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Summary

Genetically modified plants (GMP) as well as derived feed and food have to undergo a risk assessment prior to market authorisation in the EU. The particular requirements for risk assessment have been and still are particularly contested issues and only recently attempts were begun to specify requirements in greater detail. Against this backdrop the practice of risk assessment was investigated by reviewing both Directive 90/220/EEC and Novel Food dossiers. The focus thereby was on toxicity and allergenicity assessment, on how the concept of substantial equivalence was being put into practice but also included some general aspects of risk assessments.

The review revealed a number of shortcomings in both type of dossiers:

- **Shortcomings in the overall risk assessment approach: the formal structure is not based on and does not clearly distinguish between exposure assessment and hazard assessment which are both considered necessary to allow for a proper risk assessment. Substantial equivalence plays a key role in both type of applications. In contrast to its conceptual role as a starting point in risk assessment, it rather denotes a terminal stage. The claims of substantial equivalence are frequently based on field trials and compositional analysis that are not properly designed and are often not backed up by throughout and consistently applied statistical analysis.**
- **Risk assessments and safety conclusions drawn frequently cannot be entirely verified or even not verified at all on the basis of information presented in the dossiers given the lack of details in the description of tests, approaches, in data display and the tendency not to include full reports.**
- **Overall approaches in risk assessment are similar in the dossiers, differences became evident at the level of details, though. These differences, especially between dossiers pertaining the same plant species and/or aiming at similar applications might suggest differences in the soundness of the assessment procedure and clearly point to a lack of details in guidance documents.**
- **Safety conclusions are**
  - **often based on indirect evidence and/or assumption based reasoning while direct testing of toxic or allergenic properties is rather limited if conducted at all;**
  - **partly based on methods, approaches and assumptions that are questionable (e. g. homology and in vitro digestibility studies in toxicity assessment; studies and assumptions of the decision tree approach in allergenicity assessment);**
  - **not backed up by throughout compliance in safety relevant studies to a quality assurance system;**
  - **largely focussing on the novel proteins introduced only.**

- **Unintended effects of genetic modification are usually not investigated and even dismissed. Such effects are apparently believed to be reflected by conspicuous alterations of either morphological or agronomical properties or in key plant compounds. In compositional analysis, however, significant differences found are disregarded without attempts to verify or further investigate these differences in order to enhance the likelihood to detect unintended secondary effects. Whole-plant feeding studies included in the dossiers are feed conversion studies and cannot be considered toxicity studies.**

These shortcomings might not only diminish the validity of safety conclusions in scientific risk assessment but also reduce their credibility amongst stakeholders and in the general public. On the other hand EU legislative and policy documents are frequently reiterating the need for a high level of safety. With this in mind, proposals were developed aiming at further improvement and standardisation of risk assessment:

- **Overall structure of risk assessment approaches and dossiers: structure of dossiers and risk assessment should be standardised including dedicated chapters to substantial equivalence, exposure, toxicity assessment, and allergenicity assessment. The role of substantial equivalence for risk assessment should be further clarified. Detailed requirements for field trials, sampling and compositional analysis are proposed – significant differences should at least trigger repetition of the analysis including broadening the range of compounds considered.**
- **Dossiers should be "stand-alone" including full reports of all available safety relevant studies, quoted literature, statistical evaluation sheets for compositional analysis, thorough descriptions of methods and procedures applied including the type of statistical analysis.**
- **Guidance Documents should be further detailed accordingly.**
- **Safety relevant statements should specify the nature of evidence supporting the safety claim, e. g. test results, literature data or anecdotal evidence. In general, direct testing of toxic or allergenic properties should be preferred compared to approaches that rely on indications from indirect testing and assumption based reasoning. In case of toxicity assessment a minimum set of endpoints is proposed that largely resembles usually accepted endpoints in other regulatory contexts. Additional endpoints would depend on the particular exposure (especially in case of placing on the market as GMP) and on the results of preceding tests. In case of allergenicity assessment comparative IgE reactivity studies should be conducted using animal models in order to asses both sensitizing and allergenic properties. Safety relevant studies should generally by conducted according to Good Laboratory Practice. Further research should be conducted to clarify the value of studies such as homology comparisons and in vitro digestibility in toxicity assessment.**
- **Testing should be extended to include whole-plant/whole-food testing in both toxicity and allergenicity studies in order to more reliably detect unintended and detrimental effects of genetic modification.**

The first three proposals and partly also those included in the forth bullet point are deemed to assist both applicants and reviewers – the former who have to conduct the risk assessments and compile the dossiers and the later who have to evaluate the risk assessments in the process of market authorisation. The other proposals are rather aiming at providing a more appropriate factual basis for safety conclusions to be drawn.

Some of these proposals might be immediately acceptable and easily be implemented. In fact, some of the proposals of this study have already been included in most recent guidance documents issued by parallel initiative at the level of the European Commission, international organisations and elsewhere. Others might require further discussion and even to conduct additional studies, for instance the particular minimum set of toxicity endpoints. Some proposals might require the further improvement and validation of testing methods, such as whole-plant toxicity studies or even to further develop novel testing procedures, such as the application of animal models in allergenicity assessment.

Preface

This monograph was composed with the intention of providing an abridged and updated version of the content, conclusions and recommendations of two research projects carried out by the Umweltbundesamt Wien, the Inter-University Research Centre for Technology, Work and Culture (IFZ) in Graz, the ARC Seibersdorf research GmbH, the Research Center for Biotechnology, Society and the Environment (BIOGUM) at the University of Hamburg and a range of experts contracted on a personal basis including Petra Lehner, Karin Kienzl-Plochberger, and Rudolf Valenta. The main goal of these projects was to review the practice of risk assessment procedures on genetically modified plants in the EU. The main reason for this monograph was that preceding publications were published in German and were also quite comprehensive by comprising a total of four volumes (SPÖK et al., 2003a, 2003b, 2002a, 2002b), thereby rather hampering than facilitating a wider reception and more detailed discussion at the international level.

The monograph in hand is thus based on the above mentioned preceding publications and is authored by a subset of the original project team. Some chapters were composed by the original authors, whereas others were composed on the basis of the original publications by other members of the project team (for information on the authors of the individual chapters of this monograph and the expertise that served as an input into the projects see Appendix B and C; for publications issued so far see Appendix D). The work of this latter group is therefore particularly acknowledged in the following:

In the course of the preceding studies published in German, Karin Kienzl-Plochberger conducted a supplementary review of feeding studies in both type of dossiers and on field trials and compositional analysis of Novel Food dossiers (Article 4). Both co-authored the two preceding publications (SPÖK et al., 2002a, 2003a). Sandra Karner and Andreas Loinig worked on the regulatory issues. Sandra Karner also co-authored one of the preceding publications that was dealing exclusively with regulations, guidelines and experience with regulatory practice (SPÖK et al., 2003b). Although regulatory issues are not the primary focus of this monograph, her work served as a valid source when composing this monograph. Alice Schmatzberger contributed in several ways and especially in the course of a final workshop in December 2003 where the results presented here were subjected to a critical review by an international auditorium.

The authors are grateful to the Federal Ministry for Work and Labour and the Federal Ministry for Health and Women who funded the research that provided that basis for this monograph. The composition of this monograph was funded by the Federal Ministry for Agriculture, Forestry, Environment and Water Economy.

1 Introduction

1.1 Context

Toxic and allergenic properties are considered as focal aspects in the assessment of potential health risks of food derived from genetically modified organisms (GM food). In the European Union the assessment of toxic and allergenic risks has been part of a pre-market risk assessment that is mandatory for genetically modified plants as well as seeds, food, and feed derived therefrom.

Legislation aiming at regulating market authorisation and pre-market procedures have already been introduced in the 1990ies, starting with Directive 90/220/EEC for cultivation, feed, seed and processing of GMPs in 1990 and followed with the Novel Food Regulation for GM food in 1997. Up to now a total of 14 genetically modified plants (GMPs) have been authorised according to Directive 90/220/EEC and 13 GMPs were granted permission to be marketed as food products under Novel Food Regulation.[1]

In contrast to other regulatory contexts such as chemicals, plant pesticides and food additives, detailed requirements for toxicity and allergenicity assessment have not being put into concrete terms until recently[2] – hence, the wide margins for the practice of risk assessment. Consequently requirements were only put into concrete terms in the course of the particular authorisation procedures. This practice rendered the administrative procedure to be time consuming and labour intense. Given the pace of scientific progress and of the development of new GMP varieties, the different ways of interpreting EU regulations by national authorities, the pressures from industry on one hand and from public interest groups on the other hand, these particular circumstances are not likely to result in consistent procedures. Consequently questions might be raised whether the quality of risk assessments and thereby the level of safety might differ from case to case.

The practice so-far is also quite time consuming and resource-demanding for applicants. Seeking market authorisation for a new GMP variety in the EU was and still is far from being predictable.

Moreover, until recently the practice of risk assessment was not considered transparent. Due to a lack in harmonisation of information policy public access to full dossiers was and partly still is depending on the particular national authority and might even not be provided at all. Documents available and leaked to environmental and consumer groups gave rise to criticism (e. g. GREENPEACE, 1996). This is especially true for the practice of the concept of substantial equivalence (MILLSTONE et al., 1999). Recent studies on how the concept is applied in the course of risk assessment procedures revealed both lacking validity and conclusiveness in the line of reasoning and were criticised, for instance, because of the limited range of compounds analysed. Furthermore, a lack of con-

[1] All authorisations under Directive 90/220/EEC and most of the authorisations under the Novel Food Regulations were granted before 1999. Since June 1999 a de-facto moratorium had been put in place to stop market authorisation unless adequate legislation was established.

[2] During the time this study was being conducted, 2000-2003, no detailed guidance was available at all. In March 2003 the SSC issued a guidance document for risk assessment under Directive 2001/18/EC and the Novel Food Regulation. This document is presently being updated (status: May 2004).

sistency in the range of testing and methods applied as well as in statistical evaluation could be shown (e. g. NOWAK & HASLBERGER, 2000; SPELSBERG et. al., 2000; SCHENKELAARS, 2001). This again points to the wide margins of interpretation of safety requirements and to a need to put the requirements into more concrete terms. However, most of the studies cited above were carried out by evaluating publicly available summaries of applications only and did not investigate the evidence present in the full dossiers.

1.2 Terms of reference

1.2.1 The study that provides the basis for this monograph

The study described in this monograph was set against this backdrop pursuing two objectives: firstly, to thoroughly investigate the current practice of assessment of potential toxic and allergenic properties of GMPs on the basis of the full dossiers; and secondly, to come up with suggestions for further detailing and standardising risk assessment practice.

In the first part of the study Directive 90/220/EEC dossiers as well as relevant guidance documents and legislation were investigated and conclusions were drawn. The second part of the study applied the same approach to Novel Food dossiers and associated regulatory and guidance documents. The results and conclusions from both parts of the study were already published comprising three volumes with a total of 730 pages (Part 1: SPÖK et al., 2002a; Part 2: SPÖK et al., 2003a, 2003b) and one conference proceedings (SPÖK et al., 2002b).[3] To the best of our knowledge this was the first attempt of investigating and documenting the practice of risk assessment into the very details.

These reports were issued in German language only and this quickly turned out to be major hurdle when aiming at introducing the results and conclusions of the study into the discussion at the European Commission and in other international forums, as well as in other Member States.

1.2.2 This monograph

The monograph in hand attempts to make up for this hurdle, by providing a more condensed English version of the most relevant results and conclusions of both parts of the study. Unlike other abridged versions, the size of the monograph in hand provides for a sufficient level of detail that will be of interest to both scientists, technical and regulatory specialists and to the broader stakeholder community as well.

In order be as topical as possible, this condensed English version was updated in terms of references (to regulatory documents and scientific literature) and to reflect in more detail on toxicity testing and in vitro-digestibility studies.

For the sake of providing a less comprehensive and therefore more readable version this monograph does not include the quite substantial Annexes of the pre-

[3] The results were published as Volume 109, 164A, 164B in the Monograph Series and as Volume 32 in the Conference Papers Series issued by the Umweltbundesamt Wien. Spök at al. 2003a and 2003b were also published as Volumes 5/03 and 6/03 in the Series "Rote Reihe" issued by the Austrian Federal Ministry of Health and Women.

ceding German monographs describing each dossiers in detail. Readers interested in the very details of particular dossiers are referred to the above mentioned monographs (SPÖK et al., 2002a, 2003a).

1.3 Overall approach of the study

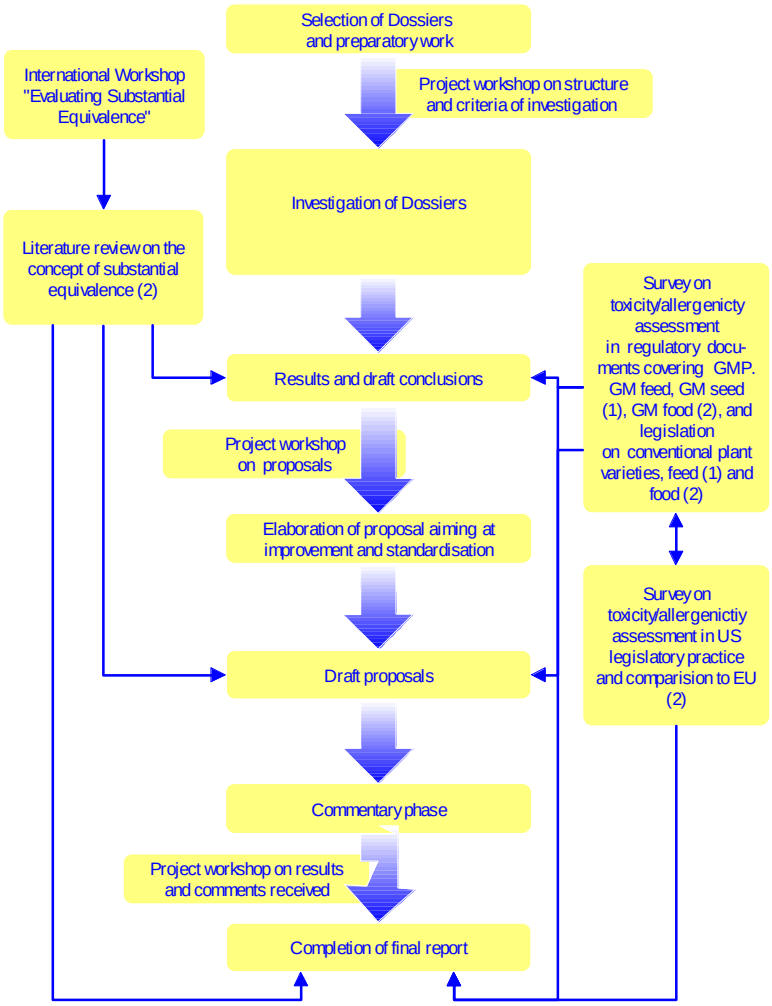
The overall approach followed in both parts of the study is outlined in Figure 1. As already mentioned largely the same approach was used for investigating both Directive 90/220/EEC and Novel Food dossiers.

1.3.1 Outline of the approach

In order to meet the first goal, the practice of toxicity and allergenicity assessment was scrutinised in selected Directive 90/220/EEC and in Novel Food dossiers as well. As substantial equivalence plays an important role in GMP risk assessment in general and in toxicity and allergenicity assessment in particular the scope of the study also included the practice of substantial equivalence as well. Thereby the focus was on compositional analysis and nutritional aspects. Furthermore, an in-depth investigation was conducted on the practice of feeding studies. Thus, two sets of four independent experts reports (one set per regulatory context) resulted form this investigation. These reports include a detailed description of the assessment procedure in each dossier, a comparison of the practice between the dossiers (mainly within one regulatory context) that was revealed and a critical evaluation of what was found to comprise assessment practice. Testing, data display, consideration of exposure, and line of reasoning were of core interest. Completeness of documents, clarity, consideration of exposure, line of reasoning, general agreement with state of the art with respect to scientific knowledge and methodical issues, and validity of safety conclusion drawn were used as criteria for evaluation.

From this a detailed picture of assessment practice emerged and possible inconsistencies between and possible shortcomings within applications become apparent. These results then served as a basis to meet the second goal of the study, namely, to develop suggestions in order to further improve and standardise GMP risk assessment. Suggestions were inspired by (i) assessment practice that was found to be not (no longer) in accordance with the state of the art in science, (ii) by comparison of dossiers (or to put in normative: comparable GMPs/applications should be assessed in a similar way in order to grant the same level of safety and to lead to comparable safety conclusions), (iii) the need to provide a valid assessment that could easily be verified by reviewers; (iv) by procedures and standards applied in other EU regulatory contexts such as plant pesticides, chemicals, food or feed additives, and herbal medicines.

Figure 1: Outline of overall project approach



The overall approach was largely identical in both parts of the study. Numbers in brackets indicate whether a particular task was carried out in the course of either Part 1 or Part 2 only.

The draft expert report (Part 1) was then subjected to critical review by external experts. The comments from the external experts were then introduced into the internal discussion and considered in the final step of completion of the final reports.

A series of internal workshops were carried out to facilitate and focus the discussion process within the project team with respect to fine-tuning of project design, elaboration of evaluation criteria, discussion of intermediary results, development of suggestions, and format of final reports.

As a parallel task to the investigation of dossiers relevant regulatory documents that are dealing with assessment of toxic or allergenic properties of GMP or with the issue of substantial equivalence are reviewed. This essentially included EU legislation, guidance as well as discussion documents of the EU and national scientific committees or international bodies (OECD, FAO, WHO), secondary literature and company documents. In addition, the authorisation requirements and

regulatory practice in the US for GMP and GM food were reviewed and compared to the EU context. The papers presented at and discussions held in the course of an international workshop "Evaluating Substantial Equivalence",[4] that was organised in the course of this study were also considered. This task furthermore includes a brief review of "downstream" legislation, i.e. screening requirements for additional authorisations including toxicity and allergenicity relevant criteria in non-GMO specific EU regulatory contexts of food, feed and new plant varieties.

The results and conclusions where then documented in three monographs, dealing with regulatory contexts and investigation of Directive 90/220/EEC dossiers (SPÖK et al., 2002a), investigation of Novel Food dossiers (SPÖK et al., 2003a), and the respective regulatory context (SPÖK et al., 2003b).[5]

1.3.2 Selection of dossiers

The selection of dossiers is of course a crucial step as general conclusions should be based on a subset of dossiers, hence, the selection criteria are described in more detail.

In case of Directive 90/220/EEC eleven dossiers were selected to reflect the various applications that were aimed at including, cultivation, processing, and import on the one hand and food, feed and non-food non-feed purposes on the other hand (see Table 1). The main reason therefore was that it was hypothesised that different applications would be associated with different exposure which in turn might be reflected by a different approach to toxicity and allergenicity assessment.

In case of Novel Food dossiers the dossiers were selected to represent applications according to both Article 4 and Article 5 of the Novel Food Regulation. Whereas the former stands for full applications according to the normal procedure, the latter offers a short-cut notification procedure for GMP/GM food that was considered substantially equivalent to conventional counterparts. Furthermore, the dossiers should be comparable, i.e. focus on a rather narrow range of GMP species, which is especially important for comparing the practice of compositional analysis that was carried out in the course of investigating/substantiating status of substantial equivalence. Eventually, the selection of dossiers was determined by the availability of full-text dossiers. Unlike Directive 90/220/EEC dossiers that were circulated to national competent authority (CA) in full text versions, Novel Food dossiers were usually distributed as summaries subsequently supplemented by the initial assessment reports of the national competent food assessment body. Consequently, full text dossiers are usually only available from the national CA to whom the application was filed initially. Unfortunately, the policies for granting access differs a lot between the national CAs. The German CAs, for instance, considered the dossiers as property of the applicants, whereas the Dutch and the British CAs were granting access to all non-confidential parts of the dossiers.

[4] The papers presented at these workshops were independently published as conference proceedings (SPÖK et al., 2002b).

[5] See also Chapter 1.2.

On the basis of this criteria and restrictions five Article 4 and seven Article 5 dossiers were selected for investigation (see Table 2).

Table 1 Overview of the Directive 90/220/EEC dossiers investigated

Dossier/ Notification number	Intended use	To EC	Before NFR[a]	Status[b]
Applications aiming at cultivation and use as feed				
RR Fodder beet A5/15 C/DK/97/01	Cultivation, seed production, feed stuff	10/97	N	Awaiting Art.21, SCP[e]
Potato EH92-527-1 C/SE/96/3501	Cultivation, technical applications, feed, fertilizer	05/98	N	Awaiting Art.21
Bt-Cotton 531 C/ES/96/02	Cultivation, feed stuff, industrial application	11/97	N	Awaiting decision of the European Council
RR Cotton 1445 C/ES/97/01	Cultivation, feed stuff (especially for poultry, sheep, catfish, and pigs)	11/97	N	Awaiting decision of the European Council
Two applications aiming at different use: 1st application at import, 2nd application at cultivation				
Maize Bt11 C/GB/96/M4/1 C/F/96/05-10	Import, processing	11/96	Y	10/98
	Cultivation	04/99	N	Awaiting Art.21, SCP[e]
RR-Maize GA21 C/GB/97/M3/2 C/ES/98/01	Import, processing for feed stuff (no cultivation, no use as food)	06/98	N	c)
	Cultivation, feed stuff	11/98	N	Awaiting Art.21, SCP
Application aiming at cultivation and use in food and feed as well[d]				
Rape Topas 19/2 C/UK/95/M5/1	Import, processing, cultivation, oil production, feed stuff	04/96	Y	10/98, for import, food and feed use only
Applications for ornamental plants aiming at cultivation only				
Carnation 66 C/NL/97/12	Cultivation, marketing as cut-flowers	08/98	N	10/98
Carnation 959A etc C/NL/97/13	Cultivation, marketing as cut-flowers	08/98	N	10/98

N... No; Y...Yes; NFR... Novel Food Regulation. [a)] Application filed before Novel Food Regulation entered into force; [b)] Status of procedure according to Directive 90/220/EEC (Date of permission, Opinion of the Scientific Committee on Plants (SCP) already issued, awaiting consultation according to Article 21 Directive 90/220/EEC); [c)] Application was withdrawn during the study; [d)] Actually also one of the Maize Bt11 dossiers would have to be categorised under this heading. For reasons of clarity the dossier was not classified twice in this table; [e)] Resubmitted under Directive 2001/18/EC and already received a favourable opinion by the EU Scientific Committee (status: 01/2004).

Thus, the investigation could largely be based on complete dossiers including the application itself as well as the initial assessment (in case of Novel Food dossiers), comments of national competent authorities and of relevant scientific committees of the EC, along with correspondence and supplementary information that was submitted on request by the applicants.

Table 2: Overview of the Directive 90/220/EEC dossiers investigated

Dossier	Food use	Submitted (country/ year)	Date of permission (status of procedure)
Maize NK603	Maize and maize derivatives	NL 2001	Favourable scientific opinion issued EFSA 4.12.2003
Maize 1507		NL 2001	Pending
Sweet Maize Bt11*#	Processed sweet maize	NL 1999	Favourable scientific opinion issued SCF 13.03.2003
Maize GA21*	Maize and maize derivatives	NL 1998	Favourable scientific opinion issued SCF 2.2.1999
Soybean 260-05	Soybean derivatives	NL 1998	Awaiting the initial assessment
Rape MS1xRF1 und MS1xRF2	Rapeseed oil; products made with rapeseed oil my include fried foods, baked products, and snack food	UK	10.06.1997
Rape Topas 19/2#		UK	09.06.1997
Rape GT73		UK	10.11.1997
Maize T25§	Maize derivates; may include maize oil, maize flour, sugar and syrup; products made with maize derivatives may include snack food, baked foods, fried foods, confectionary and soft drinks	UK	12.01.1998
Maize Bt11#		UK	30.01.1998
Maize MON809*		UK	14.10.1998
Maize MON810§		UK	10.12.1997

The bold line separates Novel Food dossiers according to Article 4 (above) from those according to Article 5 (bellow). NL... The Netherlands; UK... United Kingdom; *... Application aiming at Cultivation according to Directive 90/220/EEC pending; #... Permission granted according to Directive 90/220/EEC for import and use in food and feed (1998); §...Permission granted according to Directive 90/220/EEC for import, processing and use in feed.

1.3.3 Relevance of study results to the changed regulatory context in the EU

Part 1 of the study on Directive 90/220/EEC dossiers was started in 2000 and largely completed by the end of 2001. Part 2 of the study on Novel Food dossiers was started at the end of 2001 and was largely completed by the end of 2002.

Between start and completion of the study a number of quite substantive regulatory changes took place in the EU. Directive 2001/18/EC was completed and agreed and replaced Directive 90/220/EEC in October 2002.

Subsequently, a new Regulation on genetically modified food and feed was drafted and agreed and very recently entered into force, in April 2004 (Regulation 1829/2003). This Regulation covers areas previously included in the scope of the Novel Food Regulation (GM food) and of Directive 2001/18/EC (GM feed). In March 2003 a new guidance document aiming at facilitating risk assessment of GMPs seeking authorisation under Directive 2001/18/EC and the Novel Food Regulation was issued by the Scientific Steering Committee (SSC). Eventually, in April 2004 an updated version of this guidance was drafted by the GMO Panel of the European Food Safety Authority (EFSA, 2004).

Given the time schedule of both parts of the study only draft versions of the both the new Directive (later to become Directive 2001/18/EC) and the new Regulation on genetically modified food and feed (later to become Regulation 1829/2003) could be considered. Whereas the draft version of the new Directive was largely agreed, an agreed version of the new Regulation was not issued before December 2002. Consequently, an early draft had to be used in Part 2 (EC, 2001).[6]

The results and conclusions generated in the course of the study are, however, highly relevant to the altered regulatory context too. For, the majority of the suggestions are referring to details which are usually not specified in EC Directives or Regulations.

The SSC guidance document that was issued in March 2003 after the final report of Part 2 had been submitted is of greater interest, though. However, a preliminary analysis of the guidance (SPÖK et al., 2003c) revealed that on the one hand – compared to preceding guidance documents of 1997 (Recommendation 97/618/EC) and 1998 (SCP, 1998) – progress was largely restricted to a few areas of risk assessment. On the other hand quite a number of recommendations were simply reiterated and the document was still generally lacking a more detailed guidance.

Hence the results and conclusions of the study are still highly topical and were therefore already being discussed in the course of the UK Science Review on genetically modified organisms (GM SCIENCE REVIEW, 2003, 2004) and more recently also introduced into discussions at the European Commission and in EFSA as well.

1.4 Structure of the monograph

[6] For the sake of clarity, references given to the draft versions were updated in the course of preparing the condensed English monograph in hand.

The structure of the monograph is as follows: Chapter 2 briefly reviews the regulatory contexts in the EU and the US and thereby serves as a kind of regulatory foil of the subsequent Chapters. Chapter 1 contains the review of dossiers including the description and evaluation of assessment approaches found in Directive 90/220/EEC and Novel Food dossiers as well. This Chapter is structured to include a part on toxicity assessment, allergenicity assessment and substantial equivalence.[7] The recommendations given in Chapter 4 are based on the results of the review of dossiers described in preceding Chapter 3. The concluding Chapter 5 provides an update compared to the preceding monographs (SPÖK et al., 2003a, 2002a) by briefly discussing some of the proposals of this monograph in the context of recently issued guidance documents.

[7] The review of the application of the concept of substantial equivalence presented in this monograph focuses on Article 5 Novel Food dossiers for which full substantial equivalence is claimed. In order to be brief the results of the review of Novel Food dossiers according to Article 4, for which partial substantial equivalence is claimed, and Directive 90/220/EEC dossiers are only presented as summaries. Moreover, the review of the latter type dossiers would not change the overall picture and the conclusions presented in this monograph do consider these results too. For more details on the concept of substantial equivalence in Article 4 dossiers see SPÖK et al. (2003a), for Directive 90/220/EEC dossiers see SPÖK et al. (2002a).

2 Regulatory Context

The first part of this Chapter (Section 2.1) provides a very brief introduction into both the regulatory context of GMP, GM food and feed that applied for the dossiers investigated and into the new legislation that meanwhile entered into force. It thereby does not attempt to deliver a general description. Rather, it focuses how and to what level of detail requirements for toxicity and allergenicity assessment as well as for the application of substantial equivalence are provided either in legislation or in associated guidance documents. The second part (Section 2.2) points to relevant guidance documents of international organisations. The third part of this Chapter (Section 2.3) contrasts the EU regulatory context with the one applicable in the US.

2.1 European Union

GMPs intended to be introduced as products or in products onto the EU market must apply for a authorisation beforehand. Presently, Directive 2001/18/EC (formerly Directive 90/220/EEC) sets the general legal framework for all kind of GMP products. Since 1997 GM food is subjected to authorisation according to Sectoral Regulation (Regulation 258/97, subsequently designated as "Novel Food Regulation"). In 2004 the Novel Food Regulation was replaced in terms of GM food by Regulation 1829/2003 which at the same time replaced Directive 2001/18/EEC in terms of GM feed.

