Bioveiligheidsraad
Conseil de Biosécurité

12-02-2010

Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC/2010_0158

Title: Advice of the Belgian Biosafety Advisory Council on the application EFSA/GMO/RX-GT73 from Monsanto under Regulation (EC) No. 1829/2003

Context

The application EFSA/GMO/RX-GT73 was submitted by Monsanto on 29 June 2007 for renewal of authorisation of the glyphosate tolerant genetically modified (GM) oilseed rape GT73 for food and feed purposes (refined oil, feed materials, and food and feed additives) according to Articles 8 and 20 of Regulation (EC) No. 1829/2003[1].

Oilseed rape GT73 was lawfully placed on the market as foods produced from oilseed rape GT73 (processed oil) and as feeds produced from oilseed rape GT73 before the date of application of Regulation (EC) No. 1829/2003.

Oilseed rape GT73 was also subject previously to a notification for the placing on the market as feed containing or consisting of GT73 oilseed rape (notification C/NL/98/11 submitted under Directive 2001/18/EC); approved by Commission Decision (2005/635/EC) of 31 August 2005 [2]; Belgium has previously issued 4 scientific opinions related to this notification.

Additionally, oilseed rape GT73 has been entered on the community register of GM food and feed[3].

The application EFSA/GMO/RX-GT73 was officially acknowledged by EFSA on 28 March 2008. On the same date EFSA started the formal three-month consultation of the Member States, in accordance with Articles 6.4 and 18.4 of Regulation (EC) No. 1829/2003 (consultation of national Competent Authorities within the meaning of Directive 2001/18/EC designated by each Member State in the case of genetically modified organisms (GMOs) being part of the products).

Within the framework of this consultation, the Belgian Biosafety Advisory Council, under the supervision of a coordinator and with the assistance of its Secretariat, contacted experts chosen from the common list of experts drawn up by the Biosafety Advisory Council and the Division of Biosafety and Biotechnology to evaluate the dossier. Five experts answered positively to this request and formulated a number of comments on the dossier, which were

[1] Regulation (EC) No. 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. (OJ L 268, 18.10.2003, p.1)
[2] Commission Decision (2005/635/EC) of 31 August 2005 concerning the placing on the market, in accordance with Directive 2001/18/EC of the European Parliament and of the Council, of an oilseed rape product (*Brassica napus* L., GT73 line) genetically modified for tolerance to the herbicide glyphosate
[3] see: http://ec.europa.eu/food/dyna/gm_register/index_en.cfm

edited by the coordinator. See Annex I for an overview of all the comments and for the list of comments actually placed on the EFSAnet on 27 June 2008.

The opinion of the EFSA Scientific Panel on GMOs was adopted on 2 December 2009 (The EFSA Journal, 2009, 7 (12):1417[4]), and published together with the responses of the EFSA GMO Panel to comments submitted by the experts during the three-month consultation period.

On 17 December 2009, the opinion of EFSA was forwarded to the Belgian experts. They were invited to give comments and to react if needed to the answers given by the EFSA GMO Panel, in particular in case the comments formulated in their initial assessment of the dossier were not taken into account in the opinion of EFSA.

The comments formulated by the experts together with the opinion of EFSA, including the answers of the EFSA GMO Panel, form the basis of the advice of the Biosafety Advisory Council given below.

Scientific evaluation

1. Environmental risk assessment

The scope of this application is food and feed materials which are produced from GM oilseed rape GT73 and only includes products which contain no viable plant parts. Therefore, there are no requirements to perform an environmental risk assessment in the context of this specific application. Such an assessment has already been performed in the frame of notification C/NL/98/11submitted under Directive 2001/18/EC.

2. Molecular characterisation

An updated bioinformatics analysis was performed on the insert and adjacent genomic DNA. The Biosafety Advisory Council is of the opinion that the information received is sufficient and raises no safety concerns.

3. Food and feed safety assessment and nutritional value

3.1. Assessment of compositional analysis
The Biosafety Advisory Council is of the opinion that the information provided on the composition of the GM oilseed rape does not raise any safety concerns

3.2. Assessment of toxicity
According to the Biosafety Advisory Council no major risks were identified concerning toxicity.

3.3. Assessment of allergenicity
Oilseed rape is not a major allergen source. The potential allergenicity of the newly introduced proteins has been assessed. With regard to allergenicity, the Biosafety Advisory Council is of the opinion that the information provided is sufficient and does not raise safety concerns.

