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1 Executive Summary

This review examines recent scientific evidence concerning potential environmental and health risks of two GM maize lines – MON810 and T25. Following authorisation in the European Union, Austria has invoked national safeguard measures according to Art. 16 of Dir. 90/220/EEC for these GMOs. This analysis primarily focuses on the issues identified by Austria as specific concerns in relation to these GM maize lines and considers the Opinions of the European Commission's Scientific Committees. In this summary the environmental issues concerning the maize lines MON810 and T25 are addressed separately, whereas the issue of health risks can be considered for both GM maize lines in similar terms.

Environmental risks of MON810 maize

With regard to environmental risks of MON810 maize the following concerns are specifically addressed:

- Possible unintended effects of the genetic modification on non-target organisms
- Uncertainties concerning the resistance management by a refuge strategy
- Likelihood of the development of secondary pests
- Lack of a monitoring plan

Considering **unintended effects on non-target organisms** (Chapter 4.1.1) only a very limited number of non-target organisms were tested in the laboratory. Recent scientific studies show that a wider range of insects, including non-target Lepidoptera, may be adversely affected by MON810 maize. Additionally investigations of effects on non-target organisms at higher trophic levels indicate that adverse effects of MON810 have to be considered for a range of arthropod species, which were not included in previous risk assessments.
A comprehensive assessment of these species in relation to the regional agricultural conditions for commercial production has not been carried out. Based on current research a large number of species occurs in agricultural areas and several of these species are already classified as endangered. These species might be further threatened by the cultivation of MON810.
Existing field trial data provided with the application are inappropriate to assess these risks sufficiently because of methodological shortcomings, incompleteness of data and poor statistical evaluation of results.

Concerning **resistance management** (Chapter 4.1.2) specific information necessary for assessing the proposed management plan is missing. Baseline information about the pest biology and ecology and a workable "Insect Resistance Management" plan must be available before commercialisation of MON810 in an area with specific regional characteristics. In particular basic data regarding resistance allele frequencies and baseline susceptibilities were not gathered to date in Austria and are missing for an assessment of the management measures. Due to the differences in climate and agricultural practice, experiences gained from Bt maize cultivation in Spain cannot be expected to be applicable to the Austrian agricultural system. These differences thus indicate a need to adapt the insect resistance management strategies.

Another critical issue is the **lack of a monitoring plan** for cultivation of MON810 (Chapters 4.1.3 and 4.1.4). Firstly this conflicts with the current obligations for such products. Annex VII of Directive 2001/18/EC can be considered as agreed minimum standard for this issue. On the other hand, such a monitoring scheme would be necessary to address the **issue of secondary pests**, a question considered important by the Scientific Committee on Plants in their opinion on the safeguard measure for MON810 maize.

Environmental risks of T25 maize

With regard to environmental risks of T25 maize concerns specifically addressed in the report are

- Risks for weed communities
- Lack of a monitoring plan
- Regional aspects in combination with coexistence issues

In summary the risk assessment data available for genetically modified herbicide tolerant (GMHT) maize T25 do not fulfil the requirements for an assessment of how these new herbicide/GM plant regimes could affect weed communities (see Chapter 4.2.1). As changes in weed management are to be expected with introduction of T25 maize, a proper assessment of the **effects on weed communities** is required, based on an in-depth analysis of weeds and interactions between the GMO and target organisms of maize T25 as required both under Directive 90/220/EEC (Annex II, IV. C.3 and C.4) and Directive 2001/18/EC (Annex IIIB, D.). The insufficient control of certain weeds provided by glufosinate-ammonium and the resulting shift in weed communities has to be considered adequately. The frequent use of a non-selective herbicide such as glufosinate-ammonium will increase the pressure on weeds thus resulting in the dominance of a few species and finally the prevalence of resistant weed species. Recent records of experience indicate that in such situations failures in weed control occur with GMHT plants. This suggests that an increase or change in the proposed herbicide use and/or number of applications has to be considered for maize T25. Furthermore, the cultivation of GMHT maize T25 in combination with extensive use of a non-selective herbicide might further contribute to the decline of already endangered plant species or biotope types.

Concerning the **lack of a post-market monitoring plan** (Chapter 4.2.2) the general argument previously noted for MON810 also applies. Furthermore, long term effects of the herbicide tolerant plant cannot be evaluated independently from the respective herbicide use and effects of glufosinate-ammonium in combination with maize T25 on weed communities need to be addressed by such a monitoring plan.

Additionally there are open questions concerning **regional aspects in connection with coexistence issues** (Chapter 4.2.3). Harmonized, legally binding provisions regarding coexistence measures (including liability) of genetically modified maize and conventional or organic maize are still missing.

Potential health risks of the GM maize lines MON810 and T25

The review of the assessments of **potential health risks of the GM maize lines MON810 and T25** (Chapters 5.2 and 5.3 respectively) focuses on the assessment of potential allergic or toxic properties as well as on the comparative analysis of plant compounds (substantial equivalence). It considers the risk assessment approaches, the methods chosen, the conduct of the experiments and the evidence presented. In both cases of GM maize safety conclusions of the applicants cannot be fully verified because detailed data are missing. More importantly, a number of shortcomings and weaknesses of the risk assessments conducted by the applicants are revealed. This is particularly true for GM-maize MON 810.

There are a number of similar shortcomings in both dossiers:

- By focusing on the introduced protein only the **assessment of possible toxic and allergenic properties** does not take into account possible health risks of the whole plant as a consequence of unintended effects of the genetic modification, e.g. via upregulation of plant allergens. Furthermore, the possibility of new and unexpected toxicants and allergens is not considered. The broiler feeding study included in the MON810 dossier clearly is a feed conversion study and not a toxicity study. The importance to better address unintended effects is acknowledged by expert consultations including FAO/WHO and Codex Alimentarius. Recently the European Commission stressed the importance of

whole-food/plant studies for assessing both the potential toxic and allergenic effects of the whole plant.

- **Test proteins** derived from bacterial sources are not necessarily representative of the plant proteins in the field. A recent Australian study on GM peas revealed immunogenic effects in mice that are most likely associated with differences in posttranslational processing of the target protein between the donor and the host organism.
- The **assessments of allergenic properties** rely on homology comparisons to known allergens, in-vitro digestibility studies, a history of safe use (relevant for both GM lines), low gene expression (relevant for maize line MON810) and the absence of glycosylation (relevant for maize line T25). Recent scientific evidence has however demonstrated that these methods do not constitute reliable indicators of allergenic properties and can lead to either false positive or false negative results.
- The risk assessments did not consider inhalation as a **possible route of exposure** despite the fact that inhalation constitutes an important route for sensitization.
- Furthermore, possible **changes in the de-novo sensitizing properties** were not considered, neither of the introduced protein nor of the whole plant
- **Toxicity endpoints** were limited to acute oral (maize MON810) and 14-days repeated-dose studies in rodents (T25), which do not provide any indications of long-term effects
- **Substantial equivalence** claims are based on a limited range of compounds analysed. The parameters chosen do neither meet the OECD nor industries own consensus recommendations. In case of maize T25 compositional analysis of plant material from European field trials was essentially limited to four plant components. Statistically significant differences detected between the GM maize lines and conventional counterparts are not properly considered

In summary, risk assessment data provided cannot – in the light of recent scientific evidence - provide sufficient safety reassurance. In addition, the risk assessments provided are highly unlikely to meet the requirements of Annex II of Directive 2001/18/EC, which constitutes the current standard for a reassessment of the safeguard measures.

2 Zusammenfassung (Summary in German)

Die vorliegende Studie untersucht die wissenschaftliche Risikoabschätzung der möglichen Umweltauswirkungen und Gesundheitsrisiken von zwei gentechnisch veränderten Maislinien, MON810 und T25. Es werden dabei insbesondere neuere wissenschaftliche Erkenntnisse berücksichtigt.

Die beiden gentechnisch veränderten Maislinien haben das EU-Zulassungsverfahren auf Grundlage der Richtlinie 90/220/EWG durchlaufen. Als Ergebnis des Verfahrens wurde ihre Verwendung in der EU genehmigt. Einige Mitgliedsstaaten, darunter auch Österreich, verhängten anschließend Importverbote für diese GV-Maissorten auf der Grundlage von Artikel 16 der EU-Richtlinie 90/220/EWG.
Die vorliegende Studie untersucht die wissenschaftliche Risikoabschätzung der beiden Maislinien und konzentriert sich dabei auf jene Aspekte, die bereits in den bisherigen Stellungnahmen Österreichs aufgegriffen wurden. Bei beiden Maislinien wurden Mängel und Schwächen in der Risikoabschätzung festgestellt, weshalb Umwelt- und Gesundheitsrisiken nicht mit hinreichender Sicherheit ausgeschlossen werden können. Die Umweltrisiken stellen sich bei beiden Maislinien unterschiedlich dar und werden im Folgenden für die beiden GV-Maissorten separat analysiert. Die Gesundheitsrisiken von MON810 und T25 werden in einem gemeinsamen Abschnitt behandelt, da sowohl die jeweiligen Grundlagen für die Beurteilung, als auch Mängel und Schwächen der Risikoabschätzung ähnlich sind.

Umweltrisiken von MON810 Mais

Folgende Problembereiche in Bezug auf Umweltauswirkungen der GV-Maissorte MON810 wurden identifiziert und untersucht:

- Mögliche unerwartete Auswirkungen der GV-Sorte auf Nichtzielorganismen
- Wissenschaftliche Unsicherheiten hinsichtlich des Resistenz-Managements in Bezug auf die vorgeschlagene Strategie (Hochdosis-Refugium-Strategie)
- Die Möglichkeit des verstärkten Aufkommens von sekundären Schädlingen
- Das Fehlen eines Monitoring-Planes

Hinsichtlich möglicher **unerwarteter Auswirkungen von MON810 auf Nichtzielorganismen** (dargestellt im Kapitel 4.1.1) kann festgestellt werden, dass nur eine sehr beschränkte Anzahl von Nicht-Zielorganismen in Laboruntersuchungen hinsichtlich möglicher Auswirkungen von Bt-Toxinen untersucht worden ist. Neuere Untersuchungen zeigen, dass ein breiteres Spektrum von Insektenarten, darunter auch Nichtziel-Schmetterlingsarten, von Bt-Toxinen beeinträchtigt werden können. Neue Untersuchungen über die Auswirkungen von Bt-Toxinen auf Nicht-Zielorganismen höherer trophischer Ebenen haben auch gezeigt, dass negative Effekte von MON810 für ein ganzes Spektrum von Arthropodenarten möglich sind, welche nicht bei der Risikoabschätzung berücksichtigt worden sind.
Eine Bewertung der Risiken für diese Arten unter den Bedingungen der regionalen landwirtschaftlichen Gegebenheiten für eine kommerzielle Produktion wurde ebenfalls nicht durchgeführt. Basierend auf neueren Untersuchungen kann festgestellt werden, dass ein erheblicher Anteil der möglicherweise negativ betroffenen Arten in den landwirtschaftlich genutzten Gebieten vorkommt und einige dieser Arten bereits aufgrund anderer Umstände in ihrem Bestand gefährdet sind. Diese Arten könnten durch einen Anbau von MON810 zusätzlich gefährdet werden.
Die Ergebnisse von Feldversuchen, welche im Zuge der Anmeldung vorgelegt wurden, sind zur Einschätzung der erwähnten Risiken aus einer ganzen Reihe von Gründen nicht geeignet: Sie sind methodisch ungeeignet solche Risiken geeignet zu erfassen; die erhobenen Daten sind nicht geeignet, die gezogenen Schlussfolgerungen zu begründen, und die statistische Auswertung der Resultate ist insgesamt mangelhaft.

In Bezug auf das **Management der Resistenzentwicklung** bei Schadinsekten (dargestellt im Kapitel 4.1.2) kann festgestellt werden, dass der vorgeschlagene Management-Plan aufgrund fehlender Informationen nicht abschließend beurteilt werden kann. Konkret fehlen wesentliche, grundlegende Daten zur Schädlingsbiologie und -ökologie, speziell Daten zur Ausgangslage bei den Frequenzen von Resistenzallelen und den Empfindlichkeitsschwellenwerten der einzelnen Nicht-Ziel Arten. Außerdem sollte ein an die speziellen örtlichen Gegebenheiten angepasster und umsetzbarer Resistenzmanagement-Plan vorgelegt werden, bevor eine Kommerzialisierung von MON810 ins Auge gefasst werden kann. Aufgrund der bestehenden Unterschiede bei den klimatischen Verhältnissen und bei der landwirtschaftlichen Praxis lassen sich die in Spanien beim Anbau von MON810 gewonnenen Erkenntnisse nicht in der vorgeschlagenen Weise auf Österreich übertragen. Im Gegensatz dazu deuten die Unterschiede auf einen Bedarf in Richtung Anpassung der Management-Strategie zur Abwehr möglicher Resistenzbildung hin.

Ein weiterer wesentlicher Kritikpunkt ist das **Fehlen eines geeigneten Monitoringplans** (dargestellt im Kapitel 4.1.3 und 4.1.4). Einerseits ist ein solcher Plan nach den derzeit geltenden Vorschriften für Produkte wie MON810 obligatorisch vorzulegen. In dieser Hinsicht kann Annex VII der EU Richtlinie 2001/18/EC als allseits akzeptierter Standard gesehen werden. Andererseits ist ein derartiger Monitoringplan nötig, um die offenen Fragen bezüglich des möglicherweise vermehrten Auftretens sekundärer Schädlinge beantworten zu können. Diese Fragen wurden auch in der Stellungnahme des Scientific Committee on Plants als relevant bezeichnet.

