol*. 67(10):4701-7.

51 Lee J.H., Minn I., Park C.B., Kim S.C. (1998) Acidic peptide-mediated expression of the antimicrobial peptide buforin II as tandem repeats in *Escherichia coli*. *Protein Expr Purif.* 12(1):53-60.

52 Lee J.H., Kim M.S., Cho J.H., Kim S.C. (2002) Enhanced expression of tandem multimers of the antimicrobial peptide buforin II in *Escherichia coli* by the DEAD-box protein and trxB mutant. *Appl Microbiol Biotechnol*. 58(6):790-6.

53 Lee M.H., Roussel Y., Wilks M., Tabaqchali S. (2001) Expression of Helicobacter pylori urease subunit B gene in *Lactococcus lactis* MG1363 and its use as a vaccine delivery system against H. pylori infection in mice. *Vaccine* 19(28-29): 3927-35.

54 Lopez-Contreras A.M., Smidt H., van der Oost J., Claassen P.A., Mooibroek H., de Vos W.M. (2001) *Clostridium beijerinckii* cells expressing *Neocallimastix patriciarum* glycoside hydrolases show enhanced lichenan utilization and solvent production *Appl Environ Microbiol*. 67(11):5127-33.

55 MacNeil I.A., Tiong C.L., Minor C., August P.R., Grossman T.H., Loiacono K.A., Lynch B.A., Phillips T., Narula S., Sundaramoorthi R., Tyler A., Aldredge T., Long H., Gilman M., Holt D., Osburne M.S. (2001). Expression and isolation of antimicrobial small molecules from soil DNA libraries. *J Mol Microbiol Biotechnol*. 3(2):301-8.

56 Mauriello E.M., Duc le H., Isticato R., Cangiano G., Hong H.A., Felice M.D., Ricca E., Cutting S.M. (2004). Display of heterologous antigens on the *Bacillus subtilis* spore coat using CotC as a fusion partner. Vaccine. 22:1177-87.

57 Medaglini D, Oggioni MR, Pozzi G. (1998). Vaginal immunization with recombinant gram-positive bacteria. *Am J Reprod Immunol* 39(3): 199-208.

58 Murashima K., Kosugi A., Doi R.H. (2002) Thermostabilization of cellulosomal endoglucanase EngB from *Clostridium cellulovorans* by *in vitro* DNA recombination with non-cellulosomal endoglucanase EngD *Mol Microbiol*. 45(3):617-26.

59 Murashima K., Chen C.L., Kosugi A., Tamaru Y., Doi R.H., Wong S.L. (2002) Heterologous production of *Clostridium cellulovorans* engB, using protease-deficient *Bacillus subtilis*, and preparation of active recombinant cellulosomes *J Bacteriol*.184(1):76-81.

60 Peng RH, Xiong AS, Li X, Fan HQ, Yao QH, Guo MJ, Zhang SL. (2002) High expression of a heat-stable phytase in Pichia pastoris. Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao (Shanghai) 34(6):725-30.

61 Que Y.A., Haefliger J.A., Francioli P., Moreillon P. (2000) Expression of *Staphylococcus aureus* clumping factor A in *Lactococcus lactis subsp. cremoris* using a new shuttle vector. *Infect Immun* 68(6): 3516-22.

62 Ribeiro L.A., Azevedo V., Le Loir Y., Oliveira S.C., Dieye Y., Piard J.C., Gruss A., Langella P. (2002). Production and targeting of the *Brucella abortus* antigen L7/L12 in *Lactococcus lactis*: a first step towards food-grade live vaccines against brucellosis. *Appl Environ Microbiol,* 68(2): 910-6.

63 Simons G., Rutten G., Hornes M., Nijhuis M., van Asseldonk M. (1991) Production of prochymosin in lactococci. *Adv Exp Med B*iol 306: 115-9.

64 Sinha B., Francois P., Que Y.A., Hussain M., Heilmann C., Moreillon P., Lew D., Krause K.H., Peters G., Herrmann M. (2000) Heterologously expressed *Staphylococcus aureus* fibronectin-binding proteins are sufficient for invasion of host cells. *Infect Immun* 68(12): 6871-8.

65 Slos P., Dutot P., Reymund J., Kleinpeter P., Prozzi D., Kieny M.P., Delcour J., Mercenier A., Hols P. (1998). Production of cholera toxin B subunit in Lactobacillus. *FEMS Microbiol Lett* 169(1): 29-36.

66 Steidler L., Robinson K., Chamberlain L., Schofield K.M., Remaut E., Le Page R.W., Wells J.M. (1998) Mucosal delivery of murine interleukin-2 (IL-2) and IL-6 by recombinant strains of *Lactococcus lactis* coexpressing antigen and cytokine. *Infect Immun* 66(7): 3183-9.