3.4. Nutritional value
According to the Biosafety Advisory Council oilseed rape GT73 is as nutritious as its non-GM counterpart and a conventional oilseed rape variety.

[4] See: http://www.efsa.europa.eu/en/scdocs/scdoc/1417.htm

4. Monitoring

As the allergenicity of the whole GM oilseed has not been assessed, it is recommended to take up monitoring of allergenicity as part of the general surveillance.

Conclusion

Based on the scientific assessment of the dossier done by the Belgian experts, taking into account the opinion of EFSA, the answers of the EFSA GMO Panel to the questions raised by the Belgian experts, the answers of the applicant to the EFSA GMO Panel questions and considering the data presently available, the Biosafety Advisory Council,

Agrees with the GMO panel of EFSA that it is unlikely that the GT73 oilseed rape:
a) in the context of its proposed uses, would have any adverse effects on the environment.
b) would have any adverse effects on human or animal health.

In addition, the Biosafety Advisory Council recommends following up any unanticipated allergenicity aspects of the GM oilseed rape in monitoring systems.

p.o.

Prof. D. Reheul
President of the Belgian Biosafety Advisory Council

Annex: Full comments of experts in charge of evaluating application EFSA/GMO/RX-GT73 *and comments submitted on the EFSAnet (ref: BAC_2008_780)*

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27 June 2008

Compilation of comments of experts in charge of evaluating the application EFSA/GMO/RX-GT73
and
Comments submitted on the EFSAnet on mandate of the Biosafety Council

Mandate for the Group of Experts: mandate of the Biosafety Advisory Council (BAC) of 18 April 2008
Coordinator: René Custers
Experts: Pascal Cadot (Consultant), Armand Christophe (UGent), Jean-Pierre Hernalsteens (VUB), Peter Smet (Consultant), Nancy Terryn (UGent)
Domains of expertise of experts involved: Genetic engineering, genome analysis, transgene expression, nutrition, analysis of food/feed, immunology, alimentary allergology, toxicology, herbicide tolerance
Secretariat (SBB): Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman

INTRODUCTION

Dossier **EFSA/GMO/RX-GT73** concerns an application of the company **Monsanto** for the marketing of the genetically modified **oilseed rape GT73** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 28 March 2008.
The scope of the application is:
☐ GM plants for food use
☐ Food containing or consisting of GM plants
☒ Food produced from GM plants or containing ingredients produced from GM plants
☐ GM plants for feed use
☒ Feed produced from GM plants
☐ Import and processing (Part C of Directive 2001/18/EC)
☐ Seeds and plant propagating material for cultivation in European Union (Part C of Directive 2001/18/EC)

Depending on their expertise, the experts were asked to evaluate the genetically modified plant considered in the application on its 1) molecular, 2) allergenicity, 3) toxicity and/or 4) food and feed aspects. It was expected that the expert should evaluate if the information provided in the application is sufficient in order to state that the marketing of the genetically modified plant for its intended uses, will not raise any problems for the environment or human or animal health. If information is lacking,

the expert was asked to indicate which information should be provided and what the scientifically reasoning is behind this demand.

The comments are structured as in the "Guidance document of the scientific panel on genetically modified organisms for the risk assessment of genetically modified plants and derived food and feed" (EFSA Journal (2004), 99, 1-94). Items are left blank when no comments have been received either because the expert(s) focused on other related aspects, or because for this dossier the panel of experts who accepted to evaluate the dossier didn't have the needed expertise to review this part of the dossier.
It should be noted that all the comments received from the experts are considered in the evaluation of this dossier and in formulating the final advice of the Biosafety Advisory Council. Comments placed on the EFSAnet are indicated in grey.

List of comments received from the experts

A. GENERAL INFORMATION

Comments/Questions of the expert(s)

B. INFORMATION RELATING TO THE RECIPIENT OR (WHERE APPROPRIATE) PARENTAL PLANTS

Comments/Questions of the expert(s)

Comment 1

Complete and accurate description of the biology and ecology of oilseed rape and its relation to other *Brassicaceae* species. Although in this application no culture of the transgenic plants in the EU is involved, the risk of out-crossing is accurately considered.