Umweltrisiken von T25 Mais

Folgende Problembereiche in Bezug auf Umweltauswirkungen der GV-Maissorte T25 wurden identifiziert und untersucht:

- Risiken in Bezug auf die betroffene Unkrautflora
- Das Fehlen eines Monitoring-Planes
- Nichtberücksichtigung regionaler Aspekte mit Bezug zur Koexistenzproblematik

Betreffend die **Risiken in Bezug auf die betroffene Unkrautflora** kann zusammenfassend festgestellt werden, dass die für den Zulassungsantrag des GV-Mais T25 vorgelegten Daten zur Risikoabschätzung nicht die Voraussetzungen erfüllen, um in einer geeigneten Weise die Effekte einer Verwendung dieser GV-Maissorte auf Unkraut-Pflanzen abschätzen zu können (dargestellt im Kapitel 4.2.1).
Da durch die Verwendung dieser GV-Maissorte und insbesondere durch die damit verbundenen Änderungen in der Art der Unkrautbekämpfung aber mit solchen Effekten gerechnet werden muss, sollte eine eingehende Untersuchung derartiger Effekte im Sinne einer umfassenden Risikoabschätzung vorliegen. Eine solche Abschätzung im Einklang mit EU-Richtlinie 90/220/EEC Annex II, C.3 und C.4 sowie 2001/18/EC, Annex IIIB, D. sollte auf einer umfassenden Analyse der regionalen Unkrautflora und den Interaktionen zwischen dieser Flora und der GV-Maislinie T25 basieren. Derartige Untersuchungen liegen aber nicht vor. In diesem Zusammenhang sollte auch der Effekt einer ungenügenden Kontrollwirkung von Glufosinat-Ammonium auf die Unkrautflora und die dadurch hervorgerufenen Verschiebungen in der Zusammensetzung der Unkrautflora untersucht werden. Die häufige Verwendung eines nicht-selektiven Herbizids wie Glufosinat-Ammonium kann zur Dominanz einiger widerstandsfähiger Unkrautspezies führen und dadurch schlussendlich zur Vermehrung von resistenten Unkräutern führen. Erfahrungen mit herbizidresistenten GV-Pflanzen zeigen die Möglichkeit einer solchen Entwicklung. In einzelnen Fällen zeigt sich, dass es dadurch zu Kontrollverlusten in Bezug auf das betroffene Herbizid kommen kann. Das legt nahe, dass auch im Fall von Mais T25 mittelfristig mit einer Zunahme in der Zahl der Unkrautbehandlungen und/oder mit der Anwendung anderer Herbizide gerechnet werden muss.
Dazu kommt, dass die Verwendung der herbizidresistenten Maissorte T25 in Kombination mit der extensiven Verwendung des korrespondierenden Herbizids dazu beitragen kann, dass bereits gefährdete Pflanzen und Biotoptypen weiter unter Druck geraten.

In Bezug auf den **fehlenden Monitoringplan** (dargestellt in Kapitel 4.2.2) trifft die oben angeführte Argumentation betreffend die GV-Maissorte MON810 auch auf T25 zu. Darüber hinaus kann festgestellt werden, dass die Langzeiteffekte der herbizidresistenten Pflanzensorte nicht unabhängig von den Auswirkungen des korrespondierenden Herbizids beurteilt werden können. Speziell die Effekte der Verwendung von GV-Mais T25 in Kombination mit Glufosinat-Ammonium auf die regionale Unkrautflora sollten in einem solchen Monitoringplan angesprochen werden und im Zuge der Umsetzung untersucht werden.

Zusätzlich besteht eine ganze Reihe von **offenen Fragen zu regionalen Aspekten der Verwendung von GV-Mais T25 im Zusammenhang mit der Koexistenzproblematik** (dargestellt in Kapitel 4.2.3). In diesem Sinn ist es von besonderer Bedeutung, dass bis dato eine harmonisierte, gesetzlich verpflichtende Regelung dieser Problematik (und damit zusammenhängender Haftungsfragen) EU-weit noch aussteht. Die angestrebte Koexistenz von GV-Maissorten und konventionellen Sorten bzw. Bio-Sorten steht und fällt mit solchen Regelungen.

Mögliche Gesundheitsrisiken und substanzielle Äquivalenz der Maislinien MON810 und T25

Die Evaluierung von potentiellen Gesundheitsrisiken der Maislinien MON810 und T25 (dargestellt in den Kapiteln 5.2 sowie 5.3) konzentriert sich auf **potentielle allergene und toxische Eigenschaften** sowie auf die **vergleichende Inhaltsstoffanalyse** (substanzielle Äquivalenz). Sie bezieht sich dabei auf die Zugänge in der Risikoabschätzung, die eingesetzten Methoden und berücksichtigen Daten und auf die im Einzelfall durchgeführten Untersuchungen. Bei beiden Maislinien sind aufgrund von unvollständigen oder fehlenden Daten die Schlussfolgerungen der Antragsteller im Bezug auf die Sicherheit der Maislinien nicht vollständig nachvollziehbar und Mängel und Schwächen in der Risikoabschätzung ersichtlich, speziell im Fall Mais MON810.
Die Mängel und Schwächen sind in beiden Fällen ähnlich und lassen sich wie folgt beschreiben:

- Die Abschätzung möglicher toxischer oder allergener Risiken konzentriert sich ausschließlich auf die neu eingebrachten Proteine und blendet mögliche unbeabsichtigte Sekundäreffekte der genetischen Veränderung auf die gesamte Pflanze weitgehend aus, z.B. eine höhere Expression von endogenen Allergenen oder die Möglichkeit der Expression von neuen Allergenen oder Toxinen. Die Bedeutung von nicht-beabsichtigten Sekundäreffekten wurde mehrfach in internationalen Expertenkonsultationen unterstrichen, unter anderem auch seitens FAO/WHO und Codex Alimentarius. Kürzlich wies auch die Europäische Kommission auf die Bedeutung von toxikologischen Fütterungsstudien und Allergenitätsstudien mit der gesamten Pflanze für die Abschätzung von derartigen nicht-beabsichtigten Effekten hin.
- Aus Mikroorganismen hergestellte Testproteine sind nur bedingt repräsentativ für die Eigenschaften der analogen in GV-Pflanzen gebildeten Proteine.
  Eine unterschiedliche Prozessierung von Proteinen in Pflanzen und Mikroorganismen und damit auch die Möglichkeit von unterschiedlichen gesundheitsrelevanten Eigenschaften wurde kürzlich durch eine australische Studie mit genetisch veränderten Erbsen gezeigt. In dieser konnten immunogene Effekte des heterologen Proteins nachgewiesen werden, die mit hoher Wahrscheinlichkeit auf unterschiedliche posttranslationale Modifikationen in Ziel- und Wirtsorganismus zurückzuführen sind.
- Die Abschätzung möglicher allergener Risiken basiert im Wesentlichen auf Homologievergleichen der zusätzlichen Proteine mit bekannten Allergenen, in-vitro Verdauungsstudien und Erfahrungen mit der sicheren Anwendung der Proteine (in beiden GV-Maislinien), geringer Konzentration des Proteins in der Pflanze (GV-Mais MON810) und der Abwesenheit von Glykosylierung (GV-Mais T25). Neuere wissenschaftliche Erkenntnissen haben jedoch gezeigt, dass diese Methoden keine hinreichend verlässlichen Rückschlüsse über die allergenen Risiken ermöglichen

und sowohl zu falsch-positiven als auch zu falsch-negativen Ergebnissen führen können.
- Inhalation wird nicht als relevanter Expositionsweg berücksichtigt, obwohl diese einen wichtigen Sensibilisierungspfad darstellt.
- Mögliche Änderungen der sensibilisierenden Eigenschaften von neu eingefügten Proteinen bzw. Pflanzen wurden nicht berücksichtigt.
- Die toxikologischen Endpunkte reduzieren sich auf akute Toxizität (Mais MON810) bzw. 14-Tage subakute orale Toxizität (Mais T25) bei Nagetieren. Derartige Untersuchungen ermöglichen keine bzw. nur ungenügende Rückschlüsse auf Langzeiteffekte.
- Die Feststellung von substanzieller Äquivalenz (mit Ausnahme der zusätzlich eingeführten Eigenschaft) beruht im Wesentlichen auf vergleichenden Inhaltsstoffanalysen einer geringen Anzahl von Parametern, die weder dem OECD noch dem industrieeigenen Standard entspricht. Für Mais T25 wurden bei Feldversuchen in Europa überhaupt nur vier Inhaltsstoffe untersucht. Statistisch signifikante Unterschiede in den Vergleichsstudien wurden darüber nicht hinreichend sorgfältig analysiert.

Zusammengefasst kann gesagt werden, dass - im Lichte neuerer wissenschaftlicher Erkenntnisse - die Risikoabschätzungen in beiden Maislinien keine hinreichend verlässlichen Schlussfolgerungen bezüglich Sicherheit ermöglichen. Die Risikoabschätzungen entsprechen mit hoher Wahrscheinlichkeit auch nicht den Anforderungen des Annex II der Richtlinie 2001/18/EG.

3 Analysis of the reasoning from Austrian CAs and EU bodies concerning the import bans on GMOs issued by Austria

3.1 GM-Maize MON810

3.1.1 Introduction

An application for authorisation of the genetically modified maize MON810 according to Directive 90/220/EEC (notification C/F/95/12/02) was submitted on 24 May 1996 by the Monsanto company to the French Competent Authority. The transformation event MON810 contains the cry1A(b) gene producing a bacterial toxin thus providing protection to lepidopteran insects such as the European corn borer and a *nptII* gene conferring resistance to aminoglycoside antibiotics and thereby allowing for selection of bacteria containing the plasmid. Maize MON810 is intended to be used as any other maize, such as for the production of maize within the European Union, the import and processing of grain and maize products and their use in food, feed and industrial products. After a positive opinion by the Scientific Committee on Plants, consent was given for the placing on the market of genetically modified (GM) maize MON810 (Commission Decision of 22 April 1998). Subsequently, the placing on the market of this maize was prohibited by the Austrian Competent Authority in 1999. In 1999 the Scientific Committee on Plants and in 2004 and 2006 the EFSA GMO Panel issued opinions on the Austrian prohibition of maize MON810. In the WTO Panel report scientific experts commented on the scientific evidence submitted in support of the Austrian import bans.
The following chapter will briefly describe the main information or arguments included in these documents.

3.1.2 Information from the original dossier

The original dossier of the genetically modified maize MON810 provided by the notifier (notification C/F/95/12/02) contained an **environmental risk assessment** which comprised the following points:

a. With respect to the risk assessment parameter " *Survival, multiplication and dissemination of the GMO in the environment"* the notifier carried out field tests at 18 sites in Europe and 60 sites in the US thereby providing data on yield, agronomic characteristics, vigour, disease and insect susceptibility. For all parameters no difference was found between the GM maize and conventional maize. For the parameter "*survivability*" the germination rate and the seed dormancy were investigated and no differences were detected. For the parameter "*vegetative vigour*" visual observations in field tests showed no difference in agronomic quality, disease or insect susceptibility and no differences were observed in vegetative vigour or in any stress factors including drought, heat and frost. When evaluating "*modes and/or rate of reproduction*" field observations showed no differences in seed or plant maturity or in yield. With respect to "*dissemination*" the notifier stated that no changes in seed dissemination were expected but that no trials have been conducted to specifically study pollen production or dispersal. However, the notifier assumed that outcrossing frequency of the GM maize is unlikely to be different from conventional maize.
b. With respect to the risk assessment parameter "*Interactions of the GMOs with the environment"* no differences were observed for the parameters susceptibility to insects and diseases, survival capacity (volunteers), seed multiplication capacity (yields), seed composition analysis, and safety for birds and mammals.
c. Regarding the assessment of the "*Environmental impacts of the GMO(s)"* the notifier took into consideration the risk of resistance development of the European corn borer, the target organism of GM maize MON810 and developed strategies to

address and manage the risk of resistance. In order to provide the safety of non-target organisms data on field trials are provided (Appendix IV). However, the European field trials were carried out in France only and were designed to be efficacy trials rather than safety evaluations. Furthermore, no indication at what time the insecticide was sprayed on the conventional maize is given and no statistical evaluation was carried out. In the US trials in two out of three years only other GM lines than MON810 were used and in two years only the bug *Orius* sp. was evaluated as a non-target organism.

d. Laboratory toxicity studies using the isolated Bt protein were provided separately with only a limited range of non-target organisms (honey bees, lacewings, hymenoptera, ladybird beetles).

Concerning **health risks** the original dossier of the genetically modified maize MON810 provided by the notifier (notification C/F/95/12/02) includes an assessment of possible toxic and allergic properties and of the substantial equivalence of the GM crop. References are frequently provided to unpublished reports which are not included in the dossier[1]:

a. With respect to toxicity assessment the dossier included an acute toxicity test in mice and in-vitro digestibility studies of the CryIA(b) protein. All studies were conducted with the trypsin-resistent core of CryIA(b) produced in E.coli. Evidence showing the equivalence of the test protein to the protein expressed in maize was provided (including molecular weight, immunoreactivity and insecticidal activity; LEE et al. 1995b). The tryptic core of CryIA(b) is considered "identical to portion of the CryIA(b) protein contained in microbial formulations that have been used safely commercially for over 30 years (LEE et al. 1995a*)". Degradation rates of more than 90% within two minutes (western blot) and 74 to 90% (insecticidal activity) were observed in simulated digestive fluid (REAM 1994*).[2] Acute toxicity tests were performed for 8 or 9 days respectively. with three groups of ten male and female mice (NAYLOR 1992). Targeted doses administered to mice were 0, 400, 1000 and 4000 mg/kg body weight. Pathological changes observed were considered to be randomly distributed. It is argued that these changes are frequently observed with the strain of mice used. Significant differences were only observed in total food consumption (only one mice of the control group). Furthermore, homology studies were conducted which could not identify any "biologically significant homology" to known toxins (ASTWOOD 1995a*).

b. It was also argued that mammalian intestinal cells do not have receptors for delta-endotoxins of B.thuringiensis and therefore humans will not be susceptible to these proteins (HOFFMANN et al.1988b*, NOTEBORN and KUIPER 1995*, SACCHI et al. 1986*). In addition the dossier refers to reviews on the safety of Bt proteins (IGNOFFO 1973*, SHADDUCK 1983*, SIEGEL and SHADDUCK 1998*) as well as to the history of safe use of CryIA(b) (EPA 1988*).

c. With respect to allergenicity assessment it was argued that proteins only rarely elicit allergenic response (TAYLOR 1992*), that the source organism, B. thuringiensis has no history of causing allergy (EPA 1995*), that CryIA(b) is not stable in in-vitro digestibility tests, and that food allergens are normally present in high concentrations (TAYLOR 1992*, TAYLOR et al. 1987*, 1992*). Homology comparison did neither reveal any "biologically significant" (DOOLITTLE 1990*) nor an "immunologically significant" homology (ASTWOOD 1995b*) (minimum criterion used: 8 contiguous identical amino acids).

