



Supplement

**ASSESSMENT OF TOXIC
AND ECOTOXIC
PROPERTIES OF
NOVEL PROTEINS IN GMOS**

**Forschungsberichte der
Sektion IV**

Band 1 / 2008

Teil 2



umweltbundesamt[®]

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Impressum:

Herausgeber, Medieninhaber und Hersteller:

Bundesministerium für Gesundheit, Familie und Jugend, Sektion IV
Radetzkystraße 2, 1031 Wien

Für den Inhalt verantwortlich:

BL Mag. Ulrich Herzog

Erscheinungstermin: März 2008

Autoren:

Dr. Armin Spök
Mag. Marion Dolezel
Dr. Michael Eckerstorfer
DI Maria Freigassner
Dr. Helmut Gaugitsch
Dr. Andreas Heissenberger
Mag. Sandra Karner
Dr. Manfred Klade
Mag. Markus Proksch
Mag. Leo Schneider
Dr. Fritz Treiber
Dr. Maria Uhl

Druck: Kopierstelle des BMGFJ, Radetzkystraße 2, 1031 Wien

Bestellmöglichkeiten:

Telefon: +43-1/711 00-4700 DW
Fax: +43-1/715 58 30
E-Mail: broschuerenservice.bmgfj@bmgfj.gv.at
Internet: <http://www.bmgfj.gv.at>

ISBN 978-3-902611-15-4

Diese Studie/Broschüre ist kostenlos beim Bundesministerium für Gesundheit, Familie und Jugend, Radetzkystraße 2, 1031 Wien, erhältlich.



SUPPLEMENT

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Armin Spök
Marion Dolezel
Michael Eckerstorfer
Maria Freigassner
Helmut Gaugitsch
Andreas Heissenberger
Sandra Karner
Manfred Klade
Markus Proksch
Leo Schneider
Fritz Treiber
Maria Uhl

Vienna, 20 Dezember 2007



Preface

This document includes the more comprehensive tables which were compiled in the course of the analysis during the TOXPROT project. It is thereby considered a supplement to the final report of this project.

The tables are not intended to be self explanatory and have to be read in conjunction with the text in this report.

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Table 1: Comparison of data requirements for protein equivalence in different guidance document.

Guidance	SIPH/BS (2003)	SSC (2003)	EFSA (2006)	EFSA (2006) - GMM	EuropaBio (2003)	US EPA 2006
Remarks	e		b	b	a	c
Molecular weight	x	x	x	x	x	x
Isoelectric point	-	x	x	x	-	-
Amino acid sequence	x (d)	x	x	x	x	x
Amino acid composition	x					
Post translational modification	x	x	x	x	x	x
Immunological reactivity	x	x	x	x	x	x
Enzymatic activity	-	x	x	x	-	-
Functional analysis	x	-	-	-	x	x

a Equivalence should be established using the following analyses as appropriate

b It is essential that tested protein is equivalent to the newly expressed protein as it is expressed in the GM plant

c EPA Guidance; Bt-plant as example

d Full length amino acid sequence

e Factors should be examined

x = experiment recommended; - = no experiment recommended

SIPH/BC: Scientific Institute of Public Health and Biosafety Council; SSC: European Commission Scientific Steering Committee

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Table 2: Comparison of evidence for test protein equivalence in risk assessment dossiers.

GMP Information	Cotton 531	Maize 591227	Rape GT73	Maize GA21	Maize Bt11	Maize Bt11	Maize 603 NK	Raps HCN92	Fodder Beet	Maize 3272
Protein (s)	CryIA(c), NPTII (m)	Cry34Ab1, Cry35Ab1, PAT(o)	CP4 EPSPS, GOX	mEPSPS	Cry1Ab	Cry1Ab	CP4 EPSPS	PAT & NPTII (l)	CP4 EPSPS	AMY797E, PMI
Year (application)	1997	2005	1997	1998	Summary 1999	1999	2001	1997 (c)	1997	2007
Year (report-experiments)	1993	2003	1993	1997	1994	different studies (i)	1993 (a)	1994	1993/1996	1999/2005/2006/2007
Source of test protein	<i>E.coli</i>	<i>P.fluorescens</i>	<i>E.coli</i> (g)	<i>E.coli</i>	<i>E.coli</i>	<i>E.coli</i>	<i>E.coli</i>	<i>E.coli</i>	<i>E.coli</i>	<i>E.coli</i>
Molecular weight	SDS-PAGE	SDS-PAGE	Paper cit. no exp. shown	SDS-PAGE	SDS-PAGE	SDS-PAGE	SDS-PAGE	SDS-PAGE	-	SDS-PAGE
Isoelectric Point	-	-	-	-	-	-	-	-	-	-
Amino Acid Sequence	10 amino acid positions	N-Terminal sequencing	Comparing the deduced AAS (f)	N-Terminal sequencing	N-Terminal sequencing	N-Terminal sequencing	-	-	-	N-Terminal sequencing
Post translational modification	(k)	Carbohydrate staining	-	Carbohydrate staining	Carbohydrate staining	Carbohydrate staining	-	Glycosylation is highly unlikely (e)	-	Carbohydrate staining
Immunological reactivity	Western Blot Analysis	Western Blot Analysis	-	Western Blot Analysis	Western Blot Analysis; ELISA	Western Blot Analysis	Western Blot Analysis	Western Blot Analysis; ELISA (d)	-	Western Blot Analysis
Enzymatic activity	-	-	Kinetic characteristics	mEPSPS enzyme assay	-	-	-	PAT enzyme (d)	Taylor et al. 1996	Ceralpha amylase assay kit
Functional analysis	EC50 test-insect bioassay	-	-	mEPSPS enzyme assay (b)	Insecticidal activity (h)	Insect bioassay	-	-	-	-
Other	-	(n) Trypsinolysis	-	-	-	Equi. extension (j)	-	-	-	In-vitro digestibility

Extensive studies about the protein CP4 EPSPS (produced in E.coli), reference to different studies concerning this protein, studies available as attachment - Dossier (1993)

Functional activity comparison mentioned in results, no mention in methods (experiment implementation)

All studies end in the year 1994, Dossier present for application 1997 –UK

Cross reference to internal reports – Western Blot (ACI94-19), ELISA (AGR94-16), PAT Enzyme (AGR94-10), (AGR94-18, AGR94-08)

No experiments, only conclusions, no link to internal company report

Cited studies about CP4 EPSPS and other proteins of this family (Padgett et al., 1993a; 1991; 1988)

GOX (linage Achromobacter); CP4 EPSPS (linage Agrobacterium)

possible conformation change tested with Trypsin Digestion Assay

Attachment 1 - Protein fermentation (1992); Attachment 2 - Trypsin Digestion (1992); Attachment 3 - E.coli Protein characterisation (1994)

Equivalence extension to line MON80100 (molecular weights, specific immunological recognition)

Test was mentioned, proteins for comparison study were listed, no results or discussion of the study itself

Equivalence of neomycin phosphotransferase and phosphinothricin acetyltransferase expressed in the transgenic line HCN92 with microbially expressed forms of the proteins. MacDonald R. 1994

NTPII is mentioned as marker but no equivalence study made

Trypsinolysis in combination with bioinformatic analysis of fragments

PAT protein was also target of the biochemical equivalence studies

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Table 3: Overview of novel proteins expressed in GMPs authorised or pending for commercial application in the EU and the USA.

Protein	Donor	GMPs	Technical application	References
ACC	Tomato	Tomato 1345-4	Conversion of s-adenosylmethionine to ACC	AGBIOS Database
ACCd	<i>Pseudomonas chlororaphis</i>	Tomato DR 8338	Catalyzes metabolism of 1 1-amino-cyclopropane-1-arboxylic acid (ACC)	AGBIOS Database
ALS	<i>Nicotiana tabacum</i>	Carnation Florigene Moonlite (123.2.38)	Resistance to sulfonylurea herbicides	SNIF & Assessment Report (C/NL/04/02)
	Chimera of 2 resistant AHAS genes	Cotton DD-Ø1951A-7 (19-51A)	Tolerance to sulfonylurea herbicide	AGBIOS Database
	<i>Arabidopsis thaliana</i>	Flax CDC-FLØØ1-2 (FP967)		AGBIOS Database
APH4 (hygromycin-B-phosphotransferase)	<i>Escherichia coli</i>	Cotton SYN-IR12-7 (COT102)	Tolerance to the antibiotic hygromycin	AGBIOS Database
Barnase	N.i.	Rape MS1xRF1 and MS1xRF2	N.i.	Spök et al. 2004 (Novel Food Dossiers)
	<i>Bacillus amyloliquefaciens</i>	Rape Ms8 x Rf3	Results in male sterility	SNIF & Assessment Report (C/BE/96/01); C/BE/96/01
Barstar	<i>Bacillus amyloliquefaciens</i>	Rape Ms8 x Rf3	Results in fertility restoration	SNIF & Assessment Report (C/BE/96/01); C/BE/96/01
CMV CP	Cucumber mosaic cucumovirus	Yellow crookneck squash CZW-3	Resistance towards CMV	AGBIOS Database
CP4 EPSPS	N.i.	Cotton 1445	N.i.	Spök et al. 2004 (Directive 90/220 Dossiers C/ES/97/01)
	<i>Agrobacterium sp.</i> strain CP4	Cotton MON15985 and MON15985 x MON1445	Tolerance to glyphosate	EFSA-GMO-UK-2005-10
		Cotton MON531 x MON1445		EFSA-GMO-UK-2005-09
	N.i.	Fodder Beet A5/15	N.i.	Spök et al. 2004 (Directive 90/220 Dossiers C/DK/97/01)
	<i>Agrobacterium sp.</i> strain CP4	Maize 1507 x NK603	Tolerance to glyphosate	EFSA-GMO-UK-2004-05; EFSA-GMO-UK-2005-17
	<i>Agrobacterium tumefaciens</i> CP4	Maize MON88017		AGBIOS Database
	N.i.	Maize MON809	N.i.	Spök et al. 2004 (Novel Food Dossiers)
	<i>Agrobacterium sp.</i> strain CP4	Maize MON863 x MON810 x NK603	Tolerance to glyphosate	EFSA-GMO-BE-2004-07
		Maize MON863 x NK603		EFSA-GMO-UK-2004-06
		Maize NK603		SNIF (C/ES/03/01); C/ES/00/01; EFSA-Q-2003-002
		Maize NK603 x MON810		SNIF (C/ES/04/01), SNIF & Assessment Report (C/GB/02/M3/3); EFSA-GMO-UK-2004-01
		Rape GT73		SNIF & Assessment Report (C/NL/98/11);

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Protein	Donor	GMPs	Technical application	References
				C/NL/98/11
	N.i.	Rape GT73	N.i.	Spök et al. 2004 (Novel Food Dossiers)
	<i>Agrobacterium sp.</i> strain CP4	Sugar beet H7-1 Roundup Ready	Tolerance to glyphosate	EFSA-GMO-UK-2004-08
	N.i.	Maize NK603	N.i.	Spök et al. 2004 (Novel Food Dossiers)
Cry1Ab	<i>Bacillus thuringiensis</i> ssp. <i>kurstaki</i> strain HD-1	Maize Bt11	Resistance to certain lepidopteran insect pests	SNIF & Assessment Report (C/F/96.05.10); C/F/96/05.10
	N.i.	Maize Bt11	N.i.	Spök et al. 2004 (Directive 90/220 Dossiers C/GB/96/M4/1; C/F/96/05-10); Spök et al. 2004 (Novel Food Dossiers)
		Maize MON809		Spök et al. 2004 (Novel Food Dossiers)
		Maize MON810		Spök et al. 2004 (Novel Food Dossiers)
	<i>Bacillus thuringiensis</i> sp. <i>kurstaki</i>	Maize MON863 and MON863 x MON810	Resistance to certain lepidopteran insect pests	C/DE/02/9; EFSA-Q-2003-121; SNIF & Assessment Report (C/DE/02/9)
		Maize MON863 x MON810		C/DE/02/9; EFSA-GMO-DE-2004-03
		Maize MON863 x MON810 x NK603		EFSA-GMO-BE-2004-07
		Maize NK603 x MON810		SNIF (C/ES/04/01); SNIF & Assessment Report (C/GB/02/M3/3); EFSA-GMO-UK-2004-01
	N.i.	Sweet Maize Bt11	N.i.	Spök et al. 2004 (Novel Food Dossiers)
Cry1Ac	<i>Bacillus thuringiensis</i> sp. <i>kurstaki</i>	Cotton 236/3006-210-23	Resistance to certain lepidopteran insect pests	SNIF & Assessment Report (C/NL/04/01)
		Cotton 281-24-236/3006-210-23		EFSA-GMO-NL-2005-16
		Cotton 31807 and 31808		AGBIOS Database
	N.i.	Cotton 531	N.i.	Spök et al. 2004 (Directive 90/220 Dossiers C/ES/96/02)
	<i>Bacillus thuringiensis</i> sp. <i>kurstaki</i>	Cotton DAS-21Ø23-5 (3006-210-23)	Resistance to certain lepidopteran insect pests	AGBIOS Database
		Cotton MON15985 and MON15985 x MON1445		EFSA-GMO-UK-2005-10
		Cotton MON-15985-7		AGBIOS Database
	<i>Bacillus thuringiensis</i>	Cotton MON531 x MON1445		EFSA-GMO-UK-2005-09
	<i>Bacillus thuringiensis</i> sp. <i>kurstaki</i>	Maize DKB-89614-9 (DBT418) (trade name Bt Xtra™)		AGBIOS Database
		Tomato 5345		AGBIOS Database

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Protein	Donor	GMPs	Technical application	References
Cry1F	<i>Bacillus thuringiensis</i> sp. <i>aizawai</i>	Cotton 236/3006-210-23	Resistance to certain lepidopteran insect pests	SNIF & Assessment Report (C/NL/04/01)
		Cotton 281-24-236/3006-210-23		EFSA-GMO-NL-2005-16
		Maize 1507		C/NL/00/10; C/ES/01/01; EFSA-GMO-NL-2004-02; SNIF & Assessment Report (C/ES/01/01); SNIF & Assessment Report (C/NL/00/10); EFSA-GMO-NL-2004-02
	N.i.	Maize 1507	N.i.	Spök et al. 2004 (Novel Food Dossiers)
	<i>Bacillus thuringiensis</i> sp. <i>aizawai</i>	Maize 1507 x 59122	Resistance to certain lepidopteran insect pests	EFSA-GMO-NL-2005-15
		Maize 1507 x NK603		EFSA-GMO-UK-2004-05; EFSA-GMO-UK-2005-17
Cry2Ab	<i>Bacillus thuringiensis</i>	Cotton MON-15985-7		AGBIOS Database
Cry2Ab2	<i>Bacillus thuringiensis</i> subsp. <i>kurstaki</i>	Cotton MON15985 and MON15985 x MON1445	Insect protection	EFSA-GMO-UK-2005-10
Cry34Ab1	<i>Bacillus thuringiensis</i> strain PS149B1	Maize 1507 x 59122	Resistance to certain lepidopteran insect pests	EFSA-GMO-NL-2005-15
		Maize 59122		EFSA-GMO-NL-2005-12
		Maize DAS-59122-7		AGBIOS Database
Cry35Ab1	<i>Bacillus thuringiensis</i> strain PS149B1	Maize 1507 x 59122		EFSA-GMO-NL-2005-15
		Maize 59122		EFSA-GMO-NL-2005-12
		Maize DAS-59122-7		AGBIOS Database
Cry3A	<i>Bacillus thuringiensis</i> sp. <i>tenebrionis</i>	Russet Burbank cultivar RBMT15-101, Shepody potato cultivar SEMT15-02, Shepody potato cultivar SEMT15-15		AGBIOS Database
Cry3Bb1	<i>Bacillus thuringiensis</i> sp. <i>kumamotoensis</i>	Maize MON88017	Resistance to certain coleopteran pests	AGBIOS Database
		Maize MON863 and MON863 x MON810		SNIF & Assessment Report (C/DE/02/9); C/DE/02/9; EFSA-Q-2003-121
		Maize MON863 x MON810		EFSA-GMO-DE-2004-03; EFSA-GMO-BE-2004-07; C/DE/02/9
		Maize MON863 x NK603		EFSA-GMO-UK-2004-06
Cry9C	<i>Bacillus thuringiensis</i> sp. <i>tolworthi</i> strain BTS02618A	Maize ACS-ZMØØ4-3 (CBH-351)	Resistance to certain lepidopteran insect pests	AGBIOS Database
DAM	<i>Escherichia coli</i>	Maize 676, 678, and 680	Results in male sterility	AGBIOS Database

