Testbiotech comment: EFSA's opinion on renewal of GT73 authorization

Summary
Monsanto asked for renewal of an existing authorization of GE (genetically engineered) oilseed rape GT73 for import into the European Union. The oilseed rape is genetically modified with two genes encoding proteins conferring glyphosate tolerance. One of the proteins is CP4 EPSPS that was also incorporated in Monsanto's RR soybeans. The second protein is glyphosate oxidoreductase (GOX) which acts by breaking down glyphosate into glyoxylic acid and the metabolite AMPA.

The scope of the application covers different food uses. In December 2009 EFSA published an opinion for GT73 (EFSA 2009). The panel found no evidence that GT 73 poses any risk for animal or human health or the environment. The opinion is therefore in line with the panel's previous opinions dating from 2004 (EFSA 2004) and of June 2009 (EFSA 2009a) (where it considered Austria's import ban of GT73). Testbiotech looked just into some of the relevant health aspects. It is shown that EFSA fails in its assessment of this GE product, because

➢member states critical comments are only partly addressed
➢flawed studies on the nutritional safety of GT73 oilseed rape are considered as proof ofr the safety of GT73
➢an important study on the fate of the transgenic construct in animal tissue is not taken into account
➢compositional data of anti-nutritional compounds like tannins are not requested by EFSA
➢It is unclear if studies were performed with rape seed sprayed with glyphosate. Thus potential health effects of the residues of the spraying are not investigated thoroughly.

1. Comparative analysis (for compositional analysis and agronomic traits and GM phenotype)
Oilseed rape contains several constituents that are anti-nutritional. In the studies delivered by Monsanto and Caine et al. (2007), mainly glucosinolates are mentioned. But according to member states' comments on the application of GT73, there are also other anti-nutritional factors which were not taken into account.

For example, the Netherlands Ministry of Agriculture points to a key document published by the FAO (Anonymus). In this document, sinapine, tannins and eurucic acid are filed as anti-nutritional factors.

And according to a OECD document on low-erucic-acid rapeseed, also the following constituents, in addition to the key nutrients and toxicants, may be considered if a compositional analyses of rape seed is considered:
"Macro and trace minerals may be considered in the evaluation of low erucic acid rapeseed […]. Tannins and sinapine are considered to be minor antinutrients in low erucic acid rapeseed meal. Sinapine is the major phenolic compound in low erucic acid rapeseed which imparts a bitter taste. Phytic acid is the major form of phosphorous in plants. Although largely unavailable to the animal, phytic acid may have an impact on other mineral bioavailability." (OECD, 2001)

Referring to this document, the Netherlands Ministry of Agriculture states (EFSA 2009c):
"In 2001, a consensus document of the OECD has been published, in which a number of key nutrients and anti-nutrients has been listed that are recommended to analyse for a comparative assessment of GM oilseed rape. For several analytses mentioned in this list, no statistical analysis has been provided for GT73 oilseed rape (several amino acids, fatty acids). […] In addition, for some other nutrients, no data at all have been provided in the dossier (minerals). Without these additional data a proper evaluation of the compositional equivalence of GT73 is not possible, therefore such data should be provided."

And the Belgian Biosafety Advisory Council notes (EFSA 2009c):
“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 […]. Thus it is surprising that no values for tannins and saponins are reported.”

The member states’ requests for more data are not taken into account by the GMO panel. Therefore, important data on the compositional equivalence of GT73 oilseed rape with great importance for scientific understanding of the nutritional safety of GT73 oilseed rape are missing.

2. Food Safety assessment
In the December 2009 opinion regarding GT73 oilseed rape, EFSA states with respect to potential food safety assessment:
“The EFSA GMO Panel concludes that there is no new information provided by the applicant or in the scientific literature that would require changes of its previous scientific opinion on GM oilseed rape GT73 (EFSA, 2004).”

The panel refers to a wide range of feeding studies:
➢Three studies on rats (1994, 1995, 1996)
➢Two studies on rainbow trout (1994, 2003)
➢Two studies on quails (1993, 1994)
➢One study on lambs (2003)
➢One study on chickens (2004)
➢One study on pigs (2007)

As pointed out by member states (EFSA 2009c) and other stakeholders (Greenpeace 2004), several of the studies are either flawed or even lead to serious doubts about the safety of GT73 oilseed rape. Of the ten studies mentioned above, three are so seriously flawed that even EFSA didn’t take them into account (rat study 1994, trout study 1994, quail study 1993).

During the consultation process concerning the renewal of approval of GT73 oilseed rape member states voiced serious concerns about the standards and outcome of some of the studies that were not properly addressed by EFSA. Furthermore, Monsanto’s own technical reports indicate that also most of the other studies are flawed. The food safety of GT73 therefore cannot be claimed on the basis of the presented reports and studies.

