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GENETICALLY MODIFIED MAIZE LINE Bt176 APPROVED FOR MARKETING PURSUANT TO COUNCIL DIRECTIVE 90/220 EEC

RISK ASSESSMENT AND EVALUATION OF THE NOTIFICATION PURSUANT TO THE NORWEGIAN GENE TECHNOLOGY ACT

August 2000

Summary

Consent to market the genetically modified maize line Bt176 under Directive 90/220/EEC was given on 23 January 1997. The consent covers unrestricted use, including human food and animal feed. Based on the Norwegian Gene Technology Act and the EEA Agreement, marketing of the product was prohibited in Norway on 1 October 1997.

The maize line harbours the novel chimaeric genes, *bar*, *cryIA (b)* and *amp* (a *bla* gene) encoding herbicide tolerance, insect toxin and antibiotic resistance, respectively.

Antimicrobial agents are the foundation of modern infectious disease treatment. The fact that micro-organisms can develop resistance to antibiotics has created a situation where these drugs are losing their effectiveness because of the spread and persistence of resistant micro-organisms. The *amp* gene inserted in the genetically modified maize line encodes a broad-spectrum β-lactamase that inactivates several β-lactam antibiotics including broad-spectrum penicillins such as ampicillin, amoxicillin and carbenecillin. These are all very important for treating various bacterial infections in human and veterinary medicine. A transfer of the *amp* gene to pathogenic bacteria in such a way that the gene is successfully incorporated and expressed would therefore be highly undesirable because it might impede clinical treatment. Some results indicate that there is a risk for horizontal transfer of the *amp* gene. However, the mechanisms regulating putative horizontal gene transfer under natural conditions are inadequately described, and the information available does not allow the frequency of such events to be fully predicted. Further studies are therefore required to elucidate the potential for transfer of antibiotic resistance genes from plants to micro-organisms. Although we do not yet know whether horizontal gene transfer will significantly increase the number of antibiotic resistant pathogenic bacteria, there is no reason to accept this risk.

There are indications that the expression of insect-toxic genes from *Bacillus thuringiensis* in genetically modified plants can harm non-target insects such as the monarch butterfly. In addition, the selection for target insects that are resistant to Bt toxins may be enhanced by the introduction of plants producing Bt toxins.

Marketing of the genetically modified maize line has not been shown to benefit the community. Due to the risks and the lack of scientific knowledge on possible effects for health and the environment, the deliberate release of this line in Norway would conflict with the precautionary principle and the requirement for contribution to sustainable development.

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1. Introduction

Consent to market of the genetically modified maize (*Zea mays* L.) line Bt176 under Directive 90/220/EEC (Notification C/F/94/11-03) was given by Commission Decision 97/98/EC of 23 January 1997. The consent covers unrestricted use, including human food and animal feed.

Marketing of the product in Norway was prohibited on 1 October 1997. The Norwegian decision was made pursuant to the adaptation to Directive 90/220 EEC included in the EEA Agreement Enclosure XX no. 25, and the Act relating to the production and use of genetically modified organisms of 2 April 1993 (the Gene Technology Act).

The purpose of the Norwegian Gene Technology Act is to ensure that the production and use of genetically modified organisms (GMOs) takes place in an ethically and socially justifiable way, in accordance with the principle of sustainable development and without detrimental effects on health and the environment. When deciding whether or not to grant an application for the deliberate release of a GMO, significant emphasis must be placed on whether it represents a benefit to the community and a positive contribution to sustainable development.

The precautionary principle is important when dealing with applications for the release of genetically modified organisms into the environment under the terms of the Norwegian Gene Technology Act. In the preparatory documents to the Act (Proposition No. 8 to the Odelsting 1992-93), it is stated that: “The precautionary principle is especially important in cases of release into the environment. A reasonable degree of doubt about the risk of adverse effects will in principle be grounds for refusing the application.”

The present document contains an assessment of the risks to health and the environment related to the marketing of the product, and also takes into consideration the principle of sustainable development and social and ethical aspects in connection with the marketing. In some cases, the risk assessment refers to papers published after the Norwegian prohibition against marketing. Most of these results do not introduce new arguments, but bring more insight into the aspects of the application that led to the Norwegian decision. The assessment of potential effects of the Bt toxin on non-target insects is, however, for the most part based on papers published after the Norwegian decision.