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments/Questions of the expert(s)

Comment 1

Molecular analysis with both Southern blot and PCR, shows that GT73 contains a single inserted copy of the DNA present in the construct used for the transformation.
A small remark: it was not entirely clear to me why no additional restriction digests, like with an enzyme cutting only once or twice in the insert, could be done with the Southern analysis that would have made it clear whether it was a single or tandem insert.
There are some molecular changes at the insertion site, but these do not lead to new ORF's that could cause concern. In conclusion sufficient molecular characterization has been carried out.

Comment 2

Complete and accurate description of vector and the transformation method.

D. INFORMATION RELATING TO THE GM PLANT

D.1 DESCRIPTION OF THE TRAITS AND CHARACTERISTICS WHICH HAVE BEEN INTRODUCED OR MODIFIED

Comments/Questions of the expert(s)

Comment 1

The information in the application is accurate. Two genes encoding proteins that confer resistance to the herbicide glyphosate are inserted. These genes have been used without unexpected effects in experimental studies and in large scale cultivation of transgenic plants. The resulting resistance allows the use of glyphosate as a selective herbicide on these plants. This can be advantageous as glyphosate may be less toxic and have a lower negative impact on the environment than some other selective herbicides. This point is still under debate (Mamy *et al.*, 2008). Tolerant plants also allow the farmers to apply herbicide treatments only when required, instead of performing a systematic preventive herbicide treatment, and may therefore under optimal conditions reduce the use of herbicides.

D.2. INFORMATION ON THE SEQUENCES ACTUALLY INSERTED OR DELETED

Comments/Questions of the expert(s)

Comment 1

The data shown are consistent with the presence of a single T-DNA insert in the genome of line GT73. Unsurprisingly, the two glyphosate resistance cassettes are present intact. The absence of vector backbone sequences was demonstrated.

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comments/Questions of the expert(s)

Comment 1

The *CP4 EPSPS* gene encodes a glyphosate-insensitive EPSPS that performs the same reaction as the plant enzyme. On the other hand, the GOXv247 enzyme converts glyphosate to the novel compound aminomethylphosphonic acid. This may be relevant for the toxicological risk evaluation.
Both genes are expressed under the control of the same constitutive FMV promoter. Therefore the data proving expression of both proteins in seeds are not surprising.

D.4. INFORMATION ON HOW THE GM PLANT DIFFERS FROM THE RECIPIENT PLANT IN: REPRODUCTION, DISSEMINATION, SURVIVABILITY

Comments/Questions of the expert(s)

Comment 1

The inserted genes will not influence the reproduction or survival of the plants when no herbicide is applied. Therefore plants resulting from seeds that would be accidentally released into the environment would not be more persistent or invasive than non-transgenic oilseed rape plants.

D5. GENETIC STABILITY OF THE INSERT AND PHENOTYPIC STABILITY OF THE GM PLANT

Comments/Questions of the expert(s)

Comment 1

T-DNA inserts are known to be stably inherited. Unexpected instability above the detection level would have been noticed during the field tests preceding the release of this plant variety.

D.6. ANY CHANGE TO THE ABILITY OF THE GM PLANT TO TRANSFERR GENETIC MATERIAL TO OTHER ORGANISMS

Comments/Questions of the expert(s)

Comment 1

Due to the nature of the inserted sequences, and the resulting wild type morphology of the transgenic plants, such changes are indeed not expected.

D.7. INFORMATION ON ANY TOXIC, ALLERGENIC OR OTHER HARMFUL EFFECTS ON HUMAN OR ANIMAL HEALTH ARISING FROM THE GM FOOD/FEED

D.7.1 Comparative assessment

Comments/Questions of the expert(s)

Comment 1

The choices of the comparators for GT73 and of the varieties obtained by backcrossing are considered to be logical. No problems

Comment 2

a) Composition analysis of seed.

Proximates		**Minerals**	
moisture	X	calcium	
protein	X	copper	
fat	X	iron	
ash	X	magnesium	
carbohydrates	X	manganese	
acid detergent fiber (ADF)		phosphorus	
neutral detergent fiber (NDF)		potassium	
total detergent fiber (TDF)	X	selenium	
starch		sodium	
		zinc	
		total nitrogen	