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DFR	<i>Petunia X hybrida</i>	Carnation Florigene Moonlite (123.2.38)	Key enzymes in the anthocyanin biosynthesis pathway	SNIF & Assessment Report (C/NL/04/02)
F3'5'H	<i>Petunia X hybrida</i>	Carnation Florigene Moonlite (123.2.38)	Key enzyme in the anthocyanin biosynthesis pathway	SNIF & Assessment Report (C/NL/04/02)
GBSS	<i>Solanum tuberosum</i>	Potato EH92-527-1	Allows decrease in amylase content and a concomitant increase in amylopectin content (> 98 %) in tuber starch	EFSA-GMO-UK-2005-14
GOX	N.i.	Maize MON809	N.i.	Spök et al. 2004 (Novel Food Dossiers)
	<i>Onchrobactrum anthropi</i>	Rape GT73	Tolerance to glyphosate	C/NL/98/11
GOXv247	<i>Ochrobactrum anthropi</i>	Rape GT73		SNIF & Assessment Report (C/NL/98/11)
	N.i.	Rape GT73	N.i.	Spök et al. 2004 (Novel Food Dossiers)
GUS	<i>Escherichia coli</i>	Cotton MON15985 and MON15985 x MON1445	Selectable marker protein	EFSA-GMO-UK-2005-10
		Cotton MON-15985-7		AGBIOS Database
		Papaya 55-1 and 63-1		AGBIOS Database
mCry3A	<i>Bacillus thuringiensis</i>	Maize MIR604	Resistance to the Western and Northern Corn rootworm and other related coleopteran pests of maize	EFSA-GMO-UK-2005-11
mEPSPS		Maize GA21	Tolerance to glyphosate	EFSA-GMO-UK-2005-19
	N.i.	Maize GA21	N.i.	Spök et al. 2004 (Directive 90/220 Dossiers C/GB/97/M3/2; C/ES/98/01); Spök et al. 2004 (Novel Food Dossiers)
Nitrilase	<i>Klebsiella pneumoniae</i> sp. <i>ozanae</i>	CanolaOxy-235 (ACS-BNØ11-5)	Resistance to oxynil herbicides	AGBIOS Database
		Cotton 31807 and 31808		AGBIOS Database
No protein introduced	N.i.	Soybean 260-05	N.i.	Spök et al. 2004 (Novel Food Dossiers)
Nopaline synthase	<i>Agrobacterium tumefaciens</i> CP4	Flax CDC-FLØØ1-2 (FP967)	Catalyses the synthesis of nopaline	AGBIOS Database
NPTII	<i>Escherichia coli</i>	Canola 23-198 and 23-18-17	Resistance towards kanamycin	AGBIOS Database
		Cantaloupe A and B		AGBIOS Database
	N.i.	Cotton 1445	N.i.	Spök et al. 2004 (Directive 90/220 Dossiers C/ES/97/01)
	<i>Escherichia coli</i>	Cotton 31807 and 31808	Resistance towards kanamycin	AGBIOS Database
	N.i.	Cotton 531	N.i.	Spök et al. 2004 (Directive 90/220 Dossiers C/ES/96/02)
	N.sp.	Cotton MON15985 and MON15985 x MON1445	Resistance towards kanamycin	EFSA-GMO-UK-2005-10

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		Cotton MON531 x MON1445		EFSA-GMO-UK-2005-09
	<i>Escherichia coli</i>	Flax CDC-FLØØ1-2 (FP967)		AGBIOS Database
	N.i.	Maize MON809	N.i.	Spök et al. 2004 (Novel Food Dossiers)
	<i>Escherichia coli</i>	Maize MON863 and MON863 x MON810	Resistance towards kanamycin	SNIF & Assessment Report (C/DE/02/9); C/DE/02/9
	N.sp.	Maize MON863 and MON863 x MON810		EFSA-Q-2003-121
	<i>Escherichia coli</i>	Maize MON863 x MON810		EFSA-GMO-DE-2004-03; C/DE/02/9
		Maize MON863 x MON810 x NK603		EFSA-GMO-BE-2004-07
		Maize MON863 x NK603		EFSA-GMO-UK-2004-06
	N.sp.	Maize NK603		EFSA-Q-2003-002; C/ES/00/01
	<i>Escherichia coli</i>	Papaya 55-1 and 63-1		AGBIOS Database
	<i>Agrobacterium thumefaciens</i>	Potato EH92-527-1		EFSA-GMO-UK-2005-14
	N.i.	Potato EH92-527-1	N.i.	Spök et al. 2004 (Directive 90/220 Dossiers C/SE/96/3501)
		Rape MS1xRF1 and MS1xRF2		Spök et al. 2004 (Novel Food Dossiers)
		Rape Topas 19/2		Spök et al. 2004 (Directive 90/220 Dossiers C/UK/95/M5/1); Spök et al. 2004 (Novel Food Dossiers)
	<i>Escherichia coli</i>	Russet Burbank cultivar RBMT15-101, Shepody potato cultivar SEMT15-02, Shepody potato cultivar SEMT15-15	Resistance towards kanamycin	AGBIOS Database
		Tobacco Vector 21-41		AGBIOS Database
		Tomato 1345-4		AGBIOS Database
		Tomato 35 1 N DR		AGBIOS Database
		Tomato 5345		AGBIOS Database
		Tomato DR 8338		AGBIOS Database
		Yellow crookneck squash CZW-3		AGBIOS Database
PAT	<i>Streptomyces viridochromogenes</i> strain Tü494	Cotton 236/3006-210-23	Tolerance to glufosinate ammonium herbicide	SNIF & Assessment Report (C/NL/04/01)
		Cotton 281-24-236/3006-210-23		EFSA-GMO-NL-2005-16
	<i>Streptomyces viridochromogenes</i>	Cotton DAS-21Ø23-5 (3006-210-23)		AGBIOS Database

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	Stepromyces hygrosopicus	Cotton LLCotton25		EFSA-GMO-NL-2005-13
	<i>Streptomyces viridochromogenes</i> strain Tü494	Maize 1507		C/NL/00/10; C/ES/01/01; SNIF & Assessment Report (C/ES/01/01); SNIF & Assessment Report; EFSA-GMO-NL-2004-02
	N.i.	Maize 1507	N.i.	Spök et al. 2004 (Novel Food Dossiers)
	<i>Streptomyces viridochromogenes</i>	Maize 1507 x 59122	Tolerance to glufosinate ammonium herbicide	EFSA-GMO-NL-2005-15
	<i>Streptomyces viridochromogenes</i> strain Tü494	Maize 1507 x NK603		EFSA-GMO-UK-2004-05; EFSA-GMO-UK-2005-17
	<i>Streptomyces viridochromogenes</i>	Maize 59122		EFSA-GMO-NL-2005-12
		Maize 676, 678, and 680		AGBIOS Database
	<i>Streptomyces hygrosopicus</i>	Maize ACS-ZMØØ4-3 (CBH-351)		AGBIOS Database
	<i>Streptomyces viridochromogenes</i>	Maize Bt11		SNIF & Assessment Report (C/F/96.05.10); C/F/96/05.10
	N.i.	Maize Bt11	N.i.	Spök et al. 2004 (Directive 90/220 Dossiers C/GB/96/M4/1; C/F/96/05-10); Spök et al. 2004 (Novel Food Dossiers)
	<i>Streptomyces viridochromogenes</i>	Maize DAS-59122-7	Tolerance to glufosinate ammonium herbicide	AGBIOS Database
	Stepromyces hygrosopicus	Maize DKB-89614-9 (DBT418)		AGBIOS Database
	N.i.	Maize T25	N.i.	Spök et al. 2004 (Novel Food Dossiers)
		Rape MS1xRF1 and MS1xRF2		Spök et al. 2004 (Novel Food Dossiers)
	<i>Streptomyces viridochromogenes</i>	Rape Ms8 x Rf3	Tolerance to glufosinate ammonium herbicide	SNIF & Assessment Report (C/BE/96/01); C/BE/96/01
	N.i.	Rape Topas 19/2	N.i.	Spök et al. 2004 (Directive 90/220 Dossiers C/UK/95/M5/1); Spök et al. 2004 (Novel Food Dossiers)
	<i>Streptomyces viridochromogenes</i>	Rice LLRICE62	Tolerance to glufosinate ammonium herbicide	SNIF & Assessment Report (C/GB/03/M5/3); EFSA-GMO-UK-2004-04
	N.i.	Sweet Maize Bt11	N.i.	Spök et al. 2004 (Novel Food Dossiers)
PAT or Barstar	N.i.	Rape MS1xRF1 and MS1xRF2	N.i.	Spök et al. 2004 (Novel Food Dossiers)
PMI	<i>Escherichia coli</i>	Maize MIR604	Allows transformed corn cells to utilize mannose a sole carbon source	EFSA-GMO-UK-2005-11

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Protein	Donor	GMPs	Technical application	References
PRSV CP	Papaya ringspot potyvirus	Papaya 55-1 and 63-1	Resistance towards PRSV	AGBIOS Database
PVY CP	Potato potyvirus Y	Russet Burbank cultivar RBMT15-101, Shepody potato cultivar SEMT15-02, Shepody potato cultivar SEMT15-15	Resistance towards PVY	AGBIOS Database
QPTase	<i>Nicotiana tabaccum</i>	Tobacco Vector 21-41	Decrease in the production of nicotinic acid and thus nicotine	AGBIOS Database
RNase	<i>Bacillus amyloliquefaciens</i>	Rape Ms8 x Rf3	Tolerance to glyphosate	C/BE/96/01
S-adenosylmethionine hydrolase	<i>Escherichia coli</i> bacteriophage T3	Cantaloupe A and B	Conversion of SAM to ACC	AGBIOS Database
		Tomato 35 1 N DR		AGBIOS Database
Thioesterase	<i>Umbellularia californica</i>	Canola 23-198 and 23-18-17	Active in fatty acid biosynthetic pathway of the developing seeds	AGBIOS Database
Tn7 AAD 3' adenylyltransferase	N.sp.	Sugar beet H7-1 Roundup Ready	Resistance to spectinomycin and streptomycin	EFSA-GMO-UK-2004-08
VIP3A	<i>Bacillus thuringiensis</i>	Cotton SYN-IR12-7 (COT102)	Resistance to certain lepidopteran insect pests	AGBIOS Database
WMV2 CP	Watermelon mosaic potyvirus 2	Yellow crookneck squash CZW-3	Resistance towards WMV2	AGBIOS Database
		Yellow crookneck squash ZW-20		AGBIOS Database
ZYMV CP	Zucchini yellow mosaic potyvirus	Yellow crookneck squash CZW-3		AGBIOS Database
		Yellow crookneck squash ZW-20		AGBIOS Database

N.a.: not applicable; n.i.: not investigated; n.sp. not specified in the documents (does not necessarily mean that this information is not included in the full text risk assessment dossier).

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Table 4: Toxicity testing of heterologous proteins in GMPs authorised or pending for commercial application in the EU and the USA.

Protein	GMPs	Toxicity tests	Whole-plant animal studies	Homology comparisons	References
ALS	Carnation Florigene Moonlite (123.2.38)	Ames mutagenicity test, acute toxicity study in mice, in vitro cytotoxicity test	N.sp.	Known toxins	SNIF & Assessment Report (C/NL/04/02)
APH4 (hygromycin-B phosphotransferase)	Cotton SYN-IR12-7 (COT102)	Acute oral feeding study	N.sp.	N.sp.	AGBIOS Database
Barnase	Rape Ms8 x Rf3	N.sp.	Feeding studies	N.sp.	SNIF & Assessment Report (C/BE/96/01)
Barstar	Rape Ms8 x Rf3	N.sp.	Feeding studies	N.sp.	SNIF & Assessment Report (C/BE/96/01); C/BE/96/01
CP4 EPSPS	Cotton 1445	N.i.	Reference to a feeding study: cotton seeds fed to some 700.000 diary cows; 28-day toxicity study in rats	N.i.	Spök et al. 2004 (Directive 90/220 Dossiers C/ES/97/01)
	Cotton MON15985 and MON15985 x MON1445	Acute oral toxicity in rodents	N.sp.	Known toxins	EFSA-GMO-UK-2005-10
	Cotton MON531 x MON1445	Acute oral gavage toxicity studies in rodents	N.sp.	Known toxins	EFSA-GMO-UK-2005-09
	Fodder Beet A5/15	Acute toxicity in mice	Digestibility study in sheep	N.i.	Spök et al. 2004 (Directive 90/220 Dossiers C/DK/97/01)
	Maize 1507 x NK603	Acute toxicity study in mice	42-days poultry feeding studies	N.sp.	EFSA-GMO-UK-2004-05
	Maize 1507 x NK603	Toxicity study in mice	2-days poultry feeding studies	Known toxins	EFSA-GMO-UK-2005-17
	Maize MON88017	Acute oral toxicity in mice	N.sp.	Known toxins	AGBIOS Database
	Maize MON809	8 to 9-day acute toxicity	N.i.	N.i.	Spök et al. 2004 (Novel Food Dossiers)
	Maize MON863 x MON810 x NK603	Mouse acute gavage study	Feeding experiment in broiler chicken; 90-day oral toxicity study in rats	Known toxins	EFSA-GMO-BE-2004-07
	Maize MON863 x NK603	Mouse acute gavage study	90-day oral toxicity study in rats	Known toxins	EFSA-GMO-UK-2004-06
	Maize NK603	Acute toxicity; 90-day repeated dose rat feeding study; Acute oral toxicity in mice	Feeding study with broiler chicken; 90-day oral toxicity study in rats	Known toxins	SNIF (C/ES/03/01); C/ES/00/01; EFSA-Q-2003-002
	Maize NK603	Acute toxicity	Feed conversion study in chicken	N.i.	Spök et al. 2004 (Novel Food Dossiers)
	Maize NK603 x MON810	Acute toxicity study in mice; Acute oral gavage study	Feeding studies using broiler chicken; 42-day highly sensitive feeding study using broiler chicken	Known toxins	SNIF (C/ES/04/01); SNIF & Assessment Report (C/GB/02/M3/3); EFSA-GMO-UK-2004-01
	Rape GT73	Acute toxicity study in	N.sp.	Known toxins	SNIF & Assessment Report

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Protein	GMPs	Toxicity tests	Whole-plant animal studies	Homology comparisons	References
		mice; 7-day oral toxicity study in mice			(C/NL/98/11); C/NL/98/11
	Rape GT73	Acute toxicity	Feeding studies in rats, salmon, and quails	N.i.	Spök et al. 2004 (Novel Food Dossiers)
	Sugar beet H7-1 Roundup Ready	Acute toxicity test in mice	90-day rat feeding study	N.sp.	EFSA-GMO-UK-2004-08
Cry1Ab	Maize Bt11	Acute toxicity in mice	N.sp.	Known toxins	SNIF & Assessment Report (C/F/96.05.10); C/F/96/05.10
	Maize Bt11	Toxicity testing in various species; Acute toxicity	N.i.	N.i.	Spök et al. 2004 (Directive 90/220 Dossiers C/GB/96/M4/1; C/F/96/05-10); Spök et al. 2004 (Novel Food Dossiers)
	Maize MON809	8 to 9-day acute toxicity	N.i.	N.i.	Spök et al. 2004 (Novel Food Dossiers)
	Maize MON810	8 to 9-day acute toxicity	N.a.	N.i.	Spök et al. 2004 (Novel Food Dossiers)
	Maize MON863 and MON863 x MON810	Acute toxicity study in mice, Acute oral toxicity	Subchronic feeding studies with rats, 42-day feeding studies with chicken; 90-day oral toxicity study in rats	Known toxins	SNIF & Assessment Report (C/DE/02/9); C/DE/02/9, EFSA-Q-2003-121
	Maize MON863 x MON810	N.sp.	Highly sensitive feeding studies using broiler chickens; 90-day oral toxicity study in rats	Known toxins	EFSA-GMO-DE-2004-03, C/DE/02/9
	Maize MON863 x MON810 x NK603	Mouse acute gavage study	Feeding experiment in broiler chicken; 90-day oral toxicity study in rats	Known toxins	EFSA-GMO-BE-2004-07
	Maize NK603 x MON810	Acute toxicity study in mice; Acute oral gavage study	Feeding studies using broiler chicken; 42-day highly sensitive feeding study using broiler chicken	Known toxins	SNIF (C/ES/04/01); SNIF & Assessment Report (C/GB/02/M3/3); EFSA-GMO-UK-2004-01
	Sweet Maize Bt11	8-day acute toxicity	Feed conversion study in chicken	N.i.	Spök et al. 2004 (Novel Food Dossiers)
Cry1Ac	Cotton 236/3006-210-23	Acute toxicity study	42-day poultry feeding study	N.sp.	SNIF & Assessment Report (C/NL/04/01)
	Cotton 281-24-236/3006-210-23	Acute toxicity study in mice	42-days poultry feeding study over 42 day broiler chicken study	Known toxins	EFSA-GMO-NL-2005-16
	Cotton 531	Studies in birds and other non-target species	5-day feed conversion tests in quails	N.i.	Spök et al. 2004 (Directive 90/220 Dossiers C/ES/96/02)
	Cotton DAS-21Ø23-5 (3006-210-23)	Acute oral toxicity	N.sp.	Known toxins	AGBIOS Database
	Cotton MON15985 and MON15985 x MON1445	Acute oral toxicity in rodents	N.sp.	Known toxins	EFSA-GMO-UK-2005-10

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	Cotton MON531 x MON1445	Acute oral gavage toxicity studies in rodents	N.sp.	Known toxins	EFSA-GMO-UK-2005-09
	Maize DKB-89614-9 (DBT418)	Acute oral toxicity in mice	N.sp.	Known toxins	AGBIOS Database
Cry1F	Cotton 236/3006-210-23	Acute toxicity study	42-day poultry feeding study	N.sp.	SNIF & Assessment Report (C/NL/04/01)
	Cotton 281-24-236/3006-210-23	Acute toxicity study in mice	42-days poultry feeding study over, 42 day broiler chicken study	Known toxins	EFSA-GMO-NL-2005-16
	Maize 1507	Acute toxicity in mice, 90-day oral toxicity	42-day poultry feeding study; 90-day oral toxicity study in rats	Known toxins	SNIF & Assessment Report (C/ES/01/01), SNIF & Assessment Report (C/NL/00/10); EFSA-GMO-NL-2004-02, C/NL/00/10; C/ES/01/01
	Maize 1507	Acute toxicity	Feed conversion study in chicken	N.i.	Spök et al. 2004 (Novel Food Dossiers)
	Maize 1507 x 59122	Acute oral toxicity study in mice	42-day feeding study in broiler chicken; 90-day rat feeding study	Known toxins	EFSA-GMO-NL-2005-15
	Maize 1507 x NK603	Acute toxicity study in mice	42-days poultry feeding studies	Known toxins	EFSA-GMO-UK-2004-05; EFSA-GMO-UK-2005-17
Cry2Ab	Cotton MON-15985-7	14-day acute oral toxicity in mice	Whole plant feeding studies with channel catfish and lactating cows	N.sp.	AGBIOS Database
Cry2Ab2	Cotton MON15985 and MON15985 x MON1445	Acute oral toxicity in rodents	N.sp.	Known toxins	EFSA-GMO-UK-2005-10
Cry34Ab1	Maize 1507 x 59122	Acute oral toxicity study in mice	42-day feeding study in broiler chicken; 90-day rat feeding study	Known toxins	EFSA-GMO-NL-2005-15
	Maize 59122	Acute oral toxicity studies	42-day poultry feeding study; In vitro simulated digestibility studies; 90-day oral toxicity feeding study in rats	Known toxins	EFSA-GMO-NL-2005-12
Cry35Ab1	Maize 1507 x 59122	Acute oral toxicity study in mice	42-day feeding study in broiler chicken; 90-day rat feeding study	Known toxins	EFSA-GMO-NL-2005-15
	Maize 59122	Acute oral toxicity studies	42-day poultry feeding study; In vitro simulated digestibility studies; 90-day oral toxicity feeding study in rats	Known toxins	EFSA-GMO-NL-2005-12
Cry3A	Russet Burbank cultivar RBMT15-101, Shepody potato cultivar SEMT15-02, Shepody potato cultivar SEMT15-15	N.sp.	N.sp.	Known toxins	AGBIOS Database
Cry3Bb1	Maize MON88017	Acute oral toxicity	N.sp.	Known toxins	AGBIOS Database
	Maize MON863 and MON863 x MON810	Acute toxicity study in mice	Subchronic feeding studies with rats, 42-day feeding studies with chicken; 90-day oral toxicity study	Known toxins	SNIF & Assessment Report (C/DE/02/9); C/DE/02/9, EFSA-Q-

