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Topic 11: Gene Transfer: Mechanism and Food Safety Risks

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HORIZONTAL TRANSFER OF DNA FROM GM CROPS TO BACTERIA AND TO MAMMALIAN CELLS

Horizontal gene transfer is the movement of genetic information (DNA) between sexually unrelated organisms (different species). Concerns have been voiced regarding the possibility that DNA introduced into genetically modified (GM) crops could transfer into bacteria or cells in the animals that eat those crops, and whether there might be any risks associated with such a transfer. The purpose of this paper is to provide a framework for discussion of the scientific issues important for consideration of the potential horizontal transfer of DNA from the GM crops to bacteria or cells of the mammalian consumer. In a review of horizontal gene transfer, Dröge *et al.* (1998) concluded that circumstantial evidence (gene sequence analysis indicating evolutionary transfer) as well as direct experimental evidence indicates that the occurrence of DNA being transferred across species and propagated under natural conditions is rare at best. In this paper I will consider the evidence for direct transfer from plants to bacteria or mammals and the possible consequences were it to occur. I will first consider the steps that would be required for a gene to be transferred from food produced from GM crops to intestinal bacteria and the consequences thereof. As much of the concern in this regard is due to the feeding of GM maize to ruminants I will use this as an example. In addition, as much of the concern centers around the presence of antibiotic resistance genes in GM maize, I will refer to these genes in particular, using the *bla* gene, encoding resistance to the β-lactam ampicillin, as an example. This gene was used as a selectable marker in the generation of one of the early GM maize varieties. I will then consider other antibiotic resistance genes and their potential transfer to microorganisms in the environment. Finally I will look at the evidence for DNA uptake in the human oral cavity and the potential for transfer of bacterial DNA to mammalian cells.

Bacteria including those found in soil, in the rumen or in the intestine can receive DNA from other organisms through three mechanisms of transfer (Morrison, 1996; Davison, 1999). Only one mechanism, transformation, is relevant to the possible transfer of DNA from plants to bacteria and subsequent expression of the encoded protein product. The other two, conjugation (exchange of plasmid DNA between compatible bacteria) and transduction (viral transfer of DNA into bacteria) are specific to restricted forms of transfer and are not relevant to the potential transfer of DNA from plants. This evaluation will include assessment of the steps necessary for transfer of the DNA to occur as well as the potential consequences of the transfer if it did occur. The following issues will be presented. 1) A fragment of DNA or a plasmid containing the coding region of an antibiotic resistance gene must be available to bacteria. 2) The DNA released from the plant cells must not be highly degraded before possible transformation. 3) The DNA must integrate and stabilize in the bacteria either in a self-replicating plasmid, or in the bacterial chromosome. 4) If the *bla* gene transfers to ruminant microbes and is inserted in the correct context of a promoter to be expressed, what are the consequences? 5) If the *nptII* gene, encoding resistance to kanamycin/neomycin is transferred instead of *bla*, is there any risk? 6) Is there a risk of transferring DNA from plants to environmental microbes? 7) Is there a risk of transfer of plant DNA in the human digestive tract? 8) Is there any evidence indicating transfer of dietary DNA into mammalian cells and is there a risk if it occurs?

1. Excision of genetically modified DNA out of the maize chromosome

DNA introduced into plants to produce the GM crop is inserted into a plant chromosome and becomes an integral part of the plant genome. In order for the introduced DNA to be transferred from a plant it would have to be excised out of the chromosome. One way it could do this is by