Since the overall purpose of these legislation is to warrant protection of human and animal health and the general environment a risk assessment has to be carried out by the applicant as part of the pre-market procedure. This risk assessment has then to be reviewed by national and EU CAs (Directives 90/220/EEC or 2001/18/EC resp., Novel Food Regulation) or the EFSA (Regulation 1829/2003).

The dossiers investigated in the course of this study were submitted either under Directive 90/220/EEC or under the Novel Food Regulation. For this reason both documents along with associated decisions and guidance documents are briefly described in terms of requirements for toxicity assessment, allergenicity assessment and substantial equivalence. As both pieces of legislation are already outdated, the new legislation is also described.[8]

Table 3: Relevant EU legislative framework for GMP products

<table>
<tr><th>Application</th><th colspan="2">Relevant EU legislation</th></tr>
<tr><td>Cultivation</td><td>Up to 09/2001:</td><td>Since 10/2001:</td></tr>
<tr><td>Processing, storage, handling</td><td rowspan="3">Legislation: Directive 90/220/ECC (Directives 94/15/EC, 97/35/EC, Decision 92/146/EEC, Guidance Notes)
Guidance: SCP Guidance (1998)</td><td rowspan="3">Legislation: Directive 2001/18/EC, Decisions 2002/811/EC, 2002/623/EC</td></tr>
<tr><td>Seed</td></tr>
<tr><td>Other Purposes</td></tr>
</table>

[8] At the time of the study only draft versions of Directive 2001/18/EC and Regulation 1829/2003 were available and were therefore not systematically considered. Nevertheless, some references were given to these draft legislation in the original German Monographs. For the sake of clarity these references have been updated in preparing this monograph, so that they refer to the respective parts of the legislation presently in place.

Application	Relevant EU legislation		
Food	**Up to 05/1997:** Legislation: Directive 90/220/ECC (Directives 94/15/EC, 97/35/EC, Decision 92/146/EEC, Guidance Notes) Guidance: SCP Guidance (1998)	**From 05/1997 to 04/2004:** Regulation 258/97 (Novel Food Regulation) Guidance: **Up to 03/2003:** Recommendation 97/618/EC **Since 03/2003** SSC Guidance (2003)	**Since 04/2004:** Regulation 1829/2003 **04/ 2004:** EFSA Draft Guidance
Feed	**Up to 09/2001:** Directive 90/220/ECC (Directives 94/15/EC, 97/35/EC, Decision 92/146/EEC, Guidance Notes) Guidance: SCP Guidance (1998)	**From 10/2001 to 04/2004:** Directive 2001/18/EC, Decision 2002/811/EC, 2002/623/EC **Since 03/2003:** Guidance: SSC Guidance (2003)	**Since 04/2004:** Regulation 1829/2003

In case of legislation the dates mentioned designate the months and years the new legislation must be applied notwithstanding that different dates might apply for some of the associated Decisions mentioned in the Table.

2.1.1 Directive 90/220/EEC

Directive 90/220/EEC set the legislative framework for placing on the market of GMPs up to September 2001. Before 1997 all kind of GMPs – even those aiming at use in food products – had to seek authorisation under this Directive. Risk assessment required by this Directive (and subsequently also by Directive 2001/18/EEC) is termed "environmental risk assessment" (e.r.a.) that also includes human health effects.

The Directive itself, however, did not specify requirements in terms of toxicity or allergenicity assessment. Decision 92/146/EC required applicants to provide information on *"toxic or allergenic effects of the non-viable GMOs and/or their metabolic products"* (Annex, 40 (a)). As specified in the Explanatory Notes to the Directive the particular kind of application that is aimed at has to be considered (EC, 1992a). Elsewhere it was clarified that this *"covers effects on all classes of organisms/all vertebrate animals"* (EC, 1992b).

A Guidance issued by the SCP (1998) provides some details on toxicity assessment, ecotoxicity, substantial equivalence but not on allergenicity assessment e. g.

- **In case of insect resistant and herbicide resistant GMP *"data should be provided on the toxicity [...] of compounds with plant protective properties as expressed in GM plants".***
- ***"In the case of new metabolites appropriate toxicity studies should be carried out with respect to the assessment of animal and human safety as laid down in the Directive 91/414."***

2.1.2 Directive 2001/18/EEC

Directive 2001/18/EEC – without specifying toxicity or allergenicity assessment requirements – significantly extents the scope of the e.r.a. and explicitly refers to the precautionary principle:

- **Includes direct or indirect, immediate, delayed or cumulative effects (Annex II, Introduction and Part C, C2 (1)).**
- **Potential adverse affects should not be discount if they are unlikely to occur (Annex II, Part C, C2 (1)).**
- **Decision 2002/811/EC of 24 July 2002 establishing guidance notes supplementing Annex II to Directive 2001/18/EC of the European Parliament and of the Council on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC.**

The need to provide information on potential toxic and allergenic properties of GMOs to man and animals are more clear and more explicit compared to Directive 90/220/EEC (Directive 2001/18/EC Annex II, Part C, C2 (1); Annex III B, Part B (7); Part D (7-8)). In contrast, the concept of substantial equivalence is not mentioned in the Directive.

After the evaluation of the dossiers was completed and the recommendations were elaborated the Scientific Steering Committee issued a "Guidance Document for the Risk Assessment of Genetically Modified Plants and Derived Food and Feed" that was published in its final form in March 2003 (SSC, 2003). This document provides more details for toxicity and allergenicity assessment as well as for substantial equivalence. Very recently in April 2004 a draft update of the Guidance Document was issued by the EFSA GMO Panel. However, given the completion of the evaluation of dossiers by the end of 2002 and of the final reports in early 2003 neither the SSC Guidance Document of 2003 nor the draft update could be considered in this study.[9]

2.1.3 Novel Food Regulation

From May 1997 up to April 2004 food that is a GMO, consists of GMOs or is manufactured from GMOs had to be authorised by Regulation 258/97 (Novel Food Regulation). As with the Directive for placing on the market of GMOs (Directive 90/220/EEC, now Directive 2001/18/EC) the Novel Food Regulation required to conduct a risk assessment before market authorisation could be granted.

Whether a particular product/GMP has to undergo a normal authorisation procedure (according to Article 4) or might be eligible for a short-cut notification procedure (according to Article 5) is depending on which status of substantial equivalence will be assigned to the GMP/GM food. In case of GMPs/GM foods that are considered substantial equivalent to their conventional counterparts (and this must be confirmed e. g. by a national CA prior to submitting to the EC) an Article 5 procedure could be pursued. Otherwise the normal Article 4 procedure applied.

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[9] **In April 2004 a comprehensive commentary on the "Draft Guidance Document for the Risk Assessment of Genetically modified Plants and Derived Food and Feed" was composed and submitted to EFSA by the authors of this study. This commentary is available on request from the authors of this monograph.**

The basic structure of risk assessment and information requirements are provided in Recommendation 97/618/EC. Up to March 2003 this Guidance was the only EC document to provide details on risk assessment for GM food.

Important to this study is that this Recommendation also provided guidance on toxicity, allergenicity assessment and on how to substantiate the claim of substantial equivalence and what particular role this concept would play in guiding risk assessment.

2.1.3.1 Substantial equivalence

According to the Recommendation *"[...] substantial equivalence may be established either for the whole food or food component including the introduced 'new` change, or it might be established for the food or food component except for the specific 'new` change introduced. [...] The establishment of substantial equivalence is an analytical exercise in the assessment of the relative wholesomeness of a NF compared to an existing food or food component. [...] The analyses and data presented should [...] be tailored to the nature of the NF. Investigations should focus especially on the determination of the content of critical nutrients (both macro- and micronutrients) and any critical toxicants and anti-nutritional factors which might be either inherently present or process derived"* (Recommendation 97/618/EC, Section 3.3).

In addition, the Standing Committee on Food concluded that GM food can be considered as substantially equivalent only in case no recombinant DNA or novel proteins can be detected (EC, 1998; PETTAUER, 2002).

Likewise, in 1998, the SCP provided some additional guidance (SCP, 1998): Accordingly,

- **isogenic counterparts should be used and**
- **samples should be derived from at least two seasons, and from a number and variety of geographical locations;**
- **Compositional analysis should be accompanied by an appropriate statistical treatment;**
- **assessments should always consider known anti-nutritional, potentially toxic or allergenic compounds.**

2.1.3.2 Toxicity assessment

According to this Recommendation the toxicological as well as nutritional requirements needed to be considered on a case-by-case basis. Depending on the status of substantial equivalence three scenarios are to be considered:

- ***"substantial equivalence can be established to an accepted traditional food or food ingredient, in which case no further testing is needed;***
- ***substantial equivalence can be established except for a single or few specific traits of the NF, in which case any further assessment of safety should focus specifically on these traits;***
- ***neither partial nor total substantial equivalence can be established; in this case, the wholesomeness of the whole novel food or macronutrient has to be assessed using an appropriate combined nutritional-toxicological approach.***

If substantial equivalence to a traditional counterpart cannot be established the wholesomeness assessment has to take into account not only knowledge of the identity, chemical structure and physico-chemical properties of the NF but also aspects such as source, composition, potential intake based on the proposed use in the general diet, the potential exposure of particularly vulnerable population groups, and the likely effects of processing. The greater the predicted dietary exposure the more extensive the required toxicological testing programme will have to be." (Recommandation 97/618/EC, Section 3.7).

If the latter case applies the safety assessment based on a case-by-case evaluation must consider the following elements (ibid., Section 5 XIII):

- **consideration of the possible toxicity of the analytically identified individual chemical components;**
- **toxicity studies in vitro and in vivo including mutagenicity studies, reproduction and teratogenicity studies as well as long term feeding studies, following a tiered approach on a case-by-case basis;**
- **studies on potential allergenicity.**

"In the case of novel micro-constituents and isolated novel food components, which differ by identifiable characteristics from traditional foods, or of defined novel products obtained from genetically modified organisms, it is possible to restrict testing to only those products or substances rather than the whole NF" (ibid.).

In case of novel macro-constituents, or GMOs/GM food which are not substantially equivalent to conventional counterparts, the testing programme should generally include at least a 90 day feeding study in a rodent species. In case of in vitro mutagenicity studies the usual major endpoints should be covered. Chronic toxicity/carcinogenicity studies may deemed to be necessary.

2.1.3.3 Allergenicity assessment

In terms of assessment of allergenic properties generally the immunological reactivity of individuals who react to the traditional food counterpart should be tested in vitro and in vivo to the GMO. *"If the novel protein is expressed by genes derived from a source known to be associated with food allergy, sera of people with confirmed allergies to that source can be subjected to specific immunological tests, e. g. Western Blotting or radioallergosorbent test (RAST). If in vitro tests are negative, in vivo skin prick tests or clinically supervised double blind placebo controlled challenges in these people may be performed"* (ibid., Section 3.10). Furthermore, the allergenic potential of the host plant should be considered.

A number of factors are recommended as indicators of the potential allergenicity of novel proteins, including sequence epitope homology with known allergens, heat stability, sensitivity to pH, digestibility by gastrointestinal proteases, detectable amounts in plasma, and molecular weight. Additional evidence from premarketing human results and reports of workers' sensitisations might be relevant as well.

2.1.4 Regulation 1829/2003 on genetically modified food and feed

In April 2004 the new Regulation 1829/2003 on genetically modified food and feed entered into force and thereby replaced the Novel Food Regulation in terms of GM food and the Directive 2001/18/EC in terms of GM feed.

In contrast to the Novel Food Regulation the short-cut notification procedure was abandoned while the concept of substantial equivalence was still kept as a major guiding tool.

The scope of the Regulation was extended to cover food ingredients, food additives, and flavours that are produced from GMOs even in case the GMO is no longer detectable in the food.

Furthermore, the same provisions apply to feed and feed ingredients.

As with the Novel Food Regulation no particular requirements for toxicity or allergenicity assessment are included in the new Regulation.

Regulation 178/2002,[10] however, makes it quite clear that the extended scope of risk assessment found in Directive 2001/18/EC (see Section 2.1.2) also applies to GM food by stating that in *"determining whether any food is injurious to health, regard shall be had: (a) not only to the probable immediate and/or short-term and/or long-term effects of that food on the health of a person consuming it, but also on subsequent generations; (b) to the probable cumulative toxic effects; (c) to the particular health sensitivities of a specific category of consumers where the food is intended for that category of consumers"* (Article 14 (4)).

More detailed requirements for GM food risk assessment are still in the progress of being established. A first draft guidance document was issued by the EFSA GMO Panel in April 2004. Given the fact that the final reports of this study was completed in early 2003 this draft could not be considered.[11]

2.2 Recommendations of OECD and FAO/WHO

Besides initiatives at the EC that resulted in the Recommendation 97/618/EC and the SCP Guidance (1998) major steps have also been taken at the level of OECD, FAO, WHO and ILSI. Given the focus of this monograph only a very brief summary can be provided here. For a more detailed review and comparison of the recommendations issued by these organisations see SPÖK et al. (2003b).

2.2.1 Toxicity Assessment

With respect to some cornerstones of risk assessment the OECD (1998, 2000a) and FAO/WHO (2002) guidelines are largely in accordance with the EU Recommendation: the use of substantial equivalence; the need to consider toxicity of both the newly introduced protein and – regarding to known plant toxins – the whole GMP; different schemes of both cultivation and manufacturing should be considered as well as the possible exposure of particularly vulnerable groups.

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[10] Regulation 178/2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety.

[11] See note 9.

Whether any distinct differences can be identified largely depends on the reading of the documents. On reading of the EU Recommendation (especially Chapter XIII) is that toxicokinetics, chronic and sub-chronic toxicity, carcinogenicity, teratogenicity of novel proteins should be tested in case no substantial equivalence could be established. Another reading, however, is that this would apply only for low molecular weight substances or micro-constituents. The FAO/WHO Guideline, in contrast, is clearly suggesting these tests for novel non-protein substances only. FAO/WHO proposes sequence comparisons to known toxins and anti-nutrients, thermostability and digestibility studies. Furthermore, evidence should be provided that the gene product investigated (mostly of microbial origin) is structurally and functionally identical to the one which will eventually be consumed (of plant origin).

The EU-Recommendation and the OECD and FAO/WHO guidelines agree that potential toxic properties of the whole plant should be investigated. It is however, stressed that conventional feeding studies are of limited value. Still, the EU Recommendations suggested to carry out at least a 90-day feeding study. Alternative methods are proposed in the Recommendation and in all guidelines.

2.2.2 Allergenicity assessment

The assessment of allergenic properties is generally based on decision tree approaches. As a first step, in each of these recommendations it has to be investigated, whether or not the source organism is known to be allergenic. In contrast to the EU Recommendations the OECD recommendations (2002a) propose extended comparisons to known allergens: apart from amino acid sequence comparisons also secondary and tertiary structure should be taken into account. The particular sites of homologous sequences within the protein should also be considered. The validity of in vitro digestibility studies is however questioned.

Criticism on the approach to allergenicity assessment was partly taken into account in a subsequent FAO/WHO decision tree (2001). Comparative studies of functional and structural characteristics to known allergens are recommended. In contrast to earlier recommendations proteins from donors not known to be allergenic should nevertheless be subjected to a three step procedure comprising of homology studies, a targeted serum screen, and digestibility studies even in case of negative results of each preceding test. Proteins considered as novel and exceeding a homology of 35% or more than six subsequent amino acids respectively should be classified as allergenic and subjected to direct in vitro and in vivo testing.

2.2.3 Substantial equivalence

As a response to the demand of more detailed recommendations in terms of cultivation and methods applicable for statistical evaluation were given and OECD Consensus Documents (e. g. OECD, 2001, 2002a) specifying sets of plant-specific compounds to be analysed were issued. Databases are in the process to be established compiling data on the ranges of plant-specific compounds. Besides, novel methods are being tested which will probably be more appropriate to detect any secondary effects and which might prove to be very useful in the course of risk assessment of second generation GMPs. By the use of such methods profiles of complex mixtures of compounds e. g. mRNA, proteins, metabolites can be visualised and subjected to further comparative analysis between the

GMP and conventional counterparts. However, these methods are still not applicable in routine testing and the scientific basis is still to be developed in order to properly interpret any detected differences.

2.3 Comparison of the risk assessment of genetically modified foods in the USA and the EU

2.3.1 Regulation of genetically modified plants in the USA

In the USA a regulation of products of biotechnology was first adopted in 1986 in the "Coordinated Framework for Regulation of Biotechnology", which is still applicable today. The basis of the regulation is the assumption that the process of biotechnology in itself poses no unique or special risks and that foods developed via biotechnology should be regulated in the same way as foods developed through conventional breeding. Therefore, the same laws are applicable to genetically modified foods. Under these laws, three federal agencies – the US Department of Agriculture (USDA), the Environmental Protection Agency, and the Food and Drug Administration have primary responsibility for the regulation of products of biotechnology (McKENZIE, 2000) (see Table 4).

Table 4: Regulation of genetically modified organisms

Agency	Responsibility	Law
USDA	Plant pests, GM crop plants with potential plant pest risks, veterinary biologics	Federal Plant Pest Act (FPPA)
EPA	Microbial pesticides, plants producing toxic substances, plant-incorporated protectants (PIP)	Federal Insecticides, Fungicides, and Rodenticides Act (FIFRA), Toxic Substances Act (TSCA), Federal Food, Drug, and Cosmetic Act (FFDCA)
FDA	Food, feed, food additives, drugs, medical devices and cosmetics	Federal Food, Drug, and Cosmetic Act (FFDCA)

Source: APHIS (2003), modified.

As a consequence, most of the genetically modified plants are regulated by more than one agency (for examples see Table 5).

The USDA is responsible for protecting the US agriculture from agricultural pests and noxious weeds. Transgenic plants are regulated by USDA´s Animal and Plant Health Inspection Service (APHIS) under the Federal Plant Protection Act (FPPA) which controls the importation, transportation, and planting of any plant which may pose a pest risk to the environment. Transgenic plants which are plant pests or which carry DNA from an organism considered to be a plant pest (e. g. *Agrobacterium tumefaciens*, Cauliflower Mosaic Virus (CaMV)) are defined as "regulated articles"[12] (APHIS, 2003).

[12] Since all transgenic plants reviewed by APHIS up to now contained at least promoter sequences from CaMV they fell under the FPPA. There will be a loophole in the regulation when in the future transgenic plants with only plant-derived promoters will be developed.

Table 5: Examples of the responsibility for different genetically modified products

Organism / new trait	Agency	Responsible for
Crop plant / insect resistance	USDA EPA FDA	Safety of planting and transportation safety of the PIP for human and the environment nutritional safety
Crop plant / herbicide re-sistance	USDA EPA FDA	Safety of planting and transportation new use of the accompanying herbicide safety of consumption
Crop plant / modified oil content	USDA FDA	Safety of planting and transportation safety of consumption
Ornamental plant / herbicide resistance	USDA EPA	Safety of planting and transportation new use of the accompanying herbicide
Ornamental plant / modified flower colour	USDA	safety of planting and transportation

Source: APHIS (2003), modified.

Following field tests of a transgenic plant, a petition for non-regulated status may be submitted. The studies and data submitted in support of the petition must demonstrate that there will be no significant plant pest risk from the widespread planting (McKENZIE, 2000). Points to be considered are:

- **harm to other organisms, especially agriculturally beneficial and non-target organisms;**
- **increase in weediness in another species with which it might cross;**
- **adverse effects on the handling, processing or storage of commodities and**
- **threat to biodiversity.**

No tests requirements are laid down in the Plant Pest Act. In general, data from field experiments on the lack of toxic effects on animals (counting) as well as comparison of the nutritional composition with a conventional counterpart are considered to be sufficient.

Once a determination of non-regulated status has been made, the product and its offspring no longer require APHIS review for release and movement in the US (APHIS, 2003). The determination of the non-regulated status is the typical route to commercialisation for a transgenic plant (partially equivalent to an approval according to Part C of Directive 90/220/EEC (now: Directive 2001/18/EC).

EPA regulates the manufacture, sale and use of pesticides. Since 1994 any substance produced in a living plant through genetic engineering is registered by EPA if it is intended to control pests (the so-called plant-incorporated protectants, PIPs). EPA regulates the pesticidal protein expressed on the plant, not the plant itself. PIPs are regulated both to determine the environmental safety (Federal Insecticide, Fungicide, and Rodenticide Act, FIFRA) and to establish tolerance levels at which their present in food is safe for consumption (Federal Food, Drug, and Cosmetic Act, FFDCA) (EPA, 2003).

In general, the data requirements for PIPs are based on those for microbial pesticides. Before EPA will grant the registration of a pesticide, the applicant must show that it will not generally cause unreasonable adverse effects on man or the environment, taking into account the economical, social, and environmental costs and benefits of the use of any pesticide (FIFRA). EPA exempts a pesticide from the requirement of a tolerance level if there is reasonable certainty that no harm will result from the aggregate exposure to the pesticide chemical residue, including all anticipated dietary exposures (FFDCA). The exact data requirements have been developed on an case by case basis. The general data requirements include (EPA, 2003):

- product characterisation,
- mammalian toxicity:
  - acute oral toxicity (sub-chronic and chronic toxicity test only when long term effects can be anticipated from previous results),[13]
- effects on non-target organisms:
  - avian species (quail),
  - aquatic species (catfish and daphnia),
  - beneficial insects (honeybee, parasitic wasp, green lacewing, ladybird beetle),
  - soil organisms (springtails and earthworms),
- allergenicity potential:
  - amino acid sequence homology comparison,
  - heat/processing stability,
  - in vitro digestibility in gastric fluids,
- environmental fate, and, if appropriate,
- insect resistance management.

From the three agencies involved in the safety assessment of food and feed derived from genetically modified plants, the EPA exhibits the most stringent data requirements[14] (see also overview in Table 33, Appendix).

In 2001, EPA exempted from registration requirements pesticidal substances produced from conventional breeding of sexually compatible plants. EPA also proposed in 1994 to exempt three other categories of PIPs (e. g. PIPs based on viral coat proteins).[15] In the final rules from 2001 these exemptions have not been included but are still subject of discussions (EPA, 2003).

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[13] In the case of Bt plant incorporated protectants acute oral toxicity tests are considered sufficient since this is similar to the agency position regarding microbial Bt products.

[14] All of the Bt toxins have been re-evaluated in 2001. EPA concluded that the data submitted (s. above) support the Bt plant-incorporated protectant registrations. Concerning the amino acid sequence comparison, EPA stresses the need for more specialised comparisons including a step wise series of overlapping 8 amino acid peptides and screening for potential sites for post-translational modification (e. g. potential glycosylation sites). As valid methods become available, EPA recommended to utilise more complete analyses of the expressed proteins (e. g. MALDI TOF) to confirm the expressed amino acid sequence.

[15] In 1997, EPA also exempted from the requirement of a tolerance the replicase protein of Potato Leaf Roll Virus because of the long history of mammalian consumption of the entire plant virus particle in virus-infected plants. Viruses are ubiquitous in the agricultural environment at levels higher than will be present in the transgenic plant (EPA, 1997)

The FDA regulates food and feed from genetically modified organisms. Under the Federal Food, Drug, and Cosmetic Act (FFDCA) whole food can be marketed without registration unless it can be shown to be injurious to health. Substances added to food fall within two possible categories: food additives and substances "generally recognized as safe" (GRAS). A pre-market approval is only required for food additives. These are proteins which differ substantially in structure and function from proteins that have already been consumed (FDA, 1992). In the case of genetically modified foods, all but one newly produced protein have been found to be substantially similar to already consumed substances and therefore did not require pre-market approval as new food additives.[16] The FDA give guidance to the industry on the safety assessment of their products but the responsibility for the safety rests with the producer. Although voluntarily, all companies marketing new products from transgenic plants have made use of the consultations. Relevant safety issues addressed during the consultation procedure are:

- the source of the introduced genetic material,
- information pertaining to the agronomic and quality attributes of the plant,
- genetic analysis of the modification and stability of expected genomic traits,
- evaluation of the safety of newly introduced proteins (toxicity and allergenicity), and
- chemical analysis of important nutrients and toxicants.

Underlying this review process is the determination of whether the genetically modified food is substantially equivalent to, and as safe as, the parental species from which it was derived. This concept recognises that the new foods are variants of existing, well-accepted foods and that foods are not inherently safe.

In January 2001 the FDA proposed new regulations that would make the current practice of voluntary consultations mandatory. Manufacturers of plant-derived, bioengineered foods and animal feeds would have to notify the FDA at least 120 days before the products are marketed ("pre-market biotechnology notice"). The rationale behind this new proposal was that the FDA recognised that with future developments in genetic engineering there is a greater potential for foods to contain substances that are food additives (e. g. that do not have a history of safe use or may otherwise not satisfy the GRAS standard) (FDA, 2001). The proposal has not yet been finalised.

[16] The only genetically introduced protein which has been approved as food additive in the USA was neomycin phosphotransferase II (NPTII). The pre-market approval was applied for by Calgene to enhance confidence in the first genetically modified food product, their FLAVR SAVR-tomato.

2.3.2 Comparison of US and European regulation of food safety

The most obvious difference between the US and the European regulation of foods derived from genetically modified foods is the overall regulatory approach taken.

The basic assumption behind the regulation of genetically modified foods in the USA is that they are not inherently more risky than traditional foods. Therefore, foods from genetically modified plants are regulated according to the same regulations as traditional foods. Three agencies are responsible for the safety of crop plants for man and the environment. As a consequence, most of the genetically modified plants are regulated by more than one agency (s. Table 5). This system is relative flexible allowing the adaptation of the regulation for new products or product categories of genetic engineering. Especially the EPA uses the opportunity to consult with experts in the Scientific Advisory Panel on the data requirements for special safety issues (e. g. in the case of the potential allergenicity of StarLink maize). On the other hand the different agencies involved have to coordinate their work to avoid potential loopholes in the regulation (e. g. resistance management of herbicide resistant plants).

In the European perspective, foods from genetically modified plants might bear new risks that have to be assessed and regulated specifically. This refers to direct risks, like potential allergenicity or toxicity, but also to indirect or long-term effects on the environment and consumers, which might not be anticipated today ("precautionary principle").

While in the USA the focus is on regulating the final *product*, the EU tends to focus on the *process* of genetic engineering. This strict process-orientated approach in the EU was more and more abandoned leading to specific regulations for contained use and deliberate releases of GMOs, the Novel Food Regulation and, nowadays, the Directive on Novel Foods and Feed, regulating all aspects of food and feed production from GMOs (product-orientated approach).

But the strict product-based regulation in the USA was also broken when the EPA excluded from the registration all pesticidal substances in plants from conventional breeding (process-orientated approach).[17]

A further difference between the USA and the EU regulation is the owner of responsibility for food safety (USA: *consultations*; responsibility rests with the producer; EU: *approval*; responsibility is mainly on the agency) and the extent of *liability* (USA: no maximum limit; EU: according to national laws (Germany: about 80 Million Euro)).

Despite all the above mentioned differences in regulation, the *safety assessment tests* conducted on genetically modified foods are principally the same. The safety assessments are built on the principle of "substantial equivalence" (GM foods are best to be compared with traditional counterparts) and the risk assessment focuses on the potential negative effects the differences identified might exhibit. In the case of EPA, the most detailed data requirements for a risk assessment are provided.

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[17] With the new proposal for a mandatory consultation procedure for foods derived from biotechnology the FDA is going in the same direction, because foods derived from non-rDNA breeding methods (e. g. narrow crosses, wide crosses) will not be included in the proposed notification rule (FDA, 2001).

Differences between the US and the EU exist in the interpretation of the principle of substantial equivalence and to the extent to which a "history of safe use" in conventional foods might substitute for a safety test.

An example are high-oleic soybeans which have been found to be substantial equivalent with respect to food and feed safety to traditional foods, because soybeans with higher oleic acid content have been produced by conventional breeding and there is a lack of known toxicity of oleic acid. The safety assessment, therefore, focussed on the molecular characterisation and the compositional analysis to exclude any unexpected effects. In analysing the fatty acid profile of the transgenic soybeans, a 9,15 isomer of linoleic acid was detected. No toxic effect was attributed to this isomer because it can be found in hydrogenated soybean oils and other food stuff, such as cheese, beef, human milk and mango pulp (FDA, 1996).

These high-oleic soybeans have not yet been approved in the EU. But it might be assumed that they will fall within the third category "without substantial equivalence to conventional counterparts" leading to a safety assessment of the introduced protein, the changed fatty acid profile, potential unexpected effects as well as the exposure of consumers (aggregate exposure, vulnerable consumer groups, bioavailability of nutrients).