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			in rats		2003-121
	Maize MON863 x MON810	N.sp.	Highly sensitive feeding studies using broiler chickens; 90-day oral toxicity study in rats	Known toxins	EFSA-GMO-DE-2004-03; C/DE/02/9
	Maize MON863 x MON810 x NK603	Mouse acute gavage study	Feeding experiment in broiler chicken; 90-day oral toxicity study in rats	Known toxins	EFSA-GMO-BE-2004-07
	Maize MON863 x NK603	Mouse acute gavage study	90-day oral toxicity study in rats	Known toxins	EFSA-GMO-UK-2004-06
Cry9C	Maize ACS-ZMØØ4-3 (CBH-351)	Acute oral toxicity	N.sp.	Known toxins	AGBIOS Database
DAM	Maize 676, 678, and 680	Acute toxicity testing	N.sp.	N.sp.	AGBIOS Database
DFR	Carnation Florigene Moonlite (123.2.38)	Ames mutagenicity test, acute toxicity study in mice, in vitro cytotoxicity test	N.sp.	Known toxins	SNIF & Assessment Report (C/NL/04/02)
F3'5'H	Carnation Florigene Moonlite (123.2.38)	Ames mutagenicity test, acute toxicity study in mice, in vitro cytotoxicity test	N.sp.	Known toxins	SNIF & Assessment Report (C/NL/04/02)
GBSS	Potato EH92-527-1	N.sp.	90-day rat feeding study bovine study conducted with EH92-527-1 potato pulp	N.sp.	EFSA-GMO-UK-2005-14
GOX	Maize MON809	No marker protein expressed	N.a.	N.i.	Spök et al. 2004 (Novel Food Dossiers)
	Rape GT73	7-day oral toxicity study in mice	N.sp.	N.sp.	C/NL/98/11
GOXv247	Rape GT73	Acute toxicity study in mice	N.sp.	Known toxins	SNIF & Assessment Report (C/NL/98/11)
	Rape GT73	Acute toxicity	Feeding studies in rats, salmon, and quails	N.i.	Spök et al. 2004 (Novel Food Dossiers)
GUS	Cotton MON15985 and MON15985 x MON1445	Acute oral toxicity in rodents	N.sp.	Known toxins	EFSA-GMO-UK-2005-10
	Cotton MON-15985-7	Acute oral toxicity in mice	Whole plant feeding studies with channel catfish and lactating cows	Known toxins	AGBIOS Database
mCry3A	Maize MIR604	Acute oral toxicity in mice	49-day poultry feeding study 90-day whole food safety study in rodents	Known toxins	EFSA-GMO-UK-2005-11
mEPSPS	Maize GA21	Acute oral toxicity study in mice	49-day poultry feeding study 90-day whole food safety study in	Known toxins	EFSA-GMO-UK-2005-19

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			rodents		
	Maize GA21	Acute toxicity in mice; Acute toxicity	Performance study in broiler chicken; 90-day toxicity study in rats (kernel); feed conversion study in chicken	N.i.	Spök et al. 2004 (Directive 90/220 Dossiers C/GB/97/M3/2; C/ES/98/01); Spök et al. 2004 (Novel Food Dossiers)
Nitrilase	CanolaOxy-235 (ACS-BNØ11-5)	14-day subacute toxicity feeding study	28-day rat feeding study	Known toxins	AGBIOS Database
No protein introduced	Soybean 260-05	N.a.	Feed conversion study in chicken	N.i.	Spök et al. 2004 (Novel Food Dossiers)
NPTII	Cotton 1445	N.i.	Reference to a feeding study: cotton seeds fed to some 700.000 dairy cows; 28-day toxicity study in rats	N.i.	Spök et al. 2004 (Directive 90/220 Dossiers C/ES/97/01)
	Cotton 531	N.i.	5-day feed conversion tests in quails	N.i.	Spök et al. 2004 (Directive 90/220 Dossiers C/ES/96/02)
	Maize MON809	No marker protein expressed	N.a.	N.i.	Spök et al. 2004 (Novel Food Dossiers)
	Maize MON863 and MON863 x MON810	Acute toxicity study in mice	Subchronic feeding studies with rats, 42-day feeding studies with chicken; 90-day oral toxicity study in rats	Known toxins	SNIF & Assessment Report (C/DE/02/9); C/DE/02/9; EFSA-Q-2003-121
	Maize MON863 x MON810	N.sp.	Highly sensitive feeding studies using broiler chickens; 90-day oral toxicity study in rats	N.sp.	EFSA-GMO-DE-2004-03; C/DE/02/9
	Maize MON863 x MON810 x NK603	Mouse acute gavage study	Feeding experiment in broiler chicken; 90-day oral toxicity study in rats	Known toxins	EFSA-GMO-BE-2004-07
	Maize MON863 x NK603	Mouse acute gavage study	90-day oral toxicity study in rats	Known toxins	EFSA-GMO-UK-2004-06
	Maize NK603	N.sp.	90-day oral toxicity study in rats	N.sp.	C/ES/00/01; EFSA-Q-2003-002
	Potato EH92-527-1	Oral gavage study in mice	90-day rat feeding study bovine study conducted with EH92-527-1 potato pulp	N.sp.	EFSA-GMO-UK-2005-14
	Potato EH92-527-1	N.a.	Digestibility study in sheep	N.i.	Spök et al. 2004 (Directive 90/220 Dossiers C/SE/96/3501)
	Rape Topas 19/2	N.i.	Feed conversion study in broiler chicken	N.i.	Spök et al. 2004 (Directive 90/220 Dossiers C/UK/95/M5/1); Spök et al. 2004 (Novel Food Dossiers)
PAT	Cotton 236/3006-210-23	Acute oral toxicity	42-day poultry feeding study	Known toxins	SNIF & Assessment Report (C/NL/04/01)
	Cotton 281-24-236/3006-210-23	Acute toxicity study	42-days poultry feeding study over, 42 day broiler chicken study	Known toxins	EFSA-GMO-NL-2005-16

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	Cotton DAS-21Ø23-5 (3006-210-23)	Acute oral toxicity	N.sp.	Known toxins	AGBIOS Database
	Cotton LL Cotton25	Intravenous administration in mice	Animal feeding study in male broiler chicken	Known toxins and antinutrients	EFSA-GMO-NL-2005-13
	Maize 1507	Toxicity study with mice and rats; Acute oral toxicity in mice	42-day poultry feeding study; 90-day oral toxicity study in rats	Known toxins	SNIF & Assessment Report (C/ES/01/01); SNIF & Assessment Report (C/NL/00/10); EFSA-GMO-NL-2004-02; C/NL/00/10; C/ES/01/01
	Maize 1507	Acute toxicity	Feed conversion study in chicken	N.i.	Spök et al. 2004 (Novel Food Dossiers)
	Maize 1507 x 59122	Toxicity study in rats; acute toxicity study in mice	42-day feeding study in broiler chicken; 90-day rat feeding study	Known toxins	EFSA-GMO-NL-2005-15
	Maize 1507 x NK603	Toxicity studies in mice and rats	42-days poultry feeding studies	Known toxins	EFSA-GMO-UK-2004-05; EFSA-GMO-UK-2005-17
	Maize 59122	N.sp.	42-day poultry feeding study; in vitro simulated digestibility studies; 90-day oral toxicity feeding study in rats	Known toxins	EFSA-GMO-NL-2005-12
	Maize 676, 678, and 680	Acute toxicity testing	N.sp.	Known toxins	AGBIOS Database
	Maize Bt11	14-day repeated dose in rats; Acute toxicity in mice	N.sp.	Known toxins	SNIF & Assessment Report (C/F/96.05.10); C/F/96/05.10
	Maize Bt11	Toxicity testing in various species; Acute toxicity	N.i.	N.i.	Spök et al. 2004 (Directive 90/220 Dossiers C/GB/96/M4/1; C/F/96/05-10); Spök et al. 2004 (Novel Food Dossiers)
	Maize DKB-89614-9 (DBT418)	Acute oral toxicity in mice	N.sp.	Known toxins	AGBIOS Database
	Maize T25	14-day toxicity	Feed conversion study in chicken	N.i.	Spök et al. 2004 (Novel Food Dossiers)
	Rape Ms8 x Rf3	14-day repeated dose oral toxicity study in rats; Acute toxicity study in mice	Feeding studies	Known toxins	SNIF & Assessment Report (C/BE/96/01); C/BE/96/01
	Rape Topas 19/2	N.i.	Feed conversion study in broiler chicken	N.i.	Spök et al. 2004 (Directive 90/220 Dossiers C/UK/95/M5/1); Spök et al. 2004 (Novel Food Dossiers)
	Rice LLRICE62	Toxicity study in mice; Intravenous administration in mice	42-day feeding study in male broiler chickens; 100-day growing/finishing pigs study; 96-day growing-finishing study in swine	Known toxins and antinutrients	SNIF & Assessment Report (C/GB/03/M5/3); EFSA-GMO-UK-2004-04

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	Sweet Maize Bt11	Acute toxicity	Feed conversion study in chicken	N.i.	Spök et al. 2004 (Novel Food Dossiers)
PMI	Maize MIR604	Acute oral toxicity in mice	49-day poultry feeding study 90-day whole food safety study in rodents	Known toxins	EFSA-GMO-UK-2005-11
Tn7 AAD 3' adenylyltransferase	Sugar beet H7-1 Roundup Ready®	N.sp.	90-day rat feeding study	N.sp.	EFSA-GMO-UK-2004-08
VIP3A	Cotton SYN-IR12-7 (COT102)	Acute oral feeding study	N.sp.	N.sp.	AGBIOS Database

No information on animal tests (but occasionally information on homology comparisons to known toxins was found in the documents for CMV CP (Yellow crookneck squash CZW-3); Cry1Ac (cotton 31807 and 31808; MON-15985-7; tomato 5345); Cry34Ab1 (maize DAS-59122-7); Cry35Ab1 (maize DAS-59122-7); GUS (papaya 55-1 and 63-1); nitrilase (cotton 31807 and 31808); nopaline synthase (flax CDC-FLØØ1-2 (FP967)) ; NptII (canola 23-198 and 23-18-17; cantaloupe A and B; cotton 31807 and 31808; MON15985 and MON15985 x MON1445; MON531 x MON1445 ; flax CDC-FLØØ1-2 (FP967) ; papaya 55-1 and 63-1 ; Russet Burbank cultivar RBMT15-101, Shepody potato cultivar SEMT15-02, Shepody potato cultivar SEMT15-15 ; tobacco vector 21-41; tomato 1345-4, 35 1 N DR, 5345, DR 8338; yellow crookneck squash CZW-3); PAT (maize ACS-ZMØØ4-3 (CBH-351), DAS-59122-7) ; PRSV CP (papaya 55-1 and 63-1) ; PVY CP (Russet Burbank cultivar RBMT15-101, Shepody potato cultivar SEMT15-02, Shepody potato cultivar SEMT15-15) ; QPTase (tobacco vector 21-41);S-adenosylmethionine hydrolase (cantaloupe A and B; tomato 35 1 N DR); thioesterase (canola 23-198 and 23-18-17); WMV2 CP (yellow crookneck squash CZW-3, ZW-20); ZYMV CP (yellow crookneck squash CZW-3, ZW-20).

N.a.: not applicable; n.i.: not investigated; n.sp. not specified in the documents (does not necessarily mean that this information is not included in the full text risk assessment dossier).

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Table 5: Other toxicity relevant information for GMPs authorised or pending for commercial application in the EU and the USA.

Protein	GMPs	History of safe use / consumption	No evidence of toxic effects	High specificity	Other	References
ACC	Tomato 1345-4	–	X	–	N.sp.	AGBIOS Database
Barstar	Rape Ms8 x Rf3	–	X	X	N.sp.	SNIF & Assessment Report (C/BE/96/01); C/BE/96/01
CMV CP	Yellow crookneck squash CZW-3	X	X	–	N.sp.	AGBIOS Database
CP4 EPSP-	Cotton MON531 x MON1445	X	–	–	N.sp.	EFSA-GMO-UK-2005-09
	Maize 1507 x NK603	–	X	–	N.sp.	EFSA-GMO-UK-2004-05; EFSA-GMO-UK-2005-17
	Maize MON863 x MON810 x NK603	X	–	–	N.sp.	EFSA-GMO-BE-2004-07
	Maize MON863 x NK603	X	X	–	N.sp.	EFSA-GMO-UK-2004-06
	Maize NK603	X	X	–	The EPSPS enzyme occurs in a wide range of plants, fungi and certain micro-organisms	SNIF (C/ES/03/01); C/ES/00/01; EFSA-Q-2003-002
	Maize NK603 x MON810	X	X	–	CP4 EPSPS is present at very low levels	SNIF (C/ES/04/01); SNIF & Assessment Report (C/GB/02/M3/3); EFSA-GMO-UK-2004-01
	Rape GT73	X	–	–	N.sp.	SNIF & Assessment Report (C/NL/98/11); C/NL/98/11
Cry1Ab	Maize Bt11	–	–	–	Bt11 has been grown in the USA since 1997 with no reports of adverse effects to human health and the environment	SNIF & Assessment Report (C/F/96.05.10); C/F/96/05.10
	Maize MON863 and MON863 x MON810	X	X	–	N.sp.	SNIF & Assessment Report (C/DE/02/9); C/DE/02/9; EFSA-Q-2003-121
	Maize MON863 x MON810	X	X	–	N.sp.	EFSA-GMO-DE-2004-03; C/DE/02/9
	Maize MON863 x MON810 x NK603	X	–	X	N.sp.	EFSA-GMO-BE-2004-07
	Maize NK603 x MON810	–	X	X	A history of safe use as MON810 maize varieties expressing Cry1A(b) have been approved, marketed and consumed since 1996 in several world areas including the E.U.	SNIF (C/ES/04/01); SNIF & Assessment Report (C/GB/02/M3/3); EFSA-GMO-UK-2004-01
Cry1Ac	Cotton 236/3006-210-23	–	X	–	N.sp.	SNIF & Assessment Report (C/NL/04/01)
	Cotton 281-24-236/3006-210-23	–	X	–	N.sp.	EFSA-GMO-NL-2005-16
	Cotton 31807 and 31808	–	X	X	N.sp.	AGBIOS Database
	Cotton DAS-21023-5 (3006-210-23)	X	X	X	N.sp.	AGBIOS Database
	Cotton MON-15985-7	–	–	X	N.sp.	AGBIOS Database
	Cotton MON531 x MON1445	X	–	–	N.sp.	EFSA-GMO-UK-2005-09
	Maize DKB-89614-9 (DBT418)	X	X	X	N.sp.	AGBIOS Database

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Protein	GMPs	History of safe use / consumption	No evidence of toxic effects	High specificity	Other	References
	Tomato 5345	–	–	–	N.sp.	AGBIOS Database
Cry1F	Cotton 236/3006-210-23	–	X	–	N.sp.	SNIF & Assessment Report (C/NL/04/01)
	Cotton 281-24-236/3006-210-23	–	X	–	N.sp.	EFSA-GMO-NL-2005-16
	Maize 1507	X	X	X	N.sp.	SNIF & Assessment Report (C/ES/01/01); SNIF & Assessment Report (C/NL/00/10); EFSA-GMO-NL-2004-02; C/NL/00/10; C/ES/01/01
	Maize 1507 x 59122	X	X	–	N.sp.	EFSA-GMO-NL-2005-15
	Maize 1507 x NK603	–	X	–	N.sp.	EFSA-GMO-UK-2004-05; EFSA-GMO-UK-2005-17
Cry2Ab	Cotton MON-15985-7	–	–	X	N.sp.	AGBIOS Database
Cry2Ab2	Cotton MON15985 and MON15985 x MON1445	–	X	–	N.sp.	EFSA-GMO-UK-2005-10
Cry34Ab1	Maize 1507 x 59122	X	X	–	N.sp.	EFSA-GMO-NL-2005-15
	Maize 59122	–	X	–	N.sp.	EFSA-GMO-NL-2005-12
Cry35Ab1	Maize 1507 x 59122	X	X	–	N.sp.	EFSA-GMO-NL-2005-15
	Maize 59122	–	X	–	N.sp.	EFSA-GMO-NL-2005-12
Cry3A	Russet Burbank cultivar RBMT15-101, Shepody potato cultivar SEMT15-02, Shepody potato cultivar SEMT15-15	–	–	X	N.sp.	AGBIOS Database
Cry3Bb1	Maize MON88017	X	X	X	N.sp.	AGBIOS Database
Cry3Bb1	Maize MON863 and MON863 x MON810	X	X	–	N.sp.	SNIF & Assessment Report (C/DE/02/9); C/DE/02/9; EFSA-Q-2003-121
	Maize MON863 x MON810	X	X	–	N.sp.	EFSA-GMO-DE-2004-03; C/DE/02/9
	Maize MON863 x MON810 x NK603	X	–	X	N.sp.	EFSA-GMO-BE-2004-07
	Maize MON863 x NK603	X	X	–	N.sp.	EFSA-GMO-UK-2004-06
Cry9C	Maize ACS-ZMØØ4-3 (CBH-351)	X	X	X	N.sp.	AGBIOS Database
GBSS	Potato EH92-527-1	X	–	–	N.sp.	EFSA-GMO-UK-2005-14
GOXv247	Rape GT73	X	–	–	N.sp.	SNIF & Assessment Report (C/NL/98/11)
GUS	Cotton MON15985 and MON15985 x MON1445	–	X	–	N.sp.	EFSA-GMO-UK-2005-10
mCry3A	Maize MIR604	–	X	–	N.sp.	EFSA-GMO-UK-2005-11
Nitrilase	Cotton 31807 and 31808	–	X	X	N.sp.	AGBIOS Database
NPTII	Potato EH92-527-1	X	–	–	N.sp.	EFSA-GMO-UK-2005-14
	Canola 23-198 and 23-18-17	–	X	–	Ubiquitous in nature	AGBIOS Database