"looping out" to reconstitute a plasmid carrying the antibiotic resistance gene. As maize is usually transformed by biolistics (DNA coated particle bombardment), the entire linearized parental plasmid may be integrated into the plant DNA and could therefore be excised and ligated to form a replicative plasmid. If the transferred DNA also included the antibiotic resistance gene, a potentially complete plasmid could be reconstituted in its entirety, and could direct the synthesis of the β-lactamase protein if successfully transformed into suitable bacteria. In the presence of the ampicillin, the transformed bacteria would have a strong selective advantage over susceptible bacteria. The looping out of DNA could occur by homologous recombination within the chromosome between adjacent copies of tandemly integrated DNA. Tandem integration events are known to occur during biolistic transformation of plant cells. The entire plasmid could be reconstituted, although genetic rearrangements or deletions are common in such events as was identified in yeast cells carrying an insert with multiple plasmid copies (Graupner and Wachernagel, 1996). The inserted DNA could also be excised by illegitimate recombination, which requires no homologous sequences. In that case plasmids with varying components, including the incorporation of plant DNA, could be formed. A more likely event is the generation of linear DNA fragments by enzymatic cleavage or by chemical or physical breaking of the phosphodiester bonds in the plant chromosome resulting in a random assortment of fragments representative of the whole genome

It should be noted that most of the GM maize products have been created by transformation with selected, purified fragments of the DNA which have specific plasmid sequences such as the origin of replication removed. If the bacterial origin of replication was not included, the excised DNA could not form a replicating plasmid and therefore would behave as any other fragment of the maize genome.

In organisms where intrachromosomal homologous recombination during mitosis has been studied, such as in *Nicotiana tabacum*, the estimated frequency of occurrence appears to be between one event in 10,000 cells to less than one event in 1,000,000 cells (Peterhans *et al*., 1990). Those studies examined the frequency of repair of partial deletion mutations in copies of tandemly inserted genes in culture under carefully controlled conditions. Studies in whole plants suggest less frequent events (Assaad and Signer, 1992; Swoboda *et al*., 1994) and have also demonstrated that the insertion site of the GM DNA in the plant chromosome can markedly affect the rate of recombination. If these rates were used to calculate the maximum possible rate of formation of plasmids excised from tandem copies in the plant genome, there could be no more than one in 10,000 maize cells. However recombination within the chromosome does not indicate successful reconstitution of the plasmid, and the frequency of formation of plasmids is probably much lower. Excision by illegitimate recombination should be even less frequent. While the formation of DNA circles by recombinational looping out of integrated single or multiple tandem copies of a pUC18-derived plasmid has been demonstrated in the yeast, *Hansenula polymorpha* (Graupner and Wackernagel, 1996), a careful literature search failed to identify any studies demonstrating a similar phenomenon from maize DNA. In addition a study attempting to detect transfer of pUC19 containing the *bla* gene along with the bacterial origin of replication (*ori*) from a transgenic potato to *Erwinia chrysanthemi* also failed to demonstrate any transfer of the DNA into the plant pathogenic bacteria (Schulter *et al*., 1995).

2. Fate of DNA released from ingested maize kernels during digestion

DNA released from maize kernels, in the living plant or during feed preparation, would be exposed to plant nucleases. Once ingested the DNA would be exposed to nucleases from various

animal tissues including the salivary glands, the pancreas and the intestine, and also to nucleases from resident microbes in the rumen and the intestine. These enzymes cleave most of the DNA into small linear fragments, or even nucleotides (Beever and Kemp, 2000). The smallest fragment from pUC18 that could contain the *bla* genes is ca. 900 bp, while the fragment necessary to include the *ori* and *bla* is about 1.6 kb. It is a commonly understood that nucleases in maize cells are sufficiently active to make it necessary to use strong protein denaturing agents to eliminate nuclease activity in the laboratory to obtain high molecular weight DNA from plant cells (Ausubel, 1992). If the denaturants, which are not present in the rumen, are not used, the DNA is degraded to fragments of less than 500 bp within an hour.

If plasmid DNA was produced by recombination in the plant cells, and then released upon ingestion, it would be subjected to the same physical, chemical and nuclease activities experienced by the chromosomal DNA. Plasmids nicked or partially digested are not likely to be reform if transformed into bacteria and would therefore behave as any other randomly cleaved segment of DNA.