[1] Some of the references in this subsection (marked with asterisks) are taken from the dossier and are not included in the reference section of this review. Full references can be obtained from the original dossier.

[2] In simulated intestinal fluids CryIA(b) protein did not degrade substantially after 19,5 hours. The tryptic core of Bacillus insecticidal proteins have shown to be relatively resistant to digestion by serine proteases like trypsin, a key protease in intestinal fluid. (BIETLOT et al.1989*).

Substantial equivalence was claimed based on comparative analysis of kernel and other plant material von MON810 including hybrids based on MON810 and conventional counterparts: Means and ranges were found to be within ranges derived from conventional comparator and from literature. Field trials were conducted in the USA (1994) and Europe (France, Italy 1995). No data are included on nutritional and morphological aspects.

3.1.3 First opinion of the Scientific Committee on Plants (1998)

In its opinion of 10 February 1998 on the GM maize MON810 the Scientific Committee on Plants (SCP 1998) came to the following conclusions concerning the **environmental aspects**:

a. With respect to **gene transfer** the Committee concluded that the risk of genetic escape will be limited by poor dispersal and the absence of sexually-compatible plants either of the same or different species. Furthermore, pollen production and viability were considered to be unchanged and therefore dispersal and outcrossing should be no different from other maize varieties.
b. Considering the occurrence of GM **volunteers** the Committee stated that the risk of volunteer maize plants surviving is remote as they can be controlled by agronomic practices and non-selective herbicides in areas encountering no winter frost.
c. With respect to safety issues for **non-target organisms** no risk for adverse effects was identified to non-target herbivores including vertebrates. Risks to soil organisms and soil function through degradation of GM plant material or risks through contamination of ground water were considered to be extremely low.
d. With respect to **resistance and tolerance issues** the Committee refers to the resistance management strategy proposed by the notifier.

Concerning **health effects** the Scientific Committee on Plants (SCP) in its opinion of 10 February 1998 on the GM maize MON810 concluded that "there is no significant risk to humans or livestock following ingestion of the gene product" (SCP 1998).

a. With respect to toxicity risks the Committee based its conclusion on evidence from acute and short term toxicity studies and on homology studies to known toxins.
b. With respect to allergenic risk the Committee refers to "widespread use of the natural Btk insecticides" that "has not produced evidence of allergenic responses", to homology studies to known allergens. Critical remarks were made on the digestibility studies of the Btk protein: "the often applied in vitro methodology used to study the survival of Btk toxin can be improved. In particular, the use of the isolated protein in toxicity studies does not adequately model degradation of the same protein when fed as an integral component of the diet".

The Committee further concluded that "no significant nutritional differences could be detected between GM and non-GM materials" and that concerning maize MON810 the SCP therefore considered that **substantial equivalence** was demonstrated except for the transferred traits.

3.1.4 Reasons for the prohibition of GM maize MON810 by Austria

After consent was given to the notification of GM maize MON810 in April 1998 (Commission Decision 98/294/EG), the Austrian Competent Authority decided to prohibit the placing on the market of GM maize line MON810 on 10 June 1999 as a safeguard measure according to Article 16 of Directive 90/220/EEC. The objection of Austria was supported by the following reasons:

a. Possible unintended effects of the Bt toxin on **non-target insects**: reported adverse effects on soil collemboles, *Folsomia candida* (EPA 1995), on larvae of

Chrysoperla carnea (HILBECK et al. 1998a), on braconid parasitoids reared in Bt treated hosts (HAFEZ et al. 1997) and on non-target butterflies (LOSEY et al. 1999).

b. Uncertainty about the **effectiveness of the refuge strategy** in order to prevent the development of Bt resistance in the European Corn Borer (HUANG et al. 1999).
c. Effects of other Bt plants, e.g. cry1Ab Bt cotton in Australia, such as the increase of **secondary pests** and consequently additional use of synthetic plant protection products (FITT et al. 1994, HARDEE & BRYAN 1997, ROUSH 1997).
d. Uncertainty about the specificity of Bt plants (HILBECK et al. 1998b)

In its 1998 objection Austria focused on environmental concerns and did not explicitly address human health concerns (BMGF 1998b).

However in a subsequent communication to the Commission dated January 2004 (BMGF 2004a) Austria reiterated its objections to the GM maize event MON810 as well as to GM maize lines Bt176 and T25. The letter also summarised the main points of concern. The letter also raised additional concerns with respect to allergenic properties of Bt proteins relevant for MON810). Furthermore, general shortcomings in allergenicity and toxicity assessment under Directive 2001/18/EC and under the Novel Food Regulation were emphasised which do not comply with the requirements of Annex II of Directive 2001/18/EC.[3]
In its objections Austria also points to the precautionary principle.
In February 2004 the Austrian CA submitted a summary of supporting evidence and the original literature quoted therein to the European Commission to be used for the WTO Dispute Meeting on 14 January 2004. The objections raised earlier (BMGF 1998a, 1998b) were reiterated (BMGF 2004b). In addition, the BMGF criticised allergenicity and toxicity assessment along the lines of SPÖK et al. 2002. Digestibility studies using microbial test proteins were not considered appropriate because post translational modification could possibly affect protein properties or function (WÄTZIK et al. 2002).

3.1.5 Second opinion of the Scientific Committee on Plants (1999)

In the opinion of the Scientific Committee on Plants of 24 September 1999 on the Austrian prohibition of maize MON810 the Committee commented on the arguments cited by the Austrian Competent Authority in its prohibition of maize MON810 (SCP 1999):

a. With respect to possible unintended effects on **non-target insects** it stated that the results of laboratory studies would be difficult to interpret and extrapolate to field conditions and that such interpretation must be viewed against the comparative risk assessment of alternative spray applications of insecticides. It stated that further work would be needed to investigate and verify such effects in the field.
b. Considering the **effectiveness of the refuge strategy** the Committee emphasized that it advised on the establishment of non-Bt refuges but pointed out that due to the expected slow introduction in Europe, Bt crops would be surrounded by "natural refuges" for some time.
c. The Committee stated that monitoring also needed to cover **secondary pests.**
d. With respect to the **unspecificity of Bt plants** the Committee admitted that GM maize has the potential to be toxic to certain species of Lepidoptera and concluded that this issue must be dealt with on a species-to-species basis. However, the Committee also stated that cultivated fields are not considered as important reproductive areas for lepidopteran species and that food plants are unlikely to be exposed to significant quantities of pollen.

In accordance with the scope of Austrian submission of 1998 on environment concerns the opinion of the SCP of 24 September 1999 did not consider aspects of human health or substantial equivalence (SCP 1999).

[3] Referring to SPÖK et al.2002, 2003a, b.

3.1.6 Opinions of the EFSA GMO Panel (2004 and 2006)

In **2004** the EFSA GMO Panel responded to a request from the European Commission whether the submissions of Austria would contain "*any new or additional information affecting the Environmental Risk Assessment or re-assessment of existing information on the basis of new or additional scientific knowledge such that detailed grounds exist to consider that the above authorized GMOs …constitute a risk to human health or the environment*" and issued an opinion on the Austrian safeguard measure, according to Article 23 of Directive 2001/18/EC (EFSA 2004).
This opinion also considered the additional information provided by Austria in February 2004 in support of the national safeguard measure. This additional information contained the submission of four additional scientific papers (MORIN et al. 2003, ZWAHLEN et al. 2003a, ZWAHLEN et al. 2003b, SAXENA et al. 2002) as well a several conference abstracts.

The EFSA GMO Panel came to the following conclusions:

a. The first publication (MORIN et al. 2003) was not considered to be relevant for MON810 as it was related to the toxin Cry1Ac. The publications of ZWAHLEN et al. (2003a, 2003b) were dismissed as not having investigated effects on non-target organisms and the Panel stated that other publications had shown that persistence of Bt toxins did not occur (HOPKINS & GREGORICH 2004). The third publication of SAXENA et al. (2002) was considered not to show any evidence for an adverse effects of the Bt toxin on non-target soil organisms due to release of those toxins by roots of Bt maize. The conference abstracts provided by Austria were not regarded as evaluable on a scientific basis.
b. Furthermore, the Panel stated that regarding the impact of the Bt toxin on **non-target organisms**, previous scenarios had proven irrelevant in the lab and in field tests. The Panel referred to literature showing that the Bt toxin had no direct effect on lacewing larvae (ROMEIS et al. 2004) and showing that many arthropods were not sensitive to Cry1Ab (DUTTON et al. 2003). The Panel also stated that field tests in France had shown no effects on non-lepidopteran species, citing BOURGUET et al. (2002), and that the impact of Bt corn pollen on monarch butterfly populations was suggested to be negligible (SEARS et al. 2001).
c. With respect to **resistance development** of target pests the Panel referred to data from Bt maize planting in Spain which had shown no shifts in susceptibility after five years of Bt maize cultivation (FARINOS et al. 2004).

Consequently the Panel concluded that the evidence presented by Austria contained no new generic or local scientific information on the environmental impacts of the specified maize.

In the review of the evidence provided by Austria EFSA only discussed evidence submitted to sustain Austria's environmental concerns. The Panel dismissed the evidence provided by Austria that critically reviews and assesses the validity of toxicity assessment, allergenicity assessment and the practice of substantial equivalence in a number of Directive 90/220/EEC and Novel Food dossiers (SPÖK et al. 2002, 2003a, 2003b).[4] The panel correctly stated the overall aim of the reports but appears to have overlooked the very detailed critical review of the risk assessment information provided in the dossiers on MON810 maize (as well as on T25-maize)
EFSA pointed to a re-assessment of four Bt events by the EPA in 2001 (MENDELSON et al. 2003) that among others things reassured the lack of any human health concerns on the

[4] "Three reports from Austria […] investigated the requirements for toxicology and allergenicity related safety evaluations of genetically modified plants and derived products destined for human and/or animal consumption. The results and recommendations of these two studies are intended to contribute to national and international discussion on the improvement of safety evaluation of GM products. […] The three reports do not provide any new scientific data to indicate adverse affects on human and animal health or the environment of the maize events Bt176, MON810 and T25." (EFSA 2004)

basis of acute toxicity tests, digestibility studies and homology comparisons to known toxins.

In **2006** a second opinion from the EFSA GMO Panel, related to several GM crops subject to safeguard measures, was published (EFSA 2006), following a request by the Council of Ministers of Environment[5]. In this opinion EFSA reassessed all import bans that were still in place in 2006. EFSA explicitly stated that it did not reassess the dossiers of the original applications, whether they would comply with the most recent safety requirements laid down in Directive 2001/18/EC, Regulation 1829/2003 and the EFSA Guidance Document. Rather, EFSA considered its scope to reassess if "here is any scientific reason to believe that the continued placing on the market of the GMOs subject to the safeguard clauses are likely to cause any adverse effects for human health or the environment under the conditions of consent" (EFSA 2006).

This opinion contained the following arguments with respect to the justifications of the Austrian invoke of Article 16 of Directive 90/220/EEC.
With respect to the GM maize MON810 the Panel stated that it had assessed available data from several hybrids containing MON810 and that no adverse environmental impacts were reported from these hybrids or comparable Cry1Ab-expressing maize. The Panel further cited several studies which indicated that significant adverse environmental effects due to Bt maize cultivation were unlikely.

By reference to its evaluations of maize MON810 in hybrid applications in the context of both Directive 2001/18/EC and Regulation 1829/2003 EFSA furthermore reiterated the safety of the products in a very general way.

> "In this context [the hybrid applications, com.], the GMO Panel has assessed the molecular characterisation of the MON810 event together with data on the levels of Cry1Ab protein, compositional analysis and assessment of toxicology and allergenicity of the respective hybrids. Further, the GMO Panel has assessed available data from the literature concerning the environmental impacts of Cry1Ab" (EFSA 2006).

With respect to human health concerns, only the issue of the blaTM-1 genes was briefly discussed. In its opinion EFSA did neither mention any new evidence provided by Member States following its 2004 opinion, nor any new scientific literature in the public domain. The Panel however reaffirmed its conclusions for the previous 2004 opinion (EFSA 2006).

3.1.7 WTO Panel Report – comments from scientific experts advising the Panel

In the WTO report a small group of scientific experts were providing scientific input to questions posed by the Panel on various issues. Some of the questions were touching upon the Austrian safeguard measures. In the following replies are considered, which were targeted to relevant questions, provided answers by a balanced and fully reasoned approach, and arrived at accurate conclusions based upon a detailed analysis by experts with a relevant scientific expertise for the respective issues. The European Commission stated that considering these requirements the replies by Dr. Andow, one of the scientists from the group of experts advising the WTO Panel, specifically provided valuable scientific evidence to be taken into account (EC 2005).
Dr. Andow considered in depth the individual justifications provided by Austria in its prohibition of MON810 maize. In particular he provided information, whether there was sufficient scientific evidence available to Austria in June 1999 and in August 2003 to undertake a more objective assessment of potential risks to the environment from maize MON810 (WTO 2006).

