Biosafety Advisory Council

05-05-2008

Secretariat

O./ref.: WIV-ISP/BAC/2008_727
Email: bac@sbb.ihe.be

Title: Advice of the Belgian Biosafety Advisory Council on the applications **EFSA/GMO/UK/2005/25 and EFSA/GMO/RX/T45** from Bayer CropScience under Regulation (EC) No. 1829/2003

Context

Application EFSA/GMO/UK/2005/25

The application EFSA/GMO/UK/2005/25 was submitted by Bayer CropScience on 4 November 2005 for the marketing (import and processing) of the glufosinate-tolerant genetically modified oilseed rape T45 for food and feed applications under Regulation (EC) No. 1829/2003[1]. It was officially acknowledged by EFSA on 13 April 2007.

On the same date EFSA started the formal three-month consultation period 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 to evaluate the dossier, chosen from the common list of experts drawn up by the Biosafety Advisory Council and the Division of Biosafety and Biotechnology (SBB). Ten experts answered positively to this request, and formulated a number of comments to the dossier which were 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 11 July 2007.

[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)

The opinion of the EFSA Scientific Panel on GMOs was adopted on 30 January 2008 (The EFSA Journal, 2008, 635, 1-22)[2], and published together with the responses from the EFSA GMO Panel to comments submitted by the experts during the three-month consultation period.

On 6 March 2008 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.

Application EFSA/GMO/RX/T45

The application EFSA/GMO/RX/T45 was submitted by Bayer CropScience on 29 June 2007 for renewal of the authorisation of GM oilseed rape T45 as existing products (food additives and feed materials produced from oilseed rape T45) within the framework of Regulation (EC) No. 1829/2003. It was officially acknowledged by EFSA on 7 September 2007.

All data required for the risk assessment of this application have also been provided in application EFSA/GMO/UK/2005/25. In consequence, the Biosafety Advisory Council issues a single comprehensive advice covering both applications.

Scientific evaluation

Regarding the risk assessment in general, the Biosafety Advisory Council is of the opinion that given the fact that the T45 oilseed rape has been fully discontinued from commercial sales, it will enter the European market only as a result from factors such as volunteers or adventitious presence in conventional and/or other genetically modified varieties. Over the years any presence of T45 oilseed rape in imports to the European Union will most likely be fading out. This has some consequences for the risk assessment, as there will be no possibility for a recurrent introduction of T45 oilseed rape at a continuous (high) level. The presence of T45 oilseed rape in food and feed products is expected to be restricted to low adventitious levels in oilseed rape commodity.

[2] see: http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178690393760.htm

According to the Biosafety Advisory Council, no safety concerns were identified neither concerning the molecular characterisation nor the environment[3].
The Biosafety Advisory Council agrees with the EFSA GMO Panel that in the event that accidental spillage would occur resulting in GM plants that might establish, survive and outcross, hybrids with other oilseed rape varieties or wild relatives would not show any enhanced fitness.
The Biosafety Advisory Council nevertheless supports the view that appropriate management systems should be in place to minimize accidental loss and spillage of transgenic oilseed rape during transportation, storage and handling in the environment and processing into derived products.

The Biosafety Advisory Council is of the opinion that the compositional analysis of this genetically modified oilseed rape and the assessment of its allergenicity do not raise any health safety concerns in the context of both current applications.

As the T45 oilseed rape is shown to be substantially equivalent to relevant conventional comparators, except for the presence of the PAT protein, animal trials are not required according to the EFSA guidelines. The applicant did however perform broiler feeding trials to test the nutritional value of the T45 oilseed rape compared to relevant conventional varieties. However, owing to an insufficient power of the statistical analysis and/or the sensitivity of the trials, the results of these feeding trials were not conclusive and did not allow to draw a scientific conclusion on the nutritional value with the required level of certainty. In this particular case however, the GMO, if any, will only be present in trace amounts in EU imports, and therefore any differences in nutritional value are very unlikely to result in any effect.

General 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, and considering the data presently available,
the Biosafety Advisory Council can agree with the overall conclusion of the GMO panel of EFSA that: "it is unlikely that oilseed rape T45 will have any adverse effects on human and animal health or on the environment in the context of its intended uses".
The Biosafety Advisory Council also supports the request from EFSA that appropriate management systems should be in place to minimize accidental loss and spillage of transgenic oilseed rape during transportation, storage and handling in the environment and processing into derived products and that, within general surveillance, specific measures are introduced to actively monitor the occurrence of feral oilseed rape plants in areas where seed spillage is likely to occur.

[3] As the application doesn't imply a cultivation of the plant in EU, a full environmental assessment is not required in EFSA procedure and was not achieved.

The Biosafety Advisory Council is of the view that the power of the statistical analysis and/or the sensitivity of the tests performed on animals for toxicological and nutritional assessment need to comply with standards of good statistics in order to allow for scientifically sound conclusions with the required level of certainty. Indeed, in order to support the results of the compositional analysis, animal trials, when they are performed, must be conducted adequately.

i.o.

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

Annex : Full comments of experts in charge of evaluating application EFSA/GMO/UK/2005/25 and comments submitted on the EFSAnet (ref: BAC_2007_PT_555)

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Bioveiligheidsraad
Conseil de Biosécurité

10/07/2007

Secretariaat
Secrétariat

N./réf. : WIV-ISP/BAC_2007_PT_555
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Application EFSA/GMO/UK/2005/25
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 23 April 2007.
Coordinator: René Custers
Experts: Pascal Cadot (consultant), Armand Christophe (UGent), Rony Geers (KUL), Jean-Pierre Hernalsteens (VUB), Jean-Luc Hofs (FUSAGx), Wim Stevens (UA), Peter Smet (consultant), Nancy Terryn (UGent), Johan Van Waes (ILVO).
Domains of expertise of experts involved: genetics, genetic engineering, genome analysis, biosafety research, oilseed rape, agronomy, plant-insect relations, herbicide tolerance, outcrossing to wild relatives, effect on non-target species, horizontal gene transfer, impact on biodiversity, immunology, alimentary allergology, nutrition, animal nutrition, analysis of food/feed, additives for feed, toxicology in vitro.
Secretariat: Didier Breyer, Adinda De Schrijver, Martine Goossens

INTRODUCTION

Dossier **EFSA/GMO/UK/2005/25** concerns an application of the company **Bayer Crop Science** for the marketing of the genetically modified **oilseed rape T45** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 13 April 2007.
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)

List of comments submitted on the EFSAnet

The comments from the experts have been summarized by the coordinator. For the full comments of the Belgian experts and the bibliographic references we refer to the document given in annex 2 (ref. BAC_2007_PT_554)

A. GENERAL INFORMATION

Comments submitted on the EFSAnet

Our experts have made remarks on the fact that this dossier concerns a GMP that has been fully discontinued from commercial sales. Concerns have been raised that this type of dossier may be a waste of expert time and community money. There is a feeling that the application is only done to avoid problems like with the LLrice contamination. The fact is that the T45 oilseed rape is very unlikely to be grown any longer. T45 oilseed rape (or its transgenic allele) may however be able to enter the European market resulting from several factors such as farm saved seed, volunteers and adventitious presence in conventional and/or other genetically modified varieties such as MS8 x RF3. Over the years any presence of T45 oilseed rape in imports to the European Union will most likely be fading out.
This has some consequences for the risk assessment as there will be no possibility for a recurrent introduction of T45 oilseed rape at a continuous (high) level. In general it is not unlikely that a feral population of oilseed rape arises as a consequence of seed spillage in disturbed areas such as road sides. Chances that these are T45 plants are however small. And because of the most probable fading out of the presence of T45 oilseed it is unlikely that a T45 feral population can be renewed, or even expand. The fact that tolerance to glufosinate-ammonium does not give a selective advantage adds onto this.