Vitamins		**Amino acids**		**Fatty acids**		**Secondary metabolites**		**Antinutrients**	
A (β-carotene)		alanine	X	14:0 myristic		ferulic acid		phytic acid	
B1 (thiamine)		arginine	X	15:0 pentadecanoic					
B2 (riboflavin)		asparagine		16:0 palmitic	X	furfural		raffinose	
B3 (niacin)		aspartic acid	X	16:1 palmitoleic	X	inositol		trypsin inhibitor	
B6 (pyridoxine)		cysteine	X	18:0 stearic	X	*p*-coumaric acid		gossypol	
B9 (folic acid)		glutamic acid	X	18:1 oleic	X			malvalic acid	
C (ascorbic acid)		glycine	X	18:2 linoleic	X			sterculic acid	
E (α-tocopherol)		histidine	X	18:3 linolenic	X			dihydrosterculic acid	
		isoleucine	X	20:0 arachidic	X			sinapine	X
		leucine	X	20:1 gadoleic	X			glucosinolate	X
		lysine	X	20:2	X				
		methionine	X	22:0 behenic	X				
		phenylalanine	X	22.1 erucic acid	X				
		proline	X	24:0 lignoceric	X				
		serine	X	24:1	X				
		threonine	X						
		tryptophan	X						
		tyrosine	X						
		valine	X						

Some statistically significant differences were noted. In both years, the **protein** values for GT73 were significantly higher compared to Westar (p = 0.05). However, since these results are not consistently noted in the proximate analyses or from year-to-year, they are likely to be due to random variation. In addition, the values measured for GT73 are within the range of values reported for Canadian canola varieties (Co-Op Westar).

One significant difference was noted: in 1993, the **fat** level was significantly higher in GT73 than in Westar (p = 0.05). Since this result was not consistently noted in the proximate analyses or from year-to-year, it is likely to be due to random variation. In addition, GT73 values are within the range of values reported for Canadian canola varieties (Co-Op Westar).

There was no statistically significant difference between GT73 and its control (p = 0.05) in both years. On this basis, it is possible to reaffirm the conclusion of compositional equivalence between GT73 and conventional oilseed rape with respect to **proximate** composition.

The fact that the level of **aromatic amino acids** in GT73 is not substantially different from Westar is in accordance with the conclusion that 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) is not the rate limiting enzyme in aromatic amino acid biosynthesis in bacteria and plants (Herrmann, 1983).

The results of the **fatty acid** profile analyses support the conclusion that GT73 is compositionally equivalent to conventional oilseed rape.

GT73 and Westar are compositionally equivalent in the terms of choline esters levels in oilseed rape seed.

While it is apparent that the average level of alkyl glucosinolate in GT73 is consistently higher than the mean value for Westar, the following comments must be made:
1) all individual values are well below the 30 µmole/g limit for Canada or the 25 µmole/g limit for Europe for alkyl glucosinolates;
2) at the 95% confidence level, the values of total glucosinolates in GT73 do not exceed the 30 µmole/g seed limit
3) initial differences in glucosinolate levels between GT73 and Westar can be understood because GT73 has been selected from a line coming from a single cell of a single individual from the Westar population.

No mineral content has been determined. As a result no comparison with a control has been performed.

b) Composition analysis of toasted meal.

Proximates		**Minerals**	
moisture	X	calcium	X
protein	X	copper	X
fat	X	iron	X
ash	X	magnesium	X
carbohydrates	X	manganese	X
acid detergent fiber (ADF)		phosphorus	X

neutral detergent fiber (NDF)		potassium	X
total detergent fiber (TDF)		selenium	
starch		sodium	
		zinc	X
		total nitrogen	

Vitamins		**Amino acids**		**Fatty acids**		**Secondary metabolites**		**Antinutrients**	
A (β-carotene)		alanine	X	14:0 myristic		ferulic acid		phytic acid	X
B1 (thiamine)		arginine	X	15:0 pentadecanoic					
B2 (riboflavin)		asparagine		16:0 palmitic		furfural		raffinose	
B3 (niacin)		aspartic acid	X	16:1 palmitoleic		inositol		trypsin inhibitor	
B6 (pyridoxine)		cysteine	X	18:0 stearic		*p*-coumaric acid		gossypol	
B9 (folic acid)		glutamic acid	X	18:1 oleic				malvalic acid	
C (ascorbic acid)		glycine	X	18:2 linoleic				sterculic acid	
E (α-tocopherol)		histidine	X	18:3 linolenic				dihydrosterculic acid	
		isoleucine	X	20:0 arachidic				sinapine	
		leucine	X	20:1 gadoleic				glucosinolate	X
		lysine	X	20:2					
		methionine	X	22:0 behenic					
		phenylalanine	X	22.1 erucic acid					
		proline	X	24:0 lignoceric					
		serine	X	24:1					
		threonine	X						
		tryptophan	X						
		tyrosine	X						
		valine	X						

The levels of macronutrients (protein, ash, fat, fibre, carbohydrate), amino acids and minerals measured in GT73 toasted meal are comparable to the levels observed in Westar.