[5] "[The council] calls on the Commission to gather further scientific evidence and to further assess whether the national measures are justified and whether the authorisation of these GMOs under Directive 90/220/EEC still meets the safety requirements of Directive 2001/18/EC' (EFSA 2006).

a. Dr. Andow stated that in 2003 Austria could reasonably maintain that there is still insufficient information to know which **non-target organisms** might be at risk and therefore an objective risk assessment was not possible. He referred to the findings of SZEKACS & DARVAS from 2003 (reference not indicated) which had shown that two butterflies common in agricultural areas might be at risk. He emphasised that not all of the non-target species at risk to MON810 had been identified in Europe. Dr. Andow stated the following points which were not reflected in the SCP opinion: First additional assessments should have been conducted on lacewings and monarch butterflies in order to determine the relevance in the field as the aim of a tiered risk assessment protocol is to expose organisms to concentrations higher than considered typical in the field. Therefore experimental positives from laboratory studies should undergo additional evaluations. Both lacewings and monarchs had been adversely affected by the Cry1Ab toxin in the lab experiments. Secondly the specificity of the Cry1Ab toxin seemed to be broader than previously expected.
b. With respect to risk on **soil organisms** Dr. Andow discussed some scientific aspects that were left unconsidered by the SCP. First the actual rates and degradation processes for large proteins in soils are poorly understood. Second, the Bt toxin load in maize fields can be substantial which make large-scale effects possible. Third, it is known that the Bt toxin in the soil can have adverse effects on earthworms.
c. Referring to **resistance risk and management** Dr. Andow stated that the following points were not reflected in the SCP opinion. First the rate of market penetration of Bt maize had been faster than predicted in the US which contradicted the prediction of the SCP that market penetration would be slow. Secondly resistance would evolve locally and therefore refuges must be available wherever Bt maize is locally used and refuges are required from the beginning of Bt planting. Resistance management is the responsibility of each farmer who uses Bt maize and each farmer should be required to implement measures such as setting up of refuges.

3.1.8 References

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3.2 GM-Maize T25

3.2.1 Introduction

An application for authorisation of the genetically modified maize T25 according to Directive 90/220/EC (notification C/F/95/12/07) was submitted in May 1996 by AgrEvo France to the French Competent Authority. The transformation event T25 is a result of the genetic modification with the *pat* gene, derived from the bacterium *Saccharomyces viridochromogenes*, the bacterial replication origin *ori-Puc* and a truncated fragment of the bacterial gene *AmpR*. The *pat* gene encodes the enzyme phosphinothricin-acetyltransferase which catalyzes the conversion of phosphinothricin (PPT), the active ingredient in the glufosinate-ammonium, to an inactive form, thereby conferring tolerance to the herbicide. Maize T25 was notified to be used for the purpose of multiplication of inbred lines and seed production of lines and hybrids, cultivation, import of grain for feed and food purposes as well as industrial use.
Following a favourable opinion of the Scientific Committee on Plants in 1998 consent was given for the placing on the market of genetically modified (GM) maize T25 (Commission Decision of 22 April 1998). Subsequently, the placing on the market of this maize was prohibited by the Austrian Competent Authority in April 1998. In 2001 the Scientific Committee on Plants issued a second positive opinion on GM maize T25 replacing the original opinion of 1998.
In 2000 and in 2001 the Scientific Committee on Plants and in 2004 and 2006 the EFSA GMO Panel issued opinions on the Austrian safeguard measure concerning maize T25. In the WTO Panel report scientific experts commented on the scientific reasoning of the Austrian import bans. The following chapters will briefly describe the main information or arguments included in these documents.

3.2.2 Information from the original dossier

The original dossier of the genetically modified herbicide tolerant (GMHT) maize T25 provided by the notifier (notification C/F/95/12/07) contained an **environmental risk assessment** which comprised the following points:

a. With respect to "*Survival, Multiplication, Dissemination of the GMO in the Environment*" the notifier provided information on agronomic characteristics such as plant morphology, stand count, volunteers, flowering time, germination rate, plant height, ear height, time to pollen shed and silk emergence, crop injury due to chemical application, root and stalk lodging, both qualitatively and quantitatively. All comparisons of these parameters showed no difference between GM and non-GM plants. Furthermore, the notifier evaluated disease and pest characteristics in US field trials from 1992-1994 but found no differences.
b. With respect to the risk assessment parameter "*Interactions of the GMOs with the Environment*" the notifier included information on field tests across the US since 1992 and in Europe (France). The notifier stated that no alteration of population levels of beneficial insects, birds or other species were found although in the Appendix cited (Appendix 13) no data on this is provided.
c. Evaluation of the "*Likelihood that the GMHP becomes more persistent or more invasive*" the notifier concluded that the transformation with the *pat* gene did not alter these characteristics of maize.
d. The evaluation of the parameter "*impact on agricultural practices*" the notifier stated that a good efficacy can be achieved with one or two applications in the range of 150-450 g/ha of glufosinate-ammonium. The notifier further concluded that glufosinate tolerant corn will provide a valuable new weed management tool to corn producers not exclusive of the other herbicides. However, the data which were provided give an indication that there were efficiency problems for some weeds in the trials (Appendix 12, pages 588 and 630) and an in-depth response of weeds to the new management method was not evaluated. With respect to volunteers the notifier stated that weed management practice in crop rotation will not be modified by the introduction of the GM maize.

Concerning **health effects** the original dossier of the genetically modified herbicide tolerant maize T25 provided by the notifier (notification C/F/95/12/07) includes an assessment of possible toxic and allergic properties and of the substantial equivalence of the GM crop[6]:

a. With respect to toxicity assessment the dossier included a 14-day repeated dose oral toxicity study in rats (full report included in the dossier) and in-vitro digestibility studies using the PAT protein (RCC 1996*, incomplete draft report, completed and submitted as full report during the review process). The study was conducted according to OECD Guideline 407 (1995). Four groups of 5 male and 5 female rats were fed 50.000 and 5.000 ppm representing a 1.000 or 100 fold doses compared to normal exposure expected. Differences detected (lower glucose level in one group of males, slightly higher levels of cholesterol and phospholipids in three group and slightly higher triglyceride levels in one group of females) were interpreted as being related to dietary composition (partly because some effects were also observed in one group where no PAT protein was administered). A toxicity study has also been performed on a metabolite of phosphinothricin, N-acetyl-glufosinate (summary included in the dossier)[7].

[6] Some of the references in this subsection (marked with an asterisks) are taken from the dossier and are not included in the reference section of this review. Full references can be obtained from the original dossier.
[7] Considered outside the scope of this review.

Test protein was produced using E.coli (and for in-vitro digestibility studies also crude protein extracts from maize T14 leaves[8] were used). Evidence showing the equivalence of the test protein to the protein expressed in maize was provided (including molecular weight, immunoreactivity and insecticidal activity).
The PAT protein was shown to be both heat and acid labile (SCHULZ 1993*, included in the dossier). The protein is being degraded in in-vitro studies using gastric juices from swine, chicken and cattle (SCHNEIDER 1993*, SCHULZ 1993b*, summaries included in dossier). Degradation at pH 1,5 was complete after one hour (simulating swine, chicken and rennet fluid), takes longer at higher pH (simulating paunch digestion). Inactivation of the PAT protein present in a crude protein extract from canola [sic!] was shown to be rapid, 100% after 2,5 min. of rennet treatment and 90% after 30 min. in the bovine system. These results were considered relevant for T25 as the same protein is present in both GM plants (SCHULZ 1994c*, included in the dossier). Degradation was also demonstrated in simulated gastric fluids (SCHULZ 1994d*, full report not included in the dossier) and was observed at 5 sec. (for pure PAT protein) and at 1 min. (for a crude protein extract from leafs of T14). 100% inactivation was observed by 3 minutes (only for crude protein extract, pure PAT was not tested).

b. With respect to allergenicity assessment evidence was provided that there are no glycosylation motives in the PAT protein sequence. Sequence comparison of the PAT protein to sequence databases did not reveal any significant homology to known toxins or allergens (ECKES 1994*, included in the dossier). Inhalation was explicitly not considered as relevant route of exposure because the PAT protein is not expressed in pollen.

Substantial equivalence was claimed based on comparative analysis of silage and kernel of four transgenic lines and four isogenic controls. Compositional analysis was based on field tests in USA (2 field sites in 1994) and Europe (five sites in France, 1995) (full reports not included in the dossier). Agronomic characteristics, germination rate, flowering time, disease and pest characteristics were partly considered in the comparative studies.

3.2.3 Opinions of the Scientific Committee on Plants (1998, 2001a)

In its opinion of 10 February 1998 the Scientific Committee on Plants evaluated the possible **environmental risks** addressed in the notification of T25 maize (SCP 1998):

a. The Committee concluded that there was a limited risk of escape of GMHT maize T25 due to poor dispersal as well as absence of sexually-compatible wild relatives. As there is no detectable PAT activity in pollen, dispersal and outcrossing frequency was regarded not to be different from other maize. Therefore the risk for **genetic transfer** was considered to be remote.
b. Regarding the risk of GM **volunteers** the Committee concluded that any surviving volunteers could be controlled by agronomic practices and the use of alternative non-selective herbicides in areas free from winter frost. Therefore the risk of volunteer maize plants surviving was considered to be remote.
c. With respect to risks for **non-target organisms** the Committee concluded that no qualitative differences in susceptibility of GM and non-GM maize T25 to insects and diseases was found. However, the Committee admitted that no direct data from field experiments had been provided by the notifier. Risks to soil organisms or soil function through degradation of plant material or risk due to contamination of ground water were considered to be extremely low although no reasoning on how this conclusion was reached was given by the Committee.
d. **Resistance and tolerance issues** were considered by the Committee not to be problematic due to the low risk of gene transfer from GM maize to other plants.

[8] Another transformant from the same transformation event as T25. T14, though, contains three copies of the pat gene as opposed to T25 which contains one copy.

Concerning **health risks** the Scientific Committee on Plants concluded that "there is no significant risk to humans or livestock following ingestion of the gene product." (SCP 1998).[9]:

a. With respect to toxicity assessment the Committee based its conclusion on acute toxicity tests, on the non-pathogenic source organism *Streptomyces viridochromogenes*, and on broiler chicken studies fed on the T25 maize (no effect in growth performance and body composition).
b. With respect to allergenicity assessment the Committee draw on homology comparisons to known allergens. The Committee, however, critically mentioned that "the applied in vitro methodology to study the survival of the PAT can be improved. Similarly, use of the isolated protein in toxicity studies does not adequately model degradation of the same protein when fed as an integral component of the diet".

The Committee considered the T25 maize as **substantially equivalent** to its non-GM counterpart except for the introduced trait. Nutritional composition and investigated natural anti-nutritional factors are considered to be within the normal range. Thus the introduction to the PAT gene "does not seem to cause any negative pleiotropic effects on the characteristics of the plant relevant to its safety for human and animal consumption".

With the opinion of 5 September 2001 (SCP 2001a) the Scientific Committee on Plants replaced its previous opinion of 10 February 1998.
With respect to the environmental risk assessment the opinion regarding genetic transfer, volunteers, non-target organisms and resistance/tolerance remained unchanged from the opinion of February 1998.
With respect to health risk assessment, though, the SCP largely reiterated its conclusions from February 1998. Additional criticism was raised on the use of the isolated protein in toxicity studies which "does not adequately model degradation of the same protein when fed as an integral component of the diet."[10]

3.2.4 Reasons for the prohibition of GM maize T25

After consent had been given in order to authorize GMHT maize T25 by the European Commission in April 1998 (EC 1998), Austria prohibited the import of T25 maize (28 April 2000) according to Art. 16 of Directive 90/220/EEC. Health aspects were, however, not explicitly mentioned in the reasons provided by the Austrian Authorities (BMGF 1998a). Considering environmental aspects only, the main points supporting this import ban were the following:

a. No **realistic conditions of the use of the herbicide** and corresponding agricultural practices were used during the assessment of T25. The genetically modified plant should not be evaluated separately without the use of the herbicide. If herbicide use is inadequate or too high then there is a risk of development of resistant weed species.
b. **Long term effects** of the herbicide use should not be evaluated independently from the respective GMHT plant and long term effects of glufosinate-ammonium in combination with GMHT maize T25 were not fully investigated. There was no **monitoring program** foreseen, neither in the notification, nor in the consent, especially regarding possible long-term effects. If the notification was submitted under the new, revised Directive 2001/18/EC this would have allowed an integrative view of the herbicide use with the GMHT maize as well as possible long term effects.

[9] The Opinion also covered assessment of herbicide residues as an issue for animal and human health along with gene transfer to human or animal cells and intestinal micro-organisms. This is however, considered outside the scope of this report.
[10] See footnote 9.

c. An evaluation of **regional aspects** of the herbicide use should be considered. The use of herbicide tolerant plants should consider regional characteristics, especially in areas where the use of herbicides is actually necessary. With respect to a "good agricultural practice" a too highly dosed or locally inadequate herbicide use should be avoided in order to minimise the risk of resistance development of weeds. Additionally, approval conditions were not foreseeing a protection of ecologically sensitive areas. For this argument the study of HOPPICHLER (1999) was cited. The long term use of herbicide tolerant plants was considered to endanger **ecologically sensitive areas** and these areas should be considered within the risk assessment procedure.
d. A major further point was the risk of contamination of conventional maize – the **coexistence issue**.

3.2.5 Opinions of the Scientific Committee on Plants (2000, 2001b)

In its opinion of 30 November 2000 the Scientific Committee on Plants replied to the Austrian invoke of Article 16 of Directive 90/220/EEC (SCP 2000). The Committee stated that the Austrian document did not contain any new scientific information with relevance to the original risk assessment. In accordance with the scope of the reasons provided by Austria the Committee did not discuss any issues related to health risks or substantial equivalence.

a. With respect to the possible **genetic transfer** of the herbicide tolerant trait to wild species the Committee stated that the possibility of genetic transfer would be remote. Genetic transfer was not considered to be a biodiversity issue and herbicide tolerance transfer from maize into the environment was estimated extremely unlikely. Furthermore it was stated that the gene encoding the PAT protein was not expressed in pollen and therefore pollen dispersal and outcrossing frequency should not be different and the herbicide tolerance trait should not transfer to any other varieties of cultivated maize.
b. Regarding the potential of a **resistance development** by other species the Committee stated that any volunteer maize plants may be controlled by other agronomic practices. **The possibility of a resistance development in weed species was not considered in the opinion of the SCP.**
c. Regarding the study concerning ecological sensitive areas referred to by Austria (HOPPICHLER 1999) the Committee stated that no new scientific information relevant to the original scientific risk assessment of 1998 was provided.
d. Regarding **coexistence** the Committee stated that most released pollen is deposited close to crop plant and if separation distances developed for seed production would be observed, pollen transfer to adjacent varieties should be minimised.