3 Review of dossiers

This Chapter contains a description and evaluation of risk assessment practice found in selected Directive 90/220/EEC and Novel Food dossiers. Section 3.1 is focussing on the assessment of toxic properties, Section 3.2 on allergenic properties, whereas Section 3.3 is dealing with substantial equivalence. The overall structure of all sections is similar. Exposure is a relevant issue in all three Sections. Results presented pertain either Directive 90/220/EEC or Novel Food dossiers. If no particular reference is given to either type of dossier both types are meant. The sections on toxicity assessment and allergenicity assessment are composed of a rather descriptive part and an evaluation that explicitly assesses what was done in the dossiers on the basis of criteria described in Section 1.3.1. Section 3.3 differs insofar the review is primarily focussing on Article 5 Novel Food dossiers. For, the concept of substantial equivalence played its most important role in GM food assessment under the Novel Food Regulation as only GM food that is considered substantial equivalent to conventional counterparts is subjected to a shortcut authorisation procedure according to Article 5 of the Novel Food Regulation.[18] Moreover, the review of Article 4 dossiers (normal procedure) and of Directive 90/220/EEC dossiers essentially lead to very similar conclusions. Therefore the results of the latter type dossiers are only briefly summarised.

3.1 Assessment of toxic properties

3.1.1 General aspects

In both Directive 90/220/EEC and Novel Food dossiers full reports are usually not included (one exemption). Instead, references are frequently given to published literature or reports which are usually not included in the dossiers. Furthermore, methods and results are frequently described as summaries or as abridged versions. None of the dossiers include all the literature and reports to which reference is given. In both type of dossiers more detailed reports are occasionally submitted on request of competent authorities only, even if they had been available at the time the application was submitted.

Statements on toxic properties are sometimes scattered across the dossiers. A more uniform designation and numbering of chapters is sometimes missing. This is also true for the table of contents of the occasionally quite extensive annexes.

In contrast to Directive 90/220/EEC dossiers Novel Food dossiers (Article 4) are better structured – thereby applying the guidance given in Recommendation 97/618/EC. Recommendation 97/618/EC does not include guidance for Article 5 dossiers. However, Article 5 dossiers reviewed are apparently structured according to a similar guidance of the ACNFP.

In both type of dossiers safety relevant statements are not consequently supported by references which would enable to assess whether a claim is derived from actual testing or based on assumptions or generally accepted opinions.

[18] For more details on the Novel Food Regulation see Section 2.1.3.

Testing conducted according to Good Laboratory Practice (GLP) is scarce in Directive 90/220/EEC dossiers and was only done in one case (Bt cotton). This is contrasted by the Novel Food dossiers where compliance to GLP is more frequent.

3.1.2 Exposure

In Directive 90/220/EEC dossiers exposure is estimated to be low or even negligible. The concentration of introduced proteins in plant tissue is measured in some of the dossiers only (maize Bt11 and GA21, potato, Bt cotton). Digestibility of the newly introduced proteins on the basis of in vitro tests is frequently used to support the claim for low levels of resorption.

In case of Novel Food dossiers expression in plant tissue of introduced proteins is usually and digestibility is frequently investigated. Processing is sometimes comprehensively described. In each case it was referred to the equivalence of processing of conventional plants. Concentration of the newly introduced proteins in processed food is measured in one Article 4 dossier and two Article 5 dossiers only (see Table 6 for overview and Table 35 (Appendix) for details). Consumer exposure (GMP or food products resp., novel protein) was assessed and considered in risk assessment in case of maize GA21 only.

Table 6: Exposure related studies in Novel Food dossiers

Type of Study	Testing done[a]		Comments
	Article 4	Article 5	
Digestibility studies	4(4)	6(6)	In two dossiers references (Article 4) are given to studies carried out in the course of application procedures elsewhere. The studies are however not enclosed.
Expression in plant tissue	4(4)	5(6)	-
Concentration in processed food	1(4)	3(6)	In one dossier (Article 5) reference is given to studies carried out in the course of application procedures elsewhere. The study is however not enclosed.

a) The numbers refer to the number of dossiers including description of such tests (total number of dossiers considered or applicable in brackets). The dossier for Rape MS1xRF1 and MS1xRF2 is presumably incomplete and is therefore not considered in this table.

3.1.3 Toxicity testing

The different exposure scenarios that could be expected according to the different application intended in Directive 90/220/EEC dossiers (processing, cultivation, feed, non-food/non-feed industrial use, seed production) were not reflected in different levels of toxicity studies which is quite surprising. Whereas GM maize is intended to be consumed by farm animals in quite substantial amounts, only little exposure could be expected from cultivation of GM carnation as this is done mostly in green houses. However, as toxicity assessment generally comprises only a small section of the dossiers differences in application might not become apparent at all. Even in cases where an initial application for import is supple-

mented by a subsequent application aiming at cultivation (maize Bt11 and GA21) no differences in toxicity assessment are apparent.

Acute toxicity testing of introduced proteins is usually considered sufficient though toxicity tests are not included in each Directive 90/220/EEC dossiers investigated. In addition whole plant feeding studies are often provided. However, theses studies are usually carried out to investigate feed conversion (and thereby to support the claim of substantial equivalence) and cannot be considered as toxicity studies. Only severe toxic effects (lethal or considerable changes in body weight) would be detected by these methods. Toxicity studies would also investigate e. g. effects on organs and tissues. Additional endpoints such as chronic toxicity, mutagenicity, reproduction toxicity and carcinogenicity are not investigated at all.

The broadest range of toxicity studies was provided for Bt proteins involving chronic studies and studies in fish and birds.[19] However, it remains unclear whether the protein used in these studies is identical to the one expressed in maize Bt11. Furthermore, theses studies have probably been conducted and in the course of the registration as a plant pesticide in the USA rather than carried out to support the safety claims of the GMP dossier.

Whole plant toxicity studies were not carried out in any dossier. Long-term toxic effects of the whole plant are touched on in two applications only (maize Bt11 and fodder beet), no testing was carried out, though. In the former case it was argued that both the Btk and PAT proteins will be rapidly digested in the intestine. Hence, there should be no need for long term studies. In the latter case long-term studies are disregarded by arguing that the GMP is substantially equivalent to the parent plant. Furthermore, it is argued that for methodological reasons whole plant toxicity studies would not lead to meaningful results.

A summary of tests provided in Directive 90/220/EEC dossiers is shown in Table 34 (Appendix).

A similar picture emerged from the Novel Food dossiers: in case of newly introduced proteins usually acute oral toxicity studies were performed (in each Article 4 dossier and in four of six Article 5 dossiers). Only in two dossiers the full toxicity studies are enclosed. In these cases the testing approach is similar to the one applied for chemical products. However, a closer look revealed that certain details, comprising e. g. dosage, observation period, organs subjected to histopathological observation, do not correspond to the respective OECD guidelines.

In the rape Topas 19/2 dossier no toxicity studies were provided at all.

In the dossiers of maize 1507, maize T25, and sweet maize Bt11 only references were given to a 14 day toxicity study of the PAT protein. In the latter dossier further references to additional short-term toxicity studies, immune toxicity and subchronic toxicity testing were provided. However, these studies are not enclosed in the dossiers.

Whole-plant toxicity testing was only carried out in case of maize GA21 (subchronic 90 day study in rats). Feed conversation studies are provided in most dossier.

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[19] These studies might, however, rather have been motivated by the requirements of the US registration as plant pesticide and/or by the fact that the Bt protein is intentionally used because of its toxic properties.

Toxicity assessment in Article 4 dossiers compared to Article 5 dossiers is largely similar. In general acute oral toxicity tests of introduced proteins are conducted in mice. This is also true in the cases of e. g. sweet maize Bt11 and the Article 5 dossier of Bt11. Whereas the former is also intended to be consumed as raw vegetable the latter will be subjected to processing.

Therefore it can be concluded that testing practice is similar in Directive 90/220/EEC and Novel Food dossiers.

A summary of toxicity studies provided in the Novel Food dossiers is presented in Table 7, and in greater detail in Table 36 (Appendix).

Table 7: Toxicity studies in Novel Food dossiers

Substance investigated / type of study	Testing described[a]		Comments
	Article 4	Article 5	
Protein of target gene	4(4)	5(6)	In three cases of both Article 4 and Article 5 dossiers only references to published toxicity studies were provided. In case of maize 1507 reference was given to the review work of the US EPA.
Marker gene protein	0(0)[b]	1(1)[b]	In case of maize 1507 reference was given to the review work of the US EPA.
Whole-plant/whole-food	1(5)	0(6)	-
Other toxicity studies	2(5)	4(6)	Various sequence homology comparisons to known proteins were provided. In case of rape GT73 also eco-toxicity studies were provided.

[a] Number of dossiers including toxicity tests either as full text or summary (total number of dossiers investigated or applicable in brackets); [b] Functional marker genes are shown not be present in the plant. The dossier for Rape MS1xRF1 and MS1xRF2 is presumably incomplete and is therefore not considered in this table.

3.1.4 Line of reasoning

The assessment of toxic properties of GMPs usually comprises three elements: assessment of the toxicity of the introduced proteins, assessment of substantial equivalence of the GMP, and estimate of exposure. Homology studies comparing the amino acid sequence of introduced proteins to those of known toxins are often provided in Novel Food dossiers to support the toxicity assessment. Moreover, a broad spectrum of assumptions is presented to support safety claims.

3.1.4.1 Toxicity of introduced proteins

In both type of dossiers, applicants attempted to proof the safety of introduced proteins essentially by acute toxicity tests. This is also true for marker proteins. In certain cases additional toxicity tests were performed. In two cases toxicity tests were not considered necessary at all because the introduced proteins are

not considered novel to the particular plant (potato EH92-527-1) or to the plants in general (carnation).

Proteins used in testing are generally produced in bacteria (mainly E.coli). In case of Bt plants and also in other GMP dossiers equivalence of the test substance with plant produced Bt-protein remains unclear.[20]

Introduced DNA sequences other than target or marker gene are not considered relevant in terms of toxicity assessment by the applicant.

3.1.4.2 Substantial equivalence

Although the term substantial equivalence is not used in the toxicity chapter of each of the Directive 90/220/EEC applications, the concept seems to play a key role in supporting toxicity safety claims in each of these dossiers.[21]

Similarly, in Novel Food dossiers the conclusion of toxicological safety is generally based on the claim of (partial) substantial equivalence to conventional plants or food.

3.1.4.3 Secondary effects

Only in the maize GA21 Novel Food dossier a whole-plant sub-chronic toxicity study (maize kernel) in rats was provided.[22] Furthermore, and in contrast to other (Novel Food) dossiers, the risk assessment was based on the NOEL derived from the sub-chronic study and on an estimate of consumer exposure.

Pleiotropic or other secondary effects on plant metabolism of the genetic modification are either not properly addressed or not considered at all as a possible limitation of the substantial equivalence claim. Occasionally, from the mere absence of phenotypic changes the absence of secondary effects is concluded (maize GA21 and rape Topas 19/2, both Novel Food dossiers). Only in the Directive 90/220/EEC dossier of maize GA21 the possibility of unintended expression of endogenous genes close to the integration site is discussed.

3.1.4.4 Exposure

Exposure to man, animals and the environment was considered to be low or even negligible in most Directive 90/220/EEC dossiers. In the carnation dossiers exposure was, however, not considered at all.

Absorption of introduced proteins is believed to be low because these proteins are deemed to be easily digestible in the intestine. Digestibility is usually supported by in vitro tests. This is true for Directive 90/220/EEC and for Novel Food dossiers. Some dossiers also provided measurements of concentrations of newly introduced proteins in parts of the GMP (maize Bt11, GA21, Bt cotton 531, potato EH92-527-1).

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[20] In case of Bt GMP dossiers it was not quite clear whether Bt is already registered as a plant pesticide in the EU.

[21] Possible exemptions are the two carnation dossiers that do not make use of the substantial equivalence claim.

[22] There might also be similar studies for Bt plants. However, it was not quite clear whether the particular studies mentioned in the dossiers are applicable to the type of Bt proteins used in the GMPs.

3.1.4.5 Arguments supporting safety claims

Both Directive 90/220/EEC and Novel Food dossiers frequently contain a broad spectrum of assumptions to support safety claims. These assumptions are differing widely in soundness and plausibility.

Examples from Directive 90/220/EEC are:

- Only the newly introduced proteins are relevant for toxic properties: thereby secondary effects of gene transfer are disregarded.
- The parental line deemed to be "safe": This might be correct; nevertheless, this represents rather an assumption then factual information confirmed by rigorous testing (ROYAL SOCIETY OF CANADA, 2001).
- Proteins that are degraded in in vitro tests (e. g. simulated gastric fluid) are not considered as hazardous as they cannot act in a systemic way in vivo.
- Further examples can be found in Spök et al. (2002a, 2003a).

Some arguments refer to general opinions e. g. *"It is well established that EPSPSs [...] have been safely consumed throughout humankind's existence, and are not associated with any health concerns"* (maize GA21 Directive 90/220/EEC dossier (Cultivation), SNIF, p. 20).

Another type of arguments refers to the "absence of hazards" e. g. *"[...] no hazards of the product have been identified"* (Carnation 66 dossier), *"[...] no specific hazard has been identified in RR maize line GA21 or its progeny as a result of expression of the mEPSPS protein"*. Or: [inserted protein and terminator sequences] *"are not associated with toxic or allergenic effects related to consumption or exposure"* (Bt11 maize dossier).

Similarly, Novel Food dossiers contain arguments such as *"[...] maize has a long history of safe use world-wide"* (maize NK603 Novel Food dossier). *"The absence of hazards [...] is supported by establishing that the recipient organism, maize, has a history of safe use"* (Summary of application to the UK ACNFP, maize MON810 Novel Food dossier) without providing further support of this claim. In contrast, a more cautious wording was chosen in the sweet maize Bt11 dossier: *"[...] maize as food ingredient has not, to our knowledge, been reported to pose a health risk to humans".*

3.1.4.6 Comparison of maize Novel Food dossiers(Article 4, Article 5)

Information provided in both Article 4 and Article 5 maize dossiers was compared.

These two type of dossiers seem to differ mainly in structure and the way data are displayed. The scope of toxicity assessment and size of the toxicity chapter were largely similar and included the testing and arguments described in Section 3.1.3: acute oral toxicity of newly introduced proteins in mice, in vitro digestibility studies of newly introduced proteins, homology studies.

Deviations from this "standard" practice were found in the maize GA21 dossier (Article 4) that included a 90 day sub-chronic toxicity study of maize kernel in rats and maize T25 dossier (Article 5), that referred to a 14 day toxicity study of the PAT protein.

3.1.5 Evaluation of toxicity assessment

3.1.5.1 General aspects

The lack of formal structure, especially in case of Directive 90/220/EEC dossiers, is likely to slow down any scientific review. Toxicity relevant statements are sometimes scattered in the dossiers. The relevance of arguments and what particular conclusions should/could be drawn from them is sometimes difficult to reveal – if possible at all. References to safety relevant statements are important to verify whether a statement is based e. g. on actual testing results, literature data or on general opinions. However, such references are systematically provided in some dossiers only and – even in these dossiers – are not consequently done throughout the dossier. The fact that additional studies that are already available at the time of the application are submitted to the authorities on request only, further drag on the review process. Full reports on tests carried out are rarely enclosed in the dossiers. Instead, references, summaries containing various levels of detail are given. However, in order to verify the scientific value of both the data displayed and the claims included a review of the full reports is considered indispensable.

In case of Novel Food dossiers the overall clarity of the applications is clearly improved compared to Directive 90/220/EEC dossiers as applicants try to comply to the Recommendation 97/618/EC. However the pile of supplementary documents that are provided by applicants on request of national CAs in the course of the review procedure renders the structure of these dossiers less clear. Nevertheless, in general, observations described above for Directive 90/220/EEC dossiers also apply to Novel Food dossiers.

Typically, toxicity assessment of GMPs is based on acute toxicity tests of introduced proteins, on additional data that might be of relevance for toxic properties of the protein (especially homology studies) or for exposure (digestibility studies), and on the claim of substantial equivalence (see Sections 3.1.3 and 3.1.4). In addition, a broad range of additional arguments is provided in the dossier (see especially Section 3.1.4.5).

3.1.5.2 The relevance of substantial equivalence for toxicity studies

The concept of substantial equivalence is key in most Directive 90/220/EEC and in all Novel Food dossiers investigated.

The concept which is usually represented as a starting point for risk assessment (FAO/WHO, 2000a; CODEX ALIMENTARIUS COMMISSION, 2002) seems to be rather the most important element of toxicity assessment instead of a starting point. Toxicity studies conducted do not seem to depend on considerations of substantial equivalence. Rather they are carried out on top of substantial equivalence.

The equivalence status is also considered sufficient to either disregard secondary effects or to rely on indicators that are not considered appropriate, e. g. to conclude the absence of secondary effects on the mere basis of the absence of morphological differences (see bellow).

3.1.5.3 Disregarding secondary effects

The occurrence of secondary effects of genetic modification is a heavily contested issue. However, such effects are well acknowledged in the literature (e. g. THE ROYAL SOCIETY OF CANADA, 2001; FAO/WHO, 2000b; STIRN, 1998; FRANCK-OBERASPACH & KELLER, 1996).

Possible secondary effects that would render a GMP toxic are disregarded in most Directive 90/220/EEC and Novel Food dossiers. At least in case of Novel Food dossiers one reason for this might be that in each case full or partial substantial equivalence is claimed.

However, such effects would not necessarily appear in comparative compositional analysis or in comparing morphological criteria. For instance an increase in the concentration of a naturally occurring plant carcinogen would not necessarily be detected by these approach and methods – especially if the carcinogen has not been characterised so far. Nevertheless this would be an important health issue.

Therefore, whole-plant/whole-food toxicity studies are considered inevitable.

Feeding studies described in the dossiers can, however, definitely not be considered as whole-food toxicity studies. Rather, they are feed conversion studies and only very distinct toxic effects such as death or loss of weight would came up from this type of studies.

3.1.5.4 The relevance of acute oral toxicity testing

Toxicity testing in both 90/220/EEC Directive and Novel Food dossiers is usually restricted to acute oral toxicity of the introduced proteins. In some cases this is justified by reference to SJOBLAD et al. (1992). The authors of this paper claimed that "*if toxicity testing of a protein is considered necessary then acute exposure studies in laboratory animals should be sufficient , since – if toxic – proteins are known to act via acute mechanisms*" (p. 8). This opinion was originally published in the context of registering enzymes as plant pesticide products and was subsequently frequently quoted in various applications and guidance documents. From this assertion it can, however, not be concluded that proteins in general cannot exhibit other than acute toxic properties. Nor can it be assumed that there has been any systematic attempts already to investigate such effects. Quite in contrast, a preliminary literature search of toxic endpoints of proteins revealed that there have been no such attempts and studies – at least none are published in scientific journals.

Furthermore, there are other aspects that have to be considered: the well described sensitizing properties of proteins cannot be considered an acute toxic effect. In contrast, sensitisation is caused by long-term changes in the immune system and could not be detected by acute toxicity testing (a discussion on this issue is included in the Sections on allergenicity 3.2 and 4.2).

Proteins (e. g. exotoxins, Bt-toxin) can exhibit receptor-mediated effects. Hormonal or other long-term effects could result form this. Enzymes might also interfere with metabolism.

Oligopeptids resulting form protein digestion in the intestine can act as toxicants in various ways.

A special group of proteins, prions, are capable of chronic effects.[23]

Possible toxic effects of proteins beyond acute toxicity are also acknowledged – or at least not disregarded in the first place – by scientific advisory committees. Thus, acute toxicity testing is considered of little relevance for substances/products that might be consumed over a lifelong period. This view is shared by the SCF on food additives (SCF, 2001) and by EMEA on herbal medicines (EMEA, 1998). SCF, FDA, and JEFCA either generally require further toxicity endpoints for food enzymes or at least propose such endpoints for particular cases (UMWELTBUNDESAMT/IFZ, 2002).

3.1.5.5 In vitro digestibility studies

Besides the particular toxicity modes of proteins it is often argued that proteins by their very nature are very sensitive to the acid and protease rich environment of the stomach and would be rapidly degraded. Hence they could not exhibit any systemic effects.

However, it is not quite clear that proteins will be rapidly degraded in each case: e. g. plant protein toxins ricin (castor beans) and abrin (pinto beans) (MARQUARDT & SCHÄFER, 1994) as well as lectins are quite resistant to the action of digestive enzymes. These proteins can in fact exhibit systemic effects.

Moreover, abrin could also be absorbed percutaneously and can thereby even act as toxin (ibid.).

Hence, the question has to be dealt with how to discriminate between proteins that are susceptible to digestion and those that are more resistant. For this purpose many applications included in vitro studies.

In principle, digestibility can be investigated in vivo or by application of in vitro models. However, back in 1998 the SCP explicitly disregards the value of in vitro studies in the context of GMP risk assessment: *"Evidence of degradation of the introduced gene products should be based on data obtained in vivo by feeding the GM plant material or its derived products to the intended target animal. [...] The use of in vitro simulation of gastric and intestinal digestion of the gene product should be considered supplementary to in vivo experiments designed to measure the survival of the gene products when fed to animals as an integral part of the GM plant. Isolated proteins are known which are fully degraded in the simulated gastric system but survive gut passage intact when fed as part of a normal diet"* (SCP, 1998).

Given that recommendation it is quite surprising that in both type of dossiers, Novel Food and Directive 90/220/EEC, only in vitro studies are documented – if conducted at all.

3.1.5.6 Homology studies

Homology studies are usually conducted in the course of GMP risk assessment to check for similar properties in terms of safety. Such studies are applied in the dossiers in different ways:

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[23] It has however to be acknowledged that prions are an exemption; the mentioning of which should not mean that there is any link between the issue investigated and prions.

Firstly, homology studies are applied to compare amino acid sequences of introduced proteins to those of known toxins. A lack of homology is seen as indication for absence of toxic properties. Secondly, homology to non-toxic proteins is considered as indicator for the absence of toxic properties. Thirdly, homology studies are also applied when comparing the introduced plant-derived protein and the bacterial derived test substance. Forth, in one particular case in spite of a very high homology between two EPSPS enzymes some minor differences in the amino acids are considered sufficient to change technical properties of a particular enzyme.

The latter case is of particular importance as it points to contradictory ways of interpreting homology studies. EPSPS enzymes, for instance, that are originating from different source organisms and considered as highly homologous were shown to *"vary widely with respect to their degree of sensitivity to inhibition of glyphosate"* (PADGETTE et al., 1993 in Novel Food dossier maize NK603). This is also true in case of 99,3% homology as shown in the maize GA21 Novel Food dossier.

Given the fact that highly homologous proteins vary widely with respect to a functional property (sensitivity to glyphosate),[24] would not that contradict the frequently raised claim that homologous proteins are sharing identical or similar properties in terms of toxicity?

On the other hand, proteins that share less than 50% homology are nevertheless deemed as similar and, hence, similar (non)-toxic (Novel Food Maize NK603 dossier). Given what is said above, less than 50% homology is not considered a valuable indicator for similar toxic properties. Even if one still agrees to the general claim that homologous proteins will show similar toxic properties it has to be asked, what degree of homology is considered sufficient and what particular algorithm and what particular parameters should be used in homology comparisons to reasonably conclude similar properties in terms of toxicity? Furthermore, it would have to be asked if this approach would also serve as an indicator for toxicity endpoints beyond acute toxicity.

3.1.5.7 Test substance

Proteins that are used in toxicity testing are usually produced in bacteria, mostly in E.coli, as this is probably the easiest (and cheapest) way to produce sufficient amounts proteins for purification. Here another problem of identity and similarity comes up that would however not be detected by homology studies: plants proteins are often glycosylated whereas bacteria in general and E.coli in particular are either not or only to a very limited extent capable of glycosylation.

Some authors (e. g. UMWELTBUNDESAMT/IFZ, 2002 and also one of the commentators to this study) argue that identity of two proteins, even on the level of tertiary structure, cannot be guaranteed by methods routinely applied. As a consequence, it would not be possible to specify the test substance adequately. If the test substance is not adequately specified though, the relevance of toxicity studies could be questioned. Hence, toxicological testing on basis of bacterial proteins would have to be reconsidered.

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[24] Similarly, in case of allergenic properties the substitution of a few amino acids might change their potential to sensitise (see Chapter 3.2.3). However, in this case structural changes might be more important than functional changes.

3.1.5.8 Assumption based reasoning

Compared to other regulatory contexts, e. g. to chemicals a higher proportion of assumption based reasoning can be found in the dossiers.

Some of the arguments presented to support safety claims can easily be revealed as assumptions lacking a solid base of evidence. Quite a number of those assumptions refer to general beliefs, e. g. the parental line deemed to be "safe":

"[...] it is well established that EPSPSs [...] have been safely consumed throughout humankind's existence, and are not associated with any health concerns" **(maize GA21 dossier (Cultivation), SNIF, p. 20);** ***"[...] maize has a long history of safe use world-wide"*** **(NK603 dossier).**

as there is apparently no factual scientific basis to back up these assumptions. In the latter case harmful effects of EPSPS would not been evident in case of long-term effects. For instance the establishment of a causal connection of cancer, teratogenic or other chronic effects to a food ingredient would only be possible in the course of long-term feeding or epidemiological studies. Both type of studies usually do not exist for normal food plants.

A similar type of arguments refers to the "absence of hazard" without any clarification if and in what particular way toxic properties have been systematically investigated e. g.

"[...] no hazards of the product have been identified**" (Carnation 66 dossier);** ***"[...] no specific hazard has been identified in RR maize line GA21 or its progeny as a result of expression of the mEPSPS protein"*** **[Inserted protein and terminator sequences]** ***are not associated with toxic or allergenic effects related to consumption or exposure*****" (Bt11 maize dossier).**

Given what is said above it is not surprising that no toxic properties are evident if there had been not investigation before.

Another type of assumptions is implicit in the assessment approach. For instance, the claim that only the newly introduced proteins are relevant for toxic properties. Thereby secondary effects of the gene modification are disregarded in general or disregarded for their toxicity implications (for a brief discussion see Section 3.1.5.3). Another example is that proteins that are degraded in vitro tests (simulated gastric fluid) would give no cause for concern as they cannot act in a systemic way in vivo (for a brief discussion see Sections 3.1.5.4 and 3.1.5.5). In both cases these assumptions are not representing a kind of scientific consensus, rather they are contested in the literature and guidance documents resp.

3.1.5.9 Lack of structured risk assessment

A systematic approach to risk assessment as used for pesticides and pharmaceuticals rests on hazard assessment and estimation of exposure. If exposure is likely to exceed a dosage associated with toxic effects a toxicity risk can be concluded.

In contrast Directive 90/220/EEC dossiers (and the opinions and reviews of the competent authorities as well) are pursuing a rather unsystematic approach and are frequently mixing hazard assessment and risk assessment. As a consequence safety claims cannot easily be verified.

In cases either the hazards or the exposure is deemed negligible a low risk could be concluded without contrasting hazard and exposure. However, in principle each substance is capable to act as a toxicant – depending on the dosage. Hence, assessment of a toxicity risk would be difficult without a proper estimate of the exposure.

Disregarding exposure as negligible has in turn to be based on sound evidence. Exposure scenarios for man, animals and the environment would have to be designed. A negligible exposure would be difficult to proof though, even case of carnation as ornamental plants. For, a contact to human skin, an exposure to pollen and an environmental exposure via plant residues after disposal seems to be likely.

One way out of this would be to establish a concept that specifies that hazards of two products are identical if certain criteria apply. Accordingly the assessment approach would depend on the degree of identity or equivalence. Thereby, we arrive at the concept of substantial equivalence. In practice, however, this concept does not seem to be used as a guidance tool for risk assessment (see Section 3.1.4.2).

3.1.5.10 Quality assurance

Conducting studies according to GLP is scarce in Directive 90/220/EEC dossiers and more frequent in Novel Food dossiers.

GLP is frequently demanded in risk assessment of chemicals, plant pesticides, biocides, and medicinal products but was not required for GMO products neither according to Directive 90/220/EEC nor according to Novel Food Regulation. However, GLP would reduce reasonable doubts on data displayed and conclusions drawn in the dossiers. For, GLP would warrant that testing is comprehensively described, including all incidents and would prevent deliberate or unintentional omissions, alterations, palliation, and even distortions.

It should be emphasised that in case of safety relevant investigation the enclosure of papers published in scientific journals would not replace GLP. For, in case of papers submitted methods and conclusions are subjected to a peer review, whereas it is not considered if the published data actually corresponds to the raw data. This would only become apparent if one would repeat the experiments described. Safety studies, however, are not likely to be verified by repetition and – even if they would – a repetition might be difficult or even impossible because of the confidential nature of data and results.

A draft guidance of CODEX ALIMENTARIUS COMMISSION (2002) has recently included requirements for GLP in safety relevant testing. Raw data should be provided on request to CAs.

