

levels known to have toxic or pharmacological effects. During the long history of plants used as food, varieties have been developed that are treated and accepted as safe because they do not have obvious acute toxicity. Plant breeders may monitor substances in new varieties (e.g. solanine in potato, erucic acid and glucosinolates in canola) to ensure that concentrations in new varieties have not increased beyond acceptable levels. In other cases, processing procedures or consumption limits are needed to ensure that substances known to have toxic effects (e.g. cassava, legumes, bitter almond) are present at concentrations safe for consumption.

64. All plant breeding methods, traditional and modern, have the potential to lead to unexpected or unintended changes in concentrations of various substances in the plants. It is important that all new varieties be evaluated, in order to reduce the likelihood that unexpected changes will produce adverse health effects. In most cases of plant modification, DNA insertion takes place at random, unpredictable loci. Such random insertion may lead to unintentional changes in gene expression: first, the foreign DNA might be inserted into the coding region of a gene of the host organism, leading to a truncated or hybrid gene product whose function is altered, impaired or lost; second, it might be inserted into the regulatory region of a gene and therefore alter the gene's expression pattern; third, the foreign DNA might affect the gene of a regulatory protein thereby affecting other genes. Another issue is that plant metabolism, might be altered as an adaptation to expression of the foreign gene. None of these effects are unique to GM plants. Each could also be caused by naturally "jumping genes" and natural or induced mutations, e.g. chromosomal rearrangements. All these events may lead to more or less pronounced changes in plant metabolism. Alterations in concentrations of known plant metabolites in the new variety can be monitored using existing analytical methods.

65. The concept of substantial equivalence has been used as a tool in risk assessment. This concept involves comparing the GM organism-derived plant and its conventional counterpart with respect to their phenotypic and agronomic characteristics and their food composition, taking into particular account key nutrients, antinutrients and toxicants typical of the particular plant. Agronomic and phenotypic characteristics provide an objective assessment of the plant's health, often indicating unacceptable alterations. Analyses of key substances provide increased assurance that substances important from a nutritional or health perspective are present in acceptable concentrations.

B.5. Gene transfer: potential impacts on human health (e.g. antibiotic resistance markers)

66. Horizontal gene transfer is the non-sexual or parasexual transfer of genetic material between organisms belonging to the same or different species. Though actual evidence of its occurrence or feasibility (except among bacteria and fungi) is rare, the issue is taken seriously in the safety assessment of GMOs. This issue concerns the potential transfer of genetic material from micro-organisms and plants to other organisms. There is no scientifically valid reason to treat possible gene transfer events involving genetically modified organisms differently from those involving naturally occurring organisms (Salyers, 1997). In any case, it is the gene and the trait it confers, and whether or not it brings a reproduction or selection advantage to the recipient organism that are crucial concerns when possible impacts of potential gene transfer are being considered.

67. Since selection in favour or against a gene is important in its maintenance or proliferation, genes that confer a selective advantage deserve particular attention. However, depending on the trait, selective advantage as such does not automatically imply any harmful effects. Foreign DNA is normally linked to marker genes, in order to be able to identify cells into whose genome the DNA construct has been inserted. Two categories of selection markers commonly used are genes conferring resistance to various herbicides and certain antibiotic resistance genes of bacterial origin. The marker gene commonly used in biotechnology, kanamycin resistance, does not confer resistance to antibiotics that are in (oral) therapeutic

use. Furthermore, bacterial strains resistant to the antibiotics in question (e.g. kanamycin or ampicillin) are common in the environment and in the human intestines, and large numbers of naturally resistant bacteria are acquired when ingesting fresh food (Salysers, 1997a; Smalla et al, 1997; Sci Am, March 1998).