Other aspects mentioned in the Austrian prohibition such as the lack of a monitoring program, long term effects or regional aspects were not considered in this opinion.

On 5 September 2001 the Scientific Committee on Plants issued a new opinion on the Austrian invoke replacing its original opinion of 30 November 2000 (SCP 2001b). However, this new opinion did not state any new arguments but rather re-stated its arguments already published in its opinion of 30 November 2000.

3.2.6 Opinions of the EFSA GMO panel (2004, 2006)

On 8 July 2004 an opinion of the EFSA GMO panel on the Austrian safeguard measure invoking Article 23 of Directive 2001/18/EC was published (EFSA 2004). The European Commission had requested EFSA to investigate whether the submissions of AT contain "*any new or additional information affecting the Environmental Risk Assessment or re-assessment of existing information on the basis of new or additional scientific knowledge*

such that detailed grounds exist to consider that the above authorized GMOs …constitute a risk to human health or the environment".
The opinion did not discuss human health issues with respect to maize T25. The Panel also dismissed the evidence provided by Austria that critically reviews and assesses the validity of toxicity assessment, allergenicity assessment and the practice of substantial equivalence in a number of Directive 90/220/EEC and Novel Food dossiers including maize T25 (SPÖK et al. 2002, 2003a, 2003b).[11] The panel correctly stated the overall aim of the reports but appears to have overlooked the very detailed critical review of the risk assessment information provided in the dossiers maize MON810 and T25.
(For further comments see also Chapter 3.1.6, p.13f).

In the meantime Austria had provided new information to the Commission in order to support its safeguard measure (February 2000), namely four scientific publications for environmental aspects (MORIN et al. 2003, ZWAHLEN et al. 2003a and 2003b and SAXENA et al. 2002) as well as several conference abstracts (e.g. BIRCH et al. 2003).
In its opinion regarding the GMHT maize T25 the EFSA GMO panel stated that the ecological impact of herbicide tolerance genes would depend on the use of the herbicide and not on the transgenic event and that herbicide tolerant plants may also enable cultivation practices that could lead to an increase in-field biodiversity. Other aspects or risks regarding the Austrian justifications of the prohibition of T25 maize were not considered in this opinion.

In its second opinion on safeguard measures of Member States of 29 March 2006 (EFSA 2006) the EFSA GMO panel refers solely to the antibiotic resistance marker gene of GMHT maize T25 stating that the risk of horizontal gene transfer is very low and although the use of these genes should be avoided in GM crops the GMHT maize T25 contains only a partial gene which is therefore non-functional. Other aspects or risks specified in the Austrian justification of the prohibition of T25 maize were not considered in this opinion. Again the opinion did not discuss human health issues with respect to maize T25.

3.2.7 WTO Panel Report – comments from scientific experts advising the Panel

In the WTO Panel Report Dr. Andow, a the scientist from the group of selected experts advising the WTO Panel, considered the individual justifications provided by Austria in its prohibition of T25 maize. (For a discussion of the scientific expertise to the WTO Panel see Chapter 3.1.7, p.14)

a. With respect to the argument that no **realistic conditions of the use of herbicide** were assessed Dr. Andow considered that Austria had not provided scientific evidence that risks would exist, such as risks to non-targets or other biodiversity risks and therefore this argument cannot be used to justify the safeguard measure.
b. With respect to **the lack of a monitoring program** Dr. Andow considered this to be a risk management issue which should be justified by reference to a specific risk. He also stated that long-term and secondary effects are difficult to predict in a pre-release risk assessment and therefore it could not be used to justify a safeguard measure.
c. **Regional aspects** were also considered by Dr. Andow to be a risk management issue and should also be justified by reference to a specific risk which was not done by Austria.

[11] "Three reports from Austria […] investigated the requirements for toxicology and allergenicity related safety evaluations of genetically modified plants and derived products destined for human and/or animal consumption. The results and recommendations of these two studies are intended to contribute to national and international discussion on the improvement of safety evaluation of GM products. […] The three reports do not provide any new scientific data to indicate adverse affects on human and animal health or the environment of the maize events Bt176, MON810 and T25." (EFSA 2004)

d. Considering the **coexistence issue** Dr. Andow stated that no harm arising from pollen transfer of GMHT maize has been specified by Austria and therefore it is difficult how this reason could justify a safeguard measure.

3.2.8 References

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4 Latest scientific evidence relevant for a review of the Austrian safeguard measures - Environmental risks

4.1 GM-Maize MON810

This chapter reviews recent scientific evidence concerning the Austrian issues of concern in connection with the national safeguard measure to the genetically modified maize line MON810 notified by Monsanto France in 1996 according to Directive 90/220/EEC.
For this review the reasons originally provided in support of the prohibition, together with the opinions of the Scientific Committee on Plants and of the EFSA GMO Panel were taken into consideration.
The following issues concerning environmental concerns are addressed specifically in the following sections:

- Possible unintended effects on non-target organisms
- Uncertainties concerning the resistance management by a refuge strategy
- Likelihood of the development of secondary pests
- Lack of a monitoring plan

4.1.1 Possible unintended effects non-target organisms

a) One major argument in support of the prohibition of placing on the market of maize MON810 was the possible occurrence of unintended effects on non-target insects. It has to be emphasised that the original assessment of risks to non-target organisms was not satisfactory and the data provided were not sufficient to show that Bt maize MON810 does not have any adverse effects on non-target organisms. The original dossier of Bt maize MON810 contained results of laboratory tests of the Cry1Ab toxin which were carried out on a very limited number of non-target organisms. Additionally, the field test data which were provided in the original dossier cannot be considered as current scientific state of the art as basic information on the methods used and statistical evaluation of the results were incomplete, e.g. the evaluation of beneficial insects which was not carried out for each arthropod group separately. Only a few trials were carried out under European conditions with the aim to test the efficacy of the inserted trait rather than possible adverse effects on non-target organisms.
b) Specifically, effects on non-target Lepidoptera were not considered in the original risk assessment. Recent scientific studies have continuously supported previous indications that certain non-target Lepidoptera are at risk from the consumption of Bt maize MON810. Prolonged developmental time and reduced survival of monarch butterfly larvae were shown when exposed to pollen of Bt maize MON810 either for short or for prolonged time under field conditions, possibly resulting in up to 25% fewer surviving larvae (DIVELY et al. 2004). Also adverse effects on pupae and adults of the monarch butterfly were reported in this study thus indicating possible effects on this non-target butterfly from the cultivation of Bt maize MON810. Possible adverse effects on non-target Lepidoptera through the consumption of Bt anthers (ANDERSON et al. 2004, FELKE & LANGENBRUCH 2005) and especially the combined effects of pollen and anthers of Bt maize containing the Bt toxin (ANDERSON et al. 2005) have so far not been taken into consideration and pose a risk for non-target butterflies due to cultivation of Bt maize MON810. Also Lepidoptera other than the Monarch butterfly have been shown to suffer sublethal or lethal effects when exposed to Bt maize (VOJTECH et al. 2005, DUTTON et al. 2005) and previous indications that several common butterflies occurring in agricultural habitats could be at risk from Bt maize MON810 cultivation are supported by recent data (SZEKACS & DARVAS 2006). It is still unknown which other non-target butterflies might be adversely affected by the consumption of Bt corn pollen or anthers.

c) An assessment of risks to non-target butterflies especially relating to European agricultural conditions has not been carried out in the risk assessment. It is known that many species of butterflies are present in agricultural areas (FELKE & LANGENBRUCH 2005). Out of 215 butterfly species occurring in Austria, 152 species have been reported from agricultural areas and more than half of those species are already classified as either near threatened, vulnerable, endangered or critically endangered (TRAXLER et al. 2005) and may be further at risk from Bt maize cultivation. These species should be subject to a separate susceptibility evaluation before planting the respective Bt maize in order to be able to set risk management measures, if needed. The differential toxicity of a certain Bt toxin depending on the species was also acknowledged by the SCP in its opinion of September 1999 (SCP 1999).

d) There is evidence that Bt corn releases the Bt toxin in root exudates (SAXENA et al. 2004), decomposes slower in the soil than non-Bt plants (FLORES et al. 2005), and that the Bt toxin persists in environmental compartments such as soils for months retaining its insecticidal activity (SAXENA & STOTZKY 2002, STOTZKY 2004). Moreover, the Cry1Ab toxin can be found in several organisms thus exposing a range of non-target organisms of higher trophic levels which are present in agricultural fields (HARWOOD et al. 2005, ZWAHLEN & ANDOW 2005). Therefore adverse effects of this toxin can be expected to occur in a range of non-target arthropods. However, only a very limited number of arthropods occurring in Bt maize fields have so far been included in the risk assessment and it is unknown which arthropods that are exposed to the Cry1Ab toxin are actually adversely affected. Recent data show that non-target organisms such as parasitoids (VOJTECH et al. 2005, PRÜTZ & DETTNER 2004, PILCHER et al. 2005), hyperparasitoids (BRINK et al. 2004, PRÜTZ et al. 2004), coccinellids (SCHMIDT et al. 2004) and carabids (MEISSLE et al. 2005) do actually experience adverse effects. This evidence of adverse effects of the Cry1Ab toxin on other non-target organisms than lepidopteran species might either be due to reduced prey quality or possibly indicate that the Cry1Ab toxin is not as specific as previously thought.

4.1.2 Uncertainties concerning the resistance management by a refuge strategy

The uncertainty about the effectiveness of the proposed refuge strategy in order to prevent the development of resistance in the European corn borer was another major argument for the original Austrian prohibition of the placing on the market of Bt maize MON810. The insect resistance management (IRM) plan provided by the notifier was very general and did not give any detailed information on how it will be implemented. Although the notifier recommended a managed refuge approach and stated that a surveillance program would be implemented, no exact information on the implementation details of this program was given. With respect to susceptibility studies the notifier referred only to studies in the US and Italy. However, before introducing Bt maize, baseline susceptibilities and initial resistance allele frequencies should be investigated for each separate European corn borer population or even sub-population (see CHAUFAUX et al. 2001) as these parameters influence the rate at which resistance will evolve and may differ among different populations of this insect species (HUANG et al. 1997). IRM plans have to be carried out in combination with systematic monitoring in order to detect resistance when it occurs. As baseline susceptibilities and other ecological factors between different populations of the respective pests as well as agronomic practices might differ locally, the results of the experiences gained from Bt maize cultivation in Spain might not be applicable to other European, and especially Austrian, agricultural systems. Therefore an adaptation of the IRM strategy must be envisaged. In its opinion the SCP (1999) pointed out that the introduction of Bt maize is expected to be slow and therefore Bt crops would be surrounded by "natural refuges" for some time. However, as resistance evolves locally, refuges must be planted wherever Bt maize is locally used and should be required from the beginning of the introduction of this GM maize by

each individual farmer. Furthermore, as several varieties of MON810 maize are already approved within the European Union for cultivation it can be expected that the adoption rate of Bt maize MON810 will be high in Europe. High adoption rates of Bt maize have also been experienced in the US (CARPENTER & GIANESSI 2001) although slow introduction of this type of GM maize had been previously predicted. Therefore baseline information about the pest biology and ecology and a workable IRM plan must be at hand before commercialisation of Bt maize.

4.1.3 Likelihood of the development of secondary pests

Effects of Bt crop cultivation on other pests and the development of secondary pests and consequently the additional use of synthetic plant protection products as reported from Bt cotton cultivation in Australia were another major concern which supported the original prohibition of Bt maize MON810 by Austria. As the SCP (1999) stated in its opinion on the Austrian prohibition of maize MON810 a monitoring should also cover secondary pests. Recent investigations of Bt cotton cultivation in China have shown an extraordinary increase of other pest species such as leaf bugs (WU et al. 2002) and consequently a rise in pesticide applications (WANG et al. 2006). However, secondary pests were neither considered in the risk assessment nor in a monitoring plan.

4.1.4 Lack of a monitoring plan

According to the European Directive 2001/18/EC each notification of a GMO must contain a plan for monitoring in accordance with Annex VII with the aim to confirm the assumptions from the risk assessment and to identify the occurrence of adverse effects of the GMO or its use on human health or the environment which were not anticipated in the risk assessment. However, such a monitoring plan has not been provided by notifier. Specifically the absence of effects on non-target organisms, which was stated by the notifier in the risk assessment, as well as the possible occurrence of secondary pests should be subject to a monitoring as already suggested by the SCP (1999).