3.1.5.11 Comments on Directive 90/220/EEC dossiers from the perspective of the new Directive 2001/18/EC

Compared to Directive 90/220/EEC additional assessment requirements are included in Directive 2001/18/EC. Some of these requirements are quite clear-cut: antibiotic resistance marker genes are no longer permitted, thus potato EH92-527-1 and Bt cotton 531 would not be likely to be approved because of the antibiotic resistant gene present in the plant.

Others are more difficult to interpret, e. g. the requirement to consider harmful effects even if they are deemed unlikely. One likely reading of this provision would be that potential secondary effects of genetic modification will have to be taken into account.

3.1.5.12 Comments on Novel Food dossiers from the perspective of the new EC Regulation

The EC Regulation for genetically modified food and feed intends to establish a level of safety that is at least equivalent to the requirements of Directive 2001/18/EC. Thereby, for GMPs harbouring certain antibiotic resistant gene (such as rape Topas 19/2, rape MS1xRF1 and MS1xRF2) the risk assessment may lead to different results.

Interpreting the requirement to consider potentially harmful effects even if they are considered unlikely might mean to extend toxicity endpoints to include e. g. reproduction toxicity.

EC Regulation 178/2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority (EFSA) and laying down procedures in matters of food safety establishes risk analysis as the basis for food law in order to *"a high level of protection of human health and life"* (Article 6). This could be interpreted that risk assessment will be required to be based on comparison of exposure assessment and hazard analysis which is rather considered an exemption so far (see Section 3.1.5.9).

Article 14 states that in determining whether any food is injurious to health, regard shall be had: (a) not only to the probable immediate and/or short-term and/or long-term effects of that food on the health of a person consuming it, but also on subsequent generations; (b) to the probable cumulative toxic effects; (c) to the particular health sensitivities of a specific category of consumers where the food is intended for that category of consumers. Again this can be interpreted in favour of extending toxicity endpoints beyond acute toxic and allergenic effects.

3.2 Assessment of allergenic properties

3.2.1 General aspects

In principle allergenicity assessment of GMPs can ask for allergenic properties of introduced proteins and of the whole plant or food. Exposure is considered a relevant factor for both Directive 90/220/EEC and Novel Food dossiers.

3.2.1.1 Exposure

The question of exposure is a more complex issue in case of Directive 90/220/EEC applications as they are aiming at different usage of GMPs comprising import, processing for and use as feed or non-food/non-feed purposes, storage, handling, cultivation, seed production. Two of the applications filed before the Novel Food Regulation had entered into force were even including application in food products.

Given these different applications different exposure scenarios can be envisaged including intended and erroneous ingestion as raw/processed product by man, farm animals or wild-living animals, inhalation of dust containing plant com-

pounds by factory workers during processing or inhalation of pollen by the general population.

From this one would expect both systematic considerations of exposure and that these differences will be reflected in the design of allergenicity assessment.

However, as shown in Table 8, the various routes of exposure were not systematically considered in the dossiers. If exposure is mentioned at all, the focus lies on ingestion by man. Exposure to pollen is only mentioned in three of a total of seven dossiers (not considered here: carnation dossiers) that were aiming at cultivation, but was disregarded for reasons of pollen not spreading via air (cotton dossiers) and a presumed general lack of allergenic properties of the plant (maize GA21 dossier).

Table 8: Consideration of exposure in allergenicity assessment in the context of Directive 90/220/EEC dossiers

Application	Intended use	Exposure
Fodder beet A5/15	Cultivation, seed production, feed stuff	Different routes of exposure not explicitly considered
Potato EH92-527-1	Cultivation, seed production, starch production for industry	Different routes of exposure not explicitly considered
Maize GA21	Import, processing for feed stuff (no cultivation, no use as food)	Different routes of exposure not explicitly considered Via Pollen: not considered as maize is deemed not allergenic
	Cultivation, feed stuff	
Maize Bt11	Import, processing	Only ingestion route considered
	Cultivation	
Bt cotton 531	Cultivation, feed stuff, industrial application	Only ingestion route considered Via pollen: not considered (will not be distributed via air) Via products: oil, wool: no protein present;
RR cotton 1445	Cultivation, feed stuff (especially for poultry, sheep, catfish, and pigs)	
Rape Topas 19/ 2	Import, processing, cultivation, oil production, feed stuff	Different routes of exposure not explicitly considered
Carnation 66	Ornamental plant	Different routes of exposure not explicitly considered (exposure is however restricted according to the intended use)
Carnation 959A etc.	Ornamental plant	

As can be seen in Table 9 the different exposure routes associated with the applications are also hardly reflected in the particular design of allergenicity assessment.

In contrast to Directive 90/220/EEC dossiers the route of exposure is quite clear-cut in the context of Novel Food dossiers. According to the scope of the Novel Food Regulation ingestion is the only relevant exposure route to consider in allergenicity assessment. Nevertheless, the respiratory route might be important in terms of sensitisation.

With the possible exception of two Novel Food dossiers (maize T25, rape MS1xRF1 and MS1xRF2) only the ingestion route was considered. Usually, no consideration is given to other exposure routes or sensitization scenarios, in particular to respiratory sensitisation.

Table 9: Studies conducted and safety relevant arguments used in allergenicity assessment in the context of Directive 90/220/EEC dossiers

Application	Homology comparison	Digestibility studies	Source not known to be allergenic	Present in only small amount in the human diet	Not glycosylated	Other
Fodder beet A5/15	+	+	-	+	-	-
Potato EH92-527-1	-	+[c]	-	-	-	-
Bt cotton 531	-	-	-	-	-	-
RR cotton 1445	-	-	-	-	-	
Carnation 66	-	-	-	-	-	+[b]
Carnation 959A etc.	-	-	-	-	-	
Maize GA21	+	+	+	-	-	+[a]
	-	-	-	-	-	
Maize Bt11	-	+	-	+	+	-
	-	-	-	-	-	
Rape Topas 19/ 2	+	-	-	-	+	

Unless otherwise stated data pertain the newly introduced protein only. Allergenic properties of the whole plant was not considered. Applications above the bold line are clearly restricted to non-food purposes. Applications bellow the bold line were either intended to be used in food products (maize Bt11, rape Topas 19/2) or followed by applications according to the Novel Food Regulation (maize GA21, maize Bt11, rape Topas 19/2). [a]) Maize is not deemed allergenic ; [b]) no evidence of allergic effects in literature and after commercialisation in Australia; protein not considered as novel to the human diet; [c]) only a marker protein introduced; +... found in the dossiers; -... not found in the dossiers; n.a... not applicable.

3.2.2 Line of reasoning

3.2.2.1 Allergenic properties of introduced proteins

Allergenicity assessment in the context of both Directive 90/220/EEC and Novel Food dossiers did not include direct testing of the allergenic potential neither of the introduced proteins nor of the whole plant or food. In case of introduced proteins usually studies (exceptions: Directive 90/220/EEC dossier potato EH92-527-1, Novel Food dossier soybean 260-05) were conducted (homology comparisons to known allergens, digestibility studies) and arguments were raised that provided some indication that was deemed sufficient to assess the allergenic potential of the protein. In a number of cases it was argued on the basis of expression studies that the new protein(s) will be present in the food/human diet in very small amounts only. In one case heat stability was investigated and in an-

other case glycosylation was analysed. Table 9 provides an overview of studies found or referred to as well as safety arguments raised in Directive 90/220/EEC dossiers in the context of allergenicity assessment. Similarly, Table 10 shows studies found in or referred to in Novel Food dossiers.

Low levels of expression of the introduced protein(s) and the fact that these proteins would be present in the human diet in marginal amounts only are thereby considered as indicators of low levels of exposure. Digestibility studies, however, are not that much linked to exposure – as in case of toxicity assessment. Rather it is referred to that allergens are frequently stable against digestion, elevated temperatures and extreme pH environments. Similarly it is referred to that allergens are frequently glycosylated.

Table 10: Overview of studies in the context of allergenicity assessment in Novel Food dossiers

Application	Gene product of target gene			Gene product of marker gene			Whole plant/food
	Digestibility studies	Homology studies	Other	Digestibility studies	Homology studies	Other	
Applications acc. to Article 4							
Maize NK603	T	T	-	-	-	-	-
Maize 1507	R	R	R[a]	-	-	-	-
Sweet maize Bt11	T	T	-	T	T	-	-
Maize GA21	T	R	-	-	-	-	-
Soybean 260-05	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	T[c]
Applications acc. to Article 5							
Rape Topas 19/2	T	T	-	-	-	-	-
Rape GT73	R	R	-	-	-	-	-
Maize T25	T	T	T[b]	-	-	-	-
Maize Bt11	T	-	-	-	-	-	-
Maize MON809	R	R	-	-	-	-	-
Maize MON810	-	-	-	-	-	-	-
Total	9(10)	8(10)	n.a.	1(10)	1(10)	n.a.	1 (11)

No studies were found in the Rape MS1xRF1 and MS1xRF2 dossier. However, as this dossier was found to be incomplete (possibly due to copy errors) it was not considered for this analysis. [a]) heat stability; [b]) not glycosylated; [c]) IgE binding studies; T... actual testing conducted; R... only references provided; -... not found in the dossiers; n.a.... not applicable.

Table 11:Overview of line of reasoning found in Novel Food applications. Not included are statements pertaining the expression of the introduced protein in the GMP

Applications	Not widespread in the diet	No sequence homology detected	Easily digestible	Source not-known to be allergenic	Not glycosylated	Not novel to human diet	Other
Maize NK603	+	+	+	+	-	-	-
Maize 1507	-	+	+	+	-	-	-
Sweet Maize Bt11	+	+	+	+	+	-	-
Maize GA21	+	+	+	+	-	-	-
Soybean 260-05	-	-	-	-	-	-	+[a]
Rape MS1xRF1 and MS1xRF2[c]	-	+	-	-	-	-	+[b]
Rape Topas 19/2	-	+	+	-	-	-	-
Rape GT73	+	+	+	+	+	+	-
Maize T25	+	+	+	-	+	-	+[d]
Maize Bt11	+	+	+	-	+	-	-
Maize MON809	+	+	+	+	-	-	-
Maize MON810	+	+	+	+	-	-	-
Total	8(12)	11(12)	10(12)	7(12)	4(12)	1(12)	n.a.

[a]) Allergenic potential equivalent to parental type; [b]) no evidence of allergenic effects; [c]) no other arguments were found in the Rape MS1xRF1 and MS1xRF2 dossier. However, this dossier was found to be incomplete (possibly due to copy errors); [d]) susceptible to heat; +... found in the dossiers; -... not found in the dossiers; n.a..... not applicable.

The majority of tests were conducted by the applicants, in a number of cases, though, only references were given. Digestibility studies are thereby rarely included as full reports and some of these studies were most likely carried out for reasons other than allergenicity testing.

3.2.2.2 Allergenic properties of the whole plant/food

With the exception of the Soybean 260-05 dossier, possible secondary effects of the genetic modification that could lead to up-regulation of known allergens or to appearance of new ones are not considered at all.

The soybean dossier included a serum screen applying sera of patients already sensitised to soybean. This study was designed as a comparative analysis, comparing GMP and parental line soybean.

3.2.2.3 Approach to allergenicity assessment

Thus, the main line of reasoning in allergenicity assessment found in both type of dossiers was as follows:

The introduced protein

1. **Originates from a source not known to be allergenic.**
2. **Does not show a significant sequence homology to known allergens.**
3. **Will be easily digested in the gastrointestinal tract.**
4. **Is not glycosylated.**
5. **Is expressed in the plant/tissue in very small amounts only.**
6. **Is not considered "novel" to human diet (is already part of the human diet).**

Table 11 provides an overview of arguments invoked in allergenicity assessment in Novel Food dossiers. As can be seen, arguments No 1 to 4 largely represents the standard set of arguments.

3.2.3 Evaluation of allergenicity assessment

Allergenicity assessment in both Directive 90/220/EC and Novel Food dossiers was in almost all cases restricted to provide indirect evidence largely based on homology comparisons, digestibility studies, and analysis of glycosylation as well as on safety relevant information (e. g. allergenicity of source). The overall approach in Directive 90/220/EEC and in Novel Food dossiers is thereby largely similar. There is also little or no difference between Article 4 and Article 5 Novel Food dossiers.

In the view of the authors of this Monograph this approach does not provide a sound basis for safety claims. For, evidence presented and arguments invoked in the dossiers appears to be of questionable or limited validity in the light of recent scientific evidence as will be discussed in the following.

3.2.3.1 Allergenic properties of introduced proteins

The introduced protein originates from a source not known to be allergenic

A frequently used argument for the lack of an allergenic potential of a GMO was that the newly inserted gene does not originate from a known allergen source. Of course, it is obvious that the insertion of a known allergen into a host organism will increase the allergenic potential and should therefore be avoided.

It should however be emphasized that the route of presentation and the overall immunogenicity of an antigen is extremely important for allergic sensitization. It is therefore possible that proteins which do not behave as allergens in one source may become highly allergenic in another organism provided that they are expressed in a tissue which, for instance, easily releases the protein at the mucosa of individuals.

Furthermore, it is possible that the insertion of a non-allergenic protein derived from a source which is not allergenic into a host organism may induce the activation of allergens and thus indirectly increase the allergenic potential of the GMO (see bellow).

If considering the source organism as a relevant information, attention should be paid to progress in allergenicity research. Several dossiers contained incorrect judgments as to whether a certain source can be considered as allergenic source or not. For example, bacteria and maize were deemed non-allergenic sources although it is meanwhile well established that both are potent allergen sources (PASTORELLO et al., 2000; BUNIKOWSKI et al., 1999).

The introduced protein does not show a significant sequence homology to known allergens

Sequence comparison of the newly inserted protein with allergens submitted to the data bases are one of the instruments most frequently applied in the dossiers for allergenicity assessment.

A major criticism is related to the methodology of sequence comparisons. Both, the routinely used sequence comparison technologies such as FASTA and BLAST (PEARSON, 2000; ALTSCHUL et al., 1990; ALTSCHUL & LIPMAN, 1990) as well as new methods developed for predicting the allergenic potential of a given protein (STADLER & STADLER, 2003) will deliver wrong results in many cases. It is well known that non-allergenic isoform of allergens exist which differ only regarding a few amino acids from their allergenic counterparts. Such non-allergenic isoforms have been described for many allergen sources e. g., birch and hazel pollen (VAN REE, 2002; FERREIRA et al., 1996). Two isoform of the birch allergen, Bet v 1a and Bet v 1l differ in a few amino acids only. Bet v 1a shows a high IgE-binding activity whereas isoform Bet v 1l shows a very low IgE-binding activity. Because of their low allergenic potential such isoforms have been even suggested as candidates for allergen-specific immunotherapy (FERREIRA et al., 1998; ASTWOOD et al. 1996). Likewise, proteins with significant sequence homology to major allergens but without any allergenic activity have been described (LAFFER et al., 2003). For example, sequence homology was shown between a cytokinin-inducible periwinkle protein (T1) and 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. Despite of the significant sequence homology the periwinkle protein is immunologically distinct from the Bet v 1 allergen family and had no allergenic properties. Recombinant T1 does not induce immediate-type skin reactions in Bet v 1 allergic patients. In these cases, sequence comparisons would have identified the exemplified proteins wrongly as allergens. It must therefore be concluded that it will be quite difficult to properly interpret sequence homologies as an indicator for allergenic properties.

According to the FAO/WHO 2001 decision tree, a lack of sequence homology should lead to a serum screen. However, this was not pursued in any of the dossiers investigated.[25]

[25] The authors are of course aware that most of the dossiers investigated in the context of this project were filed before the FAO/WHO decision tree was published.

The introduced protein be easily digested in the gastrointestinal tract

In the dossiers investigated the absence of the allergenic potential of a novel protein was justified by the fact that the protein will be easily digested.

ASTWOOD et al. (1996) describe that food allergens are more stable against digestion than non-allergenic proteins and suggest to consider digestive stability as a parameter to indicate allergenicity. In contrast, HEISS et al. (1996) showed that following trypsin digestion of mugwort pollen the IgE-binding of patients' sera to profilin, an ubiquitous cross-reactive plant allergen, was completely abolished, whereas IgE reactivity to a 60 kDa component was less affected. Patients sensitised to pollen profilins react to a broad range of inhalant and nutritive allergen sources. Furthermore, a more recent paper by FU et al. (2002) food allergens and proteins without known allergenic properties are compared with respect to their stability to digestion. The results, however, did not confirm that food allergens are more stable to digestion in vitro than proteins with unproven allergenicity.

Testing for pepsin resistance of the novel protein nevertheless represents one step in the FAO/WHO 2001 decision tree for allergenicity assessment. The FAO/WHO paper, however, acknowledged that pepsin resistance or complete degradation resp. of a protein cannot be used to reliably predict allergenicity or the absence of allergenic properties resp. of proteins (FAO/WHO, 2001). This can also be illustrated by the above mentioned example of profilin. When applying the FAO/WHO 2001 decision tree to the case of profilin, a reduced allergenicity would have to be predicted. However – as described above – profilin is a potent ubiquitous cross-reactive plant allergen.

Even if food allergens are shown to be susceptible to in vitro digestion it remains to be clarified whether this is only valid for the purified protein or if it would be also true if the allergen is imbedded in a particular food or tissue (matrix effect).

Thus, in vitro digestibility of a given protein does not seem to be a reliable indicator of allergenic properties.

The introduced protein is not glycosylated

In the dossiers investigated another argument frequently raised against an allergenic potential was that the novel protein is not glycosylated.

This assertion stands, however, in sharp contrast to the fact, that non-glycosylated proteins can be important allergens. For example, profilins representing cross-reactive plant allergens are ubiquitous cytoskeletal proteins which bind to actin and which are not glycosylated (VALENTA et al., 1991). Another group of cross-reactive allergens the calcium-binding proteins are also not glycosylated (NIEDERBERGER et al., 1999). Both allergen families, profilins and calcium-binding proteins, represent potent well characterized allergens, which are tested for their IgE reactivity, for induction of basophil histamine release and immediate type skin reactions. Moreover, a lot of recombinant non-glycosylated allergens are described and compared to the natural allergens by in vitro and in vivo assays in the literature. Some of these recombinant allergens are already used in commercially available *in vitro* assays.

On the other hand there is evidence that carbohydrate components might play a role for IgE-binding and histamine release. BATANERO et al. (1999) describes carbohydrate components isolated from the major allergen of olive tree pollen,

Ole e 1, which binds IgE from sera of patients allergic to olive pollen and which induces histamine release from blood cells. These authors concluded that IgE raised against the carbohydrates could be responsible for extensive reported cross-reactivity among pollen and foods.

Given these examples and in the absence of further evidence on the role of glycosylation for allergenicity, the glycosylation of a protein does not seem to be a reliable indicator for allergenic properties.

The introduced protein is expressed in the plant/tissue in very small amounts only

Another argument frequently used in the dossiers against an allergenic potential was that the novel protein is expressed at low levels only.

Expression levels of proteins can, however, vary depending on plant growth, different developmental stages and environmental stress (MITTERMANN et al., 1995). Profilin might be a good example for the variability of allergen content in different developmental stages as well as for high expression level. This allergen represents actin-binding proteins which had been described as cross-reactive plant allergens (VALENTA et al., 1991) e. g., in birch, timothy grass, maize and tobacco. Profilin can be detected in different somatic tissues of tobacco but the expression and the content is 50-100 fold higher in mature pollen than in seed or leaf. During early stages of pollen development no profilin was detected, whereas in mature pollen large amounts of profilin were found. At the RNA level profilin transcripts could be detected in mature and germinated tobacco pollen. No transcripts were found in early pollen development, leaves and ovaries. Again a developmental up-regulation of profilin expression in mature pollen could be detected (MITTERMANN et al., 1995).

In a paper by VIETHS et al. (1994) sixteen apple varieties were analysed regarding their IgE-binding capacity to the major apple allergen. The authors showed that apple varieties with a high amount of the major apple allergen had a high IgE-binding capacity, whereas apple varieties with a low amount had a low IgE-binding capacity.

Varying allergen contents are also observed for the lipid transfer protein Pru p 3, the major allergen of peach (CARNÉS et al., 2002). The concentration of lipid transfer protein Pru p 3 in peach peel extracts was approximately seven times greater than in pulp extracts.

Given the possibility of varying expression levels of a protein depending on plant growth, different developmental stages and environmental stress proteins might become allergenic because of their increased amount.

Even a report of a Joint FAO/WHO expert consultation concluded that it is not yet possible to define a kind of expression threshold for a protein below of which a protein can be considered as safe regarding allergenicity . Thus, the level of expression should not be included in the assessment of the allergenicity of genetically modified foods (FAO/WHO, 2001). However, this does not mean that expression levels should not be considered. Quite in contrast they will be important e. g. in the course of exposure assessment.

The introduced protein is not considered "novel" to human diet (is already part of the human diet)

There is no established relationship between novelty to human diet and allergenic properties. Many well known allergens are not considered as novel proteins in human exposure. E. g. animal serum albumin which is a widespread protein in the human diet, is one of the strongest animal allergens (SPITZAUER et al., 1995). Hence, novelty of a protein either to the human diet or to the immune system does not seem to provide a proper indication of allergenic properties of a protein.

3.2.3.2 Allergenic properties of the whole plant/food

Allergenic properties of the whole plant are disregarded in almost all dossiers. The soybean 260-05 dossier might be the only exception that included a IgE-binding study comparing conventional comparator and GMP. However, in this case there is no novel protein expressed in the GMP and in the absence of this test allergenicity assessment would have to be done on the basis of reasoning only.[26]

Even in case of GMP expressing a novel protein there is evidence that allergenic potential should be considered beyond the allergenic properties of the introduced proteins. For, genetic modifications may lead to an up-regulated expression of allergens already known to be present in the plant. Alternatively, it might render "normal" proteins allergenic because of increased expression levels.

Many plant allergens were identified as pathogenesis-related proteins, expression of which is induced by stress, pathogen attack, hormones, and abiotic stimuli (HANNINEN et al., 1999; BREITENEDER et al., 2000; HOFFMANN-SOMMERGRUBER, 2001; 2002; MIDORO-HORIUTI et al., 2001).

3.2.3.3 Additional remarks

The claim that maize does not contain allergens, for instance, is contradicted by convincing evidence for allergens in maize pollen that are cross-reactive with the main allergens in grass (STAIGER et al., 1993; BROADWATER et al., 1993; PASTORELLO et al., 2000).

The paper of ROTHBARD & GEFTER (1991) (Novel Food and Directive 90/220/EEC dossier: maize GA21; Novel Food dossier MON810) is quoted with respect to IgE epitopes. Taking a closer look at this paper reveals that the paper does not deal with IgE rather with T-cells epitopes.

[26] In principle, this is also true for the potato EH92-527-1 Directive 90/220/EEC dossier. However the potato was not intended for human consumption. Rather it was primarily aiming at applications in the non-food/non-feed industry – usage (of residues) as feed were nevertheless considered.

3.3 Substantial equivalence

3.3.1 Novel Food dossiers rape (Article 5)[27]

3.3.1.1 Compositional analysis

In all dossiers compositional analysis focuses on macro-compounds of raw seeds. There is no consistency in the generation of samples between the dossiers in terms of number of years, regions and locations (see Table 12).

Table 12 Rape dossier field trials: years, regions and number of locations

Dossier	Years		Regions		Locations	
	Number	Year	Number	Geographical declaration	Number per year	Total
MS1xRF1/ MS1xRF2	4	1991	n.s.	B S F CA	11	28
		1992	n.s.	B F S	4	
		1993	n.s.	B F S UK, CA	8	
		1994	n.s.	B F UK CA	5	
Topas	4	1991	3	CA	6	19 *
		1992	2	CA	4	resp.
		1993	3	CA	6	58 **
		1994	1	CA	3	
		Co-Op 92/93	n.s.	CA	19/20	
GT73	2	1992	3	CA	7	11
		1993	2	CA	4	

... without Co-Op-trials, because these data originate from official variety monitoring programs carried out by Ag-Canada and not by the applicant; **... with Co-Op-Trials.

Abbrev.: B... Belgium, S... Sweden, F... France, CA ...Canada, UK... Great Britain; n.s.... not specified, Co-Op... Western Canadian Cooperative.

In addition to compositional comparisons of raw seeds also comparisons of crude oil, meal and/or processed products were carried out (see Table 13). These comparisons, however, include only a small number of samples and/or compounds and consider only one year.

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[27] Three Novel Food dossiers claiming substantial equivalence for rapeseed oil derived from GM rape were investigated. Data on compound analyses were compared between the three dossiers to show the different ways applicants chose to claim substantial equivalence. Furthermore composition-data of different foodstuff or raw products listed in international food composition and nutrition tables were linked to figures and tables presented in the dossiers to check for plausibility. In a second step the practice of applicants was compared to recent guidelines and/or recommendations. The OECD Task Force for the Safety of Novel Foods already published a set of "Consensus Documents" that provide lists of plant specific key nutrients, anti-nutrients and toxicants which should be considered as a minimum set of compounds in order to prove substantial equivalence. The "*Consensus Document on Key Nutrients and Key Toxicants in low Erucic Acid Rapeseed*" was published in 2001 (OECD, 2001). A Guideline on Oilseed Rape elaborated in 2001 by the European Association for Bioindustries is available also (EUROPABIO, 2001a). Comparing the practice of applicants to these recommendations shall highlight any differences between the most recent requirements and what is presented in the dossiers. It must be borne in mind however that both guidelines had not been available at the time of application. Comparing the practice among the dossiers itself is also interesting as it points to a lack of harmonization and probably even to differences in the soundness of the substantial equivalence claim.

Table 13: Rape dossiers: sample material for compositional analysis

Dossier	Raw products			Processed products		
	Seed	Meal	Oil	Refined oil	Refined hydrogen-ated oil	Meal toasted
MS1xRF1/ MS1xRF2	++	+	+	+	+	+
Topas	++	+	+	+	+	+
GT73	++		-	+	-	+

+... only one comparison, small sample, few compounds (meal: glucosinolates, other antinutritive compounds in part, amino acids in part, sporadically some minerals; oil: fatty acid profile, sterols/tocopherols in part, some physical characteristics), no information on sample origin, field trials, trial design and sampling practice; ++... comparisons of samples from two or more consecutive years (different scale),data on sample origin and partly also on field trials, trial design and sampling practice; -... not presented in the dossier; Abbrev.: n.s.... not specified.

An exact description of field trial conditions and trial design is lacking in all dossiers as well as detailed information on agricultural practice applied (see Table 14). The customary agricultural practice that can be expected when growing plants commercially does not seem to be considered. This is of particular importance with respect to herbicide resistant plants. For, single evaluation sheets of herbicide treated plants, which can occasionally be found in the dossiers, show that the application of the herbicide can clearly alter the composition of samples. However, in most comparisons it is not clear, whether herbicide had been applied. As information on herbicide application is missing, it must be assumed that non-treated plants had been used. Thus, the products used for compositional analysis differ from those included in the human diet.

Table 14: Rape dossiers: agricultural practice

Dossier	Field Trial			Control	
	Description of trial design	Description of agricultural practice	Application of herbicide	Isogenic	Others
MS1xRF1/ MS1xRF2	In part	In part	In part	+	-
Topas	+	-	-	-	+
GT73	-	-	-	+	-

+... included in the dossier/considered; -... not included in the dossier/not considered.

Sampling practice and preparation of samples are not described in detail described – if described at all (see

Table 15). Description of analytical methods applied differs in quality and detailed information on practice of analysing is generally missing. In most cases, detailed laboratory protocols are not included.

Table 15: Rape dossiers: sampling and analysing

Information provided	MS1xRF1 / MS1xRF2		Topas		GT73	
	Raw products	Processed products	Raw products	Processed products	Raw products	Processed Products
Sampling practice	-	-	In part	-	-	-
Sample storage	-	-	-	-	-	-
Sample preparing	-	In part	In part	In part	-	In part
Analyzing method	R	+	In part + R	In part	-	-
Analyzing practice	-	-	-	-	-	-
Laboratory protocols	One	Some	-	Some	-	-

+...information included in the dossier; -...information not included in the dossier; R...only references given.

Although all applicants were seeking clearance for rapeseed oil, comparative studies of raw products as well as processed products differ in the range of compounds compared. Not even the same set of major compounds is considered in the dossiers. The practice of using different units in data presentation makes it difficult or impossible to compare data across dossiers (see Table 37 and Table 38 in the Appendix). Sometimes even within the same dossiers different units are used.

Whether a proper statistical evaluation of all comparative studies had been carried out is not always evident to the reviewer or cannot be verified because of lacking information on methods and programs and of statistical evaluation sheets that are missing (see Table 39).

Only the Topas dossier contains statistical evaluations sheets (for major compounds, glucosinolates and fatty acids). Within the MS1xRF1/MS1xRF2 dossier a statistical evaluation is neither evident nor mentioned in the text. Surprisingly, statistical evaluation sheets for two comparative studies for protein, fat and glucosinolates are annexed. It remains unclear, if other compounds have been evaluated too. Within the GT73 dossier a statistical evaluation and some significant differences are mentioned in the text without any further explanation. Statistical evaluation sheets are not annexed. It remains unclear, what had been evaluated and which statistical method had been used.