One laboratory has reported studies of DNA survival in the animal intestine in which M13 phage or plasmid DNA, fed to mice was detectable in feces for up to seven hours after feeding. However, only a small fraction of the DNA was in the 1.7-kb size range; most of the recovered DNA was in the <400-bp range. No DNA was detected in intestinal bacteria and the authors noted that this "… should mitigate any concerns that one might have had in the past about the biological consequences of experiments carried out with recombinant DNA…" (Schubbert *et al*., 1994). However, it was later shown that fragments of M13 or plasmid DNA could be found in isolated cells of some mouse tissues (Schubbert *et al.*, 1997). Most or all of the cells identified by Schubbert *et al*. as containing the microbial DNA appeared to be macrophages, or other differentiated phagocytes of the immune system, which are noted for engulfing DNA, proteins and microbes. Most of the "foreign" DNA in these cells will be quickly degraded by nucleases in the endocytic compartments, though there are reports that unmethylated bacterial CpG DNA fragments, as are contained in the DNA used in those experiments, can stimulate phagocytic activity, but inhibit various processing activities within some phagocytes (Chu *et al.*, 1999) and might thereby prolonging the detectable life of the fragment.

3. Uptake of released DNA by bacteria in the rumen

Some bacteria can take up DNA from the environment by natural transformation. The DNA is bound to the cell surface, nicked and one strand is degraded. Accordingly, the remaining single strand of DNA enters the bacterium. So far, despite numerous attempts, there appears to be only one report (Orpin, *et al.*, 1986) of naturally transformable bacteria in a species of ruminant bacteria, most of which are obligate anaerobes (Salyers, 1998). While a number of bacteria can be transformed by laboratory procedure, facultative anaerobes such as *Escherichia coli* and its relatives, as well as Gram-positive enterococci, are considered to be incapable of natural transformation. However, there has been one report of the uptake of DNA by an isolate of *E. coli* obtained from water (Baur *et al.*, 1996). A variety of other bacteria have been demonstrated to have the capacity to be transformed, at various levels of efficiency, as reviewed by Lorenz and Wachernagel (1994).

In the unlikely event that a plasmid were released from a maize cell as described above, it could only replicate in *E. coli* and some of its close relatives which can utilize the specific *oriV* present on pUC18 to initiate replication. Such a plasmid would not replicate in ruminal bacteria even if

it were to be taken up by them. There are suggestions that transformation in some bacteria requires that at least two copies of a plasmid, or at least fragments of the second copy, must be introduced simultaneously into the same bacterial cell so that overlapping segments are available to regenerate the plasmid in a natural transformation (Salyers, 1998). Given the rare occurrence of the potential release of a plasmid from maize, that is a highly unlikely event. Assuming that transformation did occur, it would only be propagated if there were strong selective pressure to provide an advantage to the recipient bacteria. Bacteria carrying such plasmids would experience a high-energy toll, as pUC18 is a high copy number plasmid. Only strong selection would keep the plasmid in the rumen bacteria growing in such a competitive environment. Although β-lactam antibiotics are used to treat some infections in farm animals, they are no longer permitted to be used as feed additives in Europe.

The insertion of a linear fragment of DNA into either a chromosome or a plasmid *in vivo* will only occur during induced repair/recombination such as that induced by high levels of ultraviolet light, or gamma irradiation, or by recombination during replication of the cell. While recombination is common during meiosis, in most organisms it does not happen as frequently.

It should also be born in mind that DNA uptake in most bacteria is not sequence specific and fragments including the *bla* gene would be competing for transfer into a bacterium with the rest of the DNA in the plant genome and DNA from other sources in the diet. The maize genome is about one million times larger than pUC18 and thus only a millionth of the DNA released from transgenic maize would be plasmid DNA. An additional factor that may be critical in determining which bacterial species can accept DNA from various sources is the production of restriction enzymes that recognize combinations of nucleotide sequence and methylation patterns of DNA as targets. DNA containing the “foreign” recognition pattern will be cut and degraded (Raleigh, 1987). It is therefore likely that many bacterial species would degrade the plant or pUC18 DNA before it could be integrated.