Oilseed rape meal is rich in many of the essential minerals. Phytic acid has been demonstrated to adversely affect the uptake of phosphorus, calcium, magnesium and zinc in animal diets (Nwokolo and Bragg, 1977). High levels of phytic acid, a hexaphosphorylated inositol, reduce the availability of these essential minerals in meal.
The values for all of the minerals and phytic acid levels in GT73 meal fell within the literature ranges and correspond to Westar level.

The glucosinolate content of single meal samples from GT73 and Westar was measured. The value for alkyl glucosinolates was 10.5 µmole/g defatted meal (butenyl, pentenyl, hydroxybutenyl and hydroxypentenyl glucosinolates) and 4.7 µmole/g defatted meal for GT73 and Westar, respectively. The approximately two-fold difference in alkyl glucosinolates is possibly a result of differential loss upon processing. Q: Aren't the processed in exactly the same way?

c) Composition analysis of oil.

Vitamins		**Amino acids**		**Fatty acids**		**Secondary metabolites**		**Antinutrients**	
A (β-carotene)		alanine		14:0 myristic	X	ferulic acid		phytic acid	
B1 (thiamine)		arginine		15:0 pentadecanoic					
B2 (riboflavin)		asparagine		16:0 palmitic	X	furfural		raffinose	
B3 (niacin)		aspartic acid		16:1 palmitoleic	X	inositol		trypsin inhibitor	
B6 (pyridoxine)		cysteine		18:0 stearic	X	*p*-coumaric acid		gossypol	
B9 (folic acid)		glutamic acid		18:1 oleic	X			malvalic acid	
C (ascorbic acid)		glycine		18:2 linoleic	X			sterculic acid	
E (α-tocopherol)		histidine		18:3 alpha-linolenic	X			dihydrosterculic acid	
		isoleucine		20:0 arachidic	X			sinapine	
		leucine		20:1 gadoleic	X			glucosinolate	
		lysine		20:2					
		methionine		22:0 behenic	X				
		phenylalanine		22.1 erucic acid	X				
		proline		24:0 lignoceric	X				
		serine		24:1	X				
		threonine							
		tryptophan							
		tyrosine							
		valine							

The levels of the major fatty acids in refined, bleached, deodorized oilseed rape oil are comparable in GT73 and conventional oilseed rape and are within CODEX specifications.

D.7.2 Production of material for comparative assessment

Comments/Questions of the expert(s)

Comment 1

No problems

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

Comment 1

It is recognised in the dossier that tannins, saponins and sinapine are substances that may restrict the use of oil seed rape meal in animal foodstuffs (part II, page 12). Thus it is surprising that no values for tannins and saponins are reported. However, based on the animal feeding studies reported it is expected that the meal of rapeseed GT73 poses no extra problem compared to regular rapeseed meal. Nutritional properties of non-modified canola meal have been published (Animal Feed Resources Information System).

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

D.7.5 Product specification

Comments/Questions of the expert(s)

D.7.6 Effect of processing

Comments/Questions of the expert(s)

Comment 1

I agree with the statements. No problems expected.

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

Comment 1

I agree with the statements. No problems expected.

D.7.8 Toxicology

Comments/Questions of the expert(s)

Comment 1

Mean concentrations of:

a) GOXv247 protein measured in GT73.

Tissue	ng/mg Tissue Fresh Weight		Standard deviation
	Mean	Range	
Leaf	133	82-247	45
Seed	211	122-313	61

Please provide data based on dry weight.

b) CP4 EPSPS protein measured in GT73.

Tissue	ng/mg Tissue Fresh Weight		Standard deviation
	Mean	Range	
Leaf	27	16-70	8
Seed	28	17-37	8

For reasons of comparison the question has been raised to provide the data on the GOXv247 and CP4 EPSPS concentrations in leaf and seed in ng/mg Dry Weight.

D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

Comment 1

Safety assessment of newly expressed proteins.

a) Degradation of the GOXv247 protein in simulated gastric fluid (Ream, 1994).