The Directorate for Nature Management has prepared the document on the basis of contributions from experts in the areas that are covered. The Norwegian Agriculture Inspection Service and the Norwegian Food Control Authority have been consulted during the preparation of the document.

2. Assessment of the risks for health and the environment

This notification describes a genetically engineered *Zea mays* harbouring the novel chimaeric genes, *bar*, *cryIA (b)* and *amp* (a *bla* gene) encoding herbicide tolerance, insect toxin and antibiotic resistance, respectively.

The novel genetic sequences inserted into the *Z. mays* consist of the plasmid *pCIB3064* (harbouring the cauliflower mosaic virus (*CaMV) 35S* promoter, the *bar* gene, a *CaMV 35S* terminator, and in addition, vector sequences from the plasmid *pUC18* including an *amp* gene and an *oriV*) and the plasmid *pCIB4431* (harbouring two synthetic *cryIA (b)*, both with a *PEP-C* intron 9 and a *CaMV 35S* terminator). The genes are under the control of either a maize-derived *PEP-C* promoter or a maize-derived pollen specific (pollen:*pro*) promoter. The inserted chimaeric genes and vector sequences in *Z. mays* thus consist of DNA from three different species of bacteria and a virus.

The Norwegian authorities are primarily concerned with the risks associated with a possible horizontal spread of the *amp* gene for antibiotic resistance contained in the product, and with ecological effects of the insect toxin encoded by the *cryIA (b)* genes.

2.1 Horizontal transfer of genes encoding antibiotic resistance

The *amp* gene encodes a broad-spectrum β-lactamase that inactivates several β-lactam antibiotics including broad-spectrum penicillins such as ampicillin, amoxicillin and carbenecillin. These are all very important for the treatment of various bacterial infections in human and veterinary medicine (Kruse, 1997). The insertion of the *amp* gene therefore entails potential health and environmental impacts if the gene is transferred to bacteria. A transfer of the *amp* gene to pathogenic bacteria in such a way that the gene is successfully incorporated and expressed would be highly undesirable because it might impede clinical treatment. In order for a gene transfer to take place plant DNA has to survive passage through the gastrointestinal tract, including exposure to gastric acids and nucleases (FAO/WHO Biotechnology and Food Safety, 1996). Furthermore, the recipient micro-organisms would have to be capable of transforming, binding and taking up DNA, and finally integrating it into the host genome. For significant recombination, the integration requires at least 20 bp in a complete homologous DNA sequence at both ends of the foreign DNA. The likelihood that the foreign DNA would persist in an organism is significantly enhanced if the micro-organism is under selection pressure. To be expressed, the antibiotic resistance gene must be under the control of an appropriate bacterial promoter.

Very few studies have been conducted in soil environments on the occurrence of enzymatic mediated antibiotic resistance, the type integrated in *Z. mays* (β-lactamase) (van Elsas and Pereira, 1986; Henschke and Schmidt, 1990; Smalla *et al.,* 1993; van Elsas and Smalla, 1995). For instance, Smalla *et al.* (1993) frequently found kanamycin resistance in bacteria isolated from agricultural soils without detecting the *nptII* gene. Thus, the health and environmental impacts of a potential transfer of the *amp* gene from genetically modified *Z. mays* to soil bacteria cannot be satisfactorily assessed from the scientific studies available to date.

The detection of naturally occurring soil bacteria which are resistant to antibiotics (Schmidt *et al.*, 1990) has been used as an argument for the low impact of possible horizontal gene transfer of antibiotic resistance genes inserted in plants, in particular for the *amp* gene (Nap *et*

al., 1992). However, differences in the mechanism of antibiotic resistance in bacteria have not been assessed in these claims; for instance, Calgene´s calculations (see Redenbaugh *et al.*, 1994) were based on phenotypic estimates. Since several mechanisms mediate resistance to antibiotics (Trieu-Cuot *et al.*, 1987; Davies, 1996), the observations of phenotypic resistance (i.e. non-enzymatic) cannot predict the consequences of the large-scale introductions of antibiotic resistance traits encoded by single genes (enzymatic).