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Protein	GMPs	History of safe use / consumption	No evidence of toxic effects	High specificity	Other	References
	Cotton 31807 and 31808	–	X	X	The safety of NPTII as a food additive was evaluated by the US FDA in 1994	AGBIOS Database
	Maize MON863 and MON863 x MON810	X	X	–	N.sp.	SNIF & Assessment Report (C/DE/02/9); C/DE/02/9; EFSA-Q-2003-121
	Maize MON863 x MON810	X	X	–	N.sp.	EFSA-GMO-DE-2004-03; C/DE/02/9
	Maize MON863 x MON810 x NK603	X	–	X	Ubiquitous in the environment and is found in microbes present in food and within the human digestive system	EFSA-GMO-BE-2004-07
	Maize MON863 x NK603	X	X	–	N.sp.	EFSA-GMO-UK-2004-06
	Russet Burbank cultivar RBMT15-101, Shepody potato cultivar SEMT15-02, Shepody potato cultivar SEMT15-15	–	–	X	N.sp.	AGBIOS Database
	Tomato 1345-4	–	X	–	Widespread in nature	AGBIOS Database
	Yellow crookneck squash CZW-3	X	X	–	N.sp.	AGBIOS Database
PAT	Cotton 236/3006-210-23	–	X	X	N.sp.	SNIF & Assessment Report (C/NL/04/01)
	Maize 1507	X	X	X	N.sp.	SNIF & Assessment Report (C/ES/01/01); SNIF & Assessment Report (C/NL/00/10); EFSA-GMO-NL-2004-02; C/NL/00/10; C/ES/01/01
	Maize 1507 x 59122	X	X	–	N.sp.	EFSA-GMO-NL-2005-15
	Maize 59122	–	X	–	N.sp.	EFSA-GMO-NL-2005-12
	Maize 676, 678, and 680	–	X	–	N.sp.	AGBIOS Database
	Maize DAS-59122-7	–	X	–	N.sp.	AGBIOS Database
	Rape Ms8 x Rf3	–	X	–	N.sp.	SNIF & Assessment Report (C/BE/96/01); C/BE/96/01
	Rice LLRICE62	–	X	X	N.sp.	SNIF & Assessment Report (C/GB/03/M5/3); EFSA-GMO-UK-2004-04
PMI	Maize MIR604	–	X	–	N.sp.	EFSA-GMO-UK-2005-11
PRSV CP	Papaya 55-1 and 63-1	X	X	–	Found in all PRSV-infected plants	AGBIOS Database
PVY CP	Russet Burbank cultivar RBMT15-101, Shepody potato cultivar SEMT15-02, Shepody potato cultivar SEMT15-15	X	–	X	N.sp.	AGBIOS Database
RNase (Barnase)	Rape Ms8 x Rf3	–	X	–	N.sp.	C/BE/96/01
Thioesterase	Canola 23-198 and 23-18-17	–	X	–	Ubiquitous in nature	AGBIOS Database
WMV2 CP	Yellow crookneck squash CZW-3	X	X	–	N.sp.	AGBIOS Database

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Protein	GMPs	History of safe use / consumption	No evidence of toxic effects	High specificity	Other	References
	Yellow crookneck squash ZW-20	–	X	–	N.sp.	AGBIOS Database
ZYMV CP	Yellow crookneck squash CZW-3	X	X	–	N.sp.	AGBIOS Database
	Yellow crookneck squash ZW-20	–	X	–	N.sp.	AGBIOS Database

No relevant information was found in the documents for DAM (maize 676, 678, and 680); DFR (carnation Florigene Moonlite (123.2.38)); F3'5'H (carnation Florigene Moonlite (123.2.38)); GUS (cotton MON-15985-7; papaya 55-1 and 63-1); mEPSPS (maize GA21); Nitrilase (CanolaOxy-235 (ACS-BNØ11-5)); nopaline Synthase (flax CDC-FLØØ1-2 (FP967)); NptII (cantaloupe A and B ;; cotton MON15985 and MON15985 x MON1445 , MON531 x MON1445 ; flax CDC-FLØØ1-2 (FP967) ; maize NK603; papaya 55-1 and 63-1; tobacco vector 21-41, tomato 35 1 N DR; 5345; DR 8338); PAT (cotton 281-24-236/3006-210-23; DAS-21Ø23-5 (3006-210-23); LLCotton25, maize 1507 x NK603, ACS-ZMØØ4-3 (CBH-351), Bt11, DKB-89614-9 (DBT418)); QPTase (tobacco Vector 21-41); S-adenosylmethionine hydrolase (cantaloupe A and B, tomato 35 1 N DR); Tn7 AAD 3' adenytransferase (sugar beet H7-1 Roundup Ready); VIP3A (cotton SYN-IR12-7 (COT102)).

N.a.: not applicable; n.i.: not investigated; n.sp. not specified in the documents (does not necessarily mean that this information is not included in the full text risk assessment dossier).

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Table 6: Exposure relevant information on novel proteins in GMPs authorised or pending for commercial application in the EU and the USA.

Protein	GMPs	Digestibility	Stability	Concentration in Plant	Concentration food/feed	Reference
APH4 (hygromycin-B phosphotransferase)	Cotton SYN-IR12-7 (COT102)	N.sp.	N.sp.	LOQ (150 ng/g dw) in leaves, squares, roots, bolls, seeds and nectar, 2,3 µg/g in pollen	N.sp.	AGBIOS Database
Barstar	Rape Ms8 x Rf3	N.sp.	N.sp.	Very low amounts in seed (approx. 0,1 µg/g seed)	Not present in oil extracts	SNIF & Assessment Report (C/BE/96/01)
	Rape Ms8 x Rf3	N.sp.	N.sp.	Not Detected	Not present in oil extracts	SNIF & Assessment Report (C/BE/96/01); C/BE/96/01
CMV CP	Yellow crookneck squash CZW-3	N.sp.	N.sp.	5,9-10,8 ng/g fruit tissue	N.sp.	AGBIOS Database
CP4 EPSPS	Cotton MON15985 and MON15985 x MON1445	Digestion in SGF and SIF	N.sp.	Not detected in seed samples	MON15985: not detected in cottonseed samples MON1445: 110±6,8 µg/g fw in cottonseed samples MON 15985 x MON1445: 160±28 µg/g fw in cottonseed samples	EFSA-GMO-UK-2005-10
	Cotton MON531 x MON1445	Digestion in SGF and SIF	N.sp.	MON531: < 22,3 µg/g fw in leaf and seed tissues MON1445: 255–299 µg/g fw in leaf and seed tissues MON531 x MON1445: 286–310 µg/g fw in leaf and seed tissues fw	No detectable level of protein in refined cottonseed oil	EFSA-GMO-UK-2005-09
	Maize 1507 x NK603	Rapid degradation in SGF	Heating will lead to the rapid denaturation and degradation	N.sp.	N.sp.	EFSA-GMO-UK-2004-05; EFSA-GMO-UK-2005-17
	Maize MON88017	Rapid and complete digestion in SGF	N.sp.	150-220 µg/g dw in young leaf, 390 µg/g dw in pollen, 57 µg/g dw in forage, 70-150 µg/g dw in forage root, and 5,8 µg/g dw in grain	N.sp.	AGBIOS Database
	Maize MON863 x MON810 x NK603	Rapid digestion in in vitro SGF	N.sp.	MON863 x MON810 x NK603: 11±2,8 µg/g dw in kernels NK603: 12±1,8 µg/g dw in kernels MON863 x MON810 x NK603: 11 µg/g dw in grain and 95 µg/g dw in forage	N.sp.	EFSA-GMO-BE-2004-07
	Maize MON863 x NK603	Rapid digestion in in vitro	N.sp.	MON863 x NK603: 11±1,5	N.sp.	EFSA-GMO-UK-2004-06

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Protein	GMPs	Digestibility	Stability	Concentration in Plant	Concentration food/feed	Reference
		SGF		µg/g dw in kernels NK603: 12±1,8 µg/g dw µg/g dw in kernels Maize MON863 x NK603: 12 µg/g dw in grain, 110 µg/g dw in forage		
	Maize NK603	Rapid degradation and loss of enzymatic activity in SGF and SIF	N.sp.	Mean levels in forage: 25,5 µg/g fw for the non-replicated site and 25,9 µg/g fw for the replicated sites. Mean levels in grain: 11,0 µg/g fw for the non-replicated sites and 10,6 µg/g fw for the replicated sites.	N.sp.	SNIF (C/ES/03/01)
	Maize NK603	More than 95-98% of the protein was digested within 15 sec in the SGF. Within four hours more than 90% of the protein was digested in SIF	N.sp.	N.sp.	N.sp.	C/ES/00/01; EFSA-Q-2003-002
	Maize NK603 x MON810	Rapid degradation and loss of enzymatic activity in simulated human gastric and IF	N.sp.	Mean levels of 36,3 µg/g fw in forage and 12,7 µg/g fw in grain	N.sp.	SNIF (C/ES/04/01); SNIF & Assessment Report (C/GB/02/M3/3); EFSA-GMO-UK-2004-01
	Rape GT73	Rapid degradation under conditions which simulate mammalian digestive systems (< 15 sec) and SIF (< 10 min)	Heat and pH labile	Mean expression levels of 0,02-0,05 in seeds and 0,03 µg/g fw in leaves	N.sp.	SNIF & Assessment Report (C/NL/98/11); C/NL/98/11
	Rape GT73	In vitro study in an simulated intestine system	N.i.	Summary of study on seeds	Investigated for oil	Spök et al. 2004 (Novel Food Dossiers)
	Sugar beet H7-1 Roundup Ready	Rapid degradation under conditions which simulate mammalian digestive fluids	N.sp.	0,161-0,172 µg/mg fw in the top tissues; 0,053-0,81 µg/mg fw in the brei	N.sp.	EFSA-GMO-UK-2004-08
Cry1Ab	Maize Bt11	No complete degradation in the gastrointestinal tract	N.sp.	Maximum concentration of Cry1A(b) is found in leaves, husk and stalk (20-168 ng protein/mg total protein) and 0,4-8,2 ng protein/mg total protein in kernels. The protein is detectable in all parts of the plant. Concentrations in leaves and grain are 1,4 and 3,26	N.sp.	SNIF & Assessment Report (C/F/96.05.10); C/F/96/05.10

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Protein	GMPs	Digestibility	Stability	Concentration in Plant	Concentration food/feed	Reference
				µg /g fw		
	Maize MON863 and MON863 x MON810	Rapid degradations under conditions, which simulate mammalian digestive systems	N.sp.	0,84 µg/g fw	N.sp.	SNIF & Assessment Report (C/DE/02/9); C/DE/02/9; EFSA-Q-2003-121
	Maize MON863 x MON810	Rapid degradation under conditions which simulate mammalian digestive systems	Pepsin stability on the protein in an in vitro digestion	17,9 µg/g fw in young leaf, 7,9 µg/g fw in forage, 0,84 µg/g fw in grain, <0,08 µg/g fw in pollen	N.sp.	EFSA-GMO-DE-2004-03; C/DE/02/9
	Maize MON863 x MON810 x NK603	Rapidly degradation under conditions which simulate mammalian digestion	N.sp.	MON863 x MON810 x NK603: 0,65±0,08 µg/g dw in kernels MON810: 0,77±0,09 µg/g dw in kernels Maize MON863 x MON810 x NK603: 0,65 µg/g dw in grain and 12 µg/g dw in forage	N.sp.	EFSA-GMO-BE-2004-07
	Maize NK603 x MON810	Rapid degradation in simulated human gastric fluids	N.cp.	Mean levels of 6,06 µg/g fw in the forage and 0,73 µg/g fw in grain	N.sp.	SNIF (C/ES/04/01); SNIF & Assessment Report (C/GB/02/M3/3); EFSA-GMO-UK-2004-01
Cry1Ac	Cotton 236/3006-210-23	N.sp.	Loss of Immunoreactivity by heating	Average concentrations range from not detectable up to 1,83 ng/mg. The level on pollen was 1,45 ng/mg and not detectable in nectar	N.sp.	SNIF & Assessment Report (C/NL/04/01)
	Cotton 281-24-236/3006-210-23	Rapid degradation in SGF	Thermolabile	N.sp.	N.sp.	EFSA-GMO-NL-2005-16
	Cotton 31807 and 31808	N.sp.	N.sp.	2,5 ppm fw in seed, 2,25 ppm in meal	N.sp.	AGBIOS Database
	Cotton DAS-21023-5 (3006-210-23)	Rapid digestion (within 1 minute) in SGF	Inactivation after heating at 75°C	1,44–1,92 ng/mg dw in leaves, 1,84 ng/mg dw in squares, 1,92 ng/mg dw in flowers, 1,44 ng/mg dw in pollen, 0,77 ng/mg dw in bolls, 0,57 ng/mg dw in seeds	0,62 ng/mg fw in cottonseed, < LOD in toasted meal, hulls, or refined cottonseed	AGBIOS Database
	Cotton MON15985 and MON15985 x MON1445	Digestion in SGF and SIF	N.sp.	MON15985: 3,35±0,63 µg/g fw in seed samples MON531: 3,22±0,77 µg/g fw in seed samples	MON15985: 1,6±0,23 µg/g fw in cottonseed samples MON1445: not detected in cottonseed samples MON 15985 x MON1445: 1,5±0,095 µg/g fw in	EFSA-GMO-UK-2005-10

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Protein	GMPs	Digestibility	Stability	Concentration in Plant	Concentration food / feed	Reference
					cottonseed samples	
	Cotton MON-15985-7	Rapid digestion in SGF	N.sp.	N.sp.	Cottonseed oil is highly purified, and contains no detectable proteins	AGBIOS Database
	Cotton MON531 x MON1445	Digestion in SGF and SIF	N.sp.	MON531: 2,45–3,69 µg/g fw in eaf and seed tissue MON1445: < 0,50 µg/g fw in leaf and seed tissue MON531 x MON1445: 2,62–3,52 µg/g fw in leaf and seed tissue	No detectable level of protein in refined cottonseed oil	EFSA-GMO-UK-2005-09
	Maize DKB-89614-9 (DBT418)	N.sp.	N.sp.	459,6-1.194,4 ng/g tissue dw in harvested leaves; 36,0-42,8 ng/g tissue dw in harvested kernels; 58,0-125,4 ng/ g tissue dw in harvested stalks and 40,9-123,6 ng/g tissue dw in harvested roots	N.sp.	AGBIOS Database
	Tomato 5345	N.sp.	N.sp.	N.sp.	N.sp.	AGBIOS Database
Cry1F	Cotton 236/3006-210-23	N.sp.	Loss of Immunoreactivity by heating	The level on pollen was 0,06 ng/mg and below the limit of detection in nectar	N.sp.	SNIF & Assessment Report (C/NL/04/01)
	Cotton 281-24-236/3006-210-23	Rapid degradation in SGF	Thermolabile	N.sp.	N.sp.	EFSA-GMO-NL-2005-16
	Maize 1507	Degradation in simulated digestive fluids. In the intestinal fluid (pancreatin), Cry1F proved stable over the entire exposure of 12 minutes	When heated at 75-90°C Cry1F lost its insecticidal activity	20-29,3 ng/mg tissue dw in pollen, 1,0-6,9 ng/mg tissue dw in whole plant extract, 1,2-3,1 ng/mg tissue dw in kernels	Not detectable in the final product	SNIF & Assessment Report (C/ES/01/01), SNIF & Assessment Report (C/NL/00/10); C/ES/01/01; EFSA-GMO-NL-2004-02
	Maize 1507 x 59122	N.sp.	N.sp.	N.sp.	N.sp.	EFSA-GMO-NL-2005-15
	Maize 1507 x NK603	Rapid degradation in SGF	Heating will lead to the rapid denaturation and degradation	N.sp.	N.sp.	EFSA-GMO-UK-2004-05; EFSA-GMO-UK-2005-17
Cry2Ab	Cotton MON-15985-7	N.sp.	N.sp.	Levels in cottonseed, leaf tissue, and whole plants across all locations were 43,2±5,7 µg/g, 23,8±6,3 µg/g, 8,80±1,20 µg/g and < 0,25 µg/g	Cottonseed oil is highly purified, and contains no detectable proteins	AGBIOS Database
Cry2Ab2	Cotton MON15985 and MON15985 x MON1445	Digestion in SGF and SIF	N.sp.	MON15985: 43,2±5,7 µg/g fw in seed samples	MON15985: 44±10 µg/g fw in cottonseed samples	EFSA-GMO-UK-2005-10