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

Comments submitted on the EFSAnet

Sexual compatibility with other cultivated or wild plant species

Some comments have been raised with regard to the information given in this part of the dossier. Basically these comments come down to the fact that the possibilities for outcrossing of oilseed rape to a number of wild relatives are real and should not be minimized.

The applicant agrees that the possibility of gene flow from oilseed rape to wild relative under natural conditions has been reported, "*mostly under optimal condition"* for 4 species. Although these experiments were conducted under natural conditions, conditions were obviously not fully optimal as in a controlled lab environment. Canola is well adapted to European weather conditions (rain and temperature [12°-30°C]), and its weedy relatives as well. This fact shouldn't be minimized.

Wild radish is the most widespread and risky relative of oilseed rape. Despite the low fitness level of the *B.napus x R. raphanistrum* cross in F1, male and female fertility increase over successive backcross generations (Chèvre et al. 1997a; 1999).

Survivability

Seeds can germinate at port facilities under the environmental conditions described in the report. If 20°C is an optimum, canola can germinate and grow from 13-30°C. It is said that canola germinates easily after harvesting when it is not deep buried: this is important in the case of seed spilling in ports and crushing mill premises. The question is raised whether the reduction in the number of days of frost due to the climate change has an effect on the survivability and whether this should be taken into account for future monitoring.
Seeds will be transported in bulk via rivers. Given that canola seeds can be primed by soaking during at least 24 hours (Elias & Copeland, 1997; Zheng et al., 1998; Shekari et al., 2000), what is the survivability rate and duration of canola seeds in the water?

Dissemination

With regard to the ways and the extent of dissemination a number of relevant references are not mentioned in the dossier; for instance Lavigne et al. (1998), Rieger et al. (2002) and Thompson et al. (1999). In these publications oilseed rape pollen flow is reported over longer distances than mentioned by the applicant in the dossier.
Insect mediated pollen dispersal, in general, is strengthened by gaps between otherwise adjacent populations (Reboud, 2003). That means that hybridization rates are higher when the pollen source is moderately distant (10-20 meters) from the sink. This is exactly the type of situation one may have in an industrial area with several patches of feral oilseed rape that have arisen as a result of seed spilling. In these situations the insect mediated polled dispersal may be higher than in other situations
Some authors (Ellstrand el al., 1989; Snow et al., 2005) suggested that gene flow depends also on ecological factors such as distance, location, climate and season and should not be considered as the fact of the crop alone.
The applicant states that successful pollination decreases exponentially within increasing distance. This is true for short distances but not at the landscape scale (Devaux et al., 2007), which calls for a geometric dispersal kernel.

Special factors
Human activity (farming and especially transport (Agrisearch, 2001 and OGTR, 2002) is the major source of long distance seed dissemination. The seed is small and can be easily spilled out.

Other potential interactions….
A comment was made that biosafety can't rely upon crop pests to significantly contain GM plant reproduction, because interactions are tremendously variable and depend on environmental conditions. Sometimes pests can help, but that is all.

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments submitted on the EFSAnet

The information in this part of the dossier was regarded adequate.

D. INFORMATION RELATING TO THE GM PLANT

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

Comments submitted on the EFSAnet

The information in this part of the dossier was regarded adequate.

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

Comments submitted on the EFSAnet

Enough information is given on the sequences actually inserted and the *in silico* analysis done on newly created ORFs is in line with the EFSA guidance on this point.

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comments submitted on the EFSAnet

The information in this part of the dossier was regarded adequate.

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

Comments submitted on the EFSAnet

(a) mode or rate of reproduction

The chances of presence of any GM oilseed rape plants are very small and will be the result of accidental seed spillage. A remark is made that the closed system mentioned in the dossier may be quite relative.

A small remark: In the Belyk and MacDonald report (1995), the Net replacement Rate equation should be NRR= number seed collected x (number seed sown)$^{-1}$ instead of NRR= number seed collected x number seed sown. The reason of the nil values for HCN28 is not discussed in the dossier.

(b) Dissemination

In the last chapter of the dossier the Net Replacement Rates (NRR) are very variable according to the place and always above 1 except for HCN28. Why is it the case taking into account that no differences between cultivar types have been observed.

(c) Survivability

The applicant has a tendency to minimize the probability of seed spilling in ports facilities. However, this has already occurred: Saji et al. (2005) [only reported in the Commission Recommendation (2005)] found the existence of feral glufosinate-ammonium resistant oilseed rape in Japanese ports. As no transgenic plants have been intentionally introduced in that country, the authors suggested that the transgene would have come from imported bulk seed for industry.

The results given in MacDonald & Kuntz (2000a) are relevant in the Canadian environment but may be less relevant in the European environment, specially related to phenology and climate.

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

Comments submitted on the EFSAnet

The information in this part of the dossier was regarded adequate.

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

Comments submitted on the EFSAnet

The information in this part of the dossier was regarded adequate.

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 submitted on the EFSAnet

The remark has been made that the results of the by-site t-tests comparing the transgenic crop treated with Liberty® or with conventional herbicide (B vs C) could substantiate the claim that the application of glufosinate ammonium does not result in any secondary effects**.**

D.7.2 Production of material for comparative assessment

Comments submitted on the EFSAnet

The information in this part of the dossier was regarded adequate.

D.7.3 Selection of material and compounds for analysis

Comments submitted on the EFSAnet

The information in this part of the dossier was regarded adequate.

D.7.4 Agronomic traits

Comments submitted on the EFSAnet

The information in this part of the dossier was regarded adequate.

D.7.5 Product specification

Comments submitted on the EFSAnet

The information in this part of the dossier was regarded adequate.

D.7.6 Effect of processing

Comments submitted on the EFSAnet

The information in this part of the dossier was regarded adequate.

D.7.7 Anticipated intake/extent of use

Comments submitted on the EFSAnet

The information in this part of the dossier was regarded adequate.

D.7.8 Toxicology

Comments submitted on the EFSAnet

It is regarded unlikely that a relevant amount of the PAT protein or parts of it, will become available for uptake through the mucose of the small intestine.

D. 7.8.1 Safety assessment of newly expressed proteins

Comments submitted on the EFSAnet

The additional test by i.v. injection of the PAT protein gives additional reassurance that it does not have any acute toxicity.

D.7.8.2 Testing of new constituents other than proteins

Comments submitted on the EFSAnet

The information in this part of the dossier was regarded adequate.