More than 90% of the initially added GOXv247 protein degraded after 15 seconds incubation, as detected by western blot analysis (Ream, 1994). The enzymatic activity of the GOXv247 protein also decreased readily. More than 96% of the GOXv247 activity dissipated after 1 minute of incubation in SGF, the earliest time point measured.

b) Degradation of the GOXv247 protein in simulated intestinal fluid (Ream, 1994).

Under SIF conditions, the GOXv247 protein also degraded rapidly. More than 90% of the initially added GOXv247 protein degraded after 0.5 minutes of incubation as detected by western blot analysis of samples taken at defined intervals (Ream, 1994). The enzymatic activity of the GOXv247 protein also dissipated readily in SIF. More than 95% of the GOXv247 enzymatic activity dissipated after 60 minutes.

c) GOXv247: Acute Oral Toxicity Study in Mice (Naylor, 1994a).

The *E. coli* purified GOXv247 protein was administered by gavage to three groups of male and female mice at dose levels of 0, 1.08, 11.3, and 104 mg/kg body weight. Two additional groups of control mice were gavaged with an extract of *E. coli* protein obtained from *E. coli* containing the same vector used to produce GOXv247, but lacking the *goxv247* gene (hollow vector control). Another control group of mice was administered the carbonate buffer that was used in the test groups to dialyze the proteins (vehicle control).
No adverse effects were observed in mice to which the GOXv247 protein was administered. There were no statistically significant differences in body weight, cumulative body weight or food consumption between the vehicle controls, hollow vector controls or GOXv247 protein treated groups. No grossly observable pathologic changes were observed in mice at necropsy that were considered related to treatment.

d) Degradation of the CP4 EPSPS protein in simulated gastric fluid (Ream et al., 1993).

The data demonstrated a half-life for the CP4 EPSPS protein of less than 15 seconds in the gastric system, based on western blot analysis.

e) Degradation of the CP4 EPSPS protein in simulated intestinal fluid (Ream et al., 1993).

More than 50% of CP4 EPSPS protein was degraded in the simulated intestinal system in less than 10 minutes, based on western blot analysis.

f) CP4 EPSPS: Acute Oral Toxicity Study in Mice (Naylor, 1993).

In the study, the *E. coli*-purified CP4 EPSPS protein was administered as a single dose by gavage to groups of 10 male and 10 female CD-1 mice at dose levels up to 572 mg/kg.
There were no treatment-related effects on survival, clinical observations, body weight gain, food consumption or gross pathology. Therefore, the No Observed Effect Level (NOEL) for CP4 EPSPS was considered to be equal to or greater than 572 mg/kg, the highest dose tested.

The CP4 EPSPS content in oil seed rape is about 5 till 10 times as low as the GOXv247 content. Why then is the highest used dose in acute toxicity testing about 5 times smaller for GOXv247 compared to CP4 EPSPS?

g) Structural similarity of the GOXv247 and CP4 EPSPS proteins to known toxins

GOXv247 and CP4 EPSPS proteins were compared to peptide sequences identified as toxins from all available protein databases. The FASTA algorithm was used to conduct the amino acid homology comparison between the test proteins and all available sequenced toxins from all available electronic databases of protein sequences (Gribskov and Devereux, 1991; Pearson, 1990; Pearson and Lipman, 1988; Wilbur and Lipman, 1983).
The evidence indicates that neither the GOXv247 nor the CP4 EPSPS proteins share any significant sequence similarity with the database of known sequenced protein toxins.

D.7.8.2 Testing of new constituents other than proteins

Comments/Questions of the expert(s)

Comment 1

Although it is realised that the residue issue falls outside the scope of Regulation 1823/2003, I think that it is worthwhile to take a stand about the question whether components that are taken up by the plant without killing it due to the genetic modification, and their subsequent metabolites are to be considered as residues or rather as new constituents. Indeed, they could be accumulated in plant tissues and not be reduced by cleaning procedures. Accumulation of glyphosate and is major metabolite AMPA has been described in transgenic glyphosate-resistant soybean (Aregui et al., 2004). In the case of GT73 this may not be a problem. Major metabolites in the plant are the same as in the environment and it is claimed as a comment in the broiler feeding study that pesticide levels (which ones?) in GT73 were below the limit of detection (Part I, page 135). An “estimate of acceptable daily intakes for humans” has been made for glyphosate and AMPA (Joint Meeting of the FAO Panel of Experts on Pesticide residues in Food and the Environment and the WHO Core Assessment Group, 1997).