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4.2 GM-Maize T25

This chapter reviews recent scientific evidence concerning the Austrian issues of concern in connection with the national safeguard measure to the GM maize line T25 notified by AgrEvo France in 1996 according to Directive 90/220/EEC (Notification C/F/95/12-07). For this review the reasons originally provided in support of the prohibition, together with the opinions of the Scientific Committee on Plants and of the EFSA GMO Panel were taken into consideration.
The following issues concerning environmental concerns are addressed specifically in the following sections:

- Risks for weed communities
- Lack of a monitoring plan
- Regional aspects in combination with coexistence issues

4.2.1 Risks for weed communities

The introduction of new weed management regimes for genetically modified herbicide tolerant (GMHT) maize requires an assessment of how these new herbicide regimes could affect weed communities. However, the risk assessment data presented in the original dossier of GMHT maize T25 do not fulfil these requirements. According to the risk assessment procedure in Directive 2001/18/EC regulating the cultivation of a GMO in Europe, the assessment of adverse effects due to changes in management, including changes in agricultural practices is required (Annex II, C2.). The notifier states that "*…GTC (glufosinate tolerant corn) will provide a valuable new weed management tool to corn producers not exclusive of the other herbicides…"* (Notification C/F/95/12-07) but has not provided any data to show the behaviour of the GMHT maize T25 under European conditions with respect to agricultural practices. As weeds are the major target organisms of the GMHT maize/herbicide complex and a change in weed management is predicted, a proper assessment of the effects on weed communities is required. The notifier has failed to provide in-depth data on weeds, the target organisms, and interactions between the GMO and target organisms of GMHT maize T25 as required both under Directive 90/220/EEC (Annex II, IV. C.3 and C.4) as well as under Directive 2001/18/EC (Annex IIIB, D.).

a) Insufficient weed control / build up of herbicide resistant weeds

There is evidence that glufosinate-ammonium, the active ingredient of the non-selective herbicide used with GMHT maize T25, provides insufficient control of certain weeds, such as some grasses but also other species (O`SULLIVAN & SIKKEMA 2005, OECD 2006). Consequently, naturally resistant weed species are expected to replace those species effectively controlled by the non-selective herbicide used with the herbicide tolerant crop, leading to a shift in weed communities in the long run.
Experience from crop systems continuously using glyphosate, a different non-selective herbicide, in combination with GMHT plants as the exclusive weed management system shows that shifts in weed communities do actually occur. Selective action of a single management regime on weed assemblages can lead to profound shifts within a few years and such shifts have been reported following cropping of glyphosate tolerant plants in the US (WICKS et al. 2001 and HILGENFELD et al. 2000 cited in OWEN & ZELAYA 2005). A recent survey in the US has shown that shifts in weed communities have occurred in glyphosate-resistant cotton and soybean, crop plants where herbicide tolerant cultivars are used to a large extent (CULPEPPER 2006). There is evidence that the evolution of herbicide resistance in weeds occurs due to the strong selective pressure of a weed management system relying exclusively on a single non-selective herbicide. Currently several weed species are globally or regionally resistant to glyphosate (www.weedscience.org) and manufacturers of GMHT corn already recommend using additional pre-emergence herbicides in order to control resistant species accordingly (www.weedresistancemanagement.com).
Nevertheless, as the agricultural practice in the use of the non-selective herbicides glyphosate and glufosinate-ammonium is comparable, it can be expected that the use of glufosinate-ammonium will have a similar effect on weed communities.
In Austria the use of glufosinate-ammonium as the active ingredient in plant protection products is allowed in non-agricultural as well as in agricultural areas such as vineyards and orchards but also in crop production of potato, sugar beet, vegetables, caraway and maize. However, the major use is for pre-emergence weed control in these crops. If the existing use-pattern is extended to one or several post-emergence applications then the likelihood of the development of resistant weed populations will significantly increase.
As a consequence it can be expected that the frequent use of a non-selective herbicide such as glufosinate ammonium will increase the selective pressure on weeds as suppression of all weeds is rarely achieved thus increasing the dominance of a few species and finally the prevalence of resistant weed species.

b) Increase in herbicide use

Evidence from literature indicates that a single post-emergence application of glufosinate-ammonium alone is not sufficient to provide satisfactory weed control. Therefore either additional residual herbicides are necessary in order to control certain weed species (O`SULLIVAN & SIKKEMA 2005, HAMILL et al. 2000, BRADLEY et al. 2000) or sequential applications of glufosinate-ammonium are required for effective weed control (KRAUSZ et al. 1999). Independent research carried out by universities in the US has shown that weed control in GMHT maize by a single application of glufosinate-ammonium was inadequate and that it is recommended to spray either twice or to spray a conventional herbicide pre-emergence followed by one post-emergence application of glufosinate-ammonium (GIANESSI et al. 2002).
This has also been shown for other GMHT cropping systems, such as glyphosate-tolerant GM maize, for which the use of further, pre-emergence herbicides is recommended in order to achieve a better weed control (NURSE et al. 2006). Also manufacturers of GMHT corn already recommend using additional pre-emergence herbicides in order to control resistant species accordingly (www.weedresistancemanagement.com). It is even expected that future changes of herbicide use will be driven by the reductions in effectiveness of non-selective herbicides such as glyphosate due to weed shifts and weed resistance (YOUNG et al. 2006). The use of additional herbicides is also expected to be justified by the higher achievable corn yields when glufosinate is applied in combination with residual herbicides (HAMILL et al. 2000) as insufficient weed control reduces yield.

As the experience has shown that weed control failures are dealt with by either increasing the use rate or the number of non-selective herbicide applications (YOUNG et al. 2006), an increase in herbicide use and/or applications can also be expected for GMHT maize T25.

c) Further loss of endangered weed species

Herbicide use is a major cause of depletion of weed diversity and endangerment of weed species in Austria (TRAXLER et al. 2005a). Weed species loss in Austrian agro-habitats and also replacement of rare and endangered species by resistant neophytes is well documented. Currently 56% of the obligate weed species (i.e. those weeds that are dependent on a specific location) and 27% of the facultative weed species (i.e. those weeds that are not exclusively dependent on a specific location) are classified as almost extinct, highly endangered, endangered, potentially endangered or regionally endangered according to the red list of plants in Austria (NIKLFELD & SCHRATT-EHRENDORFER 1999). TRAXLER et al. (2005a) classified these rare weed species into biodiversity hotspots in order to show areas in which the conservation of the weed flora is considered absolutely necessary as those regions are highly important for the conservation of national diversity of agro-associated plants. Additionally, approximately three forth of the biotope type "fields, field edges, vineyards and ruderal biotopes" are classified within a certain category of endangerment (TRAXLER et al 2005b) which emphasizes the need for conservation of these biotope types which are prevalent in agriculturally used areas. The cultivation of GM maize T25 in combination with extensive use of a non-selective herbicide might therefore further contribute to the decline of already endangered weed species or biotope types.

4.2.2 Lack of a Monitoring plan

According to Directive 2001/18/EC the notification shall contain a plan for monitoring in accordance with Annex VII (Article 13, 2. e) which has to cover long term effects of the GMO on the environment and human health. The implementation of a post-market monitoring scheme to monitor changes in the management practice as well as herbicide usage due to the use of T25 maize in combination with the herbicide containing glufosinate-ammonium is an obligatory task to be carried out by the notifier. The procedures of the new directive allow an integrative view of the long-term effects due to herbicide use which would have to be considered in an adequately designed monitoring plan. As there are currently no provisions for a monitoring of herbicide use in directive 91/414/EEC it is considered as essential to monitor herbicide use and its effects in conjunction with the herbicide tolerant maize T25.
Especially long term effects of the herbicide use cannot be evaluated independently from the respective GMHT plant and effects on weed communities of glufosinate-ammonium in combination with GMHT maize T25 were not covered by the risk assessment (see point no1). Therefore the implementation of a monitoring plan is needed in order to satisfy the requirements of Directive 2001/18/EC and to take into account possible long-term effects of this GMHT plant. Furthermore, monitoring the occurrence of weeds that are not controlled by the herbicide application on GMHT maize T25 is important in order to account for possible weed resistance due to the use of glufosinate-ammonium.

4.2.3 Regional aspects in connection with coexistence issues

In case of placing on the market of GMHT maize T25 the cultivation of this maize would raise questions regarding coexistence issues within the Austrian agriculture. Since there are currently no harmonized, legally binding provisions regarding coexistence measures (including liability) of genetically modified maize and conventional or organic maize, the cultivation of T25 maize would substantially endanger the existence and development of the organic sector in Austrian agriculture. Austria has the highest share of organic agriculture within the European Union with almost 10% of the agricultural area being organically cultivated (EC 2005).

In a report to the European Council and the European Parliament the European Commission has emphasized the unacceptability of the establishment of GMO-free

regions or the limitation of GMO cultivation by setting strict measures at national or regional level and has emphasized the drafting of a proposal establishing threshold levels for seeds (EC 2004). As Commissioner Dimas has underlined in his speech at the conference on co-existence in Vienna on 5th April 2005 (Speech/06/224), member states should be able to put in place their own coexistence measures. He also supported that the Commission clarifies the measures that can or cannot be legally accepted.

So far neither this clarification was given by the European Commission nor have thresholds for seeds been established. Due to the continuation of this unclear situation the use of GMHT maize may lead to a negative impact on the Austrian agriculture and may therefore have unforeseen socio-economic implications.

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5 Latest scientific evidence relevant for a review of the Austrian safeguard measures - Health risks and Substantial Equivalence

5.1 Introduction

This chapter reviews recent scientific evidence relevant to Austrian safeguard measures for the genetically modified maize lines MON810 and T25.
The focus of this review lies on toxicity assessment, allergenicity assessment and on the application of the concept of substantial equivalence. It is based on earlier reviews of GM crop risk assessment dossiers (SPÖK et al. 2002, 2003a, 2003b, 2004) and on subsequent work (e.g. SPÖK et al. 2005).
The main issues discussed in this chapter are fairly similar for both GM events. Unlike environmental issues it does not matter that much if the introduced protein is conferring herbicide or insect tolerance as long as the GM crop are cultivated, handled, processed and used in the same or a similar way.
The review looks at three different levels of risk assessment: First, on the level of the overall approach in health risk assessment. Second, on the level of the particular methods applied to provide safety relevant data and the kind of non-experimental evidence (e.g. history of safe use) included in the dossiers. Third, on the level of the particular experiments pursued and the data provided in each dossier.
All three levels are important for drawing safety conclusions. Shortcomings and limitations can directly affect the validity of safety conclusions, can increase the scientific uncertainty or diminish the verifiability of these conclusions. Consequently, the review on the first and second level provides more general conclusions which might also be valid for the risk assessment in other dossiers. The review of the particular tests conducted and results provided in each dossier (third level) is depending on the availability of detailed descriptions and full reports. Such details are especially lacking in the maize MON810 dossier whereas more details are provided in the maize T25 dossier. While details on compositional analysis are not included in either dossier, summary tables, figures and interpretations of such are provided. Given the limitations in time and scope and the availability of detailed data the analysis on the third level largely focuses on compositional analysis.

Given the similarities in the risk assessment of both GM events reviewed, the sections on GM maize MON810 and T25 are following the same structure and are organised into four main sections:

- Toxicity assessment
- Allergenicity assessment
- Application of the concept of substantial equivalence
- Overall verifiability of the safety conclusions in the dossier.

Conclusions are provided at the end of each section.

5.2 GM-Maize MON810

5.2.1 Toxicity assessment

Toxicity assessment in the original dossier basically relies on an acute toxicity test and on in-vitro digestibility studies using a bacterial CryIA(b) Protein. In addition, the applicant provided homology comparisons with known toxic proteins and argued with the safe history of Bt toxins.
The assessment as it is documented in the dossier cannot be considered sufficient for the following reasons (along the lines of SPÖK et al. 2004).

Acute toxicity test on rodents of the CryIA(b) protein cannot provide clues for sub-chronic and chronic effects. The assumption that proteins can only act via acute mechanisms is not backed up by a solid empirical basis. This has meanwhile be acknowledged by recent Guidance documents, which ask for 28-day repeated-dose sub-acute test (EFSA 2004 and NL BIOSAFETY COUNCIL 2003).
According the British Toxicology Society studies on industrial and agrochemical indicated that for 70% of the compounds toxicological findings in the 2-year rodent test were also seen in or predicted by the 3 month subchronic test (British Toxicological Society 1994 quoted by KUIPER 2006). In other words, 30% of the effects would not even be seen or predicted in case of sub-chronic studies.

In-vitro digestibility test
Several authors discussed that choice of parameters including enzyme/test protein ratio, pepsin purity, target protein purity, detection method, buffer pH could considerably influence the outcome (FU et al. 2002, BANNON et al. 2003, THOMAS et al. 2004). This is particularly true for the pepsin/test protein ratio as shown by FU et al. (2002). Using the example of the major egg allergen ovalbumin they showed that a too high ratio of enzyme/test protein could deliver false negative results. Also, there does not seem to be a correlation between results of simulated gastric fluid assays and simulated intestinal fluid assay. Furthermore they pointed at the huge differences of enzyme/protein ratio 1:250 to 5000:1 (weight). In contrast ASTWOOD et al.(1996) recommended a ration of 13:1, a recent FAO/WHO consultation about 6.5:1 (FAO/WHO 2001). Studies on proteolytic digestion on allergenicity and nutritional studies used comparatively low rations ranging from 0,1 to 0,001. Studies conducted for the purpose of safety assessment have, however, used higher ratios ranging from 25 to 5000 (reviewed in FU et al. 2002, 2003, BANNON et al. 2003). The application of a too high enzyme/test protein ratio has also been critically raised in the context of GM crop and food authorisations pointing out the possibility of false negative results (GURIAN-SHERMAN 2003a, b). Furthermore, the interpretation of experimental results differs between authors: some have considered proteins non-stable when degraded within 30 sec, others within 30 min (reviewed in FU et al. 2002, BANNON et al. 2003).

A related issue that only recently gained attention is the biological relevance of results obtained by the in-vitro methods used. Even if a protein is degraded in the gastro-intestines the remaining fragments might be more resistant and – if exceeding a molecular weight of 1500 to 3500 Da– might be able to bind IgE (reviewed in THOMAS et al. 2004).
JENSEN-JAROLIM and UNTERSMAYR (2005) have pointed to the fact that the in-vivo situation might differ because elevated stomach pH is likely to result in a decrease of proteolytic activity, e.g. in early childhood, in elderly, or in chronic atrophic gastritis. Moreover, there are a number of pathologies, like gastritis or ulcer, where acid neutralization or inhibition is an important therapeutic goal. Acid-neutralization is state of the art during surgical care, corticosteroid or analgetic treatment. Moreover, anti-acids, H2-receptor blockers and proton pump inhibitors are increasingly consumed without prescriptions due to liberalization of the market by over-the-counter sale. Recent animal and clinical studies have confirmed that these drugs support IgE induction and sensitization to food allergens (UNTERSMAYR et al. 2003, 2005). In a way this has been recognised in the course an US EPA review (MENDELSON et al. 2003).[12]
The controversy about the relevance of in-vitro digestibility testing is also mirrored in risk assessment guidance documents providing different advice on digestibility testing (see Table 1). Critical remarks were also made by the SCP evaluating the original MON810 dossier on the digestibility studies of the Btk protein (SCP 1998a).

[12] "The digestibility test is not intended to […] imply that similar breakdown will happen in all human digestive systems." (MENDELSON et al. 2003). However, this qualification does not change the EPA's position that acute toxicity tests and in-vitro digestibility tests would provide a sufficient database to conclude the absence of toxic properties for Bt proteins.