67 Steidler L., Wells J.M., Raeymaekers A., Vandekerckhove J., Fiers W., Remaut E. (1995) Secretion of biologically active murine interleukin-2 by *Lactococcus lactis subsp. lactis*. *Appl Environ Microbiol* 61(4): 1627-9.

68 Sorokulova I.B. (1998) The safety and reactogenicity of the new probiotic subalin for volunteers. *Mikrobiol Z*. 60(1):43-6.

69 Takahashi-Ando N., Kimura M., Kakeya H., Osada H., Yamaguchi I. (2002) A novel lactonohydrolase responsible for the detoxification of zearalenone: enzyme purification and gene cloning. *Biochem J.* 365(Pt 1):1-6.

70 Tye A.J., Siu F.K., Leung T.Y., Lim B.L. (2002) Molecular cloning and the biochemical characterization of two novel phytases from *B. subtilis* 168 and *B. licheniformis*. *Appl Microbiol Biotechnol*. 59(2-3):190-7.

71 Urdaci M.C., Pinchuk I.V., Sorokulova I.B., Megraud F. (2003) Use of *Bacillus subtilis* strain CU1 as a vaccine delivery system for mucosal immunization against *Helicobacter pylori* infection in mice. FEMS Congress, Bacillus 2003 satellite symposium, Ljubljana, Slovenia.

72 Van Reenen C.A., Chikindas M.L., Van Zyl W.H., Dicks L.M. (2003) Characterization and heterologous expression of a class IIa bacteriocin, plantaricin 423 from *Lactobacillus plantarum* 423, in *Saccharomyces cerevisiae. Int J Food Microbiol*. 81(1):29-40.

73 Weng H.B., Niu B.L., Meng Z.Q., Xu M.K. (2002) Cloning and expression of the cecropin B-thanatin hybrid antimicrobial peptide in *Escherichia coli. Sheng Wu Gong Cheng Xue Bao* 18(3):352-5. Chinese.

74 Wells J.M., Robinson K., Chamberlain L.M., Schofield K.M., Le Page R.W. (1996) Lactic acid bacteria as vaccine delivery vehicles. *Antonie Van Leeuwenhoek* 70(2-4): 317-30.

75 Wolinowska R., Ceglowski P., Kok J., Venema G. (1991) Isolation, sequence and expression in *Escherichia coli, Bacillus subtilis* and *Lactococcus lactis* of the DNase (streptodornase)-encoding gene from *Streptococcus equisimilis* H46A. *Gene* 106(1): 115-9.

76 Zverlov V, Piotukh K, Dakhova O, Velikodvorskaya G, Borriss R. (1996) The multidomain xylanase A of the hyperthermophilic bacterium *Thermotoga neapolitana* is extremely thermoresistant. *Appl Microbiol Biotechnol.* 45(1-2):245-7.

77 Drouault S., Corthier G., Ehrlich S.D., Renault P. (2002) Oral treatment with *Lactococcus lacti*s expressing Staphylococcus hyicus lipase enhances lipid digestion in pigs with induced pancreatic insufficiency. *Appl Environ Microbiol* 68(6): 3166-8.

78 Arnau J., Hjerl-Hansen E., Israelsen H. (1997) Heterologous gene expression of bovine plasmin in Lactococcus lactis. *Appl Microbiol Biotechnol*. 48:331-8.

79 Drouault S., Corthier G., Ehrlich S.D., Renault P. (2000) Expression of the Staphylococcus hyicus lipase in *Lactococcus lactis*. *Appl Environ Microbiol*. 66(2):588-98.