The selection of bacterial transformants receiving antibiotic resistance genes from genetically modified *Z. mays* is uncertain as the selective forces acting upon antibiotic resistance genes in soil need to be described. Antibiotics have rarely been isolated from soil (Thomashow *et al.*, 1990). The addition of antibiotics to soil to generate a selective advantage for bacteria harbouring corresponding antibiotic resistance genes has only been modestly successful (Oliveira *et al.*, 1995). Hence, the ecology of antibiotic genes in their natural habitats remains to be described. Only an accurate perception of the selective advantage expressed by the *amp* gene will allow its possible consequences following field release to be predicted. Unfortunately, such knowledge to support the release of *Z. mays* harbouring the *amp* gene is still not available.

Knowledge of selection as a process applied to bacterial populations needs to be improved (Mayr, 1997). It should be noted that fewer than 10% of the micro-organisms present in soil are accessible to cultivation techniques and a much smaller proportion still have been characterised at the cellular level (Hugenholz and Pace, 1996; Pace, 1997). Lack of knowledge concerning the selection and population-genetic structure of bacteria in the natural environment calls for a cautious introduction of genes encoding antibiotic resistance into natural communities.

Limited and insufficient scientific knowledge exists on potential horizontal gene transfer of antibiotic resistance markers from genetically modified plants to bacteria. To our knowledge, only three field and three laboratory studies have been published (Becker *et al.*, 1994; Smalla *et al.*, 1994; Smalla, 1995; Schlüter *et al.*, 1995; Broer *et al.*, 1996; Gebhard and Smalla, 1998, 1999). These few investigations of various scientific qualities are insufficient to support the safe release into the environment of genetically modified plants harbouring antibiotic resistance genes. The main concern regarding the use of antibiotic resistance genes as marker genes in genetically modified organisms is that horizontal gene transfer of the marker genes to pathogenic bacteria may compromise the effect of antibiotic treatment. New research has shown that gene sequences are more mobile and species barriers less distinctive than previously thought. Results reported by Schubbert *et al.* (1994) indicate that unprotected DNA which is ingested may withstand digestion in the gastrointestinal tract, although it may be considerably fragmented. When DNA was orally transmitted to mice, fragments up to 1700 bp (i.e. 23% of the total length applied) were recovered from faeces and 2-4% of the DNA were detected in the gastrointestinal tract. The enormous numbers of bacteria in the intestine give rise to recipient bacteria. The use of antibiotic resistance genes as marker genes in genetically modified plants exposes these recipients to antibiotic resistance genes in the gastrointestinal tract.

The majority of field trials of genetically modified plants have been oriented towards demonstrating the efficiency and functionality of the plants for agronomy (Levin and Israeli, 1996), and have not added to our knowledge of possible incidences of horizontal gene transfer in the environment.

Several indications of gene transfer from plants (or eukaryotes) to bacteria have been reported after sequences of DNA from plants and bacteria have been compared (Wakabayashi *et al.*, 1986; Froman *et al.*, 1989; Doolittle *et al.*, 1990; Smith *et al.*, 1992; Lamour *et al.*, 1994), which implies that there is a potential for transfer of functional genes from plants to bacterial recipients. However, the fragmentary DNA sequence information available today makes it difficult to interpret DNA sequence data to assess the likelihood of gene transfer from plants to bacteria. The large-scale introduction of the *amp* gene through field grown *Z. mays* will increase the exposure of the genes to microbial recipients, thereby probably enhancing the likelihood of dissemination.

It has been claimed that genetic material is not transferred from plants to organisms other than plants (OECD, 1992; Liberman *et al.*, 1996). However, horizontal gene transfer from plants to unrelated organisms such as plant-associated fungi has been reported. The uptake of host plant DNA has been claimed for the fungus *Plasmodiophora brassicae* (Bryngelsson *et al.*, 1988; Buhariwalla and Mithen, 1995) and of the plant-harboured hygromycin gene (*hph*) for the fungus *Aspergillus niger* (Hoffmann *et al.*, 1994). Recently, transformation of *Acinetobacter* sp. BD413 with DNA from various transgenic plants carrying the *npt-II* gene was demonstrated, with detection of bacterial transformants based on recombinational repair of a deleted *npt-II* gene in the *Acinetobacter* sp. strain (Gebhard and Smalla, 1998; De Vries and Wackernagel, 1998). Studies of the field release of genetically modified sugar beet (*Beta vulgaris*) and horizontal gene transfer of plant DNA to bacteria indicate that horizontal gene transfer occurs to members of the bacterial community (Gebhard and Smalla, 1999). These examples indicate the possible transfer of DNA from *Z. mays* to other organisms. The selective advantage of the engineered genes in *Z. mays*, in particular the *amp* gene, should therefore be further elucidated before field releases can be justified.