2.1 Rat studies

Rat Study (1994)
As EFSA states correctly, Monsanto failed in keeping two different GM lines that were to be tested apart from one another. There was a mixture of GT200 and GT73 canola (in a ratio of approximately 1:1) that makes the study worthless. It was therefore not considered in EFSA’s opinions on GT73. But another fact not mentioned by EFSA is that the non-GM varieties used in this test were contaminated with GT 73 during cultivation.
According to the documents available, it is very likely the plant material that was used for testing came from the same contaminated source as in the first trout study (see below) where GM oilseed rape (canola) was grown only meters away from non-GM canola (MADGE 2009).

Rat study (1995)
In a follow-up study conducted by Monsanto the animals’ liver weights were slightly, but significantly, increased by approximately 9-16% in rats fed diets containing 15% GT73. EFSA declares these findings as “incidental”. The origin of the plant material used in this study remains unclear, and there is no indication that the control and the non-GM varieties were not contaminated with transgenic material.

The outcome of this test still leads serious to doubts by member states. Austria notes (EFSA 2009c):
“In the second rat study (Naylor 1995) only processed meal was fed. Despite this fact liver weights differed between groups. It is of special interest to investigate the meal without heat treatment as transproteins are heat labile. Further, this is an indicator for carrying out a more appropriate study design for toxicological approach and a 90 day rodent toxicity study including microscopic evaluation

is therefore highly recommended."

EFSA disregards this proposal for reasons unknown.

Rat study (1996)
Monsanto then conducted a third rat study. Its purpose was to determine whether the liver and kidney weights of rats fed GT73 fall within the range of liver and kidney weights of rats fed processed meal from nine commercial varieties of canola from Canada and oilseed rape from Europe. In other words, this study was not conducted to compare GT73 with the isogenic lines grown under similar conditions but conducted with controls from a wide range of different sources from different continents. Austria states (EFSA 2009c):
"The more varieties used, the higher is the variability and the lower the likelihood of statistically significant differences."

Furthermore, the fraction of rapeseed meal in the rats' diets was reduced from (a maximum of) 15 % to 10 %.

Predictably and according to EFSA, the study
"did not reveal any significant difference in weight gain, feed intake or organ (liver, kidney) weights between rats fed GT73 and the parental Westar line and the commercial lines."

But, as stated in a member state comment by the Belgian Biosafety Authority (EFSA 2009c), the fact "that such an effect was not observed in the second study can be explained by the use of a lower dose level in the second study (10% instead of 15% (the limit of use in monogastric diets, OECD, 2001)). In addition, in the repeated dose toxicity studies, a maximum concentration of 15% oilseed rape meal was used, whereas up to 30% will be included in feed of ruminants. Effects on such dose levels on, for instance, liver effects are not known. Therefore, the dose levels used are too low to draw conclusions on the nutritional equivalence and safety for all food/feed uses."

This third study seems therefore to be deliberately designed to mask differences that occurred in the second rat study and should not be considered.

2.2. Trout studies
Two studies on rainbow trout were done on behalf of Monsanto. The outcomes were published by Brown et al. (2003).
The first study seems to have incorporated the same kind of rapeseed meal as the first rat study (1994) as the same mixing error is reported. The authors state:
"In the first study, two lines of GTC, designated GT200 and GT73, and a parental line, Westar, were used. […] However, because of a mixing error that occurred prior to the first study, samples of seed labelled GT200 and GT73 were essentially equivalent in composition."

For this reason, the study is not considered in EFSA's opinion. But in addition, the non-GM rapeseed meal that was provided for the trial was grossly contaminated, because, the different GM and non-GM varieties were grown side by side in plots at a number of sites in Canada. Of course, this led to massive contamination of the non-GM varieties with the GM constructs present in GT73.

In an analysis done by MADGE, an Australian NGO, (MADGE, 2009) Monsanto's own technical reports are quoted to underpin this thesis. According to the document published by MADGE which investigated the conditions under which the GE canola used for the first feeding study was grown, the control samples were contaminated. But also the GE samples, meant to be used to examine potential health effects were diluted by pollen from neighbouring fields:
"However, no pollen barriers were erected. In the same trial plot area there was a plot of non-GM Westar seed which was to be used as the comparison feed for the trout. It was planted within meters of the GM feeding trial plots in some cases. This meant that the GM plants could be contaminated by the non-GM, and that the control non-GM Westar could be contaminated by the GM plants. Furthermore, Monsanto required that these GM and non-GM plots be surrounded by a 10m border of the non-GM Westar parent plant, without specifying the distance. At least one border was planted within 1.5m of the heterozygous GM canola plants, another at 2.5m. It is to be deduced that cross-pollination from these parent plants would have resulted in further reduction of the GM component

of the final seed.
What is evident is that Monsanto began with a dilute GM crop line, and surrounded the plots with material that would further dilute it. Furthermore, although not reported, it appears certain that the non-GM Westar plot used in the trials would have been GM contaminated. (...)"
There was a more profoundly bizarre twist. Strangely another GM canola line (GT200) was in duplicate side-by-side plantings, as close as 0.25m from the heterozygous GT73 line intended for the feeding trials."

There is no indication that the way of cultivating GT73 was changed for the follow-up study (apart from the exclusion of GT200). As the published study (Brown et al. 2003)- referring to both tests – states:
"All lines of canola were grown in the same field test sites in Canada …."