D.7.8.3 Information on natural food and feed constituents

Comments submitted on the EFSAnet

It is requested to clarify the paragraph on page 69 of the dossier starting with the words "Equivalence of the non-transgenics….". This paragraph does not seem to be supported by the values in table 17 (cystine is mentioned in the table; cysteine in the text; no obvious differences for histidine?).

The term "Linolenic acid (C18:3)" (page 74 and further) is poorly defined as there are 2 different "linolenic" acids with different biological effect. Non transgenic rapeseed oil contains only alpha-linolenic acid (C18:3ω3) but there are seed oils that contain gamma-linolenic acid (18:3 ω6). Is the "linolenic acid" reported in the transgenic variety exclusively alpha-linolenic acid? Does the analytical method used allow discrimination between these two isomers?

Technical Dossier, page 77: margaric acid (C14:0) should be replaced by myristic acid (C14:0).

Diets with sinapine have some effects on weight gain in growing rats and on protein utilization (Vermorel et al, 1987). Several transferases are involved in its synthesis and metabolism (Milkowski et al., 2004). Is the content of sinapine and its different derivatives (Baumert et al., 2005) the same in the meal prepared from the transgenic-and not transgenic oilseed rape?

D.7.8.4 Testing of the whole GM food/feed

Comments submitted on the EFSAnet

As the T45 oilseed rape is shown to be substantially equivalent to relevant conventional comparators, except for the presence of the PAT protein, there is no requirement to test the whole GM food/feed (see EFSA guidance document on risk assessment). Nevertheless a feeding study in broiler chickens has been performed (Stafford, 2005). We wish to point out that one our experts has commented that the observed variability between pens within treatments in this broiler feeding study makes that more than 14 replicates are necessary to find a statistically significant effect. It is also commented that a mean mortality rate of 7.9% is rather high taking into account the rather low stocking density in each pen.
Remarks of a similar nature have been forwarded by the BAC in other dossiers and the BAC would like to discuss with the EFSA GMO panel how to deal with such remarks in cases where no requirements for animal studies are triggered. In this particular case the GMO, if any, will only be present in trace amounts in EU imports, and therefore any differences in nutritional value are very unlikely to result in any effect.

D.7.9 Allergenicity

Comments submitted on the EFSAnet

It is commented that it is very unlikely that the PAT protein is a potential allergen, It has been remarked however that some are of the opinion that finding stretches of six or seven contiguous amino acids of IgE binding epitopes could trigger additional testing (Kleter et al. 2003). Bayer has screened for stretches of 8 contiguous amino acids. It has also been remarked that rapid degradation in an acid environment is not a 100% guarantee of absence of allergenicity potential.

The applicant states in the dossier that oilseed rape is not considered an allergic food. Comments were made on the allergenic potential of in particular 2S albumins of oilseed rape (see Puumalainen et al, 2006). In case the T45 oilseed rape was to enter the human food chain in relevant amounts, additional information would be requested on the effects of the genetic modification on the expression of 2S albumins.

D.7.10 Nutritional assessment of GM food/feed

Comments submitted on the EFSAnet

See earlier comments on broiler feeding trial.

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

Comments submitted on the EFSAnet

As there are already existing monitoring programs developed for the import of GM oilseed rape from North America the same recommendations are applicable.

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

Comments submitted on the EFSAnet

Not applicable. The GM plant has no target organisms and will not be cultured in the EU.

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

Comments submitted on the EFSAnet

The information in this part of the dossier was regarded adequate.

D.9.2 Selective advantage or disadvantage

Comments submitted on the EFSAnet

The introduced trait does not confer a selective advantage or disadvantage if no herbicide is applied. However it can protect the GM plant and its possible progeny against the application of a lethal dose of the herbicide. In Belgium herbicide spraying along public areas such as roads, pounds, recreation areas is prohibited by law. The dossier states that glufosinate is generally not sprayed in private industrial environments where herbicide use is allowed, but does not substantiate this.

D.9.3 Potential for gene transfer

Comments submitted on the EFSAnet

The potential for gene transfer in this particular case is estimated to be very low as a result of the limited amount of T45 in EU imports. The applicant mentions good practices for avoiding or dealing with seed spilling, appropriate plans for the eradication of volunteer oilseed rape, and the HACCP rules applied in the industry. A question has been raised on the exact content of these practices, plans or rules, or a need to provide more detailed references on this point.

D.9.4 Interactions between the GM plant and target organism

Comments submitted on the EFSAnet

Not applicable.

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

Comments submitted on the EFSAnet

The information in this part of the dossier was regarded adequate.

D.9.6 Effects on human health

Comments submitted on the EFSAnet

The information in this part of the dossier was regarded adequate.

D.9.7 Effects on animal health

Comments submitted on the EFSAnet

See comments above under testing of the whole food/feed.

D.9.8 Effects on biogeochemical processes

Comments submitted on the EFSAnet

The information in this part of the dossier was regarded adequate.

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

Comments submitted on the EFSAnet

The information in this part of the dossier was regarded adequate.

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

Comments submitted on the EFSAnet

By the nature of the transgene, no effect is expected.

D.11. ENVIRONMENTAL MONITORING PLAN

D.11.1 General

Comments submitted on the EFSAnet

The information in this part of the dossier was regarded adequate.

D.11.2 Interplay between environmental risk assessment and monitoring

Comments submitted on the EFSAnet

The applicant states that if spilling at the port or along the roadside or at a crushing facility would happen it is unlikely that a herbicide tolerant feral oilseed rape population or herbicide tolerant wild relative would establish. A comment was made stating that “unlikely” might be an underestimation of the actual chance, but that in the absence of recurrent introduction any populations are likely to regress.

D.11.3 Case-specific GM plant monitoring

Comments submitted on the EFSAnet

The information in this part of the dossier was regarded adequate.

D.11.4 General surveillance of the impact of the GM plant

Comments submitted on the EFSAnet

The general surveillance relies for a large part on the feedback that the applicant will get from federations like FEDIOL and COCERAL. The applicant also mentions that EuropaBio has set up a system for general surveillance with the operators responsible for placing agricultural commodities on the market. A question has been raised on the possibility to get some more information on the concrete practices that federations like FEDIOL apply to minimize grain spillage and clean up in port facilities (the references provided in the LMC report such as those published online (http://www.fao.org/DOCREP/005/Y1579E/ and the EU 93/43/EEC) concern only food contamination at processing facilities, in a closed environment) and concrete references on the EuropaBio general surveillance system.

D.11.5 Reporting the results of monitoring

Comments submitted on the EFSAnet

A question was raised on how much time it would take from the moment of confirmation of an adverse effect to the effective action by the management plan.