Few experimental or descriptive data exist today on the occurrence in soil environments of gene transfer processes such as natural transformation, the most likely mechanism for gene transfer from plants to bacteria (Graham and Istock, 1978; Stotzky, 1989; Lee and Stotzky, 1990; Nielsen *et al.*, 1997; Paget and Simonet, 1997). Moreover, the few studies reported have been limited to soil microcosms (Trevors, 1988), only a few model bacteria being analysed. How far these data are relevant for predicting effects on natural environments is difficult to assess. Natural transformation requires access to free DNA. Studies have shown that chromosomal DNA from different types of organisms can survive in the environment for thousands of years (Pääbo *et al.*, 1988). Plasmids are also able to survive longer than previously assumed, just how long being greatly dependent on the environmental conditions (Recorbet *et al.*, 1993). Evidence has been presented (Lorenz and Wackernagel, 1987; Ogram *et al.*, 1988, 1994; Romanowski *et al.*, 1991; Paget *et al.*, 1992) indicating that introduced DNA can be stabilised by binding to mineral surfaces in the soil. Both bacterial and plant DNA have been shown to persist in soil for weeks or months (Recorbet *et al.*, 1993; Romanowski *et al.*, 1993; Paget and Simonet, 1994; Gebhard and Smalla, 1998). This is also expected to be the case for DNA of *Z. mays*. DNA bound to clay has been shown to retain an ability to transform bacteria (Stewart *et al.*, 1991; Khanna and Stotzky, 1992; Paget *et al.*, 1992; Gallori *et al.*, 1994), thus demonstrating the biological activity and availability of DNA released into soil. DNA released from genetically modified *Z. mays* will probably not differ substantially from bacterial DNA and will therefore contribute to the transient reservoir of genetic elements available to bacteria in the soil.

The uptake, by natural transformation, of free DNA exposed to recipient bacteria in their environment is controlled during the regulation of bacterial competence (Stewart and Carlson,

1986; Stewart, 1989). Several bacteria present in natural habitats have been shown to be transformable (Lorenz and Wackernagel, 1994; Paget and Simonet, 1994). Surprisingly, *Escherichia coli* was recently also found to be naturally transformable (Baur *et al.*, 1996). Uptake of DNA can be non-specific with regard to its sequence in some bacteria (Stewart, 1989; Dubnau, 1991; Lorenz *et al.*, 1992; Lorenz and Wackernagel, 1994). Thus, DNA from *Z. mays* is assumed to be taken up in the cytoplasm of these bacteria. This is supported by studies which indicate that the successful uptake of genetically modified plant DNA in competent bacteria is detected when sequence homology to the plant DNA is present in the bacteria (Frostegaard *et al.*, 1996; Gebhard and Smalla, 1998; K. Smalla, pers. comm.).

Restriction enzymes attack double-stranded DNA (Cerritelli *et al.*, 1989), whereas most competent bacteria are believed to take up single-stranded DNA by transformation (Solomon and Grossman, 1996). Gene transfer by natural transformation therefore seems not to be strongly affected by restriction enzymes (Lorenz *et al.*, 1992, Lorenz and Wackernagel 1994; Vulic *et al.*, 1997). It has also been proposed that the presence of large amounts of DNA can overcome the restriction system (Bickle and Krüger, 1993) in bacteria (Wilkins, 1995). Even if restriction enzymes are highly active, DNA fragments of approximately 250 bp are expected to be present after digestion with restriction enzymes that recognise specific 4-base pair sequences (Maynard Smith, 1990), and larger fragments, possibly encoding functional traits, are expected to be present after digestion by restriction enzymes that recognise 6-base pair sequences.