PATENTS CITED

- Patent WO 97/28693, registered by Lesaffre.
- Patent 87/201670 Gist-brocades
- Patent EP 0 921 190 A2, 1999, Oriental Yeast CO LTD, Japan.
- Patent WO 98/03630 Spain.
- US Pat 6,468,567 B1, 2002, Cerveceria Polar, Venezuela.
- US Pat 0138874 2002 Mukerji ("Elongase").
- US Pat 6,451,572, 2002, Lei, Xingen.
- US Pat No. 6,455,284:Metabolically engineered E. coli for enhanced production of oxaloacetate-derived biochemicals. University of Georgia Research Foundation, 2002.
- US Pat No. 6,451,564: Methods for producing L-isoleucine, Massachusetts Institute Technology, 2002.
- US Pat No. 6,355,454: Process for the fermentative production of L-amino acids using coryneform bacteria. Degussa Huls AG, 2002.
- US Pat No. 6,372,479: Fusarium sporotrichioides strains for production of ?carotene. US Government, 2002.
- US Pat No. 5,910,433: Keto group-introducing enzyme, DNA coding therefor and method for producing keto-carotenoids. Kirin Beer Kabushiki Kaisha, 1999.
- US Pat No. 6,372,457: Process and materials for production of glucosamine. Arikon Life Sciences LLC, 2002.
- US Pat No. 6,329,183: Polyhydroxyalkanoate production from polyols. Metabolix Inc, 2001.
- US Pat No. 6,271,007: Yeast strains for the production of xylitol, Xyrofin Oy, 2001.
- US Pat No. 6,114,157: Method for increasing total production of 4-hydroxybenzoic acid by biofermentation. General Electric Company, 2000.
- US Pat No. 6,136,576: Method for the recombinant production of 1,3-propanediol. Genencor International, 2000.
- US Pat No. 5,424,202: Ethanol production by recombinant hosts. University of Florida, 1995.
- US Pat No. 5,616,469: Bacterial cell transformants for production of cis, cis-muconic acid and catechol, Purdue Research Foundation, 1997.

SUMMARY AND CONCLUSIONS

The question asked in the title of this document, "GMOs and food: is it possible to identify and assess health benefits" has been illustrated by four cases, from which a general conclusion can be drawn. It leads to the notion that the potential benefits of GMOs should be evaluated in parallel with their risks. Let us first examine the conclusions that we reached for each of the four cases.

- The introduction of new varieties of **insect resistant plants** would have a dual beneficial effect on health by reducing exposure of consumers both to insecticides and to mycotoxins.

  While being careful not to make any hasty generalisations, it does seem that, for these two points, a reduction in exposure is indeed observed. Thus, first of all, for example, a significant reduction in the use of pesticide products (including insecticides) has accompanied the introduction of insect-resistant plant varieties in North America and the Far East. Secondly, given that resistance to insects is correlated with lower sensitivity of the plants to moulds, a few indisputable results demonstrate lower contamination of some of these plants (corn) by mycotoxins, such as fumonisins. With respect to this second point, the value of this lower level of mycotoxin contamination is suggested by observation of better growth in pigs and chickens fed with insect-resistant corn than with conventional corn.

  It would thus appear consistent to consider that the use of these insect-resistant varieties may have a beneficial effect on health. However, it is difficult to say that these benefits are guaranteed and general. Although the toxicity of insecticides and mycotoxins is well documented, there are few epidemiological studies making it possible to measure their real impact on consumer health. It is therefore very difficult at the current time to assess the effects of the reduction in exposure to these substances that may be result from the introduction of insect-resistant varieties. For insecticides, the benefit to the farmer handling these products is not in any doubt, but this benefit cannot be demonstrated for consumers at the current time. In the case of mycotoxins, it would be important to compare the long-term effect of insecticide treatments with resistant crop varieties.

- The introduction of **varieties tolerant to a specific herbicide** would be likely to favour the use of herbicides less dangerous to health than are used when growing conventional varieties.

  This question has been dealt with in the case of glyphosate-tolerant sugar beet. Weed control of conventional varieties involves 7 main herbicides which, at the concentrations used, are considered not to present any health risks. In the specific case of sugar beet, the purification and crystallisation processes lead to an absence of detectable levels of herbicides in white sugar. Therefore one cannot expect that reducing the use of these herbicides would have a direct benefit on consumers of sugar beet sugar.

  However, the question still remains for the farmer and, indirectly, for the environment. In comparison with other herbicides, glyphosate, given its physicochemical characteristics (low liposolubility, low volatility) presents a more limited risk to the farmer. Conversely, its greater solubility and stability in aqueous medium make it a pesticide which could present greater disadvantages for the environment. The impact of this pollution on the health of populations is still to be determined. It should be noted that in these comparisons between herbicides, one must take into account not only the properties of the active substance itself, but also those of the additives present in the formulations.

- The **case of "golden rice"** is emblematic although still insufficiently documented. The creation of this variety of rice enriched in a vitamin A precursor is the subject of intense debate. For some authors, it demonstrates that transgenic plants represent a technological advance likely to provide solutions to the serious problems posed by nutrition in developing countries. For others, this rice could in no way compensate for vitamin A deficiencies, which could be remedied by other means if a proactive policy were to be implemented.

  The debate primarily concerns the quantity of rice that one would need to ingest to compensate for the vitamin deficiency. The study presented here reveals the uncertainty affecting the assessment of this quantity and seeks the causes of this uncertainty. It can be seen that, depending on the hypothesis retained, the daily rice intake required to significantly remedy vitamin A deficiencies ranges from 90 to 4500 g. The mean daily rice intake in the considered countries being 250 to 300 g, this range obviously allows all the protagonists to produce figures that fit in with their own particular point of view.