Table 1: Diverging opinions on in-vitro digestibility testing in guidance documents (SPÖK et al. 2004; including unpublished results)

Source	Testing Requirements
SCP (1998b)	Discouraged, in-vivo tests suggested instead
EU Dossiers (import, cultivation, food, feed) 1995 to 2001 Update: to 2005	Routinely conducted (SGF, SIF etc.) Very similar (prelim. results)
SSC (2000)	Limited validity (best case situation only)
OECD (2000)	"May not always provide clear evidence"
NL Biosafety Council (2003)	Encouraged; ex-vivo or in –vivo on a case-by-case basis
EFSA (2004)	Encouraged
Codex Alimentarius (2003)	Encouraged

Source of test protein

For conducting studies on toxic as well as allergenic properties of novel proteins in GM crops test proteins are almost exclusively produced from microbes, frequently using *E.coli* strains but also *Bacillus* and *Pseudomonas spp*.

Companies state that the need to purify gram amounts of test protein from GM plants (usually the target protein is expressed at µg or even ng level) would put excessive cost on applicants and that these proteins can easily be expressed at high levels in microbes. Using test proteins from microbes would in principle be acceptable if the proteins produced in the GM crop and the microbe would be identical or at least equivalent with respect to properties investigated in the test. Critics have, however, pointed to several differences that might occur to the protein in cases the same gene is expressed in plants and microbes (GURIAN-SHERMAN 2003b, FREESE and SCHUBERT 2004, SPÖK et al. 2004). Differences might occur on the level of DNA sequence during transformation and in RNA splicing eventually resulting in an altered amino acid sequence. Posttranslational processing including proteolytic processing, glycosylation, acetylation, phosphorylation, methylation and folding might also differ between plants and microbes. Any structural changes between the plant and the test protein may not necessarily but could be safety relevant as these changes could alter immunological and/or functional properties and thereby influence properties relevant to toxicity. In particular, glycosylation pattern might be completely absent in bacteria but not in plants. Glycosylation could play a role for immunogenic properties.

Tests on equivalency of test and plant protein are being conducted (GURIAN-SHERMAN 2003b, FREESE and SCHUBERT 2004, SPÖK et al. 2004). The questions still remains, though, whether the methods routinely applied /required might be sufficient to detect all possibly relevant differences.

A recent paper of PRESCOTT et al. (2005) shows that these concerns are not merely theoretically considerations. The authors described the safety relevant health effects in mice fed on alpha-amylase inhibitor-1 from a common bean expressed in peas (Pisum sativum L.). These effects included $CD4^+$ Th_2 cell-mediated inflammation and elucidated immunoreactivity to concurrently consumed heterogeneous food antigens in feeding studies of mice. No such health impacts were observed with the native bean alpha-amylase inhibitor-1 and effects were therefore attributed to differences in glycosylation and/or other modifications of the protein by the heterologous host. This case would represent the first incident in GM crops to empirically confirm the safety relevance of differences between donor and host organisms in protein processing. From this a need

can be concluded for reviewing and refining equivalence testing and/or more seriously considering the possibility of obtaining test proteins from plants.

Whole-plant/food toxicity testing
A whole-plant/food toxicity study was not included in the original MON810 dossier and was not requested by the SCP (nor by the Scientific Committee on Food in the context of the Novel Food authorisation). However the toxicity assessment provided in the original dossier did not consider sub-acute, sub-chronic or chronic effects of the introduced protein. Neither did it cover possibly unintended effects on the whole plant that might lead to altered toxic properties. Given these limitations[13], whole food/plant studies are considered indispensable.

Reliance on history of safe use
As in this dossier applicants are often arguing with a "history of safe use". However, in case of food plants and in the absence of epidemiological data, only very severe effects would have been detected. Chronic effects, such as a moderate increase in cancer rates would not have been detected. With food products there is not only a lack of epidemiological evidence but also a lack of data on exposure.
This problem has recently also been acknowledged in the course of the WTO dispute on EC measures in regard to biotech products:

> [Dr. Nutti][14] states that exposure to human diet that has no indication of adverse effects would be a criteria to take into account. Notwithstanding the ethical question of performing pre marketing toxicity tests on humans, that statement is not scientifically sound for anything else than acute toxicological risk, as explained by the European Communities in the section of general and methodological issues, on surveillance and food safety. Standard epidemiology says that, in the absence of exposure data with respect to chronic conditions, there is simply no way of ascertaining any effect – or lack thereof – on human health (EC 2005, Par. 873).

5.2.2 Allergenicity testing

Allergenicity testing in case of the MON810 dossiers is limited to the introduced CryIA(b) protein and consists of in-vitro-digestibility tests and homology comparisons to known allergens. History of safe use of Bt proteins in general and low expression levels are also mentioned to support the safety claim.
As discussed in detail in SPÖK et al. (2005) these methods do not provide any direct evidence of allergic properties and not at all on sensitizing properties. Furthermore, the methods and evidence used cannot be considered as reliable indicators of allergic properties for a number of reasons stated in the following.
First, the work of HEISS et al. (1996), YAGAMI et al.(2000), KENNA and EVANS (2000), FU et al.(2002), and others did not confirm the correlation of allergenic properties and proteolytic stability postulated by ASTWOOD et al.(1996) and others. YAGAMI et al.(2000) did not find food allergens to be more stable than proteins with no confirmed allergic properties. KENNA and EVANS (2000) and FU et al.(2002) could not find a correlation at all. Thus, both false positive and false negative results in safety testing might be possible. Furthermore, the differences in the design of in-vitro studies (discussed above) cast considerable doubt whether these experiments provide meaningful data at all.
Second, the routinely used sequence comparison technologies such as FASTA and BLAST (PEARSON 2000, ALTSCHUL et al. 1990a, b) as well as all of the new methods developed more specifically for predicting the allergenic potential of a given protein (HILEMAN et al. 2002, STADLER & STADLER 2003, SOERIA-ATMADJA et al. 2004, BJÖRKLUND et al. 2005) would provide the false positive and false negative results in many cases. Results of the comparisons might differ depending on the parameters set (e.g. substitution matrix and gap penalties) (BÖRKLUND et. al. 2005). More importantly, thought, it is well known that non-allergenic isoforms of allergens exist which differ by only a few amino acids compared to their allergenic counterparts. Such non-allergenic isoforms have been

[13] Partly described above, for a more detailed discussion see SPÖK et al. (2004).
[14] Comment: one of the scientific experts heard by the Panel.

described for many allergen sources (e.g. birch, hazel pollen) (BREITENEDER et al. 1993, FERREIRA et al. 1996). Isoform Bet v 1a (of five recombinant Bet v 1 isoforms which differ only in few amino acids) shows a high IgE-binding activity, whereas isoforms Bet v 1d and Bet v 1l show a very low IgE-binding activity. Because of their low allergenic potential such isoforms have even been suggested as candidates for allergen-specific immunotherapy (FERREIRA et al. 1996). Likewise, proteins with significant sequence homology to major allergens but without any allergenic activity have been described. For example, a cytokinin-inducible periwinkle protein (T1) was found to exhibit significant sequence homology with pathogenesis-related proteins and the Bet v 1 allergen family. The amino acid sequences of the periwinkle protein (T1) and the major birch pollen allergen showed 40,4% sequence identity, but in spite of the sequence homology the periwinkle protein showed immunologically distinct features from the Bet v 1 allergen family and exhibited no allergenic properties (LAFFER et al. 2003). These examples show that sequence comparisons could identify proteins wrongly as allergens. On the other hand new allergens are discovered continuously and it is hence clear that the databases containing allergen sequences are incomplete at present. Accordingly, allergenic potential cannot be excluded with sufficient certainty in case no sequence homology has been found. Furthermore, the validity of the homology comparison would depend on the database used. The homology comparison referred to in the MON810 dossier dates back to 1990 and 1995 respectively. Given the pace of immunological research a more recent comparative analysis would have included five times more sequences from allergenic proteins (MARI 2005).
Third, expression levels of certain allergens can vary depending on plant growth, developmental stages, tissue-specific expression and environmental stress. An example of the variability of allergen contents in different developmental stages and tissues is the allergen profilin. Profilins represent actin-binding proteins which have been described as cross-reactive plant allergens (VALENTA et al. 1991). Profilin can be detected in different somatic plant tissues, but its expression and content were 50- to 100-fold higher in mature pollen than in seed or leaf. During early stages of pollen development no profilin could be detected by Northern blotting, whereas in mature pollen large amounts of profilin were found (MITTERMANN et al. 1995). Another study has investigated sixteen apple varieties regarding their contents of the major apple allergen, Mal d 1 (VIETHS et al. 1994). The authors showed that certain apple strains contained larger amounts of Mal d 1 than others and concluded that the IgE-binding potency of different apple strains correlated with the amount of the allergen. Similar findings were made for the lipid transfer protein, Pru p3, the major allergen of peach (CARNÉS et al. 2002). The concentration of lipid transfer protein Pru p3 in peach peel extracts was approximately 7 times greater than in pulp extracts (CARNÉS et al. 2002). Thus it can be concluded that the expression level of a given protein cannot always be related to its potential allergenicity. Furthermore, expression levels of proven allergens may greatly vary in different strains, tissues and developmental stages, and can be influenced by a variety of factors. This has also been acknowledged by a Joint FAO/WHO expert consultation which concluded that it is not possible to link potential allergenicity of a given protein to its expression level (FAO/WHO 2001).

Another major shortcoming of the approach lies in its focus on the ability of the introduced proteins to exhibit allergenic responses in already sensitised individuals. This approach does not consider the de-novo sensitising properties, i.e. the ability to sensitise. Equally important, allergenicity assessment of the introduced protein should be complemented by an assessment of the whole-plant as described in SPÖK et al. 2005. Many plant allergens belong to a family of pathogenesis related proteins, the expression of which could be upregulated by infections, hormones and other stressors (HANNINEN et al. 1999; BREITENEDER et al. 1993; HOFFMANN-SOMMERGRUBER 2002; MIDORO-HORIUTI et al. 2001).
This again has been acknowledged in the submissions of the European Commission in the WTO dispute:

> *"Even if a given protein per se does not represent an allergen, its expression in another host organism may indirectly upregulate the expression of potential allergens. It is therefore recommended to compare the engineered plant/plant*

> *product with that of the parent/wildtype plant/plant product regarding IgE reactivity to establish whether the transgenic organism represents a more potent allergen source than the parent/wildtype organism for already sensitized patients. The potentially increased ability of the transgenic organism versus the parent/wildtype organism to induce de novo IgE responses (i.e. allergic sensitization) needs to be compared by immunization experiments.*" (EC 2005, Par. 716)

In this document the European Commission - arguing along the lines of an FAO/WHO consultation on allergenicity assessment (FAO/WHO 2001) – puts also emphasis on serum testing which is considered to be "widely accepted in unclear situations nowadays" (EC 2005, Par. 398).

Requests during an authorisation procedure for "*additional screening of the transgenic proteins for binding to sera from allergy patients, and for testing potential changes in the intrinsic allergenicity of maize caused by the genetic modification, using whole plant extracts for these studies"* were therefore considered reasonable and "*in accordance with the decision tree approach recommended by the FAO/WHO Expert Consultation {FAO/WHO, 2001} and are also mentioned in the Codex guidelines as methods and tools that may be considered as scientific knowledge and technology evolves*." (EC 2005, Par. 470-474)[15]

A whole-plant study, which was not conducted with GM-Maize MON810, would also consider other important exposure routes that have been previously dismissed by GMO panel (inhalation of pollen as well as dust e.g. during handling and processing of the plants). GM crops may, however, exhibit allergenic activity also via other routes, particularly in case of large scale cultivation and processing. For example, pollens represent much more potent and frequent allergen sources than plant-derived food and it should, therefore, be considered that GM crops may also release allergens via pollen production and hence cause respiratory sensitization. Furthermore, processing of maize may lead to respiratory sensitization in bakers who are exposed to flour. In this context it has been reported that soybean dust caused severe outbreaks of asthma in Barcelona, Spain when the soybeans were unloaded in the city harbour (CODINA et a. 1999, ANTO et al. 1993). Many of the responsible soybean hull allergens were identified and characterized (ANTO et al. 1993). Another example for respiratory sensitization has been described in employees who are exposed to papain (NOVEY et al. 1980). The cross-reactivity of IgE antibodies of papain with latex allergens has been reported using sera of latex-exposed and papain-exposed people (BAUR et al. 1995). Hypersensitivity to papain was found in approximately 1% of an allergic population using skin prick tests and IgE measurements and was confirmed by oral challenge (MANSFIELD et al. 1985). These results suggest that other exposure and sensitization scenarios be considered when assessing GMPs or other products rather than focusing on the gastrointestinal route only.

Further uncertainty also arises from consideration of the work of VAZQUEZ et. al. (1999a, b; 2000), MORENO-FIERROS et al. (2003) and PRESCOTT et al. (2005). Vazquez, Moreno-Fierros and co-workers could show Cry1Ab to act as an adjuvant, e.g. it enhances the mucosal and/or the systemic antibody response to a protein which is co-administered with the Cry protein. After intraperitoneal or intragastric administration of Cry1Ab to mice at relatively high dosage, IgG, IgM and mucosal IgA response were induced, but no IgE response was observed. EFSA has so far stated that as maize is not a common allergenic food, and only a rare cause of occupational allergy, the adjuvant effect of Cry proteins, observed after high dosage intragastric or intranasal administration will not raise any concerns regarding allergenicity. Prescott and co-workers could show immunogenic effects in mice fed on alpha-amylase inhibitor-1 from a common bean expressed in peas (already described above). The relevance of these studies for allergenicity assessment is still not fully understood and a contested issue in GMO risk assessment.

[15] Monsanto Roundup Ready corn (NK603) C/ES/00/01 (EC chronology 76), Question 39bis Given the information before the Panel, including the notification and additional letter from Monsanto providing additional information (previously referenced and EC-76/At.11-12), was additional information regarding allergenicity studies and PCR tests requested by Austria (EC-76/At.44) necessary or useful to ensure that conclusions of the safety assessment were valid?