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Conseil de Biosécurité

27/06/2007

Secretariaat
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Compilation of comments of experts in charge of evaluating the application EFSA/GMO/UK/2005/25

Mandate for the Group of Experts: mandate of the Biosafety Advisory Council (BAC) of 23 April 2007.
Coordinator: René Custers
Experts: Pascal Cadot (consultant), Armand Christophe (UGent), Rony Geers (KUL), Jean-Pierre Hernalsteens (VUB), Jean-Luc Hofs (FUSAGx), Wim Stevens (UA), Peter Smet (consultant), Nancy Terryn (UGent), Johan Van Waes (ILVO).
Domains of expertise of experts involved: genetics, genetic engineering, genome analysis, biosafety research, oilseed rape, agronomy, plant-insect relations, herbicide tolerance, outcrossing to wild relatives, effect on non-target species, horizontal gene transfer, impact on biodiversity, immunology, alimentary allergology, nutrition, animal nutrition, analysis of food/feed, additives for feed, toxicology in vitro.
Secretariat: Didier Breyer, Adinda De Schrijver, Martine Goossens

INTRODUCTION

Dossier **EFSA/GMO/UK/2005/25** concerns an application of the company **Bayer Crop Science** for the marketing of the genetically modified **oilseed rape T45** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 13 April 2007.
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) environmental, 3) allergenicity, 4) toxicity and/or 5) 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.

List of comments received from the experts

A. GENERAL INFORMATION

Comments/Questions of the expert(s)

Comment 1

1. In the Technical Dossier it is stated that T45 has been fully discontinued from commercial sales. In the background related to discontinuation, low commercial sales were mentioned (page 10).

Q1.1: Were there any other reasons for the discontinuation of sales and destruction of the stocks?

Q1.2: Why is there an application for the import in Europe considering that all stocks were destroyed?

2. Published methods based on TAP protein determination seem not to be sensitive enough to detect small amounts of T45 oilseed rapeseeds in a batch of conventional rapeseeds (1).

Q2: **Is there a method that enables the detection of small amounts of T45 Oilseed rapeseeds in the presence of non-GMO rapeseeds? What is the limit of sensitivity?**

Comment 2

Sufficient information is provided in the dossier.

Comment 3

It is well known that rapeseed easily spreads. During springtime these plants appear nearly everywhere. Not only in open fields, but also in municipal areas, near (rail)roads, etc…

Some of my concerns are the following:

- What about contamination of classic rapeseed fields with the T45 variant?
- T45 rapeseed is L-glufosinate (= herbicide) tolerant. Keeping in mind that rapeseed spreads easily, aren't you creating a super weed?

To my point of view, stringent criteria for production and transport of this type of rapeseed should be imposed.

Comment 4

This application deals with the approval for import of seeds of herbicide tolerant canola, event T45 intended for feed and food use, not for cultivation.

This product contains the same technology and expresses the same protein accordingly as already previously approved events for food and feed purposes; the only (even more positive) difference is that T45 does not contain/express an antibiotic resistance marker.

The event T45 has been fully discontinued from commercial sales in Canada where it is grown since 2005 season. So this application deals with a fading out GM crop. Therefore the T45 line grown historically in Canada/US on a very limited surface already will only enter the European Union (EU) by import at low level in commodity rapeseed. Therefore after 2006 the presence of T45 in the canola commodity will be limited to trace adventitious levels only.

The authors state that "However, it can not be overemphasized that regardless of the efficacy of a product discontinuation strategy no system can assure the complete absence of an allele after it has been introduced into the commodity due to several factors like farm saved seed, volunteers, adventitious presence in certified seed and holding grains in storage at farmer level".

It is my feeling that this application would not be done if it was not for the LLrice "contamination" story, and that the applicant wants to avoid a similar problem. Nevertheless I had the feeling throughout the work at the application that this exercise is a bit a waste of expert time and community money. Maybe EFSA should consider proposing that in cases like this, a fast track evaluation should be put in place, when a crop/trait combination that has already been approved in the EU (like here the herbicide tolerant canola, based on the PAT enzyme) a new event can be approved faster? This does not mean less data will be needed, but the evaluation can be done by a smaller team.

Comment 5

The scope of this application is the import of oilseeds and derived products of T45 for food, feed and industrial uses, and no authorization for growing is requested in the member states of the European union. However I can imagine that there will follow very soon a request for cultivation in Europe due to the potential of this modified oilseed rape. Therefore I refer to the general description of the product (point 8) : The applicant mentions that agricultural production of oil seed rape requires weed control and successful weed control depends upon combinations of management practices. Growing Liberty Link oilseed rape allows to use an effective post-emergence herbicide and avoid the precautionary pre-emergence herbicide treatments.

Why is the applicant given all these detailed information for cultivation if he is not interested in cultivation in Europe. The applicant knows very well that the culture of oil seed rape is increasing in Europe and that there is a potential for Liberty Link varieties. So as agronomical expert I will also give some comment in this questionnaire, related to cultivation and the agronomical value.

Comment from the coordinator: As mentioned in comment 4, the event T45 has been fully discontinued from commercial sales in Canada and will never be cultivated in the EU. In that respect, comment 5 does not seem to be very pertinent.

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

Comments/Questions of the expert(s)

Comment 1

Sufficient information is provided in the dossier.

Comment 2

B2b Sexual compatibility with other cultivated or wild plant species

Lack of information:

1) In table 2, it would be suitable to list which crosses have succeeded thanks to tissue culture. The term "many" is not accurate. *B.rapa* x *B. napus* and *B. napus* x *Raphanus raphanistrum* and reciprocal occur naturally. Frequency of progeny should be given for each level. Other references report higher outcrossing levels: spontaneous hybrids of *B.rapa* x *B. napus* have an average success rate of 6.5% within 10m distance from the male parent (Warwick et al. 2003).

2) The report agrees that the possibility of gene flow from oilseed rape to wild relative under natural conditions has been reported, "*mostly under optimal condition"* for 4 species. Although these experiments were conducted under natural conditions, conditions were obviously not fully optimal as

in a controlled lab environment. Canola is well adapted to European weather conditions (rain and temperature [12°-30°C]), and its weedy relatives as well. This fact shouldn't be minimized.
3) If the fitness of interspecific F1 hybrids is reduced compared to the parents, backcrosses of hybrids between *B.napus* and some wild species have an increased fitness. In the case of *B.napus* x *B. rapa*, fitness decrease till F2 and further stages. The *B. napus* x *B. incana* hybrid has a low reproduction rate which reach zero at the F4 level. Nevertheless the hybridization between *B.napus* and *R. raphanistrum* is of major concern: despite the low fitness level in F1, male and female fertility increase over successive backcross generations (Chèvre et al. 1997a; 1999). **Thus wild radish is the most widespread and risky wild relative of canola**. In another hand, as the report concludes, *Sinapis arvensis* rarely hybridizes with canola (Lefol et al. 1996b).

B3 Survivability
Lack of information: **Presence of a risk**
(b) Seeds can germinate at port facilities under the environmental conditions described in the report.
If 20°C is an optimum, canola can germinate and grow from 13-30°C. It is said that canola germinates easily after harvesting when it is not deep buried: this is important in the case of seed spilling in ports and crushing mills premises. The reduction in the number of days of frost due to the climate change should be taken into account for future monitoring.
Question: Seeds will be transported in bulk via rivers. Given that canola seeds can be primed by soaking during at least 24 hours (Elias & Copeland, 1997; Zheng et al., 1998; Shekari et al., 2000), what is the survivability rate and duration of canola seeds in the water?