It is believed that the difference in the nucleotide sequence is the main barrier to the stable introduction of heterologous DNA in bacteria (Baron *et al.*, 1968; Kondorosi *et al.*, 1980; Rayssiguier *et al.*, 1989; Lorenz and Wackernagel, 1994; Matic *et al.*, 1995, 1996; Vulic *et al.*, 1997). Recombination between diverged DNA can, however, be obtained with at least a 24% difference in sequence homology (Dowson *et al.*, 1989, 1990; Maynard Smith, 1990; Maynard Smith *et al.*, 1991; Spratt *et al.*, 1989, 1992). Vulic *et al.* (1997) observed decreasing recombination frequencies with increasing sequence divergence in enterobacteria. Similar observations have been reported for *Bacillus* spp. (Zawadzki *et al.*, 1995). However, bacteria with mutations in their genes involved in DNA repair generate less stringent homology requirements for recombination to exist (Rayssiguier *et al.*, 1989; Harris *et al.*, 1994; Hanada *et al.*, 1997), and it has been proposed that an adjustable species barrier may be present in DNA repair-deficient strains (Young, 1992). Interestingly, Vulic *et al.* (1997) demonstrated directly that an increased frequency of recombination was obtained with diverged DNA in bacteria with mutations in their mismatch system. Unexpectedly, it has recently been reported that up to 1% of natural isolates of *E. coli* and *Salmonella* sp. display such mutations (LeClerc *et al.*, 1996).

The regulation of the stringency for recombination in bacteria with diverged DNA, such as genetically engineered DNA from *Z. mays*, is uncertain. There are indications of less need for homology for recombination in bacteria grown under adverse conditions (Taddei *et al.*, 1995; Feng *et al.*, 1996). This could mean that the low frequencies of recombination in bacteria seen with diverged DNA in laboratory studies may not reflect the situation present in natural habitats of the bacteria. The signals and mechanisms regulating horizontal gene transfer under natural conditions may remain undetected under laboratory conditions, thus generating false estimates of how frequently transfer takes place (van Elsas *et al.*, 1988; Schäfer *et al.*, 1993; Mel and Mekalanos, 1996; Lilley and Bailey, 1997; Troxler *et al.*, 1997).

An increasing amount of evidence indicates that gene transfer between species is a significant mechanism for their evolution (Halter *et al.*, 1989; Coffey *et al.*, 1993; Lenski, 1993; Maynard Smith *et al.*, 1993; Zhou *et al.*, 1993; Bowler *et al.*, 1994; Guttman and Dykhuizen, 1994; Nelson and Selander, 1994; Kapur *et al.*, 1995; Feil *et al.*, 1996; Zhou *et al.*, 1997).

DNA with homology to bacterial DNA has been introduced as a result of the genetic engineering of *Z. mays*. An increased possibility for stabilisation of the genetically modified plant DNA (including the *amp* gene conferring antibiotic resistance) in bacterial recipients is therefore to be expected.

Homology of the inserted antibiotic resistance gene in the plant to relate antibiotic resistance genes in soil bacteria cannot be excluded either. The minimum length of homology of DNA required in *E. coli* for homologous recombination is approximately 20 base pairs (Shen and Huang, 1986), and short regions of homology can mediate recombination which includes incorporation of adjacent non-homologous sequences (Harris-Warrick and Lederberg, 1978; Duncan *et al.*, 1978; Stuy and Walter, 1981; Ikeda *et al.*, 1982; Marvo *et al.*, 1983). Thus, the chimaeric constructs present in *Z. mays* containing homology to bacterial sequences may facilitate the transfer of their adjacent genes to bacteria.

Short repetitive sequences are commonly found dispersed in bacterial genomes (Mazel *et al.*, 1990; Bruijn, 1992; Lupski and Weinstock, 1992). If they are present in the engineered plant DNA and are recombined to homologous sequences in recipient bacteria, these sequences may mediate the transfer of adjacent non-homologous plant genes to bacteria. The notification does not provide documentation of the absence of such sequences in the prokaryotic DNA from four bacterial species utilised to construct the genetically engineered *Z. mays*.