With respect to compositional comparisons of processed products a statistical evaluation is missing in all dossiers.

3.3.1.2 Evaluation

All applicants state, that their rape seed oil is substantial equivalent to conventional ones. This assertion seems to be the main safety argument and not the starting point of risk assessment as laid down in recent OECD documents and Explanatory Notes to the Novel Food Regulation (OECD, 2002b; Recommendation 97/618/EC; EC, 2000).[28]

Comparative studies: As the Novel Food Regulation also requires to consider nutritional value, all applicants claim, that their oil has the same nutritional quality than conventional rape seed oils. This statement is, however, backed up by the assertion of substantial equivalence based on comparisons of major components of raw products, only. More extensive comparisons of components of processed oil and a nutritional assessment are lacking in all dossiers. Some data on refined oils are included, though, as part of processing studies which have been carried out by each applicant to demonstrate equivalent processing properties of the transgenic oil. These studies had been carried out with a small number of samples from only one or a few locations collected in one season and did either include a rather poor description of measurement practice only or even none.

Comparative studies are not considered representative. Furthermore, the set of compounds compared does not include all components of nutritional importance and important nutritional indexes are even missing (see Table 16 and Table 17). Thus, is has to be concluded that the claim of "same nutritional value" is not based on sound analysis and data.

Table 16: Rape dossiers: differences in composition analysis of refined oil[a]

Compound	MS1xRF1 / MS1xRF 2	Topas	GT73
Minerals	Ca, Mg, Fe, Cu	P	-
Heavy metals	-	-	+
Arsenic	-	-	+
Lead	-	-	+

[a]) Considered in all dossiers: Fatty acids (two times 13, once 12), Tocopherols (α, β, γ), Sterols (Brassica-, Campe-, β-Sitosterol), Free fatty acids.+... considered in the dossier; -... not considered in the dossier.

Table 17: Rape dossiers: compounds analysed in refined oil compared to compounds listed in SOUCI et al. (2000)

SOUCI et. al (2000)	MS1xRF1 / MS1xRF2	Topas 19 / 2	GT73
Oleic acid g/100g	+	+	+
Linoleic acid g/100g	+	+	+
Linolenic acid g/100g	+	+	+
Vitamin-E-Activity mg/100g	-	-	-

[28] For example Recommendation 97/618/EG states: *"If a new food or food component is found to be substantially equivalent to an existing food or food component, it can be treated in the same manner with respect to safety, keeping in mind that establishment of substantial equivalence is not a safety or nutritional assessment in itself, but an approach to compare a potential new food with its conventional counterpart"* (EC, 1997).

SOUCI et. al (2000)	MS1xRF1 / MS1xRF2	Topas 19/2	GT73
Total Tocopherols mg/100g	-	-	-
α-Tocopherol mg/100g	+	+	+
χ-Tocopherol mg/100g	+	+	+
δ-Tocopherol mg/100g	+	+	+
Vitamin K µg/100g	-	-	-
Retinol equivalent µg/100g	-	-	-
β-Carotene mg/100g	-	-	-
Selenium µg	-	-	-
Sterols total mg/100g	-	-	-
Brassicasterol mg/100g	+	+	+
Campesterol mg/100g	+	+	+
Sitosterol mg/100g	+	+	+
Stigmasterol mg/100g	-	-	-

+... considered in the dossier; -... not considered in the dossier.

In all dossiers the following shortcomings could be detected:

- **information on origins of sample material and descriptions of field trials is lacking; small samples (pooled samples from 4 locations at maximum, sometimes no information on sampling provided at all – possibility that only single samples had been analysed), only samples from one year collected;**
- **data on nutritionally relevant compounds like vitamin A and K as well as on carotenoids or selenium lacking;[29]**
- **nutritional indexes like the ratio of saturated to poly-unsaturated fatty acids (P/S-ratio) or the ratio of mono-unsaturated to poly-unsaturated fatty acids (MUFA/PUFA-ratio) lacking;[30]**
- **correlation of vitamin E and poly-unsaturated fatty acids as a marker for the sensitivity to oxidation lacking;[31]**

- **data on stigmasterol lacking;**
- **data on hydrogenated oil lacking (GT73 only);**
- **Nevertheless it has to be mentioned that the range of fatty acids, vitamins and sterols considered in all three rape dossiers correspond to compounds proposed in the OECD Consensus Document (OECD, 2001: C16:0, C16:1, C18:0, C18:1, C18:2, C18:3, C20:1, C20:2, C22:0, C22:1, C24:0 , Tocopherols, Sterols , Pigments (Chlorophyll).**

All applicants are focussing on comparisons of components in seeds. If seeds are found to be substantial equivalent all following products are concluded to be substantial equivalent as well. The statement of substantial equivalence is thereby mainly based on comparisons of protein, fat, glucosinolates and fatty acids. Other macro and micro components are, in general, less frequently considered or even not considered at all. The same is true for anti-nutrients. One dossier contains data on sinapin, another one on phytic acid (in toasted meal; see Table 18). This (narrow) set of compounds seems to be sufficient, though, for claiming equivalence for refined oil, as most of the components of raw seeds will not be present in significant amounts in the final product. But with respect to seeds the chosen set of compounds seems to be not comprehensive enough to justify substantial equivalence – especially for reliably detecting possible unintended effects that must not be evident by significant changes in levels of macro compounds.

Furthermore some significant differences in at least one of the composition comparisons are reported in each dossier but never triggered a repetition of the analysis including broadening of the range of compounds in order to more reliably exclude unintended effects. However, this is regarded as necessary in order to properly justify the claim of substantial equivalence. In contrast, differences were attributed to naturally occurring ranges, effects from backcrossing, climate conditions etc.

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[29] Selenium is considered important from a nutrition point of view. A complex interactive system of different antioxidants and enzymes protects the oxidation-sensitive parts of the human body (especially cell membranes and genes). The major substance protecting body lipids is vitamin E which can scavenge free radicals and disrupt already ongoing chain-reactions and thus hinder or minimize oxidative damage of body lipids, mainly membrane lipids. By doing so vitamin E itself looses its antioxidant capacity and needs to be regenerated. The enzyme glutathione peroxidase plays a prominent role in the regeneration of vitamin E and Selenium is an integral part of this enzyme. If a food product contains both vitamin E and selenium it improves the body's resistance to oxidative stress more than a product only containing one of these substances.

[30] When analysing the dietary habits of humans in the so called industrialised world an over-consumption of fat in general is evident. The fat quality is however, often inadequate and does not meet recommendations given by the scientific nutrition societies. Reason for this inadequacy is a too high – proportion of saturated fatty acids (SFA) in human diets and a relatively too low proportion of mono-unsaturated (MUFA) and poly-unsaturated fatty acids (PUFA). Saturated fats are thus, playing a major role in human diets and are one of the main factors responsible for the development of coronary heart diseases and some kinds of cancer.

Within the group of edible oils rapeseed oil has the lowest proportion of saturated fatty acids and together with safflower oil and soy oil a significant proportion of α-linolenic acid. Like all PUFA α-linolenic acid has a lowering effect on blood cholesterol levels and furthermore it can counteract the agglutination of platelets and thus prevent thromboses. Rapeseed oil contains a high proportion of oleic acid which provides manifold applications in food as oleic acid is neutral with respect to cardiovascular effects.

[31] Rapeseed oil contains on mean of 23 g α-tocopherol equivalents per 100 g oil (15-24 g/100 g) or 65 g total tocopherols per 100g oil (spectrum: 39-69 g/100g) which is good compared to many other oils. (SOUCI et al., 2000). A high concentration of tocopherols is considered a quality criterion for oils high in unsaturated fatty acids, as vitamin E inhibits oxidation and thus preserves the quality of the oil.

Moreover, the claim of substantial equivalence is based on a poor description of sampling and of the data collection practice as well. It also remains unclear, if statistical evaluations had been properly carried out.

Thus, given the incomplete data presented none of the dossiers can be accurately verified.

More observations are stated bellow:

- **Field trials:** Field trial conditions, design and practice are insufficiently described in all dossiers. Especially the GT73 dossier does not contain any information on field trials. Topas and GT73: only data on Canadian samples, no data from samples from European test fields included. Comparisons over more years vary significantly in number of locations and geographical regions.
- **Controls:** Data on "controls" within the Topas dossier are derived from different traditional varieties (not isogenic) (see Table 14). This pool of comparators also varies in the consecutive years. As the set of control lines varies significantly such an approach must be regarded not suitable for reliably detecting possible changes in composition. FAO/WHO recommends: "*The comparator used to detect unintended effects for all critical components should ideally be the near isogenic parenteral line grown under identical conditions*" (FAO/WHO, 2000a). Correspondingly, in the OECD Consensus Document on rape an isogenic or near isogenic line is recommended for comparative purposes (OECD, 2002a).
- **Herbicide application:** In general herbicide untreated material has been used for compositional analysis. Apparently only within the MS1xRF1/MS1xRF2 dossier three single comparative studies have been carried out with herbicide treated products. A few single evaluation sheets mentioned herbicide application but further comments are not included and the results of these sites are not summarised and presented or explained in detail. However, from these sheets it is evident, that application of herbicide can noticeably alter the composition of samples. Conclusions drawn upon comparisons with herbicide untreated material are therefore considered questionable given that the expected commercial use will in any case include herbicide application. It can therefore be concluded, that the products used in compositional analysis and for which substantial equivalence had been established are different to the ones consumers will be exposed to.
- **Sampling:** Only the Topas dossier contains some information on sampling practice and preparation, but this not continuously provided for all comparisons throughout the dossier.
- **Methods:** Detailed descriptions and/or references to commonly accepted and published analysing methods can be found within the Topas dossier for most of the analyses done (but not for all). The MS1xRF1/MS1xRF2 dossier contains references to commonly applied methods for most of the analyses whereas one cannot find any information or references within the GT73 dossier. If methods are not described in detail or not referenced adequately the quality of the analyses can however not be accurately assessed.
- **Measurement practice:** Information on practice is lacking in all dossiers. Thus it remains unclear, if the values specified are at least means resulting from double testing. It could also be possible, that specified values are the result of a single analysis which cannot be considered state of the art. There is also no mentioning in the dossiers, if analyses had been carried out by apply-

ing a blindfold testing design. Furthermore, no reference is made whether or not GLP is applied.

- **Statistics:** With regard to statistics only the Topas dossier contains comprehensive information on applied methods as well as on software used. Statistical evaluations sheets, however, show that the data pools for statistical evaluations do not always correspond. For some component comparisons results from some locations are not included in the respective statistical evaluation whereas for others the results of that location are included. No explanation is provided. Consequently it cannot be excluded, that values that did not fit had simply been dismissed. The MS1xRF1/MS1xRF2 dossier contains statistical evaluation sheets of fat, protein and glucosinolates for two single comparison studies but no further information on the method applied. The GT73 dossier does not contain statistical evaluation sheets. From the latter two dossiers it remains unclear, which comparisons had been subjected to statistical evaluation and which methods had been applied. In conclusion the statistical evaluation are more or less questionable in all dossiers, for, information is lacking that would enable to verify that statistics had been carried out in a proper way and is actually state of the art.

Table 18: Rape dossiers: Differences in composition analysis of raw seeds [a]

Compounds	MS1xRF1 / MS1xRF2	Topas	GT73
Fibres	-	+	+
Ash	-	+	+
Carbohydrates	-	-	+
Sinapin	-	-	+
Tocopherols	-	+	-
Sterols	-	+	-

[a)] *Considered in all dossiers: Protein, Fat, Fatty acids, Amino acids, Glucosinolates. .+... considered in the dossier; -... not considered in the dossier.*

3.3.1.3 Comparing the dossiers to recent Consensus Documents

The Consensus Document on rape (OECD, 2001) does not contain proposals for components to be considered for establishing substantial equivalence of rape seed. Instead, it lists key compounds, anti-nutrients and toxicants to be considered in feed. Unfortunately the units proposed in the Consensus Document do not always correspond to the ones applicants were using. The rape document of EuropaBio (EUROPABIO, 2001a) contains proposals for components to be considered for establishing substantial equivalence in raw products. In this document, no units are specified at all. Compared to the OECD Consensus Document the industry Guideline contends itself with a smaller set of compounds, but includes vitamin E. Vitamin E is listed in the OECD document as to be considered in refined oil but not in feed. With regard to amino acids glutamic acid and aspartic acid are listed in the EuropaBio document, whereas both amino acids are not mentioned in the OECD document.

Table 19 provides an overview on what compounds were considered by the applicants to substantiate the claim of substantial equivalence compared to what is presently considered as the minimum set of compounds.[32] It must be borne in mind, that both guidelines had not been available at the time of application. Where proposed units are not met, the respective units specified in the dossiers are presented in the table. As far as glucosinolates and amino acids are concerned, one can find different practices of specifying quantities even within the same dossier.

Table 19: Compounds analysed compared to compounds listed in OECD Consensus Document and EuropaBio Guideline

OECD		EuropaBio	MS1xRF1 / MS1xRF2	Topas 19 / 2	GT73
Protein %		+	+	+	+
Fat %		+	+	+	+
Fibres %		-	-	+	+
	ADF %	+	-	-	-
	NDF %	+	-	-	-
Ash %		+	-	+	+
Amino acids (16)[a] %		+ [k]	+ [c] [k]	+ [g] [l]	+ [h] [k]
Fatty acids (11)[b] % of total fatty acids		+ [m]	+ [d] [n]	+ [m]	+ [d] [m]
Glucosinolates µmol/g oil-free meal		+	+	+	+
	3-butenyl	Optional	+ [e]	+	[i]
	4-pentenyl	Optional	+ [e]	+	[i]
	2-hydroxy-3-butenyl	Optional	+ [e]	+	[i]
	2-hydroxy-4-pentenyl	Optional	+ [e]	+	[i]
Minerals %					
	Ca	+	+ [f]	-	+ [f]
	K	+	+ [f]	-	
	Mg	+	+ [f]	-	+
	Na	+	-	-	+
	P	+	+ [f]	-	+
Trace elements mg/kg				-	
	Co	-	+	-	-
	Cu	-	+	-	+
	Fe	-	+		+
	J	-	-	-	-
	Mn	-	+	-	+

[32] An important qualification may be that these components are proposed for feed.

OECD		EuropaBio	MS1xRF1 / MS1xRF2	Topas 19 / 2	GT73
	Se	-	+	-	-
	Zn	-	+	-	+
Tannins % meal		-	-	-	-
Sinapin % meal		-	-	-	+ [j)]
Phytic acid % meal		+	-	-	+

Numbers in brackets in the boxes for amino acids and fatty acids show the number of different amino/fatty acids analysed and compared. [a]) Ala, Arg, Cys, Gly, Iso, Leu, Lys, His, Met, Phe, Pro, Ser, Thr, Try, Tyr, Val; EurobaBio in addition: Asp, Glu; [b]) like refined oil; [c]) mg/g seed; [d]) % oil; [e]) in part; [f]) ppm; [g]) nmol/g seed; [h]) g/100g DW and g/100g protein; [i]) Alkyl-Glucosinolates total; [j]) mg/g oil-free meal; [k]) 18; [l]) 24; [m]) 11; [n]) 12,11,10,9,7. .+... considered; -... not considered.

Source: OECD (2001), EuropaBio (2001a), results from the review of dossiers.

3.3.2 Novel Food dossiers maize (Article 5)[33]

3.3.2.1 Compositional analysis

Compositional analysis is carried out with raw material only. Comparisons focus on macro compounds in raw seeds. In addition, one can find also comparisons of raw plant material or silage in some dossiers, whereas analyses of processed products are missing (see Table 20). There is no consistency in the generation of samples between the dossiers in terms of number of years, regions and locations (see Table 21 und

Table 22).

Table 20: Maize dossiers: sample material for compositional analysis

Dossier	Raw products			Processed products
	Kernel	Plantmaterial	Silage	
T25	+	-	+	-
Bt11	+(+)	-	-	-
MON810	++	+	-	-
MON809	+	+	-	-

+... samples from one year; +(+)... samples form one year and samples from green house; ++... samples from two years.

Table 21: Maize dossiers: years, regions and locations – comparisons of kernel

Dossier	Year		Region		Locations
T25	1	1994	2	Illinois, Indiana	2
Bt11	1	1994 major comp.	5	3 US-North, 2 US-South	12
		1994 AA/FA	2	Illinois, Wisconsin	2
		1995 Vit/Min green-house	3	Wisconsin, Ohio, Iowa	3
MON810	2	1994	n.s.	USA	6
		1995	n.s.	France	3
		1995-2nd generation	2	France, Italy	2
MON809	1	1994	n.s.	USA	6

AA... amino acids; FA... fatty acids; n.s.... not specified.

[33] Four Novel Food dossiers claiming substantial equivalence for maize products derived from GM maize were investigated in the same way as described for the rape dossiers (see Section 3.3.1). Similar to previous Section a OECD Consensus Document "*Consensus Document on Compositional Considerations for New Varieties of Maize: Key Food and Feed Nutrients, Anti-Nutrients and Secondary Plant Metabolites*" published in 2002 (OECD, 2002a) and a Guideline elaborated by EuropaBio (EUROPABIO, 2001b) was used in the analysis.

Table 22: Maize dossiers: years, regions and locations – comparisons of plant material

Dossier	Year		Region		Locations
T25	1	1994	2	Illinois, Indiana	2
Bt11	-	-	-	-	-
MON810	1	1995	n.s.	France	3
		1995-2nd generation	n.s.	France, Italy	2
MON809	1	1994	n.s.	France (3), Italy (1)	4

n.s.... not specified.

Detailed descriptions of field trial conditions and trial design as well as detailed information on agricultural practice are lacking in all dossiers. Controls used are either isogenic non-modified varieties, "similar" varieties or parental lines (see Table 23). The agricultural practice used does not seem to correspond to the practice that can be expected when growing plants commercially. The T25 dossiers includes quite a number of single result sheets, also one (!) of a herbicide treated sample (silage) which shows that the application of the herbicide has altered the composition remarkably in some of the compounds analysed even beyond the variation range of conventional counterparts used. Apparently, all other analyses have been conducted with untreated samples. Thus, products used for compositional analysis differ from the ones that will be included in the human diet.

Table 23: Maize dossiers: field trials and controls

Dossier	Field Trial			Control		
	Description of trial design	Description of agricultural practice	Application of herbicide	Isogenic	Similar	Parental
T25	In part	-	n.s.	+	+	-
Bt11	In part	-	n.s.	+	-	-
MON810	-	-	n.r.	-	+	-
MON809	-	-	n.s.	-	+	-

n.s... not specified; n.r.... not relevant. .+... control used; -... not included (field trials)/not used (controls).

Sampling practice and preparation of samples are not described in detail or even not described at all (see

Table 24). Description of analytical methods used is of different quality even within the same dossier (covers the whole range of annexing detailed descriptions of the methods to cases where methods are not mentioned at all). Information on practice of analysing is generally missing. Laboratory protocols are not annexed in most cases.

Table 24: Maize dossiers: sampling and analyzing

Information provided	T25	Bt11	MON810	MON809
Sampling practice	+	In part	In part	In part
Sample storage	-	-	-	-
Sample preparing	In part	In part	In part	In part
Analysing method	+	References but not for all	References	References
Analysing practice	In part	In part	-	-
Laboratory protocols	+	-	-	-

.+...provided in the dossier; -... not provided in the dossier.

Although all applications were seeking clearance for processed products, comparative studies have only been restricted to raw products and are different range of compounds analysed. When compared between dossiers, not even major compounds are corresponding to each other. The different units used by applicants to present data on amino acids renders it almost impossible to compare data across dossiers (see Table 41 and Table 42).[34]

In three dossiers a statistical evaluation is mentioned either in the text or as a footnote to a table. Methods are in general not described in detail (software, confidence level). In some cases it remains unclear, what actually had been subjected to statistical evaluation. Apparently, only in the T25 dossier statistical evaluations of all comparisons had been carried out. Statistical evaluation sheets or summaries of the statistical evaluations are generally not included.

3.3.2.2 Evaluation

All applicants claim, that their maize products are substantially equivalent to conventional products. This assertion seems to be the main safety argument and not the starting point of risk assessment as laid down in recent OECD documents and some explanatory notes to the Novel Food Regulation to structure dossiers and processes (OECD, 2002b; Recommendation 97/618/EC; EC, 2000).

With the exception of MON810 all dossiers contain data from one growing season only which cannot be considered representative.

Data on the composition of major maize products are not included in any case. This is in contrast the proposals of the recent OECD Consensus Document on maize which specifies not only compounds to be considered in kernels but also compounds to be considered in processed products (refined oil, grits, flocks, meal, starch).

[34] In the Bt11 dossier vitamins are specified in mg/lb, which is unusual and also complicates comparing the values to literature data or to values in other dossiers. In many tables units and/or reference magnitudes are completely missing. Occasionally they can be found in the text, sometimes one can extract them from other data presentations within the same dossier and sometimes units and/or reference magnitudes remain unclear. Units that are not specified or difficult to reveal are not deemed appropriate, are hampering evaluation and even may render it impossible to evaluate the data.

The claim of substantial equivalence of final products is based on compositional comparisons of raw seeds;[35] focusing in general on a few macro components (but not on a complete set). Only the Bt11 dossier contains data on some micro compounds (see Table 25). Data on components that significantly influence the nutritional value are lacking in all dossiers. In all cases the set of compounds however must, nevertheless be regarded as too narrow to justify substantial equivalence of raw products and – with the exception of syrup[36] – also of processed products. Especially, in order to increase the likelihood of detecting possible unintended effects the narrow set of compounds used is not considered appropriate. For, unintended effects need not necessarily trigger striking changes in levels of macro components.

Table 25: Maize dossiers: similarities and differences – compositional analysis

Not considered in all dossiers[a]	T25	Bt11	MON810	MON809
Nitrogen total	-	+	-	-
Carbohydrates	+	-	+	+
Starch	-	+	-	-
Fibres	+	+	-	-
ADF	-	-	+	-
NDF	-	-	+	-
Cellulose	-	+	-	-
Xanthophylls	-	+	-	-
Vitamin B1	-	+	-	-
Vitamin B2	-	+	-	-
Niacin	-	+	-	-
Folic acid	-	+	-	-
Cu, Mg, Mn, Zn	-	+	-	-

[a]) considered in all dossiers: protein, fat, fatty acids, amino acids, ash, moisture. .+... considered in the dossier; -... not considered in the dossier.

Some significant differences were reported in each dossier but did not trigger a repetition of the analysis including a broadening of the range of compounds in any case. This would however be a more appropriate procedure for detecting any unintended effects.

The claim of substantial equivalence is based on poorly described sampling and data collection followed by incomplete data presentation and therefore cannot be accurately verified in all cases. On the basis of information provided it is often not clear, if statistic evaluations had been carried out in a proper way and is actually state of the art.

[35] In some cases also on raw plant material, but plant material is not used in human nutrition therefore these comparisons is not considered in this Chapter.

[36] Starch and all products derived thereof usually do not contain components of the raw seed anymore.

As the Novel Food Regulation also requires to consider nutritional value, all applicants claim, that their maize products do have the same nutritional quality than conventional products. This claim is based on the assertion of substantial equivalence that in turn is based on comparisons of major components of raw products only. Data of processed products and further nutritional assessments are missing in all dossiers. Thus, the claim of "same nutritional value" is not based on sound data and testing.

Moreover, additional observations on possible shortcomings and differences between the dossiers are described in the following:

- **Field trials:** No representative geographical distribution of samples in order to reflect naturally occurring geographical variances (except in Bt11 dossier in one year). T25 dossier: only data on 2 locations in USA for only one year (samples from Europe are not considered, although existing);[37]) Bt11 dossier: amino acid comparison has been carried out with samples from only 2 locations in USA. Samples from greenhouse are not appropriate for protein and amino acid comparisons. Field trial conditions, as well as design and practice are not described in sufficient detail in all dossiers. In case of MON809 and MON810 descriptions of practice of field trials are even lacking.
- **Controls:** Isogenic controls only in Bt11 dossier and partially in T25 dossier.
- **Herbicide application:** In three cases applicants were seeking clearance for herbicide resistant maize but used herbicide untreated material in almost all comparisons. Only in the T25 dossier some data on herbicide-treated samples are included. An in-depth survey of these data reveals that the difference in composition of treated and untreated samples is larger compared to samples and controls. Given that herbicide will be used in commercial application in any case, conclusions drawn on data derived without herbicide treatment are therefore considered questionable. Products used for compositional analysis and for which substantial equivalence is claimed are not thus different from those included in the human diet.
- **Sampling:** Some information on sampling practice and preparation is included in all dossiers, but not throughout dossiers and therefore must be regarded as insufficient (e. g. to reproduce tests). In the T25 dossier contradicting information on sampling practice and preparation is provided and discrepancies can even not be resolved with the help of the annexed raw data.
- **Measurement practice:** Some information on measurement practice is included in the T25 and Bt11 dossier but completely missing in the others. Laboratory protocols are only included in the T25 dossier. The rest of the dossiers does neither included raw data nor conclusive summaries.
- **Statistics:** In the MON810 dossier a statistical evaluation is neither mentioned nor evident. In the rest of the dossiers, information on statistics is more or less precise but no dossier includes statistical evaluation sheets. It remains unclear which comparisons have been subjected to a statistical evaluation. Detailed information on methods, software and confidence limits are only provided in the Bt11 dossier. Hence, it cannot be verified that statistical evaluations had been carried out properly.

[37] There are data available on Europe as well, because they are mentioned in the corresponding Directive 90/220/EEC dossier.

- **Processed products: None of the dossiers contains data on processed products. The claim of "same nutritional value" is based on assumptions only and not backed by sound data. For, comprehensive data on nutritional relevant components or nutritional indexes are lacking in all cases.**

3.3.2.3 Comparison of dossiers to recent Consensus Documents

The review of the dossiers also revealed diverging definitions of "proximates" (see Table 26). The OECD Consensus Document on maize (OECD, 2002a) defines "proximates" as: protein, fat, fibres, ADF, NDF, ash and carbohydrates. In contrast, the EuropaBio Guidelines consider "proximates" to be limited to protein, fat and ash. In three of the dossiers the expression "proximates" is also used. In the Bt11 dossier the expression "kernel quality" or "grain property" is used instead. No applicant defines "proximates" as broad as the OECD Consensus Document. Again, this points to a need for standardisation.

Table 26: Maize dossiers: different interpretations of "proximates"

	Protein	Fat	CH	Fibres	ADF	NDF	Ash
OECD	+	+	+	+	+	+	+
EuropaBio	+	+	-	-	-	-	+
T25	+	+	+	+	-	-	+
MON809	+	+	+	-	-	-	+
MON810 1994	+	+	+	-	-	-	+
MON810 1995	+	+	+	-	+	+	+
Bt11	"Grain properties" (protein, fat, starch, fibre) or "kernel quality" (total nitrogen, ash, starch, cellulose, xanthophylls, fatty acids, amino acids)						

CH... Carbohydrates. .+... considered in the dossier; -... not considered in the dossier.

Unfortunately, the OECD Consensus Document does not specify measurement units for fatty acids, amino acids and some antinutritive substances. Moreover, some literature ranges mentioned in this document are specified in differing units. These parts of the Consensus Document therefore need, to be revised. In the maize Guidelines of EuropaBio (EUROPABIO, 2001b) units are generally not specified. Compared to the OECD Consensus Document the set of compounds proposed by industry is more narrow. The OECD document also recommends to consider secondary plant metabolites, which are not mentioned at all in the EuropaBio Guidelines.

Table 27 provides an overview the compounds analysed in order to substantiate the substantial equivalence claim and what is compared to what is presently considered as minimum requirements. It must be borne in mind, that both guidelines have not been available at the time of application.