So there is no way of securing that Monsanto didn't simply compare a GM variety with different highly contaminated non-GM varieties. The study therefore cannot be considered as long as there is a proof that the non-GM varieties were indeed GM-free.

2.3 Pig study (2007)
Recently a further study was conducted and published. Pigs received diets containing 15% processed meal derived from GM oilseed rape GT73, its control or two commercial reference oilseed rape lines (Caine et al., 2007). According to EFSA, the study
"did not indicate any impact of the genetic modification on animal performance" (EFSA 2009b).

But as in the second rat study (1995), liver weights of animals fed GT73 oilseed rape were higher than in the other groups (though not statistically significant), whereas carcass yield of was lowest of the four groups tested. Regarding Roundup Ready Canola (RRC), the study says:
"Pigs receiving the PCC and RRC diets had heavier (P = 0.002) liver weights than those pigs fed the COM1 and COM2 diets (2.06 and 2.05 kg vs. 1.95 and 1.96 kg, respectively)."

In another study were GT73, isogenic control and non-GM varieties were fed to lambs (Stanford et al., 2003), liver weights were not measured, but only observed by appearance.

2.4 Quail studies
A study was conducted on quails that were fed GT73 and commercial non-GM varieties and a control, respectively. The quails were fed for only 5 days (!) on diets which included 20% unprocessed meal. As in the first rat study and the first trout study, GT73 was co-mingled with GT200. The study is therefore not considered by EFSA.

A follow-up experiment was conducted, again for only five days. Despite the narrow timescale, the second study did reveal that the
"…quails in the GT73 group exhibited a slight reduction in body weight gain during the exposure period (day 0 – day 5). However, there was no reduction in body weight gain for the entire test duration (day 0 – day 8)." (EFSA 2004)

EFSA concludes:
"All birds appeared normal when the study was terminated three days later."

So the GT73 quails suffered weight loss during the time they were fed GT73, and only recovered in the subsequent three days when they were no longer fed GT73. Yet the findings are ignored by EFSA in all three opinions.
Proper results of the study would have been of high interest as, unlike in most of the other feeding studies, unprocessed GT73 oilseed rape meal was used.

2. 5. Chicken study (2004)
Rapidly growing broilers were used to compare diets containing GT73 oilseed rape with the parental and six commercially available oilseed rape varieties (Taylor et al., 2004). According to EFSA:
"No significant differences were observed in the performance parameters (growth, carcass fat pad, breast meat, thighs, legs, wings, chill weight; percentage of moisture, protein and fat in breast or thigh meat) between the GT73 and parental oilseed rape groups."

As for the cultivation of the RR canola, the published study states:
“Oilseed of glyphosate-tolerant (Roundup Ready event RT73) and non-transgenic control canola varieties was commercially produced and harvested in 1999 in Manitoba, Canada.”

A technical report by Monsanto reveals that the non-GM canola varieties used for this study were contaminated with the RR gene construct (Monsanto report 2000-01-43-10, document available at Testbiotech). According to Monsanto,
“this finding was not unexpected given the prevalence of commercial RR canola in Canada (Country of material origin).”

It is well known that in Canada it is nearly impossible to cultivate GE free oilseed rape (canola) or even to get GE free canola seed (Friesen et al 2003).

Furthermore, the animals were fed very large amounts of both corn and soy. It’s not mentioned whether these were GM varieties, containing the same GM genes as the GM canola. Also in this study, the pigs’ meals were prepared at different temperatures.
It’s evident that this study is flawed and can’t be considered as proof for the safety of GT73.

3. Other studies relevant for safety considerations
Apart from the nutritional studies, there is at least one other study that should have been considered by EFSA.

In a study by Sharma et al. (2006), fragments of GM canola DNA (CP4 EPSPS transgene) were found in the digestive tissues of sheep and pigs and in the liver and kidney of pigs. In none of its opinions regarding GT73 does the GMO panel refer to this important study and its implication for animal or human safety.

4. Conclusion
The results of animal feeding studies are substantially flawed by the design (duration, number of animals, choice of parameters and controls) as well as by contaminations.

Some of the studies show some significant differences between GE and conventional rape seed. These effects might be comparable to effects observed with a GE pea expressing an amylase prohibitor: Several studies in pigs, sheep, rats and poultry did not reveal severe health reactions, but only some differences in weight gain (several parameters), resulting in some unclear overall outcomes regarding health risks. Only targeted studies on immunological reactions of mice revealed a substantial health risk of these GE products (see for review Valenta&Spök, 2008). In the case of GT73 several questions were raised by member states concerning immunological reactions and the GOX protein. There were no testing with wholesome GE oilseed GT73 to investigate potential immune reactions.

Other open questions relate to compositional analyses and the residues of glyphosate. As far as we can see, the impact of GE rape seed sprayed with glyphosate was not investigated thoroughly, but would be relevant in the light of some recent studies (Benachour et al, 2008).

Testbiotech concludes that health risks of GT73 have to be subjected to further investigations before any market authorisation for food and feed can be given. The opinion of EFSA should be rejected by the EU Commission.

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