Because of the relatively low rate of intrachromosomal recombination and the low probability of reconstituting a replication competent plasmid, it would be more likely that the pUC18 *bla* DNA might be taken up by bacterial cells as a linear fragment of the plant chromosomes rather than as a plasmid. The linear fragments could be incorporated into the bacterial genome by illegitimate recombination, which occurs at even lower frequencies than homologous recombination and which is as likely to integrate a fragment of a gene as an intact gene. The only exception would be if, by chance the bacterium taking up the pUC18 *bla* DNA already had a copy of the plasmid or marker gene or one of its relatives to provide a homologous sequence for recombination. That would be similar to an event described in section 6 (Gebhard and Smalla, 1998).

4. Consequences of the acquisition of ampicillin resistance genes by ruminant bacteria

If rumen bacteria were transformed would the *bla* gene be expressed in ruminant bacteria? Expression is only likely to occur in *E. coli* and close relatives, but not in any rumen anaerobes so far tested because of significant differences in promoter sequences (Salyers, 1998). In any other bacterium, in order to be expressed, it would have to acquire a new promoter by the insertion, in the proper context, of an existing bacterial promoter. Because there is no direct evidence of transfer of any plant genes into bacteria under natural conditions in a way that would allow expression of the gene product, the arguments for and against that possibility are only theoretical. The possibility of such a transfer occurring within the digestive tract of an animal

is even more difficult to prove. However, scientists at the University of Leeds are attempting to do just that and recently presented their preliminary results (Coughlan, 2000). To date they have not been able to isolate gut bacteria that have incorporated or expressed the *bla* gene after chickens had been fed GM maize for five days. They have also added pUC18 to silage effluent, saliva and rumen fluid taken from sheep but found no uptake by bacteria in the normal flora of the rumen, saliva or silage. The scientists also plan to test the fate of the *bla* gene when the maize is fed to sheep.

Although the risk of rumen bacteria acquiring and expressing the ampicillin resistance *bla* gene from transgenic maize is extremely low, it is not zero. We must therefore consider the consequences of such acquisition. In particular, how will such bacteria add to the already high incidence of ampicillin resistant bacteria in natural isolates? Estimates that over 70% of *E. coli* isolated from diseased calves and nearly 20% from diseased adult cattle are ampicillin resistant and that over 60% of *Salmonella typhimurium* from adult cattle are similarly resistant have been published (Martel *et al*., 1995; Salyers, 1998). However, this is not universally seen and wide ranges of the frequency of resistance have been reported, down to as little as 1% (Courvalin, 1998). In groundwater in rural Tennessee 69% of isolates, mostly enterics, were ampicillin resistant (McKeon *et al*., 1995) and almost all children studied in Mexico carry ampicillin resistant *E. coli* strains (Calva *et al*., 1996). Although levels of resistance do vary, ampicillin resistance is widespread among *E. coli* and enteric bacteria. Thus it seems more rational to be concerned about the overuse of antibiotics, both on the farm and in human medicine, than to worry about the unproven and probably infinitesimally small risk that new copies of the *bla* gene could enter bacteria from transgenic maize feed.

It should also be noted that the *bla* gene on pUC18 encodes one of the early forms of β-lactamase and since then they have undergone extensive evolution to achieve more potent and broad spectrum antibiotic resistance forms (Medeiros, 1997). It is true that many of these new genes have been derived by mutation of the original *bla* gene which was cloned to create the parental cloning plasmid from which pUC18 was derived. While similar mutations could occur in a ruminant organism which may acquire the *bla* gene from GM corn, such evolution would take a decade or two with intensive selection (Salyers, 1998).