  A reasonable conclusion would be that it is too early to say whether the varieties currently available will be able to provide a solution to the problems of vitamin A deficiency, but that the studies on “golden rice” show that the conception and development of transgenic plants for nutritional purposes, notably for the benefit of developing countries, is not a utopic idea. In any case, this approach remains exemplary in its concept.

- Whilst the first three chapters concern transgenic plants, the fourth concerns another category of GMOs, **"Genetically-Modified Microorganisns"** or GMMs. While a number of products produced by GMM technology are already authorised in the pharmaceutical and agrifood industries, here we are talking about microorganisms (bacteria, yeasts, moulds) which, in living state, could be used in pharmaceutical preparations, be ingested for nutritional purposes with foods or drinks (dairy products, in particular) or be used in various bioremediation processes. Although such microorganisms are not yet available on the market, they could soon be the subject of marketing applications. This chapter is mainly presented as an inventory of the studies under way on these GMMs, an assessment of their benefits and risks remaining to be carried out. For those that could be used in pharmaceutical preparations, assessment of their risks and benefits should be incorporated in the now classic process of assessment of medicinal products.

Analysis of these four "textbook cases" reveals that there are indeed data in existence suggesting that the GMOs considered may offer benefits to human health but that quantification of these benefits is difficult to carry out, especially for first-generation GMOs, which were not conceived to modify the nutritional composition. In the majority of cases, this quantitative assessment appears to be very difficult, not to say out of reach. However, if we look closely, it may actually present fewer difficulties than the theoretically widely employed method of risk assessment. Let us look at this point again.

Traditionally, the assessment of nutritional risks first of all includes identification of the dangers related to a product or microorganism and then consideration of the probability of exposure to this product or this microorganism and the possible consequences of this exposure. This approach applies without any real difficulty when we are considering the risks related to the presence of a substance of known toxicity or a pathogenic bacterium in a food. In the case of GMOs intended for consumption, the difficulty is much greater since, to date, no health problems, either with respect to toxicity or allergenicity, have been specifically attributed to a GM food placed on the market[21]. This does not exclude the possibility that a risk may exist but has not yet been accurately identified, far less quantified. This explains why there are quite often differences of opinion in the assessment of GMO risks, with all the experts not necessarily having the same perception of the degree of precaution to be applied in response to this unquantifiable risk.

In view of this fundamental difficulty in assessing the risks related to the consumption of GMOs, assessment of the benefits may appear to be simpler. In fact the benefits, in contrast with the dangers, are identified. If we take the case of mycotoxins, for example, we know their biological effects and we know that they can contaminate plants used in animal and human nutrition. We should therefore, in theory, be able to attempt to quantitatively assess the risk of exposure to these toxins and the reduction of this risk resulting from the introduction of insect-resistant plant varieties. We would then tend towards a quantitative assessment of this benefit to health. This is probably the approach that one should take prior to any assessment of a marketing authorisation application for a GMO supposed to provide benefits in terms of health. These potential benefits to health will obviously not be the only factors to be taken into account in the final decision. The factors that could concern the various players involved in the production process or the environment must also be taken into consideration, but these are not matters covered by the scope of Afssa's expertise, at least not in the context of its current missions.

This assessment of the benefits will very quickly become a necessity if the "first-generation" GMOs manage to establish themselves on the market. In fact whilst, as we have seen, these GMOs conceived primarily for economic purposes probably only present a limited benefit in terms of health, it is evident that, in the near future GMOs designed to present a direct value in terms of nutrition or prevention of diseases will emerge. We are thus seeing varieties of hypoallergenic rice and soja, proteaginous plants containing a greater quantity of essential amino acids, oleaginous plants in which the fatty acid composition is being modified to obtain higher-quality cooking oils or "functional foods", making it possible to envisage oral vaccination forms. In addition, transgenic plants currently under development could be capable of combating abiotic stresses (excessive salinity and drought, in particular) and could help to better protect the environment and make certain crops possible on soils today considered to be unsuitable for agriculture.

For all these "second-generation" GMOs, it will be important to conduct, in parallel and on a case-by-case basis, an assessment of the health benefits, either direct or indirect via protection of the environment, at the same time as a risk assessment. It will also be necessary to consider the possibility of obtaining the same benefits using other strategies. We will thus tend, for these new foods, towards analysis of the benefit/risk ratio, comparable to that prevailing prior to marketing of a drug.

[21] The placing on the market of GMOs and products produced by GMPs is subject to express authorisation, granted on a case-by-case basis at European level. In France, as in each Member state of the European Union, specialist expert committees examine each application for a marketing authorisation and assess the risks related to the introduction of these products into our diet.