4 Dissemination
Lack of references and information:
(a) Ways and extent of dissemination
1) The major part of pollen flow falls within a short distance. The study of Lavigne et al. (1998) is a good reference about that matter but is not mentioned in table 3. This study finds an average hybridization rate of 0.1% at 9 m from the source and shows that the rate strongly depends on the size of the receiver and other factors. However, canola pollen flow can travel further as described in the report: in Rieger et al. (2002) with 0.01% at 3 km and in Thompson et al. (1999) with 5% at 4 km on male sterile canola.
2) Insect mediated pollen dispersal, in general, is strengthened by gaps between otherwise adjacent populations (Reboud, 2003). That means that hybridization rates are higher when the pollen source is moderately distant (10-20 meters) from the sink. This statement fits totally in the case of an adventitious seed spilling siring a small feral population in an industrial area containing several patches of feral canola.
3) Some authors (Ellstrand el al., 1989; Snow et al., 2005) suggested that gene flow depends also on ecological factors such as distance, location, climate and season and should not be considered as the fact of the crop alone.
4) The report states that successful pollination decreases exponentially within increasing distance. This is true for short distances but not at the landscape scale (Devaux et al., 2007), which calls for a geometric dispersal kernel.
5) Ellstrand reported that gene flow was ubiquitous and evolutionarily important for the recipient populations for almost all of the major crops and canola is a good example (Ellstrand et al, 1999; Ellstrand, 2002). **The risk exists; it should be taken cautiously and certainly not minimized**.
(b) Special factors
Human activity (farming, transport) is the major source of long distance seed dissemination. The seed is small and can be easily spilled out.

7 Other potential interactions….

Comment:

The enumeration of adverse pests would discourage the most enthusiastic organic farmer; but nevertheless on a feral plant there are enough viable seeds to keep on natural reproduction. In fact there are three major limiting factors in canola "organic" or natural growing: Nitrogen, weeds and early stage insect pests. These last ones are most critical from emergence till 3-4 true leaves. The **depredation risk** is high in a cultivated field **but could be less in a feral population** where different growing stages and different species can be found (see more information on Cetiom, Itab, Inra websites). Authors of the T45 technical dossier further recognize that insects as the flea beetles may be (only) occasionally detrimental (page 28 of 113). Biosafety can't rely upon crop pests to significantly contain GM plant reproduction: interactions are tremendously variable and depend on environmental conditions. Sometimes it helps, that is all.

About invasiveness per se, populations of canola may be displaced to let the place free for climax, but the report doesn't mention how long it takes to reach it. Moreover and however, this point concerns industrial areas in a lesser extent. In Australia (Agrisearch, 2001; OGTR, 2002), surveys recorded incidences of feral canola, in small numbers per spot, within 5 m of the roadsides in a range between 9% and 31% of the cases. Nevertheless, these results suggest more an **external (lorries) seed dispersal** source than a plant-to-plant dissemination.

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments/Questions of the expert(s)

Comment 1

Sufficient information is provided in the dossier. The *Agrobacterium*-mediated protoplast transformation method that is used is classical and generally gives predictable results.

Comment 2

Similar trait has been approved before and adequate molecular techniques were used to characterise the transformation event and insertion.

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

Sufficient information is provided in the dossier. The strategy that was used to obtain the phosphinothricin-tolerant plants was used repeatedly in different crops with good results and without adverse effects.

Comment 2

No indication of unintended effect.

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

Comments/Questions of the expert(s)

Comment 1
The inserted segment is completely defined. The intensity of the hybridising bands in several Southern blots is weak, and some are not clearly visible. Nevertheless, taken together, the data that are shown are sufficient to confirm the presence of the expected sequence.

Comment 2
The *Brassica napus* event T45 specific integration sequences including the Right Border flanking sequences (911 bp) and the Left Border flanking sequences (994 bp) were determined (Vandermarliere & De Beuckeleer, 2004) and sequence alignments were done. No relevant homologies were found. I just wondered whether as new sequences appear every day in the database these searches have been done recently. Last reference I found is 2004, but I guess it must have been monitored? I did a search myself and anyway didn't find a relevant match.
To confirm the absence of cryptic expression in the flanking plant DNA / insert junction regions of oilseed rape event T45 an *in silico* analysis was performed. There are rearrangements at the DNA at the site of insertion (small deletion). Newly created open reading frames ORF-5, ORF-10, ORF-21 and ORF-34 were subjected as query sequences to a BLASTp search. The BLASTp similarity search revealed no meaningful sequence similarity with published proteins.
Also the nature of the 5', ATG and 3' region did not match with known consensus sequences for transcriptionaly active ORF's.
A small remark, I wonder if it is seen as standard procedure not to check for example expression of an ORF like ORF21 which is newly created and 108bp long? This does not mean I ask for this experiment to be done, just wonder if there are general guidelines that help the applicant when he should check more in detail and when not.

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comments/Questions of the expert(s)

Comment 1
Sufficient information is provided in the dossier to prove expression of the PAT enzyme. The expression, at a significant level, of other spurious open reading frames, resulting from the integration of the T-DNA should indeed be extremely unlikely.

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

Only a single very well defined new protein, conferring phosphinothricin tolerance, is expressed in the transgenic plants. Therefore the reproduction, dissemination and survivability of the GM plant will not differ from non-GM plants of the same genotype unless phosphinothricin is applied. The GM plants are tolerant to the application of a dose of this herbicide that is lethal for non-GM plants.

Comment 2

(a) mode or rate of reproduction

1) Seed spillage: the closed system put in place in the case of handling and transport is quite relative. The information provided in the LMC (2003) report **doesn't respond accurately** to the risk management in port facilities and transport by road (see D8).

2) Nowadays, only one authorisation for a similar cultivar is still valid: MS8/RF3 which is additionally male sterile; Topas 19/2 has been withdrawn and is no longer valid.

3) In Belyk and MacDonald confidential report (1995b), the Net replacement Rate equation should be NRR= number seed collected x (number seed sown)$^{-1}$ instead of NRR= number seed collected x number seed sown. The reason of the nil values for HCN28 is not discussed in the reports.

(b) Dissemination

If no difference between cultivar types has been observed, the last report chapter shown that Net Replacement Rates (NRR) are very variable according to the place and always above 1 except for HCN28. Dissemination is comparable to conventional types but NRR are sometimes (as in Indian Head) relatively high (even if it is less than the certified multiplication levels - 300 fold). An average NRR of 50 is still significant for undisturbed soils and can favour plant multiplication.

(c) Survivability

1) **Spilling incidents** in the case of the seed import could be **assimilated to recurrent introduction**. The effect of such introduction should be studied. There is a lack of information about localized dispersal of transgene in general. Recurrent release acts as a migration pressure (Haygood et al. 2003) and in the case of a neutral (if there is no selective pressure on the Pat gene) allele, it could be assimilated in a small recipient population and **could drift to fixation** (Andow & Zwahlen, 2006). The genetic assimilation would evolve even faster when the allele is selectively advantageous, under recurrent introduction and global dispersal.

2) The T45 report minimizes the probability of seed spilling in ports facilities. However, these incidents already occurred: Saji et al. (2005) [only reported in the Commission Recommendation (2005)] found the **existence of feral glufosinate-ammonium resistant canola in Japanese ports**. As no transgenic plants have been intentionally introduced in that country, the authors suggested that the transgene would have come from imported bulk seed for industry.