The genetic mechanisms of homologous recombination in *E. coli* are still not completely elucidated (Kowalczykowski *et al.*, 1994; Vulic *et al.*, 1997), and illegitimate recombination (not requiring sequence homology) events are known to occur in various organisms (Ehrlich *et al.*, 1993; Mayerhofer *et al.*, 1991; Phillips and Morgan, 1994). For instance, Brakstad and Mæland (1997) have proposed that antibiotic resistance (methicilin) in staphylococci emerges after horizontal gene transfer and illegitimate recombination. The production of all genetically modified plants is currently based on illegitimate recombination events with random sites of insertion of the genes into the plant genome. Hence, the general success of artificial gene transfer over a broad range of species indicates the feasibility for natural horizontal gene transfer in the environment. The field release of *Z. mays* will generate large numbers of antibiotic resistance genes in the environment and may thereby enhance their likelihood of amplification after putative illegitimate recombination.

Small changes in a DNA sequence can change the host spectrum of a transferable genetic element. This is important when recombinant shuttle vectors, commonly employed tools in recombinant work, are used. They are able to be reproduced in prokaryotes and eukaryotes and to spread across normal taxonomic boundaries. The genetically modified *Z. mays* has integrated several bacterial plasmids that have originated by vegetative replication from pUC 18 (*ori*V). Insertion of the *ori*V into the *Z. mays* genome may facilitate the stabilisation of recircularised DNA fragments of *Z. mays* taken up in bacterial recipients by plasmid rescue (Andre *et al.*, 1986; Koncz *et al.*, 1990). Thus, the engineered *Z. mays* DNA has increased the chance of dissemination taking place into bacterial communities. Furthermore, there is a greater likelihood that the *amp* gene inserted into the *Z. mays*, if it is transferred to bacteria, will be expressed since it has a bacterial promoter and does not contain introns. The

cauliflower mosaic virus 35S promoter, present in the *Z. mays*, has also been shown to be active in bacteria (Assad and Signer, 1990). Thus, in addition to an increased likelihood for stabilisation if transferred to bacteria, the genes inserted into the *Z. mays* are also more likely to be expressed in the bacteria.

In the context of the ample evidence of horizontal gene transfer in bacteria (see references above), the increased likelihood of stabilisation and expression of the antibiotic resistance genes in bacteria raises questions regarding the biosafety of the genetically engineered *Z. mays*. Virtually no descriptions of the mechanisms regulating horizontal gene transfer under natural conditions exist today, and the frequency of such events is therefore difficult to assess.

The insertion of the *amp* gene into *Z. mays* raises concern over the potential health impact of this gene, particularly the possible transfer of this trait to bacteria of medical importance (Kok *et al.*, 1994; Kruse, 1996). Horizontal gene transfers, in which bacteria are involved, are known to occur frequently (Buchanan-Wollaston *et al.*, 1987; Heinemann and Sprague, 1989; Stachel and Zambryski, 1989; Davies, 1990, 1994, 1996; Fry and Day, 1990; Stewart and Sinigalliano, 1990; Heinemann, 1991; Mazodier and Davies, 1991; Sprague, 1991; Veal *et al.*, 1992; Wellington and van Elsas, 1992; Heitman and Lopes-Pila, 1993; Salyers *et al.*, 1995; Bundock and Hooykaas, 1996; Harding, 1996; Schlüter and Potrykus, 1996; Schmidt and Hankelen, 1996; Wöstemeyer *et al.*, 1997). Recently, the concept of adaptive mutation in bacteria, in which gene transfer has been implicated, has received increased focus (Cairns *et al.*, 1988; Galitski and Roth, 1995; Radicella *et al.*, 1995). Any transfer of antibiotic resistance traits to bacteria is highly undesirable as it may impede the treatment of infectious diseases caused by pathogenic bacteria. Pathogenic bacteria originating in the soil are known to occur and numerous soil bacteria are opportunistic pathogens in humans (Courvalin, 1994).