68. In the case of antibiotic resistance genes, there is good reason to think that genetically modified strains pose much less of a threat than naturally occurring resistant bacterial strains, because the former represent old, narrow-spectrum and less mobilizable resistance genes not involved in the present problems in hospitals (Salysers, 1997b). Marker genes conferring resistance to antibiotics for therapeutic use should be avoided in viable GM micro-organisms in food. If the GM micro-organism includes marker genes, information should be provided to show that these genes do not provide a selective advantage in the gut or influence the existing microflora under either typical and extreme conditions (e.g. consumers taking medication). If the marker does provide a selective advantage in the gut, the consequences for the consumer must be defined. Genetic exchange between living bacteria by conjugation is known to occur very broadly, even across genus or family limits. Certain bacteria can also take up bare DNA from their surrounding fluid and even sometimes integrate it in their genome - a phenomenon called natural transformation. However, that occurs relatively rarely and only with DNA from the same or closely related bacterium species (Salysers, 1997a).

69. *In vivo* gene transfer of DNA from GM plants to bacteria, while hypothetically possible, is a remote possibility. This is because a number of unlikely events must occur sequentially. These events include the availability of the right kind of DNA, the type of bacteria, the ability of these bacteria to take up DNA and be transformed by that DNA and the competitiveness of the transformed bacteria. At present there is no evidence that these events occur in the bacteria normally found in human or animal digestive tracts, and the probability of transfer of antibiotic resistance traits does not present a concern. However, in evaluation, the following should be taken into account:

a) **Fate of the antibiotic resistance gene DNA**

DNA, including the genetic material encoding for the antibiotic resistance trait, is not normally exposed to the environment outside of the plant's tissue. However, once the plant cell wall and membranes have been disrupted, DNA released from the plant tissue is primarily degraded by the plant's own nucleases. In addition, DNAses and enzymes found in the digestive environment of the gastrointestinal (GI) tract degrade any remaining intact DNA into small pieces. These pieces encode little of the original information for antibiotic resistance.

b) **Uptake by bacteria**

Under idealised laboratory conditions, DNA from GM plants has been shown to transform bacteria commonly found in soil at low frequencies (de Vries, 1998). However, as noted a) above, the genetic material encoding antibiotic selection markers are normally contained in the plant cells and are not exposed to the outside environment. In fact, soil has been shown to inhibit transformation efficiencies, and the occurrence of gene transfer from plants to soil micro-organisms is considered to be so low as to be irrelevant (Nielsen, 1997; Schluter, 1995). It is known that opportunistic pathogenic micro-organisms of soil origin with various resistances to antibiotics can emerge, even in the absence of exposure to plant tissue. However, the medical implications, both human and animal, of gene transfer of antibiotic resistance genes in soil has not been documented.

In vivo gene transfer to populations of bacteria normally found in the gut is also extremely low. Neither the small pieces of DNA produced by gastrointestinal digestion of plant tissue, nor any small amounts of larger-sized DNA that may have escaped digestion, have been shown to become incorporated into, or transform, the normal flora found in the gut (Syvanen, 1999).

c) **Establishment of stable foreign DNA fragments in a bacterial cell**

Stable integration of foreign DNA into the host genetic material occurs by means of recombination. The frequency of these events occurring in either soil or gastrointestinal environments (paragraph b) is exceptionally low. The stable establishment of foreign DNA fragments within a bacterial population is dependent upon a number of events, including the relative competitiveness of any transformed bacteria with naturally occurring bacteria. It is unlikely that any transferred trait would be stably maintained, or expressed, without selective pressure (e.g. the presence of antibiotic).

d) **Successful expression of the transferred antibiotic resistance gene**

For this to occur, the regulatory segments required for gene expression must be present in an appropriate arrangement and be recognised in the new host cell.

70. The sequential occurrence of these individually rare events needed to establish *in vivo* gene transfer is highly improbable (Droege et al., 1998). Nevertheless, *in principle*, introduced genes should be restricted to those genes required to confer the desired trait, while avoiding use of marker genes that may confer resistance to therapeutically relevant antibiotics (German Central Advisory Committee for Biological Safety, 1999).