D.7.8.3 Information on natural food and feed constituents

Comments/Questions of the expert(s)

Comment 1

In contrast to part II, page 12, saponins and tannins are no longer mentioned here (part II, page 22).

D.7.8.4 Testing of the whole GM food/feed

Comments/Questions of the expert(s)

Comment 1

Based on several animal feeding experiments and on the history of human consumption, GT73 seems to be safe.

Comment 2

a) 42-day feeding study with broiler chickens (Stanisiewski *et al.*, 2001; Stanisiewski *et al.*, 2002; Taylor *et al.*, 2004)

Sixty two out of the 800 total birds died during the study. The distribution of the broilers that died from day 7 to study termination was random across treatments (deaths per treatments averaged 7.8% and ranged from 2% to 17% across all treatments). Most of the apparent causes of death were identified at necropsy and occur commonly in chickens (sudden death syndrome and ascites). The highest mortality occurred in the treatment group fed the parental control (17%), followed by the test line (13%). The mortality in this study was slightly higher than expected; the majority of deaths occurred in the males, which can be expected since males are heavier and grow faster than females. A possible explanation for the high mortality rate could be that canola meal was incorporated into diets at an upper extreme (25% wt/wt canola meal during the first 20 d and 20% wt/wt canola meal thereafter) relative to industry practice (12-15%). However, broilers in all treatment groups were in good health based on twice daily pen observations. The starting and final body weights of the chicks were normal and the average body weight gain/bird values were comparable between treatments.

In both Stanisiewski *et al*., 2001 and Stanisiewski *et al*., 2002 only the CP4 EPSPS protein is mentioned. What about the GOXv247?
In Taylor *et al*., 2004 both are mentioned.

b) 90-day rat feeding study (author).

No further testing is needed.

c) 4-week feeding studies with rats (Naylor, 1994b; Naylor, 1995; Naylor, 1996).

Glucosinolates and their reactive metabolites have been implicated as possible causes of liver hypertrophy in rodents fed oilseed rape meal (Vermorel *et al*., 1988).

d) 10-week and 8-week feeding study with trout fed processed oilseed rape meal (Brown *et al.*, 1994; Brown *et al.*, 1996, *Brown et al., 2003*).

Processed oilseed rape meal derived from GT73 and Westar seed (a conventional oilseed rape variety with similar background genetics to GT73) was administered in the diet to rainbow trout at graded levels of substitution (5, 10, 15 or 20% of the dry diet) for approximately 10 weeks. Additionally, one diet contained no oilseed rape meal (diet control).

Survival of fish in all groups was very good in the study with only a few deaths occurring randomly in some groups fed GT73 or Westar. Fish fed diets containing oilseed rape meal exhibited slightly lower weight gain and feed efficiencies than fish fed the diet control. Overall, fish fed any level of processed oilseed rape meal exhibited lower weight gain and feed efficiency than fish fed the diet control.
In summary, there were no adverse effects observed in the 10 week trout feeding study with GT73.

e) 5-day feeding studie with quail (Campbell and Beavers, 1994; Campbell *et al*., 1994).

Unprocessed oilseed rape meal derived from GT73 and Westar seed (a conventional oilseed rape variety with similar background genetics to GT73) was fed to bobwhite quail of mixed sex at a level of 20% of the dry diet (w/w) for 5 days (Campbell *et al*., 1994). Afterwards, each control and treatment group was switched to basal (unsupplemented) diets for the 3 final days of the study. Additionally, one group of quail was fed a diet containing no oilseed rape meal for 8 days (diet control).
There was no mortality observed during the study.
For birds receiving Glyphosate tolerant canola seed meal there was a slight reduction in body weight gain during the exposure period (Day 0 to Day 5). However, there were no apparent effects on total body weight gain over the duration of the test pay 0 to Day 8).

f) swine feeding studie (Aalhus *et al*., 2003).

One hundred fortyfour pigs were fed grower and finisher diets formulated with oilseed rape meal derived from GT73, a conventional oilseed rape variety with similar background genetics to GT73 (*i.e.* the control) and two commercially available oilseed rape varieties.
Average daily gains, daily feed intakes, and feed conversion efficiencies were similar when feeding GT73 and control diets, but some differences from the commercial diets were noted ($p \geq 0.05$), particularly during the finisher phase. Postmortem pH and temperature declines were similar across diets indicating postmortem metabolism proceeded normally in carcasses from all diets. Carcass and meat quality evaluation indicated only small differences amongst dietary treatment, and no differences between the GT73 and control diets.

g) lamb feeding studie (Stanford *et al*., 2003; Stanford *et al*., 2002).