5. Transfer of other antibiotic resistance genes

I would now like to consider concerns over the acquisition of genes other than ampicillin resistance by ruminant bacteria. One example is the *nptII* gene encoding resistance to kanamycin/neomycin. This gene is the most common antibiotic marker gene used in GM plants for selection of resistant (transformed) plant cells. In most cases the *nptII* gene contains a plant, not bacterial, promoter and would therefore have to be inserted next to the appropriate bacterial promoter in order to be expressed in bacteria. Kanamycin, and its close relative, neomycin, are infrequently used antibiotics, neither is unique for any use and they are rarely administered orally. Thus selective pressure would be minimal for development of resistant bacteria. They are also not used widely in agriculture. In addition, bacteria with kanamycin resistance genes are common in the environment, including *nptII* from Tn5 as well as other genes (Leff, *et al.*, 1993; Smalla, *et al.*, 1993). The safety of using *nptII* in transgenic plants was reviewed by Nap *et al*. (1992). In a 1993 workshop of the WHO, it was concluded that the use of the *aad* (spectinomycin/streptomycin resistance) and *nptII* marker genes in GM plants present no health risks to people. The European Commission's Scientific Committee for Food and the European Scientific Committee on Animal Nutrition (1998) determined that the risk of gene (*aad* and *nptII*)

transfer is negligible, and if it were to occur, there would be no significant health impact. Malik and Saroha (1999) provide a comprehensive review of the use of a variety of markers for GM plants, their potential for transfer and the abundance of resistance genes endemic in the environment. Less commonly used antibiotic resistance marker genes include those providing resistance to hygromycin, spectinomycin, tetracycline and chloramphenicol. Those genes used as markers for the GM plant varieties are also common in environmental isolates of bacteria and therefore the risk of transfer from plants containing those genes is minute. Most of the antibiotic targets of various marker genes are rarely used, or not by oral dose.

Some discussion of relative risks for a variety of practices is worth considering. The risk of corrupting the usefulness of pharmaceutical antibiotics by having the currently used marker genes in GM crops is considerably below the risk posed by using clinically important antibiotics widely as prophylactic agents for humans or to enhance the growth of farm animals. Some countries have allowed the use of such as the critically important antibiotics as avoparcin, a vancomycin analog, as a prophylactic agent. Vancomycin is one of the newest and best antibiotics for a number of systemic infections. Resistance to vancomycin has now been reported in *Enterococcus faecium* from hospital, farm animal and animal feed sources in the US and EU (Wood, 1999; Davies and Roberts, 1999; Schwalbe *et al.,* 1999). Widespread use of clinically important antibiotics provides strong selection pressure for dissemination of bacteria with resistance to the more important antibiotics. While the effect of using antibiotics in animal feed have been debated, there is growing evidence that it can lead to increased incidence of clinically important resistant bacteria (Wegener *et al*., 1999).

6. Transfer of antibiotic resistant genes to microorganisms in the environment

What about the transfer of antibiotic resistance genes to microorganisms in the environment? Although some soil microbes might be naturally competent for transformation and DNA attached to plant or soil material might be more resistant to nucleases, such DNA might not be readily available for uptake by bacteria. However, Sikorski *et al*., (1998) have shown that when non-sterile soil was loaded with *Pseudomonas stutzeri* together with its plasmid or chromosomal DNA, transformants with both DNAs were obtained. Although this was extremely time-dependent, presumably due to the presence of soil nucleases, addition of cells three days after DNA loading still yielded 3% of the initial number of transformants. Nielsen *et al*. (1997) determined that soil type, nutrient conditions, the presence or absence of other soil microbes had significant effects on the transformation efficiency of *Acinetobacter calcoaceticus* in soil microcosms.

Although no one has been able to show that soil bacteria take up expressible antibiotic resistance genes when exposed to transgenic plant material under natural conditions, Gebhard and Smalla (1998) were able to detect horizontal gene transfer under laboratory conditions using *Acinetobacter calcoaceticus* BD413 which had been transformed with a deletion mutant of the Tn5 *nptII* gene. This species has an inherently high transformation frequency. They used a high frequency recombinational repair assay between the bacterial *nptII* deletion mutant gene, and an intact *nptII* gene in transgenic potato DNA. Transformation of the bacteria with 5 μg of naked plant DNA yielded 100 transformants with neomycin resistance. A further experiment with purified DNA including the *nptII* gene from GM sugar beets and competent *Acinetobacter calcoaceticus* BD413 (carrying the deletion mutant of *nptII* as a plasmid, rather than as a chromosomal insert) was performed in a soil microcosm. Kanamycin resistant bacteria were recovered from the experiments performed with sterile soil, but not when the soil was not pre-