Table 27: Compounds analysed compared to compounds listed in OECD Consensus Document and EuropaBio-Guideline

OECD	Europa-Bio	T25	Bt11	MON810	MON809
Protein % DW	+	+	+	+	+
Fat % DW	+	+	+	+	+
Carbohydrates % DW	+	+		+	+
Fibres % DW	Optional	+	+	-	-
ADF % DW	Optional	-	+	+	-
NDF % DW	Optional	-	-	+	-
Ash % DW	+	+	+	+	+
Amino acids (18)[a] % n.c.	+	+	-	+	+
Fatty acids (5)[b] % n.c.	+	+	-	+	+
Minerals mg/100g					
Ca	+	-	-	-	-
K	+	-	-	-	-
Mg	+	-	+ % n.s.	-	-
Na	+	-	-	-	-
P	+	-	-	-	-
Trace elements mg/100g					
Cu	-	-	+ % n.s.	-	-
Fe	-	-	-	-	-
Se	-	-	-	-	-
Zn	-	-	+ % n.s.	-	-
Vitamins mg/kg					
Retinolequivalent	-	-	-	-	-
Vit B1	+	-	+ mg/lb	-	-
Vit B2	+	-	+ mg/lb	-	-
Vit B6	-	-	-	-	-
Vit E	+	-	-	-	-
Folate total	+	-	+ mg/lb	-	-
Niacin	-	-	+ mg/lb	-	-
others					
Phytic acid % DW	+	-	-	-	-
Raffinose % n.c.		-	-	-	-
Furfural ppm		-	-	-	-
Ferulic acid % n.c.	-	-	-	-	-
p-Coumaric acid n.c.	-	-	-	-	-

Where units proposed by OECD are not met, the respective units specified in the dossiers are stated. If units are not specified the abbreviation n.c. (for "not clear") is used. [a]) Ala, Arg, Asp, Cys, Iso, His, Glu, Gly, Leu, Lys, Met, Phe, Pro, Ser, Thr, Tyr, Try, Val; [b]) Palmitic, stearic, oleic, linolic and linolenic acid; DW... dry weight; [c]) 1 lb is equivalent to 0,453 kg; n.c... not clear; n.s.... not specified. .+... considered; -... not considered.

3.3.3 Novel Food dossiers (Article 4)

All applications under Article 4 of the Novel Food Regulation investigated in the course of the study (see Section 1.3.2 for full list of dossiers investigated) referred to partial substantial equivalence, that means the substantial equivalence is claimed except one clearly defined property – the novel properties caused by the gene introduced (causing e. g. herbicide or insect resistance). For reasons of comparisons, these dossiers were included in the review too. The results and conclusions drawn from this review are however quite similar to those of Article 5 dossiers (full substantial equivalence). Thus, only a brief summary is provided in this monograph, pointing out similarities and differences found. For a full account on Article 5 dossiers see Spök et al. (2003a).

In compositional analysis a broader set of compounds was analysed compared to Article 5 (notification) dossiers. Unlike Article 4 maize dossiers, the Article 5 dossiers also include analysis of anti-nutrients, minerals, and vitamins. Description of agricultural practice, design of field trials, sampling, and storage of samples are sparse – similar to the Article 5 dossiers. The number of sites and replicates also varies between the Article 4 dossiers. In none of the dossiers the same set of compounds was determined for two successive seasons.

Macro nutrients were determined in each dossier, micro nutrients, vitamins and minerals in most of the application dossiers. Comparative studies frequently referred to older literature.

Some more similarities to Article 5 dossiers: Presentation of data differs a lot between dossiers and could include raw data or be restricted to diagrams – without specifying numerical data. Significant differences occurring in only one or two compounds are not considered as relevant by the applicants. Analysis was thus not repeated. In some cases, no information was provided on the statistical analysis applied.

3.3.4 Directive 90/220/EEC dossiers

Reviewing and evaluation of data for nine Directive 90/220/EEC dossiers[38] revealed that each dossier not only includes comparisons of plant components and the subsequent conclusion "no major difference" or "substantial equivalence" of raw products (wording depends on the date of the application) but that substantial equivalence seems to be one of the main safety arguments. This is true irrespective of the intended use of the GMP – only two of the Directive 90/220/EEC applications were including food use.[39]

Despite their diversity in the intended use covering such different applications as import, processing, cultivation and use in food, feed, or non-food/non-feed industrial products no correlation can be established between the nature and extent of compositional analysis and the intended use of the GMP (see Table 29).

Overall, the review of Directive 920/220/EEC dossiers leads to very similar results as the review of Novel Food dossiers. Therefore, the results of the former review are only presented as a brief summary. A more systematic comparison of the dossiers is presented in Table 28 and Table 29 including also some details.

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[38] See Section 1.3.2.

[39] These applications had been submitted before the Novel Food Regulation entered into force.

Only two dossiers included food use (maize Bt11; rape Topas). For, these two GMP also the respective Novel Food dossiers (maize products and rapeseed oil) were investigated.[40]

Compositional analyses are largely restricted to macronutrients, plant specific anti-nutrients and/or toxins. A detailed characterisation of macro components however is only rarely conducted. Data on substantial equivalence in Directive 90/220/EEC dossiers of maize Bt11 and rape Topas 19/2 are completely identical with those presented in the respective Novel Food dossier, which is interesting as the Directive 90/220/EEC dossiers of maize Bt11 does not include application in food.

Substantial equivalence is referred to in each dossier in order to argue for the safety of the particular GMP. Plant compounds selected in compositional analysis are however, considered neither comprehensive enough to justify substantial equivalence nor sufficient to detect possible unintended effects. In each dossier some significant differences between the GMP and conventional counterparts were reported or could be revealed in the course of in-depth analysis of data – at least for single comparisons. However, these differences did not lead to a repetition of the analyses including broadening the range of compounds. In contrast, these differences were attributed to naturally occurring ranges, effects from backcrossing, climate conditions etc. As a consequence of comparing mean values of different cultivation sites the variance of analysed compounds is sometimes quite high, and might even cover any unintended effects.

Detailed descriptions of agricultural practice, storage, sampling, analysing practise are lacking in most cases as well as detailed data on results of compositional analysis (no single evaluation sheets or lab protocols). Detailed explanations on summaries of compound analysis are frequently lacking or fragmentary only. Annexing statistical evaluation sheets seems to be rather an exception. On the basis of information provide and data presented, substantial equivalence cannot be verified in most cases. In case of herbicide resistant plants it is often not clear if the herbicide was actually applied during cultivation.

Nutritional aspects in general and especially with respect to substantial equivalence (e. g. vitamin profiles, characterisation of fibre, characterisation of protein) do not play a role and are only occasionally considered.

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[40] The Novel Food dossiers, however, contained no additional analyses or further data compared with the Directive 90/220/EEC dossiers. Data on consumption and a prognosis of the expected quantity in food use are missing, although this would be required by the Novel Food Regulation for a proper assessment of exposure and of the potential influence on nutritional habits. Compositional analyses are largely restricted to (a few) macronutrients, accompanied by comparisons of some plant specific anti-nutrients and toxins (varying in quantity and quality of analyses). A detailed characterisation of macro compounds is however rarely conducted. The latter is true for both Directive 90/220/EEC and Novel Food dossiers.

Table 28: Evaluation of compositional analysis in Directive 90/220/EEC dossiers

GMP	Compositional comparisons/ set of compounds	DQ	ES	Diff	SE
Maize Bt11	Starch, protein, oil, fibres	+/-	-	+	+
	Like above + total nitrogen, ash, cellulose, xanthophylls, fatty acids, amino acids, Cu, Mg, Mn, Zn, vitamin B1, vitamin B2, niacin, folic acid	+/-	-	+	+
Rape Topas 19/2	Oil, protein, glucosinolates, erucic acid, fatty acids, soluble amino acids	-	+	+	+
Bt-cotton 531	Protein, fat, carbohydrates, ash, gossypol, malvalic acid, sterculic acid, fatty acids	-	-	+	+
RR-cotton 1445	Protein, fat, carbohydrates, ash, gossypol, fatty acids, amino acids	-	-	+	+
RR-maize GA21	Protein, fat, carbohydrates, ash, fatty acids, amino acids, Ca, P	+/-	-	+	+
	See above	+/-	-	+	+
RR-fodder beet A5/15	Protein, ash, fibres, sugar, invert sugar, nitrogen, Na, K, saponins	-	-	+	+
Potato EH92-527-1	Protein, fat, ash, carbohydrates, mono- poly-saccharides, glycoalkaloids, nitrate, chlorogenic acid, vitamin C, Na, K, Ca, Mg, P, Fe, Zn, Cu, Mn, Cd	+/-	+	+	+

DQ... Quality of data documentation and presentation; ES... single evaluation sheets and statistical data sheets annexed, Diff... significant differences (at least for single comparisons); SE ... claiming substantial equivalence . +... sufficient/included/detected/claimed; +/-... in part; -... not sufficient/not included/not detected/not claimed.

Table 29: Comparison of analyses in Directive 90/220/EEC dossiers

Component	Bt11 maize import	Bt11 maize cultivation	RR maize GA21 import	RR maize GA 21 cultivation	Bt cotton 531	RR cotton 1445	RR fodder beet A5/15	Rape Topas 19/2	Potato EH92-527-1
Protein	+	+	+	+	+	+	+	+	+
Oil	+	+	-	-	-	-	-	+	-
Fat	-	-	+	+	+	+	-	-	+
Fibre	+	+	-	-	-	-	+	-	-
Ash	-	+	+	+	+	+	+	-	+
Fatty Acids	-	+	+	+	+	+	-	+	-
Amino Acids	-	+	+	+	+	+	-	Partially	-
Sugar	-	-	-	-	-	-	-	+	+
Anti-nutritive substances	-		-	-	+	+	+	+	+
Minerals	-	+	-	-	-	-	+	-	+
Vitamins	-	+	-	-	-	-	-	-	+

+... considered in the dossier; -... not considered in the dossier.

3.3.5 Parallel findings in all three type of dossiers

Detailed descriptions of conditions of cultivation, of sampling as well as information on storage and preparation of samples are lacking in most cases. Sampling is highly varying in terms of years of cultivation, number of sites and geographic regions. In some cases – especially regarding Novel Food dossiers – it is even not considered adequate (only one single season, just a few sites, no representative geographical distribution of field trails).

In case of herbicide resistant GMPs it is sometimes not clear, if the herbicide was applied in the field trials or – as in most cases – it is evident that comparisons were done with plants not exposed to the corresponding herbicide.

Descriptions of analysing methods (e. g. method-sensitivity and/or method-errors) and information on measurement practise (e. g. pool samples, single samples, double or single measurements, coding practice) are often lacking or not sufficiently comprehensive in order to reproduce the tests. Explanations on summaries of analysis presented are often missing or only fragmentary. No dossier contains all laboratory protocols and all testing results.

The accurateness of the statistical evaluation is not verifiable as necessary information (kind of statistic tests applied, confidence levels, software) is often lacking or incomplete. The inclusion of statistical sheets, that would allow to trace the factual basis of certain statements, is rather an exception than the rule. Apparently not all compositional comparisons had been subjected to a statistical evaluation. Hence, it cannot be concluded, that in each case the statistical evaluation is actually state of the art. Only two dossiers (the Directive 90/220/EEC dossier and the Novel Food dossier of rape Topas) include detailed statistical evaluation sheets.

With respect to Novel Food dossiers, compositional comparisons focus on raw products used for food processing. All applicants argue that if a raw product is substantial equivalent to a conventional counterpart any processed food will be equivalent as well. Processed products were only considered in the Novel Food rape dossiers, though, to a rather limited extend (small sample, few compounds). Final food products were not investigated for the presence of rDNA or the respective gene product in any Novel Food dossier. As far as final products are concerned one must conclude that neither exhaustive data on composition nor on the influence of processing on the composition of food products derived from GMP can be found within the dossiers. Consequently the claim of substantial equivalence for final products cannot be justified by experimental data in any dossier.

Nutritional considerations (e. g. vitamin profiles, characterisation of fibres, analyses of different types of proteins with different nutritional values) apparently do not play a role in the characterisation and/or assessment of GM food as such analyses are either lacking or carried out only occasionally. Thus it must be concluded, that an assessment of the equivalence of the nutritional value of GM food does not take place. For properly assessing exposure, data on consumption and use of food products would be necessary as well as reliable estimations of future trends but comprehensive and sound data on consumption are missing in all dossiers and estimations of future trends are generally not being done.

Based upon insufficient data on field trails, sampling and analysing as well as on non-traceable statements and striking differences in some comparative studies substantial equivalence was claimed in all cases for plant material as well as for final food products (in the Novel Food dossiers). The concept of substantial equivalence seems to be used rather as a terminal stage in risk assessment than as a decision tool. For, substantial equivalence is always used to argue for the safety of the particular GMP/GM food. From a safety point of view however, the compounds chosen in compositional analysis does not seem to be comprehensive enough to justify substantial equivalence and/or to detect possible unintended secondary effects. In each dossier some significant differences were reported or could be detected at least for single comparisons in the course of in-depth review of data presented. In no case, however, did such differences trigger a repetition of analyses possibly broadening the set of compounds for reliably excluding unintended effects. This is nevertheless, regarded as necessary in order to properly justify the claim of substantial equivalence and to clarify the relevance of this observations. In contrast, differences were brushed aside and explained as natural ranges, effects from backcrossing, caused by climate conditions etc. Furthermore, as a consequence of comparing mean values of different cultivation sites the variance of compounds is sometimes quite high and might mask any unintended secondary effects.

In general the procedures applied and documented do not deem to ensure a sufficient degree of product safety. With respect to the Novel Food dossiers and indented food-uses within in Directive 90/220/EEC dossiers a proper assessment of the nutritional value is lacking at all.

4 Proposals

The recommendations given in this Chapter are based on the results of the review of dossiers described in the preceding Chapter. Firstly, recommendations are derived from particular practice that is considered no longer appropriate with respect to the state of the art in science. Secondly, the recommendations are based on two presumptions that (i) comparable things should be subjected to a similar level of safety testing and that (ii) risk assessment should be fully verifiable on the basis of the dossiers. Thirdly, the recommendations were inspired by analogies to procedures and standards in other EU regulatory contexts.

Recommendations given are aiming at improvements of risk assessment by either pointing to improvements and standardisation of procedures or pointing to the need to clarify particular questions or improve the scientific database.

In case of issues that are already discussed in Section 3.1.5 discussions are not repeated and only references are given to the preceding subchapters.

4.1 Assessment of toxic properties

4.1.1 General aspects

4.1.1.1 Dossiers should be stand-alone and include all available studies

Dossiers should include full reports of studies to which reference is given in order to be fully verifiable (see Sections 3.1.1 and 3.1.5.1). This is not only state of the art in other regulatory contexts – it is also recommended for GMOs and GM food and feed in the SCP Guidance (SCP, 1998) and in Regulation 1829/2003.[41]

Similarly, <u>all</u> available studies should be submitted along with the application.

In case of pesticide producing plants a copy of the approval as a plant pesticide should be included (see Section 4.1.1.5). In case of pending applications the present status of the procedure should be indicated.

4.1.1.2 Format and structure of applications should be further developed

Recommendation 97/618/EC has proven a good tool for structuring Novel Food applications and warrant clarity. This is especially true when comparing to Directive 90/220/EEC applications that are lacking of a more elaborated guidance document.[42]

In order to make it easier to review the applications the design requirements for the applications should be further detailed (for both type of dossiers). It should be more easily recognised which questions were dealt with and are substantiated and which were not. Hence, the Guidance has to be detailed including a descrip-

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[41] ***"The application shall be accompanied by the following: [...] a copy of the studies, including, where available, independent, peer-reviewed studies, which have been carried out and any other material which is available to demonstrate that the food complies with the criteria referred to in Article 4(1)"*** **(Article 5 (7) e).**

[42] **Note that the part on Directive 90/220/EEC dossiers was completed at the end of 2001 far in advance of Commission Decisions 2002/623/EC and 2002/811/EC) specifying some more details.**

tion of appropriate toxicity endpoints and an application form supplemented with comments for the applicant should be elaborated. Such application forms are available for plant pesticides, biocides and chemicals.

The form should also include a detailed guidance of toxicity endpoints required. Furthermore, information should be included on e. g.

a) Measurement of concentration of newly introduced proteins in different processed products. Specification of the testing carried out and reference to the full report. A summary should be enclosed in the application and the full report should be enclosed in the annexes.

b) Measurement of concentration of newly introduced proteins in different food products. Details should be specified analogous to a).

c) Measurement of digestibility in vitro. Details should be specified analogous to a).

d) Measurement of digestibility in vivo. Details should be specified analogous to a).

e) Acute oral toxicity testing of the newly introduced proteins. Details should be specified analogous to a). In addition the source of the protein should be specified (plant/bacteria). In case of bacterial proteins investigations of the equivalence of the plant and the bacterial protein should described. Details should be provided for species used in testing, according to which guidance the testing was done (method); dosage.

f) Mutagenicity testing of the newly introduced proteins. Details should be specified analogous to e).

g) Sub chronic toxicity testing of the processed product. Details should be specified analogous to e).

h) Investigation of possible secondary effects.

For Directive 2001/18/EC applications eco-toxicological endpoints for both the introduced proteins and the whole-plant should also be included.

Chapters should follow a standardised numbering system. Additional documents submitted at a later stage should also include a table of contents. The application including the annexes will most likely comprise a pile of paper and should therefore be accompanied by a separate summary version.

If substantial equivalent is an issue relevant statements and testing should be concentrated in a separate chapter. As substantial equivalence is considered a starting point for risk assessment (FAO/WHO, 2002; CODEX ALIMENTARIUS COMMISSION, 2002) it should also be clearly indicated if the investigation of substantial equivalence has led to any further testing.

Hazard assessment, exposure analysis providing the basis for risk assessment and the risk assessment itself should be dealt with in separate chapters. An intermingling of these three steps of risk analysis – as was revealed in the dossiers investigated – should be avoided. For Directive 2001/18/EC the latter chapter should include:

a) Evaluation of the potential consequences of each adverse effect, if it occurs (Annex II, C2.2).

b) Evaluation of the likelihood of the occurrence of each identified potential adverse effect (Annex II, C2.3).

c) Estimation of the risk posed by each identified characteristic of the GMO(s) (Annex II, C2.4).

d) Determination of the overall risk of the GMO(s) (Annex II, C2.6).

e) Identification of any new risks to human health and the environment that may arise from the release of the GMO(s) in question as compared to the release of the corresponding non-modified organism(s), based on the environmental risk assessment carried out in accordance with Annex II (Annex VI, 4).

f) The description of the methods used or the reference to standardised or internationally recognised methods shall also be mentioned in the dossier, together with the name of the body or bodies responsible for carrying out the studies (Annex III).

This should also be implemented in a similar way for GM Food dossiers. Novel Food dossier maize GA21 provides an example that this can be done.

Moreover, a list of questions should be developed and included in a more detailed guidance for applicants that would ask for exposure relevant data such as concentration of gene products in different tissues (parts) of the plant as well as in the respective products; concentration and fate of gene products in soil, water and air, data on digestibility in the intestinal tract etc. Different routes for contact, e. g. ingestion, inhalation, skin contact should be considered as well.

4.1.1.3 Clarification of the validity of safety claims

As described in Sections 3.1.4.5 and 3.1.5.8 a number of safety claims refer to general opinions rather than to results from safety testing. In practice these two kind of evidences are intermingled and therefore require considerable efforts to separate them in order to uncover the nature or the evidence supporting the safety claim.

A more transparent way of specifying the nature of evidence would be:

- Each relevant safety claim should be supported by a study (or a paper) that is referred to in the text and included in the annexes.
- If such references are lacking or false the application should be rejected.
- Safety relevant claims should primarily be supported by data from actual testing that should be enclosed. Unless the applicant can justify that these data cannot be compiled or in cases they can be replaced by other data. In the latter case this should be justified in the application.
- Assumption based reasoning and their limited validity should be an issue in the guidance document.
- Research should be conducted in order to reduce those grey areas between assumptions, presuppositions, general beliefs and scientific opinions, e. g. on the validity of homology of proteins for toxicity assessment.

4.1.1.4 GLP

Unlike in GMO regulation GLP is presently a standard requirement for chemicals, medicinal products, cosmetic products, feed additives, biocides and plant pesticides in the EU (EC, 1999) – in certain contexts GLP has been required since about 20 years. For reasons given in Section 3.1.5.10 GLP should be required for GMO products as well.

Hence, GLP should not only apply for laboratory studies but also to field trials and compositional analysis.

Besides increasing verifiability, validity and credibility there might be even an incentive for applicants as testing done in industry laboratories is usually accepted by authorities if carried out according to GLP.

4.1.1.5 Plants producing pesticides

In case of plants producing a pesticide these substances should be subjected to a similar scrutiny as chemical or biological pesticides[43]. The pesticide active component as such should require authorisation as a plant pesticide and should be assessed by competent authorities experienced with plant pesticides. The problem of pesticide residue should be assessed by those authorities as well. Ideally this should be done prior or at least in parallel to the GMP application. The competent authorities assessing the GMP should limit their assessment to the special situation that the pesticide is produced inside the plant.

A similar provision is already included in Regulation 1829/2003 (Article 5 (6)9.

4.1.2 Exposure

4.1.2.1 Exposure scenarios

Directive 2001/18/EC applications could – in principle aim at very different applications of GMPs including cultivation, import, processing, and storage. Hence, exposure of both man and environment might differ a lot between applications.

Therefore and especially if the particulars of testing requirements would depend on the particular exposure (e. g. in case a decision tree approach would be established), exposure scenarios for the various products and applications should be established; for instance worst-case exposure or depending on experience and availability of more realistic parameter also more realistic worst-case scenarios.

4.1.2.2 Estimate of human exposure

A proper estimate of the exposure that can be used for risk assessment considerations should include the following information:

- **The kind of products that are intended for human/feed consumption should be specified and a description of processing should be given.**
- **The concentration of the newly introduced proteins should be measured on the basis of these products.**
- **Consumption of the products should be estimated.**

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[43] **Exemptions from this general requirements might be made e. g. in case of herbicide resistance genes used as markers only.**

4.1.2.3 Need for clarification of the value of in vitro digestibility studies

Despite a strong recommendation of the SCP (1998) only in vitro digestibility studies are carried out – no in vivo studies are described. More recent guidance documents are providing contradicting recommendations on this issue.

Hence there is need for clarification whether or not and to what extent in vivo studies should be carried out in addition to in vitro studies and if so which methods should be applied.

4.1.3 Toxicity testing

4.1.3.1 Exposure depending scope

In case of placing on the market of a GMP according to Directive 2001/18/EC the particular application could differ a lot. The authorisation could be sought for e. g. mass cultivation and processing in industry of food or feed plants on the one hand and for import and handling of non-food and non-feed plants on the other hand. Even cut-flowers have to be considered. Accordingly exposure scenarios might also differ.

In some EU regulatory contexts the scope of toxicity testing is depending on the particular exposure expected. E. g. pharmaceuticals that will only be applied for a short period (e. g. diagnostic agents) no chronic toxicity studies are required.

In case of chemicals the scope of toxicity testing will depend on the annual tonnage of substance that will be marketed. However, in the new REACH system that will replace the present chemical policy the scope of toxicity testing is likely to depend on the particular properties and applications (AHLERS et. al., 2001).

In biocide legislation – representing a more recent regulatory concept minimum data requirements in terms of toxicity and ecotoxicity are established. Depending on the results of these tests and on the particular exposure additional tests may be required.

The latter approach – combining minimum requirements and case-by-case requirements – seems to be a valid concept for GMPs. Such a minimum set would be justified by the fact that in case of GMPs amounts and geographical distribution tend to be very high and are – in contrast to chemicals and biocides – even unlimited in principle. Depending on the result from the minimum set and the particular exposure expected additional testing might be necessary.

If one would like to define these additional tests in advance exposure, for instance for particular categories of GMPs and as a step towards a decision tree, exposure scenarios would have to be developed. Point of departure should be worst-case scenarios. Depending on the available experience these scenarios will be more or less realistic.

Furthermore, the scope of testing might depend on the (range of) organisms that are likely to be exposed. Plant pesticide agents, for instances, have to be investigated for teratogenic properties for man but not for other less closely related animals. A widespread distribution (in geographical terms as well as in terms of

applications) in contrast to a local and restricted application might also be a relevant information to consider.[44]

In case of GMP food or feed products the range of likely exposure scenarios is more narrow. However, depending on the particular use as raw or cooked food, the particular amount of protein present in the food/feed and the estimated mean and maximum intake toxicity testing requirements might also include flexible elements.

4.1.3.2 Scope of toxicity testing

The toxicity assessment practice revealed in the context of this study, acute oral toxicity testing, with introduced proteins is not considered sufficient for a number of reasons discussed in Section 3.1.5.4.

The claim that proteins can only act in an acute way is only supported by a very poor scientific database. Moreover the question of whole-plant toxicity is not dealt with at all in the dossiers (Section 3.1.5.3).

On the other hand the question, there is presently no empirical evidence of GMPs or introduced proteins showing toxic properties. Therefore, the question what would constitute an appropriate basis for toxicity assessment cannot be considered a pure scientific problem. Rather it has to be dealt with as a question of value judgements and therefore be subjected to negotiations among stakeholders. Given the scope of this report only some clues can be provided.

From a toxicological point of view GMPs and food derived from GMP should be considered in the same way as products the safety of which is not a priori considered as negligible. In these cases the potential hazard has to be characterised by an appropriate testing regime.

There are three reasons to pursue this approach. Firstly, the safety requirements included in the new legislation (Directive 2001/18/EC and Regulation 1829/2003) have become more stringent compared to legislation that was in place so far. Secondly, to break the vicious circle that will not allow to improve the present database of toxicological properties. Thirdly, in order to establish a similar level of safety compared to proteinaceous substances in other regulatory contexts.

Concerning the first point: Directive 90/220/EEC and the Novel Food Regulation does not provide any clues, however, back in 1998 the SCP already proposed to applicants to conduct whole-plant or whole-food(feed) toxicity studies (SCP, 1998). In the new Directive 2001/18/EC requirements for risk assessment are even more precautionary compared to the preceding legislation. For instance it is stated that *"it is important not to discount any potential adverse effect on the basis that it is unlikely to occur"* (Annex II C 2.1). From this provision and from the fact that some of the national CAs have already urged for toxicity endpoints beyond acute toxicity a need for additional endpoints can be concluded.

Concerning the second point: as mentioned above, certain toxicological properties will only become evident in case of systematic testing, e. g. carcinogenicity and reproduction toxicity. If a toxic property would not be followed by immediate or serious health impacts but rather increase the occurrence of a non-rare tu-

[44] This would probably not be important in case of cultivation as once authorised there are no principal and legal restrictions in terms of area or harvest.

mour, e. g. liver tumour for, let's say, 10 percent, this would not become apparent without from normal practitioner's observations. If these tests are not legally required there will be no incentive for conducting such tests. If not conducted there will be no chance of establishing an empirical database of toxic properties and, hence, the testing regime will continue to lack an appropriate empirical basis.

Thus, if acute toxicity and allergenicity assessment are deemed sufficient the database on possible long term effects and other toxicological endpoints will not be improved. Hence the presently used argument of no evidence of harm would neither be verified nor falsified.

Medicinal products might serve as an example: Up to the 1960ies it was not considered as possible that agents that are not considered toxicants in a classical sense could cause considerable harm even if applied bellow the toxicity safety limit. In 1960 the first case of teratogene effects of a substance of so-far low toxicity, thalidomide, was discovered. As a result, the safety testing regime of medicinal products was reconsidered and extended.

As already discussed elsewhere in this Chapter secondary effects of the genetic modification are quite likely and might also render the toxic properties of the whole plant. These changes might be toxicologically neutral or not and might be discovered in the course of comparative compositional analysis or not.

However, toxic effects of any kind might not necessarily be apparent from these studies and can be of significance, e. g. in case of a significant increase in the concentration of a carcinogenic substance.

Concerning the third point means to apply a level of safety that is considered acceptable for products that are comparable to the case of GMPs: For instances, authorisation procedures of herbal medicines that have already been marketed for a long time, could serve as an example for the possible case of secondary effects. Authorisation of such products is granted for and guidance for risk assessment are issued by the European Agency for the Evaluation of Medicinal Products (EMEA). Some of the concepts and terms used in theses Guidance Documents are essentially similar to GMP regulations. For instance, *"essential similar herbal medicines"* and *"long history of medicinal use"* (EMEA, 1999). In case of these medicinal products with a long history of safe use it is assumed that effects are already described in the literature or in pharmacopoeia. Nevertheless, even these well-characterised products are required to undergo toxicity testing (EMEA, 1998) beyond those toxicity data that can be more easily found in clinical medicine, e. g. reproduction toxicity, genotoxicity, and carcinogenicity.[45]

Herbal medicines that are considered as "essentially similar" to products that are already authorised can be subjected to a short-cut authorisation procedure. Essential similarity is thereby assessed via analysis of plant compounds.

This situation somehow resembles the one of GMPs with the possible exception that herbal medicines are applied under supervision of a doctor. Furthermore, these products are subjected to an extended period of medical surveillance. Thus, a history of safe use seems to be much better documented compared to food or plants.

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[45] Neither single or repeated dose effects, nor studies of immunotoxicity or topical effects are required to be investigated in animal studies in case of well characterised products with a long history of safe use.

Given what is said above a toxicity testing regime comparable to the one of herbal medicines should be introduced as a standard requirement for GMPs and GM products used for food/feed purposes, including

- 90-day subchronic toxicity
- Mutagenicity and cytogenetic effects
- Teratogenicity and general reproduction toxicity
- Carcinogenicity.