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Protein	GMPs	Digestibility	Stability	Concentration in Plant	Concentration food/feed	Reference
				MON531. < LOD (=2,31) µg/g fw in seed samples	MON1445: not detected in cottonseed samples MON 15985 x MON1445: 45±5,7 µg/g fw in cottonseed samples	
Cry34Ab1	Maize 59122	Rapid degradation SGF and highly susceptible to proteolytic digestion	Rapid degradation when heated	N.sp.	N.sp.	EFSA-GMO-NL-2005-12
Cry35Ab1	Maize 59122	Rapid degradation SGF and highly susceptible to proteolytic digestion	Rapid degradation when heated	N.sp.	N.sp.	EFSA-GMO-NL-2005-12
Cry3A	Russet Burbank cultivar RBMT15-101, Shepody potato cultivar SEMT15-02, Shepody potato cultivar SEMT15-15	N.sp.	N.sp.	20-63 µg/g fw leaf tissue, 0,08-0,38 µg/g in tuber tissue	N.sp.	AGBIOS Database
Cry3Bb1	Maize MON88017	Rapid digestion in SGF	N.sp.	260-570 µg/g dw in young leaf, 25 µg/g dw in pollen, 380 µg/g dw in silk, 95 µg/g dw in forage, 130 µg/g dw in forage root, 100-370 µg/g dw in root tissues, 15 µg/g dw in grain, 88 µg/g dw in stover and 220-500 µg/g dw in the whole plant	N.sp.	AGBIOS Database
	Maize MON863 and MON863 x MON810	Rapid degradations under conditions, which simulate mammalian digestive systems	N.sp.	42-70 µg/g fw in Mon863 and 1,1 µg/g fw in MON863 x MON810	N.sp.	SNIF & Assessment Report (C/DE/02/9); C/DE/02/9<, EFSA-Q-2003-121
	Maize MON863 x MON810	Rapid degradation under conditions which simulate mammalian digestive systems	Pepsin stability on the protein in an in vitro digestion	46,7 µg/g fw in young leaf, 23,6 µg/g fw in forage, 61,1 µg/g fw in grain, 79,6 µg/g fw in pollen, 17,7 µg/g fw in mature root, 22,0 µg/g fw in over-season root	N.sp.	EFSA-GMO-DE-2004-03; C/DE/02/9
	Maize MON863 x MON810 x NK603	Rapid digestion in in vitro SGF	N.sp.	MON863 x MON810 x NK603: 37±11 µg/g dw in kernels MON863: 29±3,9 µg/g dw in kernels Maize MON863 x MON810 x NK603: 37 µg/g dw in grain and 45 µg/g dw in forage	N.sp.	EFSA-GMO-BE-2004-07
	Maize MON863 x NK603	Rapid digestion in in vitro SGF	N.sp.	MON863 x NK603: 34±5,4 µg/g dw in kernels	N.sp.	EFSA-GMO-UK-2004-06

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Protein	GMPs	Digestibility	Stability	Concentration in Plant	Concentration food/feed	Reference
				MON863: 29±3,9 µg/g dw in kernels Maize MON863 x NK603: 34 µg/g dw in grain, 40 µg/g dw in forage		
Cry9C	Maize ACS-ZMØØ4-3 (CBH-351)	Rapid digestion in SGF	N.sp.	N.sp.	N.sp.	AGBIOS Database
GOX	Maize MON809	+	N.i.	Different part of the plant including maize kernels	N.i.	Spök et al. 2004 (Novel Food Dossiers)
GOXv247	Rape GT73	Rapid degradation under conditions which simulate mammalian digestive systems	Heat and pH labile	Mean expression levels of 0,15-0,19 in seeds and 0,11 µg/ g fw in leaves	N.sp.	SNIF & Assessment Report (C/NL/98/11)
	Rape GT73	N.i.	N.i.	Summary of study on seeds	Investigated for oil	Spök et al. 2004 (Novel Food Dossiers)
GUS	Cotton MON15985 and MON15985 x MON1445	Digestion in SGF and SIF	N.sp.	MON15985: 58,8±13,0 µg/g fw in seed samples MON531: < LOD (=4,56) µg/g fw in seed samples	MON15985: 46±13 µg/g fw in cottonseed samples MON1445: not detected in cottonseed samples MON 15985 x MON1445: 45±16 µg/g fw in cottonseed samples	EFSA-GMO-UK-2005-10
	Cotton MON-15985-7	Degradation to non-detectable levels in 15 seconds in SGF and lost 91% of its enzymatic activity after 2 hours digestion in SIF	N.sp.	Concentrations in cottonseed and leaf tissue were 106±32 µg/g, and 58,8±13,0 µg/g	Cottonseed oil is highly purified, and contains no detectable proteins ⁹³	AGBIOS Database
mCry3A	Maize MIR604	Rapidly degraded in SGF	Incubation for 30 minutes at 65°C against WCRW. At 95°C the protein was completely inactivated	Quantifiable levels were detected in all plant tissues except pollen	N.sp.	EFSA-GMO-UK-2005-11
mEPSPS	Maize GA21	Rapid degradation in SGF	Sensitive to heat	< LOQ-15 µg/g fw (< 0,4–71 µg/g dw) in leaves, roots and whole plants; 4-7 µg/g fw (5-10 µg/g dw) in kernels and 168 µg/g fw in pollen	N.sp.	EFSA-GMO-UK-2005-19

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Protein	GMPs	Digestibility	Stability	Concentration in Plant	Concentration food/feed	Reference
Nitrilase	Canola Oxy-235 (ACS-BNØ11-5)	Rapid inactivation in mammalian stomach and intestinal fluids by enzymatic degradation and pH-mediated proteolysis	N.sp.	1.000 ng/mg total protein in leaf, <10 ng/mg total protein in seed	N.sp.	AGBIOS Database
	Cotton 31807 and 31808	N.sp.	N.sp.	0,25 ppm fw in seed, 1,4 ppm in meal	N.sp.	AGBIOS Database
Nopaline synthase	Flax CDC-FLØØ1-2 (FP967)	N.sp.	N.sp.	In roots 0,05-0,63 mg/g fw at the seedling stage, and 0,07-0,47 mg/g fw at pre-flowering In stems 0,01-0,33 mg/g fw at the seedling stage, and 0,02-0,12 mg/g fw at pre-flowering In leaves 0,05-2,4 mg/g fw at the seedling stage, and 0,23-0,72 mg/g fw at pre-flowering	N.sp.	AGBIOS Database
NPTII	Potato EH92-527-1	Rapid degradation in rumens	Detectable in pulp, raw tuber and leaves but not in starch samples. Tubers contained approx. 31 ppb or ng/g fw (6,82 ng/mg protein)	N.sp.	N.sp.	EFSA-GMO-UK-2005-14
	Canola 23-198 and 23-18-17	NPTII enzyme was found to degrade rapidly in simulated mammalian gastric and intestinal fluids	N.sp.	N.sp.	N.sp.	AGBIOS Database
	Cotton 31807 and 31808	N.sp.	N.sp.	2,5 ppm fw in seed, 13,5 ppm in meal	N.sp.	AGBIOS Database
	Cotton MON15985 and MON15985 x MON1445	Digestion in SGF and SIF	N.sp.	MON15985: 10,8±1,2 µg/g fw) in seed samples MON531: 9,92±2,19 µg/g fw) in seed samples	MON15985: 5,5±0,59 µg/g fw in cottonseed samples MON1445: 16±2,0 µg/g fw in cottonseed samples MON 15985 x MON1445: 17±2,6 µg/g fw in cottonseed samples	EFSA-GMO-UK-2005-10
	Cotton MON531 x MON1445	Digestion in SGF and SIF	N.sp.	MON531: 10,9–13,2 µg/g fw in leaf and seed tissue MON1445: 29,0–35,1 µg/g fw in leaf and seed tissue MON531 x MON1445: 31,0–51,8 µg/g fw in leaf and	No detectable level of protein in refined cottonseed oil	EFSA-GMO-UK-2005-09

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Protein	GMPs	Digestibility	Stability	Concentration in Plant	Concentration food/feed	Reference
				seed tissue		
	Maize MON809	+	N.i.	Different part of the plant including maize kernels	N.i.	Spök et al. 2004 (Novel Food Dossiers)
	Maize MON863 and MON863 x MON810	Rapid degradations under conditions, which simulate mammalian digestive systems	N.sp.	< LOD–61 µg/g fw	N.sp.	SNIF & Assessment Report (C/DE/02/9); C/DE/02/9; EFSA-Q-2003-121
	Maize MON863 x MON810	Rapid degradation under conditions which simulate mammalian digestive systems	Pepsin stability on the protein in an in vitro digestion	1,6 µg/g fw in young leaf, 0,19 µg/g fw in forage, <0,076 µg/g fw in grain	N.sp.	EFSA-GMO-DE-2004-03; C/DE/02/9
	Maize MON863 x MON810 x NK603	Rapid digestion in in vitro SGF	N.sp.	< LOD (0,21 µg/g fw) in grain	N.sp.	EFSA-GMO-BE-2004-07
	Maize MON863 x NK603	Rapid digestion in in vitro SGF	N.sp.	< LOD (0,21 µg/g fw) in grain	N.sp.	EFSA-GMO-UK-2004-06
	Rape Topas 19/2	Study in gastric liquids from cattle and pigs	N.i.	Study on posttranslational processing in plant	Report on NPTII in processed seeds; report on NPTII in processed rape (oil and flour)	Spök et al. 2004 (Directive 90/220 Dossiers C/UK/95/M5/1); Spök et al. 2004 (Novel Food Dossiers)
	Russet Burbank cultivar RBMT15-101, Shepody potato cultivar SEMT15-02, Shepody potato cultivar SEMT15-15	N.sp.	N.sp.	0,003-0,01 µg/g fw (equivalent to < 0.001% of the total tuber protein)	N.sp.	AGBIOS Database
	Tomato 1345-4	N.sp.	N.sp.	0,72-2,36 µg/mg fw	N.sp.	AGBIOS Database
	Yellow crookneck squash CZW-3	N.sp.	N.sp.	44 µg/g fruit tissue	N.sp.	AGBIOS Database
PAT	Cotton 236/3006-210-23	Rapidly degradation upon exposure to mixtures of digestive tract enzymes	Heat and pH labile	Concentrations range from not detectable up to 0,54 ng/mg. The level on pollen was 0,05 ng/mg while in nectar it was undetectable	N.sp.	SNIF & Assessment Report (C/NL/04/01)
	Cotton 281-24-236/3006-210-23	Rapid degradation in SGF	Thermolabile	N.sp.	N.sp.	EFSA-GMO-NL-2005-16
	Cotton DAS-21Ø23-5 (3006-210-23)	Rapid digestion (within 1 minute) in SGF	N.sp.	< LOQ in seeds (0,06 ng/mg) and cottonseed oil (0.09 ng/mg)	< LOQ in cottonseed oil (0.09 ng/mg)	AGBIOS Database
	Cotton LLCotton25	Rapid degradation and denaturation in gastric and intestinal fluids of domestic animals and humans	Heating degrades the protein but not the DNA	8 µg/g fw in roots, 37 µg/g fw in stems, 70 µg/g fw in seed and 53-130 µg/g fw in leaves	N.sp.	EFSA-GMO-NL-2005-13
	Maize 1507	Rapid degradation (within 5 sec) upon exposure to	Heating will lead to the rapid degradation and	<LOD-136,8 pg/µg TEP in leaves, <LOD-38,0 pg/µg	N.sp.	SNIF & Assessment Report (C/ES/01/01); SNIF &

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Protein	GMPs	Digestibility	Stability	Concentration in Plant	Concentration food/feed	Reference
		mixtures of digestive tract enzymes	denaturation	TEP in whole plant extracts, <LOD in kernels. Mean values of 42 µg/µg TEP for leaves, <LOD in whole plants, <LOD for kernels		Assessment Report; EFSA-GMO-NL-2004-02; C/NL/00/10; C/ES/01/01
	Maize 1507 x NK603	Rapid degradation in SGF	Heating will lead to the rapid denaturation and degradation	< LOQ in grain (0,075 ng/mg dw)	N.sp.	EFSA-GMO-UK-2004-05; EFSA-GMO-UK-2005-17
	Maize 59122	Rapid degradation SGF and highly susceptible to proteolytic digestion	Rapid degradation when heated	N.sp.	N.sp.	EFSA-GMO-NL-2005-12
	Maize 676, 678, and 680	N.sp.	N.sp.	Line 676: 204-278 µg/g total protein in leaf tissue, < 19,2 µg/g total protein in grain Line 678: 601-717 µg/g total protein in leaf tissue, < 14,8 µg/g total protein Line 680: < LOD (20 µg/g total protein) in grain and leaf tissue	N.sp.	AGBIOS Database
	Maize Bt11	N.sp.	Not stable under processing conditions	Detectable levels of PAT were found in leaf, tassel and silk extracts. No PAT was found in root, pollen and kernel 0,000008% fresh weight and 0,00016% of total maize grain protein	N.sp.	SNIF & Assessment Report (C/F/96.05.10); C/F/96/05.10
	Maize Bt11	+	N.i.	Different part of the plant including maize kernels	N.i.	Spök et al. 2004 (Directive 90/220 Dossiers C/GB/96/M4/1; C/F/96/05-10); Spök et al. 2004 (Novel Food Dossiers)
	Maize DKB-89614-9 (DBT418)	N.sp.	N.sp.	501,8-1.099,4 µg/g tissue dw in leaves, 66-77 µg/g tissue dw in stalks, 27,5-69,5 µg/g tissue dw in roots, 3,1-6,0 µg/g tissue dw in kernels	N.sp.	AGBIOS Database
	Maize T25	+	N.i.	Study of PAT protein in different parts of the plant and products (starch, oil flour, semolina)	Study of PAT protein in different parts of the plant and products (starch, oil flour, semolina)	Spök et al. 2004 (Novel Food Dossiers)
	Rape Ms8 x Rf3	Digestion within 30 seconds	N.sp.	0,001% of total extractable	Not present in oil extracts	SNIF & Assessment Report

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Protein	GMPs	Digestibility	Stability	Concentration in Plant	Concentration food/feed	Reference
		in SGF. In SIF digestion into smaller fragments within seconds, but the complete degradation of a 7kDa fragment was archived within 5 minutes of incubation		protein in Ms8 x Rf3		(C/BE/96/01); C/BE/96/01
	Rape Topas 19/2	Study in gastric liquids from cattle and pigs	N.i.	Study on posttranslation processing in plant	Report on activity of PAT in processed/unprocessed seeds; report on PAT in processed rape (oil and flour)	Spök et al. 2004 (Directive 90/220 Dossiers C/UK/95/M5/1); Spök et al. 2004 (Novel Food Dossiers)
	Rice LLRICE62	Rapid degradation and denaturation in gastric and SIF of domestic animals and humans	N.sp.	12 µg/g fw in roots = 0,23 % of total crude protein, 30 µg/g fw in stems = 0,19 % of total crude protein, 90 µg/g fw in leaves = 0,13 % of total crude protein Average concentrations of 75,3 mg/kg fw in straw, 12,1 mg/g fw in grain, 1,6 mg/kg fw in hulls and 42 mg/kg fw in bran	N.sp.	SNIF & Assessment Report (C/GB/03/M5/3); EFSA-GMO-UK-2004-04
	Sweet Maize Bt11	Digestibility study on an equivalent PAT protein derived from bar gene	N.i.	+	N.i.	Spök et al. 2004 (Novel Food Dossiers)
PMI	Maize MIR604	Rapidly degraded in SGF	Incubation for 30 minutes at 65°C inactivated the protein	< LOD-0,4 µg/g fw	N.sp.	EFSA-GMO-UK-2005-11
PVY CP	Russet Burbank cultivar RBMT15-101, Shepody potato cultivar SEMT15-02, Shepody potato cultivar SEMT15-15	N.sp.	N.sp.	< LOD (2 ng/mg fresh leaf tissue)	N.sp.	AGBIOS Database
Thioesterase	Canola 23-198 and 23-18-17	Full degradation into inactive peptides and amino acids following protease digestion	Not heat stable	< 0.0008% of total protein in seeds and leaves	N.sp.	AGBIOS Database
VIP3A	Cotton SYN-IR12-7 (COT102)	Rapid digestion (within 2 minutes) in a mammalian gut environment	N.sp.	12,11µg/g at the 4-leaf stage, 5-5,28 µg/g at first bloom and first open boll, 1,75 µg/g at pre-harvest In leaves: 15,31 µg/g at 4-leaf stage, 17,55 µg/g at squaring, 9,73 µg/g at first bloom, 5,29 µg/g at peak	N.sp.	AGBIOS Database

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Protein	GMPs	Digestibility	Stability	Concentration in Plant	Concentration food/feed	Reference
				bloom, 8,82 µg/g at first open boll, 4.73 µg/g at pre-harvest. Maximum of 136 µg/g was detected in leaves at squaring In roots: 1,39 µg/g at 4-leaf stage, 1,5 µg/g at peak bloom, 1,18 µg/g at pre-harvest In bolls: 1,0 µg/g at peak bloom, < LOQ (< 0,45 µg/g) at pre-harvest) 2-4 µg/g in squares, 3 µg/g in seeds, and 1,1 µg/g in pollen		
WMV2 CP	Yellow crookneck squash CZW-3	N.sp.	N.sp.	5,9-10,8 ng/g fruit tissue	N.sp.	AGBIOS Database
	Yellow crookneck squash ZW-20	N.sp.	N.sp.	47-112 ng/g fw	N.sp.	AGBIOS Database
ZYMV CP	Yellow crookneck squash CZW-3	N.sp.	N.sp.	5,9-10,8 ng/g fruit tissue	N.sp.	AGBIOS Database
	Yellow crookneck squash ZW-20	N.sp.	N.sp.	73–1.218 ng/g fw	N.sp.	AGBIOS Database

No exposure relevant information could be obtained in case of : Cantaloupe A and B, Carnation Florigene Moonlite (123.2.38), Flax CDC-FLØØ1-2 (FP967), Maize 676, 678, and 680, Maize ACS-ZMØØ4-3 (CBH-351), Maize DAS-59122-7, Maize NK603, Papaya 55-1 and 63-1, Potato EH92-527-1, Rape GT73, Rape Ms8 x Rf3, Sugar beet H7-1 Roundup Ready, Tobacco Vector 21-41, Tomato 35 1 N DR, Tomato 5345, Tomato DR 8338.