sterilized, demonstrating the small likelihood of such an event occurring under natural conditions (Nielsen *et al.*, 2000). Additionally, transformants with *nptII* in the chromosome were quite stable compared to the near complete loss over 31 days of those bacteria carrying the *nptII* gene on the plasmid. Whether successful transformation of this type could occur in nature needs to be tested, as all studies on gene transfer from plants to soil microorganisms *in situ* have shown that if such events occur at all they do so with extremely low frequencies, often below the limit of detection (Dröge *et al.*, 1998). It is essential to remember that all of the experiments by Nielsen and Smalla which demonstrated successful transformation from plant DNA were mediated through homologous recombinational events which require identical sequences flanking the DNA target and insertion sites.

Finally it should be born in mind that there would be no selective pressure for genes such as *bla* and *nptII* in transformed soil bacteria. Therefore it is unlikely that the traits would be maintained. It should also be noted that resistance to antibiotics and herbicides are wide spread among soil microbes, because of selection pressure from naturally occurring antibiotic producing fungi and because of repeated application of herbicides.

7. Uptake of DNA in the human oral cavity and intestine

What about uptake of DNA from transgenic foods in the oral cavity? This is one of the most complex and heterogeneous microbial habitats in the human body. Additionally, certain oral bacteria are naturally competent. Recently, Mercer *et al*. (1999) have shown that very high concentrations of plasmid DNA (1 μg from 1 ml of 10^9 cells/ml), exposed to degradation for short periods of time by filter sterilized human saliva *in vitro*, were able to transform the naturally competent oral bacterium *Streptococcus gordonii*. The conditions of the experiment were heavily weighted in favor of transformation as high quantities of supercoiled plasmid were added to the system containing a large population of competent bacteria. In order to extrapolate the frequency of occurrence of transforming similar populations of oral bacterial with replicative plasmids formed from GM corn, the quantity of DNA in corn, and the rate of possible plasmid formation must be considered. A reasonable highest possible estimate for the quantity of such plasmids is likely to be a few hundred per corn plant. Therefore any attempt to transform the same bacterial population as was used by Mercer using corn DNA would probably fail to produce any recoverable colonies. In addition, attempts to transform a natural oral microfloral population with such a low concentration of plasmid in a massive quantity of typical maize plant DNA would not be expected to produce any positive transformants. Due to safety considerations *in vivo* tests equivalent to those performed by Mercer would not be performed with human subjects because of the possibility of transferring the erythromycin resistance gene used in their experiments to endogenous bacteria. That experiment would not be equivalent to using corn DNA containing either the *bla* gene, or *nptII*. Further, one key feature not replicated by their experiments is the effect of an endogenous bacterial population on the survival and transmission of the plasmid.

8. Transfer of dietary DNA to mammalian cells

Direct introduction of DNA from bacteria to mammalian cells has been reported in very few instances. Schaffner (1980) reported that plasmids carrying tandem copies of the SV40 virus genome could be transferred at a very low frequency from *E. coli* to mammalian cells *in vitro* by exposing them to a bacterial suspension. The viral sequence elements in those plasmids are adapted to mammalian cells and might therefore be expected to survive and possibly function. Some may argue that one should assume that DNA could get into the epithelial cells lining the

gut. However without selective pressure it is highly unlikely that genes taken up by these cells would be expressed even if integrated into the genome. In addition, the epithelial cells are short lived and would slough off after a few days to be replaced by untransformed cells. While a number of pathogenic strains of bacteria and viruses have the ability to transfer their DNA into host mammalian cells, the mechanisms required for such transfer are not available in GM plants. Evaluation of studies relating to such transfer is therefore uninformative for these considerations.