Food additives might serve as another example as discussed in Section 3.1.5.4 and might lead to similar endpoints. The NptII protein was registered as a food additive at the US FDA and thereby represents an example that the authorisation regime for food additives is also applicable to introduced proteins of GMPs.[46]

It has, however, to be acknowledged that no methods are available that can routinely applied to investigate toxicity of whole-plants. On the other hand, whole-plant studies aiming at investigating sub-chronic toxicity are already applied as in case of Maize GA21 Novel Food dossier.

In the context of GMP authorisation according to Directive 2001/18/EC the particular endpoints should depend on the particular exposure. The minimum set for each introduced protein should comprise

- Acute oral toxicity
- 90-day subchronic toxicity
- Mutagenicity and cytogenetic effects
- Teratogenicity and general reproduction toxicity.

Appropriate endpoints for ecotoxicity assessment might be acute toxicity of animals that will be fed on these plants and acute toxicity of fish, daphnia, algae and worms.

These endpoints should be investigated for each newly introduced protein. In case of whole plant testing the same endpoints would apply.

This scope of toxicity assessment roughly corresponds to the base set in EU chemical legislation. Depending on the particular application of the GMP further testing might be necessary. Likewise, the results of the minimum set might trigger further testing.

4.1.3.3 Developing and validation of testing methods

In accordance with current practice in other regulatory contexts, e. g. for plant pesticides, testing methods should be developed in order to enable a valid, reproducible and comparable conclusion with respect to the different toxicity endpoints.

[46] Monsanto was voluntarily seeking a food additive registration including the need to pass a much more stringent test regime, in order to get official confirmation for the safety of the products (STIRN, 1998). The FDA requirements would probably be of interest when detailing a toxicity testing regime for GMPs.

Thereby, toxicity safety claims on the basis of feed conversion studies that were frequently found in the dossiers would be avoided.

4.1.3.4 Further clarification of the value of homology studies

Homology comparisons seem to lead to very different and almost contradicting conclusions as discussed in Section 3.1.5.6. Moreover, with respect to allergenic properties an exchange of only a few amino acid residues could render a protein allergenic/non-allergenic and could also fundamentally change functional characteristics of a protein (summarised in UMWELTBUNDESAMT/IFZ, 2002). Hence, the value of homology studies has to be critically evaluated and criteria have to be developed what would constitute a sufficiently homologue protein in terms of toxicity assessment.

4.1.3.5 Equivalence of bacterial and plant derived proteins as test substances

As discussed in Section 3.1.5.7 proteins applied in toxicity testing are derived from bacterial sources in almost all cases. Given existing evidence on different routes and mechanisms of protein processing, especially posttranslational glycosylation in bacteria, fungi and plants identity or equivalence of a given plant protein produced in microbes cannot automatically be expected and has to be demonstrated. However, as the demonstration of full identity between two proteins might be require quite substantive and costly studies (extensively discussed in UMWELTBUNDESAMT/IFZ, 2002) either clear-cut criteria have to be developed what would constitute a sufficient degree of equivalence or proteins would have to be purified from the GMPs.

4.1.4 Comparing the scope of toxicity testing

According to a rough estimate based on personal experience the costs for a more comprehensive toxicity testing programme of GMPs are likely to amount 0,5 to 3 Mio. € whereas the exact number would depend on the particular programme that could – e. g. in case of non-food or non-feed purposes – also depend on the particular exposure (see also Section 4.1.3).

A comparison to other regulatory contexts reveals that this would largely be within the range of standard costs for toxicity testing.

Table 30: Comparison of costs for toxicity testing

Regulatory Context	Costs [Mio.€]
Chemicals (base set 1-100 T/year)	0,1
Chemicals (base set < 1000 T/year)	2
Food additives	2
Pesticides	3-4
Biocides	2-3

Source: The costs are estimates according to the experience of Heinz Hofer who conducted the part of the study on toxicity assessment.

4.1.5 Review of risk assessment by the Competent Authorities

Provided more elaborated structure requirements for dossiers (see also Section 4.1.1) applications can more easily be checked by the CAs for complying with formal requirements in the first place.

In case of plant pesticides a stepwise review procedure is established that could also be applied for GMP dossiers:

1. **Check for completeness of the dossier**
2. **Assessment of each part of the dossier**
3. **Summarising assessment of the particular topics (e. g. human toxicity, ecological impacts)**
4. **Overall summary of risk assessment.**

4.1.6 Open questions

In the course of investigating the dossiers a lack of scientific knowledge and of appropriate testing methods became apparent. Suggestions for further research and method development were partly already touched upon in previous Sections and will briefly discussed here.

4.1.6.1 The role of substantial equivalence for toxicity testing

The role of substantial equivalence for toxicity testing should be further clarified and a more detailed guidance should be elaborated. It should be fully transparent what degree of equivalence would be followed by what type of toxicity testing regimen. Similarly, the consequences for toxicity testing in case of significant differences in compositional analysis should be clarified.

4.1.6.2 Development of exposure scenarios

As discussed above exposure scenarios for different categories and applications of GMPs in the context of Directive 2001/18/EC should be developed in order to establish the additional requirements for toxicity testing (see Section 4.1.2).

4.1.6.3 Development of appropriate methods for toxicity testing

Conventional methods for toxicity testing that are routinely used, e. g. for assessing chemicals, cannot be readily applied for whole-plant studies. Conventional methods focus on single substances and can therefore apply high dosages of the test substance. A safety factor can be derived by comparing the actual intake/dosage of human to the lowest dosage causing toxic effects. In case of food additives, this safety factor should be no less than 100 in order to conclude a substance as sufficiently safe and to grant market authorisation.

In case of whole-plant or food the dosage applied in testing cannot exceed the usual intake orders of magnitude, because otherwise problems might appear from nutritional imbalance. Hence, safety factors are rather low. As a matter of fact, toxicity assessment must be conceptualised differently in case of whole-plant testing.

Consequently, in interpreting the results of such studies one have to carefully differentiate between toxic effects and effects of nutritional imbalance – this is not considered an easy task.

Whole-food studies that are used to test for possible toxic properties of the introduced protein are probably less useful if the protein in question would be digested in the intestine (in vivo). This method will, moreover, be less sensitive as the introduced protein is likely to be present at a maximum of 1% of plant tissue. The concentration in the test diet might be even lower.

On the other hand, whole-plant toxicity studies have already been conducted in the course of GMP risk assessment (maize GA21 Novel Food dossier; STIRN, 1998). Furthermore, there is a longstanding experience of whole-food toxicity testing of irradiated food.

It should therefore be possible to develop an appropriate testing regime. Main goal would be to analyse available methods of toxicity feeding studies and to adopt these methods to the particular demands of GMP risk assessment. The final goal should thereby be to establish internationally acknowledged procedures of toxicity testing and assessment. To pursue this goal it would probably be more feasible to aim at comparative toxicity studies comparing the GMP and the conventional counterpart instead of aiming at absolute toxicity assessments.

4.1.6.4 Review of present knowledge on toxicity modes of proteins

Subsequent to the discussion in Sections 3.1.5.2, 3.1.5.4 and 4.1.3 the claim that acute toxicity is not only an appropriate but also sufficient endpoint for toxicity testing is supported by a very poor scientific database only. There is definitely a need for further research in order to improve the knowledge on toxicity modes of enzymes.

Therefore, extensive literature studies should be carried out in order to more systematically investigate the available scientific database on protein toxicity. Thereby observations on possible toxic modes of proteins should be compiled and analysed. Of special interest would be whether proteins have already been investigated for chronic toxicity, mutagenicity, carcinogenicity and reproduction toxicity.

4.1.6.5 Development of criteria for the use of homology studies in toxicity assessment

Following the discussion in Sections 3.1.5.6 and 4.1.3 the value of homology studies in toxicity assessment should be reconsidered and more detailed guidance should be given on how to conduct homology studies and criteria should be developed that will help to interpret such studies in the course of risk assessment.

4.2 Assessment of allergenic properties

4.2.1 General remarks

At present, allergenicity assessment of GMPs is almost exclusively restricted to assessing the allergenic potential of introduced proteins. This is reflected by the approach found in the dossiers investigated and in recent decision tree approaches proposed for allergenicity assessment of GMOs, such as the FAO/WHO decision tree (FAO/WHO, 2001). The characterization of the novel protein in terms of allergenicity and allergenic reactivity is important – without a doubt. For, the insertion of an allergen will of course increase the allergenic potential.

However, as discussed in Section 3.2.3 this presently applied approach is not considered sufficient. On the one hand the scientific basis of a number of biochemical and exposure related characteristics of proteins that have been used as indicators for allergenic properties is increasingly contradicted in the light of recent scientific evidence. On the other hand there is evidence of secondary effects of genetic modification along with evidence on plant allergen inducement by environmental effects.

Thus, an obligatory investigation of whole plants is proposed in addition to the investigation of the novel proteins. This investigation could comprise a two-steps procedure, an IgE binding study applying sera from allergic patients and immunisation studies in mice.

4.2.2 Allergenicity testing

As a first, step patients already suffering from allergy would be investigated whether they would also be allergic against the GMP. Thereby it is tested whether antibodies present in those patients would react or cross-react with plant antigens.

For this purpose a comparative serum screen should be conducted applying sera from allergic patients. The IgE reactivity to extracts from GMPs and the wildtype should be compared. If the IgE reactivity of the GMP is higher than the one of the parental line the GMO this would indicate an increased allergenic activity compared to the parental line and represent an allergenic risk for already sensitised individuals. GMPs with similar IgE reactivity compared with the parental line would represent a similar allergenic risk compared to the wildtype.

The latter category of GMPs and GMPs not showing IgE reactivity should be analysed in the next step for de-novo sensitisation and induction of allergic reactions. Thereby, the potential to sensitise those who have not been allergic beforehand will be tested. To pursue this goal immunisations studies with extracts prepared from the GMP and from the parental line should be performed in Balb/c mice and the immunisation profile and the allergenic activity should be compared (VRTALA et al., 1998). If the allergenicity of the GMP is higher than the one of the parental line this would indicate an increased risk to induce IgE responses. GMPs with similar or even decreased allergenicity compared to the parental line would represent a similar or even lower allergenic risk with respect to the parental line.

The paper of VRTALA et al. (1998) shows that immunisation of Balb/c mice could induce an immunological reaction that is comparable to allergic patients in terms of epitope range and immunogenicity of the allergen.

In both steps appropriate extracts (e. g. tissue, pollen) depending on the exposure scenario should be used.

A potential limiting factor in the first step is the quality and availability of a sufficient number of sera. Furthermore, both type and number of sera that should be used in a particular test would remain to be decided. For, it would be of limited use, to investigate maize plants using birch IgE antigens. It would be more reasonable though, to use sera of patients allergic against various grains.

The particular strengths of this approach is that thereby secondary effects would also be considered and that indirect and partly very weak indications for allergenic properties would be replaced by or at least complemented by direct allergenicity testing. Furthermore, this approach could be applied on isolated proteins as well.

It could be an issue for further research projects to elaborate the experimental protocols necessary for the testing approach described including preparation of protein extracts, selection criteria of appropriate sera, protocols for animal immunisation, and evaluation of IgE antibody production.

4.2.3 Questions that could not be addressed

If allergenicity assessment of GMP is to be re-designed a number of additional questions should also be dealt with. Those questions came up in the course of the project, but could not be further discussed given the scope and time limitations of the study.

- Presently used approaches are focussing on IgE mediated type I reactions which comprise most of allergic reactions to food. However, there are also several other hypersensitivity diseases such as celiac disease, cellular-mediated reactions and immune complex diseases which are currently not considered in GMP or GM food risk assessment.
- Allergenicity assessment in Novel Food and in Directive 90/220/EEC dossiers is largely restricted to the ingestion route. In case of Novel Food dossiers the intake will depend on the particular protein or plant in question and might vary considerably. In case of future 2001/18/EC dossiers exposure might differ a lot depending on the particular aim on the application that could be either one or more of the following: import, processing, storage, cultivation. If and to what extend should allergenicity assessment depend on the particular exposure scenario?
- More generally it should also be asked whether uniform allergenicity assessment requirement should also be introduced for both GMPs and conventional plants.

4.3 Substantial equivalence

4.3.1 General aspects

On the basis of the results of the review, a number of more general recommendations are drawn aiming at detailing and standardising the practice of field trials and compositional analysis carried out in the context of substantial equivalence. These recommendations shall make easier the evaluation of dossiers, by checking in a more systematic way comprehensiveness and acceptability of tests displayed in the dossiers.

It is desirable that dossiers are composed in a similar and reasoned way. With regard to substantial equivalence, two chapters are recommended. One should focus on exposure (levels of expression, detailed description of processing, degradation and/or removal of rDNA/protein, processing studies, data on current consumption and likely trends, possible scenarios for future consumption, characterizing of dietary habits, proposal for post marketing surveillance plans). The second chapter should include extensive compositional comparisons of raw products supplemented by less extensive comparisons of the composition of the major final products.

- **In general all dossiers should be stand-alone. Dossiers should include all referenced reports and publications available at time of application as well as statistical evaluation sheets to avoid preselection of studies.**
- **In case of Bt- or herbicide resistant GMP these dossiers should include the approval of the plant protection product.**
- **All safety testing should be performed according to GLP. GLP ensures, that presented data actually reflect the raw data.**
- **In general only validated and generally accepted methods should be applied and a detailed description of material and methods should be provided for each analysis. Measurement errors and limits of measurements should be specified.**

4.3.2 Field trials and sampling

- **The trial design should follow a standardized model. Field trials should be carried out on at least six different sites located in typical geographical regions relevant for commercial cultivation and during at least two growing seasons.**
- **In the event of non typical climate conditions, an additional seasons should be considered. Climate conditions should be reported anyway.**
- **Greenhouse trials should only be considered as supplements but not as substitute for field trials.**
- **In order to thoroughly and comparatively investigate different cultivars, all factors that might affect this investigation have to be considered. Time of cultivation and harvesting, on-site cultivation conditions, characteristics of the experimental plots (size, number, replications etc.) should be described in detail.**
- **If different maturity stages are required for different products sampling should be carried out accordingly.**

- **The agricultural practice on the trial fields should be very close or even identical to those in normal agricultural practice of the particular crop. This will include the application of the herbicide in case of herbicide resistant crops – in appropriate amounts and frequencies. In case of insect resistant crops an appropriate infestation rate should be considered when selecting the particular test sites/regions.**
- **Any deviation from standard procedures should be justified and described in detail.**
- **As it is rather difficult to distinguish between natural variation and variations probably induced by the inserted genes, only isogenic lines should be used as conventional counterparts.**
- **Sampling should be described in detail for each site.**
- **It is recommended to use coded samples; at least analysis should be done in duplicate for each particular sample and investigated compound as well.**
- **In case of herbicide resistant GMPs all analyses should be carried out with treated samples.**
- **Conditions and length of storage of samples as well as sample preparation and methods used in sample preparation should be described.**

4.3.3 Exposure

- **The expression of the introduced genes should be measured in the particular part of the unprocessed plant that will be used in food manufacturing.**
- **The different ways of processing should be described in detail.**
- **Processing studies should be carried out which should reflect commercial practice as good as possible. These studies shall compare processing properties of the GMP to those of conventional counterparts. The final products of these studies can be used for compositional comparisons and nutritional characterisation (see below). Therefore one has to consider also that samples used are representative. Results of compositional comparisons however should be presented in the chapter "composition".**
- **Occasional testing of final products should show degradation, removal or decrease in concentration of rDNA, corresponding proteins and/or metabolites. The use of profiling methods – as soon as available and validated – should be considered.**
- **Recent data on consumption in general and on consumption per capita should be compiled to show a comprehensive picture and the current use of comparable traditional products.**
- **Trends in consumption should be estimated to properly assess exposure and impact on human nutrition. Different consumption scenarios should be considered.**
- **When assessing different consumption scenarios, "heavy users" should be taken into account anyway. At least two scenarios, a maximum scenario and a standard scenario should be considered.**
- **Figures on cultivation and data on import quantities and on production can be seen as supplementary information to exposure assessment but cannot replace data on effective per capita consumption. It would be interesting and ease the prognosis of future trends to show data on cultiva-**

tion/import/production over the last years to get an overview on recent developments.

- It seems vital to gather data on human consumption in a systematic way to estimate exposure more accurately. It would therefore of utmost importance, to review and consolidate existing consumption databases and make it available e. g. in form of an open database established and run by FAO/WHO, OECD or by EFSA.
- In order to elucidate possible negative and positive effects of GMP/GM food a research-oriented monitoring/post marketing surveillance is recommended to assess the impact of approved GMP/GM food on human nutrition and health. Ideally data should be gathered by the authorisation holder and evaluated by an independent scientific body. A proposal, how applicants plan to do this monitoring/post marketing surveillance should be an integral part of the dossier.

4.3.4 Compositional analyses

In the view of the authors one cannot exclude nutritional considerations when talking about compositional analyses and substantial equivalence. From the nutritional point of view far more than just the levels of macro compounds and a few micro components must be considered to effectively characterise the nutritional value of a food product derived of transgenic plants. For example, the Novel Food Regulation as well as the Recommendation 97/618 enforce implicitly to consider nutritional aspects within the evaluation of Novel Food as both mention, that the use of the Novel Food on the long run must not provoke or favour nutritional deficiencies not even within the most sensitive population group. Moreover, if substantial equivalence is claimed and accepted, no further (nutritional and safety) testing is required by the Novel Food Regulation. Therefore nutritional aspects should be a vital part of compositional comparisons done to show substantial equivalence and will not be treated separately but as part of compositional analyses in this Chapter.

- Compositional comparisons of raw products should be carried out extensively and should be accompanied by less extensive comparisons of the most relevant intermediate and final products. The latter could be derived from the processing studies. Comparisons of final products should include, however, all substances of nutritional relevance and nutritional indexes (e. g. P/S-ratio) should be considered as well.
- Harmonised lists of key components (nutrients, plant specific characteristic compounds and anti-nutrients as well as known toxins or potential toxic plant-metabolites) should be established for each particular crop. These lists should in any case include not only major compounds and the fatty acid and amino acid profile but also micro components having a positive or negative impact on human health or are characteristic for the particular product (e. g. vitamins, minerals, secondary plant metabolites etc). Also a characterisation of major and other components should be done, where applicable (protein fractions, fibre characterisation, different types of a certain vitamin etc.). Likewise, content and ranges of these key components should be agreed for each particular crop.
- The use of units for presenting data need to be standardised.

- With respect to major compounds beyond protein, fat, carbohydrates, ash and fibre also starch and soluble sugars should be considered. Moisture content or dry substance should be specified anyway. Referring to fibres, a division in soluble and insoluble fibre fractions should be done.
- Irrespective of the intended use amino acids and fatty acids should be considered. Referring to amino acids all essential and semi-essential amino acids should be taken in account. Referring to fatty acids at least C16:0, C18:0, C18:1, C18:2, C18:3 should be considered, accompanied by typical fatty acids to be found in a particular plant/food.
- As far as vitamins (including pro-vitamins like carotenoids), minerals and trace elements are concerned, at least those nutrients should be considered for which the particular plant/food is a "good source". Referring to Vitamin E, K, folic acid and carotenoids a characterisation of the different possible types should be done as the different types of these vitamins/pro-vitamins show different biological activities and data presentation of these substances should include the value for the respective equivalent (e. g. retinol equivalent, tocopherol equivalent etc).
- With respect to micro compounds the unit "ppm" (as specified in OECD Consensus Documents) seems not to be adequate as the presence of these substances as well as human requirements can differ considerably (e. g. 100 g wheat kernels contain 70µg retinol equivalents, 1,6 mg tocopherol equivalents and 502 mg potassium). Furthermore the units used in international databanks are usually mg/100g or µg/100g respectively. Therefore it would be more expressive and better for comparison purposes, if these units were considered within the dossiers instead of ppm.
- Referring to other micro components than vitamins and minerals – especially secondary plant metabolites and pigments – not much knowledge on what kind of different substances do exist and how they influence human health and especially on contents in plants/food has been gathered so far. Nevertheless it is recommended, at least to consider known typical secondary plant metabolites with positive health effects in compositional comparisons. Also phytosterols and phospholipids should be considered.
- In plants a variety of antinutritive substances can occur. These do not immediately trigger negative health effects but can negatively affect human health on the long run (e. g. by decreasing the bioavailability of nutrients). Such substances are for example phytic acid, oxalic acid, coumarinic acid, trpysin inhibitors or raffinose. Although knowledge on antinutritive substances is poor, known antinutrients should be considered in compositional comparisons. Known toxins however must be considered anyway.
- Raw data, sound summaries including mean and ranges as well as data for each single site should be included in dossiers. In the course of comparative studies always the same reference values should be applied.
- Appropriate statistical methods should be applied for each comparison. For each statistical evaluation the method applied, the software used and the confidence level should be specified. Statistical evaluations should include all results from each site. Data pools for statistically evaluating each compound should be consistent in order to avoid that "unfitting" results are rejected so as to not endanger the hypothesis of equivalence.

- **The results of the statistical evaluation should be presented in a summarised form and explained in detail. All statistical evaluation sheets should be annexed.**
- **In case of significant differences analyses should be repeated by extending the set of compounds compared for reliably excluding secondary effects going back to the genetic modification.**
- **Reference values for comparative studies should be based on modern high-yielding varieties rather than using literature ranges that have been published more than 10 years ago.**
- **In order to improve the basis of comparative analysis, all relevant data on the nutritional aspects of each particular compound and the natural variability in content (e. g. depending on the nature of the site, climate, agricultural management and cultivars used) should be systematically collected in international databases.**
- **In order to overcome the current methodological limits of compositional analysis, new methods that are not focussing on particular compounds but on whole profiles of compounds (e. g. DNA array, mRNA fingerprinting, proteomics, chemical-fingerprinting) should be further developed for an additional use in routine testing as soon as possible.**

5 Results and Proposals in Context of Recent regulatory developments

This Chapter aims at providing a brief update in order to discuss the results and proposals of this monograph in the context of recent developments and further guidance documents that were issued after completion of the studies underlying this monograph.

The review of Directive 90/220/EEC and Novel Food dossiers in essence revealed shortcomings of GMO risk assessment in several respects that are interrelated to each other:

1. **Risk assessments described in the dossiers and safety conclusions drawn can frequently not be (fully) verified on the basis of information presented in the dossiers and are not backed up by a consistently applied statistical analysis and by compliance to a quality assurance system.**

   The lack of details in the description of tests, approaches, in data display and the tendency not to include full reports (e. g. toxicity studies) complicates verification if not rendering it impossible. The value of methods and approaches is sometimes questionable (e. g. homology and digestibility studies in toxicity assessment), some studies are often missing (e. g. processing studies in compositional analysis and toxicity assessment; see also point 2) or not properly designed (e. g. compositional analysis), and (direct) testing is rarely being done (see also point 2). This might not only weaken the scientific basis of risk assessments but also diminish the credibility of safety claims which – due to their highly contested nature – are often under close scrutiny of both evaluators and stakeholders.

   Statistical analysis is especially important for composition analysis, however it frequently remains unclear whether statistics are consistently applied or applied at all. GLP while being an agreed standard requirement in other regularly contexts is rarely applied in risk assessments. GLP is considered vital to enhance credibility and cannot be replaced by annexing publications in scientific journals.

2. **Assumption based reasoning is not only supplementing but frequently replacing safety testing – moreover, there is a lack of direct testing in general.**

   For instance, in toxicity assessment applicants frequently refer to anecdotal evidence or common knowledge when claiming a history of safe use (e. g. in the absence of appropriate epidemiological studies). In compositional analysis, significant differences detected are disregarded without any attempts to verify these differences by repeating the experiment and by broadening the range of compounds in order to enhance the likelihood to detect unintended secondary effects.

   Compared to other regulatory contexts such as plant pesticides, food additives and herbal medicines toxicity endpoints considered in the dossiers are not deemed sufficient for products that will be produced and consumed in large amounts and for a lifetime. This is not only true for toxicity assessment as mentioned above but also for allergenicity assessment. It was shown that the presently applied approach to allergenicity assessment is

largely based on indirect evidences each of which is considered questionable in the light of recent scientific evidence.

3. There is a lack of detailed guidance for risk assessment.

   The differences in risk assessment between dossiers, especially between those pertaining the same plant species and/or aiming at similar applications are pointing to a lack of details in guidance documents at the EU level or even to the absence of guidance at all. This is true – albeit to a different extent – for all three aspects investigated, toxicity assessment, allergenicity assessment and substantial equivalence. In the absence of such guidance the specific requirements have to be discussed and agreed among the CAs of Member States and the EC for each application separately.

4. Unintended effects of genetic modification are largely disregarded.

   With a very few exceptions possible unintended effects are not investigated. Hence, unintended effects would only become evident in case of conspicuous alterations in morphological or agronomical properties or in key compounds – thereby comprising a rather widely-meshed safety net.

5. Shortcomings in the overall risk assessment approach.

   The formal structure does not clearly distinguish between exposure assessment and hazard assessment which are both considered necessary to allow for a proper risk assessment. Little attention is thereby given to exposure assessment, in general. Substantial equivalence seems to be rather the final step instead of being the starting point in risk assessment as laid out in conceptual documents.

These diagnoses of shortcomings subsequently served as the basis for developing proposals in order to improve and standardise GMO risk assessment.

It has to be acknowledged that these results and proposals are based on dossiers submitted between 1995 (or even before) and 1998 (only three of the dossiers investigated were submitted between 1999 and 2001). Furthermore, most of the proposals in this monograph were developed between 2000 and early 2002. However, in recent years a couple of updated and more detailed guidance documents were issued by EC (SSC, 2000, 2003), EFSA (2004), OECD (2000b), FAO/WHO (2001), CODEX ALIMENTARIUS (2003), ILSI (2003), INSTITUTE OF PUBLIC HEALTH AND THE BIOSAFETY COUNCIL (2003), and Industry (e. g. EUROPABIO, 2003). Likewise important studies have only recently been completed, for instance ROYAL SOCIETY OF CANADA (2001), ROYAL SOCIETY (2003), and most recently the conclusions of the UK GM Science Review (GM SCIENCE REVIEW 2003, 2004) and ENTRANSFOOD were published (VAN DEN EEDE et al., 2004; CELLINI et al., 2004, KÖNIG et al., 2004).

Given this sequence of events it is obvious that most of these documents could not be considered in the course of the study that build the basis of this monograph. It is also obvious that a systematic comparison of proposals cannot be provided in the course of preparing this abridged English version.

However, in order to prove the relevance and timeliness of the recommendations of this monograph a brief discussion of these recent proposals will be provided. Generally, a scanning of recent guidance documents reveals that some of the

proposals of this monograph have meanwhile been included.[47] Certain requirements for risk assessment have also been specified in more detail compared to preceding guidance documents. Others are simply reiterated, though, leaving it as vague as before. Again others are largely providing advice that contradicts those developed in this monograph.

Some illustrating evidence will be provided from a comparison of recommendations in this monograph to those of the currently available official EC guidance document (SSC, 2003).[48] In addition, by drawing on two particular proposals, toxicity endpoints of introduced proteins and digestibility studies diverging views and requirements will become apparent that point to different interpretations of uncertainties underlying these proposals.

Some similarities and differences in both proposals are specified in the following:

- The SSC Guidance – in accordance to proposals of this monograph – stressed that dossiers should be **complete documents** that contain all information required for a full risk assessment (SSC, 2003, p. 6).
- GLP is however only demanded for toxicological studies (ibid., p. 18).
- The SSC Guidance is still ambiguous whether **toxicological testing of the introduced proteins** is actually required in any case, regardless of the equivalence status, knowledge base and explicit concerns raised. However, in case toxicity testing is deemed necessary a 28-day repeated dose oral toxicity study is recommended. Acute toxicity is explicitly disregarded as not providing relevant information (ibid., p. 19).[49] Additional toxicity endpoints are especially required in case of novel metabolites and according to the requirements concerning food additives which is largely consistent with earlier recommendations (Recommendation 97/618/EC) but not in accordance to the proposals presented here (see also bellow).
- The possibility of **secondary effects** is widely acknowledged. However, possible secondary effects are to be evaluated only (i) if there are clear indications of such effects depending on the nature of the genetic modification (SSC, 2003, p. 14), (ii) *"if the composition is modified substantially, or (iii) if there are any uncertainties on the equivalence"* (ibid., p. 20). In these cases a 90 day feeding study in rodents is encouraged. Hence, the Guidance seems to argue for a much more limited evaluation of whole plants compared to this monograph.

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[47] Some of this consideration have been presented and discussed in the context of the ICABR Conference 2003 (SPÖK et al., 2003c) and of the Workshop "Scrutinising GMO Risk Assessment" and where also introduced in a commentary on the EFSA Draft Guidance Document for the risk assessment of genetically modified plants and derived foods and feed that was issued in April 2004 (SPÖK et al., 2004).

[48] This Guidance Document was issued in March 2003. Meanwhile, in April 2004, an updated draft version of this document was issued by EFSA (EFSA 2004). However, the proposals discussed here are quite similar in both documents. Moreover, the EFSA guidance is still under revision. Thus reference is given to the SSC Guidance Document.