N.a.: not applicable; n.i.: not investigated; n.sp. not specified in the documents (does not necessarily mean that this information is not included in the full text risk assessment dossier).

Table 7: Opinions of the SCF on the use and safety of enzyme preparations.

Enzyme	Application	EU Regulatory Context	Opinion of the Scientific Committee
Enzyme preparation based on thrombin:fibrinogen from cattle and/or pigs*	Food additive: reconstituting food	Directive 95/2/EC	AFC: 25 April 2005
Urease from <i>Lactobacillus fermentum</i> *	Wine production	Regulation 822/87 amended by Regulation 536/97, Art. 4 (c); presently Regulation 1622/2000	SCF: 10 December 1998
Papain from papaya fruit (additional information)	Meat tenderising agent	Not made explicit in the opinion a, b	SCF: 20 September 1997
Papain from papaya fruit			SCF: 2 June 1995
Invertase from <i>Saccharomyces cerevisiae</i>	Food additive	Presently Directive 95/2/EC	SCF: 23 September 1994
Chymosin A from <i>E.coli</i> , chymosin B from <i>Kluyveromyces lactis</i> , chymosin B from <i>Aspergillus niger</i> *	Cheese	Not made explicit in the opinion a	SCF: 25 June and 10 December 1993
Lactoperoxidase and glucose oxidase (activated lactoperoxidase system)	Food additive: anti-microbial effects on milk	Presently Directive 95/2/EC	SCF: 19 June 1992

^{a)} Not made explicit whether these enzymes are considered additives or processing aids.

^{b)} Injected into animals before slaughtering.

^{*}) Granted a positive opinion; N. r. not revealed

Table 8: Enzymes authorised as food additives under Directive 95/2/EC.

Enzyme	E No.	Application	References
Invertase	E1103	N.sp.	Annex 1 „Food additives generally permitted for use in foodstuff not referred to in article 2 (3)“
Lysozyme	E1105	Ripened cheese	Annex 3 „Conditionally permitted preservatives and antioxidants“, part C „Other preservatives“

N.sp. ... not specified.

Table 9: Enzymes evaluated by JECFA for use in food

	Substance Name	Production organism	Donor organism
1.	alpha-Acetolactate Decarboxylase	<i>Bacillus brevis</i>	<i>Bacillus subtilis</i>
2.	alpha-Amylase and Glucoamylase .	<i>Aspergillus oryzae</i> var.	N.a.
3.	alpha-Amylase	<i>Aspergillus oryzae</i> var.	N.a.
4.	alpha-Amylase	<i>Bacillus megaterium</i>	<i>Bacillus subtilis</i>
5.	alpha-Amylase	<i>Bacillus stearothermophilus</i>	N.a.
6.	alpha-Amylase	<i>Bacillus stearothermophilus</i>	<i>Bacillus subtilis</i>
7.	alpha-Amylase	<i>Bacillus subtilis</i>	N.a.
8.	Amyloglucosidase	<i>Aspergillus niger</i> var.	N.a.
9.	Avian Pepsin	N.sp.	N.a.
10.	beta-Glucanase	<i>Aspergillus niger</i> var.	N.a.
11.	beta-Glucanase	<i>Trichoderma harzianum</i>	N.a.
12.	Bromelain	N.sp.	N.a.
13.	Carbohydase	<i>Aspergillus awamori</i> , var.	N.a.
14.	Carbohydase	<i>Aspergillus niger</i> , var.	N.a.
15.	Carbohydase	<i>Bacillus licheniformis</i>	N.a.
16.	Carbohydase	<i>Rhizopus oryzae</i> , var.	N.a.
17.	Carbohydase	<i>Saccharomyces</i> sp.	N.a.
18.	Catalase	<i>Bovine Liver</i>	N.a.
19.	Catalase	<i>Micrococcus lysodeicticus</i>	N.a.
20.	Cellulase	<i>Penicillium funiculosum</i>	N.a.
21.	Cellulase	<i>Trichoderma longibrachiatum</i>	N.a.
22.	Chymosin A	<i>Escherichia coli</i> K-12 containing the prochymosin A gene	N.sp.
23.	Chymosin B	<i>Aspergillus niger</i> var. <i>awamori</i> containing the prochymosin B gene	N.sp.
24.	Chymosin B	<i>Kluyveromyces lactis</i> containing the prochymosin B gene	N.sp.
25.	Ficin	N.sp.	N.a.
26.	Glucose Isomerase	<i>Actinoplanes missouriensis</i>	N.a.
27.	Glucose Isomerase	<i>Bacillus coagulans</i>	N.a.
28.	Glucose Isomerase	<i>Streptomyces olivaceus</i>	N.a.
29.	Glucose Isomerase	<i>Streptomyces olivochromogen</i>	N.a.
30.	Glucose Isomerase	<i>Streptomyces rubiginosus</i>	N.a.
31.	Glucose Isomerase	<i>Streptomyces violaceoniger</i>	N.a.
32.	Glucose Oxidase and Catalase	<i>Aspergillus niger</i> var.	N.a.
33.	Hemicellulase	<i>Aspergillus niger</i> var.	N.a.
34.	Lipase	Animal	N.a.
35.	Lipase	<i>Aspergillus oryzae</i> var.	N.a.
36.	Malt Carbohydase	N.sp.	N.a.
37.	Maltogenic Amylase	<i>Bacillus stearothermophilus</i>	<i>Bacillus subtilis</i>
38.	Mixed Microbial Carbohydase and Protease	<i>Bacillus subtilis</i> var.	N.a.
39.	Papain	N.sp.	N.a.
40.	Pectinase	<i>Aspergillus niger</i> var.	N.a.
41.	Pepsin	<i>Hog Stomach</i>	N.a.

	Substance Name	Production organism	Donor organism
42.	Protease	<i>Aspergillus oryzae, var.</i>	N.a.
43.	Protease	<i>Streptomyces fradiae</i>	N.a.
44.	Pullulanase	<i>Klebsiella aerogenes</i>	N.a.
45.	Rennet	N.sp.	N.a.
46.	Rennet Bovine	N.sp.	N.a.
47.	Rennet	<i>Bacillus cereus</i>	N.a.
48.	Rennet	<i>Endothia parasitica</i>	N.a.
49.	Rennet	<i>Rhizomucor species</i>	N.a.
50.	Trypsin	N.sp.	N.a.

Source: JECFA: Compendium of food additives specifications. N.a. ... Not applicable; N.sp. ... Not specified

Table 10: Comparison of information requirements for enzymes in different guidelines and regulations

	SCFa	FDA / GRASb	JECFAc	AMFEPd
Characterisation of protein				
Principal enzyme activities	Systematic names EC numbers	Systematic names EC numbers CAS Registry Number Common and/or trade names	Systematic names EC number	Systematic names EC number
Enzyme Activity	Units/Weight or Units/Volume or (Quantity of enzyme preparation to be added to a given quantity of food)	Units Enzymatic function Mode of action Substrate specificity Molecular weightg Isoelectric pointg Kinetic propertiesg Specific activityg Temperatureh pHh Inorganic ionsh	Units/Weight Reaction catalysed Detailed description of suitable methods for each evaluated enzyme is provided	International units or manufacturer units
Subsidiary enzymatic activities	List of subsidiary enzymatic activities „whether they perform a useful function or not” (page 15)	Other major enzymatic activities	Secondary enzyme activities	N.sp.

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	SCFa	FDA / GRASb	JECFAc	AMFEPd
recombinant enzymes from GMM:	N.sp.	Description of permanent or transient structural modifications (by chemical or genetic engineering) Comparative analysis i: Enzymatic activity Kinetic parameters Amino acid composition Amino sugar composition Amino acid sequence (full or partial) Molecular weight Isoelectric point Gel-migration Chromatographic (or similar) properties	molecular weight; isoelectric point; substrate specificity; reaction kinetics; activity as a function of pH and temperature; amino acid composition; amino acid sequence; a peptide map; and DNA base sequence coding for the enzyme.	N.sp.
animal sources	Identification of animal and part of the animal Source must comply with meat inspection requirements	Should be characterised; should comply with the General requirements for enzyme preparations set forth in the Food and Chemicals Codex 3rd edition, 1981	Sources must comply with meat-inspection requirements and be handled in accordance with good hygienic practice	N.sp.
plant sources	Identification of plant and part of the plant	Should be identified	No residues harmful to health must be left in the processed finished food under normal conditions of use	N.sp.
microbial sources (including GMM) ¹	Must be discrete stable strains and well characterised (to enable to be assigned unique identities as the sources of the enzyme preparation)	Should be taxonomically and genetically identified	Must be discrete and stable strains or variants which are sufficiently well characterised to enable them to be assigned unique identities as the sources of the enzyme preparations which are the subject of individual specifications	Taxonomy of the MO should be precisely defined.
GMM	Information about host organism	All procedures and steps involved in the construction	Host microorganisms should be taxonomically	Host organism should be

¹ Including wild-type strains, „variants” ? or strains resulting from selection procedures or genetic modification (page 25).

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	SCFa	FDA / GRASb	JECFAc	AMFEPd
		of the production organism should be thoroughly described Genetic stability Growth properties	and genetically characterised Identification at the level of genus and species is usually adequate for those that have been determined to be safe and suitable ² Documentation that the host microorganism is non-pathogenic and non-toxicogenic should be provided Production microorganism should be characterised with respect to the introduced DNA, its genetic stability, and its growth properties	described
Vector	Must be free of harmful sequences, non-conjugative, and non-mobilizable Identity and biology Size Restriction map Possible full DNA sequence Genes found on the vector Marker genes	Should be genetically identified and characterised	characterised and a description of its construction provided genetic material does not contain genes coding for virulence factors, protein toxins, or enzymes that may be involved in the synthesis of mycotoxins or any other toxic or undesirable substances As with the citation of strains, the citation of specific expression plasmids is generally unnecessary and may impose unnecessary restrictions on the development of enzyme preparations.	should be described
introduced DNA	DNA sequence(s) introduced Number of inserted genes Type of regulation (promoter activity) Gene product(s) Origin and pedigree of the genetic construct	Introduced DNA	N.sp.	should be described
Donor organism	Identification of the donor organism	Should be taxonomically and genetically identified and characterised	The source of the DNA encoding the enzyme of interest should be identified where possible.	should be described

² According to 53rd JECFA meeting, identification at the strain level may impose unnecessary constraints on the development of production organisms. The committee further concluded that in the case of a non-pathogenic, non-toxicogenic strain that belongs to a species that includes pathogenic and toxicogenic strains (e. g. *Escherichia coli*), there should be a requirement in the monograph that the strain be non-pathogenic and non-toxicogenic. Citation of a suitable strain number may be included by way of example.

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	SCFa	FDA / GRASb	JECFAc	AMFEPd
Manufacturing process				
Method of manufacture	In case of MO: information on fermentation media information on fermentation conditions	Should be thoroughly described In case of GMM: Fermentation process Components of fermentation medium should be identified	N.sp.	N.sp.
Purification procedure	Information on purification procedure	Should be thoroughly described In case of GMM: Isolation of the enzyme including all physical and chemical treatment should be described	N.sp.	N.sp.
Usage				
	Technological function of the enzyme	N.sp.	N.sp.	N.sp.
	Types of foodstuff in which the enzyme is intended to be used	N.sp.	N.sp.	N.sp.
	Maximum amount of enzyme preparation to be used in each foodstuff	N.sp.	N.sp.	N.sp.
Stability and fate in food				
	Amount of enzyme preparation in the final food	N.sp.	N.sp.	N.sp.
	Main reaction products and possible reaction products not considered normal constituents of the diet, formed during production and storage	N.sp.	N.sp.	N.sp.
	Possible effects on nutrients	N.sp.	N.sp.	N.sp.
Toxicological requirements				
Source: edible parts of animals and plants	No toxicological tests are required	According to FDA 1997 ⁱ	Specified in: Principles for the safety assessment of food additives and contaminants in food. WHO Environmental Health Criteria, No. 70, Geneva, World Health Organization,	N.sp.

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	SCFa	FDA / GRASb	JECFAc	AMFEPd
Source: not considered as normal part of the diet	Some toxicological testing may be required („unless other satisfactory documentation for safety in use is provided")	According to FDA 1997 ⁱ	1987 Enzyme preparations are grouped into classes I to V depending on their production organism	N.sp.
Source: MO	Test should be performed on a batch from the final purified product, before addition of carriers, diluents etc. in accordance to EC/OECD guidelines ³ 90-day oral toxicity test in rodent species test for gene mutations in bacteria test for chromosomal aberrations (preferably in-vitro)	According to FDA 1997 ⁱ	Normally, acute and short-term toxigenicity tests are required. In case of MO the do not normally occur in food, also long term test have to be provided. In case of fungi or bacteria from genus that might produce (myco)toxins specific test have to be carried out. In case of testing of genetic toxicity, teratology and mutagenicity are also required	„[...] extensive animal feeding trials of the enzyme preparatioN. Evidence of non-pathogenicity of the production strain should be provided either by literature or by actual experiments such as biological tests of the strain in rodents.“
Source: GMM	N.sp.	According to FDA 1997 ⁱ ; Transformable DNA coding for toxins and/or proteins that inactivate therapeutic antibiotics should not be present	All enzyme preparations should be evaluated for their potential to elicit allergic reactions. As a general rule, if the food is known to cause an allergic reaction in humans, its use as a source of DNA encoding the enzyme of interest should be avoided. In exceptional cases, where there is a demonstrated need to use an allergenic source of DNA, documentation should be provided indicating that the enzyme is not associated with the allergic reaction. The most common allergenic foods on a world-wide basis are fish, crustacea, peanuts, tree nuts, soybeans, milk, eggs, and wheat Rev.f: „In case of GMM the need for an evaluation for allergenic potential of the gene products encoded should be assessed.“ No need to further assess the allergic potential in case the DNA sequence is comparable to that coding for an enzyme with a long history of safe use in food” (page 19).	N.sp.
Exemptions from the toxicological requirements				

³ Modifications of the standard test protocols may be necessary due to e. g. the enzymatic activities of certain enzyme preparations and will be accepted if they are supported by arguments.

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	SCFa	FDA / GRASb	JECFAc	AMFEPd
	If other enzyme preparations from the particular strain have thoroughly been tested before and if the manufacturing process does not significantly differ Full testing battery may be waived	N. i.	N.sp.	N.sp.
	If the production MO Has a long history of safety in food use, and Belongs to a species where no toxins are produced, and The particular strain is of well documented origin Acceptance of enzyme preparation without specific toxicological testing may be justified	N. i.	N.sp.	N.sp.
	If a production organism of an already approved enzyme preparation is replaced by a mutant strain A modified, less comprehensive test procedure may be possible	N. i.	N.sp.	N.sp.
	In case of immobilised enzymes: if the immobilisation techniques and the enzyme preparation have already been approved on the basis of adequate toxicity testing and if it is ensured that the leakage of components of the immobilisation system is within acceptable limits The combinatorial product may not be subjected to any additional testing	N. i.	N.sp.	Where no leakage of either carrier / immobilisation agent or enzyme can be demonstrated in validated assays, toxicological tests are not necessary.
	In case of non-toxin producing GMO: If high purity and specificity of the enzyme product could be demonstrated Full toxicity testing may not be needed	N. i.	N.sp.	N.sp.

^a SCF, (1992)

^b FDA (1993)

^c Prepared at the 35th JECFA (1989) and published in FNP 49 (1990) and in FNP 52 (1992), superseding general specifications prepared at the 25th JECFA (1981) and published in FNP 19 (1981) and in FNP 31/2 (1984). Amended at the 55th JECFA (1999) and partially published in FNP 52 Add 7 (1999). For Enzymes from GMM: Prepared at the 53rd JECFA (1998) and published in FNP 52 Add 6 (1998), superseding Appendix prepared at the 37th JECFA (1990) and published FNP 52 (1992). Amended at the 55th JECFA (1999) and partially published in FNP 52 Add 7 (1999).

^d AMFEP, (1992)

^e SCOOP Task 7.4 preliminary results.

^f Amendments decided at the 57th JECFA (2001).

^g If available or necessary to support the enzyme identity.

^h If such characteristics are relevant for the intended use of the enzyme preparation.

ⁱ If an enzyme normally produced in one organism is expressed in another organism through molecular cloning, especially in case of gene transfer to genetically distant organisms.

^j The extent of toxicological testing of food additives is depending on the on the assignment of a „concern level”, on structural features and on an estimation of exposure. Minimum testing requirements are recommended for each concern level as well as each structural and exposure group (FDA, 1997). However, these requirements are included in the investigations of this section.

N.sp. ... Not specified; N. i.... Not investigated.

Table 11: Comparison of information requirements of SCF and AFC for food additives and food enzymes.

Reference / Type of document	EFSA (2005) – Draft Guidance a	SCF (1992)	SCF (2001)	Codex Alimentarius (2003)
General information				
Risk assessment context	GMM used in/as food/feed, proteins and non-protein substances from GMM used in/as food/feed GM food and feed: Regulation 1829/2003, GMOs: Directive 2001/18/EC Food additives: Directive 89/107/EEC, flavourings: Directive 88/388/EEC, feed additives: Regulation 1831/2003	Enzymes from MO and GMM Enzymes as food additives: Directive 89/107/EEC; enzymes as processing aids (not harmonised so far)	Substances used as food additives (flavourings excluded) Directive 89/107/EEC	Foods from GMM, no environmental risks “safety of substances produced by microorganisms that are used as additives or processing aids, including enzymes for use in food production” (p.27)m
Description of product/protein				
Conventional product	X ^d	?	N.i.	X
Production process	X	X	N.i.	X
Technical application	X	X	N.i.	X
Protein	X	X	N.i.	X the gene product's function the phenotypic description of the new trait(s)
Principal enzymatic activity	X	X	N.i.	N.r.
Subsidiary enzymatic activity	X	X	N.i.	N.r.
IUB number	N.r.	X	N.i.	N.r.
Temperature range	X	N.sp.	N.i.	N.r.
pH range	X	N.sp.	N.i.	N.r.
Substrate specificity	X	N.sp.	N.i.	N.r.
Possible reaction products	X	N.sp.	N.i.	N.r.
Amino acid sequence	X	N.sp.	N.i.	N.r.
Protein database entry number	N.r.	N.sp.	N.i.	N.r.
Molecular Weight (kDa)	X	N.sp.	N.i.	N.r.