3) The results given in MacDonald & Kuntz (2000a) are relevant in the Canadian environment but less in the European one, specially related to phenology and climate.

Comment 3

No differences.

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

Comments/Questions of the expert(s)

Comment 1
The data presented prove the genetic stability of the GM plant. This is what is logically expected for plants with a single-copy T-DNA insert.
This is not important in this case, as this plant will not be cultured in the EU.

Comment 2
As mentioned in 2a "information concerning reproduction" oil seed rape is both self-pollination (70%) and cross-pollination (30%). So how can the genotypic and phenotypic stability be verified?

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
By the nature of the inserted sequence, such changes should not be expected. However, if crossing-out of the transgene to a related plant would occur, its presence would provide a selective advantage when phosphinothricin is applied. This is very unlikely, as the GM plants will not cultured in the EU.

Comment 2
(b) plant to plant gene transfer
iii. Consequence of gene flow: Potential effect on biodiversity
- In a recurrent introduction context due to recurrent seed spilling, feral population can be renewed and expand. It is true that Pat gene does not have an effect on that unless glufosinate ammonium is used to control weeds within the expansion area.
- However special care must be taken with *B. napus* x *R. raphanistrum* as mentioned in section B.

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
By-site t-test results comparing transgenic crops treated by Liberty® or conventional herbicide could be calculated from the existing databases but are not given. Such results could substantiate the claim (Technical Dossier page78) that application of glufosinate ammonium does not result in any secondary effects.

Q: Can the results of the by-site t-tests comparing transgenic crops treated by Liberty® or conventional herbicide (B vs C) be given?

Comment 2
Substantial equivalence has been demonstrated for the food properties of the seed derived from T45 plants. Comparisons of the chemical composition of key nutritional elements between transgenic and non-transgenic oilseed rape confirm equivalence, except for the PAT protein. In crude and refined oil PAT is not detectable. In addition safety reviews in several countries have found the PAT protein to be safe. Also the main T45 derived product will be the bio-diesel and the food use of the canola oil derived from T45 will be limited.

D.7.2 Production of material for comparative assessment

Comments/Questions of the expert(s)

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

Comment 1
The information given by the applicant is sufficient to conclude that there are no agronomical and nutritional differences between T45 and the reference, with the exception for the tolerance for glufosinate ammonium.
A supplementary question: What is the agronomical value of the GMO oilseed rape compared to the best actual varieties in the market?

D.7.5 Product specification

Comments/Questions of the expert(s)

D.7.6 Effect of processing

Comments/Questions of the expert(s)

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

Comment 1
The culture of the GM was discontinued in the production area outside the EU. The aim of this application is to avoid problems, if a low proportion of GM seeds of this line would still be present in oilseed rapeseeds that are imported in the EU for processing. Processing will mainly be for bio-fuel production, not for human food use.

D.7.8 Toxicology

Comments/Questions of the expert(s)

Comment 1
Due to the nature of the insert, no toxic effect must be expected. This is confirmed by a long experience of safe use of GM plants expressing the same protein.

Comment 2
Digestibility
* *In vitro* digestibility in simulated gastric fluid (SGF) (Esdaile, 2002b):
The PAT protein was digested very rapidly with no residual protein visible at 30 seconds of incubation with SGF, in the presence of pepsin, at pH 2,0 and 37°C.
* *In vitro* digestibility in simulated intestinal fluid (SIF) (Esdaile, 2002a):
In less than 30 seconds of incubation at 37°C with SIF, in the presence of pancreatin at pH 7,5, all remaining fragments were completely digested.
Conclusion: It is unlikely that significant amounts of the PAT protein or fragments of it, will become available for uptake through the mucosa of the small intestine.

D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

Comment 1
The safety of the newly expressed protein is confirmed by a long experience of safe use of transgenic plant of different species expressing it.

Comment 2
Acute toxicity
* Acute toxicity by intravenous injection in the mouse (Kennel, 2003):
There were no mortalities or treatment-related toxic effects in mice after an acute intravenous administration of PAT protein at 1 or 10 mg/kg body weight.
Conclusion: Keeping in mind the rapid digestion of the PAT protein, it was very interesting to conduct this test by i.v. injection. Even though this digestion was by-passed, no adverse effects

contributable to the PAT protein were seen. So, this protein can be regarded as having no acute toxicity.

Comment 3
Safety reviews in several countries have found the PAT protein to be safe.

D.7.8.2 Testing of new constituents other than proteins

Comments/Questions of the expert(s)

Comment 1
It has been shown that the composition of the metabolites of glufosinate ammonium are different in transgenic plants with the *pat*-gene than in their conventional counterparts (2, 3).
Q: Are metabolites of glufosinate ammonium present in seed meal and if so, has their toxicological profile been determined?

Comment 2
Due to the nature of the genetic modification the presence of such components is not expected.

D.7.8.3 Information on natural food and feed constituents

Comments/Questions of the expert(s)

Comment 1
The paragraph on page 69 of the Technical Dossier starting with the words "Equivalence of the non-transgenics…." is not clear and does not seem to be supported by the values in Table 17 (cystine is mentioned in the table; cysteine in the text; no obvious differences for histidine???…).
Q: Clarify the paragraph on page 69 of the technical dossier starting with the words "Equivalence of the non-transgenics…."

The term "Linolenic acid (C18:3)" (page 74 and further) is poorly defined as there are 2 different "linolenic" acids with different biological effect. Non transgenic rapeseed oil contains only alpha-linolenic acid (C18:3ω3) but there are seed oils that contain gamma-linolenic acid (18:3ω6).
Q: Is the "linolenic acid" reported in the transgenic variety exclusively alpha-linolenic acid? Does the analytical method used allow discrimination between these two isomers?

Technical Dossier, page 77: margaric acid (C14:0) should be replaced by myristic acid (C14:0).

Diets with sinapine have some effects on weight gain in growing rats and on protein utilization (4). Several transferases are involved in its synthesis and metabolism (5).
Q: Is the content of sinapine and its different derivatives (6) the same in the meal prepared from the transgenic-and not transgenic oilseed rape?

D.7.8.4 Testing of the whole GM food/feed

Comments/Questions of the expert(s)

Comment 1
Human consumption: Refined edible canola (=rapeseed) oil consists of purified triacylglycerols (96-97%) and does not contain any detectable protein. So, no test on the rat (13-week feeding study) was conducted. This seems reasonable to me.

Animal consumption: The processing of the rapeseed meal reduces the PAT content. In roasted meal only a trace amount (below the limit of quantification) of PAT protein was detected. Nevertheless, a 42-day feeding study in broiler chickens was conducted.

Analytical data concerning protein content can be found in table 35 on page 84 of the technical dossier.

* 42-day feeding study in broiler chickens (Stafford, 2005):
No abnormal effects were seen in broiler chickens following 42 days exposure to T45 rapeseed.

Conclusion: The amount of PAT protein present in feed is rather small and no adverse effects are seen in a 42-day feeding study using broiler chickens. According to these data, the use of T45 rapeseed meal for animal consumption seems to be safe.

Comment 2
Not necessary, substantial equivalent. Anyway the applicants did it with positive outcome.