Two research groups have reported the intracellular uptake of orally or gastrically presented exogenous DNA by mammalian cells of the gastrointestinal tract. Schubbert *et al*. (1998) have reported a number of experiments where large doses of purified bacterial plasmid or M13 bacteriophage DNA were gavaged into the stomachs of mice and the DNA was detected in various cells and tissues at intervals after dosing. Most (all?) of the cells with detectable foreign DNA appear to be differentiated phagocytic cells (macrophages, dendritic cells, B cells, Kupffer cells) which naturally engulf and degrade macromolecules. By *in situ* hybridization with M13 or plasmid specific fluorescent probes a few individual cells were identified in fetal or neonatal mice following high dose exposure of the pregnant dams. In one case, there appeared to be a double hybridization on what appeared to be sister chromatids of a single cell, indicating a possible integration and duplication of the chromosome. However, in no case was there evidence that clones of cells containing the M13 or plasmid DNA were formed, or that germinal tissue had been transformed. The relative high frequency of cells that contained the foreign DNA is probably related to the occurrence of unmethylated CpG sequences in those DNA constructs which would stimulate macrophages and other immune cells to phagocytose the fragments (Beever and Kemp, 2000). Klotz and Einspanier (1998) tested bovine tissue samples for the presence of a fragment of an abundant soybean chloroplast gene by specific polymerase chain reaction (PCR). Collected white blood cells isolated from animals fed soybean concentrate did contain a detectable fragment of this gene, but a fragment of a single copy gene was not detected in the same samples. The demonstration of fragments of dietary DNA in phagocytic cells of mammals should be expected as a natural occurrence. These cells provide immune surveillance of the digestive tract and other tissues, and recirculate, frequently to the liver as a normal mechanism of removing debris. The rare appearance of bacterial DNA fragments in a few fetal or neonatal cells should likewise not be of concern as it indicates that a few macromolecules have crossed the placenta and been engulfed by phagocytes of the fetus. The apparent association and possible insertion of a small fragment of bacterial DNA in those terminally differentiated cells is probably not an unusual occurrence

9. Conclusions

The above evidence supports the conclusion that while horizontal gene transfer can and has occurred, such events are rare and need to be viewed in the context of evolutionary time. However, even very rare events may have an ecological impact if the transferred gene alters the fitness of the recipient bacteria or cell. Hence the genes encoded by the transferred DNA in the GM plant should be the focus of biosafety considerations rather than the transfer process itself. Careful analysis is needed to determine whether any proposed GM product presents a realistic potential hazard that needs to be evaluated in terms of a risk assessment. In the cases referred to in this paper, *bla* and other antibiotic resistance marker genes including *nptII*, the chances of increasing the ecological fitness of any bacteria acquiring the genes from transgenic plant material is remote. The incidence of natural populations of bacteria having homologous or alternative antibiotic resistance genes is already very high. The hypothetical risk that any bacteria could be transformed with these genes from plants and express them is extremely low and there

is no evidence that an equivalent event has occurred even under ideal laboratory conditions. Finally, there is no known risk associated with the remote possibility that mammalian cells could be transformed with these genes and express the proteins. Expression of these proteins by a few leukocytes, or epithelial cells, will not affect the performance of antibiotics used for killing pathogenic bacteria in an infection. Other genes that might be transferred from GM plants to bacteria or to mammalian cells are equally as unlikely to be transferred or expressed. The safety of the expressed proteins from those genes is carefully evaluated in the safety assessments conducted for registration of those crops. The introduced (GM) genes represent a few of the 20,000 to 80,000 genes contained in the crop plants. The probability of transferring any of the GM genes is no greater than of transferring any of the other genes in the plants. Based on the evidence, the possibility of transferring any gene from a plant to a bacterium or a mammalian cell is remote. In the final analysis, all genes proposed for addition to crops used for feed or food are assessed on a case-by-case basis. Key consideration in the registration process is a thorough assessment of the safety of the proteins which are coded by the genes. If the protein products of the genes are safe, the potential exposure that might result from the production of the protein by microbes in the unlikely event of horizontal transfer, should not pose a risk to consumers or the environment.

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