Assessment of Toxic and Ecotoxic Properties of Novel Proteins in GMOs – Supplement

Reference / Type of document	EFSA (2005) – Draft Guidance a	SCF (1992)	SCF (2001)	Codex Alimentarius (2003)
Post-translational modification	X	N.sp.	N.i.	N.r. (see below)
Amino acid sequence modifications	?	?	?	Possible changes in posttranslational modification Critical for structure – function relationship
Production organism	X ^f	X	X	X
Hazard identification				
Assumptions made:	Proteins are considered of lower toxicity compared to non-protein substances (implicit)	N.sp.	N.sp.	N.sp.
Toxicity relevant data		h)		
Homology comparisons	“To proteins known to cause adverse effect e.g. toxins” (p.35) “To proteins exerting a normal metabolic or structural function can also contribute valuable information” (p.35) Database and methodology should be specified	N.r.	N.r.	To known protein toxins and anti-nutrients
Source organisms	X ^g	X	X	X
Test substance	If the protein is produced from an alternative source, the structural, biochemical and functional equivalence of the substitute protein to the newly expressed GMM protein must be demonstrated	Batch of final purified product before addition of carriers, diluents etc.	Test material should conform to the proposed or existing specification. If not then the specifications of the test material should be given and it should be indicated whether this is representative of the substance intended for the market.	From recombinant-DNA microorganisms, the food product if the substance is secreted, or, if necessary, the synthesis or production of the substance from an alternative source

Assessment of Toxic and Ecotoxic Properties of Novel Proteins in GMOs – Supplement

Reference / Type of document	EFSA (2005) – Draft Guidance a	SCF (1992)	SCF (2001)	Codex Alimentarius (2003)
Equivalence indicators in case of proteins from alternative sources	E.g. comparisons of the molecular weight, the isoelectric point, amino acid sequence, post-translational modification, immunological reactivity and, in the case of enzymes, the enzymatic activity.	N.sp.		“structurally, functionally, and biochemically equivalent to that produced in the recombinant-DNA microorganisms” (p.41)
Other heterologous proteins present	??	N.sp.	N.r.	
Information requested besides toxicity test (including anecdotal evidence)	History of safe consumption by humans/animals (product) History of safe use (GMM) History of use (donor)	History of safety in food use (MO)	l)	
Exemptions from testing:	If both the GMM and the protein do “have a history of safe consumption by humans and animals, specific toxicity testing might not be required” (p. 35) “If the applicant considers that a decision on safety can be taken without conducting a repeated dosing study or that other tests are more appropriate, the reason for this decision must be given.” (p.36)	If other enzyme preparations from the particular strain have thoroughly been tested before and if the manufacturing process does not significantly differ Full testing battery may be waived ¹	In case e.g. substances are normal constituents of the diet or of the body, or may be metabolised to such or toxicological data on close homologues or other structure activity considerations are available or in case of intake considerations the full core set might not be necessary. (p.14)	
Other	N.r.	N.r.		“Additional in-vivo or in-vitro studies may be needed on a case-by-case basis to assess the toxicity of expressed substances, taking into account the potential accumulation of any substances, toxic metabolites or antibiotics that might result from the genetic modification.” (p.42).
Toxicity tests				

Assessment of Toxic and Ecotoxic Properties of Novel Proteins in GMOs – Supplement

Reference / Type of document	EFSA (2005) – Draft Guidance a	SCF (1992)	SCF (2001)	Codex Alimentarius (2003)
Toxicity tests mandatory	-	?		“When a substance is new to foods or food processing, the use of conventional toxicology studies or other applicable studies on the new substance will be necessary”.(p.41) “In case of proteins: [...] appropriate oral toxicity studies may be carried out in cases where the protein is present in the food, but is not closely similar to proteins that have been safely consumed in food, and has not previously been consumed safely in food, and taking into account its biological function in microorganisms where known” (p.41.)
Toxicokinetics	N.r.	N.r.	Toxicokinetic and metabolism studies (whole animal studies using single and repeat dosing)	N.r.
Acute oral toxicity	N.r.	N.r.	N.r.	N.r.
Subacute oral toxicity	Duration of subchronic toxicity studies not specified	N.r.	14 or 28-day studies can provide an indication of target organs and help in selection of appropriate doses for the 90-day studies	N.r.
Oral subchronic		90-day oral study in rodents	At least 90-day oral studies in two animals (rodent and non-rodent)	N.r.
Oral chronic	c)	N.r.	Preferably oral studies in rat and mouse covering the majority of the lifespan (25 month and 18 or 12 months). Combined chronic toxicity /carcinogenicity studies are acceptable.	N.r.
Carcinogenicity		Nr.		

Assessment of Toxic and Ecotoxic Properties of Novel Proteins in GMOs – Supplement

Reference / Type of document	EFSA (2005) – Draft Guidance a	SCF (1992)	SCF (2001)	Codex Alimentarius (2003)
Genotoxicity		Gene mutation in bacteria Chromosomal aberrations (preferably in-vitro)	Gene mutation in bacteria Gene mutation in mammalian cells (in-vitro) Chromosomal aberrations in mammalian cells (in-vitro)k	N.r.
Reproduction or developmental toxicity		N.r.	Oral multigenerational reproduction toxicity study in one laboratory species (usually rat) Oral Developmental toxicity studies in two laboratory species – usually rat or mouse and rabbit	N.r.
Other		N.r.	Immunotoxicity, neurotoxicity, human volunteer studies, predictive mechanistic studies etc. might be appropriate on a case-by-case basis.	N.r.
Reference to standard	OECD	EC/OECD ⁱ	EC/OECD	OECD
GLP	X	N.r.	X	X
Hazard characterisation				
ADI (acceptable daily intake)	N.r.	Possibly allocated on the basis of the NOEL from the subchronic rodent study	X	N.r.
NOAEL (no-observed adverse effect level)/NOEL (no-observed effect level)	N.r.	N.r.	X	N.r.
Exposure assessment				
Average intake (in case of food/feed)	N.r.	N.r.	“Information should be provided on known or	“Information on the anticipated exposure of consumers to the

Assessment of Toxic and Ecotoxic Properties of Novel Proteins in GMOs – Supplement

Reference / Type of document	EFSA (2005) – Draft Guidance a	SCF (1992)	SCF (2001)	Codex Alimentarius (2003)
EDI (maximum expected intake) and/or other worst-case assumptions	N.r.	N.r.	anticipated human exposure to the proposed additive from food, including amount (e.g. maximum and average intake or exposure), frequency and other factors influencing exposure. Information should also be given on any other sources of human exposure to the same substance (e.g. from drinking water, consumer products, etc.)" (p.9)	substance, the potential intake and dietary impact of the substance should be provided. [...]Current dietary exposure and possible effects on population sub-groups should also be considered." (p.41)
Concentration in source tissue and/or final food/feed products	Expression of novel protein in the GMM	Maximum amount of enzyme preparation to be used in each foodstuff Amount of enzyme preparation in the final food	N.r.	"The safety assessment of the expressed substance should take into account its function and concentration in the food. "(p.41)
Stability	"under processing and storage conditions and the expected treatment of the food/feed" (p.35) "influences of temperature, particularly of heat treatments, and pH changes should normally be examined and potential modification(s) of the proteins (e.g. denaturation) and/or production of stable protein fragments generated through such treatments should be characterised."b	Main reaction products and possible reaction products not considered normal constituents of the diet during production and storage Possible effects on nutrients	The stability and any degradation products or reaction products appearing as a result of processing, storage and preparation of foods containing the substance.	To heat or processing
Digestibility	Resistance to proteolytic enzymes (e.g. pepsin), e.g. by in-vitro standardised tests "Stable breakdown products should be characterised and evaluated with regard to the hazard linked to their biological activity" (p.35)	N.r.	N.r.	Appropriate representative gastric and intestinal model systems
Risk characterisation				

Assessment of Toxic and Ecotoxic Properties of Novel Proteins in GMOs – Supplement

Reference / Type of document	EFSA (2005) – Draft Guidance a	SCF (1992)	SCF (2001)	Codex Alimentarius (2003)
Handling of uncertainty, ambiguity and ignorance	Assumptions made in the risk assessment, nature and magnitude of uncertainties associated with establishing the risks should be clearly explained (p.57)	N.sp.	N.sp.	N.r.
Other	N.r.	Possibly defining acceptable conditions of use		N.r.
Other characteristics				
Overall approach (to toxicity testing)	Case-by-case, depending on source, function/activity and history of human/animal consumption Comparison to conventional counterpart should guide the safety assessment (elaborated in p.31-32), even in the case of a purified single substance	??	Core-toxicity test battery; additional tests on a case-by-case basis	“This approach is based on the principle that the safety of foods produced using recombinant-DNA microorganisms is assessed relative to the conventional counterparts that have a history of safe use, not only for the food produced using a recombinant-DNA microorganism, but also for the microorganism itself” (p.29).
Role of anecdotal evidence				N.sp.
Role of experience (history of safe use)	History of human/animal consumption” is generally guiding the evaluation, toxicity testing requirements and information on the GMM “For newly expressed proteins with an insufficient body of knowledge and, in particular, if the data available suggest any cause for concern, specific toxicity studies should be carried out.”(p.35)	??	In case of additives from MO: history of safe use of MO in food	“The criterion for establishing the safety of microorganisms used in the production of foods where there is no history of safe use is beyond the scope of the current document.” (p.27) “Recipient microorganisms should have a history of safe use in food production or safe consumption in foods.” (p.36)

N.r. ... not required, N.sp. ... not specified; ??: unclear

a) Quotations refer to p.34/35 unless differing page numbers are given

b) Protein fragments could be of toxicological relevance.

c) Depending on the outcome of the subchronic study.

d) "identity of the product according to its principal function (i.e. specification of the category of product to which it belongs), the name, the chemical definition, the chemical name, synonyms, trade names and abbreviations, if any" (p.30),

e) For applicability of this approach to proteins from GMM see discussion in the report..

f) Not required in case of QPS.

g) Not required in case of self-cloning.

h) Requirements specified in this table applies to microorganisms only (including GMM). In case of enzymes from edible parts of plant or animal no toxicological tests are required. In case parts are not considered as normal part of the diet some toxicological testing may be required "unless other satisfactory documentation for safety in use is provided".

i) Modifications of the standard test protocols may be necessary due to e. g. the enzymatic activities of certain enzyme preparations and will be accepted if they are supported by arguments.

j) If the production MO (i) has a long history of safety in food use, and (ii) belongs to a species where no toxins are produced, and (iii) the particular strain is of well documented origin. Acceptance of enzyme preparation without specific toxicological testing may be justified if a production organism of an already approved enzyme preparation is replaced by a mutant strain. A modified, less comprehensive test procedure may be possible in case of immobilised enzymes: if the immobilisation techniques and the enzyme preparation have already been approved on the basis of adequate toxicity testing and if it is ensured that the leakage of components of the immobilisation system is within acceptable limits. The combinatorial product may not be subjected to any additional testing in case of non-toxin producing GMO. If high purity and specificity of the enzyme product could be demonstrated full toxicity testing may not be needed

k) Positive results in any of the tests will normally require further assessment of genotoxicity in-vivo.

l) In case of MO: if it has a history of safe use in food, then 9.1.3 and 9.1.4 are not required.

9.1.2 Information from previous human exposure to the product or its source.

9.1.3 Pathogenicity of the organism(s).

9.1.4 Toxicology of the organism(s).

9.1.5 Specification of the production process (see 4).

9.1.6 Specification of the end product (see 3).

In cases where the microorganism(s) and/or their metabolites remain the final product, information should also be provided on:

9.2.1 Safety of the microorganism(s) and/or their metabolites in the final product (see 2.1).

9.2.2 Any special procedures for preparation and cooking to ensure safety. In the case of additives produced by microbiological processes using genetically modified microorganisms (GMMs), information should also be provided on:

9.3.1 Source of the transgenic DNA and its effect on the properties the host organism(s).

9.3.2 Genetic stability of the GMMs.

9.3.3 Probability of transfer of inserted genetic material to human flora and its likely consequences.

9.3.5 Ability of the GMMs to survive in and colonise the human gut.

m) Reference is provided to the Joint FAO/WHO Expert Committee on Food Additives (JECFA) is revising guidelines for General Specifications and Considerations for Enzyme Preparations used in food processing.

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C/ES/01/01: Opinion on the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the notification for the placing on the market of insect-tolerant genetically modified maize 1507 for import, feed and industrial processing and cultivation, under Part C of Directive 2001/18/EC from Pioneer Hi-Bred International/Mycogen Seeds. (Question No EFSA-Q-2004-072). Opinion adopted on 19 January 2005.

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Tables of Part B Assessment of ecotoxicity

Table S3. Studies for the estimation of exposure of non-target organisms to the respective novel proteins as provided by the notifiers of GMPs

Table S4. Overview of ecotoxicological studies provided by the notifiers in order to demonstrate the safety of Cry-toxins of GMOs intended for cultivation within the European Union (except MON863)

Table S6. Studies for the assessment of degradation of the respective novel proteins in soil as provided by the notifiers of GMPs

Table S3. Studies for the estimation of exposure of non-target organisms to the respective novel proteins as provided by the notifiers of GMPs

GMP	protein	notification	Exposure estimation study conducted by the notifier	Exposure estimations
Single events				
Maize Bt11	Cry1Ab	C/F/96/05-10	None	-
Maize MON810	Cry1Ab	C/F/95/12-02	None	-
Maize MON863 (MON863 x MON810)	Cry3Bb1	C/DE/02/9	Astwood et al. (2001)	Maximum predicted environmental concentrations (MPEC) based on expression levels from Argentina (Dudin et al. 2001) for Plant feeding organisms = 93 µg protein/g For soil dwelling organisms = 13,3 mg/kg Measured NOECs exceed the MPEC 3-140 fold
			Anonymous (2003) cited in Responses to EFSA questions, Jan 2004	Calculation of maximum possible Bt protein in soil amended with 18 t chicken manure/ha: 0,7 mg Bt protein/kg soil (0,7 µg Bt protein/g soil)
Maize 1507	Cry1F	C/ES/01/01	Wolt & Conlan (2001)	published data on pollen dispersal and expression levels of Cry1F in pollen of 1507 maize Hazard characterisation: LC ₅₀ values for 15 lepidopteran pest species (Dow internal data) and the monarch butterfly larvae, variation in sensitivity to Cry1F = 3 orders of magnitude; 0,67µg/g = high end sensitivity (conservative endpoint) Exposure estimation: toxin concentration in pollen = 32 µg/g Pollen PEC of Cry1F on host plant leaf: 0,00003- 0,96 µg Cry1F protein/g leaf (0-60 m from field edge)

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Cotton MON531	Cry1Ac	C/ES/96/02	None	
Maize 59122	Cry34Ab1, Cry35Ab1	EFSA/GMO/ NL/ 2005/23	Poletika (2003)	high end exposure estimates (HEEE): 90% upper bound on reported mean for each tissue; estimated environmental concentration (EEC) based on expression levels of GM maize from Chile locations; for different taxa (phytophages, higher trophic levels, pollinators, detritivores, incidentals, aquatic habitats) compared with effects data for different species (NOEC, NOEL)
Stacked events				
Maize MON810xNK6 03	Cry1Ab	EFSA/GMO/ NL/ 2005/26	None	-
Maize 1507xNK603	Cry1F	EFSA/GMO/ UK/ 2005/17	None	-
Maize 1507x59122	Cry1F, Cry34Ab1, Cry35Ab1	EFSA/GMO/ NL/ 2005/28	None	-
Maize 59122x1507x NK603	Cry1F Cry34Ab1, Cry35Ab1	EFSA/GMO/ UK/ 2006/30	None	-

Table S4. Overview of ecotoxicological studies provided by the notifiers in order to demonstrate the safety of Cry-toxins of GMOs intended for cultivation within the European Union (except MON863)

GMP	Protein	Notification	Studies (Author, Year)	Test organism	Test substance	Study availability
Single events						
Maize Bt11 ⁴	Cry1Ab	C/F/96/05-10	Maggi & Sims (1994a, 1994b)	Honey bee larvae, adults	Purified Btk	Monsanto study – summary only
			Hoxter & Lynn (1992a)	Green lacewing larvae (<i>Chrysopa carnea</i>)	Activated Btk protein	Monsanto study – summary only
			Hoxter & Lynn (1992b)	Parasitic Hymenoptera (<i>Brachymeria intermedia</i>)	Activated Btk protein	Monsanto study – summary only
			Hoxter & Lynn (1992c)	Ladybird beetles (<i>Hippodamia convergens</i>)	Activated Btk protein	Monsanto study – summary only
			Collins (1994)	Collembola (<i>Folsomia candida</i>)	Lyophilized protein (from Bt176)	Attached in update 2003
			Garvey (1994)	Earthworms (<i>Eisenia foetida</i>)	Lyophilized protein (from Bt176)	Attached in update 2003
Maize MON810	Cry1Ab	C/F/95/12-02	Maggi & Sims (1994a, b)	Honey bee larvae, adults	Purified Btk protein	attached
			Hoxter & Lynn (1992a)	Green lacewing larvae (<i>Chrysopa carnea</i>)	Activated Btk protein	attached
			Hoxter & Lynn (1992b)	Parasitic Hymenoptera (<i>Brachymeria intermedia</i>)	Activated Btk protein	attached
			Hoxter & Lynn (1992c)	Ladybird beetles (<i>Hippodamia convergens</i>)	Activated Btk protein	attached
			Sims (1994)	Honey bees	Cry1Ab insecticidal protein	not attached
Maize MON863	Cry3Bb1	C/DE/02/9	Gallagher et al. (1999)	Northern Bobwhite quail (<i>Colinus virginianus</i>)	Bt protein 11231 in corn grain	attached as Appendix 27, cited in Astwood et al. (2001)
			Campbell & Beavers (1994)	Northern Bobwhite Quail (<i>Colinus virginianus</i>)	MON80187 meal	not attached, cited in App. 32