Four diets with the same total protein content were prepared including oilseed rape meal (6.5% on dry weight basis) from four different sources (GT73, a conventional oilseed rape variety with similar background genetics to GT73, *i.e.* the control, and two commercially available oilseed rape varieties). Diet did not affect ($p > 0.05$) average daily gain or feed efficiency. Carcass yield grade was higher ($p < 0.05$) for the two commercial diets than for GT73 or the control, although carcass composition did not differ ($p > 0.05$) between GT73 and the control. The oilseed rape source did not affect ($p > 0.05$) meat tenderness, as determined by shear force, drip loss or intramuscular fat content. Meat colour of GT73 fed lambs did not differ from that of all other treatment groups. In this study, including oilseed rape meal prepared from GT73 did not alter diet digestibility, feed efficiency, growth performance, carcass characteristics or meat quality of lambs.

For both the pig and lamb study, it would have been interesting to incorporate a group, fed with a diet containing no oilseed rape meal.
At this moment, no further testing is needed.

D.7.9 Allergenicity

Comments/Questions of the expert(s)

Comment 1

Assessment of the allergenicity of the newly expressed proteins.
CP4EPSPS has already been defined as allergy safe by EFSA. There is no new data to contest this statement. GOXv247 is not likely to be allergenic.

Assessment of the allergenicity of the whole GM plant or crop.
The applicant did not evaluate the potential allergenicity of oilseed rape GT73, mainly on the basis that oilseed rape is not an allergen source. However, rapeseed allergy has been recently described and 2S albumin has been demonstrated as being an allergen of oilseed rape (Poikonen, 2008; Puumalainen, 2006). The 2S albumins are seed pan-allergens. Of note, the determination of oilseed rape allergenicity in the aforementioned references relied on skin testing with crushed seeds, which is not a form consumed by humans. Therefore, it might be argued that oilseed rape being only used to make refined oils in human diet, and refined oils being claimed to be devoid of proteins, conversely to crude oils, this rules out the possibility of allergic reaction against oilseed rape allergens. However, traces of proteins in quantities enough to induce allergic reactions were found in refined peanut oil (Olszewski, 1998), which shows that it might be possible to react after ingestion of refined oil.
Therefore, although there is probably no allergy risk in the overwhelming majority of allergic population, it might be relevant to determine the levels of 2S albumin, but also of vicillin (another known seed pan-allergen family) in oilseed rape GT73, as compared to a natural counterpart. This is relevant particularly because the introduction of the new traits might have influenced the expression levels of these allergens in the GMO plant.

D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

Comment 1

As to humans, no change in dietary intake of rapeseed oil or nutritional imbalances by using GT73 oil is expected. No unanticipated effects were found in the animal feeding studies.

D.7.11 Post-market monitoring of GM food/feed

Comments/Questions of the expert(s)

D.8. MECHANISM OF INTERACTION BETWEEN THE GM PLANT AND TARGET ORGANISMS (IF APPLICABLE)

Not applicable

D.9. POTENTIAL CHANGES IN THE INTERACTIONS BETWEEN THE GM PLANT WITH THE BIOTIC ENVIRONMENT RESULTING FROM THE GENETIC MODIFICATION

D.9.1. Persistence and invasiveness

Not applicable

D.9.2 Selective advantage or disadvantage

Not applicable

D.9.3 Potential for gene transfer

Not applicable

D.9.4 Interactions between the GM plant and target organism

Not applicable

D.9.5 Interactions of the GM plant with non-target organism

Not applicable

D.9.6 Effects on human health

Comments/Questions of the expert(s)

Comment 1

GT73 oil and food products in which it is incorporated are likely to be as safe and nutritious as their counterparts derived from conventional canola oil.

D.9.7 Effects on animal health

Comments/Questions of the expert(s)

Comment 1

No adverse effects of GT73 meal different from those of non-transgenic canola meal are expected.

D.9.8 Effects on biogeochemical processes

Not applicable

D.9.9 Impacts of the specific cultivation, management and harvesting techniques

Not applicable

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

Not applicable

D.11. ENVIRONMENTAL MONITORING PLAN

Not applicable

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