D.7.9 Allergenicity

Comments/Questions of the expert(s)

Comment 1
Allergenicity of the newly introduced protein:
In line with the claim by the applicant, there is no evidence that PAT proteins are potential allergens.

Allergenicity of the whole GM plant or crop:
Allergy to the seeds of oilseed rape has been described. Children have been said to show IgE-mediated reactions against those seeds (1), the responsible allergens being 2S albumins (2). However, the sensitization pathways are unknown, and reactions might result from cross-reactivity.
Given the scope of the application, as mentioned in paragraph 7, page 18 of the technical dossier: 1) "The purpose of this application is to ensure that the potential low level presence does not result in an impediment to the import of oilseed rape into the EU", and 2) "The Canadian harvested seeds will be imported and mainly channelled to designated bio- diesel manufactures located in the vicinity North-European ports for bio–diesel production. The food use of the canola oil derived from T45 will therefore be likely to be limited", in such context, the application should not represent allergy risk. However, if T45 has to enter the food chain more directly, titration of 2S albumins, potent cross-reactive seed allergens, should be carried out for comparison between T45 seeds and their traditional

counterparts. Indeed, the introduction of a new trait might influence the expression levels of other genes, among them possibly 2S albumins.
Within the scope, it is also indicated that "The by products of the processing; canola meal and hulls will be used for animal feeding". In that respect, some cases of occupational asthma due to oilseed rape dust have been described in workers of the feed industry (3,4). The responsible allergens, however, are unknown. Therefore, vigilance should be applied when handling this dust or meal in order to detect and report any sign of increased allergenicity of T45 by-products.

Comment 2

Bayer Crop Science states that the PAT protein is non allergic. This statement is based on a study of the amino acid sequence. The total amino acid sequence of the PAT protein was compared to that of known toxins and allergens listed in 7 large public databases. No evidence for any known allergens was found. Matches of 8 contiguous amino acids were considered appropriate criteria for Bayer; furthermore PAT is not stable in an acidic environment, which was taken as an argument for non allergenicity. It have to be remembered however, that other labile proteins such as Mal d 1 can cause symptoms (oral allergy syndrome) in humans.

Some allergenic proteins have been found in similar plants. This means that the plant as such can not be considered as non allergic.

1. Kleter GA, Peijnenburg AA. (2003) (RIKILT Institute of Food Safety, PO Box 230, NL 6700 AE Wageningen, the Netherlands) :

"Mitochondria of cytoplasmic male sterile crop plants contain novel, chimeric open reading frames. In addition, a number of crops carry endogenous double-stranded ribonucleic acid (dsRNA). In this study, the novel proteins encoded by these genetic components were screened for the presence of potential binding sites (epitopes) of allergy-associated IgE antibodies, as was previously done with transgenic proteins from genetically modified crops. The procedure entails the identification of stretches of at least six contiguous amino acids that are shared by novel proteins and known allergenic proteins. These stretches are further checked for potential linear IgE-binding epitopes. Of the 16 novel protein sequences screened in this study, nine contained stretches of six or
seven amino acids that were also present in allergenic proteins. Four cases of similarity are of special interest, given the predicted antigenicity of the identical stretch within the allergenic and novel protein, the IgE-binding by a peptide containing an identical stretch reported in literature, or the multiple incidence of identical stretches of the same allergen within a novel protein.
These selected stretches are present in novel proteins derived from oilseed rape and radish (ORF138), rice (dsRNA), and fava bean (dsRNA), and warrant further clinical testing. The frequency of positive outcomes and the sizes of the identical stretches were comparable to those previously found for transgenic proteins in genetically modified crops. It is discussed whether novel proteins from conventional crops should be subject to an assessment of potential allergenicity, a procedure which is currently mandatory for transgenic proteins from genetically modified crops."

2. Puumalainen TJ et al (2006) (National Public Health Institute, Helsinki, Finland) :

" BACKGROUND: Children with IgE-mediated allergy to foods frequently react to seeds of oilseed rape (Brassica napus ssp. oleifera) and turnip rape (Brassica rapa ssp. oleifera) in skin prick tests (SPTs). Sensitization pathways are not known.

OBJECTIVE: We identified possible major allergens in oilseed rape and turnip rape using sera from 72 atopic children (mean age, 3.3 years) with positive SPT responses to oilseed rape and turnip rape. METHODS: Allergens from oilseed rape and turnip rape seed extracts were purified by using gel filtration and cation exchange chromatography and characterized by means of reversed-phase chromatography, N-terminal amino acid sequencing, and matrix-assisted laser desorption ionization time-of-flight mass spectrometry. IgE binding of sera from 72 children with positive SPT reactions to oilseed rape and turnip rape and 72 age- and sex-matched atopic control subjects with negative SPT responses were analyzed by means of IgE ELISA and immunoblotting. In vivo reactivity of the purified allergens was tested with SPTs in 6 children. RESULTS: In IgE immunoblotting and IgE ELISA major reactivity was to a group of homologous, approximately 9.5- to 14.5-kd proteins. These allergens were identified as 2S albumins, also known as napins, by means of N-terminal amino acid sequencing. In ELISA approximately 80% of the patients had IgE to purified napins from both plants. In SPTs purified napins caused positive reactions in all 6 children tested. CONCLUSIONS: This study shows that 2S albumins in oilseed rape and turnip rape are new potential food allergens. Further studies are needed to clarify the routes of exposure and mechanisms of sensitization"

D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

Comment 1
Due to the nature of the genetic modification no alteration of the nutritional value of the GM crop is expected.

Comment 2
Substantially equivalent.

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

Comments/Questions of the expert(s)

Comment 1
As there are already existing monitoring programs developed for the import of GM Canola from North America the same recommendations are applicable.

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

Comments/Questions of the expert(s)

Comment 1
Not applicable. The GM plant has no target organisms and will not be cultured in the EU.

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

Comments/Questions of the expert(s)

Comment 1

Cultivated oilseed rape can survive in the environment, but is not an invasive plant. Agricultural traits, such as reduced shedding of its seeds due to a higher mechanical strength of the pods reduce its fitness in the natural environment. Persistence and invasiveness will not be altered by the genetic modification that was applied.

Comment 2

Accidental spillage of seeds identified as T45 will be managed consistently with methods established Safety reviews in several countries have found the PAT protein to be safe, like for the previously approved GM oilseed rape event GT 73 (Commission Recommendation, 2005).
The only potential adverse effect associated with import could be the establishment of feral herbicide tolerance oilseed rape populations with a possible outcross of the herbicide-tolerance to commercial oilseed rape fields or to wild *Brassica* relatives, if imported seed is spilled in transit. These transit areas are predominantly situated in seaport harbours or along the River Rhine, as most oilseed rape crushers plants handling overseas imported grains are situated in these areas. In the unlikely event that spillage, germination and flowering of a GM derived oilseed rape plant occurred in the ports and associated crushing plants, their location in industrial areas makes it highly unlikely that gene transfer to non-GM *Brassicae* would occur.
Due to the limited exposure, and this only at import facilities or processing plants, it is unlikely that a possible spill of ACS-BNØØ8-2 will have any influence on the environment.