⁴ Same studies as provided for MON810 (in Bt11 notification a letter from Monsanto is cited in Appendix 9 with an authorization for Novartis Seeds to refer to the studies conducted by Monsanto)

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GMP	Protein	Notification	Studies (Author, Year)	Test organism	Test substance	Study availability
			Li & Robinson (1999)	Channel catfish (<i>Ictalurus punctatus</i>)	Corn lines MON853 and MON859	attached as Appendix 28, cited in Astwood et al. (2001)
			Jackson et al. (1995)	Channel Catfish (<i>Ictalurus punctatus</i>)	Corn line MON801	not attached, cited in Appendix 32
			Drottar & Krueger (1999)	Cladocera (<i>Daphnia magna</i>)	Bt protein 11098 in corn pollen	not attached, cited in Astwood et al. (2001) and App. 32
			Teixeira (1999)	Collembola (<i>Folsomia candida</i>)	Corn tissue containing Bt protein 11098	attached as Appendix II, cited in Astwood et al. (2001) and App. 32
			Palmer & Krueger (1999a)	Green lacewing larvae (<i>Chrysoperla carnea</i>)	Bt protein 11231	not attached, cited in Astwood et al. (2001) and App. 32
			Palmer & Krueger (1999c)	Parasitic hymenoptera (<i>Nasonia vitripennis</i>)	Bt protein 11231	not attached, cited in Astwood et al. (2001) and App. 32
			Maggi (1999a, 1999b)	Honeybee adults, larvae	Bt protein 11231	not attached, cited in Astwood et al. (2001) and App. 32
			Hoxter et al. (1999)	Earthworm	Bt protein 11231	not attached, cited in Astwood et al. (2001)
			Sindermann et al. (2002)	Earthworm (<i>Eisenia fetida</i>)	Bt protein 11089	attached as Appendix I, cited in App. 32
			Bryan et al. (2001)	Ladybird beetles (<i>Hippodamia convergens</i>)	Corn pollen expressing Cry3Bb1	not attached, cited in Astwood et al. (2001) and App. 32
			Duan et al. (2001a, 2001b)	Ladybird beetles (adult, larval) (<i>Coleomegilla maculata</i>)	Corn pollen expressing Cry3Bb1	not attached, cited in Astwood et al. (2001) and App. 32
			Palmer & Krueger (1999b)	Ladybird beetle (<i>Hippodamia convergens</i>)	Bt protein 11231	not attached, cited in Astwood et al. (2001) and App. 32
			Sears & Matilla (2001)	Monarch butterfly larvae (<i>Danaus plexippus</i>)	Corn pollen expressing Cry3Bb1	not attached, cited in App. 32
			Duan et al. (2002)	Not indicated	Cry3Bb1 protein in transgenic	not attached, cited in

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GMP	Protein	Notification	Studies (Author, Year)	Test organism	Test substance	Study availability
					corn MON863	App. 32
			Head (2001, 2002)	Not indicated	Corn rootworm protected transgenic corn	not attached, cited in App. 32
			Head et al. (2001)	Not indicated	Cry3Bb1 protein	not attached, cited in App. 32
	Cry1Ab		Graves & Swigert (1997)	Cladocera (<i>Daphnia magna</i>)	Corn pollen containing the CryIA(b) protein	not attached, cited in App. 32
			Halliday (1997)	Collembola (<i>Folsomia candida</i>)	Corn tissue expressing CryIA(b) protein	not attached, cited in App. 32
			Sims & Martin (1996)	Collembola (<i>Folsomia candida</i> , <i>Xenilla grisea</i>)	CryIA(b), CryIA(c), CryIIA, CryIIIA insecticidal proteins	not attached, cited in App. 32
			Hoxter & Lynn (1992a)	Green lacewing larvae	Activated Btk HD-1 protein	not attached, cited in App. 32
			Hoxter & Lynn (1992b)	Parasitic hymenoptera (Brachymeria intermedia)	Activated Btk HD-1 protein	not attached, cited in App. 32
			Hoxter & Lynn (1992c)	Ladybird beetles	Activated Btk HD-1 protein	not attached, cited in App. 32
			Maggi & Sims (1994a, 1994b)	Honey bee larvae, adults	B.t.k. Endotoxin proteins	not attached, cited in App. 32
			Palmer & Beavers (1995)	Earthworm	CryIA(b) protein	not attached, cited in App. 32
			Sims (1994)	Not indicated	Insecticidal protein of Bt. var. kurstaki (HD-1)	not attached, cited in App. 32
Maize 1507	Cry1F	C/ES/01/01	Hoxter et al. (1999a)	Green lacewing larvae (<i>Chrysoperla carnea</i>)	Microbially-derived Cry1F protein	attached
			Hoxter et al. (1999b)	Ladybird beetle (<i>Hippodamia convergens</i>)	Microbially-derived Cry1F protein	attached
			Hoxter et al. (1999c)	Parasitic hymenoptera (<i>Nasonia vitripennis</i>)	Microbially-derived Cry1F protein	attached
			Hoxter et al. (1999d)	Earthworms (<i>Eisenia fetida</i>)	Microbially-derived Cry1F protein	attached
			Maggi (1999)	Honey bee larvae (<i>Apis mellifera</i>)	1507 maize pollen	attached

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GMP	Protein	Notification	Studies (Author, Year)	Test organism	Test substance	Study availability
			Bystrak (2000)	Monarch Butterfly (<i>Danaus plexippus</i>)	Microbially-derived Cry1F protein	not attached
			Halliday (1998a)	Collembola (<i>Folsomia candida</i>)	Microbially-derived Cry1F protein	not attached
			Drottar & Krueger (1999)	Cladocera (<i>Daphnia magna</i>) (pollen)	Microbially-derived Cry1F protein and pollen of 1507 maize	not attached
			Gallagher et al. (1999)	Northern Bobwhite quail (<i>Colinus virginianus</i>)	Grounded grain of 1507 maize	not attached
Maize 59122	Cry34Ab1 Cry35Ab1	EFSA/GMO /NL/2005/ 23	All studies in Poletika (2003):			attached
			Bryan et al. (2000a)	Ladybird beetles (<i>Hippodamia convergens</i>)	PS149B1 binary insecticidal crystal protein	not attached
			Porch & Krueger (2001)	Parasitic hymenoptera (<i>Nasonia vitripennis</i>)	PS149B1 binary insecticidal crystal protein	not attached
			Maggi (2001)	Honeybee larvae	PS149B1 binary insecticidal crystal protein	not attached
			Sindermann et al. (2001)	Green lacewing larvae (<i>Chrysoperla carnea</i>)	PS149B1 binary insecticidal crystal protein	not attached
			Teixeira (2001)	Collembola (<i>Folsomia candida</i>)	PS149B1 binary insecticidal crystal protein	not attached
			Bryan et al. (2000b)	Earthworms (<i>Eisenia fetida</i>)	PS149B1 binary insecticidal crystal protein	not attached
			Marino & Yaroeh (2001)	Daphnids (<i>Daphnia magna</i>)	PS149B1 binary insecticidal crystal protein	not attached
			Marino & Yaroeh (2002)	Rainbow trout (<i>Oncorhynchus mykiss</i>)	PS149B1 binary insecticidal crystal protein	not attached
			Higgins (2003)	Ladybird beetles (<i>Coleomegilla maculata</i>)	Cry34/35Ab1 protein	not attached
			Sears (2003)	Monarch butterfly larvae (<i>Danaus plexippus</i>)	Bt corn pollen	not attached

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GMP	Protein	Notification	Studies (Author, Year)	Test organism	Test substance	Study availability
Cotton MON531	Cry1Ac	C/ES/96/02	Palmer & Beavers (1993a)	Parasitic hymenoptera (<i>Nasonia vitripennis</i>)	B..t.k. HD-73-Protein	not attached
			Palmer & Beavers (1993b)	Ladybird beetles (<i>Hippodamia convergens</i>)	B.t.k. HD-73-Protein	not attached
			Palmer & Beavers (1993c)	Green lacewing larvae (<i>Chysopa carnea</i>)	B.t.k. HD-73-Protein	not attached
			Maggi (1993a, 1993b)	Honeybee larvae, adults	purified Btk endotoxin proteins	not attached
			Cambell & Beavers (1993)	Northern Bobwhite quail (<i>Colinus virginianus</i>)	cottonseed meal	not attached
Stacked events						
Maize MON810 x NK603	Cry1Ab	EFSA/GMO /NL/2005/ 26	Maggi & Sims (1994a, 1994b)	Honey bee adult, larvae	Tryptic Btk HD-1 protein (Cry1Ab)	attached
			Hoxter & Lynn (1992a)	Green lacewing larvae (<i>Chrysoperla carnea</i>)	Tryptic Btk HD-1 protein (Cry1Ab)	attached
			Hoxter & Lynn (1992b)	Ladybird beetle (<i>Hippodamia convergens</i>)	Tryptic Btk HD-1 protein (Cry1Ab)	attached
			Hoxter & Lynn (1992c)	Parasitic hymenoptera (<i>Brachymeria intermedia</i>)	Tryptic Btk HD-1 protein (Cry1Ab)	attached
			Palmer & Beavers (1995)	Earthworms (<i>Eisenia fetida</i>)	Tryptic Btk HD-1 protein (Cry1Ab)	attached
			Halliday (1997)	Collembola (<i>Folsomia candida</i>)	MON810 maize containing Cry1Ab toxin	attached
			Graves & Swigert (1996)	Daphnids (<i>Daphnia magna</i>)	Pollen of Bt11	attached
Maize 1507xNK 603	Cry1F	EFSA/GMO /UK/2005/ 17	Evans (1998) (see toxicology assessment)	sensitive (ECB, <i>Heliothis virescens</i> , <i>Spodoptera frugiperda</i>) & insensitive (<i>Agrotis ipsilon</i> , <i>Helicoverpa zea</i>) insects	bacterial Cry1F, plant Cry1F	attached
Maize 1507x591 22		EFSA/GMO /NL/2005/ 28	Refers to assessment of maize 59122 (EFSA opinion and responses by the notifier to EFSA) and maize 1507 (C/ES/01/01)			

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GMP	Protein	Notification	Studies (Author, Year)	Test organism	Test substance	Study availability
	Cry1F		Hoxter et al. (1999a)	Green lacewing larvae (<i>Chrysoperla carnea</i>)	Cry1F delta-endotoxin	attached
	Hoxter et al. (1999b)		Parasitic wasp (<i>Nasonia vitripennis</i>)	Cry1F delta-endotoxin	attached	
	Hoxter et al. (1999c)		Earthworms (<i>Eisenia fetida</i>)	Cry1F delta-endotoxin	attached	
	Maggi (1999)		Honey bees	microbial Cry1F delta-endotoxin; maize pollen expressing Cry1F	attached	
	Halliday (1998b)		Collembola (<i>Folsomia candida</i>)	Bacterially expressed Cry1F protein	attached	
	Gallagher et al. (1999)		Northern Bobwhite quail (<i>Colinus virginianus</i>)	corn expressing Cry1F Bt delta-endotoxin	attached	
	Drottar & Krueger (1999)		Cladocera (<i>Daphnia magna</i>)	Cry1F delta-endotoxin	attached	
	Sinderman et al. (2001)		Green lacewing larvae (<i>Chrysoperla carnea</i>)	PS149B1 binary crystal protein	attached	
	Bryan et al. (2000a)		Ladybird beetle (<i>Hippodamia convergens</i>)	PS149B1 binary crystal protein	attached	
	Higgins (2003)		Ladybird beetle (<i>Coleomegilla maculata</i>)	Cry34Ab1/Cry 35Ab1 proteins	attached	
	Porch & Krueger (2001)		Parasitic wasp (<i>Nasonia vitripennis</i>)	PS149B1 binary crystal protein	attached	
	Maggi (2001)		Honey bees	PS149B1 binary crystal protein; pollen expressing PS149B1; individual PS149B1 proteins	attached	
	Bryan et al. (2000b)		Earthworms (<i>Eisenia fetida</i>)	PS149B1 binary crystal protein	attached	
	Texeira (2001)		Collembola (<i>Folsomia candida</i>)	PS149B1 binary crystal protein	attached	
	Vinall (2005)		Ground beetles (<i>Poecilus cupreus</i>)	Cry34Ab1/Cry35Ab1 proteins	attached	
	Marino & Yaroeh (2001)		Daphnids (<i>Daphnia magna</i>)	PS149B1 binary crystal protein	attached	
	Marino & Yaroeh (2002)		Rainbow trout (<i>Oncorhynchus mykiss</i>)	PS149B1 binary crystal protein	attached	
Maize 59122x 1507x NK603	Cry1F, Cry34/35Ab1	EFSA/GMO /UK/2006/30	Notifier refers to safety assessment of Cry1F to Notification of 1507 maize C/ES/01/01; and of Cry34/35Ab1 to notification of 59122 maize (EFSA/GMO/NL/2005/23)			

Table S6. Studies for the assessment of degradation of the respective novel proteins in soil as provided by the notifiers of GMPs

GMP	Protein	Notification	Protein degradation study in soil	Test system / conditions	Results degradation study
Single events					
Maize Bt11	Cry1Ab	C/F/96/05-10	Anonymous: Annex 12 of dossier (Fate of the Btk protein in transgenic plant material and soil)	Stalk and leaf tissue placed on soil surface or incorporated into soil; Soil spiked with purified Btk protein ELISA, protein assay, Western Blot, insecticidal activity assay (ECB) in weekly intervals for 3 weeks	After 3 weeks no Bt protein detectable in leaves but some in stalk, no insecticidal activity
Maize MON810	Cry1Ab	C/F/95/12-02	None		
Maize MON863 (MON863 x MON810)	Cry3Bb1	C/DE/02/9	DUBELMAN et al. (2002): study not attached to notification	Lyophilized corn shoot and root tissues and purified Cry3Bb1 dissolved protein mixed with 3 soils, incubated at 25°C for 2 months Cry3Bb1 concentration measured by ELISA (9 samples)	Maize tissues: DT ₅₀ = 0.9-2.3 days DT ₉₀ = 7.4-50 days Purified protein: DT ₉₀ = 4-5.2 days
			MARTIN et al. (2000, cited in ASTWOOD et al. 2001), study not attached to notification	Bt produced Cry3Bb1 protein (11098) in soil; Insect bioassays and ELISA	DT ₅₀ = 2.37-2.76 days DT ₉₀ = 7.87-9.16 days
Maize 1507	Cry1F	C/ES/01/01	HALLIDAY (1998b)	Purified Cry1F protein added to soil; incubated under laboratory	DT ₅₀ = 3.13 days

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				conditions for 28 days; Bioassay with neonate tobacco budworm <i>Heliothis virescens</i>	Weight after 6 days : EC ₅₀ Cry1F+soil = 0.17 (d 0) – 3.87 (d 28) Cry1F alone = 0.04 (d 0) – 0.1 (d 28)
Cotton MON531	Cry1Ac	C/ES/96/02	None		
Maize 59122	Cry34A b1, Cry35A b1	EFSA/GMO/ NL/ 2005/23	HERMAN et al. (2000)	purified bacterially produced protein mixed with soil and incubated under laboratory conditions for 28 days; larval bioassays with corn rootworm (<i>Diabrotica undecimpunctata howardi</i>)	DT ₅₀ = 3.2 days
			HERMAN et al. (2002a)	Microbially produced toxins (powder mixture) incubated with different soils for 28 days (samples at 0, 1, 3, 7, 14 and 28 d); ; insect bioassays (southern corn rootworm)	DT ₅₀ = 3.2 d (only initial phase of degradation) DT ₅₀ = 2.4 d (bi-phasic model) DT ₅₀ = 1.7 d Linear explanatory model (shift-log model)
Stacked events					
Maize MON810xNK603	Cry1Ab	EFSA/GMO / NL/ 2005/26	PALM et al. (1994)	Purified toxin (Cry1Ab, Cry1Ac); Bt cotton leaves (crushed to fine pieces) incubated in 3 different soils; samples at 7-day intervals for 4 weeks; ELISA	DT ₅₀ /DT ₉₀ not indicated According to notifier comparable to Sims & Holden (1996)
			SIMS & HOLDEN (1996)	Bt maize (lyophilized, ground, finely sieved) incubated in Drummer loam soil for 43 days (samples at d 0, 7, 14, 21, 30, 43); Insect bioassays (<i>H. virescens</i>)	corn tissue with soil contact: DT ₅₀ = 1.6 days, DT ₉₀ = 15 days corn tissue without soil contact DT ₅₀ = 25.6 days. DT ₉₀ = 40.7 days

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Maize 1507xNK603	Cry1F	EFSA/GMO/ UK/ 2005/17	refers to assessment of 1507 maize (EFSA opinion)		
Maize 1507x59122	Cry1F Cry34Ab 1 Cry35Ab 1	EFSA/GMO / NL/ 2005/28	refers to several EFSA opinions		
Maize 59122x1507x NK603	Cry1F Cry34Ab 1 Cry35Ab 1	EFSA/GMO / UK/ 2006/30	refers to single event assessments by EFSA and other regulative authorities		

Source Documents Part B

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- WOLT & CONLAN (2001): Non-target exposure and risk assessment for cultivation of 1507 maize in Europe. Monsanto internal study.



This document includes the more comprehensive tables which were compiled in the course of the analysis during the TOXPROT project. It is thereby considered a supplement to the final report of this project. The tables are not intended to be self explanatory and have to be read in conjunction with the report in part 1.



**Bestelltelefon:
01 / 711 00-4700**