We are facing a serious health problem due to increasing antibiotic resistance among pathogenic micro-organisms. A Norwegian study demonstrated that *E. coli* with unusual resistance patterns could be isolated from iceberg lettuce imported from Spain that had been identified as the source of a *Shigella* outbreak (Kapperud, 1995). The Norwegian health authorities pursue a restricted policy regarding the introduction and use of antimicrobial agents. These findings reflect the differences in the distribution of antibiotic restriction genes between north and south Europe (Dornbusch and the European Study Group, 1990).

The results presented above indicate that no final conclusions can be drawn regarding the potential transfer of antibiotic resistance marker genes from genetically modified plants to micro-organisms in the intestine or the environment. Although we do not yet know whether horizontal gene transfer will significantly increase the number of antibiotic resistant pathogenic bacteria, there is no reason to accept this risk. Furthermore, technology is available today to avoid the presence of genes encoding antibiotic resistance in genetically modified plants.

2.2 Effects of Bt toxin on non-target organisms

The *cryIA (b)* genes inserted into maize line Bt176 are aimed at the European corn borer (*Ostrinia nubilalis*), which is a major pest in maize fields. Recent results have, however, raised concerns that the toxin produced by these genes may also affect other organisms.

The insecticidal crystalline prototoxins produced by several subspecies of *Bacillus thuringiensis* are activated in the midgut of susceptible insect larvae by solubilisation in the high pH and cleavage by specific proteases. These two activating processes are thought to be involved in the specificity of the Bt toxins. The truncated forms that have been genetically engineered into plants, however, express the active toxins rather than the inactive prototoxins. This could imply that non-target insect larvae would be more exposed to the active Bt toxins.

Hilbeck *et al*. (1998a) demonstrated that *Crysoperla carnea* larvae raised on two prey species that had eaten transgenic *Cry1A (b)*-expressing maize leaves showed a significantly higher mortality rate than *C. carnea* larvae raised on prey that had eaten non-transformed leaves. These results were supported by a study showing that continuous exposure to *Cry1Ab* toxin resulted in significantly higher mortality levels in immature *C. carnea* (Hilbeck *et al*., 1998b). Losey *et al.* (1999) observed that larvae of the monarch butterfly (*Danaus plexippus)* which ingested pollen from Bt plants had significantly higher mortality rates.

When those portions of the plants that are not being harvested are ploughed into the soil, the accumulated toxin may bind on clays and become resistant to microbial degradation for several months (Crecchio and Stotzky, 1997; Koskella and Stotzky, 1997). The persistence of these active toxins could lead to accumulation in the soil, thereby contributing to a hazard for non-target insects.

2.3 Development of Bt toxin resistance in target insects

It has been assumed that development of resistance to the Bt toxins would not be a problem because of the rapid degradation of the toxins in the environment. The continuous expression of the toxins in Bt plants may, however, increase the probability of development of resistance in target insects as a result of the selection pressure. Accumulation of the toxins in soil (see above) may also enhance the selection of resistant target organisms.

Development of resistance to Bt toxins in insects has been achieved both in laboratories and field trials. This has been documented in diamondback moths (*Plutella xylostella*) exposed to externally applied Bt toxin (Tabashnik *et al.*, 1991, 1992), and in the Colorado potato beetle (*Leptinotarsa decemlineata*) (Commandeur and Komen, 1992). Gould *et al*. (1992, 1993) noticed that tobacco budworms (*Heliothis virescens*) exposed to the *Cry IA (c*) Bt toxin in the laboratory for 20 generations evolved resistance to not only this Bt toxin but also other forms. Cross-resistance may make the management of Bt resistance in target insects difficult.

Several methods to avoid or control Bt resistant insects have been proposed. The high-dose/refuge strategy is the most widely acknowledged one for Bt maize. This strategy combines high expression of Bt toxin in the transgenic cultivars with refuges planted with non-transgenic maize, and relies on the resistance being a recessive or partially recessive trait. A report (Huang *et al*., 1999) from a laboratory trial indicated, however, that Bt resistance in the European corn borer may be inherited as an incompletely dominant autosomal gene, which may diminish the usefulness of the high-dose/refuge strategy.

The results presented above indicate that there is a risk for development of Bt resistant insects as a consequence of widespread cultivation of Bt maize. Biocontrol by Bt toxins has been a notable alternative to the use of chemically synthesised pesticides since the early 1960´s. If resistance towards Bt toxins in target pests evolves as a result of the widespread cultivation of

Bt plants, serious consequences could also result for farms that depend on Bt sprays for pest management in non-transgenic cultivars.