D.9.2 Selective advantage or disadvantage

Comments/Questions of the expert(s)

Comment 1

The introduced trait does not confer a selective advantage or disadvantage if no herbicide is applied. However it can protect the GM plant and its possible progeny against the application of a lethal dose of the herbicide.

Comment 2

In the LMC report (page 6), it is said that in France there is a high probability to use trucks for seed transport. Consequently the likelihood of seed escape while in transit is not so “very low” as it is mentioned in the report.
Question:
In Belgium, herbicide spraying along public areas as roads, pounds, recreation areas… is prohibited by law. What is the situation in the other European countries concerned with the introduction?
The applicant should provide accurate guideline references about herbicide use in public and industrial areas.

Question:
It is said that glufosinate is generally not sprayed in industrial environments which are private or restricted areas where (any) herbicide control is allowed.
Concerning glufosinate sprays, are there specific recommendations suggesting not to apply such products in ports or crushing facilities and their surroundings?
The applicant should provide guideline references, as well.

Comment 3
In the report it is mentioned that "Agronomic performance shows no disadvantage". Are there real data to prove this?

D.9.3 Potential for gene transfer

Comments/Questions of the expert(s)

Comment 1
I agree with the detailed data presented in the dossier. *Brassica napus* can hybridize with different other cruciferous plants. However, very specific conditions are often required for this hybridisation and the viability of the resulting hybrids is often reduced.
In addition, the GM crop will not be cultured in the EU, making gene transfer even less likely.

Comment 2
Pat gene doesn't improve gene flow between cultivated and wild parents but the risk of hybridization is not diminished for all that. Gene flow is far to be negligible and is sometimes problematic in pedigree seed conventional canola containing more than 0.25% of GM herbicide-tolerance traits (Friesen et al., 2003). Although gene leakage is small (even through containing measures), the probability of escape within some generations (e.g. 10) could be significant (Haygood et al. 2004).

Questions:
1) After some (probably limited) investigations about HACCP in France and Belgium, no accurate guides of good practices or specific rules have been found about seed spilling (in the scope of gene transfer).
2) What are the so-called appropriate plans for eradicating volunteer oilseed rape as mentioned in point 5(b) of the annexes of the Commission Recommendation 2005/637/EC?
3) If the EU crushing industry and port facilities are HACCP compliant, what are the specific HACCP rules applying about that matter? The applicant should provide guideline references.

Comment 3
Very unlikely, the bulk of crosspollination has been observed to occur over very short distances. There are no canola fields close to the harbour and the regions of seed crushing.

D.9.4 Interactions between the GM plant and target organism

Comments/Questions of the expert(s)

Comment 1
Not applicable.

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

Comments/Questions of the expert(s)

Comment 1
Not applicable.

D.9.6 Effects on human health

Comments/Questions of the expert(s)

Comment 1
By the nature of the transgene, no effect is expected.

Comment 2
Safety reviews in several countries have found the PAT protein to be safe.

D.9.7 Effects on animal health

Comments/Questions of the expert(s)

Comment 1
One trial with broilers is reported (Stafford, 2005), comparing three treatments (A, B, C) with 14 pens (7 males, 7 females) per treatment and 10 chickens per pen, i.e. 140 birds per treatment. Body weight was measured individually, but statistically analysed at pen level, as was also the case for feed intake. Taking into account the observed variability between pens within treatments, 42 replicates in stead of 14 replicates are necessary to find out whether or not treatment has a statistically significant effect on body weight, and 24 replicates are necessary to find a statistically significant effect of treatment on FCR (Berndtson, 1991). A mean mortality rate of 7.9% is reported, which is rather high taking into account the rather low stocking density in each pen.

Comment 2
By the nature of the transgene, no effect is expected.

D.9.8 Effects on biogeochemical processes

Comments/Questions of the expert(s)

Comment 1
By the nature of the transgene, no effect is expected.

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

Comments/Questions of the expert(s)

Comment 1
The applicant mentions here again that T45 varieties will be grown principally in Canada and enters the EU by import as commodity rapeseed. Again here a lot of advantages of Liberty Link oilseed rape varieties are given by the applicant; so I can imagine that there will be soon a request for cultivation in Europe and then we have to compare the GMO T45 varieties with the best actual varieties in the market (Value for Cultivation and Use Trials in the frame work of the EU-legislation).

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

Comments/Questions of the expert(s)

Comment 1
By the nature of the transgene, no effect is expected.

D.11. ENVIRONMENTAL MONITORING PLAN

D.11.1 General

Comments/Questions of the expert(s)

D.11.2 Interplay between environmental risk assessment and monitoring

Comments/Questions of the expert(s)

Comment 1
Question:
Same as 9.3. The applicant should provide guideline references; specially the description of procedures.
Given the references provided in the comments (Chèvre et al. 1997a; 1999; Saji et al., 2005; Andow & Zwahlen, 2006) the point 11.2 could be altered as follows:
"If herbicide-tolerant oilseed rape were to spill at the port or along the roadside or at a crushing facility, it is **probable [and not "unlikely"]** it would establish herbicide-tolerant feral oilseed rape population or herbicide-tolerant wild relative, **but in the absence of recurrent introduction these populations are likely to regress"**.

Comment 2
Any presence of the derived plants in the EU would be a consequence of seed spillage during handling and transport and is unlikely given the closed system in place. Anyway a more then adequate monitoring plan has been put in place.

Comment 3
The proposed risk assessment is satisfactory taken into account that the scope of application is only to import oilseeds and derived products.

D.11.3 Case-specific GM plant monitoring

Comments/Questions of the expert(s)

Comment 1
These case-specific GM plant monitoring must be conducted within long term public research programs.

D.11.4 General surveillance of the impact of the GM plant

Comments/Questions of the expert(s)

Comment 1
More details should be provided about existing measures to minimize grain spillage and clean-up practices in the frame of "good handling and manufacture practices and environmental systems". A review is necessary but training programs would be most certainly useful. The references provided in the LMC report such as those published online (http://www.fao.org/DOCREP/005/Y1579E/ and the EU 93/43/EEC) concern only food contamination at processing facilities, in a closed environment.

About the primary sources of surveillance, FEDIOL is supposed to provide information. To my knowledge, there is no specific risk analysis of the oilseed rape transformation chain aiming GM seed (at the date of 22/12/2006). FEDIOL provides general surveillance procedures only. The code of practice is at present under revision but the March 2002 issue doesn't mention measures to be taken in relation to GM crops and seed spilling.

Questions:
1) Could it be possible to get more information on that matter as available public information is likely to be scarce?
2) Does EuropaBio already clearly provide specific information about surveillance and risk management on already introduced and deregulated GM traits? If yes, please provide tangible references.

After the introduction of any trait, assessment of risks to biological diversity needs to be conducted on a long term and large-scale basis. NRC (2002) recommends monitoring by trained observers to detect unexpected effects. Imports of GM seed must be targeted by these measures

D.11.5 Reporting the results of monitoring

Comments/Questions of the expert(s)

Comment 1
Question:
Achievement rapidity is one of the key issues of a surveillance procedure. What could be the delay from the confirmation of an adverse effect to the effective action upon the management plan?

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