[49] *"An acute, single dose test with a 14-day observation period, is inadequate to detect possible toxicity arising from repeated dosing. Furthermore, this test does not provide information on the dose-response relationship and is designed to examine only a few endpoints (mortality, morbidity, clinical observation and gross necropsy) and not the broad range of endpoints required to be investigated in repeated dose studies, such as haematology, clinical chemistry, urine analysis, organ weights and histopathological examination of organs and tissues"* (SSC 2003, p. 19).

- **Homology studies for toxicity assessment are still encouraged – further guidance how to properly avoid the hurdles of those studies remains, however, to be specified (ibid., p. 18).**
- **Identity testing of proteins from plants and microorganisms has now to be demonstrated by providing additional information including posttranslational modification and immunological activity.**
- **In vitro digestibility studies are still required – this contrasts earlier critique (see below).**
- **The assessment of potential allergenic properties is still based on indirect evidences that render the appearance of allergenic properties of newly introduced proteins less likely. Direct testing of both the introduced protein and the whole plant is only encouraged in case the source organisms is known to be allergenic – an approach criticised in this monograph as not providing an appropriate level of safety.**
- **The importance of a proper description of field trials is also emphasised in the Guidance. Recommendations pertaining number of locations, growing seasons, geographical spreading and replicates, statistical analysis, nature of baseline data are similar to those given here.**
- **Compositional analysis should be carried out on the raw commodity – analysis of processed products is not routinely required and should be carried out only on a case-by-case basis and when scientifically justified (ibid., p. 13). The latter proposals contrast those of this monograph that suggests to generally carry out studies on processed products. For the selection of plant specific compounds the Guidance refers to the OECD consensus documents mentioned above. In case of statistically significant differences further investigation are recommended.**

In order to take a closer look at some proposals for toxicity assessment in recent guidance documents, toxicity endpoints proposed for introduced proteins and the view on in vitro digestibility studies, are compared.

Acute toxicity is advocated by EUROPABIO (2003), and to a certain extent also by the Belgium INSTITUTE OF PUBLIC HEALTH AND THE BIOSAFETY COUNCIL (2003). The latter of which, the SSC and the EFSA Guidance also advocated a 28 day repeated dose toxicity (SSC, 2003; EFSA, 2004). ILSI (2003) and the ROYAL SOCIETY OF CANADA (2001) proposed a 90 day sub-chronic toxicity (see Table 31).

Toxicity endpoints beyond acute/sub-acute/sub-chronic oral toxicity are rarely required in this type of documents. The Draft Guidance recommended immunotoxicity studies to be carried out depending on the outcome of the 28 day study. The Belgium Institute of Public Health and the Biosafety Council, for instance, proposed tests for eye and dermal irritation.

Mutagenicity, carcinogenicity, teratogenicity, and reproductive toxicity are, in general, not required for proteins in GMO risk assessment guidance. In this case the same argument on the lack of scientific data as described above applies. These endpoints are, nevertheless, routinely required for food additives, chemicals, and plant pesticides.

Table 31: Suggestions for vs. practice of toxicity endpoints of introduced proteins in GMP

Source	Toxicity endpoints (introduced proteins)
SCF (1997)	Not specified
SCP (1998)	Not specified
In practice (Directive 90/220/EEC dossiers submitted 1995-1998)	14 day acute toxicity, single dose
In practice (Novel Food dossiers submitted 1995-2001)	8 to 14 day acute toxicity, single dose
ROYAL SOCIETY OF CANADA (2001)	90 day, sub-chronic, repeated dose
SSC (2003)	28 day repeated dose (not required in any case)
EUROPABIO (2003)	Acute toxicity
INSTITUTE OF PUBLIC HEALTH AND THE BIOSAFETY COUNCIL (2003)	28 day acute toxicity, repeated dose
ILSI (2003)	90 day, sub-chronic, repeated dose
EFSA (2004)	28 day repeated dose (not required in any case)

Proposals on the use of in vitro digestibility studies for novel proteins are even more diverging: In vitro digestibility studies have been criticised by OECD and SCP (OECD, 2000b; SCP, 1998) and suggested by FAO or SSC (FAO/WHO, 2001; SSC, 2003) or even disregarded in general by SSC (SSC, 2000) (see Table 32).

Table 32: Different views on the use of in vitro digestibility studies in guidance documents

"Evidence of degradation of the introduced gene products should be based on data obtained in vivo by feeding the GM plant material or its derived products to the intended target animal. […] The use of in vitro simulation of gastric and intestinal digestion of the gene product should be considered supplementary to in vivo experiments designed to measure the survival of the gene products when fed to animals as an integral part of the GM plant. Isolated proteins are known which are fully degraded in the simulated gastric system but survive gut passage intact when fed as part of a normal diet" (SCP, 1998).
"There are arguments that the currently used tests of gastric and intestinal protein resistance to gastric and intestinal hydrolysis represent a ‚best case' situation and do not reflect the digestive capacity of the very young and those with pancreatic and severe gastric disorders. The fact that a protein is digested does not preclude for some eventual pharmacological/toxicological properties of derived peptides. Such properties have been observed for conventional foods and thus should be anticipated in all foods, whether they are derived from GM protein or from non-GM protein" (SSC, 2000).
"[…] the simulated gastric fluid (SGF) test, an artificial system for testing proteins' digestibility, does not mimic exactly the physiological conditions in the digestive tract. Such testing may not always provide clear evidence of the possible toxic or allergenic potential of peptides formed as breakdown products in the test system" (OECD, 2000b, p. 14).

Table 32 continued.

"Tests normally used to assess toxicity include in vitro digestibility of the protein which is used to compare the properties of the novel gene product to the characteristics of known proteins. This test is not intended to detect any potential toxicity in the very young, the elderly, and that segment of the population which is unable to produce stomach acid. Test methods should be designed to evaluate potential risks for those subjects when the gene product is similar to a chemical that is implicated in having unique toxicity when not digested" **(OECD, 2000b, p. 26-27).** *"Data concerning the resistance of the novel protein to proteolytic enzymes (e. g. pepsin) should be obtained, e. g. by in vitro investigations using appropriate and validated tests"* **(SSC, 2003, p. 19).** *"An in vitro digestibility assay in simulated gastric and/or intestinal fluids is required. It is important to note the at resistance to in vitro digestion is not a toxicity endpoint by itself, but simply an indication that the protein warrants closer examination and perhaps different types of testing. On a case-by-case basis, also an ex-vivo gastric fluid test (e. g. pig, cattle, dog) or in vivo models may be required"* **(DUTCH BIOSAFETY COUNCIL, 2003 p. 11).**

Of course there would be much more to comment on similarities and differences of proposals in this monograph compared to recent guidance documents. This general comparison however might serve to illustrate two general points.

First, the recommendations summarised in this paper go quite beyond the most recent official Guidance Document in various aspects and raise some general questions as well, which should be addressed in order to improve the current practice.

Second, given these diverging recommendations it becomes quite obvious, that still a number of questions, e.g. what constitutes a proper toxicity and allergenicity assessment for GM food as well as how to apply the concept of substantial equivalence remain open and scientific consensus has not been established yet.

While clearly not being able to bring about a scientific consensus, this monograph will hopefully contribute to the discussion by highlighting shortcomings in the practice of GMO risk assessment, identifying open questions and providing suggestions for further improving and standardising of risk assessment in the highly contested area of genetically modified plants and food.

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7 Appendix

A Tables

Table 33: Comparison of the data requirements related to toxicity and allergenicity assessment by the three responsible agencies in the USA

	USDA	EPA	FDA
Responsible for	Planting, transportation and importation of GMP	Plant-incorporated protectants (PIPs)	Safety of foods and feed from GMP
Basis of regulation	No significant plant pest risk should result from the widespread planting of GMP	Safety of PIPs for environment and human health (analogous to biochemical and microbial pesticides)	Foods and feed from GMP should be as safe and nutritious as their conventional counterparts
<u>Toxicity:</u> a) Impact / organisms b) Methods	a) Potential impact on non-target organisms, including beneficial organisms and endangered species b) Field observations, nutritional composition	a) Potential impact on non-target organisms (beneficial organisms, birds, fish, honey bees, invertebrates) b) Acute oral toxicity with a maximum hazard dose (2-5 gm/kg body weight), Amino acid sequence homology with known protein toxins	a) Potential impact on human and animals b) Concept of substantial equivalence (OECD): nutritional composition of GMP, known toxicants, feeding tests with high doses, "history of safe use" of the gene product
<u>Allergenicity:</u> a) Aim b) Methods	a) Worker safety b) Evaluation of potential allergenicity based on a literature survey	a) Food safety of PIPs b) IFBC-concept: Amino acid sequence homology with known allergens, In vitro digestibility in simulated gastric and intestinal digestive fluids, Stability to heat and processing	a) Food safety (except for PIPs) b) The allergenic substance is not present in the new food or amino acid sequence homology and in vitro digestibility

Table 34: Toxicity testing in Directive 90/220 dossiers

Dossier		Target/Marker Proteins	Tests described			
GMP	Intended Use		Toxicity testing (target protein)	Toxicity testing (marker protein)	Other tests	Type of feeding study (whole plant)
Fodder Beet A5/15	Cultivation, seed production, feed stuff	CP4 EPSP-Synthase/n.a.	Acute toxicity in mice (summary and literature references) Further references to published studies	n.a.	Homology comparisons	Digestibility study in sheep
Potato EH92-527-1	Cultivation, feed stuff, fertilizer, technical application	n.a./NptII	n.a.	Literature reference given to an acute toxicity study of the introduced protein	n.i.	n.i.
Maize GA21	Import, processing for feed stuff (no cultivation, no use as food)	Modified mEPSP-Synthase/n.a.	Acute toxicity in mice (summary)	n.a.	n.i.	Performance study in broiler chicken
	Cultivation, feed stuff		Toxicity assessment identical to 1st application (see above)	n.a.	n.i.	Identical to 1st application (see above)

Dossier		Target/Marker Proteins	Tests described			
GMP	Intended Use		Toxicity testing (target protein)	Toxicity testing (marker protein)	Other tests	Type of feeding study (whole plant)
Maize Bt11	Import, processing	Cry1Ab, PAT/n.a.	Toxicity testing of an E.coli Btk protein in various insects[a] Toxicity testing in bees, other insects, soil organisms, aquatic invertebratae, and mice[c] Chronic toxicity in laboratory animals, birds, and fish using a Bacillus thuringiensis suspension[b] Only references provided Only references provided on toxic properties of the PAT protein	n.a.	n.i.	n.i.
Maize Bt11	Cultivation	Cry1Ab, PAT/n.a.	Toxicity testing in various species (summary (abridged version) References to toxicity testing in literature given	n.i.	Equivalence of plant and bacterial Btk protein	n.i.
Cotton 531	Cultivation, feed stuff, industrial application	Cry1Ac/NptII	References to studies in the context of the US registration of Cry1A(c) protein in birds and other non-target species Acute toxicity in mice ($LD_{50;}$ application includes summary only; full report provided on request)	n.i.	n.i.	Feed conversion tests in quails (5 days) did not show any toxic effects (based on EPA, OECD Guidelines and ASTM standard)

Dossier		Target/Marker Proteins	Tests described			
GMP	Intended Use		Toxicity testing (target protein)	Toxicity testing (marker protein)	Other tests	Type of feeding study (whole plant)
Cotton 1445	Cultivation, feed stuff (especially for poultry, sheep, catfish, and pigs)	CP4 EPSP-Synthase/ NptII	n.i.	n.i.	n.i.	Reference to a feeding study: cotton seeds fed to some 700.000 diary cows 28-d toxicity study in rats (identity of the test substance not fully clear, probably RR cotton; no histophysiological investigation!)
Rape Topas 19/2	Import, processing, cultivation, oil production, feed stuff	PAT/NptII	n.i.	n.i.	n.i.	Feed conversion study in broiler chicken

GMP… genetically modified plant; n.a… not applicable; n.i… .not included in the dossier;; EPSPS… 5-enolpyruvylshikimate-3-phosphate Synthase. a) The sequence of the Cry1Ab gene was modified; b) Not clear if the Btk protein is identical to the gene product of maize Bt11; c) It remains unclear which Btk protein had been used.

No toxicity studies were included in the carnation dossiers.

Table 35: Exposure relevant studies in the course of toxicity testing in Novel Food dossiers

Dossier		Tests described			
GVP	Target / marker proteins	Di-gesti–bility	Expression in plant tis-sue	Concentra-tion in final food	Comments
Maize NK603	CP4-EPSPS/n.a.	+	+a	n.i.	a) Report not enclosed.
Maize 1507	Cry1F, PAT/n.a.	+b	+c	n.i.	b) For both proteins only summaries are provided. c) Different parts of the plant including maize kernel.
Sweet Maize Bt11	Cry1Ab, PAT/n.a.	+d	+e	n.i.	d) Digestibility study on an equivalent PAT protein derived from bar gene. e) Different part of the plant including maize kernel.
Maize GA21	mEPSPS/n.a.	+	+f	+g	f) Plant including maize kernel. g) Only submitted on request.
Soybean 260-05	No protein introduced[a]	n.a.	h	n.i.	h) Prove that none of the genes introduced is being expressed.
Rape MS1xRF1 and MS1xRF2[i]	Barnase, PAT or barstar, PAT/NptII	n.i.	n.i.	n.i.	i) Dossier presumably incomplete.
Rape Topas 19/2	PAT/NptII	+j	k	+l	j) Study in gastric liquids from cattle and pigs; only coversheet of report provided. k) Study on posttranslational processing in plant of both target and marker protein. l) Report on activity of PAT in processed/unprocessed seeds, re-port on NptII in processed seeds; report on both proteins in processed rape (oil and flour) (parts of all three studies are missing due to confidentiality reasons).
Rape GT73	CP4 EPSPS, GOXv247/n.a.	+m	+n	+o	m) CP4-EPSPS: including in vitro study in an simulated intestine system. n) Summary of study on seeds (both proteins).

Dossier		Tests described			
GVP	Target / marker proteins	Di-gesti–bility	Expression in plant tis-sue	Concentra-tion in final food	Comments
					o) Investigated for oil.
Maize T25	PAT/n.a.	+	+p	+p	p) Study of PAT protein in different parts of the plant and prod-ucts (starch, oil, flour, semolina); prove that marker gene is not expressed in the plant.
Maize Bt11	Cry1Ab, PAT/n.a.	+q	+r	n.i.	q) Provided for both proteins, in case of Btk protein only refer-ence given to digestibility study on an equivalent PAT protein de-rived from bar gene. r) Different part of the plant including maize kernel.
Maize MON809	Cry1Ab, CP4 EPSPS/Gox, NptII	+s	+t	n.i.	s) Only summaries provided, both proteins were also studied in an simulated intestine system. t) Different part of the plant including maize kernel (only sum-mary provided).
Maize MON810	Cry1Ab/n.a.	+u	+v	n.i.	u) Only summaries provided, including data generated by a simulated intestine system. v) Different part of the plant including maize kernel (only sum-mary provided).

n.a.... not applicable; n.i.... not included in the dossier.

Digestibility studies are carried out in vitro using simulated gastric (intestine) liquid (with the exception of rape 19/2).

Table 36: Toxicity testing in Novel Food dossiers

Dossier		Tests described				
GVP	Target / marker proteins	Toxicity testing (target gene product)	Toxicity testing (marker gene product)	Source of test substance	Other studies	Type of feeding study (whole plant)
Maize NK603	CP4 EPSPS/n.a.	Acute toxicity (full report, GLP; but max. dosage 50mg/kg!)	n.a	E.coli	n.i.	Feed conversion study in chicken
Maize 1507	Cry1F, PAT/n.a.	Acute toxicity EPA review of toxicity studies PAT: Acute toxicity; reference to 14 day study in rats	n.a	Chimeric Cry1F/Cry1Ab protein from Pseudomonas sp.	n.i.	Feed conversion study in chicken
Sweet Maize Bt11	Cry1Ab, PAT/n.a.	8 day acute toxicity References for further toxicity studies PAT: Acute toxicity (according to EPA Guidelines) Reference to a 14 day study	n.a.	E.coli HD-1 tryptic core protein E.coli	Homology studies to known toxic proteins	Feed conversion study in chicken
Maize GA21	mEPSPS/n.a.	Acute toxicity (presumably identical to NK603)	n.a	Modified protein in E.coli	Homology studies to known toxic proteins	90 day toxicity study in rats (kernel); not fully in accordance with testing standards Feed conversion study in chicken
Soybean 260-05	No protein introduced[a]	n.a.	n.a.	n.a.	n.i.	Feed conversion study in chicken and pigs
Rape MS1xRF1	Barnase, PAT or-barstar,	n.i. (dossier presumably in-	n.i.	n.i.	n.i.	n.i.

Dossier		Tests described				
GVP	Target / marker proteins	Toxicity testing (target gene product)	Toxicity testing (marker gene product)	Source of test substance	Other studies	Type of feeding study (whole plant)
and MS1xRF2	PAT/NptII	complete)				
Rape Topas 19/2	PAT/NptII	n.i.	n.i.	n.i.	n.i.	n.i.
Rape GT73	CP4 EPSPS, GOXv247/n.a.	Acute toxicity (EPSPS and GOX)	n.a.	E.coli	Homology studies to known toxic proteins	Feeding studies in rats, salmon, and quails (to prove SA)
Maize T25	PAT/n.a.	14 day toxicity (according to OECD Guidelines)	n.a.	Not specified	Homology studies to known toxic proteins	Feed conversion study in chicken
Maize Bt11	Cry1Ab, PAT/n.a.	Acute toxicity (reference only) References for further toxicity studies PAT: Acute toxicity (according to EPA guidelines)	n.a.	Not specified; E.coli	n.i.	n.i.
Maize MON809	Cry1Ab, CP4 EPSPS/Gox, NptII	8 to 9 day acute toxicity	No marker protein expressed	E.coli (Cry1Ab, CP4 EPSPS)	Homology studies to known toxic proteins	n.i.
Maize MON810	Cry1Ab/n.a.	8 to 9 day acute toxicity	n.a.	E.coli	Homology studies to known toxic proteins	n.i.

GMP… genetically modified plant; n.a…. not applicable; n.i…. not included in the dossier; EPSPS… 5-enolpyruvylshikimate-3-phosphate Synthase; a) Gene knock-out via anti-sense DNA.

Table 37: Rape dossiers: compounds analysed and units used – raw seed

	MS1xRF1 / MS1xRF2		Topas		GT73	
Major components		**Unit**		**Unit**		**Unit**
Moisture	+	%	-	-	+	%
Protein	+	% seed and %meal	+	% oil-free dry weight seeds	+	% dry-weight and % oil-free meal
Fat	+	% seed	+	% dry-weight	+	% dry-weight
Fibres	-	-	+	% oil-free dry-weight	+	% dry-weight
Ash	-	-	+	% oil-free dry-weight	+	% dry-weight
Carbohydrates	-	-	-	-	calc	% dry-weight
Other components						
Fatty Acids	+	% oil (12,11,10,9,7)	+	% fatty acids total (11)	+	% oil (11)
Amino Acids	+	mg/g seed (17)	+	nmol/g seed (24)	+	g/100g dry-weight and g/100g Protein (18)
Glucosinolates	+	µmol/g seed; µmol/g oil-free meal and µmol/100g fat-free basis	+	µmol/g oil-free dry-weight	+	µmol/g oil-free meal
Sinapin	-	-	-	-	+	mg/g oil-free meal
Tocopherols	-	-	+	mg/100g oil	-	-
Sterols	-	-	+	mg/100g oil	-	-

Numbers in brackets in the box for amino acids and fatty acids show the number of different amino and fatty acids analysed and compared.

Table 38: Rape dossiers: compounds analysed and units used – refined oil

Components	MS1xRF1 / MS1xRF2		Topas		GT73	
		Unit		Unit		Unit
Fatty Acids	+	% (13)	+	% (12)	+	% (13)
Tocopherols	+	mg/100g	+	mg/100g	+	mg/100g
Sterols	+	mg/100g	+	mg/100g	+	mg/100g
Minerals						
Ca	+	ppm	-	-	-	-
Mg	+	ppm	-	-	-	-
Fe	+	ppm	-	-	-	-
Cu	+	ppm	-	-	-	-
P	-	-	+	ppm		
Other compounds						
Chlorophyll	+	ppm	+	ppm	-	-
Moisture	-	-	-	-	+	%
Free fatty acids	+	%	+	%	+	%
Heavy metals	-	-	-	-	+	mg/kg
Arsenic	-	-	-	-	+	mg/kg
Lead	-	-	-	-	+	mg/kg

Numbers in brackets in the box for fatty acids show the number of different fatty acids analysed and compared.

Table 39: Rape dossiers: statistics

Statistical information provided / Assessment of statistics	MS1xRF1 / MS1xRF2	Topas	GT73
Statistical evaluation processed products	No	No	No
Statistical evaluation raw products	At least partially	At least partially	At least partially
Method	Analyses of Variance F-Test	Only for the year 1993 mentioned: ONE-WAY ANOVA	-
Software	-	Only for 1993 mentioned: Statistical/W© Software	-
Evaluation sheets annexed	For 2 studies and 3 compounds	Detailed for 2 years, summary for other years	-
Conclusions plausible	No	In part	No
Possibility to check for plausibility of drawn conclusions	No	In part	No
Uncertainties	Unclear, which comparisons had been subjected to a statistical evaluation	Not all compound comparisons had been subjected to a statistical evaluation; different data-pools used	Significant differences mentioned within the text; unclear, what had been evaluated and how

Table 40: Maize dossiers: statistics

Statistical information provided / Assessment of statistics	T25	Bt11	MON810	MON809
Statistical evaluation mentioned	+	+	-	+
All comparisons statistically evaluated	+	-	-	Unclear
Method	ANOVA – no further information	Different – depending on comparison: "means comparison" (without further explanation); Variance- + Pair-Analyses, t-test	-	Double t-test
Software	Not specified	Different depending on comparison: STAT-ITCF; SAS; MS Excel	-	Not specified
Evaluation sheets annexed	-	-	-	-
Conclusions plausible	Partly	Partly	No	No
Possibility to check for plausibility of drawn conclusions	Partly because of loads of raw data	No	No	No
Uncertainties	Herbicide application not considered	Not all compounds have been subjected to statistical evaluation; results of pair-analyses are lacking; no information on herbicide application	Apparently no statistical evaluation	Significant differences only mentioned in tables of kernel comparisons (no additional information); apparently no statistical evaluation of plant material comparisons; no information on herbicide application;

Table 41: Maize dossiers: compounds and chosen units – kernels

Compounds	T25		Bt11		MON810		MON809	
		Unit		Unit		Unit		Unit
Major components								
Protein	+	% DW	+	% n.s.	+	% DW.	+	% DW
Nitrogen total	-	-	+	% DW	-	-	-	-
Fat	+	% DW	+	% n.s.	+	% DW	+	% DW
Carbohydrates	+	% DW	-	-	+	% DW	+	% DW
Starch	-	-	+	% DW	-	-	-	-
Ash	+	% DW	+	% DW	+	% DW	+	% DW
Fibres	+	% DW	+	% n.s.	-	-	-	-
ADF	-	-	-	-	+	% DW.	-	-
NDF	-	-	-	-	+	% DW	-	-
Moisture	+	%	+	%	+	%	+	%
Other components								
Fatty Acids	+	% fat	+	% n.s.	+	% fat	+	-
Amino Acids	+	% MM	+	g/kg DS	+	% protein	+	-
Cellulose	-	-	+	% DW	-	-	-	-
Xanthophylls	-	-	+	mg/kg DW	-	-	-	-
Vitamin B1	-	-	+	mg/lb	-	-	-	-
Vitamin B2	-	-	+	mg/lb	-	-	-	-
Niacin	-	-	+	mg/lb	-	-	-	-
Folic Acid	-	-	+	mg/lb	-	-	-	-
Cu, Mg, Mn, Zn	-	-	+	% n.s.	-	-	-	-

DW… dry weight; DS… dry substance; MM… moist mass; n.s.… not specified.

[a] *1 lb is equivalent to 0,453 kg.*

Table 42: Maize dossiers: compounds and chosen units – plant material/silage

Compounds	T25		Bt11		MON810		MON809	
		Unit		Unit		Unit		Unit
Major components								
Protein	+	% DW	-	-	+	% DW	+	% DW
Fat	+	% DW	-	-	+	% DW	+	% DW
Carbohydrates	+	% DW	-	-	+	% DW	+	% DW
Starch	-	-	-	-	+	% DW	-	-
Ash	+	% DW	-	-	+	% DW	+	% DW
Fibres	-	-	-	-	+	% DW	-	-
ADF	+	% DW	-	-	+	% DW	+	% DW
NDF	+	% DW	-	-	+	% DW	+	% DW
Moisture	+	%	-	-	+	% DW.	-	-
Other components								
Soluble sugars	-	-	-	-	+	% DW	-	-
Phytic acid	+	% DW	-	-	-	-	-	-

B Contributors to this monograph

Chapter 1 Introduction	**Armin Spök**
Chapter 2 Regulatory Context	**Sections 2.1 and 2.2 Armin Spök (including work of Sandra Karner in Spök et al. 2003b); Section 2.3 Susanne Stirn**
Chapter 3 Review of dossiers	**Section 3.1 Armin Spök (based on the work of Heinz Hofer on toxicological and general issues included in Spök et al. 2002a, 2003a); Section 3.2 Rudolf Valenta and Armin Spök; Section 3.3 Petra Lehner (including work of Karin Kienzl-Plochberger)**
Chapter 4 Proposals	**Section 4.1 Armin Spök (based on the work of Heinz Hofer on toxicological and general issues included in Spök et al. 2002a, 2003a); Section 4.2 Rudolf Valenta and Armin Spök; Section 0 Petra Lehner (including work of Karin Kienzl-Plochberger)**
Chapter 5 Results and Proposals in Context	**Armin Spök**
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C Expert reports and testimonials

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E Abbreviations and acronyms

ACNFP	Advisory Committee on Novel Foods and Processes (UK)
ADF	Acid Detergent Fibre
APHIS	Animal and Plant Health Inspection Service
ARC	Austrian Research Centers, Seibersdorf, Austria
BIOGUM	Research Center for Biotechnology, Society and the Environment at the University of Hamburg, Germany
Bt protein	Endotoxin of Bacillus thuringiensis
Btk protein	Endotoxin of Bacillus thuringiensis kurstaki
CA	Competent Authority
CaMV	Cauliflower Mosaic Virus
CFSAN	Center for Food Safety and Applied Nutrition, USA
Co-Op	Western Canadian Cooperative Rapeseed Test
CP4 EPSPS	5-Enolpyruvylshikimat-3-Phosphate-Synthase of Agrobacterium tumefaciens strain CP4
DNA	Deoxyribonucleic Acid
EEC	European Economic Community
EFSA	European Food Safety Authority
E.coli	Escherichia coli
EC	European Commission
EMEA	European Agency for the Evaluation of Medicinal Products
EPA	Environment Protection Agency (USA)
EU	European Union
EuropaBio	European Association for Bioindustries
e.r.a.	Environmental Risk Assessment
FAO	Food and Agriculture Organisation
FDA	Food and Drug Administration, USA
FFDCA	Federal Food, Drug, and Cosmetic Act, USA
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act, USA
FPPA	Federal Plant Pest Act, USA
GLP	Good Laboratory Practices
GOX	Glyphosate Oxidoreductase
GRAS	Generally Recognized as Safe
GM	Genetically Modified
GMO	Genetically Modified Organism
GMP	Genetically Modified Plant

IFBC	International Food Biotechnology Council
IFZ	Inter-University Research Centre for Technology, Work and Culture, Graz, Austria
IgE	Immunoglobulin E
ILSI	International Life Science Institute
JEFCA	Joint FAO/WHO Expert Committee on Food Additives
kDa	Kilodalton
mRNA	messenger-RNA
MUFA	Monounsaturated Fatty Acids
NDF	Neutral Detergent Fibre
NF	Novel Food
NOEL	No Observed Effect Level
OECD	Organisation for Economic Co-operation and Development
P/S-ratio	Ratio of polyunsaturated to saturated fatty acids
PAT	Phosphinothricin acetyltransferase
PIP	Plant-Incorporated Protectants
PPM	Parts Per Million
PUFA	Polyunsaturated Fatty Acids
RAST	Radio-Allergo-Sorbent-Test
rDNA	ribosomal DNA
RNA	Ribonucleic Acid
RR	Roundup Ready
SCF	Scientific Committee on Food, EU
SCP	Scientific Committee on Plants, EU
SGF	Simulated Gastric Fluid
SSC	Scientific Steering Committee
SNIF	Short Notification Information Format
TSCA	Toxic Substances Act
UK	United Kingdom
US	United States
USA	United States of America
USDA	US-Department of Agriculture
WHO	World Health Organization