2.4 Gene flow to wild plants and crops

Maize is a mainly wind-pollinated, annual species. Only sweet corn (*Zea mays saccharata*) is cultivated in Norway. The very limited commercial cultivation of this maize form makes hybridisation with traditional maize crops, or volunteer maize plants, improbable. Furthermore, there are no cross-compatible wild or weedy relatives of maize in Europe, and gene flow from the genetically modified maize line Bt176 is therefore not likely to happen (Harding and Harris, 1994).

2.5 Changes in herbicide use

As mentioned above, maize is only cultivated to a very small extent in Norway. The herbicides pyridate, clopyralid and cyanazin are approved for use against weeds in maize fields.

Gluphosinate, which is not approved for use against weeds in maize in Norway, inhibits the enzyme glutamine synthetase. This will lead to accumulation of ammonium ions at a level that is toxic to the plant and to decreased photosynthesis due to an increased level of glyoxylate (Pesticide Manual, 1994; Norwegian Agricultural Inspection Service, 1996). Before gluphosinate can be approved for use in the genetically modified cultivar, Maximum Residue Levels (MRL) must be established based on the acceptable daily intake (ADI), the average food intake and the highest known residue levels from relevant trials (Norwegian Agricultural Inspection Service, 1997; UNEP/FAO/WHO/, 1989). Residue data have to be generated according to national guidelines. Data on the metabolism of gluphosinate in the transgenic maize line will also be needed.

To assess the impact on health and the environment of introducing genetically modified maize with gluphosinate tolerance, the present use of herbicides and the expected use on the genetically modified cultivar should be compared. There is also a need for a more comprehensive study focusing on the long-term effects on the environment caused by the introduction of herbicide-tolerant plants such as maize line Bt176.

3. Sustainable development

The risk that horizontal gene transfer from plants to micro-organisms may contribute to enhanced antibiotic resistance implies that deliberate release of genetically modified plants with antibiotic resistance marker genes does not comply with the need for sustainable development. The ampicillin resistance gene has no function in the product. Since techniques to avoid or remove marker genes encoding antibiotic resistance exist, there is no reason to accept the risks for an increase in antibiotic resistance in pathogenic micro-organisms.

The application contains no information substantiating that marketing of the genetically modified maize will make a positive contribution to sustainable development. The use of gene technology in such a way as to reduce the need for chemicals in agriculture is in keeping with

the principle of sustainable development if it also satisfies the requirements for safety as regards health and the environment. However, insufficient data are available on the expected impact on herbicide use that will result from marketing the gluphosinate-tolerant maize line. Furthermore, introduction of herbicide-tolerant plants may in the long run impede the evolution of weed management strategies that depend less on chemical herbicides.

Development of resistance in target insects to the Bt toxin expressed by maize line Bt176 would presumably also affect the use of foliage sprays containing the same Bt toxin. The result could be a future need for more environmentally harmful insecticides, which would not be in accordance with sustainable development.

4. Benefit to the community

Since the European corn borer is not a problem in Norwegian maize fields, there is no need for the product for pest management in Norway. To assess the possible benefits to the community, it would be interesting to compare herbicide residues in the genetically modified maize and non-modified maize. Such data have, however, not been presented. In sum, it has not been substantiated that marketing of the genetically modified maize will result in benefits to the community that could outweigh the risks of negative consequences.

5. Ethical aspects

Marketing of genetically modified plants that cause risks for ecological damage, or an impeded treatment of infectious diseases, also raises ethical concerns in cases where no major benefit is expected that can outweigh the risks.

6. Conclusion

Deliberate release in Norway of the genetically modified maize line Bt176 would entail a risk of adverse effects to health and the environment. The risks and the lack of sufficient knowledge about horizontal gene transfer of antibiotic resistance genes and the possible undesirable effects of the Bt toxin on both target and non-target insects implies that marketing of the product would conflict with the precautionary principle and the requirement for sustainable development. No benefits to the community, or other aspects, can outweigh the risks associated with marketing the product.

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