5.2.3 Substantial equivalence

Substantial equivalence is claimed for MON810 essentially on the basis of a compositional analysis that has several shortcomings:
First, only a very limited range of parameters is measured. Only proximates, amino acids and fatty acids are included in the comparative analysis. Micronutrients and other important ingredients are not considered. Furthermore, even the proximates are interpreted differently, i.e. not necessarily including fibres, ADF and NDF (in the case of 1994 field trials). This investigated set of ingredients must be considered as too narrow when compared to the OECD and to the EUROPABIO consensus documents (see Table 2).
Second, differences are detected between MON810 and the control (e.g. for glutamine, leucin, proline, ADF, NDF, C18:1 and C18:2 fatty acids, starch, protein).[16] These differences are not considered relevant, as still inside literature ranges. In one case the literature range used was exceeded (protein, US trials), so an older literature range (1976) was used in order to "normalise" the deviation. None of the differences were considered a reason to repeat or extend the comparative analysis.
Third, it appears that no isogenic control line was used.
If compositional analysis is being used as an indicator for unintended effects, the number of substances measured is very small. If used for nutritional assessment, certain proximates and micronutrients are missing.

Table 2: Compounds analysed in the MON810 dossier compared to compounds listed in the OECD Consensus Document and the EuropaBio-Guideline

Plant ingredient	OECD (2002)	EuropaBio (2001)	MON810
Protein	+	+	+
Fat	+	+	+
Carbohydrates	+	+	+
Fibre	+	Optional	-
ADF	+	Optional	+
NDF	+	Optional	+
Ash	+	+	+
Amino acids (18)[a]	+	+	+
Fatty acids (5)[b]	+	+	+
Bulk minerals			
Ca	+	+	-
K	+	+	-
Mg	+	+	-
Na	+	+	-
P	+	+	-
Trace minerals			
Cu	+	-	-
Fe	+	-	-
Se	+	-	-

[16] It is not mentioned and it can also not be concluded from the information provided, whether a statistical analysis was conducted. Given the lack of detailed information provided in the dossier it is not entirely clear whether these differences are statistically significant.

Plant ingredient	OECD (2002)	EuropaBio (2001)	MON810
Zn	+	-	-
Vitamins			
Retinol equivalent	+	-	-
Vit B1	+	+	-
Vit B2	+	+	-
Vit B6	+	-	-
Vit E	+	+	-
Folic acid total	+	+	-
Niacin	+	-	-
Others			
Phytic acid	+	+	-
Raffinose	+		-
Furfural	+		-
Ferulic acid	+	-	-
p-Coumaric acid	+	-	-

Shaded areas indicate that the respective data were not provided in the maize MON810 dossier.

[a]) Ala, Arg, Asp, Cys, Iso, His, Glu, Gly, Leu, Lys, Met, Phe, Pro, Ser, Thr, Tyr, Try, Val; [b]) Palmitic, stearic, oleic, linoleic and linolenic acid;

ADF…acid detergent fibre; NDF…neutral detergent fibre; +… considered; -… not considered.

5.2.4 Dossier does not include detailed reports

As a general disadvantage, the detailed reports on which the above mentioned safety conclusions in the MON810 dossiers are based, are not included in the dossier and according to the knowledge of the author, have not been available to the risk assessors at the European Commission and at national level. The extent of which some of the methodological criticism (such as the one on in-vitro digestibility studies) actually applies, can therefore only be judged after evaluation of the detailed reports.
A more accurate evaluation of field trials and comparative analysis would also only be possible if the full reports would be provided. On the basis of the information provided the safety conclusions cannot be fully verified for a number of reasons:

- Composition analysis is documented in summary tables, detailed data are missing
- Sometimes it is not clear which units of measurement are used in the tables
- Two different methods are used to analyse plant material. They do not enable comparisons in each case
- It remains unclear whether and how a statistical analysis was conducted.
- Sampling is not properly described (e.g. how many kernels/plants have been pooled for one sample), storage of proteins are not properly described
- Not stated whether single- or repeated measures are conducted
- No/too little description of field trial design, conditions and practice of cultivation
- Literature ranges are missing for plant material and carbohydrates (kernel only)
- Reported ranges are partly drawing on an unpublished study of the applicant
- Mean values are sometimes missing

5.2.5 Conclusions

The discussion in the preceding sections reveals a number of shortcomings and a lack of verifiability. Toxicity assessment does not consider effects beyond acute toxic of the introduced protein. The assessment of the allergenic potential is based on methods and evidence that cannot be considered reliable. The approach used is even less appropriate to assess any de-novo sensitizing properties. Inhalation of dust and pollen is not considered at all as an exposure route. The possibility that toxic or allergenic properties might appear is not considered at all.
Field trials and compositional analysis are not fully verifiable and do not appear to be properly conducted.
In light of the most recent guidance provided, the information included in the dossier would also not be sufficient for a market authorisation under Directive 2001/18/EC or Regulation 1829/2003.
The fact that EFSA has repeatedly been re-evaluating the maize MON810 case as stated in its opinion of 2006 (EFSA 2006) does appear to provide any substantial reassurance.
The safety studies quoted in the dossier of the hybrid applications are essentially the same as in the original dossier, mainly unpublished studies conducted by the company during the first half of the 1990ies.
In sum from the data provided in the dossier of maize MON810 and in the light of recent evidence from scientific literature, it is neither possible to fully verify all aspects of the risk assessment conducted by the applicant nor to conclude a sufficient degree of safety.

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5.3 GM-Maize T25

This section reviews recent scientific evidence relevant to the Austrian safeguard measure for placing on the market of genetically modified maize line T25. As described in the introduction to this chapter (p. 34), many health risk assessment issues are similar in both dossiers. To avoid redundancy this sections only includes issues specific for maize T25. In case an approach or method has already been discussed reference is given to preceding subsections.

5.3.1 Toxicity assessment

Toxicity assessment in the original dossier basically relies on a 14-day repeated dose toxicity study in rats, on in-vitro digestibility tests (simulating human, swine, ruminant and chicken digestion), and tests of heat and acid lability using a bacterial PAT protein. In addition, the applicant argued with the safe history of the PAT protein.
This assessment cannot be considered sufficient for the following reasons (along the lines of SPÖK et al. 2004).

Repeated-dose toxicity study of the PAT protein
The 14-day repeated-dose toxicity study included in the final dossier was conducted according to OECD norms and was included in the dossier as a full report.
This type of study is clearly better suited than acute toxicity studies. However for reasons discussed in the previous section on maize MON810 (Chapter 3.2, p.27ff and in Spök et al. 2004, 2003, 2002) these studies are neither considering appropriate endpoints for standard toxicity testing of GM crops nor of the introduced protein as such.
Even recent Guidance documents ask for 28-day repeated-dose study (EFSA 2004 and NL BIOSAFETY COUNCIL 2003).

In-vitro digestibility test
With respect to in-vitro digestibility studies see discussion of maize MON810, Chapter 5.2, p.34ff.

Source of test protein
With respect to in-vitro digestibility studies see discussion of maize MON810, Chapter 5.2, p.34ff.

Reliance on history of safe use
With respect to in-vitro digestibility studies see discussion of maize MON810, Chapter 5.2, p.34ff.

Whole-plant/food toxicity
The dossier does not include a toxicity study of the whole plant. According to the knowledge of the author no such study on maize T25 has been published in the scientific literature.
The 42-day broiler chicken study included in the dossier is clearly a feed conversion study and must not be confused with toxicity studies. Only very severe toxic effects would have shown up in such a study. This has also been pointed out by the European Commission (EC 2005, Par. 603).
The occurrence of secondary effects of genetic modification is meanwhile well acknowledged in the scientific literature and in guidance documents of national and international bodies (e.g. FAO/WHO 2000, THE ROYAL SOCIETY OF CANADA 2001, CODEX ALIMENTARIUS COMMISSION 2003 also commented in HASLBERGER 2003, SAP 2005). However, such effects would not necessarily show-up in comparative compositional analysis (with an even lower probability if, as in this case, only a small number of plant component are analysed) or in comparing morphological criteria. In this regard the limitations of compositional analysis and the relevance of whole/food plant studies have also been acknowledged by the European Commission:

"Although realising the limitations of such studies, chronic toxicology studies are needed especially in the screening for unintended effects of GMOs because there are presently no better methods sufficiently developed." (EC 2005, Par. 902)
"*The determination of the nutrients-toxicants (substantial equivalence) can not detect all unintended effects (products) […] Whole food studies can and must be used to complement other safety testing approaches."* (EC 2005, Par. 907)

5.3.2 Allergenicity assessment

Evidence was provided showing the absence of glycosylation motifs in the PAT protein sequence, on homology comparisons to known allergens and on the above mentioned in-vitro digestibility studies.
The assessment is evaluated in the following along the lines of SPÖK et al. (2005).
With respect to homology and in-vitro digestibility studies see discussion of the maize MON810 dossier, Chapter 5.2, p.34ff.
Glycosylation of proteins could play a role in immunogenicity but can definitely not be considered a reliable indicator of allergenic properties. For instance, profilins represent highly cross-reactive allergens but are not glycosylated (VALENTA et al. 1991). Also another group of cross-reactive allergens belonging to calcium-binding proteins are not glycosylated (NIEDERBERGER et al. 1999). Both families, profilins and calcium-binding proteins, represent potent well characterized allergens, which have been tested for their allergenic activity in great detail. Although a few studies indicate that carbohydrate components might contribute to allergenic activity, there is now increasing evidence that carbohydrates are involved in IgE recognition but are poor elicitors of allergic reactions (VAN DER VEEN et al. 1997, MARI et al. 1999, MARI 2002, VON REE 2002). Only few reports are available describing carbohydrate components that bind IgE from sera of allergic patients and induce histamine release from blood cells (BATANERO et al. 1999, BUBLIN et al. 2003).
With respect to allergenic properties of the whole GMO and other exposure routes see discussion of the maize MON810 dossier, p.34ff.

5.3.3 Substantial equivalence

Substantial equivalence is claimed for T25 essentially on the basis of a compositional analysis that has several short comings.
First, only a very limited range of parameters is measured. Only proximates, amino acids and fatty acids are included in the comparative analysis of the US, only proximates are analysed in case of the European field trials (without ash). Micronutrients and other important ingredients are not considered (see Table *23*).
Second, statistically significant differences are detected in compositional analysis of the US trials between T25 and the control in kernel for fat, carbohydrates, arginine, lysine, histidine, and in silage for fat, protein, ADF, NDF, phytate (and also several parameters of silage of treated samples). These differences are not considered relevant either because still within literature ranges or because they are not consistently detected in all comparisons. Fewer differences were found in European trials. However, these comparisons were limited to four compounds (cellulose, fat, nitrogen, starch).
If compositional analysis is being used as an indicator for unintended effects, the number of substances measured is very small. If also used for nutritional assessment, certain proximates and micronutrients are still missing.
If compositional analysis is being used as an indicator for unintended effects, the number of substances measured is very small. If also used for nutritional assessment, certain proximates, minerals, and vitamins are missing. Carotinoide and lutein would have been important as well.

Table 3: Compounds spectrum analysed in the T25 dossier compared to compounds listed in the OECD Consensus Document and the EuropaBio-Guideline.

Plant ingredient	OECD (2002)	EuropaBio (2001)	T25
Protein	+	+	+
Fat	+	+	+
Carbohydrates	+	+	+
Fibres	+	Optional	+
ADF	+	Optional	+
NDF	+	Optional	+
Ash	+	+	+
Amino acids (18)[a]	+	+	+
Fatty acids (5)[b]	+	+	+
Bulk minerals			
Ca	+	+	-
K	+	+	-
Mg	+	+	-
Na	+	+	-
P	+	+	-
Trace minerals			
Cu	+	-	-
Fe	+	-	-
Se	+	-	-
Zn	+	-	-
Vitamins			
Retinolequivalent	+	-	-
Vit B1	+	+	-
Vit B2	+	+	-
Vit B6	+	-	-
Vit E	+	+	-
Folic acid	+	+	-
Niacin	+	-	-
Others			
Phytic acid	+	+	+
Raffinose	+		-
Furfural	+		-
Ferulic acid	+	-	-
p-Coumaric acid	+	-	-

The table is based on the data from US field trials. Analysis from European field trials was limited to proximates but lacking ash, NDF and ADF. Shaded areas indicate that the respective data were not provided in the maize T25 dossier.

[a]) Ala, Arg, Asp, Cys, Iso, His, Glu, Gly, Leu, Lys, Met, Phe, Pro, Ser, Thr, Tyr, Try, Val; [b]) Palmitic, stearic, oleic, linoleic and linolenic acid;

ADF…acid detergent fibre; NDF…neutral detergent fibre; +… considered; -… not considered.

5.3.4 Dossier does not include detailed reports

In the maize T25 dossier detailed descriptions are missing for the in-vitro digestibility studies as well as for field trials and comparative analysis. The latter of which cannot be fully verified for a number of reasons:

- Composition analysis is provided in bar charts only, the precise figures are missing
- Statistical evaluation sheets are not included
- Only in case of silage it is clear that herbicide-treated plants were analysed
- Field trial design, conditions of cultivation, measuring practice, sampling and treatment of samples is not properly described
- Literature ranges are missing for some compounds (amino acids, fatty acids, phytate)

5.3.5 Conclusions

The discussion above reveals a number of shortcomings and a lack of verifiability. Toxicity assessment does not consider effects beyond a 14-day study of the introduced protein. The assessment of the allergenic potential is based on methods and evidence that cannot be considered sufficiently reliable. The approach used is even less appropriate to assess any de-novo sensitizing properties. Inhalation of dust and pollen is not considered as an exposure route. The possibility of toxic or allergenic properties of the whole-plant is not considered at all.
Field trials and compositional analysis are not fully verifiable and it is not clear whether they are properly conducted.
In light of the most recent guidance provided, the information included in the dossier would also not be sufficient for a market authorisation under Directive 2001/18/EC or Regulation 1829/2003.
In summary, from the data provided in the dossier of maize T25 and in the light of recent evidence from scientific literature, it is neither possible to fully verify all aspects of the risk assessment conducted by the applicant nor to conclude a sufficient degree of safety.

5.3.6 References

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